Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Review on the Treatment of Urinary Tract Infection without the use of Antibiotics

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Received: 26 Dec 2019 Revised: 28 Jan 2020 Accepted: 02 Mar 2020

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ABSTRACT

Antibiotics plays crucial role in curing various diseases in effective ways by showing fastest results but it gives some negative impacts also. So as 'prevention is better than cure' replacing them by some herbal or products which are alternatives of antibiotics, can be a savior for all. In today's busy scheduled life no one have the patience to wait for the diseases to cure slowly but in effective and no risk manner that's why the nature after being the biggest storage room of natural medicines is ignored. According to most research, the pH of urine is a large factor in how hospitable the urinary tract will be to the bacteria that causes a UTI. Because not only our diet but also cleanliness of bathroom or toilets, washing clothes perfectly and the way in which the urinary tract is nurtured , directly affects the pH of our urine. We can manipulate this balance to be beneficial for UTI fighting compounds already present in the body by maintaining the ph. A healthy pH is often a high one that allows the "bad bacteria blocking" compound siderocalin to do its job effectively. Polyphenol, a type of antioxidant, also help this "blocking" process starting all the way back in the digestive system. Keeping watch the general alternatives of antibiotics using simple home and herbal remedies, having Foods and drinks high in antioxidants and with a slight acidic taste can help keep everything in check. The outcomes of this work can help more efficiently for the people belongs to the rural places and also these processes will play the lead role to spread awareness in under developed areas of the country .

Keywords: - Alternatives of Antibiotics, pH of urine, UTI, siderocalin, polyphenols, antioxidant, blocking.

INTRODUCTION

Urinary Tract Infection (UTI)

UTI can be defined as infection in any part of the urinary system, which includes kidney, ureters, bladder and urethra. When it affects the lower urinary tract it is known as cystitis (Rane A, 2013) due to which the person might feel like need to pee a lot and hurt when peeing. Lower belly pain and bloody urine can also be seen. When it affects the upper urinary tract it is known as pyelonephritis, which can cause fever, chills, nausea, and pain in upper back or side. Another type of UTI, which can cause a discharge and burning when peeing, is urethritis, occurs when it affects urethra. (WebMD, 2019)

Alternatives of Antibiotics for Treating UTI

Antibiotics, also known as anti-bacterial, are substances produced by bacteria mostly and some fungi that have the ability to destroy or slow down the growth of bacteria. (MedicalNewsToday, 2019) Antibiotics constitute a range of powerful drugs and are used to efficiently treat diseases caused by bacteria. Since antibiotics have no effect on virus therefore antibiotics cannot treat viral infections. Antibiotics are powerful drugs that fight certain bacterial infections and can save lives when used properly. The mode of action of antibiotics is that they either stop bacteria from reproducing or destroy them before bacteria can multiply and cause symptoms; it helps the immune system in typically killing them. White blood cells (WBCs) attack harmful bacteria and, even if symptoms do occur, the immune system can usually cope and fight off the infection. Sometimes, however, the number of harmful bacteria is so high that our immune system cannot fight them all; in this case the role of antibiotics comes into play. (Medical News Today).

The commonly prescribed antibiotics for treating UTI are Bactrim, Amoxicillin, Amphicillin and Cipro (Bally, 2017).Antibiotics are an effective treatment for UTIs, since UTI is a kind of bacterial infection... However, our body can often resolve minor, uncomplicated UTIs on its own without the help of antibiotics. By some estimates, 25–42 percent of uncomplicated UTI infections clear on their own. In these cases, people can try a range of home remedies to speed up recovery. (MedicalNewsToday, 2018)

REASON FOR AN ALTERNATIVE OF ANTIBIOTIC TO TREAT UTI

While antibiotics can usually treat UTIs quickly and effectively, there are many cons of using antibiotics like people can be allergic to them, and their use can carry certain risks. For instance, an estimated 22 percent of the women receiving treatment for uncomplicated UTIs develop a vaginal *Candida* infection, which is a type of fungal infection. (Medical News Today, 2018). Other side effects of antibiotics as UTI treatments include: nausea and vomiting, diarrhea, rash, headache, abnormal liver function tests. More severe risks of using antibiotics include creating stronger resistant strains of bacteria.

Over time, some species of bacteria have undergone mutations and became resistant to traditional antibiotics. The major causative organism of UTI is *E. coli* and there are several species of *E. coli* that are showing increasing drug resistance. Every time we use an antibiotic, there is an increased risk of the bacteria developing resistance to it because of its adaption to survive by mutation. This is even more likely to happen when we do not follow the doctor's instructions to complete the full prescribed course of treatment. As a result, doctors are trying to reduce the use of antibiotics, especially when other treatments may be effective or when illnesses can resolve on their own. (Medical News Today, 2018).

Another severe risk of using antibiotics is it damages the good bacteria also. The human body contains a community of bacteria, viruses, and fungi that live harmoniously and help with bodily functions. Antibiotics may destroy some of these bacteria, which could increase the likelihood of occurrence of other infections. (Medical NewsToday, 2018). To take the place of use of antibiotics there are a lot of alternatives, which can be more effective in treating and preventing UTIs in a no or a less risk manner. They are easily available and easy to follow, some of which are helpful intreatment and some which may be helpful in control of the most common deadliest diseases UTI.

Phytoproducts

As the name suggests, phytoproducts are the products that are derived from the plants. Since plant and plant products are easily available and have a very little or no risk they can be the most effective and an easy alternative in treating and preventing UTI. There are many phytoproducts that have been attractiveoptions for treating and preventing UTI. Some of them are discussed below.

Berry Products

Cranberries are a group of evergreen dwarf shrubs and they are the member of healthy family and are related to blueberries, bilberries and lingberries. Cranberries in a form of juice or tablets are widely used and even sometimes it is taken as a whole which helps in prevention of UTI .Now the question arises how it prevents, the answer to this is, the mechanism of action of cranberry includes inhibition of bacterial (mainly *E. coli*) adhesion to uroepithelial cells (Sobota, 1984). When the adhesion is blocked, bacteria are not able to invade the mucosal surface of the urinary tract, thus it helps in preventing UTI. In a study conducted by Singh et al. in a group of patients, after 12 weeks of receiving cranberry extracts, when compared to placebo, bacterial adhesion decreased. Cranberry extracts were also superior to placebo in terms of urine pH reduction and prevention of UTI symptoms such as dysuria, bacteriuria, and pyuria (Singh, 2016).

In another Cochrane Review, the authors concluded that cranberry juice did not decrease the number of UTIs and thus cranberry has no significant benefit in preventing UTI (Singh, 2016). Thus there has been always different opinion of the use effectiveness of cranberries in preventing UTI. In another Cochrane report (Jespon, 2008), some evidence was found that cranberry juice may decrease the number of symptomatic UTIs over a 12-month period, particularly for women with rUTIs. Its effectiveness for other groups was less certain. Since then, new clinical trials on cranberry products have been conducted to find out the effectiveness of cranberry in preventing different types of UTI. Another group led by Foxman.Assessed the therapeutic effects of cranberry juice and the risk of UTI after gynecologic surgery wherein the patient was catheterized. In this randomized, double-blinded, placebo-controlled trial, 160 patients received cranberry capsules or placebo. The results showed that in the cranberry treatment group, the incidence of UTI was significantly lower than in the placebo group. Furthermore, among women after elective benign gynecological surgery with catheter placement, the use of cranberry capsules reduced the risk of UTI during the postoperative period by half (Foxman, 2015).

Maki *et al.* estimated the effect of cranberry juice consumption on the occurrence of UTI episodes in women with a recent history of UTI. What they did was, for 24 weeks, 185 women received 240 ml of cranberry juice, while another 185 women were given a placebo beverage. The results showed that 1 in 3.2 incidences, clinical UTI was prevented through cranberry intervention (Maki K C, 2016). Another group of workers i.e. Takahashi et al. also conducted a randomized, double-blinded study and demonstrated that cranberry beverage is superior to placebo in terms of UTI prevention, but this was only observed in a group of female patients over 50 years of age. They concluded that the efficiency of cranberry products still remains controversial since it reduced the risk of only certain kinds of UTI and that to only in a limited population (Takahashi S, 2017). In contrast, cranberry juice did not significantly reduce UTI risk compared with placebo in a study conducted by Stapleton et al. in which 176 premenopausal women with a

recent history of UTI were randomized (120 to cranberry juice and 56 to placebo) and followed up for a median of 168 days, although a trend of protective effect was observed in this study (Stapleton, 2012)

The overall results suggest that cranberry products may be an option (since its use is controversial) for prevention of UTI in healthy, non-pregnant patients, as well as in patients after gynecological surgery when a catheter was placed. However, these findings still need confirmation because the conducted studies did not involve a large enough number of participants. Lysat*e al*, in their meta-analysis on cranberries and UTIs stated that a number of publications may report conflicting conclusion due to the fact that recommendations are mainly directed to women with rUTI and that they include outcome from various populations which also can cause differences in the results (Liska, 2016).

Bearberries or *Arctostaphylos uva-ursi* is an herb that is used to treat UTI, contains tannins and has antiseptic properties to treat recurrent UTI of lower urinary tract and provides relief from the symptoms. In a double blind placebo study of 57 women with recurrent UTIs,it was seen that this diuretic astringent which can also heal the mucous membranes of the urinary tract can only effectively work in the starting phase of infection and suppress further infection. These berries can work for irritable bladder, bacterial vaginitis and Ulcerative cystitis.But this should not be taken for longer period of time as it can have major side effects like nausea,cancer,indigestion and death. Therefore it's not given to people who suffer from any kidney or liver diseases, pregnant women and children.

Blueberries are rich in antioxidants. These antioxidants neutralize the free radicals which can hence prevent many life threatening diseases such as cardiovascular disease and cancer. Blueberries help with urinary tract infection. Just like cranberries, blueberries also prevent the adhesion of bacteria to the uroepithelial cells. They also help with the symptoms of UTI. According to a previous search, 9 trials of cranberry products were able to reduce the chances of symptomatic UTIs in 12 months compared with placebo or control but relevant results for blueberry were not identified.

Herbal Therapies

Most of the medicinal herbs have antibacterial property that makes them ideal to treat urinary tract infections. Some herbs work by interfere with the attachment of bacteria to the epithelial lining. This has been shown by *Agropyron repens* and *Zea mays.* In a study conducted by Rafsanjany *et al*, it was shown that instead of acting on the bacteria, the plants, java tea, birch and nettles act on the host cell to lessen the adhesion of the bacteria. *Lactuca indica* prevents the bacterial uptake. (Lüthje , 2016). In a study, under in vitro conditions, herbal preparations exhibited antibacterial activity against uropathogens. A clinical trial was conducted using a preparation containing *Armoracia rusticana* and *Tropaeoli majoris* and it was found tohave a prophylactic effect on recurrent UTI. (Lüthje , 2016). *Moringa oleifera* also has inhibitory effect against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Proteus mirabilis* (Goel , 2015).

The leaves and stamen of *Nelumbo nucifera* has antibacterial property. The flowers of this plant work against gram negative bacteria that cause UTI. The leaves, flower and rhizome possess antioxidant properties. It also has anti-viral properties in its seeds and leaves and can be used to treat herpes zoster virus. (Goel , 2015). *Nymphea nouchali* is a safe choice as it has no toxicity and has antibacterial properties. It works against *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Xanthomonas campestris*, *Streptococcus mutans*, *Staphylococcus aureus*, *Lactobacillus casei*, *Lactobacillus acidophilus* and *Bacillus subtilis.* It also has anti-inflammatory property (Goel , 2015).

Agathosma betulina: Leaves of this herb are used to treat inflammation in genitourinary tract and is also used for urine that is unusually acidic as well as for incontinence. Due to the essential oil present in the herb, it possesses antiseptic property. The underside of the leaves contains oil glands that has diosphenol and monoterpene that helps in treating UTI. It is also a disinfectant for the urinary tract (Geetha , 2011). *Echinacea purpurea:* It has anti-inflammatory property

and provides relief against the burning sensation that occurs with UTI. This is either taken as a capsule or tea or tincture. This can have side effects so it is supposed to be used with caution (Geetha , 2011)

Equisetum arvense is effective in eliminating kidney stones. It has diuretic property which is attributes to it containing equisetonin. Due to its diuretic property as well as being astringent and having tissue rebuilding properties, it is used to treat UTI (Geetha , 2011). *Taraxacum* is a diuretic herb that is rich in antioxidants, vitamins and minerals. The leaves and roots of this herb are dried and taken as coffee or tea. This improves the immune system (Pulipati et.al. , 2017). Golden seal is a natural method of treating UTI. This is due to the presence of an alkaloid called berberine which has antibacterial property. It reduces the attachment of the bacteria to the epithelial cells of the urinary tract. It can either be used as an antiseptic or ingested in the form of tea (Health communities)

FRUITS

Pineapple

It is used to lessen the symptoms of UTI. This is possible due to the presence of an enzyme called Bromeliad that is anti-inflammatory. It is recommended to take pineapple juice. Canned pineapples are discouraged as it contains preservatives (Pulipati et.al. , 2017).

Grape Fruit

The seeds of grapefruit have antifungal, antibacterial and antiviral properties. The grapefruit seed extract which is derived from the pulp and seed of grapefruit has antibacterial property similar to synthetic antibiotics. This is effective against both gram negative and gram positive bacteria and is broad spectrum in its activity (Pulipati et.al. , 2017)

Onion

Onions have antibacterial property. In a test it was shown that onions work against *pseudomonas aeruginosa* which is multi drug resistant and one of the common member of *Pseudomonas* that is responsible for causing UTI. They tested this by creating a mixture of onion extract with hydrogen peroxide (Hamad et.al. 2016).

Garlic

Garlic has antiviral, antibacterial and antifungal properties. This is due to the compound diallyl-thiosulphate or allicin and sulfur containing compounds. Interstitial cystitis is a chronic condition that causes bladder pain. Garlic has antioxidant, anti-inflammatory and immune modulatory effects that help in the treatment of this condition. Not many studies have been done over the impact of garlic on UTI, but one study showed that in an experimental set up of UTI model; garlic had a significant impact in the attenuation of the virulence of *Pseudomonas aeruginosa.* Garlic is used for non-E.coli UTI (Mansour et.al, 2014).

SPICES

Ginseng

Ginseng is an herb that contains anti-microbial activity. It is anti-cancerous, anti-inflammatory, and has immune-modulatory effects. Ginseng contains several components that vary according to the year of cultivation and the processing method used, such as heating, drying, and steaming, which induce different degrees of pharmacological activities. It is effective against many UTI causing bacteria and is specifically effective against *pseudomonas aeruginosa* which is one of the common causative agents of UTI (Kim et.al, 2018)

Clove

The essential oil of this is used to protect against yeast infection and is also used for UTI. It has anti-inflammatory properties and works quickly. It is also used to improve immunity. (Pulipati et.al. S, 2017)

Oregano

This plant ha antibacterial properties and is used in the form of essential oils. The antibacterial property is due to the presence of caracole which is a monoterpene. It inhibits the growth of *Escherichia coli* and *pseudomonas aeruginosa*. It is also effective against infections caused by *Streptococcus sp.* (Pulipati et.al., 2017).

Cinnamon and Coriander Seed

Being an anti-inflammatory, anti-oxidant, anti-microbial, anti-diabetic and anti-tumour agent cinnamon can work against *Staphylococcus aureus* and *E.coli*. It reduces the inflammation causing pain during urination. Three tablespoons coriander, boiled in water, drink as tea, once per day can stops bacteria breeding. It's a cooling herb which helps to reduce painful urination (Biovitallabs et.al, 2015)

Herbal Therapy with Canephron N

Another alternative of non-antibiotic approach to UTI treatment is the herbal therapy i.e. which involves administration of herbs and its products .The most common example of herbal therapy is a product named Canephron N which have already been approved in many countries . This product contains century herbs, lovage roots and rosemary leaves. The main properties of Canephron –N are that it has diuretic, spasmolytic, anti-inflammatory, antibacterial and nephron-protective properties, and is also considered to be safe in both pregnancy and during breastfeeding, unlike cranberry. (Naber.et.al, 2013).

The efficiency of Canephron N in the preventing UTI in high-risk female patients those who were undergoing urodynamic studies (UDS). Therein, women with at least one risk factor for UTI received those after UDS either 3 g of fosfomycin trometamol (FT) single dose or 5 ml of Canephron N taken orally three times daily for 1 week. There was no statistically significant difference in UTI incidence between the two groups. Since there was no statistical difference in UTI incidence between the groups, the authors came to a conclusion that prophylaxis of UTI after UDS with this phyto-drug (Canephron –N) may be a good alternative to antibiotics administered after UDS in high-risk female patients (Miotla et.al, 2018).

A double-blinded, multicenter, non-inferiority study comparing the efficiency of UTI treatment with Canephron N to standard antibiotic therapy with fosfomycin trometamol (FT). A large cohort of female patients with symptoms of acute uncomplicated lower UTI was randomly allocated into two groups receiving either Canephron N or 3grams of fosfomycin trometamol (FT) single dose with corresponding matched placebo. The results were very promising, as only 16.5% of patients treated with Canephron N required additional antibiotic treatment as compared to 10.2% in the fosfomycin group. This implies nearly 83.5% of the patients treated with the phyto-drug didn't require the further administration of antibiotics. An additional advantage of using Canephron N, fewer gastrointestinal side effects, such as diarrhea and abdominal pain, were observed. Unfortunately, in the group that was given the phyto-drug however, five episodes of pyelonephritis occurred (mainly during the first days of therapy), as compared to one episode of pyelonephritis in the fosfomycin group (Wagenlehner,et.al. 2018).

Essential Oils

Fighting UTIs with essential oils can be tricky. Using a diffuser to inhale essential oils is the recommended method. The urinary tract is normally a sterile area, so you don't want to introduce anything foreign into the area. If you choose to apply essential oils, you must dilute them before you apply them to your skin. To dilute an essential oil, place 1 to 5 drops in 1 ounce of carrier oil. Carrier oils include: sweet almond oil, coconut oil, sunflower oil ,olive oil. Essential oils should not be applied to the mucous membranes of the vagina or urethra. This can irritate the female

parts (Healthline,et.al. 2017). Essential oils shouldn't apply essential oils directly to the skin; always dilute them in carrier oil. The mixture of essential oil and carrier oil can be applied to areas around the inner thighs, moons- pubis, and outside the labia. You can also try blending a few of your favorite oils and using them in a hot compress placed on your lower abdomen. To do this, dilute one drop of essential oil with one drop of carrier oil. You can use essential oils dropped into a diffuser for inhaling. Essential oils are meant to be inhaled in aromatherapy.

One essential oil that's helpful for any type of bacterial infection is a blend from Young Living called Citrus Fresh. This oil blends many different types of citrus oils, including orange peel, tangerine peel, grapefruit peel, lemon peel, and spearmint leaf extract. The blend of citrus oils is a powerful anti-bacterial agent. Tea Tree Oil is antibacterial and is used to treat UTI and also helps with the pain associated with it. This is not ingested and instead is mixed in bath water that cleans the urethral opening. It can also be rubbed on the abdomen and can be used by mixing it with sandalwood oil (Pulipati et.al., 2017)

Treatment Using Common Ingredients

Water

Drinking water is a cheap and safe method of treating UTI. Increased fluid intake prevents cystitis due to the decreased attachment of the bacteria to the uroepethilium and by reducing nutrients required for its growth. Water dilutes the urine. It cleanses the bladder by flushing the bacteria out of the urinary tract and retains the electrolytes and vital nutrients (Hooton et al, 2018; Medical News Today). A study conducted on participants with urinary catheters found that if output of urine is low then it increased their risk of getting UTI. It is recommended to drink 6 to 8 ounce glasses of water a day (Medical News Today).

Baking Soda

This is a household ingredient used mostly in baking. It works by neutralizing the acid in the urine. Drinking of 1/2 to 1 teaspoon of baking soda with water in empty stomach can neutralizes the acid present in urine. By detoxifying kidney it prevents damage and spreading of infection. Despite being natural, it can also be dangerous and cause brain damage or brain bleeding. An overdose of baking soda can cause nausea, abdominal pain and vomiting. So the recommended doses should be taken by the person (Healthline,et.al. 2017)

Apple Cider Vinegar

Apple cider vinegar is the vinegar produced from apple juice after fermentation. It is rich in enzymes and minerals like calcium, magnesium, potassium and iron. It is a highly acidic liquid that is excellent for balancing out the pH of our body. UTI infection usually occurs in non-acidic areas, so to make it unwelcomed in body, a glass of water with tablespoon of apple cider vinegar is recommended. It can prevent the bacteria to multiply and grow within urinary tract. A study conducted in 2018 showed that apple cider vinegar can inhibit the growth of *E.coli* but this test was conducted in petri dishes and not on humans. (Women's Health, 2018). Besides that, the immune system also becomes stronger due to increase in body detoxification.

Vitamins

Vitamin C (ascorbic acid) is found in a variety of food items and is also sold as a nutritional supplement. As a non-antibiotic prophylaxis for UTI, vitamin C supplementation has two action mechanisms i.e.;- urine acidification and a bacteriostatic effect ,which is mediated by reduction of urinary nitrates into reactive nitrogen oxides but its protective value on UTI in non –pregnant patients is limited, hence should not be promoted.Vitamin D is fat soluble and is obtained from exposure to sunlight. There are very few foods that provide vitamin D. It acts as an inducer of antibacterial innate immune responses. It affects and prevents UTI mostly found in case of males.Vitamin E is also fat soluble and is known to repair damaged cells. Administration of vitamin E improves the symptoms in acute phase of UTI and hence is recommended to be taken from the start.

Probiotics

Probiotics include the live micro-organisms (bacteria and yeast) that provide health benefits when consumed.These beneficial bacteria are mostly *Lactobacillus sp* and *Bifidobacterium sp.* They can be obtained from yoghurt, fermented food and supplements in the form of powder, capsule or tablet. The probiotics need to be resistant to bile acid and gastric acid so that they can reach the intestines and produce the beneficial effects. The vagina is a potential reservoir for the bacteria causing UTIs, so probiotics can be used in treating as well as preventing UTI. Changes in the micro-biota of vagina, with reduction of colonies of protective *Lactobacillus* spp. are associated with an increased risk of UTI. These changes can be induced by antimicrobial therapy, hormone level changes due to menopause or usage of contraceptives and even by UTI itself (Stapleton, et.al. 2016). The use of either oral or intra-vaginal probiotics restore the natural vaginal micro-biota and thus in this way it helps in treating UTI. Therefore, probiotics seems to be a promising approach to reducing antibiotic consumption and to decreasing antimicrobial resistance. They inhibit the growth of the uropathogens by binding to the uroepithelial cells and also secrete biosurfactants.

In a double-blind, non-inferiority trial conducted by Beerepoot *et al.* 252 postmenopausal women with UTIs were randomized to receive 12 months of either antibiotic prophylaxis with trimethoprim-sulfamethoxazole or oral probiotic capsules containing 10^9 colony-forming units of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14. The results of the study showed that supplementation with 480 mg *L. rhamnosus* GR-1 and L. reuteri RC-14 significantly decreased the mean number of recurrences in patients with uncomplicated UTIs when compared with trimethoprim-sulfamethoxazole administration.

In another double-blinded study, Stapleton et al. investigated premenopausal women with a history of rUTIs, by giving them daily either Lactin-V (*Lactobacillus crispatus* strains) or placebo for 5 days, then once weekly for 10 weeks. Results of this study showed a significant reduction of UTI episodes in patients who received intra-vaginal *Lactobacillus* treatment, compared to the placebo group. Herein, Lactin-V treatment resulted in prolonged colonization with *L. crispatus* strains and it reduced the frequency of UTI by around 50% among participants. (Stapleton,et.al. 2011). Lactobacilli may especially be useful for women with histories of recurrent, complicated UTIs or on prolonged antibiotic use. Probiotics are safe in terms of causing antibiotic resistance and may offer other health benefits due to vaginal re-colonization with Lactobacilli. However, more comprehensive research is still needed before recommending probiotics as alternatives to antibiotics (Gupta,et.al. 2017).

Alternative Treatments

D-Mannose

D-Mannose is a naturally occurring sugar(monosaccharide) that is found in a number of fruits ,including apples, blueberries and cranberries, peaches broccoli green beans.(WebMD)The urinary tract mucus membrane is coated with proteins that interfere with the adhesion of bacteria (Caretto,et.al. 2017). D-Mannose can be rapidly absorbed and excreted by the urinary tract and can prevent the adhesion of type 1 bacterial fimbria to the euro-epithelium and these results in elimination of bacteria from the body during urination. This is a bacterial virulence factor promoting UTI—especially caused by *E. coli* (Altarac,et.al. 2014). .This sugar is the reason that cranberry is recommended for preventing UTI. (Michaels,et.al. 1983) Taking it during a time when it is felt that we are most prone to UTI, this can help in preventing the development of UTI. Commercially a lot of different D-Mannose products are available .D-Mannose is available in capsules and powdered form, and the form to use completely depends on the preference. (Healthline, 2019).

The basic advantage of using it is that even in large quantities this sugar does not cause any adverse side effects and unlike other sugars it cannot be metabolized by us so it is safe for diabetics and others who are having sugar-free diet. It is also safe for aged persons and children. It has a faster action i.e. the symptoms relief can be seen very soon as quick as the following day and nearly most of the symptoms are generally resolved after almost 48 hours of

administration of D-Mannose. Kranjcec *et al did a case study*. The results of the case study showed that the risk of UTI was significantly reduced by D-mannose with a low risk of side effects. The authors therefore concluded that D-mannose may be useful for UTI prevention, although more research is still required (Kranjčec, et.al. 2014).

Nonsteroidal Anti-Inflammatory Drugs (NSAID)
NSAID are members of a drug class that reduces pain, decreases fever, and prevents blood clots and in higher doses decreases inflammation. (Bally, 2017). Prostaglandins are the chemical that are produced by the cells of the body and have many important functions. One of them is, they promote inflammation that is required for healing but it also results in fever and pain. The other functions include it support the blood clotting and protects the lining of the stomach from the damaging effects of hydrochloric acid.

Prostaglandins are produced within the cells by the help of the biocatalyst cyclooxygenase (COX). NSAIDs block the COX enzymes and reduce prostaglandins throughout the body. As a result, inflammation, pain and fever are reduced. The symptoms of UTIs are mostly connected to the inflammatory reaction of the urinary tract due to a significant increase in urinary prostaglandin production (Farkas, 1980). Since NSAIDs can inhibit the biosynthesis of prostaglandins (Vane, 1998), they can be useful in alleviating the symptoms of UTI. The most common NSAID for UTI are Diclofenac (Healthline, 2019). However, it is still not clear whether they can replace antibiotics in the treatment and/or prevention of UTIs.

Replacing antibiotics with NSAIDs for the treatment of uncomplicated UTI may be at the cost of prolongation of symptoms and increased risk of pyelonephritis. Due to that fact, in uncomplicated UTI it is preferable to delay the use of antibiotic while closely monitoring the patient rather than completely resigning from antimicrobial treatment. In this strategy it is also important to share a decision-making process with a patient and to know his/her expectations towards the treatment (Kronenberg, 2017). Thus, NSAIDs have been studied only as a treatment option for UTIs, but not for prevention of rUTIs.

Hormonal Treatment

Estrogens
Estrogen , the female hormone is not only responsible for regulating the female reproductive system, but also for stimulating the proliferation of lactobacilli, reducing vaginal pH and decreasing vaginal *Entero bacteriaceae* colonization (Caretto, 2017). Estrogen enhances the antimicrobial capacity of the uro-epithelium, it inhibits bacterial multiplication and by strengthening the epithelial integrity, it prevents bacteria from reaching deeper levels of the uro-epithelium (Luthje, 2013) In post-menopausal women with low estrogen levels suffer from recurrent infections because there is changes in the urinary tract which make it more vulnerable to infection, so stimulating these mechanisms is essentially beneficial in such cases. Hence, it is suggested that the use of topical estrogens can reduce the risk of recurrent infections. However, the efficiency of estrogens in UTI prevention still remains disputable.

A literature shows the reduction of UTI incidence after topical intra-vaginal estrogen use (Raz, 1993; Eriksen,1999) and failure in restoring vaginal micro-biota and lowering UTI risk (Raz, 1993; Brown, 2001; Jackson, 2004). In the 2008 Cochrane Review on estrogens for preventing rUTIs in postmenopausal women, the authors concluded that vaginal estrogens reduced the number of UTIs in postmenopausal women with rUTIs, but the results depended on the type of estrogen and the duration of treatment (Perrotta, 2008). No new clinical trials on vaginal estrogens have been conducted within the last years. Moreover, oral estrogens are considered to be ineffective in UTI prevention and they are connected to a vast number of adverse effects such as breast tenderness or vaginal bleeding (Simon, 2018). However, recently, an oral estrogen agonist/antagonist, ospemifene, was introduced. It is intended to be used to treat moderate-to-severe dyspareunia due to vulvo-vaginal atrophy and it has no major adverse effects on the breast, bone or cardiovascular systems of patients. Hence, ospemifene could be a promising new non-antibiotic option for UTI

prevention in postmenopausal patients, especially in those with additional vulvo-vaginal atrophy (Schiavi, 2018). However, no valid data on its possible preventive effect are available at present. Further well-structured research on the use of ospemifene in UTI is, therefore, needed.

Immunotherapy

To avoid the problems related to overuse of antibiotics for recurrent UTI, immunostimulation with bacterial extracts has been shown to prevent recurrent UTIs and to be very safe (Meredith,et.al. 2009). In immunotherapy, bacterial extracts are used in the management of UTI because they are able to stimulate the host's immune system through the activation of monocyte-derived dendritic cells to produce antibodies and cytokines (Schmidhammer,et.al. 2002). The bacterial extracts can be an fascinating option for prevention of UTI because they can be used an alternative to antibiotic prophylaxis in patients with UTI since antibiotics often only provide a palliative treatment which is effective against the acute episode, but may not always be able to prevent the disease from recurring or becoming chronic.

The predominant pathogen in both complicated and non-complicated UTIs is Escherichia coli, which is responsible for about 80% of UTIs, followed by *Staphylococcus aurous*, *Proteus mirabilis* and *Klebsiella sp.* (Meredith, et.al. 2009). The biotechnology-derived bacterial extract OM89 is an immunoactive product which has been manufactured from 18 selected and standardized strains of Escherichia coli is an oral immunotherapeutic drug on the market for UTIs with documented Phases II and III clinical trials. Strains are cultured separately in industrial fermenters, killed and fractionated. The extract is then purified and lyophilized to provide the final product. By carrying out the SDS-PAGE it has been found that the product consists mainly of acidic proteins, peptides and amino acids., OM-89 contains in minor quantities other components, i.e. hydrolyzed and thus detoxified lipo polysaccharides molecules, lipid a molecules, sugars and fatty acids which could also be responsible for its activity. (Meredith, et.al. 2009).

Uromune is another bacterial extract with a potential benefit when used in UTI management. Uromune is a sublingual spray consisting of inactivated bacteria—*Escherichia coli, Klebsiellapneumoniae, Proteus vulgaris, and Enterococcus faecalis.* The mechanism of action of Uromune is based on a theory that stimulation of the sublingual mucosa will lead to an activation of an immune response in the urinary tract mucosa (Yang,et.al. 2018)

DISCUSSION

The repeated use of antibiotics leads to antibiotic resistant strains which become difficult to cure. So to lower the use of antibiotics, a search for alternatives was done. With the development of medicine science we are ignoring our ancient medicinal remedies which are beneficial to cure a disease with no risk manner. May be antibiotic medications are effective as compared to any treatments but still as a root is the main part in growth and development of a plant, every disease has it's own medication formulae. Going through the whole studies, it can be established that antibiotic alternatives can may be the effective way to treat several diseases. The products produced from plant or plant parts i.e:- by using berry products which have diaphoretic, astringent and antiseptic properties the treatment of UTI can be reduced or cured. Medicinal herbs and spices, which can found easily in home or around the nature has the greatest role in treating this disease. Not only the phytoproducts but also some products made from chemicals, vitamins are also plays vast roles. Irrespective of them all daily diet and nutrition, maintaining personal hygiene , taking care the way of clothing, everyday urination habits and birth controlling by spreading awareness in the use of spermicidal contraception , avoiding diaphragm and spermicidal condoms ,the mostly found deadliest disease can be controlled. Obtained formulas from this study may be an effective solution for the people who are suffering or who are on the way to suffer

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Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Selection of Die Casting Process Parameters using Artificial Neural Network and Taguchi Method

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Received: 31 Dec 2019 Revised: 03 Feb 2020 Accepted: 05 Mar 2020

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ABSTRACT

Improvement of the procedure parameters of the Aluminum die casting process is discussed in the present paper. The quality issue during the process was porosity and the potential components that cause it are recognized by reason impact investigation. A variance analysis (ANOVA) is aimed at discovering the components that have huge impacts on porosity. Pressure of plunger used in die casting system and the liquid aluminum temperature are determined as significant factors after study. Such process parameters and porosity are then modeled and fitted with the Artificial Neural Network (ANN) back propagation to forecast or monitor output parameters by optimizing the input parameters.

Keywords: ANOVA; ANN; Die Casting; Taguchi.

INTRODUCTION

The selection and optimisation of the manufacturing process is always considered to be an important task to improve the performance of the product and to achieve several other objectives. This territory of research has increased developing consideration in many assembling associations in the course of the most recent couple of decades. The work was done to simplify the process of die casting and to improve casting efficiency. Using soft computing technologies and Taguchi techniques is an effective way of achieving optimization. Complexity relation between parameters, the better ability is to use soft computing devices. For this purpose, the ANOVA and Taguchi methods can be used to define and pick these process parameters and also to find the optimum level of the selected parameters. The present work follows a joined methodology of choosing the noteworthy variables for the quality issue the porosity of the die cast components and the back propagation of the ANN modeling, in order to optimize the operation.

The paper was broken down into four sections. The first section presents the context of the problem and the related literature in the field of metal casting soft computing applications and ANOVA-based optimization. The second part deals with the objective of the work, relevant details on the case study and identification of significant factors through ANOVA. In part three, the advancement and preparing of ANN with gathered information from the case issue is exhibited alongside examination and enhancement of the issue. At last the ends got from the present work have been displayed. The primary advances are as per the following:

- To define and pick the parameters of the casting process using the variance analysis approach and the ANN
- Build a predictive density model using selected parameters from the Taguchi method with the aid of the MATLAB Artificial Neural Network.
- Study of the regression to compare with the performance of the ANN.

Application parameters for die casting

The cause and effect diagram designed and shown below is specific for the casting process which takes place in the ALCAST Ltd, Ranchi.

(a) Similar to casting system die parameters.
(b) The shot sleeve parameters were related to.
(c) The parameters to which it refers. Parameters concerning cast metal.

Problem Identification and ANOVA

The experimental data were obtained in 1978 from an ISO-accredited production unit dealing with components of aluminum ammunition for defense area. They die cast using cold-chamber machines before machining the components to the desired specification. To analyze and investigate the interrelationships between the different die casting parameters, with machine having suitable data acquisition, monitoring and control system.

Orthogonal Array

Orthogonal Array (OA) is a factorial matrix that confirms an equilibrated comparison of any variable or interaction factor. Many designed experiment use orthogonal arrays to find the factor level combination for use and analyse the data for each experiment. It is a matrix in which rows represent the factor amount in respective run, and column represents a different factor which can be modified from each run. This array is known as orthogonal, since all columns can be assessed separately. The assignment (design) methods for orthogonal array experiments and calculations are related with data analysis. Table 1 represents various parameters and its value at particular level.

Requirement for Orthogonal Array

An L8 orthogonal array with one test per trial (four tests versus four tests) makes the experimenter 90% sure (confident) of detecting a change of approximately.

Experimental Data collection

The experimental process is conducted using a measuring cylinder. Water is poured into the measuring cylinder and the initial reading is noted down. Now the work piece is immersed into the cylinder. The water level rises. We note the final water level reading. Now we subtract the initial reading from the final reading to get the volume of the work piece. The density is calculated by dividing the mass of the work piece by its volume.

$$\text{density}, \rho = \frac{\text{mass}(m)}{\text{volume}(v)} \quad (1)$$

The volumes of eight specimens are found out by using the measuring cylinders and subsequently their densities are found out. The table 3 given below shows the readings. ANOVA of Taguchi L8 OA: The OA is carried out by measuring the numbers of squares for each column. Factor A, B can be allocated to first and second column respectively of the L8 OA. The sums of squares for factor A, column 1 is

$$SS_A = \frac{(A1-A2)2}{N}$$

A1 and A2 are the quantities of the data associated with the first and second stages of factor A, respectively. **Result**

Using ANOVA

ANOVA results show F-ratios for different sources. Also, standard F-values for different confidence level is also shown. It can be said with 90% confidence that level A and D are most affecting parameters. At 95% confidence level, A is the significant parameter. At 99% confidence level no parameter is found to be significant enough. For present research, 90% confidence level is enough and hence, we are selecting level A (temperature) and D (pressure) as the two significant parameters.

ANN an Optimisation method

Artificial Neural Network composed of artificial neurons and it is a basic model of ccomputing that interconnect and are the basic element that processes neural network. It is a model of data processing and it is learnt through experiences or by data preparation. Neural networks are ideal if an algorithmic solution to a problem cannot be formulated but can obtain multiple experimental result from action of the network.

Figure 2 shows forward network with multi-layer feed model and is built to optimise or predict the problem raised with density of die casting. From observation of network, two different input are temperature of melting and density of casting and output is hydraulic pressure. Since model purpose is to determine value of controlling factors for a specified density value of casting. Therefore it is used as input parameter to determine unknown hydraulic pressure. [v] and [w] represents input and output hidden vectors of weight respectively. Input and output data set are necessary for training artificial neural network found from experimental data. For future performance prediction the weight values, which provide the minimum error, are to be chosen.

Experimental Data Collection and Training

For parameter Temperature of melting, density of casting and hydraulic pressure the experimental data was collected from die casting unit. The real values obtained are standardized, so that the values range from 0 to 1. Neural network training's main function is to determine weights, which signify mapping from input to output space. Normalization of input and output parameters are required as it has different unit of measurement and will stop saturation of sigmoid function. Data collected (x) have been standardized with use of reference (2)

$$\frac{X - min.value}{max.value - min.value} \quad (2)$$

System Optimization Implementation

The experimental data were broken down into three sets to test and verify the model. Next, 70 percent of the observations are used for network training and the weight values are changed to reduce their error. A further fifteen percent of data will be considered in authenticating the generalization of the network and to break further training before overfitting. The last fifteen percent will be utilised as a fully independent ANN generalization check and it will not impact on the training and will therefore offer an objective measure of network performance. Correctness of the estimate of output by ANN is confirmed by giving the set of test data to network.

Validation

Regression analysis is used to validate the data. Experimental data on all parameters are used to investigate this, as previously collected. The aim of analysis is to provide estimated data for hydraulic pressure, casting density and temperature of melting based on data set. Regression Analysis: z versus x, y. (Using MINITAB 13.1)
Where, x, y, z represents temperature, density and pressure respectively. Regression equation is

$$z = 0.369 + 0.047\,x + 0.002\,y \qquad (3)$$

CONCLUSION

A prediction / optimization model based on ANN for an aluminium die casting process was addressed in the present work. First, the statistical ANOVA technique has been used to find major controlling factor from five which affect casting density. Then these factors will be used for ANN modeling. Hundreds of experimental data were collected and reused for training, validation and testing at the rate of 70, 15 and 15 percent respectively. To obtain proper casting density, the corresponding settings of melt temperature, hydraulic pressure can be determined from neural grid. Real data were tested to determine the predictive error. The measured values were found to be very similar to those expected values compared to the current process, many outputs can be considered to include the casting's mechanical properties for optimization during ANN simulation

ACKNOWLEDGEMENTS

The authors are thankful to ALCAST Ltd, Ranchi for cooperating in the research during the case study.

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Table.1. Casting parameters and their levels.

Destination for the Casting Parameter	Parameters	1st Level	2nd Level
A	Temperature (ºc)	605	728
B	Forward velocity of plunger (m/s)	0.03	0.6
C	Return velocity of plunger (m/s)	1.1	3.9
D	Plunger Pressure, bar	121	290
E	Cavity Filling Time(micro-sec)	35	135

Table.2. Orthogonal Array of experiment (L8 array)

Experiment	A	B	C	D	E	Ex1	Ex2
1	1	1	1	1	1	1	1
2	1	1	1	2	2	2	2
3	1	2	2	1	1	2	2
4	1	2	2	2	2	1	1
5	2	1	2	1	2	1	2
6	2	1	2	2	1	2	1
7	2	2	1	1	2	2	1
8	2	2	1	2	1	1	2

A: Temperature, B: Plunger Velocity first stage, C: Plunger velocity second stage, D: Pressure, E: Filling Time, Ex1and Ex2: Dummy Columns

Table 3. Densities of Eight samples of castings for L8 OA in gm/cm³

gm/cm³	Process1	Process 2	Process 3	Process 4	Process 5	Process 6	Process 7	Process 8
Sample1	2	2	2.226	2.064	2.318	2.141	2.064	2
Sample2	2.064	2.318	2	2.141	2.318	2.419	2.480	2.226
Sample3	2	2	2	2.318	2.064	2.141	2.318	2.226
Sample 4	2.064	2.064	2	2.226	2.141	2.480	2.419	2
Sample 5	2.064	2	2.226	2.064	2	2.419	2.419	2.480
Sample 6	2.480	2.480	2.318	2.064	2.318	2	2.141	2.419
Sample 7	2.318	2.064	2	2.141	2.480	2.495	2.480	2.419
Sample 8	2.419	2.419	2.480	2.480	2.419	2.480	2	2.480

Table.4 Die casting density ANOVA. (Using ANOVA method)

A1	69.24
A2	73.466
B1	70.671
B2	72.035
C1	72.353
C2	70.353
D1	69.949
D2	72.757
E1	71.277
E2	71.429
(Ex1)1	71.056
(Ex1)2	71.65
(Ex2)1	70.824
(Ex2)2	71.882

Table.5. Sum of square (SS)

SS_A	SS_B	SS_C	SS_D	SS_E	SS_{Ex1}	SS_{Ex2}
2.232	0.232	0.50	0.985	0.0028	0.044	0.124

Sum of square error

$SS_e = SS_{Ex1} + SS_{Ex2}$

SSe = 0.044+0.124 = 0.168

SS_T = Total sum of square

$SS_T = SS_A + SS_B + SS_C + SS_D + SS_E + SSe$

SS_T = 4.1198

Table 6. Die casting density ANOVA

Source	SS	DOF	V	F ratio	90% Confidence	95% Confidence	99% Confidence
A	2.232	1	2.232	26.57	8.53	18.5	88.5
B	0.232	1	0.232	2.76	8.53	18.5	88.5
C	0.50	1	0.50	5.952	8.53	18.5	88.5
D	0.985	1	0.985	11.726	8.53	18.5	88.5
E	0.0028	1	0.0028	0.03	8.53	18.5	88.5
E	0.168	2	0.084				
T	4.1198	7	0.588				

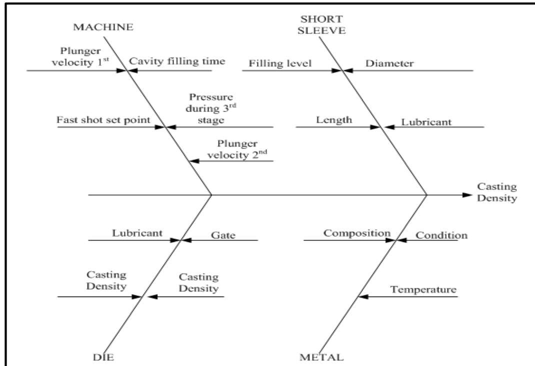

Fig. 1: Cause and effect diagram for casting process

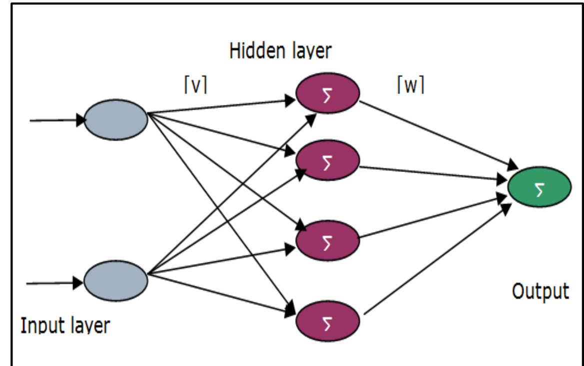

Fig.2. Optimisation of Aluminum die casting density using ANN

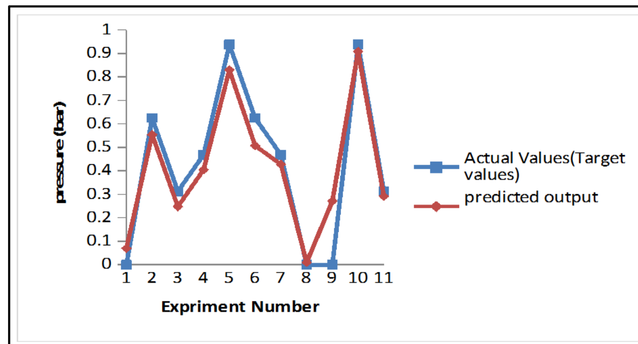

Fig. 3: Predicted Vs Actual output pressure

RESEARCH ARTICLE

Heavy Metal Concentration in Coastal Water and Sediment of TamilNadu Coast, India

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Received: 02 Jan 2020 Revised: 05 Feb 2020 Accepted: 07 Mar 2020

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ABSTRACT

The present study was carried out to determine heavy metal distribution *viz.,* Cd, Cu, Cr, Ni, Pb, Fe and Zn in costal water and sediments of the Eastern coastal region *i.e.*Chennai, Cuddalore, Nagapatinam, Rameswaram and Tuticorin Coasts of Tamilnadu. The sampling was carried out in all seasons from 2014. The present analysis showed that the concentration of cadmium, copper and chromium increased with respect to the seasons (Post-monsoon, summer, Pre-monsoon and monsoon). A high level accumulation of lead was observed in samples collected from Cuddalore irrespective of seasons. And the level of Fe and Zn found to be very high in Tuticorin in all the seasons studied. The concentration of heavy metals in the samples from the collected area indicated that the high level of heavy metal accumulation could be due to industrialization and anthropogenic activities.

Keywords: Heavy metals, Seasonal variation, Coastal Waters, Sediments, Tamil Nadu Coast of India.

INTRODUCTION

In the environment, heavy Metals are naturally occurring and vary in concentrations across the earth. Heavy metals are not harmful to the environment, because they play an essential role in tissue metabolism and growth of plants and animals. Metals like Cu, Zn, Fe, Co, MO, and Ni *etc.,* are essential and at the same time it becomes toxic when their level exceeds the limit, and Cd, Pb, and Hg are classified as toxic because of their harmful effect even at low concentrations (Michael *et al.,* 2010). The distribution of metals within the aquatic coastal environment is managed by complex processes of substantial exchange affected by various natural and anthropogenic activities (Ip*et al.,* 2007). Metals are natural components of earth and are present in all environments, but human activities have drastically altered its concentration (Guerzoni*et al.,* 1984). Since heavy metals are toxic, non-degradable in the environment, and

the contamination with sediments tends to have a great impacton coastal marine ecosystem (Gracia*et al*, 2008). Industrialization and modern developments along the coastal sidescontributes more heavy metals into the coastal environment. Also, the anthropogenic changes in the coastal environment like land reclamation, dredging and aquaculture cause the metal pollutions (Antonio and Ricardo, 2003). The majority of the industries along the coastal area discharge the chemical effluents into the aquatic environment which in turn cause changes in habitat, species distribution, and bio-geo chemical cycles. The distribution of heavy metals in the water bodies has been recognized as a major factor for biological risks (Ananthan*et al.*, 2006; Karthikeyan *et al*, 2007). Since last twenty five years, high levels of heavy metals and their compounds, both inorganic and organic, have been released into the coastal environment by anthropogenic activities (Komarnicki, 2005).

Higher heavy metals concentrations in the coastal marine environment considered to be an indicators of anthropogenic impact and potential risk to the natural environment. Therefore, it is important to evaluate the track of these heavy metals in coastal ecosystem (Gibson, 1994). The mode of transfer and distribution of toxic metals between the sediment and water columns is of great importance. Once heavy metals introduced into the aquatic environment, it redistributed throughout the water column, and finally gets accumulated in sediments (Eugenia *et al.*, 2004). Heavy metal levels in sediments helps in identifying the history and sources of pollution. Several researches demonstrated that water & sediments from coastal areas, which are greatly contaminated by heavy metals, therefore the present study was conducted to evaluate the heavy metal distribution in the east coastal region of Tamil Nadu.

MATERIALS AND METHODS

Study area
Sampling

Total of 5 stations/ coastal cities belong to Tamilnadu coasts were selected for this study. Around 20 costal water and sediment samples were collected from these selected sites in all four seasons (Post-monsoon (January to March), Summer (April to June), Pre- Monsoon (July to September), Monsoon (October to December) during the year 2014. All the sea water and sea surface sediment samples were collected from shoreline of each coastal station. Sea water samples were collected from 0 -20 cm below the sea surface water in sterile 2500 mL bottles. 250 g of sea surface sediments were collected from the shoreline of the coast by sterile spatula and were stored in sterile polythene bags (Vignesh*et al.*, 2012). All the samples, including different edible crabs were stored in ice box at 4 °C until further process. All samples were kept in ice boxes and processed within 12 h of collection. For heavy metal analysis, the water samples were acidified immediately with concentrated nitric acid and maintained the pH below 2 (Vignesh*et al.*, 2013; 2014).The latitude and longitude of sampling stations were recorded by global position system (GPS) (Model - Garmin GPS Etrex 30). The sampling stations were: Chennai (S1), Cuddalore (S2), Nagappattinam (S3), Rameswaram (S4) and Tuticorin (S5) (Fig; 1).

Trace metal analysis

One liter of water was filtered through a 0.45 μm nitrocellulose membrane filter paper and adjusted to pH 2 with HNO_3 taken in a separatory funnel. Ten mL (3% w/v) of a freshly prepared solution of amino-pyrolidine-dithio-carbamate (APDC) was added into the funnel, and the mixture was shaken by a mechanical shaker for 10 min. Furthermore, 25 mL of methyl-isobutyl-ketone (MIBK) was added to this mixture and shaken for 15 minutes. The phases were allowed to separate and top organic phase was collected. The bottom aqueous phase again shaken with 25 mL of MIBK, and the organic phase was obtained and pooled with the previous phase. The pooled organic phase was mixed with 2 mL of 50% HNO_3, and shaken vigorously for 10 min to separate the bottom acid layer (Brooks *et al.*, 1967; Vignesh*et al.*, 2012b).

Sea sediment samples were air-dried and smaller than (>) 63 µm in size were retained in pre-cleaned properly. On the other hand, the dried sediment and crab samples were crushed by agate mortar and pestle. Both the samples were treated with an aqua - regia mixture (i.e. $HCl:HNO_3$= 3:1) in Teflon bomb and were incubated at 140 °C for 2-3 days after dried and sieved samples. After incubation, the reaction mixture was filtered with Whatman No.1 filter paper. The trace metals in the sea water, sea sediment and crab samples were determined by the atomic absorption spectrophotometry (GBC SensAA - AAS, Australia) in flame mode. A blank determination was done using the same procedure but without water samples (Brooks *et al.*, 1967; Vignesh*et al.*, 2012b).

RESULT AND DISCUSSION

Cadmium (Cd)

Cd is highly toxic to freshwater and marine organisms except for some diatoms and It's bio-accumulative through the food chain. It has been demonstrated as a highly toxic metal to wildlife and carcinogenic to humans Cadmium (Palanichamy and Rajendran, 2000; Ronald, 2000). The concentration of Cd in the coastal seawater can range from Post-Monsoon Below detection Limit (BDL) - 0.51 mg l^{-1}. It was found minimum BDL at Chennai during Post-monsoon and maximum 0.51 mg l^{-1} at Rameshwaram during summer as shown in the Table-1. Similarly the concentration of Cd in sediments ranges from 0.06-1.72 mg kg^{-1}, and minimum 0.06 mg kg^{-1} at Chennai during Post-monsoon and maximum 1.72 mg kg^{-1} at Rameshwaram during Monsoon season as shown in the Figure-3.

Lead (Pb)

Pb is also known as a snowballing metabolic poison. Its concentration in the coastal regions has been altered by human activities (Fernandez *et al.*, 2008). Pb concentration in coastal environment can be attributed by the sources like automotive exhausts, domestic sewage, agricultural runoff,power-plant operation, loading and unloading of cargo as well as dredging activities in harbor zones, and leaching from antifouling paints used in fisherman boats and leakage or un-burnt leaded diesel and petrol from boats (Abu-Hilal, 1987; Batley and Brockbank, 1990). The concentration of Pb in the coastal seawater can range from 0.03-1.18 mg l^{-1}, It was found minimum 0.03 mg l^{-1} at Chennai during Post-Monsoon season and maximum 1.18 mg l^{-1} at Rameshwaram during Monsoon season as shown in the Table-1. Similarly the concentration of Pb in sediments range from 0.38-2.83 mg kg^{-1}, it was found to be minimum 0.38 mg kg^{-1} at Cuddalore during Pre-Monsoon season and maximum 2.83 mg kg^{-1} as shown in the Figure-3.

Chromium (Cr)

The effluent of the metal finishing industry, corrosion of building materials, domestic and Municipal sewage play an important role in increasing the Cr concentration in the marine environment (Maanan*et al.*, 2004). Land run off during monsoon season also increasing Cr concentration. Elemental Cr is used as a marker of metal industry. Moreover, in addition to iron and steel industries, sewages also contribute equally to the contamination of Cr (Raman and Ganapati, 1983). The concentration of Cr in the coastal seawater can range from BDL- 0.24 mg l^{-1}. It was found minimum BDL at Chennai during Post-monsoon and maximum 0.24 mg l^{-1} at Rameshwaram during Summer as shown in the Table-1. Similarly the concentration of Cr in sediments ranges from 0.10-0.95 mg kg^{-1}, and minimum 0.10 mg kg^{-1} at Chennai during Pre-monsoon and maximum 0.95 mg kg^{-1} at Rameshwaram during Summer season as shown in the Figure-3.

Nickel (Ni)

Ni is known to be a nutritional requirement for many eukaryotic and prokaryotic organisms, which is necessary for plants to metabolize urea (Thomson, 1982). Higher concentration Ni along the coastal region due to the discharge of industrial effluents and domestic sewage, land runoff and its gradual diffusion on the nearby coastal region. Moreover, lower values of Ni show that low intense activity of chemical weathering of the source rock which is a common phenomenon in the environment. The petroleum related activities also bring Ni a0.05 mg l^{-1} at Chennai during Post-monsoon and maximum 1.26 mg l^{-1} at Tuticorin during Monsoon as shown in the Table-1. Similarly the concentration of Ni in sediments ranges from 0nd contaminate the environment (Achyuthan, 2002). The concentration of Ni in the coastal seawater can range from 0.05-1.28 mg l^{-1}. It was found to be minimum .22-2.66 mg kg^{1}, and minimum 0.22 mg kg^{-1} at Chennai during Post-monsoon and maximum 2.66 mg kg^{-1} at Tuticorin during Monsoon season as shown in the Figure-3.

Zinc (Zn)

Zn is present in all organisms and also essential trace element for metabolic processes. The highest concentration of Zn observed in the coastal environment is from the domestic sewage, municipal waste, the coal powered thermal power plant, atmospheric deposition of fly ash, anthropogenic sources and dredging and dumping of sediments (Ashokkumar, 2011). The concentration of Zn in the coastal seawater can range from 0.22-8.28 mg l^{-1}. It was found to be minimum 0.22 mg l^{-1} at Chennai during Post-monsoon and maximum 8.28 mg l^{-1} at Tuticorin during summer as shown in the Table-1. Similarly the concentration of Zn in sediments ranges from 3.98-13.56 mg kg^{-1}, and minimum 3.58 mg kg^{-1} at Nagapatinam during Pre-monsoon and maximum 13.56 mg kg^{-1} at Tuticorin during Monsoon season as shown in the Figure-3.

Iron (Fe)

Sewage waste, domestic and river flow and land runoff implement the Fe content in water and sediment. Organic content and sediment texture are a reason for metal load in the marine environment. Higher Fe concentration than the present study has been recorded by (Jonathan and Ram, 2003) in the coastal sediments of Tuticorin. The concentration of Fe in the coastal seawater can range from 0.63-2.79 mg l^{-1}. It was found minimum 0.63 mg l^{-1}at Chennai during Post-monsoon and maximum 2.79 mg l^{-1} at Tuticorin during Monsoon as shown in the Table-1. Similarly the concentration of Fe in sediments ranges from 4.89-15.34 mg kg^{-1}, and minimum 4.89 mg kg^{-1} at Rameshwaram during Pre-Monsoon and maximum 15.34 mg kg^{-1} at Tuticorin during Monsoon season as shown in the Figure-3.

Copper (Cu)

Cu is a micronutrient for aquatic life, but it becomes toxic at higher levels. The observed high concentrations in the coastal transacts are attributed by industrial effluents, Industrial water coolant discharge, Combustion of coal in Power Plants, Municipal domestic sewage and harbour activities-ore handling. Cu is a common ingredient in antibiofouling paints which are applied on the surface ships and in offshore engineering (Raman and Ganapati, 1983). The concentration of Cu in the coastal seawater can range from 0.09-1.65 mg l^{-1}. It was found to be minimum 0.09 mg l^{-1}at Chennai during Post-monsoon and maximum 1.65 mg l^{-1} at Chennai during Monsoon as shown in the Table-1. Similarly the concentration of Cu in sediments ranges from 1.10-2.92 mg kg^{-1}, and minimum 1.10 mg kg^{-1} at Nagapatinam during Pre-Monsoon and maximum 2.92 mg kg^{-1} at Tuticorin during Pre-Monsoon season as shown in the Figure-3.

CONCLUSION

Observing the results obtained during the present study, it is evident that the Monsoon plays a prominent role in the distribution of heavy metals. There is a seasonal dependent noticeable change in the metal concentration. The accumulation of heavy metals found to be high in the near shore sediment mainly due to the land based activities and during Pre-Monsoon maximum level of lead has been observed in Cuddalore. Maximum level of Fe and Zn has been noticed in Tuticorin irrespective of seasons. Due to the expansion of Industries, Chennai, Cuddalore and Tuticorin Port Trust, Thermal power station, Copper smelter Industry, Petrochemicals, Alkali Industry and other allied small industries around coastal areas will be the main source for the anthropogenic input in future. The gradual rise in heavy metal levels in the sea creatures has a chance to enter human life through food web. Therefore coastal region should be given great attention to control the anthropogenic input into the coastal environment. Continuous monitoring of near shore coastal area recommended and proper monitoring is required for the effluent treatment in the industry.

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Table 1: Seawater mg l^{-1} all seasons and stations

S.No	Season/Stations	Cd	Cr	Cu	Fe	Ni	Pb	Zn
1	POMS1	0.01	0.01	0.09	1.17	0.05	0.03	0.22
2	POMS2	0.18	0.01	0.12	0.63	0.1	0.13	0.31
3	POMS3	0.09	0.01	0.07	0.72	0.22	0.08	0.45
4	POMS4	0.01	0.01	0.17	0.82	0.01	0.22	0.28
5	POMS5	0.42	0.02	0.22	2.35	0.18	0.11	0.58
6	SS1	0.08	0.01	1.1	1.23	0.05	0.07	0.75
7	SS2	0.23	0.05	0.5	0.98	0.13	0.11	0.6
8	SS3	0.12	0.1	0.64	1.22	0.19	0.05	0.37
9	SS4	0.07	0.09	0.32	0.75	0.09	0.19	0.28
10	SS5	0.51	0.21	0.45	2.24	0.21	0.09	0.62
11	PMS1	0.06	0.01	1.15	1.11	0.01	0.08	0.51
12	PMS2	0.15	0.01	0.3	1.09	0.09	0.15	5.32
13	PMS3	0.17	0.05	0.41	1.15	0.13	0.09	0.37
14	PMS4	0.01	0.07	0.55	0.98	0.11	0.15	8.22
15	PMS5	0.23	0.1	0.68	1.35	0.16	0.21	0.22

16	MS1	0.1	0.06	0.3	1.61	0.12	0.08	0.65
17	MS2	0.18	0.01	0.25	1.1	0.18	1.18	5.12
18	MS3	0.2	0.1	0.33	1.31	0.11	1.17	0.9
19	MS4	0.26	0.15	0.41	1.11	0.08	0.79	7.91
20	MS5	0.51	0.24	0.65	2.79	0.18	0.25	0.75

Table 2. Seawater mg l^{-1} all seasons and stations

S.No	Elements	Postmonsoon	Summer	Premomsoon	Monsoon
1	Cd	0.142±0.170499	0.202±0.088204	0.124±0.088204	0.25±0.156205
2	Cr	0.012±0.004472	0.092±0.038987	0.048±0.038987	0.112±0.088148
3	Cu	0.134±0.061074	0.602±0.330106	0.618±0.330106	0.388±0.157544
4	Fe	1.138±0.707792	1.284±0.135204	1.136±0.135204	1.584±0.705181
5	Ni	0.112±0.087579	0.134±0.056569	0.1±0.056569	0.134±0.044497
6	Pb	0.114±0.070214	0.102±0.052726	0.136±0.052726	0.694±0.5114
7	Zn	0.368±0.145499	0.524±3.655485	2.928±3.655485	3.066±3.300595

Table 3. Sediment mg kg^{-1} all season and stations

±S.No	Season/Stations	Cd	Cr	Cu	Fe	Ni	Pb	Zn
1	POMS1	0.06	0.18	1.55	9.13	0.22	0.98	10.52
2	POMS2	0.25	0.52	1.85	8.1	0.41	1.1	11.68
3	POMS3	0.88	0.36	1.63	9.8	0.65	0.78	9.62
4	POMS4	0.6	0.18	1.55	7.6	0.7	0.75	5.2
5	POMS5	1.1	0.85	2.8	11.82	0.56	1.66	10.9
6	SS1	0.57	0.21	1.34	8.65	0.88	1.12	11.68
7	SS2	1.23	0.38	1.77	7.9	1.1	1.81	12.48
8	SS3	1.05	0.33	1.91	11.12	0.91	0.95	10.22
9	SS4	0.82	0.55	1.81	10.81	0.8	0.83	7.63
10	SS5	1.44	0.95	3.62	14.68	1.95	2.1	11.18
11	PMS1	0.32	0.1	1.55	8.45	0.25	0.38	7.14
12	PMS2	0.48	0.23	1.83	10.15	0.87	0.75	5.18
13	PMS3	0.37	0.55	1.1	6.41	0.92	1.13	3.98
14	PMS4	0.29	0.48	2.11	4.89	0.81	1.97	6.44
15	PMS5	0.94	0.28	2.92	11.58	0.76	1.24	8.34
16	MS1	0.65	0.16	1.56	10.16	1.22	0.92	8.82
17	MS2	0.81	0.25	1.76	11.21	1.42	0.83	7.71
18	MS3	0.55	0.6	1.35	8.7	0.78	1.23	5.08
19	MS4	0.43	0.55	1.9	7.61	0.66	1.87	7.65
20	MS5	1.72	0.74	2.87	15.34	2.1	2.14	13.56

Table 4. Sediment mg kg^{-1} all season and stations

S.No	Elements	Postmonsoon	Summer	Premomsoon	Monsoon
1	Cd	0.578±0.43014	1.022±0.340544	0.124±0.088204	0.832±0.515577
2	Cr	0.418±0.280036	0.484±0.287715	0.048±0.038987	0.46±0.245051
3	Cu	1.876±0.530924	2.09±0.882695	0.618±0.330106	1.888±0.586916
4	Fe	9.29±1.655204	10.632±2.648031	1.136±0.135204	10.604±2.982588
5	Ni	0.508±0.195115	1.128±0.472515	0.1±0.056569	1.236±0.574526
6	Pb	1.054±0.368212	1.362±0.560509	0.136±0.052726	1.398±0.581524
7	Zn	9.584±2.560367	10.638±1.870513	2.928±3.655485	8.564±3.111612

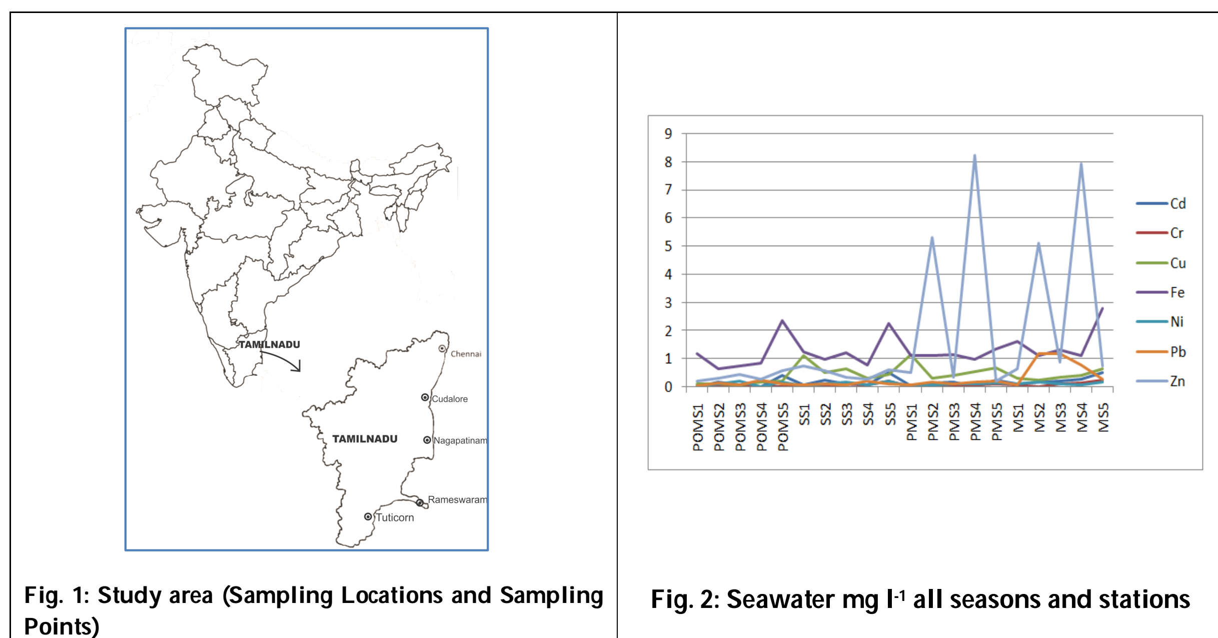

Fig. 1: Study area (Sampling Locations and Sampling Points)

Fig. 2: Seawater mg l^{-1} all seasons and stations

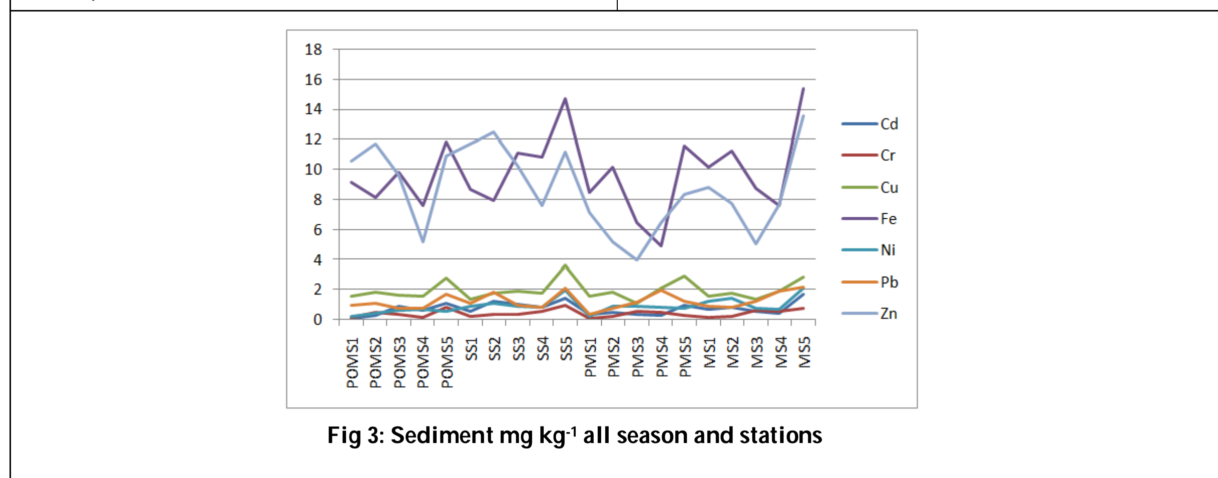

Fig 3: Sediment mg kg^{-1} all season and stations

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

A Review Study on Acne Caused by *Propionibacterium*

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Received: 28 Dec 2019 Revised: 30 Jan 2020 Accepted: 02 Mar 2020

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ABSTRACT

Acne is world's most common skin disease. It is a inflammatory disease and mostly teens and adult get affected from the increased sebum production, inflammation and bacterial colonization of hair follicles on face, chest, back, shoulder and neck by *Propionibacterium acne*. Acne can persist into adulthood with determinate effects on self-esteem of the affected which an eventually leads to depression. Effective approaches towards the treatment of acne and to know and understand its pathogenesis. Acne affects skin having dense sebaceous follicles in areas including face, chest and back. Acne is not life threatening but severe acne can affect psychological status and social activities. The present review focuses on an epidemiology, etiology, pathogenesis, diagnosis, differential diagnosis and management of acne with the pharmaceutical dosage forms of oral and topical administrations. Various medicines for acne treatment includes benzoyl peroxide, antibiotics, antiseborrheic medications, sulfur and sodium Sulphacetamide, anti-androgen medications, salicylic acid, hormonal treatments, alpha hydroxy acid, retinoids, azelaic acid, keratolytic soaps and nicotinamide. Currently laser and light devices and minor subcision surgery have been also performed for acne treatment. This review paper lays emphasis on a brief idea on acne treatments, preventions, and acne causing bacteria *Propionibacterium acnes*. Youngsters who lose their confidence it results in depression and some committed suicide. Youngsters can able to get idea how to prevent and treat against acne.

Keywords: Colonisation, Inflammation, Sebum, Pathogenesis.

INTRODUCTION

Acne is the one of the most common skin disease and affecting teens and young adults. Acne occurs when the pores of the skin become clogged, inflamed and get infected. Acne starts in the skin's sebaceous gland. These glands secrete an oily substance called "sebum". The sebum normally travels through a tiny hair follicle from the gland to the skin surface the sebaceous gland present at the basal part of the hair follicle, sometime the sebum becomes trapped, mix with the dead skin cells and bacteria. The areas of the skin that have the largest number of functional oil glands, such as the face, neck, back, shoulders and nose are often affected [Abolnik et al, 1995].

We have found that there are two types of acne differentiated on the basis of inflammations a) Non-Inflammatory Acne and b) Inflammatory Acne. Non-Inflammatory Acne does not cause inflammation and sever damage to the skin. It is closer to the surface of the skin. In this cases most found acne types are i) Blackheads, which is medically termed as "Opened Comedones". It occurs when a pore is clogged by a combination of sebum and dead skin cells and coloration is due to the exposure of sebum with the air and ii) Whiteheads which is medically known as "Closed Comedones".These are small, whitish or flesh coloured spots. In case of Inflammatory Acne we have found that it may cause inflammation and sever damage to the skin as it does consists of swelling, redness, and pores that are deeply clogged with bacteria, oil, and dead skin cells. These are found to be cause by different reasons and those are i) Papules which are small, pus-filled red bumps appear on the skin surface. It is a types of inflammes blemish. Papules developed due to irritation in blackheads and whiteheads it damage some of the surrounding skin. The damage leads to inflammation [Bruggemann, H, 2005]. ii)Pustules which has found to be are larger, tender bumps with a circular center. The center is filled with whitish or yellowish pus,with red or pink base. iii) Nodules are found as hard, painful, inflamed lumps located deep within the skin. They look like larger, deeper papules.This type of acne lesion develops when clogged pores damage tissues and cells deep beneath the skin's surface. Nodules are a severe form of acne blemish [Bruggemann, H et al. 2004].

The last one is the iv) Cyst which are found to be the most occurred and very large, soft, painful, red or white lumps situated deep in the skin. They are filled with pus [Bruggemann, H, 2005].These are largest forms of acne and their formation usually results from a severe. *Propionibacterium acne* is the name of the bacteria that live on the skin and contribute to the infection of pimples. This is originally identified as Bacillus acne [Parte, NCBI]. *Pronionibacterium* is a gram-positive, an aerobic, non-sporulating rod shaped bacteria [Cheung et.al,1975]. These bacteria commensals of human and other animals They are able to synthesize propionic acid by using unusual transcarboxylase enzymes [Cheung et al.1975]. This bacteria produces numbers of virulence factors and well known as immunomo adulatory and inflammatory properties. It has ability to generate Propionic acid that is why it was named *Propionibacterium acnes.* Skin cells, sebum and hair clumps together into a plug and a microcomedone can develop into larger structures, called Comedones. This is infected with bacteria and swelling results [Bojar, R., and Holland, K, 2004]. The formation of acne has found to undergo into various stages including closing of comedones (whiteheads) and appear like a small, flesh-coloured structures then it rezults into opened (blackheads) comedones, these are known as Non-inflammatory acnes. The comedones rupture and the follicular materials become dispersed in the dermis rather than on the skin surface. Depending on the rupture or damage of the comedone wall various inflammatory acne are produced that is Papules, Pustules, Nodules, Cysts. This organism forms part of oral cavity, large intestine, the external ear canal [Brook and Frozier 1991] and skin where these bacteria predominates.

It is anaerobic bacteria and can survive gor 8 months under anaerobic conditions without sub- culture it could also survive in human tissue at low oxidation potentials [Csukas et al 2004]. The bacteria *Propionibacterium acne* are sub divided into 2 types, 1) Type-1 and 2) Type-2. These sub divisions was first described by (Johnson and Cummins 1972) on the basis of cell wall agglutination tests and the organism's wall sugars. Type-1 and Type-2 was discriminated biochemically, but type-2 unable to frement sorbitol [Cummins 1975]. Immunofluroscence microscopy using specific antisera and molecular techniques including recA sequence analysis [Mc Dowell et al.2005] and

random amplification of polymorphic DNA (Perry et al.2003) have also used to distinguish between type-I and type-II. It has ability to generate Propionic acid that is why it was named *Propionibacterium acnes.* Skin cells, sebum and hair clump together into a plug and a microcomedone can develop into larger structures, called Comedous .This is infected with bacteria and welling results.

CAUSES of ACNE

Acne is the most commonly linked to the changes in hormone levels during puberty. The glands begin to produce too much sebum and excess sebum mixes with dead skin cells and form plug in the follicles. Hormones affects the grease producing glands next to the hair follicles in the skin, and it cause them to produce larger amount of oil. The frequency of acne depend on the strain of bacteria. Hormonal changes - A range of factors triggers acne, but the main cause is rise in androgen levels. In women, it gets converted into estrogen .Rise in androgen levels cause the growth of oil glands. The enlarged gland produces more sebum. Sebum can break down cellular walls inside the pores, causing the bacteria to grow. In males, rise in the production of Testosterone level stimulates the production of sebum and cause enlargememt of pores and cause infection.

Cosmetic products like Creams or Oily substance come in contact with sebum and trigger more oil production from Sebaceous Glands. Certain medications which have found to be the most hazards includes Steroid medications, Lithium and some anti-epileptic drugs cause acne infections. Stress is the most common quality studies have been demonstrated that may stress can cause acne. Acne in women can be caused due to Periods, Pregnancy, Polycystic ovary syndrome and also the changes occurred during puberty, menstrual cycle and menopose. It has also found that acne can be continued through generation after generations. Food supplements can also be the common reason for acne in all over the world, which includes pizza, chocolate, greasy and fried foods, and junk food. These foods may not be good for overall health as it contains various forms of preservatives and other chemicals, as a result of this, acne got worse in presence of these chemicals. Studies show dairy products and high glycemic foods, can trigger acnes.

INFECTIONS and DISEASE ASSOCIATIONS

Propionibacterium acnes causing a range of infection as well as associated with numbers of inflammatory conditions.It is primarily recognised for its role in acne vulgaris and contribute to the inflammatory phase of the conditions (Leyden 2001). A total of 94 propionibacterium isolates(12%)identified in 92 patients were considered to cause infection.The significant infections caused by propionibacterium species were associated with the blood in 15 patients,central nervous system in 11 patients,lymph glands in 10 patients,absence in 8 patients,joints in 7 patients,wounds in 7 patients,cysts in 6 patients and sinuses in 5 patients.Antimicrobial therapy was administered to 83 patients,47 patients this therapy was given in conjugation with surgical drainage or correction. Surgical drainage was performed in 9 patients,5 patients died.

These organisms can occasionally cause serious infections.Antibiotic therapy address against *Propionobacterium acnes* has been a backbone of treatment for more than 40 years(Brook and Fraizer 1991). First clinically relevant changes in Propionobacterium acnes antibiotic sensitivity were found in USA, shortly after the introduction of topical formulations of erythromycin and tetracycline.It cause mutuations in the gene encoding 23S and 16S subunits of ribosomal RNA (Eady et al.2003). *Propionibacterium acnes* DNA has been isolated from lymph nodes of patients with sarcoidosis (Eishi et al. 2002). *Propionibacterium acnes* normally resides in peripheral lung tissue and mediastinal lumph nodes .Recovery of *Propionibacterium acnes* from microdiscectomy material removed for the treatment of sciatria has suggested on associated between the organisms and sciatica.It cause infection in bones and joints , mouth, eyes and brains ,inflammation of the prostate leading to cancer , SAPHO (synovits, acne, pustulosis, hypoerostosis,

asteitis) syndrame, sarcoidosis and sciatica. In table 1 the various reasons and remedies are being listed for the acne caused be *Propionibacterium acnes.*

IMMUNOLIGICAL PROPERTIES and VIRULENCE

Propionibacterium acnes produces a number of exocellular enzymes (Hoeffer 1977; Holland et al.1981;Inglaums et al.1981,Kabongo Muawlia 1982) and metabiotics that can directly damage host tissue [Allaker et al. 1987]. *Propionibacterium acnes* derived components posses chemoattractant properties (Webster and Leyden 1980;Puluerer et al.1988).Pretreatment with heat-killed cells of *Propionibacterium acnes* has been shown to provide protection against infections and anti-tuneor activity in a variety of animal models(Eady and Ingham 1944). *Propionibacterium acne* has ability to produce bioactive exocellular products and there intractions with the immune system.

GENETICS

Genetic predisposition has been considered an important predisposing factor, influencing the number, size and activity of the sebaceous glands. Its influence on hormonal control has also been observed, as well as on the process of follicular hyperkeratinization and on innate immunity. In one study, adult women with acne reported first-degree relatives with acne in adulthood [Goulden et al.1999].

HORMONES

The role of androgens in the etiopathogenesis of acne vulgaris is well established. Testosterone, Dehydroepiandrosterone Sulfate (SDHEA) and Dihydrotestosterone (DHT) stimulate sebaceous gland growth and sebum production. Estrogens have the opposite effect, that is, they inhibit the secretion of androgens, modulate genes involved in the growth of the sebaceous gland and inhibit their function. The activity of the sebaceous gland therefore depends on the estrogen/androgen ratio [Zouboulis et al.2016]. In relation to AFA and hormones, the following are outstanding: 1) Increased sensitivity of the sebaceous gland to androgenic hormones: As in acne vulgaris, in AFA there is an increase in the number and sensitivity of the receptors located in sebocytes and keratinocytes to circulating androgenic hormones [Beylot et.al,2014].

2) Increased peripheral hormonal conversion: sebocytes and keratinocytes present an enzymatic system capable of locally producing testosterone and DHT. Hyperactivity and abnormal activity of enzymes related to the metabolism of androgenic hormones such as 5-alpha reductase, 3-beta-hydroxysteroid dehydrogenase and 17-hydroxysteroid dehydrogenase, with increased pre-hormone peripheral conversion (SDHEA, androstenedione and testosterone) into more potent androgenic hormones (testosterone and DHT) [Ribeiro.et.al.2015]. DHT is 5 to 10 times more potent than its precursor, testosterone, and less likely to be metabolized by aromatase into estrogen.20 3) Worsening of the disease in the premenstrual period in 60 to 70% of women, as well as in premenopausal, pregnancy and during the use of progestin-only contraceptives. In these periods there is a relative increase of the hormones with greater androgenic activity, in relation to estradiol [Dreno et.al.2014].4) Other hormones, besides androgens and estrogens, regulate the production of sebum: the sebaceous gland is a neuroendocrine organ and the production of sebum can also be stimulated, in periods of stress, by neuropeptides and hormones such as melanocortins and corticotropin-releasing hormone (CRH) [Albuquerque et al.2014]. CRH increases the expression of 3β-hydroxy-steroid dehydrogenase mRNA, the enzyme responsible for the conversion of dihydroepiandrosterone (DHEA) to testosterone. Also neuropeptides, histamine, retinoids, vitamin D and insulin-like growth factor 1 (IGF-1) have been described as regulators of sebum production.

CHANGES IN SEBUM

Qualitative changes were verified in the components of sebum in the skin with acne. There is a relative decrease of Linoleic Acid (AL), an Essential Fatty Acid (EFA), protector of the glandular epithelial wall [Melnik and Zouboulis 2013]. There is also the peroxidation of squalene by the combined action of P. acnes and ultraviolet radiation. These alterations and the presence of free fatty acids resulting from the hydrolysis of triglycerides, through the action of lipases released by P. acnes, cause damage the epithelium, increasing infundibular keratinization and dermal inflammation [Bhat at al. 2017].

FOLLICULAR HYPERKERATINIZATIN

There is an abnormal proliferation of keratinocytes, stimulated by pro-inflammatory cytokines, such as interleukin-1 alpha (IL-1 alpha). This cytokine is triggered by the activation of Toll-like receptors or TLR 2 and 4, related to innate immunity, which recognize molecular patterns present in P. acnes, as well as sebaceous hyper-secretion and squalene peroxidation.14 The formation of the micro-comedone is also preceded by a mononuclear infiltrate formed mainly by CD4+ T lymphocytes and CD68+ macrophages, which corroborates to the hypothesis of the participation of the inflammatory process in the early stages of acne [Das et al. 2014].

BACTERIAL COLONIZATION

The main bacterium involved in the pathogenesis of acne is P. acnes. It is a Gram-positive anaerobic bacterium that makes up the microbiome of the skin, being located preferably in the seborrheic areas. In skin with acne there is an exaggerated growth of the population of P. acnes [Beylot et al.2014]. *P. acnes* are involved in several mechanisms: stimulation of follicular hyper-keratinization; alteration of the sebaceous composition; and inflammatory response through TLR activation. In addition, it produces several enzymes, such as lipases, proteases, hyaluronidases, endoglyceramidase, sialidase/neuroaminidase, proteinase and 5 cAMP factors, which contribute to follicular rupture and tissue degradation [Ribeiro et al. 2015]. Immuno-inflammatory processes Upon recognition of the P. acnes molecular patterns, TLR 2 and 4 are activated, triggering an inflammatory cascade through the nuclear pathway NFk β, with production of pro-inflammatory cytokines or interleukins and tumor necrosis factor alpha (TNF-alpha) that recruit neutrophils and macrophages, maintaining the inflammatory cycle. Activation of TLR leads to the release of antimicrobial peptides such as beta-defensins 1 and 2, cathelecidins and granulolysins. A second nuclear pathway is also activated - pathway AP1, with release of metalloproteinases 1, 3 and 9 that degrade the extracellular dermal matrix and are associated with the formation of scars [Dreno et al. 2016]

DIET

Studies have shown that consumption of high glycemic and dairy foods increases insulin and IGF1 levels. The gonads and sebaceous glands have receptors for both hormones which stimulate the production of androgens, such as testosterone, and inhibit the action of aromatase that converts testosterone to estradiol. The nutritional status of the cell is recognized initially by the transcription factors FOX01 and the signaling pathway mTORC1 [Melnik et al.,2017]. High glycemic load foods, dairy products, increased insulin and IGF1 stimulate mTORC1, triggering processes such as: increased protein and lipid synthesis, cell proliferation, cell differentiation with acroinfundibular hyper-proliferation of keratinocytes, sebaceous gland hyperplasia, increased sebaceous lipogenesis, insulin resistance and increased body mass index [Melnik et al.,2017]. In addition to the diet rich in foods with a high glycemic load and the consumption of dairy products, worsening of acne by the use of dietary supplements for muscle mass gains that are rich in branched-chain amino acids and peptides derived from whey is observed in daily practice [Pontes et.al,2014]

SIGN and SYMPTOMS

Scars

Acne scars are caused by inflammation within the dermal layer of skin and are estimated to affect 95% of people with acne vulgaris. The scar is created by abnormal healing following dermal inflammation. Scarring is most likely to take place with severe acne, but may occur with any form of acne vulgaris. There are 2 types of Scars have been found 1) Atrophic acne scars 2) Hypertropic scars

Pigmentation

Postinflammatory hyperpigmentation (PIH) is usually the result of nodular acne. These acne often leave behind an inflamed darkened mark after the original acne lesion has resolved. This inflammation stimulates specialized pigment producing skin cells (known as melanocytes) to produce more melanin pigment which leads to the skin's darkened appearance [Chandra M et al.2012].

TREATMENTS

TREATMENT OF ADULT FEMALE ACNE

AFA is a therapeutic challenge because it presents a tendency to relapse, even after cycles of oral antibiotics or isotretinoin [Pontes et al. 2013]. The typical evolution of AFA, with frequent relapses, makes maintenance treatment essential [Dréno et al.2014]. In choosing the treatment, numerous factors must be taken into account: acne severity, response to previous treatments, psychosocial impact, possibility of pregnancy, slow response to treatment and increased risk of sensitive skin irritation. Individual preferences and costs are also important factors [Kircik 2014]. In the Brazilian population, the diversity of phototypes, with a large contingent of patients prone to post-inflammatory hyperchromia, as well as climatic variation limit therapeutic options, contributing to their complexity.

TOPICAL TREATMENT

Topical treatments are the most widely used and effective option for treating moderate AFA and for maintenance treatment. Tolerability and efficacy may contribute to greater adherence to treatment, good outcomes, and patient satisfaction [Bagatin,et.al.2017]. Therapeutic regimens employing resources directed against two or more fundamental pathogenic factors are the main strategy in cases of mild to moderate intensity of acne [Sato, et.al,2006]. Retinoids Adapalene: The efficacy and tolerability of adapalene 0.3% compared to the vehicle in women aged 18-41 was evaluated in an analysis of data obtained in two studies. There was a significant reduction at the 12th week (P = 0.045) of the inflammatory lesions (-61%) and non-inflammatory lesions (-51%). The main side effects were dryness and "skin discomfort" [Plovanich,et.al, 2015]. The concentration of 0.1% has also been studied with positive results and good tolerance [Lessner,et.al. 2014] Tretinoin: Increased efficacy of AFA treatment was demonstrated when tretinoin at 0.025% concentration was associated with spironolactone. In that same study, another group used 0.1% adapalene in cream associated with spironolactone with similar results. Other authors have shown improvement in inflammatory acne in adolescents and adults when using topical tretinoin at concentrations of 0.04% to 0.1% in micro-capsulated presentations [Kim and Del Rosso,2014].

ANTIBIOTICS

Topical antibiotics have a direct anti-inflammatory action reducing perifollicular lymphocytic infiltrate. Due to the significant increase of P. acnes strains resistant to clindamycin and erythromycin, the use of these substances alone is contra-indicated [Krunic et.al. 2014]. In adult women, benzoyl peroxide can induce irritative contact dermatitis or skin dryness, the extent of which is related to the amount and type of product, concentration, and vehicle. Therefore,

concentrations of benzoyl peroxide above 5% are not recommended for use in adult women [Krunic et.al. 2008]. It can also cause photosensitivity and bleaching of clothing.

AZELAIC ACID

Azelaic acid 20%, applied twice a day, was evaluated in a study for the treatment of AFA in 241 women, alone or in combination with various treatments (including adapalene, benzoyl peroxide, cosmeceuticals, isotretinoin and oral hormonal contraceptives). The study concluded that topical treatment improved the Dermatol ogy Quality of Life Index (DLQI) and reduced the severity of acne.51Azelaic Acid 15% gel, applied twice daily, was also effective in reducing post-inflammatory pigmentation [Layton et al. 2017]. There was a significant reduction in the expression of TLR-2 in the skin of adult females with facial acne who used azelaic acid 15% gel or combined oral contraceptive (drospirenone + ethinylestradiol). The authors suggested a possible anti-inflammatory effect of oral contraceptive and azelaic acid in AFA via modulation of this receptor[de Bastos et.al. 2014] According to the AFA review, published in 2013, azelaic acid (20% cream or 15% gel) is recommended as the first line of treatment in monotherapy for non-inflammatory and inflammatory acne. Azelaic acid shows similar efficacy to other topical therapies in the treatment of mild to moderate acne and is associated with a favorable tolerability profile and high rates of satisfaction. Finally, it is unlikely that systemic side effects occur with azelaic acid, making it safe for use during pregnancy and breastfeeding [Fox et.al. 2016]. Azelaic acid represents an important option for women of childbearing age and with a desire to become pregnant as it is considered safe by the Food and Drug Administration (FDA).

DAPSONE

Dapsone 5% gel was used as treatment twice daily in black adult females with good efficacy and tolerance. In patients with acne vulgaris, it has been shown to be useful when combined with doxycycline and then alone as maintenance for long periods, with the advantage of having no risk for bacterial resistance [Cooper et.al. 2012, Melnik et.al. 2010] .

ASSOCIATIONS

RETINOIDS + BENZOYL PEROXIDE

The efficacy and safety of adapalene 0.1% combined with 2.5% benzoyl peroxide gel compared to the vehicle in women 25 years of age or older were analyzed by meta-analysis of data extracted from subgroups from three multi-center, phase 2 and 3, randomized, parallel and double-blind studies. A rapid onset of action was demonstrated, with a significant reduction in lesions from the first week of use; the combination was considered effective, safe and tolerated well in both the population under 18 years and over 25 years, with similar indices.46

RETINOIDS + ANTIBIOTICS

The tretinoin-clindamicyn combination has been studied for the treatment of acne; a later analysis included only data from adult females. The combination proved to be more effective in reducing inflammatory and non-inflammatory lesions than the isolated tretinoin or clindamycin or the carrier substance [Nickle et.al. 2014].

ANTIBIOTICS + BENZOYL PEROXIDE

The combination of benzoyl peroxide 3.75% + clindamycin 1.2% was studied for long-term use (up to 24 weeks) in 20 adult females. At week 12, inflammatory lesions decreased by 70% and non-inflammatory lesions decreased by 58%. In the 24th week, the improvement was 93% and 90%, respectively. There were no serious adverse events. This study allowed to evaluate the safety of longterm use as well as demonstrated continuous improvement [Sbidian et.al. 2016].

MECHANICAL PROCEDURES

Intralesional infiltration with corticosteroid: The corticosteroid of choice for this minimally invasive procedure is triamcinolone acetonide. It is indicated in nodulocystic acne, in a concentration of 2.5mg/mL, diluted in distilled water, in single application, with improvement in 48 to 72 hours. In the presence of multiple lesions, 5mg per application should not be exceeded to avoid systemic absorption [Bagatin et.al. 2014]. It is useful in the treatment of inflammatory nodules, even during the use of oral isotretinoin, since it accelerates regression and relieves pain rapidly in these lesions. It is also indicated in the approach of hypertrophic and keloid scars, with total or partial regression [de Souza et.al. 2017]. Manual extraction of comedones: Removal of comedones, particularly open ones, may be useful by unclogging the follicular opening, facilitating the penetration of topical keratolytic products and contributing to the reduction of inflammation. In addition, it provides a positive impact on the quality of life, prevents the manipulation of the lesions in an inadequate way by the patient and/ or laymen [Rademaker, 2010]. Eletrocauterization of macro-comedones: The electrocautery of closed macro-comedones should be performed with great care not to reach excessive depth and cause scarring. It is useful since macro-comedones frequently evolve to inflammatory lesions. In addition, it avoids manipulation that causes exulcerations and inflammation [Rademaker, 2010].

DRAINING of CYSTS and ABSCESSES

It is a necessary procedure when there is fluctuation in these lesions, associated with the use of oral antibiotics, because it reduces the period of evolution and the scarring development.

MICRODERMABRASION

Microdermabrasion is a very superficial exfoliation method equivalent to a superficial peeling. It is based on the blasting of aluminum crystals until the appearance of mild erythema. After 2 to 3 days there is a fine peeling. The advantage over chemical peels is lack of burning, but the result may be poorer. It is most indicated in the preparation of the skin for the treatment of superficial atrophic scars. It is a simple, safe procedure and when carried out serially in several weekly sessions can induce reorganization and increase the density of dermal collagen [Brzezinski et.al. 2017, Rademaker, 2013]. Chemical peels, lights and lasers The superficial chemical peels have keratolytic action, causing superficial exfoliation due to its epidermal effects that are useful for comedonal and mild inflammatory forms. The most commonly used agents in the treatment of active acne are Jessner's solution, 10% trichloroacetic acid (TCA) or 20% in aqueous solution, 30% salicylic in hydro-alcoholic solution or polyethylene glycol, 70% glycolic or other gel concentrations of natrosol, with partially buffered pH and 50% pyruvic acid. Salicylic and pyruvic acids have demonstrated a significant reduction in sebum content in the skin with acne through sebummetry [Hansen et.al. 2016]. Several comparative studies have shown similar efficacy among the agents, with varying differences in tolerability [Sladden and Harman, 2007].

All can cause burning, erythema and desquamation after 3 to 5 days, lasting from 7 to 15 days. Unpredictable immediate reactions may occur, particularly with glycolic acid, which requires more care and observation of symptoms and signs until their removal. Such reactions include edema, vesiculation and undesirable bleaching due to epidermolysis. In this situation, immediate neutralization with 10% sodium bicarbonate in aqueous solution and removal of the agent should be performed. In general, they are safe procedures, without late complications; except when exaggerated, unpredictable immediate reactions occur that may cause post-inflammatory hyperpigmentation. The average peels are performed with the combination of a keratolytic agent - Jessner's solution (no removal) or 70% glycolic acid (removed as soon as there is mild burning or the appearance of erythema) - and TCA 35% in aqueous solution applied immediately after. They are indicated for the treatment of superficial atrophic scars in isolated semi-annual applications or associated with other procedures at different times, particularly when they are deep

[Halvorsen, etl.al. 2011, Marron et.al. 2013, Huang and Cheng, 2017]. In this situation, the peel acts as a preparation of the skin, making its surface more homogeneous. The application of blue or red light emitting diodes (LEDs) has an anti-inflammatory effect faster than topical and/or systemic treatments. One study showed that this effect was similar to salicylic acid peel. The associated lights in the early stages of treatment improved inflammation and adhesion. No adverse event has been recorded [Etminan et al. 2013, Alhusayen et.al, 2013 Titus and Hodge, 2014]. Photodynamic Therapy (PDT), as it is better known, has been proposed as a therapeutic option for acne, although with many doubts and scanty evidence [Puri 2014, Margolis et.al. 2010].

A review of randomized, controlled studies of light-based therapies for acne treatment concluded that none of the studies demonstrated efficacy; it is necessary to carry out well-designed studies that employ standardized outcome measures as well as comparison with usual drug treatments [Marczyk et.al. 2014, Lee et.al.2016]. At the moment there is no laser technology whose target is the sebaceous gland and that can destroy it, leading to healing. Two suggestions have been discussed. One associates an integrated cooling and vacuum system applied to the skin followed by the application of 3 to 4 pulses of the 1540-nm medium-erbium: glass infrared laser. An open, uncontrolled study including 12 patients with mild to moderate acne used this method in 4 to 6 sessions, with a 2-week interval. The improvement scores were 3.6 and 2.0, on a scale of 0 to 4, after one and 3 months of the last session, without adverse events [Sampaio and Bagatin, 2008, El-Domyati et.al. 2016]. Another recently developed method is the use of a suspension of inert gold micro-particles with silica center applied with 8-minute massage on the face followed by 800nm laser application. There are 3 sessions, with intervals of 2 weeks. There are reports of improvement of up to 61% of inflammatory lesions [Levy and Zeichner 2012, Shinkai et.al. 2016].

LASER and LIGHT DEVICES

The reports showed that photodynamic therapy, light emitting diode therapy and combination of pneumatic energy and light has been successfully used with traditional therapies for treatment of acne [Mahajan and Garg ,2003, Boyraz and Mustak, 2013]. For the treatment of acne combination product Isolaz™ (Aesthera, Pleasanton, CA, USA) uses a vacuum with broad band light source has been shown to be effective in 11 patients treated at 3 week intervals and decreases inflammatory and noninflammatory lesions [Rademaker et.al, 2014, Monfrecola et.al, 2016].

ALTERNATIVE MEDICINE

Various natural products have been investigated for treatment of acne [Dhaked et.al. 2016, Dreno et.al, 2009]. Topically administered Azelaic acid (20%) twice daily for six months has been shown to be effective for treatment of mild to moderate acne [Draelos et.al, 2016, Schmitt et.al. 2011]. It is similar effective as 5% topical benzoyl peroxide, 0.05% isotretinoin and 2% erythromycin [Cetinözman et.al. 2014]. Sometimes azelaic acid may cause skin irritation [Kim et.al,2015, Thielitz et.al. 2007]. A topical application of tea tree oil is also effective for acne treatment [Morales-Cardona et.al. 2013]. A vaccine has been tested successfully against inflammatory acne in mice but has not yet been proven to be effective in humans [Chularojanamontri et.al. 2014, Wohlrab and Kreft, 2007]. In 2007, the first genome sequencing of a P. acnes bacteriophage (PA6) occurred which could enhance the development of a potential bacteriophage therapy to treat acne and might improve the problems associated with long term antibiotic therapy and bacterial resistance [Araviiskaia et.al. 2016, Larsen et.al, 2017].

CONCLUSION

AFA has been considered a particular subtype of acne, distinct from acne vulgaris or adolescent acne, not only for differences in clinical status and etiopathogenesis, but also for its chronicity, which may last until the postmenopausal period. Some characteristics such as more sensitive and less oily skin, and multiple etiopathogenic factors, such as new work rate in women's lives, stress, sleep disorders, dietary supplements and certain types of

contraceptive methods make management more complex. Recent findings on its chronicity, involving TLR stimulation, demonstrate that control of the innate immune response has contributed to understanding the mechanism of action of the drugs used in its treatment. For all these issues, AFA is a challenge in clinical practices and should be further understood.

ACKNOWLEDGEMENTS

We would like to thank Rama Devi Women's University , Bhubaneswar, Odisha and Centurion University of Tehnology and Management, Parlakhemundi, Odisha, to support us for all of our work.

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Table 1: Infections where *Propionibacterium acnes* was found to be the causing agent

SI.No.	Infection	Predisposing factors	Reference
1	Discitis	Surgery	Chia and Nakata(1966) and Harris et al (2005).
2	Spondylodiscitis	Epidural catheterization	Halkic et al (2001) and Hernandez-Palazon et al (2003).
3	Central Nervous System	Neurosurgical procesures	Critchely and Strachan (1966) and Ghalayini et al (2004).
4	Endocardiscitis	Prosthetic aortic valve	Gunthard et al. (1994).
5	Osteomyelitis	Lumban puncture	Abolnik et al. (1995)
6	Endophthalmitis	Postoperative	Benz et al.(2004)
7	Joint infections	Prosthetic hip	Junney et al. (1999)

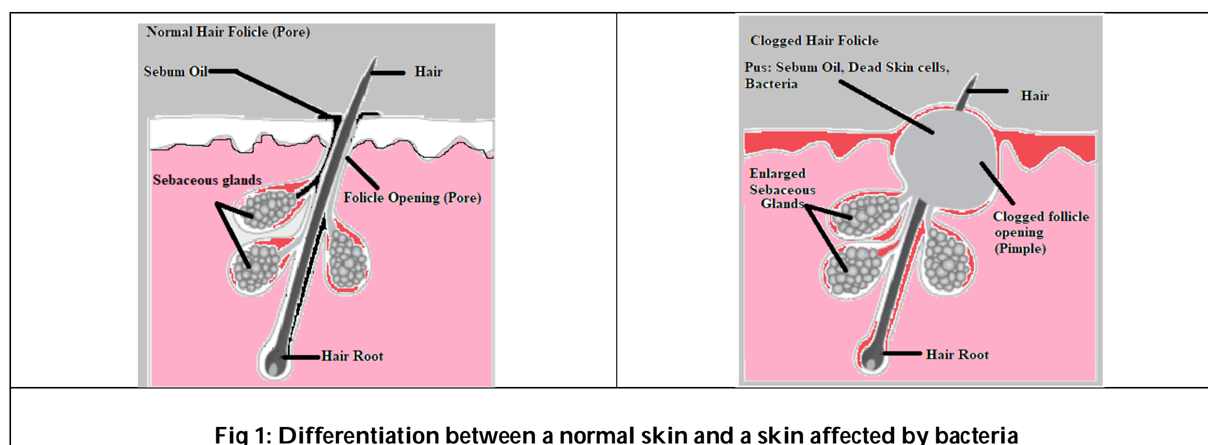

Fig 1: Differentiation between a normal skin and a skin affected by bacteria

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Ultrasonic Investigation of Dextran with Glycine at Various Temperatures and Frequencies

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Received: 26 Dec 2019 Revised: 29 Jan 2020 Accepted: 04 Mar 2020

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ABSTRACT

The density (ϱ) viscosity (η) and ultrasonic speed (U), at 303 K, 308K, 313 K, 318K and 323K have been measured in the systems of dextran with glycine at various frequenciesin aqueous medium.The acoustic parameters such as adiabatic compressibility (β), acoustic impedance (Z), relaxation time (τ), intermolecular free length (L_f) and Gibb's free energy (ΔG), are calculated. The solute-solvent interactions for the dextran (1%) have been studied at five various temperatures ranges from 303 K to 323K and at four different frequencies i.e. 1MHz, 5MHz, 9MHz, 12MHz.The variation of thermo acoustic parameterwith different temperature and frequency leads to the analysis of molecular motion and various types of inter-molecular interaction and their strength of the constituent between solute (dextran 1%) and solvent (glycine 2(M)). The results have been interpreted in the light of structural rearrangement occurs in the aqueous dextran solution.

Key Words: Acoustic impedance, adiabatic compressibility, Gibb's free energy, intermolecular free length and relaxation time.

INTRODUCTION

Ultrasonic speed of a fluid is basically related to binding forces among the atoms or molecules. The authors [1] have qualitatively inferred the degree of molecular association in fluids by the use of sound speed data. The measurements of ultrasonic speed have been effectivelyworking in appreciative the environment of molecular interaction in pure fluids, binary and ternary solutions [2]. However, the calculated parameters from ultrasonicspeed provide a better insight into the molecular environment of fluids [3]. In our work we have calculated acoustic parameters such asadiabatic compressibility (β), acoustic impedance (Z), relaxation time (τ), intermolecular free length (L_f) and Gibb's free energy (ΔG), of polymer dextran (1%) have been studied at five various temperatures ranges from 303 K to 323K

and at four frequencies i.e. 1, 5, 9, 12MHz. in solvent 2(M) glycine.We have chosen a polymer dextran as a solute with2(M) glycine as a solvent. This is the only polymer which is water soluble. It has involved a different region of examinations by analysts due to its flexible pharmaceutical, biomedical and modern application [4-6].

MATERIALS AND METHODS

Freshly prepared distilled water has been used for 2(M) glycine as solvent for preparing dextran solution. Dextran of 70,000 Da used as solute, and methods are same as reported in my earlier paper [7-8].

Theoretical aspect

The ϱ, η and U, have been measured and using these experimental data the thermo acoustic parameters β, Z, τ, L_f and ΔG, were calculated using standard formula [9].

RESULTS AND DISCUSSION

The ϱand ηof dextran with glycine at temperature 303K, 308K, 313K, 318K and 323Kare represented in Table-1. The ultrasonic speed increases with rise in temperature at a particular frequency (fig.1). This is due to the structural changes taking place in the polymer solution by addition of glycine, resulting association in the constituent. The association is due to dipole are induced due to permanent dipoles are present in the water molecules. For a constant temperature, ultrasonic speed falls with rise in frequency. The fall in speed is an indication of existence of low molecular association among the dextran and glycine due to increase in agitation between molecules give rise to decrease in speed at higher frequency (fig.2) [10].

The β decreases with rise in temperature at a given frequency and β rises with increase in frequency at a given temperature, which shows a reverse trend to velocity variation. This type of behaviour may be due to breaking up of associated clusters of dextran releasing several dipoles, which in turn induces dipole moment in glycine resulting dipole- induced dipole interaction [11]. Z increases with rise in temperature [fig-5]. The solute-solvent molecules collide with each other more frequently hence opposition offered by the solution to the propagation of ultrasonic wave through it increase i.eZ increase with rise in temperature. This indicates the likelihood of strong molecular interaction among the components of the solution at a given frequency. Z decreases with the rise in frequency. This indicates the possibility of weak interaction among unlike molecules at a given temperature [12].

The variation of τ of dextran with glycine at different temperatures in different frequencies. The fig.7 indicates the relaxation time falls with increasing temperature. This is due to the increase in temperature cause more number of collision and hence decreasing the collision time leading to less τ. The variation of τ with frequency is almost parallel to frequency axis (fig.8) indicating very small change in τ with increase in frequency [13]. Rise in temperature, $L_f$increases (Fig.9) due to thermal energy is utilised for to rise in volumeof the system hence increase in L_f. Further, in higher frequency range intermolecular gap gradually increases which leads to decrease in velocity that is observed. As association of cooperating molecules disagrees with frequency of the ultrasonic wave [14]. The variation of ΔGof dextran with glycine at different temperature falls with increasing temperature. The trend of variation is same at all frequencies as if the frequency changes does not influence Gibb's free energy considerably [15].

CONCLUSION

From the above discussion it is understandable that, there exist a molecular interaction among the solute and solvents and weak molecular interaction (like dipole-dipole, dipole-induced dipole and dispersive forces) were found to exist.

ACKNOWLEDGEMENTS

The author is sincerely thanks to the staffs Centurion University of Technology and Management, Odisha andABIT Cuttack for their strategic help and encouragement.

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Table. 1-The experimental values of density (ϱ) and viscosity (η) at 303K, 308K, 313K and 318K ofsolution.

T in kelvin	(ϱ) $Kg.m^{-3}$	(η) 10^{-3} $N.s.m^{-2}$
303	1057.86	1.25
308	1055.85	1.12
313	1053.22	1.04
318	1050.80	0.96
323	1047.77	0.87

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Table: 2. values of U andβat different temperature and frequencies

T in kelvin	U $m.s^{-1}$				β (10^{10} m^2 N^{-1})			
	1MHz	5MHz	9MHz	12 MHz	1MHz	5MHz	9MHz	12 MHz
303	1616.00	1607.00	1606.00	1604.00	3.62	3.66	3.67	3.67
308	1623.50	1618.00	1616.00	1614.00	3.59	3.62	3.63	3.64
313	1629.00	1623.50	1622.00	1620.60	3.58	3.60	3.61	3.62
318	1633.40	1630.00	1628.00	1626.60	3.57	3.58	3.59	3.60
323	1638.00	1633.00	1632.00	1630.00	3.56	3.58	3.58	3.59

Table: 3. values of Zand τat different temperature and frequencies

T in kelvin	Z (×10^4)kg·m^2·s^{-1}				τ(x 10^{10})s			
	1MHz	5MHz	9MHz	12 MHz	1MHz	5MHz	9MHz	12 MHz
303	1.71	1.70	1.70	1.70	6.04	6.11	6.11	6.13
308	1.71	1.71	1.71	1.70	5.36	5.39	5.41	5.42
313	1.72	1.71	1.71	1.71	4.98	5.01	5.02	5.03
318	1.72	1.71	1.71	1.71	4.58	4.59	4.61	4.61
323	1.72	1.71	1.71	1.71	4.10	4.13	4.13	4.14

Table: 4 values of $L_f$and ΔGat different temperature and frequencies

T in kelvin	L_f(10^{-10} m)				ΔG(10^{-20} K.J.mol^{-1})			
	1MHz	5MHz	9MHz	12 MHz	1MHz	5MHz	9MHz	12 MHz
303	3.77	3.80	3.80	3.80	243.14	245.17	245.39	245.85
308	3.79	3.81	3.81	3.82	228.05	229.30	229.76	230.21
313	3.82	3.83	3.83	3.84	221.05	222.32	222.67	222.99
318	3.84	3.85	3.86	3.86	211.48	212.27	212.74	213.07
323	3.87	3.88	3.89	3.89	196.79	197.98	198.22	198.69

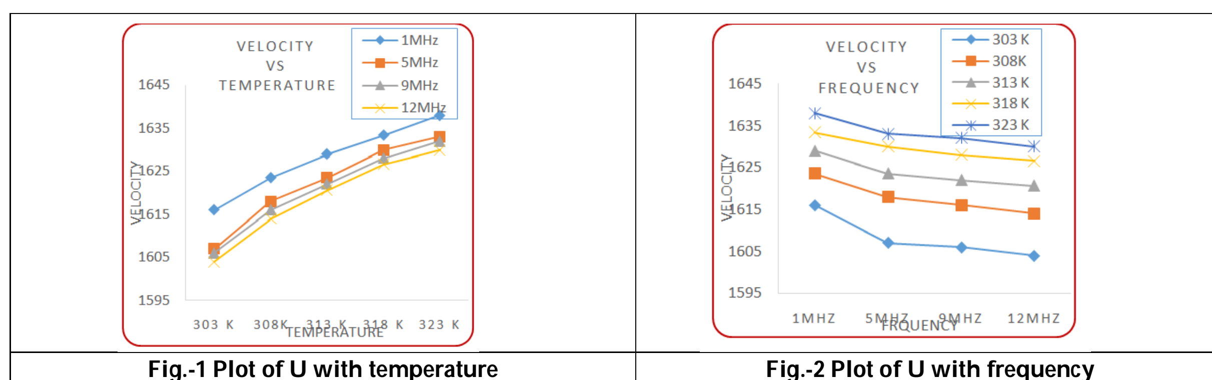

Fig.-1 Plot of U with temperature | Fig.-2 Plot of U with frequency

Fig.-3 Plot of βwith temperature	**Fig.-4 Plot of βwith frequency**
Fig.-5Plot of Zwith temperature	**Fig.-6Plotof Zwith frequency**
Fig.-7 Variation ofτ with temperature	**Fig.-8 Variation ofτ with frequency**
Fig.-9 Plot of $L_f$with temperature	**Fig.-10 Plot of $L_f$with frequency**

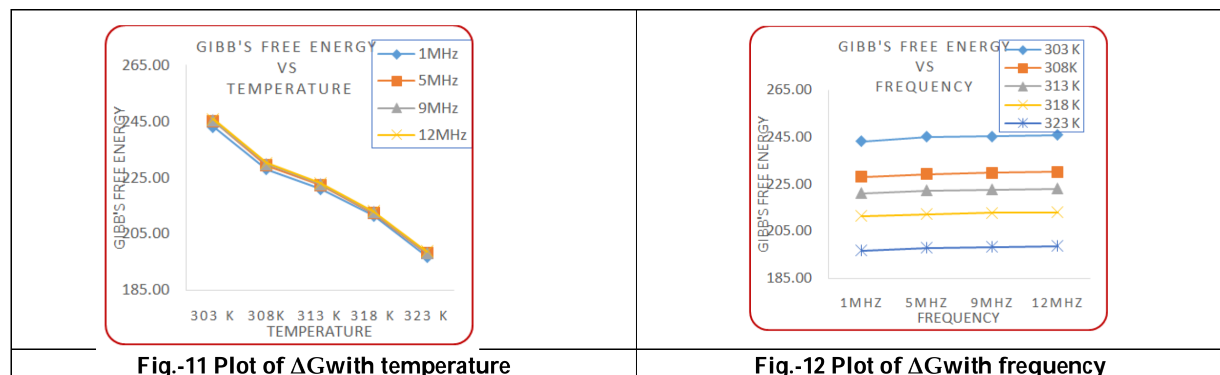

Fig.-11 Plot of ΔGwith temperature	Fig.-12 Plot of ΔGwith frequency

RESEARCH ARTICLE

Identification and Classification of Disease in Mango Leaves using Modified FRFCM Algorithm and APSO Based LLWNN Machine Learning Approach

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Received: 24 Dec 2019 Revised: 27 Jan 2020 Accepted: 29 Feb 2020

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ABSTRACT

Agricultural productivity of mango highly depends on economy of India. Detection of mango plant leaf disease by automatic technique is beneficial since it reduces a large monitoring work in large crop farms. This research work presents a novel APSO (Accelerated Particle Swarm Optimization) based Local Linear Wavelet Neural Network(LLWNN)for classification of diseased mango leaf. The modified FRFCM (Fast and Robust Fuzzy C Means) technique is employed for segmentation to identify the anthracnose diseases from the mango leaf image. Further the GLCM (Gray level co-occurrence matrix) feature extraction is employed for feature extraction and the extracted features are fed as input to the APSO-LLWNN model for classification. The weights of the LLWNN model are optimized by the APSO algorithm to improve the performance of the model. The results obtained from the APSO-LLWNN model has been compared with LLWNN, PSO-LLWNN and PSO-RBFNN is presented. It is proposed to detect and classify the disease from the mango leaf by taking high resolution image.

Keywords: Radial basis function neural network, Particle Swarm Optimization, Fuzzy C Means, Support Vector Machine, Local Linear Wavelet Neural Network.

INTRODUCTION

The agricultural becomes a big feeding source and business in today's world. Indian economy is basically dependent on agricultural productivity in rural belts. The disease in the crop in any form harms the growth of economy of the farmers; therefore detection of disease at early stage is essential. The current method for plant disease detection is

accomplished through eye observation by experts which is time consuming as well as cost of detection is very high when larger farms are taken into account. In such conditions automatic detection of the diseases for the plant leaves will provide a unique solution to the farmers as well as agricultural personals. Leaf disease detection [1] has been an extensive research in the present scenario. There are various machine learning techniques such as the Convolutional Neural Network [2], the Artificial Neural Network [3], the Back Propagation Neural Network [4], the Support Vector Machine [5] and other image processing methods [6, 7] that have been used for detection.

Image segmentation algorithms based on Fuzzy c means algorithm [8] has been proposed for brain tumor detection [9] breast cancer detection [10] etc. FCM based genetic algorithm [11, 12], Wavelet transform [13], K-means clustering [14], spectral clustering [15], utilized to have intensity in homogeneities in image partitioning. Particularly FCM based segmentation algorithms for agricultural diseases detection are not proposed by researchers. The aim of the research work to identify of mango leaf disease using FCM algorithm based segmentation and classification of diseases using local linear wavelet neural network model (LLWNN). The contribution of the work as follows:

- A novel modified FRFCM Segmentation technique is proposed to detect different leaf diseases which will help the farmers at the rural belt to know in advance the effect of disease.
- A wavelet based local linear neural network classifier has been proposed for classification of mango leaf disease in which the weights are updated by APSO meta- heuristic algorithm.

The rest of the paper is organized as follows section 2 presents related work and back ground of research work, section 3 presents the material and methods which includes the explanation of the proposed APSO-LLWNN model, and details of FRFCM segmentation, section 4 presents results and discussion and section -5 presents conclusion followed by reference.

Related work

Savita N. Ghaiwat et al. [16] Proposed imaging methods for the identification of plant-leaf diseases. Sanjay B. et al. [17] were proposed Vision-based masking detection algorithm to increase the recognition rate of the plant leaf disease detection classification process. Mrunalini R. et al., [18] suggested clustering of K-means and artificial intelligence in crop diseases. S. Arivazhagan et al. [19] proposed classifier SVM and "co-occurrence" color method for the detection of insalubrious plant area. Anand H. Kulkarni et al. [20] suggested filter Gabor and classifier ANN for the identification and classification of plant diseases. Sabah Bashir et al. [21] used K-means, Bayes classifier, Smita Naikwadi et al. [22] proposed image processing algorithm, Piyush Chaudhary et al .[23] proposed an image smoothing median filter and a detection and classification threshold. Iqbal et al. researched diseases of citrus plant leaves [24]. Golhani et al. [25] uses neural networks to classify the disease from the plant's hyper spectral images. The Deep Convolutional Neural Network (DCNN) proposed by Ma et al. [26] classifies the anthracnose powdery mildew, and target leaf spots.

Ferentinos [27] has proposed a VGG -CNN, Kaur et al. [29] utilized computer vision concepts for the identification and classification of the plant leaves [30]. Picon et al. have considered DCNN for "fungal" diseases in wheat plant. "Google Net" and "Cifar10" network was proposed by Zhang et al. [31] for the maize leaf images classification. U. P. Singh et al. [32] proposed Multiclass CNN for classification of Anthracnose mango leave disease and achieved an accuracy of 97.13%, K. Srunitha et al. [33] uses multiclass SVM for classification of unhealthy region of mango leaf and attains an accuracy of 96%, Md. Rasel Mia et al. [34] proposed ANN and SVM and attained 87.5% accuracy, Jayaprakash Sethupathy et al. [35] proposed open CV based disease identification of mango leaves, Sampada Gulavnai et al.[36] employed deep CNN for detection and attained an accuracy of 91%. Bashir et al.[37] proposed LLWNN for load forecasting, Chen et al.[38]uses a LLWNN for time sequence prediction etc. To improve the classification performance, the hybrid meta-heuristic algorithm with machine learning model APSO-LLWNN has been proposed.

The FCM algorithm is able to reserve additional evidence of the image but the problem is noise reduction capability. To get rid of this crisis, FCM_S proposed by Ahmed et al. [8] with spatial information which reduces the noise from MR images, but takes extra computational time. Chen and Zhang [39] considered spatial term, but FCM_S not capable to reduce Gaussian noise. Enhanced FCM algorithm (EnFCM) proposed by Szilagyi et al. [40] , a fast and robust FCM (FRFCM), has been proposed by Tao Lei et al. [41] to improve the performance of the segmentation and noise reduction from MRI images. Inspired by the robustness of the FRFCM algorithm, and to improve further segmentation capability, we have developed modified FRFCM segmentation technique for mango leaf disease detection. The LLWNN model employs different layers to smooth images in order to improve the noise-immunity. Therefore, the proposed APSO-LLWNN is more robust for noisy images is suitable for good classification results.

MATERIALS AND METHODS

Implementation of Research Work

The flow chart of research diagram indicates the step by step accomplishment of the research work. Further the block diagram shows the flow of algorithm application for detection and classification of leaf disease. In the first phase (i) the input image is segmented by wavelet transform, in the second stage (ii) the GLCM technique is employed to extract the features from images (iv) The local linear wavelet neural network has been trained by APSO algorithm to differentiate the leaves from the healthy surrounding. The comparison results from LLWNN, PSO-LLWNN and proposed APSO-LLWNN has been presents. The design and development of the research work flow includes the following functionality.

a) The proposed design is to address the issues of diseases due to climate change
b) The proposed segmentation scheme will address detection of diseases problems and noise removal from images without difficulties.
c) The proposed designed model will provide a complete platform which will try to cope with all these problems.
d) In addition, it will also offer quality of service in detecting and classifying leaf diseases without facing much problem.

Proposed APSO Based Local Linear Wavelet Neural Network Model:

In this work, we present a complete statistical model identification framework in order to classify the leaf disease utilizing by APSO based LLWNN model. The weights are simplified by APSO algorithm The feature data points $x_1, x_2, ...x_n$ are inputs and $\psi_1, \psi_2 -- \psi_N$ are the wavelet activation function in the hidden layers and the activation function of the nth hidden layered neuron is mentioned by a wavelet Kernel as

$$\psi_n(x) = |a_i|^{\frac{1}{2}} \phi\left(\frac{x - b_i}{a_i}\right) \quad \text{.....} (1)$$

Where the parameters a, b are the scaling and translation parameters, respectively

$$y_n = \sum_{i=1}^{N} (w_{i0} + w_{i1}x_1 + ...w_{iN}x_N)\psi_n(x) \quad \text{.....}(2)$$

The objective is to minimize the error and objective function is the mean square error which is specified by

$$MSE(e) = \frac{1}{N}\sum_{n=1}^{N}(d_n - y_n)^2 \quad \text{.....}(3)$$

Where "d" represents the desired vector.

Weight Optimization by APSO

In the expedited "particle swarm optimization" (APSO) [108,109], the "velocity vector" is given as

$$v_i^{p+1} = v_i^p + \alpha \in_n + \beta\left[g^b - x_i^p\right] \quad (4)$$

Where g^b is the gbest, v is the velocity of the particle and p is the indices of iteration and α, β are the controlling parameter taken as between [0,1]. The position equation is updated as

$$x_i^{p+1} = x_i^p + v_i^{p+1} \quad (5)$$

In order to increase the convergence performance capability the position equation is updated as

$$x_i^{p+1} = (1-\beta)x_i^p + \beta g^p + \alpha \in_n \quad (6)$$

With the updated position equation the weights of the APSO-LLWNN will be updated to enhance the performance of the proposed model.

lgorithm for weight optimization by APSO

1. Initializing weights of the model
2. With random position and velocity vectors assign the weights
2. Calculate fitness function for each particle's position
3. Choose best fitness gbest= g^p
4. Until convergence obtained do updating of velocity and position equation.

Modified FRFCM Segmentation

The FRFCM [41] segmentation utilizes a median filter "membership partition matrix" of the objective function. The partition function of objective function has been modified by employing a "weiner filter" and the fuzzy factor has been modified with a logarithmic term to improve the performance of the FRFCM algorithm
The objective function with local information is specified by

$$J_L = \sum_{l=1}^{n}\sum_{k=1}^{c} u_{kl}^t \left\| x_l - v_k \right\|^2 + \sum_{l=1}^{n}\sum_{k=1}^{c} Z_{kl} \quad (7)$$

Where the fuzzy aspect is given by

$$Z_{kl} = \sum_{\substack{k \in N_v \\ l \neq k}} \frac{1}{d_{lk}+1}(1-u_{kl})^t \left\| x_l - v_k \right\|^2 \quad (8)$$

Wherever x_l is the gray value of the kth pixel, u_{kl} signifies the fuzzy membership value of the lth pixel, and c indicates the "cluster centre" and t concludes the "fuzziness" of the significant partition. The fuzzy separation matrix is specified by

$$u_{kl} = \frac{1}{\sum_{j=1}^{c}\left(\frac{\|x_l - v_k\|^2 + G_{kl}}{\|x_l - v_j\|^2 + G_{jl}}\right)^{\frac{1}{t-1}}} \quad \text{.....(9)}$$

and $$v_k = \frac{\sum_{k=1}^{n} u_{kl}^t x_l}{\sum_{l=1}^{n} u_{kl}} \quad \text{.....(10)}$$

From equation (9), it is established that the factor G_{kv} controls the noise and image details, but computational time increases..

To reduce the "computational" intricacy, the "membership partition matrix" is adapted as

$$G'_{kl} = \sum_{\substack{r \in N_v \\ l \neq r}} \exp\left(\frac{\gamma^{\tau}}{d_{lr}+1}\right) u_{kr}^t \|x_r - v_k\|^2 \quad \text{..... (11)}$$

Where u_{kr} the neighbors of is u_{kl} , γ is gray assessment of image and " τ "is the smoothness factor between 0 and 1. Further, utilizing the "morphological" reconstruction operations like dialation and erosion, the renovation of the image is considered as " γ_p ", which is specified by

$$\gamma_p = R_b^C(f) \quad \text{.....(12)}$$

Where R_b^C signifies the "morphological closing" reconstruction which is efficient for noise elimination and f denotes an original image. The "membership partition" matrix is given by

$$u_{kp} = \frac{1}{\sum_{j=1}^{c}\left(\frac{\|\gamma_p - v_k\|^2 + G'_{kp}}{\|\gamma_p - v_j\|^2 + G'_{jp}}\right)^{\frac{1}{t-1}}} \quad \text{..... (13)}$$

and $$v_k = \frac{\sum_{k=1}^{c} u_{kp}^t \gamma^p}{\sum_{p=1}^{s} u_{kp}} \quad \text{.....(14)}$$

Now, we can mark the membership separation matrix in the form as $U_1 = [u_{kp}]^{c\times s}$. Further, allowing for "convergence speed" of the algorithms with the performance of the separation matrix U .We utilize a wiener filter and the new "membership" separation matrix is given by

$$U^y = wiener[U_1] \quad \text{.....(15)}$$

RESULTS AND DISCUSSION

ATA SET

Alternaria tenuissima disease appears as brownish spots particularly seen in northern India. Anthracnose is also known as blossom blight, leaf spot, fruit rot and twig blight. The disease is present all mango area of India. Symptoms are Leaf spot. Due to the fungus attacks "brown" or "dark circular" or irregular spots are formed on the "leaves" and such leaves are crinkled. We have considered the Anthracnose mango leaf disease for the research work which contributes 85% of the total entity. The dataset considered from the plant Village dataset [42] repository. A total of the real-time 1130 images of Mango leaves containing healthy and non-healthy were taken from the plant Village dataset.

Segmentation Results

We have considered Anthracnose mango leaf disease for the research from The dataset considered from the plant Village dataset [42] repository of image of size 256×256.and the segmentation results are presented from Fig.4 - Fig.7 Table 2 provides the computational time and segmentation accuracy of the eight algorithms including MFRFCM algorithm. The proposed MFRFCM technique good capability of detection of anthracnose disease from the mango leaves. In some cases the computational time revealed the nearly similar; still the proposed MFRFCM segmentation is selected due to strong ability of detection of diseases and reduction of noise

GLCM Feature Extraction

The features extracted using GLCM [9,103] technique and presented in Table 3

Performance measure and computational time

The proposed models were implemented Matlab2019a programming language for simulating training and testing process. The images were considered with the ratio of 75% images for training and 25% images for testing. The images are undergone the process of "segmentation" and "feature extraction" prior to process of training. The extracted features are fed as input to the proposed model for relegation task. .These results show the accuracy for all the approaches selected for the classification of diseased and healthy Mango leaves. The training and testing results obtained from the APSO-LLWNN model has been compared with LLWNN, PSO-LLWNN and PSO-RBFNN are presented in Table-4 and MSE results are shown in Fig. 8

The accurateness obtained for leaf image data set with PSO-RBFNN, LLWNN, PSO-LLWNN, APSO-LLWNN are 94.52%, 96.78%, 98.35%, 99.82% respectively. FromFig.8, it is found that that the planned APSO-LLWNN took nearly400 iterations to "converge". The PSO-RBFNN takes near about 600 iterations, while the LLWNN and PSO-LLWNN takes 800 and 500 iterations to congregate. It is found from the "MSE" consequences that, the accuracy in PSO-RBFNN and LLWNN is close to each other, but the convergence is faster in case of APSO + LLWNN classifier. Computation time also plays an important role in terms of performance of a classifier. The calculation time taken by PSO-RBFNN, LLWNN, PSO-LLWNN, APSO-LLWNN for each phase has been calculated on behalf of the leaf dataset and the attained values are 28.34 sec, 30.21 sec, ,19.38 sec and 11.42 sec.

With the proposed Modified FRFCM segmentation method, the leaf diseases were detected from the image and partitioned into the healthy and non-healthy leaf. From these discussions, it is specious that the proposed APSO-LLWNN model algorithm provides loftier optimized results regarding accuracy and calculation time and classified into healthy and non-healthy anthracnose diseases.

CONCLUSION

In this investigate work, the mango leaves are considered for the intention of segmentation and classification. The modified fast and robust "FCM" based segmentation technique has been employed for "segmentation". It is observed that the modified "FRFCM" acquires superior value of segmentation accuracy in comparison to NDFCM, FLICM, FRFCM, EnFCM, FCM_S1 and FCM_S2 and FCM algorithms which substantiates the removal of noise from the leaf image. The texture features are extracted with GLCM feature extraction method. We have considered different features which are utilized as input to the classifier models for the classification purpose. The APSO algorithm has been employed for weights updation of LLWNN replica. To authenticate the robustness of MASCA+PSO hybrid algorithm, the classification results are compared with PSO-RBFNN, LLWNN, PSO-LLWNN classifiers and presented. The convergence rate is faster in terms of APSO-LLWNN model as compared to other mentioned classifiers. The proposed classifier has revealed decent skill in classifying the healthy and non-healthy anthracnose diseases from the mango leaf dataset.

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Table 1: Details of Mango leaf image category

Sl.No	Type of image	class	Images considered
1	Non healthy Mango leaf	C1	510
2	Healthy mango leaf	C2	620

Table 2: Segmentation Accuracy and Performance Evaluation

Algorithm	Accuracy in%	Computational time in sec
FCM	93.27	5.14
FCM S1	95.91	3.42
FCM S2	96.82	3.13
En FCM	97.11	2.25
FLIFCM	97.62	9.89
NDFCM	98.34	2.78
FRFCM	98.81	1.54
MFRFCM	99.96	1.26

Table 3: Feature Extraction

Sl.No	Features	Normalized Feature values
1	Correlation	0.1469
2	Coarseness	0.3425
3	Skewness	0.5757
4	Kurtosis	0.2406
5	Energy	0.1224
6	Directional Moment	0.0322
7	Inverse Difference Moment	0.0711

Table 4.Performance measure

Classifier	Accuracy in (%)		Computational time in sec
	Training	Testing	
PSO-RBFNN	94.52	93.14	28.34
PSO-LLWNN	98.35	96.22	19.38
APSO-LLWNN	**99.82**	**98.87**	**11.42**
LLWNN	96.78	94.97	30.21

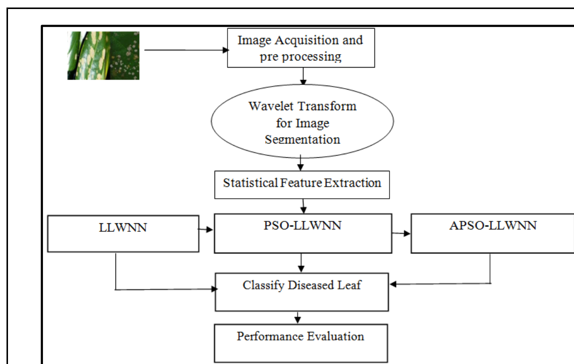

Fig. 1: Work flow diagram of research

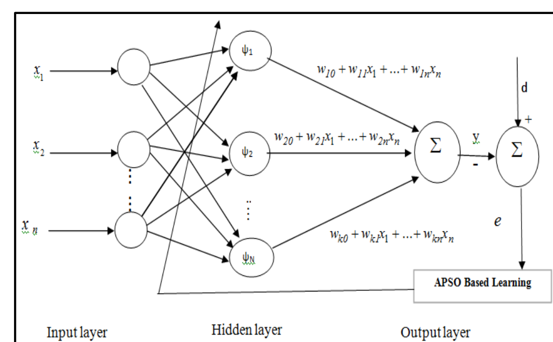

Fig. 2: APSO–LLWNN Model

Fig. 3: Leaf Spot Anthracose Disease

Fig. 4: Segmentation of Anthracose Disease utilizing NDFCM Algorithm

Fig. 5: Segmentation of Anthracose Disease utilizing FLICM Algorithm

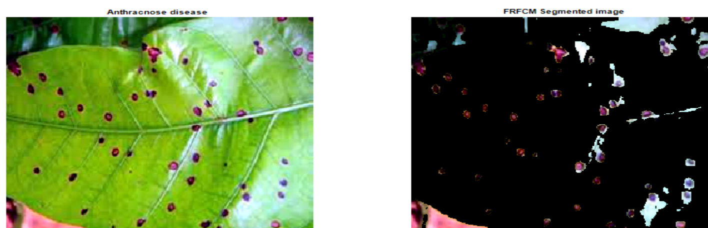

Fig. 6: Segmentation of Anthracose Disease utilizing FRFCM Algorithm

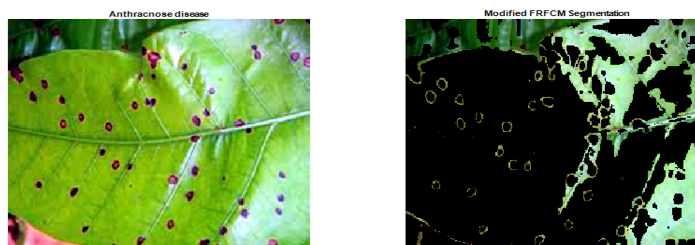

Fig. 7: Segmentation of Anthracose Disease utilizing Modified FRFCM Algorithm

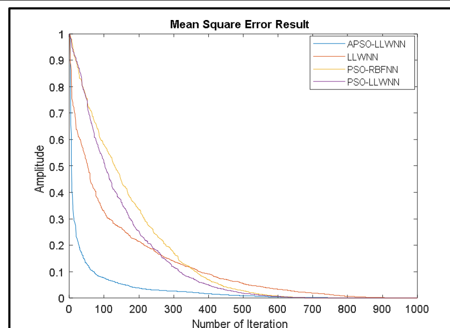

Fig. 8: Mean Square Error Result

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

On Algebraic Binding Number of Simple Graphs

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Received: 08 Jan 2020 Revised: 10 Feb 2020 Accepted: 13 Mar 2020

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ABSTRACT

The neighborhood of a certain vertex u in a simple graph Γ consists all adjacent vertices of it .denoted by $N(u)$, the binding number of a graph Γ denoted by $B(\Gamma)$, where $B(\Gamma)$ is the minimum of $\frac{|N(S)|}{|S|}$, where S is a proper subset of $V(\Gamma)$ and $N(S)$ is the union of $N(u)$ for all u in S. In this article, we will investigate and determine the binding number of certain simple graphs by classic methods, then we will develop a new method using linear algebra to find the binding number of certain simple graphs.

Keywords: Simple graph, binding numbers, characteristic polynomial.

INTRODUCTION

The relationship between graph theory and matrix theory is very tangible, using matrices as a tool in graph theory convert the study of graphs properties easily handling. Definitions of graph theory (F.Harory [3], N.Biggs [2])

Definition 1.1

A graph Γ is an unordered triple of a non-empty set of vertices $V(\Gamma)$ and prescribed of set of edges of $E(\Gamma)$ and an incident function $\Psi(\Gamma)$.

Definition 1.2

The end vertices of an edge is said to be incident with its edge and vice versa. The distinct vertices which are incident with common edges are called adjacent.

Definition 1.3

A graph Γ is called simple if it has no loops and no parallel edges.

Definition 1.4
The valancy of vertex is the number of edges incident to it, if the valancy of every vertex in the graph equal to k, then the graph is called k-regular.

Definition 1.5
A graph Γ is called complete graph denoted by k_n, if every vertices in it is adjacent with all rest vertices.

Definition 1.6
A graph Γ is connected if there is at least one path between any two vertices in it.

Definition 1.7
A tree is a connected graph with no cycles.

Definition 1.8
A complete bigraph is a graph consists of two a disjoint sets of vertices , in which every vertex in the first set adjacent with all vertices in the second set, denoted by $k_{m,n}$.

Definition 1.9
A cycle graph C_n is closed path with n vertices.

Definition 1.10
A star graph $S\left(1,n\right)$ is a tree with one root and n end vertices.

Definition 1.11
A wheel graph $W\left(1,n\right)$ is a graph consists of the union of C_n and $S\left(1,n\right)$.

Definition 1.12
The neighborhood of a vertex u in Γ is the set $N\left(u\right)$ which is consists of all vertices in Γ adjacent with u .

Definition 1.13
The binding number of a simple graph Γ, denoted by $B\left(\Gamma\right)$; where $B\left(\Gamma\right)=\min\left(\frac{|N\left(S\right)|}{|S|}\right)$, where $S\neq\phi$, $S\subseteq V\left(\Gamma\right)$, and $N\left(S\right)=\bigcup N\left(u\right)$ for all $u\in S$.

Definition 1.14
The adjacency matrix $A\left(\Gamma\right)=\left[a_{ij}\right]_{n\times n}$ of graph Γ with n vertices is an $n\times n$ matrix in which; $a_{ij}=\begin{cases}1, & u_i \text{ adjacent to} u_j \\ 0, & \text{otherwise}\end{cases}$.

Definition 1.15
The characteristic polynomial of a graph Γ, denoted by $P\left(\Gamma\right)$ the determinant of the matrix $\left[\lambda I-A\left(\Gamma\right)\right]$.

Binding Numbers of Graphs
In the following sequel, we will investigate and find the binding numbers well-known graphs and we will construct the relations between binding numbers of simple graph and characteristics polynomial of graphs, and we will develop algebraic methods to determine the binding numbers of certain simple graph.

Binding numbers of graphs by classic method.

Theorem 2.1.1: D. Woodal [4]

Let C_n be a cycle graph. Then the binding number of C_n equals 1 if n is even and $\frac{n-1}{n-2}$ if n is odd.

Theorem 2.1.2: **(**V. Aytac and Z.Berberler [1]**)**

Thee binding number of path graph P_n is 1 if n is even and $\frac{n-1}{n-2}$ if n is odd.

Theorem 2.1.3: D. Woodal [4]

Let k_n be a complete graph. Then $B(k_n)=n-1$, whereas $n>1$.

Theorem 2.1.4: D. Woodal [4]

Let $k_{m,n}$ be a bigraph. Then $B(k_{m,n})=\frac{n}{m}+\frac{m}{n}$.

Theorem 2.1.5

Let $S(1,n)$ be a star graph with $n+1$ vertices. Then $B(S(1,n))=\frac{1}{n}$.

Proof: Let $S(1,n)$ be a star graph with one root and n vertices , so the root vertex is adjacent with all end vertices. Thus S consists of n vertices and so $N(S)$ consist of all the root. So $B(S(1,n))=\frac{1}{n}$.

Theorem 2.1.6:

Let Γ be a wheel graph $W(1,n)$. Then

$$B(\Gamma)=\begin{cases}\frac{1+n}{n}, & \text{if } n \text{ is even} \\ \frac{n^2-2}{n^2-2n}, & \text{if } n \text{ is odd}\end{cases}.$$

Proof: Let Γ be a wheel graph $W(1,n)$ with $n+1$ vertices. Since Γ consists of $S(1,n)\cup C_n$ and $B(V)=B(S(1,n))+n\,B(C_n)$ (V. Aytac and Z.Berberler [1]**)**, so $B(\Gamma)=\frac{1+n}{n}$ if n is even and $B(\Gamma)=\frac{n^2-2}{n^2-2n}$ if n is even by Theorem 2.1.15 and Theorem 2.1.1.

Binding number of graphs using characteristic polynomial of graphs.

Let Γ be a simple graph and let A be the adjacency matrix of Γ, and $\det[\lambda I - A(\Gamma)]=a_0\lambda^n+a_1\lambda^{n-1}+\cdots+a_n$ be the characteristics polynomial Γ.

Theorem 2.2.1:

Let C_n be a cycle graph with n vertices. The binding number of C_n is $|a_0|$ if n is even and $\frac{|a_2|-1}{|a_2|}$ if n is odd.

Proof: Let C_n be a cycle graph with n vertices and n edges, let $\mathrm{P}(C_n)=a_0\lambda^n+a_1\lambda^{n-1}+\cdots+a_n$. By Theorem 2.1.1, $B(C_n)=1$ if n is even and $\frac{n-1}{n-2}$ if n is odd, so $B(C_n)=a_0$ if n is even, and since $|E(C_n)|=a_2=n$ (N. Biggs [2]), then $B(C_n)=\frac{|a_2|-1}{|a_2|}$ if n is odd.

Theorem 2.2.2:

The binding number of path graph $P(n)$ equals a_0 if n is even and $\frac{|a_2|-1}{|a_2|-2}$ if n is odd.

Proof: Let the characteristics polynomial of $P(n)$ is $\lambda^n+(n-2)\lambda^{n-2}$, so a_0 if n is even and $\frac{|a_2|-1}{|a_2|-2}$ if n is odd by Theorem 2.1.2 and N. Biggs [2].

Theorem 2.2.3:

Let k_n be a complete graph with n vertices. Then the binding number of k_n is $|a_n|$.

Proof: Let k_n be a complete graph with n vertices. Since the characteristics polynomial of k_n is $(\lambda+1)^{n-1}(\lambda^2-n+1)$ Wrikat [5], so $|a_n|=n-1$, and by Theorem 2.1.3, $B(k_n)=|a_n|$.

Theorem 2.2.4:

The binding number of star graph $B(S(n,1))=\frac{1}{|a_2|}$.

Proof: Let $S(n,1)$ be a star graph with $n+1$ vertices, the characteristics polynomial of $S(n,1)$ is $\lambda^{n-2}(\lambda^2-n+1)$ Wrikat [5], so $|a_2|=n$, so by Theorem 2.1.5, $B(S(n,1))=\frac{1}{|a_2|}$.

Theorem 2.2.5:

The binding number of wheel graph $W(1,n)$ equals $\frac{1+|a_2|}{|a_2|}$ if n is even and $\frac{1}{|a_2|}+\frac{|a_2|-1}{|a_2|-2}$ if n is odd.

Proof: Let Γ be a wheel graph $W(1,n)$, so $\Gamma=S(1,n)\cup C_n$. Thus $B(\Gamma)=B(S(1,n))+n\,B(C_n)$, then $B(\Gamma)=\frac{1+|a_2|}{|a_2|}$ if n is even and $B(\Gamma)=\frac{1}{|a_2|}+\frac{|a_2|-1}{|a_2|-2}$ if n is odd by Theorem 2.1.5, Theorem 2.2.1 and Theorem 2.2.4.

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RESEARCH ARTICLE

Evaluation of Antimicrobial Activity of Some Selected Woody and Fleshy Mushrooms Collected from Khurda District of Odisha, India

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ABSTRACT

An experiment of plate culture and agar cup well diffusion method was conducted for evaluating the bioactive potential of 20 different types of naturally growing mushrooms collected from different locations of Khurda district of Odisha, India. Agar cup well diffusion method was undertaken to evaluate the bioactive potential of 20 different types of naturally growing mushrooms collected from natural habitats. These mushroom samples belong to genus *Clavaria, Coltricia, Coniophora, Daldinia, Fomitopsis, Geastrum, Grifola, Langermannia, Lentinus, Lycoperdon, Microporous, Peniophora, Pisolithus, Polyporus, Schizophyllum, Suillus, Trametes* and *Tricholoma.* Solvent extracts were prepared by using distilled water, methanol, ethyl alcohol, isopropanol, ethyl acetate, acetone and chloroform. The concentrated extracts were used and placed on the media plates by agar well method, inoculated with bacteria and fungus separately and incubated for 24hr and 4 days, respectively. The observation data recorded for formation of inhibition zone around the well confirmed the antibacterial properties of *Lentinus fusipes, Lentinus torulosus, Suillus luteus* and *Langermannia gigantea.* Among tested mushroom species, no one found with antifungal properties. From the experimental results of the efficacy of the selected mushroom samples against PBC-1 (gram +ve) and PBC-2 (gram -ve) bacteria it can be concluded that mushroom samples used in the present investigation might have certain bioactive compounds. It is also evident that the solvent system used in this process have the capability to extract antimicrobial compound in it.

Keywords: Bioactive compound, Potential, well-diffusion.

INTRODUCTION

Mushrooms are known as functional food and endowed with nutraceuticals and pharmaceutical properties. They are also the source of bioactive properties like antimicrobial, antitumor, antiviral and anti-inflammatory etc.[1-3]. Mushrooms have been prescribed for treatment of various human diseases such as gastrointestinal disorder,

bleeding, high blood pressure and various microbial infections [4]. Many of them known as a suitable source of antibiotics. *Lycoperdom*, *Tricholoma*, *Fomitopsis*, *Trametes*, *Schizophyllum* and others have found to have good antimicrobial activity [5]. *Suillus* and *Clavaria* have confirmed to possess wider antibacterial properties along with *Lentinus* that produces bioactive metabolites [6-8].The demand of traditional medicine also known as green medicine, encourages for the search of new sources of such compounds. Mushrooms are good alternative for chemosynthetic drugs and are being used as curing agent for many diseases [9-11]. In view above aspects the unexplored environment of Khurda district was surveyed for its mushroom diversity to evaluate their potential against test microorganisms under study. The mushrooms of local environment were collected and evaluated in the laboratory for their efficacy against some gram +ve and gram –ve bacteria as well as fungi.

MATERIALS AND METHODS

Macro-fungi and test organism

In this study, 20 mushrooms species were selected belonging to different genera and categorised into fleshy and woody type. Samples were collected from different localities of Khurda district of Odisha and were identified morphologically. Taxonomically 9 mushrooms namely –*Clavaria vermiculari*, *Lentinus fusipes*, *Lentinus torulosus*, *Tricholoma lobayense*, *Geastrum fimbriatum*, *Pisolithus arrhizus*, *Suillus luteus*, *Langermannia gigantea*, and *Lycoperdon pyriformi* were placed under fleshy mushroorm while *Coltricia cinamomia*, *Coniophora puteana*, *Daldinia concentrica*, *Fomitopsi spinicola*, *Grifola frondosa*, *Lentinus betulina*, *Microporous xanthopus*, *Peniophora incarnata*, *Polyporous sulphurous*, *Schizophyllum commune* and *Trametes versicolor* were considered as woody type. These 20 species were used for the antimicrobial screening against 4 bacteria namely PBC-1& MBC-1 (gram +ve), PBC-2& PBC-3 (gram –ve) as well as against a fungal strain belonging to *Fusarium* sp.

Preparation of extracts

Mushrooms were dried properly at a controlled temperature of 50ºC in a hot air oven. Dried mushrooms were powdered by using mixture grinder and treated with seven different solvents including distilled water, methanol, ethyl alcohol, isopropanol, ethyl acetate, acetone and chloroform. To obtain active compounds, solutions were prepared with a ratio 0.05 g/ml in shaking condition at 120rpm for 24 hours and filtered before the residue and solvent were collected separately. Respective extractions were carried out till clear solvent was obtained. Extracts containing crude compounds were evaporated and obtained in dried condition and stored in a refrigerator in air tight containers for analysis of antimicrobial properties [12].

Antimicrobial assay

The antimicrobial activity of crude extracts of the selected 20 mushrooms species under study were determined by using well diffusion method [13]. Media plates were prepared containing Nutrient agar (NA) and Sabouraud dextrose agar for evaluation of their antibacterial and antifungal activity respectively. 1ml of sterile distilled water was used for the dilution of solid mushroom extracts. Simultaneously sterilised distilled water was used as control. Fresh broth cultures of test bacteria/fungi were prepared and spreaded over Nutrient agar and Sabouraud dextrose agar medium through spread plate method. Wells (6mm diameter) were made on the surface of media plates through cork borer and extracts were poured into the well using micro pipette. Plates were incubated for 24hr period at 37ºC and observed for halo zone formation for antibacterial assay. Diluted samples were also poured into well made in Sabouraud dextrose agar (SDA) plate and antifungal activity of extracts were tested. For antifungal assay, plates were incubated for a period of 48hr at 28 ºC and observations were recorded.

RESULTS

All the mushrooms were screened for the presence of antimicrobial properties against different bacterial organisms belonging to gram +ve and gram –ve and one fungal strain of *Fusarium* sp. The experimental results of the present study revealed the bioactive potential of almost all mushrooms tested except *Pisolithus arrhizus, Lycoperdon pyriformi, Coltricia cinamomia, Daldinia concentric, Fomitopsi spinicola, Lentinus betulina* and *Polyporous sulphurous.* Interestingly, no mushroom extracts could show any bioactive inhibitory zone against bacteria PBC-3 and the fungal strain. *Lentinu storulosus, Suillus luteus* were found to be active against both gram –ve and gram +ve bacteria. Observations depicted in Table-1(A&B) clearly indicates the poor antibacterial properties of woody mushrooms tested in the present study. However, water extract of few woody mushrooms such as *Grifola frondosa*, *Schizophyllum commune* and *Trametes versicolor* exhibited the inhibition zone against gram –ve bacteria PBC-2. All the woody mushroom extracts were found with -ve result as tested. During the study some fleshy mushrooms were recorded with antimicrobial properties which are presented in Table-2 (A&B). The evaluation data of all 20 species revealed that four mushroom species showed a clear zone of inhibition against PBC-1 and PBC-2 through different solvent system (methanol, ethyl alcohol and acetone) and the measurement of said zones are depicted in Table-3. Methanolic and ethanolic extract of *Lentinus fusipes* showed better result against PBC-1(gram +ve) as the inhibition zone was measured to be 20 mm and 15 mm respectively [Fig:1 (A&B)]. Ethyl alcohol extracts of *Lentinus torulosus* showed inhibitory zone of 14 mm and 13 mm against PBC-1 and PBC-2 bacteria respectively [Fig:1 (C&D)]. It is further interesting to note that bioactive compounds of antibacterial nature could be extracted with methanol, ethyl alcohol, isopropanol and acetone for the present investigation. Different extracts of *Suillus luteus* was inhibitory against PBC-1, PBC-2 and MBC-I test microorganisms. However, a clear zone of inhibition (14 mm and 13 mm) were found in methanolic and acetone extract against PBC-1 [Fig:1 (E&F)] respectively. *Langermania gigantea* showed inhibition zone (12 mm) against PBC-1 (gram +ve) by acetone extract (Fig:1 G), while all other extracts were insignificant.

DISCUSSION

Mushrooms are well known for their bioactivity and medicinal uses, mostly for their antimicrobial activity. The present investigationon 20 macro-fungi for their antimicrobial potential in different solvent extracts revealed that the woody mushrooms under study did not show any antibacterial properties, although some woody mushroom were earlier reported as good source of antibacterial phytoconstituents [14,15]. It may be due to the different bacterial strains, solvent used and/or quantity of test material. Role of different solvent system for the extraction of bioactive compounds from different kinds of mushrooms has been reported [3]. *Suillus luteus* as reported to have good activity as per earlier literature [8]. Bioactivity of *Lentinus* species is significant and corroborated with earlier reports [6,7]. Similarly, different extracts of *Coniophora puteana* prepared in isopropanol and ethyl acetate exhibited distinct antibacterial activity against PBC-2 and MBC-3, respectively. It was interesting to note that bioactive compounds of antibacterial nature could be extracted with methanol, ethyl alcohol, isopropanol, ethyl acetate and acetone during the present investigation. Further it was observed that better antimicrobial results could be recorded in methanolic and acetone extracts as compared to other solvents.The findings of the present study can be treated as base line for future isolation of antimicrobial compounds in large scale for industrial purpose.

Antibacterial activity of different mushrooms against PBC-2 (gram -ve) showed significant results in different solvents used for the study. It indicates that either these mushrooms are rich source of antibacterial compounds useful against PBC-2 or different solvents used in this experiment might be helpful in extracting different compounds having bioactivity against PBC-2. Further study is required for the mass scale extraction of those bioactive substances and their purification and characterization may be recommended for broad spectrum antimicrobial analysis. However, the present preliminary data on antibacterial properties of woody and fleshy mushrooms against gram –ve and +ve bacteria might be helpful to proceed further for the detailed study on this aspect.

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Table1(A):Antibacterial and antifungal activity of woody mushrooms.

Solvent system		**Mushroom species**					
		Coltricia cinamomia	***Coniophora puteana***	***Daldinia concentrica***	***Fomitopsis pinicola***	***Grifola frondosa***	***Lentius betulina***
Water	PBC-1	-	-	-	-	-	-
	PBC-2	-	-	-	-	-	-
	MBC-1	-	-	-	-	-	-
	PBC-3	-	-	-	-	-	-
	F1	-	-	-	-	-	-
Methanol	PBC-1	-	-	-	-	-	-
	PBC-2	-	-	-	-	-	-
	MBC-1	-	-	-	-	-	-
	PBC-3	-	-	-	-	-	-

	F1	-	-	-	-	-	-
Ethyl alcohol	PBC-1	-	-	-	-	-	-
	PBC-2	-	-	-	-	-	-
	MBC-1	-	-	-	-	-	-
	PBC-3	-	-	-	-	-	-
	F1	-	-	-	-	-	-
Isopropanol	PBC-1	-	-	-	-	-	-
	PBC-2	-	+	-	-	-	-
	MBC-1	-	-	-	-	-	-
	PBC-3	-	-	-	-	-	-
	F1	-	-	-	-	-	-
Ethyl acetate	PBC-1	-	-	-	-	-	-
	PBC-2	-	-	-	-	-	-
	MBC-1	-	+	-	-	-	-
	PBC-3	-	-	-	-	-	-
	F1	-	-	-	-	-	-
Acetone	PBC-1	-	-	-	-	-	-
	PBC-2	-	-	-	-	-	-
	MBC-1	-	-	-	-	-	-
	PBC-3	-	-	-	-	-	-
	F1	-	-	-	-	-	-
Chloroform	PBC-1	-	-	-	-	-	-
	PBC-2	-	-	-	-	-	-
	MBC-1	-	-	-	-	-	-
	PBC-3	-	-	-	-	-	-
	F1	-	-	-	-	-	-

(+) indicates partial of zone of inhibition and (-) shows no activities. PBC-1(gram +ve) bacteria, PBC-2 (gram -ve) bacteria, MBC-1 (gram +ve) bacteria, PBC-3 (gram -ve) bacteria and F1:- *Fusarium* sp.

Table1(B): Antibacterial and antifungal activity of woody mushrooms.

Solvent system		**Mushroom species**				
		Microporus xanthopus	***Peniophora incarnata***	***Polyporus sulphureus***	***Schizophyllum commune***	***Trametes versicolor***
Water	PBC-1	-	-	-	-	-
	PBC-2	-	-	-	+	+
	MBC-1	-	-	-	-	-
	PBC-3	-	-	-	-	-
	F1	-	-	-	-	-
Methanol	PBC-1	-	-	-	-	-
	PBC-2	-	-	-	-	-
	MBC-1	-	-	-	-	-
	PBC-3	-	-	-	-	-
	F1	-	-	-	-	-

Ethyl alcohol	PBC-1	-	-	-	-	-
	PBC-2	-	-	-	-	-
	MBC-1	-	-	-	-	-
	PBC-3	-	-	-	-	-
	F1	-	-	-	-	-
Isopropanol	PBC-1	-	-	-	-	-
	PBC-2	-	+	-	-	-
	MBC-1	-	-	-	-	-
	PBC-3	-	-	-	-	-
	F1	-	-	-	-	-
Ethyl acetate	PBC-1	-	-	-	-	-
	PBC-2	+	-	-	-	-
	MBC-1	-	-	-	-	-
	PBC-3	-	-	-	-	-
	F1	-	-	-	-	-
Acetone	PBC-1	-	-	-	-	-
	PBC-2	-	-	-	-	-
	MBC-1	-	-	-	-	-
	PBC-3	-	-	-	-	-
	F1	-	-	-	-	-
Chloroform	PBC-1	-	-	-	-	-
	PBC-2	-	-	-	-	-
	MBC-1	-	-	-	-	-
	PBC-3	-	-	-	-	-
	F1	-	-	-	-	-

(+) indicates partial of zone of inhibition and (-) shows no activities. PBC-1(gram +ve) bacteria, PBC-2 (gram -ve) bacteria, MBC-1 (gram +ve) bacteria, PBC-3 (gram -ve) bacteria and F1:- *Fusarium* sp.

Table2(A): Antibacterial and antifungal activity of fleshy mushrooms grows on soil, leaf litter and grassland.

Solvent system		**Mushroom species**				
		Clavaria vermicularis	***Lentinus fusipes***	***Lentinus torulosus***	***Tricholoma lobayense***	***Geastrum fimbriatum***
Water	PBC-1	-	-	-	-	-
	PBC-2	-	-	-	-	-
	MBC-1	-	-	-	-	-
	PBC-3	-	-	-	-	-
	F1	-	-	-	-	-
Methanol	PBC-1	-	++	-	-	-
	PBC-2	-	-	-	-	-
	MBC-1	-	-	-	-	-
	PBC-3	-	-	-	-	-
	F1	-	-	-	-	-
Ethyl	PBC-1	-	++	++	-	-

alcohol	PBC-2	-	-	++	-	-
	MBC-1	-	-	-	-	+
	PBC-3	-	-	-	-	-
	F1	-	-	-	-	-
Isopropanol	PBC-1	-	-	-	+	-
	PBC-2	-	-	-	-	-
	MBC-1	-	-	-	-	-
	PBC-3	-	-	-	-	-
	F1	-	-	-	-	-
Ethyl acetate	PBC-1	-	-	+	-	-
	PBC-2	-	-	-	-	-
	MBC-1	-	-	-	-	-
	PBC-3	-	-	-	-	-
	F1	-	-	-	-	-
Acetone	PBC-1	-	-	-	-	-
	PBC-2	-	-	-	-	-
	MBC-1	-	-	-	-	-
	PBC-3	-	-	-	-	-
	F1	-	-	-	-	-
Chloroform	PBC-1	+	-	-	-	-
	PBC-2	-	-	-	-	-
	MBC-1	-	-	-	-	-
	PBC-3	-	-	-	-	-
	F1	-	-	-	-	-

(++) indicates clear zone of inhibition whereas (+) indicates partial of zone of inhibition and (-) shows no activities. PBC-1(gram +ve) bacteria, PBC-2 (gram -ve) bacteria, MBC-1 (gram +ve) bacteria, PBC-3 (gram -ve) bacteria and F1:- *Fusarium* sp.

Table 2(B): Antibacterial and antifungal activity of fleshy mushrooms grows on soil, leaf litter and grassland.

Solvent system		Mushroom species			
		Pisolithus arhizus	*Suillus luteus*	*Langermannia gigantea*	*Lycoperdon pyriforme*
Water	PBC-1	-	-	-	-
	PBC-2	-	-	-	-
	MBC-1	-	-	-	-
	PBC-3	-	-	-	-
	F1	-	-	-	-
Methanol	PBC-1	-	++	-	-
	PBC-2	-	-	-	-
	MBC-1	-	+	-	-
	PBC-3	-	-	-	-
	F1	-	-	-	-

Ethyl alcohol	PBC-1	-	-	-	-
	PBC-2	-	+	-	-
	MBC-1	-	+	-	-
	PBC-3	-	-	-	-
	F1	-	-	-	-
Isopropanol	PBC-1	-	+	-	-
	PBC-2	-	-	-	-
	MBC-1	-	-	-	-
	PBC-3	-	-	-	-
	F1	-	-	-	-
Ethyl acetate	PBC-1	-	-	-	-
	PBC-2	-	-	-	-
	MBC-1	-	-	-	-
	PBC-3	-	-	-	-
	F1	-	-	-	-
Acetone	PBC-1	-	++	++	-
	PBC-2	-	-	-	-
	MBC-1	-	-	-	-
	PBC-3	-	-	-	-
	F1	-	-	-	-
Chloroform	PBC-1	-	-	-	-
	PBC-2	-	-	-	-
	MBC-1	-	-	-	-
	PBC-3	-	-	-	-
	F1	-	-	-	-

(++) indicates clear zone of inhibition whereas (+) indicates partial of zone of inhibition and (-) shows no activities. PBC-1(gram +ve) bacteria, PBC-2 (gram -ve) bacteria, MBC-1 (gram +ve) bacteria, PBC-3 (gram -ve) bacteria and F1:- *Fusarium* sp.

Table 3: Zone of inhibition (in mm) of 4 mushroom species in different solvent extract against different test organismPBC-1(gram +ve) and PBC-2 (gram -ve) bacteria.

Solvent system		Mushroom species			
		Lentinus fusipes	*Lentinus torulosus*	*Suillus luteus*	*Langermannia gigantea*
Methanol	PBC-1	20	-	14	-
	PBC-2	-	-	-	-
Ethyl alcohol	PBC-1	15	14	-	-
	PBC-2	-	13	-	-
Acetone	PBC-1	-	-	13	12
	PBC-2	-	-	-	-

A. *Lentinus fusipes* B. *Lentinus fusipes* C. *Lentinus torulosus*

D. *Lentinus torulosus* E. *Suillus luteus* F. *Suillus luteus*

G.Langermania gigantea

Figure 1. Four mushroom presenting clear zone of inhibition by different solvent extracts

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

An Overview of Ajwain (*Trachyspermum ammi*)

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Received: 05 Jan 2020 Revised: 07 Feb 2020 Accepted: 09 Mar 2020

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ABSTRACT

Trachyspermum ammi (L.) Sprague or commonly Ajwain is a herbaceous herb belonging to the family Apiaceae and vastly grows in Egypt, Iran, Pakistan, Afghanistan, and India as well as European region. Known as Zenyan or Nankhah in medical and pharmaceutical manuscript of medieval Persia, seeds of Ajwain were highly administered by traditional healers and traditionally employed for different ailments. Due to its various chemical constituents, the herb seeds were also evaluated for its numerous pharmacological properties. Accordingly, current work was carried out to review the traditional and modern pharmacological properties of Ajwain regarding current and medieval reports. To this, respective databases were searched for the terms 'Trachyspermum ammi', 'Carum copticum', 'Ajwain' and 'Ajowan' without limitation up to early 2013. Ajwain seeds revealed to possess antiseptic, stimulant, carminative, diuretic, anesthetic, antimicrobial, antiviral, nematicidal, antiulcer, antihypertensive, antitussive, bronchodilatory, antiplatelet and hepatoprotective as well as antihyperlipidemic effects, many of those were remarked by early Persian physicians. With reference to these pharmacological activities, Ajwain seeds can be a good candidate for to be applied in clinical practice. However, in spite of various experimental and animal studies, lack of comprehensive clinical trials aimed on regarded effects is still remained to reconfirm the traditional knowledge.

Keywords: *Trachyspermum ammi* (L.), herbaceous, pharmacological, clinical, traditional.

INTRODUCTION

Known as Ajwain, *Trachyspermum ammi* (L.) Sprague is an annual herbaceous plant belonging to the highly valued medicinally important family, Apiaceae [1]. It is said that the herb is widely grown in arid and semi-arid regions where the soil involve high amount of salts [2]. Ajwain has an erect and striate stem involving glabrous or minutely pubescent properties which may grow up to 90 cm tall [3]. Ajwain is widely distributed and cultivated in various regions such as Iran, Pakistan, Afghanistan, and India as well as Europe while it is indigenous to Egypt [4]. The herb is generally grown in October–November and should be harvested in May–June [5, 6]. Usually grayish brown seeds or fruits of Ajwain are considered for medical and nutritional purposes [5]

A number of chemical constituents have been reported for the herb. Fiber (11.9%), carbohydrates (24.6%), tannins, glycosides, moisture (8.9%), protein (17.1%), fat (21.1%), saponins, flavones and other components (7.1%) involving calcium, phosphorous, iron, cobalt, copper, iodine, manganese, thiamine, riboflavin and nicotinic acid are of reported phytochemical constituents of Ajwain [6–8]. In the alcoholic extraction process, a large amount of saponin has been derived [6]. Similar to the most species of the family Apiaceae, Ajwain is famous for its brownish essential oil. Apparently, presence of an Ajowan essential oil is responsible for its odor and taste. Hence fruits of Ajwain accumulate up to 5% essential oil in its compartments [9]. However, some investigation reported the yield of fruits essential oil up to 9% which may be considerable [10]. Usually, Thymol is the main Ajwain essential oil constituent and may be yielded from 35% to 60% [11, 12].

The non-thymol fraction (Thymene) contains Paracymene, Gamma-terpinene, Alpha-pinene, Betapinene, α-terpinene, Styrene, Delta-3-carene, Betaphyllanderene, terpinene-4-ol and Carvacrol [6, 13]. On the other hand, in an investigation, carvone (46.2%), limonene (38.1%) and dillapiole (8.9%) were introduced as principal oil constituents [14]. Also oleic, linoleic, palmitic, petroselinic acid, resin acids are isolated from fruits of Ajwain [7]. New glycosyl constituents such as 6-hydroxycarvacrol 2-O-β-D-Glucopyranoside and 3, 5-Dihydroxytoluene 3-O-β-D-Galactopyranoside are recently reported from fruits of Ajwain [15]. Also, a steroid like substance and a compound namely 6-O-β Glucopyranosyloxy thymol has been isolated from the fruits [16]. Water-soluble extract of Ajwain fruit revealed to involve many compounds such as a new Monoterpenoid, 3, 7-Dimethyloct-3(10)-ene-1, 2, 6, 7-tetrol; new Monoterpenoid Glucosides namely (2S, 6Z)-3, 7-Dimethyloct3(10)-ene-1, 2, 6, 7-Tetrol 1-O-β-D-Glucopyranoside and 6-Hydroxythymol 3-O-β-D-Glucopyranoside; new aromatic compound glucosides as 2-Methyl-3-Buten-2ol-β-D-Glucopyanoside Benzyl-β-D-Glucopyranoside and Glucide namely (3R)-2-Hydroxymethyl butane1,2,3,4-tetrol [11, 17]. Other glucosides such as 1-DeoxyL-Erythritol and 1-Deoxypentitol and also nucleosides as adenosine and uridine were isolated from Ajwain fruits [11].

Ajwain was vastly applied by medieval practitioners and it also exhibited different pharmacological effects regarding various chemical ingredients. Accordingly, current paper was aimed to review the clinical applications of the herb as well as most cited pharmacological properties in current medicine. To this, respective databases were searched for the terms "Trachyspermum ammi", "Carum copticum", "Ajwain" and "Ajowan" without limitation up to early 2013. Information on the herb was gathered via electronic search using Pubmed, Scopus, Google scholar and SID (for articles in Persian language) as well as medical and pharmaceutical manuscripts of Persian medicine.

Applications of Ajwain in Medieval and Traditional Persian Medicine

Ajwain has been commonly used in traditional medicine systems for a variety of medicinal and pharmacological aspects [18]. In Traditional Persian Medicine (TPM), Ajwain was well known from thousands of years. Persian practitioners usually used seeds of Ajwain as the most useful part of the herb [19]. According to its temperament, Ajwain is hot and dry in the third degree and also possesses some bitterness and acridity [20]. Oral application of seed was reported to be useful for paralysis, tremor and palsy as well as other neural disorders in the field of

neurology [20]. Persian practitioners also applied the eye and ear drop formulated from seeds of Ajwain in order to control the infected conditions and correct the auditory weakness [21].

In the field of respiratory, Ajwain was said to be effective on cough, pleurisy and dysphonia [19]. Fruits were widely administered for liver spleen as well as gastrointestinal disorders such as nausea, vomiting, reflux, abdominal cramps and loss of appetite [20]. They were also said to be beneficial in stomach troubles and possess stimulant and carminative properties [21]. Ajwain was reported as an anthelmintic medicine and also antidote for various natural toxic agents [21]. It was also believed to be beneficial for dissolving the calculi and stones if taken with wine. Persian practitioners also considered the seeds as an aphrodisiac, galactagogue and diuretic agent [2o]

As a cosmetic agent, local administration of Ajwain as a paint results in yellowish complexion on the skin. It was also incorporated in medicine prepared for pityriasis and leukoderma and plastered with honey in cases of all types of ecchymosis [19, 20]. Persian practitioners also used the seeds in the form of fumigation for the female genital disorders. In the field of toxicology, it was reported that bathing the affected part with the decoction of Ajwain seeds alleviates the pain caused by scorpion's bite [19]. Also it was used for the reduction of undesired effects related to the opioid withdrawal. Ajwain was also introduced as a potent analgesic and anti-inflammatory agent. Therefore it was applied on the affected area solely of in combination with egg white or honey. Persian practitioners used Ajwain in chronic fevers and gripes [20, 21]. Hydrosol and oil extracted from the seeds of Ajwain was also used for medical purposes. Of those, management of paralysis, palsy, tremor and neurological disorders such as neuropathic pain as well as chronic pains are cited in Persian medical and pharmaceutical manuscripts [20, 22]. The Ajwain hydrosol combining with Borage and Cinnamom used Ajwain in chronic fevers and gripes.

Current Pharmacological Findings

Analgesic and Antinociceptive Effects In order to evaluate the analgesic and antinociceptive activity of Ajwain, an In vivo investigation was carried out using a Tail-flick Analgesiometer Device [24]. The study revealed that the ethanolic extract significantly increase in Tail-Flick Latency (TFL) within 2 hours postdrug administration. An experimental trial study has also been carried out to compare the antinociceptive effect of the hydroalcoholic extract of Ajwain with morphine sulphate using formalin test. Findings revealed that Ajwain extract exhibited antinociceptive effect on both early and late phases [25]. Similar study has been done on the Ajwain total essential oil which was significantly effective on the late phase of formalin test [26] and it may be due to the presence of thymol in essential oil. In addition, under a randomized controlled placebo control clinical trial, the herb essential oil was assayed for the analgesic effect in neuropathic feet burn. Results revealed that Ajwain essential oil significantly reduced the feet burn compared to placebo [27].

Antibacterial and Antifungal Activities

To assay the antibacterial efficacy of Ajwain, acetone and aqueous extracts were tested against *Enterococcus faecalis, Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella flexneri*, and *Staphylococcus aureus* using agar diffusion assay [28]. The study showed that acetone extract shows more activity compared to the aqueous extract. In another study, ethanolic extract of Ajwain possessed antibacterial activity against eight strains of *Helicobacter pylori* [29]. Also methanolic extract of Ajwain exhibited bactericidal activity against 11 species at 2mg/well in agar well-diffusion method. It was measured by Diameter of Inhibition Zones (DIZ). DIZ was over 15mm against *Staphylococcus aureus* and *Staphylococcus epidermidis*; 10–14 mm against *Pseudomonas aeruginosa* and *Bacillus pumiilus* against *Escherichia coli*, *Klebsiella pneumonia* as well as *Bordetella bronchiseptica.* On the other hand, no activity wasreported against *Pseudomonas fluorescens* and *Micrococcus luteus* [30]. As Ajwain may have large amounts of Thymol or Carvacrol in its total essential oil, mentioned phenolic compounds are reported to be either bactericidal or bacteriostatic agents depending on the concentration [31]. In order to assess the antifungal

activity of Ajwain, total essential oil extracted from seeds was subjected for fungicidal effect and showed proper effect on *Aspergillus niger* and *Curvularia ovoidea* at 5000 ppm as minimum inhibitory concentration [32].

Insecticidal Assessment

It is reported that the essential oil extracted from the seeds of Ajwain can exhibit insecticidal activity in the oviposition step as well as egg hatching and developmental inhibitory activities against Callosobruchus chinensis [33, 34].

Anthelmintic Activity

Antifilarial activity assessment of the Ajwain methanolic extract was done as an in vitro assay against adult bovine filarial Setaria digitata worms. In that investigation, a bioassay-guided fractionation was prepared by introducing the crude extract to flash chromatography. HPLC analysis was done for both crude extract and active fraction [35]. Active fraction and also crude extract exhibited significant activity against adult *S. digitata* by both worm motility and MTT [3-(4, 5-dimethylthiazol2-yl)-2, 5-diphenyltetrazolium bromide] reduction assays. The isolated active principle which was identified as a phenolic monoterpene was structurally charac terized by IR, H-NMR and MS analysis. The compound was then evaluated for in vivo antifilarial activity against the human filarial worm *Brugia malayi.* Results revealed in vivo macrofilaricidal activity and female worm sterility against B. malayi [35]. Antihelmintic activity of Ajwain was carried out by considering the Haemonchus contortus in sheep and Ascaris lumbricoides in humans.

Results were due to loss of energy reserves by interference with the energy metabolism of parasites through potentiating the ATPase activity. Ajwain has also been reported to exhibit cholinergic activity with peristaltic movements of gut. Hence this fact may help in expulsion of intestinal parasites and be a contributory factor to its anthelmintic activity [36, 37]. Ajwain was also evaluated for its nematicidal activity. A survey was done on the total essential oil components of Ajwain that showed significant nematicidal activity against pinewood nematode, *Bursaphelenchus xylophilus.* Nematicidal activity of Ajwain essential oils LC50 values was measured as 0.431mg/ml [38] and it was mainly attributed to the activity of Thymol and Carvacrol [39].

Antiplatelet Activity

Antiplatelet activity has been done on the dried ethereal extract of Ajwain. Therefore, in an in vitro study with human blood samples, Ajwain seeds inhibited the platelet aggregation induced by arachidonic acid, collagen and epinephrine [40].

EffectsAnti-inflammatory

Ajwain was also evaluated for exhibiting anti– inflammatory effect. Accordingly, both total alcoholic extract and total aqueous extract possess in vivo significant anti–inflammatory effect [41].

Antitussive and Bronchodilatory Effects

Antitussive effect of Ajwain has been reported in traditional medical manuscripts. In this regard, in a study the mentioned clinical effect of aerosols related to two different concentrations of aqueous and macerated extracts of Ajwain seeds as well as Carvacrol, codeine, and saline were evaluated by counting the number of coughs produced. According to the results, both concentrations of Ajwain seeds revealed significant reduction of cough number which may be a result of its potent antitussive effect [42]. Relative studies showed the inhibitory effect of both Ajwain extract and essential oil on Histamine (H1) receptors of isolated guinea-pig tracheal chains [43]. In another study, in

the field of respiratory, bronchodilatory effects of different fractions of Ajwain essential were examined. Results showed that the relaxant and bronchodilatory effect of essential oil fractions may be due to the amount of Carvacrol [44]. The bronchodilatory effect of decocted extract of Ajwain on the asthmatic patients' airways was examined in a subsequent trial study. According to the results, the extract has a relatively bronchodilatory effect on asthmatic airways compared to the effect of Theophylline at concentrations used [45].

Diuretic and Anti-lithiasis Activity

Ajwain was attributed to have diuretic and anti-lithiasis activity in ethnopharmacological reports. Accordingly, a human study was performed and in which, seeds of Ajwain were decocted in milk and given orally to volunteers suffering from urinary stone for a nine days period. The results were reported satisfactory against pure ca-oxalate stone [46].

Antihyperlipidemic Properties

Another activity which has been proved for Ajwain is the antihyperlipidemic property. An in vivo study revealed that Ajwain seeds powder is extensively effective on lipid profile and can decrease total cholesterol, LDL-cholesterol, triglycerides and total lipids. Moreover, organic extract of seeds reduced atherogenic index and increased the level of HDL-cholesterol in albino rabbits [47].

Detoxification Activity

Detoxification of aflatoxins by seed extract of Ajwain can support the related traditional reports. Hence in anexperimental study, Ajwain seed extract exhibited the maximum degradation of aflatoxin G1 [48].

Antioxidant Properties

The antioxidant and ameliorative property of Ajwain extract has been evaluated on hexachlorocyclohexane induced oxidative stress and toxicity in an in vivo investigation. Accordingly, results revealed that the dietary Ajwain extract would reduce the toxicity resulted from hepatic free radical stress [49].

Antiviral Effects

For the evaluation of Ajwain antiviral activity, an in vitro assay was carried out on the methanolic extract of the herb which showed significant inhibitory effects on Hepatitis C Virus (HCV) protease [50].

Spermicidal Activity

Spermicidal activities of Ajwain essential oil was determined via an in vitro study where it was revealed that the volatile oil possessed potent spermicidal action [51]. Therefore, the oil may be considered as a natural contraceptive agent. 3.14 Hepatoprotective Effects Along with the potent antioxidant activity, the Ajwain methanolic extract revealed to exhibit in vivo hepatoprotective activity with eighty percent protection against a normally-lethal dose of paracetamol in mice. The extract also possessed preventive effects against CCl4-induced prolongation of pentobarbital sleeping time as well as equilibrating the level of hepatic enzymes, Alkaline Phosphatase (ALP) and Aminotransferases (AST and ALT) during liver damage [52].

Antiulcer Activity

Using different ulcer models, Ajwain ethanolic extract resulted in significant ulcer index decrease in animal pre-treated with and also exhibited ulcer protection in all models. Overall, the extract reduced the ulcerative lesions compared to control group of animal model [53]

Antihypertensive and Antispasmodic Activity

Ajwain was evaluated for the potentiality of antihypertensive and antispasmodic activity. In the related investigation, the aqueous-methanolic extract of the seeds caused a dose dependent decrease in arterial blood pressure in anaesthetized animal models. Furthermore, inhibitory effect on the K+-induced contractions was seen in isolated rabbit aorta and jejunum preparations during the application of Ajwain extract. These findings prove the potential antihypertensive and antispasmodic activity of Ajwain [52].

Digestive Stimulant Activity

Traditional practitioners recommended the herb as a digestive stimulant medicine [20]. It is now proved that Ajwain can increase the secretion of gastric acid, bile acids and activity of digestive enzymes. It may also reduce the food transient time [54, 55]. As the enzyme modulatory activity, Ajwain reinforced the pancreatic lipase and amylase effectiveness, which may support the digestive stimulant activity [56].

Estrogenic Activity

The total phytoestrogen content of dry Ajwain seed was determined as 473 ppm. In this regard, the herb is the second highest in the list of plants tested for total phytoestrogen content [57]. It should be noted that the herb has been traditionally used as a galactagogue [20].

Toxicity and Teratogenicity

It was reported that Ajwain showed teratogenicity in rat fetuses. Therefore it may be harmful to be intake during pregnancy [58].

CONCLUSION

With reference to the mentioned pharmacological activities, Ajwain seeds can be used for clinical applications. However, in spite of various experimental and animal studies, lack of comprehensive clinical trials aimed on regarded effects still remains to reconfirm the traditional knowledge.

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RESEARCH ARTICLE

VEDIC ANTI-CANCER - VAC How to Make

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Received: 02 Jan 2020 Revised: 04 Feb 2020 Accepted: 06 Mar 2020

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ABSTRACT

Indians of late are finding new vigour in announcing nuggets of ancient sciences. And India administration in her various echelons are exhibiting select indulgence. Cancer i.e., malignancy and metastasis is however conspicuous by absence in her great and small treatise. Apporting credits and heralds are being noted. We herein report one of our works that has been officially in public motion since c. 2000 (NIH-NCAAM). Vedic ingredients are used to combat such emperor of maladies. It is called VAC : vedic anti-cancer. Prophylaxis is also being indicated. It is novel, comprehensive and forward looking. Technology adoptable.

Key words: Atox; ATAMCOX; VAC – vedic anti cancer; MRC – metastasis reversal concepts; Herbal anti-cancer; Cancer Prevention; Palliative Care.

INTRODUCTION

Indian civilisation has been in continuum since 5000-7000 yrs before present, while most other arose and waned. Indian civilisation is also known as Vedic (plural) civilisation alias Sanatan savyata (perennial\hydespas civilisation). Mahatma Gandhi the father of the mighty Indian nation has said "India lives in her villages". Swami Vivekanada said "the soul of (such plural-rural) India is religion". The Adi Sankracharya has opined that "religion/God rest & arise from Texts". Among the religious texts Chanda Upanisada (metrical aphorisms) says "Tat twam see/asi" (though thee art). It has been hailed (unassailably over last millennia) as the "mahavyakhya" (great explanation). The mahavyakhya tacitly makes anthropomorphs/mind as the divine entity. Among the non-religious plural texts (i.e., secular Vedic) are the gamut called Ayurveda (plural life). Thus the texts be religious and as well secular. Hence, the indo ancient texts are plural (Vedic). Mahatmaa Gandhi in his days of leadership (freedom movement), had staunchly preferred herbal/ayurvedic medicine, to the exception of all [1]. Ayurveda is India's national school of medicine. Pandit (the erudite) Madam Mohan Malaviya was inspired by such way of life. We are aware of our heritage. And, stand inspired.

Deepak Bhattacharya

This communication arises from related works [2,3] and more due continuous social service from such remote of rural India about a low profile; conscientious slow flow effort; having begun in 1981 (started with male, geriatric colorectal carcinoma, end stage, dual colostomy, with complete recovery). By 2015 the the below mentioned (a) formula emerged as a fixed dose. So also emerged the (b) process of making (c) packaging (d) consumption i.e., prescription\ medication (e) clinical superintendence (f) contradictions. Its primary role being (i) prevention/ prophylaxis (ii) anti-metastasis (iii) reduces all the side effects of the entire range of Chemotherapy (iv) shore up anti-cancer\carcinoma treatments and assistance during inter treatment periods. It is safe, broad spectrum, effective, easy to make, economic, non-toxic, synergistic with allopathy (up-regulates, better targeting – least physiological wandering - complements & supplements). It is called as VAC – vedic anti- cancer.

VAC uses Vedic i.e, Ayurvedic ingredients and a very modern process. In other words VAC can be made via ayurvedic system and also via modern pharmacological methods. However, the modern method(s) prove to yield more accuracy in the end product, repeatability, with batch to batch identicality, therapeutics and prognosis. VAC aims at patient centric care with family welfare at heart. VAC in the ripe end of the bench-to-bed stage (translational medicine). Allopathic and or a Ayurvedic\Natural VAC can easily be made within months. VAC can be used as complement; as a supplement and as well as a medicament. Table – 1 gives the ingredients.

Process

Incineration means burning in modern furnace for 2hrs at 1000^{0}C., followed by instant quenching by pouring ascorbic\hydroxyl group of acids (pure\crude-natural) *ad libitum* on red hot metal. Repeating at best 2 times (2nd round requiring higher temp and shorter period of in-furnace stay). Related hydroxlylic group of acids yield near similar results while widening the spectrum of the eficacy. Such quenching (vital step) inflicts deep infraction of the metal matrix, metal reformation failure, atomization. Neither metal – nor whole ash. Unique intermediary state. Becomes non-toxic; non cytotoxic; non corrosive. Marked by purification, clatherinisation, adsorption of $+^{ve}$ ions (non electrons) from the quenching fluids into the clatherined vacuoles (of the depleted metal matrix), mass gain, good ion affinity, soluble in blood (also aminos), chromatic changes, long shelf life, volume gain with weight reduction, good bulk density, free flowing, amorphous, salt (ash) of that metal or alloy (not rust), electron less/depleted, crystalline, sandy type, semi-conductor property even at RT and also above 35+^{0}C. May have application also in chip making! Poikilotherms are noted to avoid. Also seem to thwart fungus and moss. Thus may also have some use in anti-barnacle\anti-fouling applications.
Quenching Sources: Wide Spectrum Composition Effect

Ascorbic acid; Ellagitanins; Gallagic group of acids; Juices from Ayurvedic\indo native Pomegranate; Jamboo; Bataabi; Nimboo; Amla; Kamla; Narangi; etc., bio-similars.
Note: each of these inflict a variation in the end compound. Very much needed by the carcinoma afflicted and or CT insulted physiology

Dose

Each gelatin filled cap contains 300-500mg of mixed powders (Table-2). Oral, 1cap BD or TID daily during CT cycle period, 60days post CT tapered down to status 1 cap per week for one year (52 weeks) as all clear maintenance prophylactic therapy. Calculated at 60-65 Kg, 5’-6’ (height), indo native (vegetarian & non vegi).

Application

As a stand alone mono therapy VAC\ATAMCOX is very effective in slowing down and\or stopping primary cancer\carcinoma progression. Yeomanly effective in reversing metastasis when administered concurrently with CT.

With concurrent low dose MDT chemotherapy effective in all cell lines; reverses secondary cancer (metastasis) from bones very efficiently and to various degrees from most other organs/cell lines (failure not reported as yet). Sterling in earlier stages. Breast; bone; blood; liver very well indicated. Post surgery of benign tumors and or post needle biopsies (thwarts cancerisation and or metastasis).

RESULTS

Super safe & very effective as (i) an anti-metastasis (ii) superbly synergistic with chemotherapy (CT) – making the combination as ultra potent (iii) near complete down turn of the side effects and the contradictions of even the most toxic CT (iv) complements-and-supplements CT and all other regimens of conventional therapies (v) synergic also with MDT (vi) synergic with all conventional regimes (vii) does not seek to replace CT nor make it redundant or anything alike – extended course of CT cycle become viable with concurrent intake of VAC\ATAMCOX (viii) broad spectrum efficacy (ix) well indicated in irritable bowl syndrome & gut inflammation syndromes (x) prophylactic (post surgery; needling) vis-à-vis metastasis in all stages & conditions i.e., preventive role is prominent (xi) significant evidence based results within 60-120days (xii) Hb, platelet, RBC and TLC counts return to normal range even during CT cycle periods (xiii) down turn in treatment cost & time (xiv) contra indicated in soriasis; icterus causing group; arthritis. (xv) Generally salutary physiologically.

Constituent No 1-to-3 sufficient as potent anti-metastasis & CT' & MDT's side effect reducing APIs. In cancer, internal oozing carcinomas (outpour exosomes) and the circulating load of exosomes (gone wrong mitochondria) be the pathological causes for inflammation. Constituent No.1-to-3 subsume the exosomes (anatgonise) – which all wane and eventually become untraceable in liquid biopsy. Are also, very well indicated in Leukemias (excellent synergy with triosin kinase inhibitors with gradual retraction of TKIs noted). Constituent No 4-to-7 makes ATAMCOX \ VAC holistic; physiologically salutary; anti-constipation; anti-fibrosis; numerous; all age- stage condition applicable food type medicament; compatible & useful in stomach, liver & gastric stages.

Mode of Action

Evidently becauses Warberg's mechanism. Proton is donated (from the electron depleted constituents No.1-3) as the 'off switch' and electrons from the gone wrong i.e., askew mitrochondria is trapped i.e., apoptosis occurs. The metals being poly each donation is cell line and or differentiation specific; and also use various gateways. Well deffirentiated cancerous cells have high affilinity for trace elements of low conductivity. Thus are well aspected (traget oriented); finally ssists cell wall fjording of\by drug loaded moities. Concurrent CT proves lethal.

Contra Indications

Pulmonary cancers; embolic pathologies; cardio thoracic or pulmonary disorders; High pro-thrombin; Deep vein thrombosis, etc.

Ancient Texts (pan global)

In the acknowledged, accomplished, ancient, traditional schools of health care sciences viz., Indo-Greek [4]; Ayurveda [5,6]; Sino-Nipponese (Chinese/Japanese) [7]; the term 'cancer' and or the description amounting to 'metastasis' as the modern sciences is not noted. Even regional-vernacular health care treatise do not indicate anything alike [8]. These two terms came to be used from around datum *c.*1850 (Industrial Revolution) or in *c.* 1950 (post IInd World War), respectively. Allopathy (chemicals that inflict symptom's opposite effects) the European model of medicine (arose from Greek civilisation 6-5th *c.* B.C.) has evolved out of the 'Surgeon Barber Associations' [9], the Chinese medicine system [10] neither used the terms 'cancer\metastasis' nor metal ashes.

Vedic Vigyan & Modern Vigyan: The Connection (via VAC).

Warburg's hypothesis was postulated by the Nobel laureate Otto Heinrich Warburg of Germany in 1924 [11]. He was a clinician, a son of a Physicist. Clinician Mr. Warburg had hypothesized that tumor and subsequent cancer (or even the as known now) direct malignancy alias carcinoma are caused by the fact that all cells mainly generate energy (a sub micro part of the cell) by using its organelle later got to be known as 'mitochondria'. The very term mitochondria suggest that it chemically produces power (pico-volts) to cause cell mitosis (division). Once the required amounts of chemical energy is produced the mitochondria switches off and cell division stops. It is a loop. In tumor and cancer such loop mechanism is noted to be in failed state (Warburg's hypothesis). In other words, mitochondria continues with power production (streaming electrons) resulting in incessant division of cells due to absence of any reciprocal donor. The mitochondrias needs to switch off. VAC switches off the gone wrong mitochondria.

DISCUSSION

Following our usage, public talks & averments a US based company [12] have started using iron/ferrous ash under trade name "injectafer' & 'venofer' being indicated for anaemia (which is pronounced in status cancer, and bone marrow suppression is a hall mark of chemotherapy). And whereas, the metal ashes and their making process pre-existed in the pan-Indo Ayurveda texts, including specific use in anaemia and in debilitated status [13]. Solid pill forms apart even suspension/s (Lauhasava-aristha) pre existed [14]. However, these products and or the ingredients as in T-1 were never used vis-a-vis cancer, carcinoma, tumor and least against metastasis and or for reducing the side effects of chemotherapy or radio-therapy or for palliative [15-16]. We have used vedic ingredients vis-a-vis such killer pathologies for the first time (pan global comparative basis). We have used modern pharma technique of making.

CONCLUSION

VAC\ATOX\ATAMCOX are the various names of the development stages of VAC. It uses vedic ingredients and modern pharmaceutical making process. High QC, repeatability, diagnosis-therapy-prognosis clinically monitorable. Such making process is also inspired by vedic texts. Superbly useful in status cancer; as a palliative; as a anti-anemia; as a end of life stage medicament.

ACKNOWLEDGEMENTS

Author wishes to than them who all have encouraged to make such an submission & facilitated publication. Hope indians shall copy this method to combat cancer more successfully, more holistically. The author stands by to provide all-full support. There are no commercial bindings. No limitations. Dedicated to my late father Sri Haraprasanna Bhattacharya – who being afflicted for over a 2 decades had recovered from end stage of colo rectal with double permanent colostomy (either getting removed). Thanks to IJONS for supporting ground breaking frank reports.

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Table 1: Gives the ingredients.

Sl. No.	Oriental-Name in Sanskrit #	Occidental name in * English (part used)	Designed Therapeutics **	Availability
1	Lauha Vasma	Ferrous\Iron ash	Anti-Metastasis & Anti Cancer; Salutary effect on Hematology & Hemodynamics	Whole of Indian Ayurvedic bulk & raw drug market & in Large Medical Colleges (Rasa Sastra Dept). Legal.
2	Tambra Vasma	Copper ash	Anti- Cancer; Anti Metastasis & Anti-Carcinoma; Anti-Hematoma; anti angiogenesis; etc..	
3	Kansa Vasma	Bell metal ash	Anti – IBS; Salutary effect on Gastric chamber; Gut; Micro nutrient marshalling; anti angiogenesis; etc.	
Note - i	Constituents 1-to-3 sufficient as anti-metastasis & CT' & MDT's side effect reducing formulation			
Note - ii	There are a number of modern medicines that uses ashes alias vasmas, The term 'vasma' is a technical term of Sanskrit linguaphone			

Deepak Bhattacharya

Table .2. Gives the ashes (vasmas) preparation process via modern pharma methods

Sl No. as in Table - I	Occidental name in English	Ascorbic Acid Quenching	Incineration & Quenching	Pulverisation	Per Cap 500mg ± 10%
1	Ferrous ash (incinerated, 10000C, 2hrs.)	When Red Hot – Quenching ad libitum	2 times	100 – 200 mesh	200-350mg
2	Copper ash (- do -)	(- do -)	2 times	-do-	50-150mg
3	Bell metal ash (- do -)	(- do -)	2 times	-do-	25-100mg

Note - i

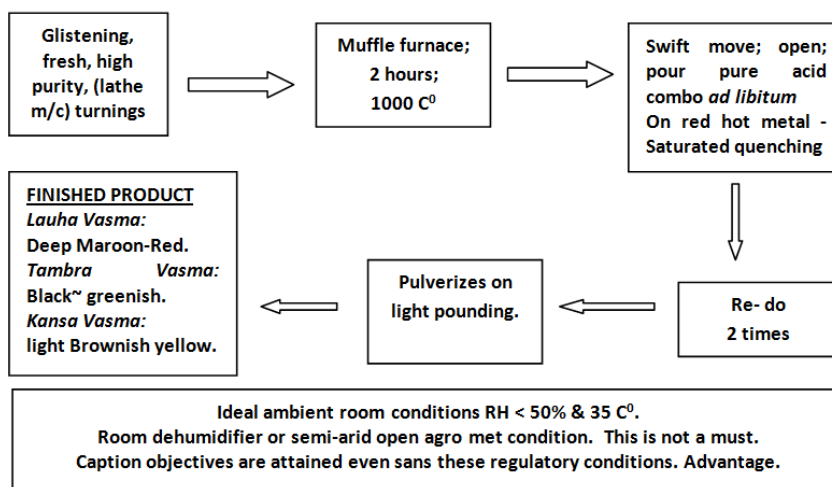

Fig.1.Schematic Diagram of the Making Process

Indian Journal of Natural Sciences

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Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Mushroom Diversity of Sundergarh District in the State of Odisha, India

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Received: 09 Jan 2020 Revised: 11 Feb 2020 Accepted: 14 Mar 2020

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ABSTRACT

Sundergarh, a district of Odisha in India, situated between 21^0 36' & 22^0 32' north latitude and 83^0 32' & 85^0 22' east longitude at an elevation of 243 m height with a much diversified ecosystem. Mushrooms which form a significant component of the ecosystem has been meagerly studied as regards to the biodiversity studies of Odisha. These are the organisms which are cosmopolitan in distribution and occur seasonally in various habitats like humus rich soils, decaying plant litter and wood logs in forests as well as in meadows and even in sandy and other soils. To give an important and relevant feature for diversity of macrofungi of the study area, an extensive survey work was carried out from January 2016 to December 2019 in 15 different places such as scrub patches, dry deciduous forests, crop fields, and urban places in and around Sundergarh. A total of 76 species of mushrooms were collected which taxonomically belonged to 48 genera and 33 families. The results of the present investigation revealed that this area has 18 edible mushrooms and 6 species had medicinal uses. Among others 20 species were unfit for consumption and remaining 32 species were unknown for their usability. *Agaricus campestris, Agaricus bisporus* and *Termitomyces microcarpus* were most abundant mushrooms found in the study area. *Ganoderma lucidum* and *Trametes versicolor* were the two important mushroom species widely used by local people for curing different ailments.

Keywords: Ecosystem, diversity, mushroom, Sundergarh, Odisha

INTRODUCTION

The structures that are commonly known as mushroom are nothing else but the fruiting bodies of those organisms that the mycologists call higher fungi or macromycetes, even though the dimension of caps of some mushrooms

might be only a few millimeters across [1]. The diversity of macro-fungi and their natural beauty occupy prime place in the biological world and India has been a cradle for these species. Defining the number of fungi on earth has been a point of discussion and several studies have focused on enumerating the world's fungal diversity [2]. Mushrooms are seasonal fungi, which occupy the diverse niches in nature in the forest ecosystem. They predominantly occur during the rainy season as also during spring when the snow melts. Mushrooms are in fact the 'fruit' of the underground fungal mycelium. They are macromycetes forming macroscopic fruiting bodies such as agarics, boletes, jelly fungi, coral fungi, stink horns, bracket fungi, puffballs and bird's nest fungi. They are fleshy, sub-fleshy, or sometimes leathery and woody bearing their fertile surface either on lamellae or on lining of the tubes, opening out by means of pores. The lamellate members are called agarics and the tube bearing poroid members, as boletes and polypores.

Mushrooms are considered as a very strange group, very difficult to study and understood which may be due to its hidden nature and frequently sporadic and short-lived sporocarps. Therefore, they have largely been untended and overlooked in national and international nature conservation actions. However, through the research, our erudition of macrofungi has crucially increased. It is now highly sensible to assess the adjacent status and future for macro fungi species in their diversity by human activities such as land management [3]. Mushrooms are all around in nature and they remain the earliest form of fungi known to human being [4]. The controversy of fungal diversity, its scope and conservation, has attracted more attention in the last 10-15 years [5]. Mushrooms appear to be collected and consumed during almost the entire year, but most fungi are collected during the rainy seasons, prescribed the importance of rainfall patterns in fungal diversity [6].

Till date there are no detailed reports on survey and documentation of mushroom in the region of Odisha, India, although mushroom diversity in northern part of Odisha is smeary exploited from Similipal Biosphere Reserves (SBR) and its nearby areas by some mushroom workers [7-8]. Occurrences of mushrooms have also been meagerly studied in different forest areas and some special green vegetation of Odisha [9-10]. There are many reserve forests, several green moist microhabitats, protected green campus and forest area in Sundergarh district of Odisha, which creating a suitable environment for growth of mushrooms and variously utilized. Therefore, a systematic survey was carried out during the present investigation to study the occurrence of different type of mushroom in Sundergarh district of Odisha and their economic importance.

MATERIALS AND METHODS

Study Area

Sundergarh is situated between 21^{0} 36' & 22^{0} 32' north latitude and 83^{0} 32' & 85^{0} 22' east longitude and has a geographical area of 9,712 km^2 receiving an average annual rainfall of 1230 mm. It experiences moderately hot humid climate (55%) with the maximum and minimum temperature of $43^{0}C$ and $12^{0}C$ respectively. The area of study includes Hemgiri, Lephripara, Tangarpali, Sadar, Bonai, Subdega, Balisankara, Baragaon, Kutra, Ujalpur and Gopalpur forest division as well as the hill sides. The present field survey has covered all most all areas of dry deciduous vegetation and humus rich soil. The survey work was done in two rounds, once in winter during the month of November-January and again during the month of June-August for collection in rainy season.

Collection and Identification of specimens

Various species of mushrooms show variety of pileus, and stipe structure, which vary from month to month. Sampling was done by using 20 m × 20 m quadrat and total of 100 plots were selected for study. A systematic and seasonal survey of different forest and other habitats rich with organic matters in the district of Sundergarh were undertaken during Jan 2016 to December 2019. Equipments such as a light and wide bottomed basket, a knife, a tall

walking stick, waterproof boot, rain coat, food and snacks, a map and a GPS and a digital camera were used during the investigation [11]. The full bloomed and complete sporocarps of fresh specimens were photographed and collected from their natural habitats. They were kept in sterilized polyethylene packets separately and sealed with staples. The period and locality of collection, nature of substrate, odour, colour of fresh specimen and nature of latex, if present were noted. Their ecological habitats and occurrence in solitary or groups were recorded. The morphological investigation of macrofungi fructification were done visually with respect to size, diameter and length of cap (pileus) and stalk (stipe), colour and its variation during maturity, colour of gills and its mode of attachment to stem and other details of ascocarp or basidiocarp. Thin sections of gill and hymenium layer was cut, stained in cotton blue and mounted in lactophenol for microscopic studies of collected specimens. Microscopic observations included size, colour and nature of basidium or ascus, size, colour and number of spores, colour of spore mass and the presence or absence of cystidia. Simultaneously a spore print was prepared by placing the pileus downwards where a black and white paper (half white and half black) was covered with bell jar [12]. Further biochemical spot test and other necessary processing were also carried out [13].Collected specimens were dried, preserved in paper or polythene bags and numbered [14]. All the basidiomycetes fruit body collections were preserved mostly dried and by using chlorobenzene and a few were kept in formalin-glycerol-ethanol.

RESULTS AND DISCUSSION

The results of the present study on mushroom diversity of Sundergarh district of Odisha revealed that out of a total numbers of 104 mushroom species collected from the area 76 species were taxonomically studied and identified (Table 1; Fig. 1, Fig. 2). Among 76 species 63 species were identified up to species level and 12 species up to their genus level only. These 76 identified species were bio-documented by denoting their morphological identifying characters, structure and colour of fruiting bodies, stipe length, thickness and spores. The species were included under 33 families and 48 genera while reviewing the earlier literature it could be ascertained that the study area harboured 18 edible mushrooms and 6 mushrooms with medicinal values (Table 2; Fig. 3). The other 20 species were found unsuitable for consumption and 30 species remained unknown for their use. Hence, the area is rich and highly diversified with respect to mushroom flora. Field survey was carried out from June-October and results revealed greater occurrence of mushrooms during the months of July, August and September. Least number of mushroom species was found in October. Mushroom species like *Leucocoprinus cretatus* and *Pluteus podospileus* were observed and reported in the month of June, while *Schizophyllum commune, Termitomyces microcarpus, Ganoderma lucidum* and *Macrolepiota procera* were found to occur during the rainy season.

Mushrooms are well-known for their ethnic uses and considered as good source of food. In this investigation, mushroom flora of the present area under study has been surveyed with special reference to their diversity and uses. The observation of rich biodiversity of mushrooms in the study sites providing a healthy and appropriate condition for macro fungal growth can be attributed the favourable climatic factor in the area. A total 104 species of mushrooms were collected from 11 different localities of the district which were preserved in the laboratory for their detailed taxonomic and other studies. A remarkable number of species were reported during the present investigation as compared to the earlier findings [8-10] in Odisha. Exploration of edibility and curative properties of some of the mushroom samples collected from Gopalpur forest area are the uniqueness of this piece of work.

Odisha with varied habitat and diverse ecological conditions harbours wide varieties of mushrooms. The present study on mushroom diversity in Sundergarh district of Odisha revealed that Gopalpur and Bonai forest area were the two places showing high species richness of mushrooms which might be due to rich vegetation of moist deciduous pattern. It is evident that mushroom diversity seems to be higher in moist forest area as compared to other habitats which are affected seriously by environmental factor like light, temperature and humidity. Diversity can be considered as a parameter for monitoring the ecological succession and other adverse effect of urbanization and industrialization leading to environmental pollution. The diversity of mushroom is gradually depleting in a quick

rate due to deforestation, urbanization, climate change and unsystematic exploitation through collection of wild species of mushrooms. This unfair and uncanny situation demands an urgent need to collect, document and conserve these lower plants. The rich diversity of mushrooms in the study area not only need conservation measures but also further studies on their edibility and medicinal properties may throw new lights for their sustainable use and conservation.

ACKNOWLEDGMENTS

The authors are thankful to the authorities of Centurion University of Technology and Management, Odisha for providing necessary Laboratory facilities during the investigation.

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Table 1. List of Mushrooms found on the Study Area

Sl. No.	Name of the Species	Family
1	*Agaricus bisporous* (J.E.Lange) Emil J. Imbach	Agaricaceae
2	*Agaricus campestris* L.	Agaricaceae
3	*Agaricus placomyces* Peck	Agaricaceae

4	*Agaricus silvicola* (Vittad) Peck.	Agaricaceae
5	*Agaricus trisulpharatus* Berk	Agaricaceae
6	*Agrocybe praecox* (Pers.) Fauod	Strophariaceae
7	*Amanita multisquamosa* Peck	Amanitaceae
8	*Bjerkandera adusta* (Willd.) P.Karst.	Haplopilaceae
9	*Boletus impolitus* Fries	Boletaceae
10	*Boletus* sp.	Boletaceae
11	*Bovista longispora* Kreisel	Lycoperdaceae
12	*Calvatia cyathiformis* (Bosc.) Morgan	Lycoperdaceae
13	*Calvatia utriformis* (Bull.) Jaap.	Lycoperdaceae
14	*Chlorophylum molybditis* (G.May.) Massee	Agaricaceae
15	*Coniophora puteana* (Schum. ex Fries) Karst	Boletaceae
16	*Coprinopsis lagopus* (Fr.) Readhead,Vilgalys & Moncalvo	Psathyrellaceae
17	*Coprinus comatus* (O.F. Mull.) Pers.	Coprinaceae
18	*Coprinus disseminates* (Pers) Gray	Coprinaceae
19	*Dacryopinax spathularia* Schweien & G.W. Martin	Dacrymycetaceae
20	*Daldinia concentrica* (Bolton) Cesati and de Notaris	Xylariaceae
21	*Entoloma unicolar* (Scop) Fr.	Entolomaceae
22	*Fomitopsis pinicola* (Sw.) P. Karst	Polyporaceae
23	*Ganoderma australe* (Fr) Pat.	Ganodermataceae
24	*Ganoderma lucidum* (Curtis) P. Karst	Ganodermataceae
25	*Grifola frondosa* Dicks. ex Fr.	Meripilaceae
26	*Hydnum repandum* L.	Hydnaceae
27	*Hygrophorous* sp.	Hygrophoraceae
28	*Lactarius deliciosus* (L.) Gray	Russulaceae
29	*Lactarius resimus* (Fr.)Fr.	Russulaceae
30	*Lactarius* sp.1	Russulaceae
31	*Lactarius* sp.2	Russulaceae
32	*Lactarius* sp.3	Russulaceae
33	*Lentinus polychrous* Lev.	Polyporaceae
34	*Lentinus* sp.	Polyporaceae
35	*Lepiota americana* Pk.	Agaricaceae
36	*Lepiota* sp.	Agaricaceae
37	*Lepiota viriditincta* (Berk. & Broome) Sacc.	Agaricaceae
38	*Lepista luscina* (Fr. ex Fr.) Sing.	Tricholomataceae
39	*Leucocoprinus birnbaumi* (Corda) Singer	Agaricaceae
40	*Leucocoprinus cretatus* Bull.	Agaricaceae
41	*Macrolepiota procera* Scop. ex Fr.	Lepiotaceae
42	*Marasmius haematocephalus* (Mont.) Fr.	Marasmiaceae
43	*Marasmius oraedes* (Bolton) Fr.	Marasmiaceae
44	*Marasmius rotula* (Scop) Fr.	Marasmiaceae
45	*Marasmius* sp.	Marasmiaceae
46	*Microporous xanthopus* P. Beauv	Polyporaceae
47	*Mitrula phaloides* Fr.	Helotiaceae
48	*Mollisia cineria* (Batsch) P. Karst	Hymenochaetaceae
49	*Mycena belliae* (Johnst.) P.D. Orton	Mycenaceae
50	*Mycena* sp.1	Mycenaceae

51	*Mycena* sp.2	Mycenaceae
52	*Nigroporous* sp.	Polyporaceae
53	*Peniophora incarnata* (Fr.) Karst.	Peniophoraceae
54	*Phelinus tuberculosus* (Baumg.) Niemela	Hymenochaetaceae
55	*Pholiota nameco* (T. Ito) S. Ito & S. Imai	Strophariaceae
56	*Pleurotus djamer* (Rumph. ex Fr.) Boedijn	Pleurotaceae
57	*Pleurotus eryngyi* (DC.) Quel	Pleurotaceae
58	*Pluteus podospileus* Sacc. & Cub	Pluteaceae
59	*Podoscypha petaloides* (Berk.) Pat.	Meruliaceae
60	*Polyporous* sp.	Polyporaceae
61	*Psathyrella piluliformis* (Bull.) P.D. Orton	Psathyrellaceae
62	*Psathyrella prona* (Fr.) Gillet	Psathyrellaceae
63	*Pycnoporous cinnabarinus* (Jacq.) Fr.	Polyporaceae
64	*Ramaria* sp.	Gomphaceae
65	*Resupinatus cinerascens* (Cleland) Grgur.	Tricholomataceae
66	*Russula sanguinaria* (Velen.) Bon	Russulaceae
67	*Schizophyllum commune* Fries, Spirin and Zmitr.	Schizophyllaceae
68	*Termitomyces medius* R. Heim	Lyophyllaceae
69	*Termitomyces microcarpus* (Berk. and Broome) Heim	Lyophyllaceae
70	*Trametes suaveolens* (L.) Fr.	Polyporaceae
71	*Trametes versicolor* (L.) Lloyd.	Polyporaceae
72	*Trametopsis cervina* Schwein.	Polyporaceae
73	*Tremella fuciformis* Berk.	Tremellaceae
74	*Tricholoma boudieri* Barla	Tricholomataceae
75	*Volvariella volvacea* (Bul. ex Fr.) Singer	Pluteaceae
76	*Xylaria longipes* Nitschke	Xylariaceae

Table 2: Economic importance of collected mushrooms

Sl. No.	Importance	Total number of Species
1	Edible	18
2	Non edible or poisonous or unknown importance	20
3	Medicinal	6

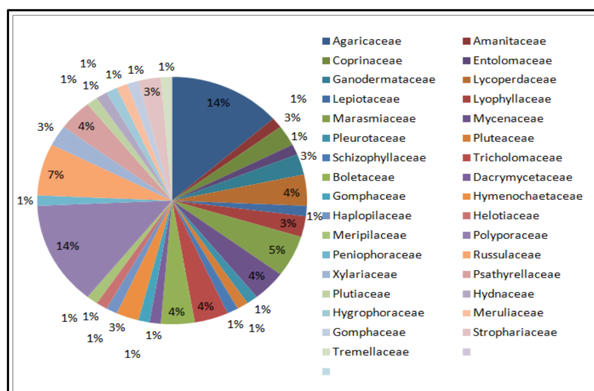

Fig. 1. Statistics of the representation of the mushroom families in the area

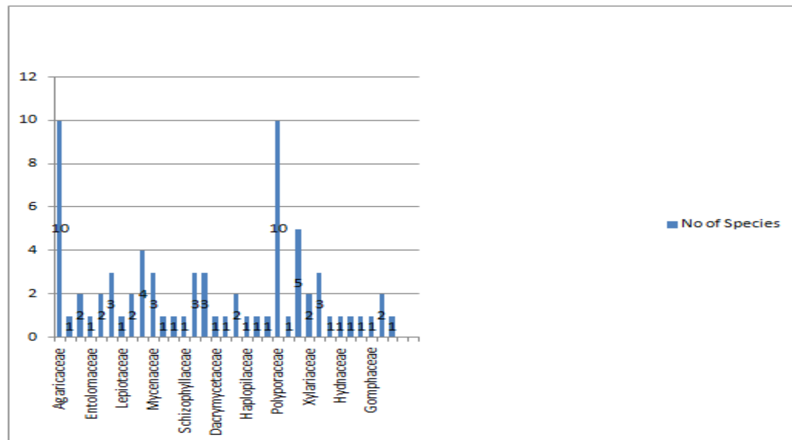

Fig. 2. Statistics of the distribution of the mushroom species among the families

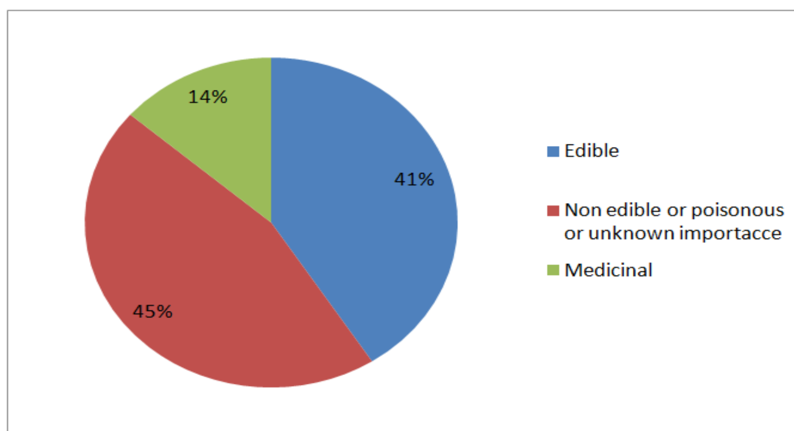

Fig. 3. Showing the economic importance of the documented mushroom species

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Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Accurate Vehicle Number Plate Identification Using Raspberry Pi

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Received: 06 Jan 2020 Revised: 09 Feb 2020 Accepted: 11 Mar 2020

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ABSTRACT

Essentially video surveillance system as well as monitoring systems is used for security purposes. Yet movable object detection is a challenging part of video surveillance. For home security, military uses, financial security, automated teller machine (ATM), traffic control etc. video surveillance system is used. Now a days has become increasingly realistic because of lower costs of high quality video surveillance systems, detecting human activity and monitoring. Automated processes accordingly were designed for multiple detection activities, but the job of detecting vehicles parked illegally was largely left to the human supervisory system operators. The most interesting and challenging research area from the past few years is the identification of vehicles by their number plates. It is found that vehicle number plates are of various shapes and sizes and have different colours in different countries. This paper proposes a method for detecting and distinguishing the number plate of a vehicle that will help to detect number plate of approved and unauthorized vehicles. It offers a successful morphological operation and the method of detection of Sobel edges. This strategy is simplified by using the bounding box method to segment all the numerical block letters and numbers used. Model matching method is used to identify numbers and characters after segmentation of numbers and characters present on number plate.

Keywords: Vehicle number, video surveillance, Sobel edges, morphological operation, Raspberry Pi.

INTRODUCTION

The most commonly pronounced word in the electronics area is automation. The quest for technology has brought many changes to existing technologies. Generally, an automatic license plate recognition (ALPR) system is made up of five modules;

a) The identification of plate
b) Segmentation
c) Classification
d) Plate Recognition
e) CR Segmentation modules

Followings are the different challenges to design an ALPR system.

i. First, Image License Plate Localisation.
ii. Secondly, segmentation of character from localized license plate

Eventually, acknowledgment of characters taken.

iii. By conducting edge extraction, morphological administrators and Sobel administrator, the most wellknown a nswers for tag restriction in computerize images are.
iv. Edge discovery administrator Sobel gives positive results on the picture. The containment of tags by 3 morph ologically dependent methods is not defenseless to clamor but rather fair in execution.
v. The cycle of separating characters comes after the tag constraint. Normal processes for dividing character dep end on the investigation and thresholding of histograms. Some approaches suggested late are the use of falsifi ed neural systems.
vi. Character acknowledgment process is the last step of the ANPR structure. To handle various varieties found i n characters crosswise over different tags, certain pretreatment steps, such as standardization and skew adjus tment, may involve the portioned character.Such extra steps end up being useful, as the calculation time need ed is significantly reduced.
vii. There are two major technological criteria for automatic plate recognition
   1.The quality of the license plate recognition algorithms.
   2. The quality of the image acquisition (camera and the illumination conditions)
viii. The better algorithms are:
   1. Recognition accuracy is higher.
   2. The processing speed is higher.
   3.Wider is the selection of image quality that can be used on Faster is the pace of processing
ix. One NPR programming can read plate from a specific nation only. This is because the architectural arrangem ent of the plate and presentation, text styles and grammar were essential components of the LPR system.With out earlier plate geometry details (character distribution, spacing of characters, plate color, dimensional ratios , etc.), the algorithm may not even locate the plate in the captured picture.
x. The image acquisition technique shall assess the image quality of the license plate with which the detection algorithms must work. Higher the quality of the photographs obtained, the greater the precision that can be achieved. A well captured image has the following properties:
   a) Effective spatial resolution
   b) Better endurance
   c) High contrast
   d) Good lighting conditions
   e) The Good View Angle

The paper aims to design a system which automatically captures the image of a vehicle's number plate and these deta ils have been checked using the Raspberry Pi authentication processor. The device also alerts authorities if any unauthorized number plate picture is identified using buzzer alarm system.This systems uses an onboard computer, commonly referred to as the processor Raspberry Pi. It serves as the core of the project. This onboard computer can interact effectively with the output and input modules that are being used. The Raspberry Pi is a single board credit card sizeddevice built by the Raspberry Pi Foundation in the UK.The Raspberry Pi has a Broadcom BCM2835 system on a chip (SoC), which includes an ARM1176JZFS 700 MHz processor, Video Core IV GPU, and was originally shipped with 256 megabytes of RAM, later upgraded to 512 MB. It does not include a built-in hard disk or solid-state drive, but uses an SD card for booting and long-term storage. The computer that can perform the task is a processor running Raspberry Pi. When any vehicle passes through the device, the camera captures the image of each vehicle's number late. The numeral plate information image is fed to the Raspberry Pi processor as input. It is the duty of the Processor to verify the authentication information for each vehicle. Once the details of the vehicle are

Understood then the processor uses stepper motor to operate the doors.The device also warns the user by buzzer war ning whenever an unwanted number plate picture has been identified. Raspberry Pi processor is programmed using the embedded ' Linux ' feature to perform this task.

HARDWARE USED
RASPBERRY PI

The Raspberry Pi is a credit-card-sized single-board computer developed in the UK by the Raspberry. The Raspberry Pi is manufactured by approved manufacturing deals with Newark element 14 (Premier Farnell), RS Com ponents and man Ego.

i. The Raspberry Pi has a chipbased Broadcom BCM2835 device (SoC) with an ARM1176JZFS 700 MHz processor (the firmware provides a range of "Turbo" modes so that the user can attempt to clock up to 1 GHz;, without affecting the warranty), Video Core IV GPU was initially supplied with 256 megabytes of RAM, and later upgraded to 512 MB.
ii. Itdoesn't include a builtin hard disk drive or a solidstate drive, but uses an SD card to boot and store long-term.

Raspberry Pi does not have a realtime clock, so the operating system must use a network time server or ask the u ser for time information at boot time to access the time and date for file time and date stamping.However, a real-time clock (such as the DS1307) with battery backup can be added via the I²C interface

- A 900MHz quad-core ARM Cortex-A7 CPU
- 1 GB RAM
- 4 USB Ports
- 40 GPIO Pins
- Full HDMI Port
- Ethernet Port
- Combined 3.5mm audio jack and composite video
- Camera interface(CSI)
- Display interface(DSI)
- Micro SD card slot
- Video Core IV 3D graphics core

iii. The ARM1176JZFS processor provides an integer core implementing the ARM11 ARM architecturev6.
iv. ARM1176TM applications processors are widely deployed in devices ranging from smartphones to digital TV to e Readers, providing media and browser functionality, a safe computing environment and up to 1GHz efficiency in low cost designs..The ARM1176JZS Processor features ARM Trust Zone Safe Application Technology and ARM Jazelle Powerful Java Embedded Technology.vailable tightly coupled memories simplify ARM9TM processor mig ration and realtime architecture while AMBA 3 AXITM interfaces improve the performance of memory bus.DVFS support allows performance optimisation below the ARM11TM processor architecture's best-in-class static and dynamic nominal capacity.

USB CAMERA

An USB camera is a video camera that feeds or streams its image to a computer network in real time, or via a comput er. When the machine "captures" the video stream can be saved, accessed or transmitted to other networks through systems such as the internet and sent as an attachment.The video stream may be saved, accessed or sent there when it

is sent to a remote location. A USB cam is usually connected to, or built into, computer hardware, such as laptops, by a USB cable, or similar.

LCD

Liquid crystals do not specifically emit light, but instead use backlight to generate color images or monochromeLCDs to show arbitrary images or fixed images with low information content that can be displayed or obscured, such as preset words, digits and 7 segment displays, as in digital clock displays. They use the same basic technology, except that a large number of small pixels make up arbitrary images, while other displays have larger components.LCDs are used in a wide range of applications including TV instrument panels for computer monitors, cock pit screens for aircraft and indoor and outdoor signage. Small LCD screens are common in portable consumer devices including digital cameras, watches, calculators, and mobile phones. Also used on consumer electronic goods such as DVD play ers, video game consoles, and clocks are LCD displays.

BUZZER

A buzzer or beeper is an electronic, electromechanical audio signalling system. Typical uses of buzzers and beepers include warning systems, timers and user input confirmation like a mouse click or a keystroke.

STEPPER MOTOR

A stepper motor is an electric DC motor, which divides a full rotation into several equal phases. The position of the motor can then be ordered to shift and hold at one of these steps without any input sensor, as long as the torque and speed of the motor are carefully measured to the application.

ETHERNET CABLE

Ethernet is a family of computer networking technologies that are widely used in local area networks, metropolitan area networks and wide area networking.

POWER CABLE

A power cable is an arrangement of one or more electrical conductors which is usually held together with an overall sheath. The assembly is used for electric power transmission. Power cables can be mounted within houses, buried in The ground, run overhead, or exposed as permanent wiring. To portable devices, mobile equipment and machinery, versatile power cables are used.

SOFTWARE USED
MATLAB 14.0

MATLAB (matrix laboratory) is a multiparadigm computer numerical system and programming language of the four the generation

SUPPORT PACKAGE FOR RASPBERRY PI

Support package is required to simulate code in MATLAB using raspberry pi. As support package for each controller never comes inbuilt that the user has to download.

METHODOLOGY

ALGORITHM

- Load the raspi object, the imgfile, and the registered number file.
- Show the original picture,resize,conversion to grey scale and binary image
- Properties extraction and boundarybox creation.
- Number of objects-extraction.
- Comparison of each object with each cell in imgfile.
- Show final output

PROCEDURE

- Raspi object is created first in command window of MATLAB.
  - Inorder to make connections the pin configuration of raspberry pi is required.
  - Accordingly, the connections should be made.
  - Interfacing with LCD:
    -->RS-27
    -->EN-22
    -->R/W-Gnd
    -->D0-5,D1-6,D2-13,D3-19,D4-26,D5-21,D6-20,D7-16
- Interfacing with Stepper motor:
  -->5th
  -25,6th-24,7th-23,8th-17
- Interfacing with Buzzer: 12th pin
- To check whether the interfacing with camera is correct or not open the simulink input model, type simulink in command prompt, go to simulink support package for raspberry pi, place SDL Video to see the video captured by webcam.
- For continuous video make the range as infinity and run the model.
- After the confirmation stop the simulation.
- Run the main code.
- After successful simulation all the image files will be visible. And extracted numbers will be shown.
- For correct matching the recognized number will be displayed on LCD and stepper motor will rotate to open the gate.
- For incorrect matching the buzzer will beep and not authenticate message will be displayed on LCD.

RESULTS DISCUSSION

After successful simulation, all the image files will be visible. And extracted numbers will be shown. Detection of all the plates in the current camera frame is required. Two broad categories in which they can be defined are:

- ✓ Segmentation
- ✓ Classification

Segmentation

- ✓ Dividing the image into multiple segments is a form of segmentation. This process is designed to simplify the image for analysis and make extraction of the function easier.

- ✓ The high number of vertical edges is an important feature that can be extracted from Number Plates.
- ✓ But before that, a convenient pre-processing of the current image needs to be done::
  1. Gray Scale conversion: The Red, Green, and Blue elements are isolated from each pixel's 24-bit color value.To convert the image captured into Gray Scale image: picture=rgb2gray(picture);
  2. Conversion to binary scale: All 0-255 components of grayscale images are separated and a binary image is achieved i.e. a black and white image. To convert the image from grayscale to binary: Thres hold = graythresh (picture); picture =~im2bw(picture,threshold)

Classification

- This technique is used to differentiate the possible area of the license plate from the image issued. The main objective of such schemes is to confine the area 8 of the license plate to images taken from the camera mounted on the Raspberry Pi2.
- Image quality is an important part of this technique and preprocessing helps to improve the transparency of the image. Number plates usually appear to have high contrast (black-and-yellow or black-and-white) areas in the file.
- Numbers and letters are positioned in the same line (i.e. at the same vertical level) resulting in repeated horizontal variations in strength. This provides the basis for horizontal measurement of shifts in horizontal pressure. This provides the basis for detecting shifts in horizontal strength as it is predicted that the rows comprising the number plate will display sharp variations.
- This sharp difference is due to the contrast between the letters and their context.

Principles of Character Segmentation

- The method of character segmentation serves as a bridge between modules for plate detection and optical character recognition. The main function is to segment the characters in the selected applicant area (extracted license plate),so that each character can be sent individually for identification to the optical charact er recognition module
- Standardized or standardized in fancy format, the conditions of the license plate are important criteria for efficient segmentation, since if the numbers are of fancy type, the conditions of the license plate as mentioned above..
- Once the license plate is found, the actual characters will be collected. A license plate as mentioned above has high intensity variability regions.
- It is sometimes observed that different texts may be present along with license numbers which need to be deleted. By separate measurements we found that the volume of white on black for the license plate regions is similar for the numerical regions and falls within a certain range
- Morphological technique is used to eliminate small white areas that avoid changes to the selection. Eventually, actual characters are removed to proceed through the recognition system of optical characteres. Segmentation is one of the most critical processes in the identification of automatic plate numbers, because all further steps rely on it.
- If the segmentation fails, a character may be wrongly split into two parts or two characters may be misfused, resulting in the loss of the following identification levels. The second segmentation process consists of Strengthening segments. In addition to the character, the section of a plate often includes unnecessary elementssuch as noise due to shadows or faults in camera equipment as well as redundant space on the sides of characters.

Preprocessing Stage

We search the plate vertically and horizontally and miss those rows and columns that have too much black and white.This is explained because those areas containing the numbers have black areas within a specific range. Experiments find it range to be between 0.2 and 0.8 times the amount of pixels horizontally and vertically.

The method of character segmentation takes the extracted region of the license plate from the previous module as the input.The input is an image colored with JPEG. We only function binary images for our process and There fore the first part of segmentation is binarization of the image as shown in segmentation. Extraction of numbersis then necessary one by one. The boundary box is designed to obtain full rows and columns according to each character's shape and size.Every character must be marked before that. And so they remove each of the characters

```
[L,Ne]=bwlabel(picture);
propied=regionprops(L,'BoundingBox');
rectangle('Position',propied(n).BoundingBox,'EdgeColor','g','LineWidth',2)
```

RESULTS

For correct matching the recognized number will be displayed on LCD and stepper motor will rotate to open the gate.

- For incorrect matching the buzzer will beep and not authenticate message will be displayed on LCD.

CONCLUSION

We got the desired efficiency. The concentrates are given for the correct location of the number plate area to segment all the numbers and letters to mark each number separately. A device able to identify registration numbers can be useful in regular circumstances. The key features of the proposed system are controlled stability plasticity behavior (optional external test input), managed reliability threshold (optional external validation input), self-assessment of output reliability, high reliability based on multiple feedback. This system was designed using a modular approach that allows for easy updating and/ or removal of different sub-modules, thereby making it potentially suitable for a wide range of vision applications.The system's performance makes it a valid option among its competitors particularly in those situations where applications' cost has to be held at reasonable levels.

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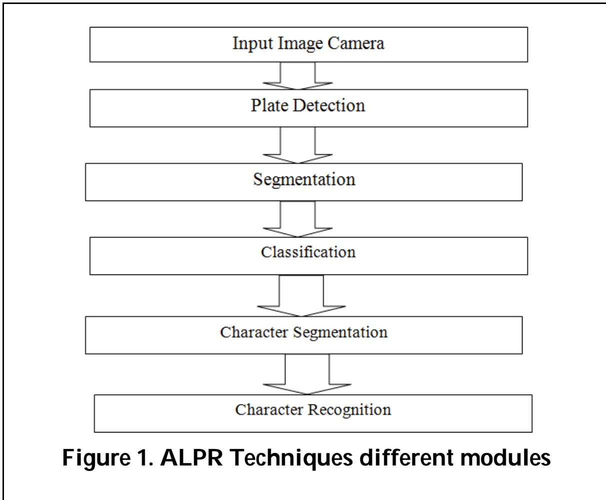

Figure 1. ALPR Techniques different modules

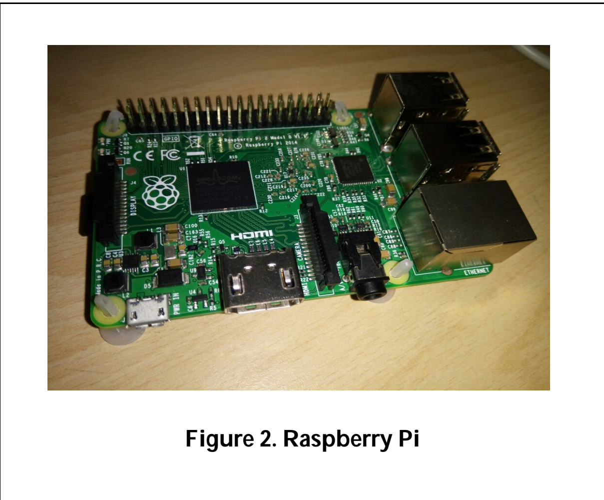

Figure 2. Raspberry Pi

Figure 3. Photo of USB Camera

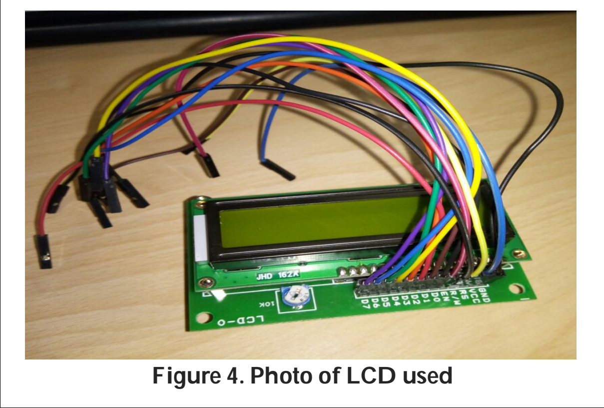

Figure 4. Photo of LCD used

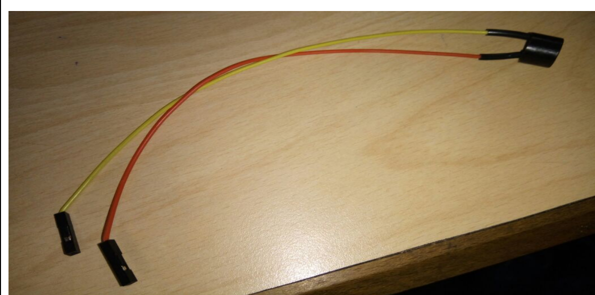

Figure 5. Photo of Buzzer used

Figure 6 (a) Photo of Stepper Motor used

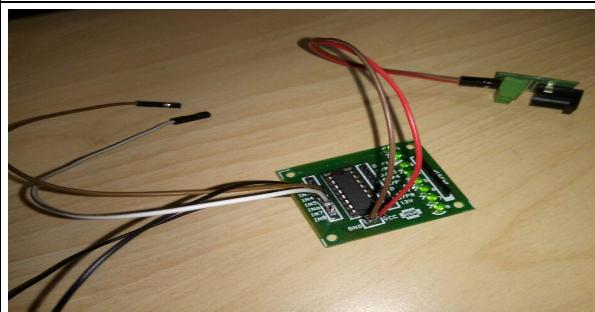

Figure 6(b). Photo of Steeper Motor Driver used

Figure 7.photo of ethernet cable used

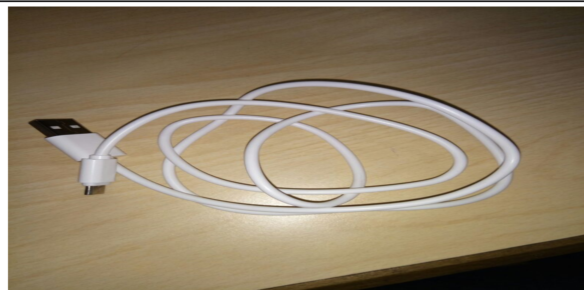

Figure 8. Photo of Power Cable used

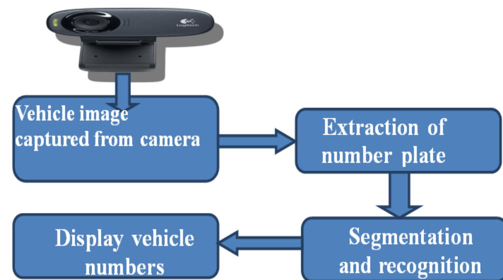

Figure 9. Review block diagram

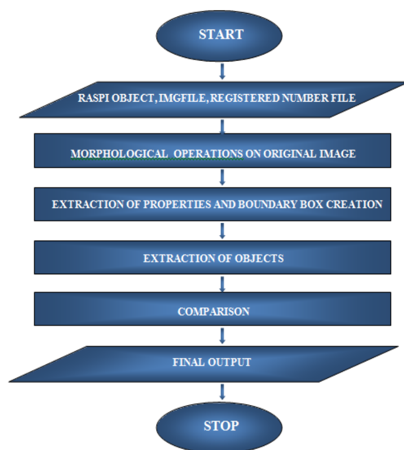

Figure 10. Flow chart

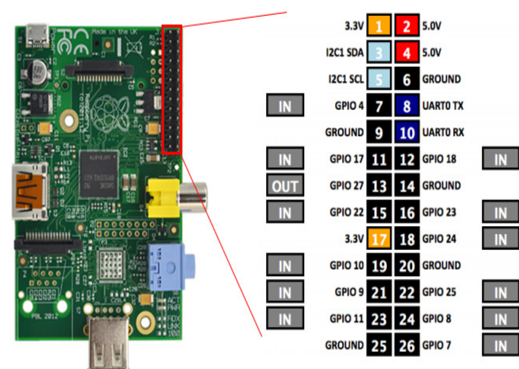

Figure 11. Pin configuration of Raspberry Pi

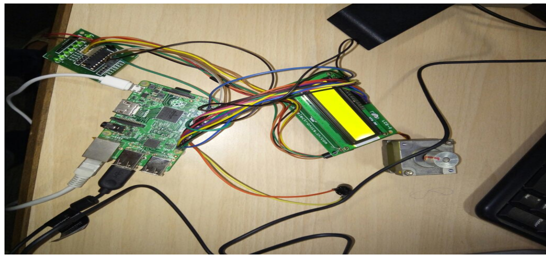

Figure 12. Connections

Figure 13.The grayscale image

Figure 14. The binaryscale image

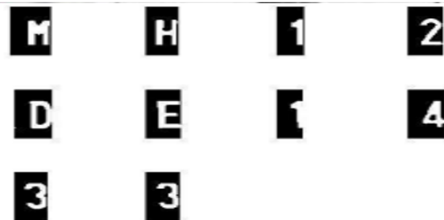

Figure 15. Extracted Characters' Image

Figure 16. Final output

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Phenanthrene Toxicity in Tadpoles of *Polypedates maculatus* (Anura, Dicroglossidae) with Special Reference to Growth, Life History and Genotoxicity

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Received: 10 Jan 2020 Revised: 12 Feb 2020 Accepted: 14 Mar 2020

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ABSTRACT

Phenanthrene (PHE), a tricyclic aromatic hydrocarbon (molecular formula $C_{14}H_{10}$) belongs to a cohort of pollutants referred to as polycyclic aromatic hydrocarbons (PAHs). Amongst the 16 enlisted priority PAHs by USEPA, PHE is one of the most predominant members and often occur as the principal constituent of PAH contaminated aquatic ecosystems. The lipophilic property of PAHs facilitates accumulation of PHE in fatty tissues by altering the membrane permeability and other associated enzymes. In this study, we evaluated the toxicity of PHE on the tadpoles of *Polypedates maculatus*. The acute toxic concentrations (LC_{50}) of PHE in the tadpoles were primarily determined using four different concentrations viz., 3, 5, 7 and 9 mg/L. Further, to analyze the survival, life history parameters and genotoxicity, different sub-lethal concentrations were used as percentages of 96-h LC_{50} concentration. Exposure to increasing concentrations of PHE significantly increased the mortality of the tadpoles and altered the life history parameters. PHE also significantly induced the formation of micronuclei in the erythrocytes of the exposed tadpoles, which is considered as the biomarker of genotoxicity. In recent times, the amphibian population is declining at an alarming rate and PHE along with the other potent PAHs could be one of the possible contributing factors towards this decline. The adverse effects of PHE observed in the present study can be extrapolated to other aquatic biota.

Keywords: Genotoxicity, Life history, Micronucleus, Phenanthrene, *Polypedates maculatus*..

INTRODUCTION

The incomplete combustion as well as partial pyrolysis of all organic substances found in nature lead to the production of an environmentally concerned group of emerging pollutants known as polycyclic aromatic hydrocarbons, PAHs (1). They are enlisted among the persistent organic pollutants (POPs) in the Convention on Long range Transboundary Air Pollution Protocol (2). PAHs are composed of two or more aromatic hydrocarbon rings in their structure and usually found as complex mixtures in different proportions (3). Among all PAHs, PHE is one of the most abundant compounds and is found predominantly in coal tar sealants (4), emission particle of municipal waste incinerator (5), diesel exhaust particulates (6), soil and sediments (7). PHE is a tricyclic petrogenic PAH which is regarded as a major contributor to the total PAH content of the environment (8) and is frequently detected in the aquatic ecosystems (9). In recent years, the presence of PHE in surface water has been detected worldwide (10) and their levels are increasing due to their source multiplication (11). PHE concentrations in the environment near oil exploitations sites could go up to 1.46 mg/L (12). The lipophilic property of PAHs facilitates accumulation in fatty tissues (1) by altering the membrane permeability and other associated enzymes (13).

PHE can cause lethal and sub-lethal toxicities in aquatic organisms such as fishes (14, 15, 16) molluscs (17, 18) annelids (19) among others. PHE exposure has been reported to affect the survival, growth, development and reproductive parameters of two species of meiobenthic copepods, *Schizopera knabeni* and *Nitocra lacustris* (20). A known cocktail mixture of naphthalene, PHE and pyrene induced significant mortality of *Rana temporaria* eggs (21). It is also known to induce genetic instability, chromosomal aberrations and DNA damages in a number of aquatic organisms (9, 18, 22). Anuran amphibians are the keystone species in many aquatic ecosystems. They are usually sensitive towards environmental alterations in both aquatic and terrestrial ecosystems and hence are regarded as the excellent bio-indicators of environmental contamination (23). Their unique characteristics like highly permeable integument (24), biphasic lifestyle (25) and high conversion efficiency (4) make them extremely susceptible to the anthropogenic contaminants. However, literature regarding the toxicity of PHE on an anuran amphibian system is very infrequent (26). In recent times, the amphibian populations are declining at an alarming rate due to several environmental factors (27) and PHE along with other PAHs could possibly be one amongst them (27, 28).

Therefore, the present study is aimed to assess the lethal and sub-lethal effects of PHE in the anuran tadpoles of *Polypedates maculatus* (Anura, Dicroglossidae) with special reference to growth, survival and genotoxicity. The study has greater relevance as no data regarding the PHE toxicity on the selected species is reported till date.

MATERIALS AND METHODS

Animal collection and acclimatization

The collection and acclimatization of the tadpoles of *Polypedates maculatus* were done as earlier (29). Briefly, the tadpoles were collected from a captive breeding pond located within the Assam University campus using plastic nets with proper care. The tadpoles belonging to Gosner 26-30 stages (30) were segregated from the collected stock and were acclimated to the laboratory condition in aged well water with aeration for at least 48-h. The remaining tadpoles were carefully released at the same breeding site. During acclimatization, the tadpoles were fed with crushed fish food pellets (Amrit Feeds, Kolkata, India) *ad libitum*. All the experiments were performed at 26±1ºC and 12-h/12-h light/dark cycles in silicone-coated polypropylene tubs. During the experiments, the pH of the water medium varied between 7.1-7.5 and the measured dissolved oxygen content was always ≥ 8.2 mg/L. The study has institutional ethical clearance vide approval number IEC-AUS/2015-032.

Acute toxicity assay

To determine the LC_{50} concentrations of PHE in the tadpoles of *P. maculatus*, acute survival assay was performed. For the assay, tadpoles of of gosner 26-30 developmental stage were randomly assigned to four treatment groups (10 tadpoles/group) exposed with PHE (3, 5, 7 and 9 mg/L) and two control groups (negative and vehicle control) in triplicates (30 tadpoles for each treatment point) containing 2L of aged well water. DMSO (<0.01%) was used as a vehicle solvent and was procured from Himedia Laboratories, Mumbai, India. The experimental tubs were inspected every 24-h over a period of 96-h to assessed the survival response in terms of mortality of the tadpoles and the dead animals found were carefully removed. A tadpole was considered dead when it did not react to the mechanical stimulus and floating motionlessly without any body movements. The LC_{50} values at different exposure times were then determined using probit analysis.

Survival and life history

Survival and developmental plasticity of the tadpoles was studied following the methods described earlier (31). The survivability and developmental plasticity were assessed following exposure to sub-lethal concentrations 5% (161 µg/L), 10% (323 µg/L), 20% (646 µg/L), and 30% (969 µg/L) of 96-h LC_{50} concentration. The study was performed in 48-h static renewals protocol with 10 tadpoles in each experimental tub in triplicates (n= 30) containing 2L of aerated aged well water. The tubs were monitored at every 24-h to record the number of surviving animals till the day at which the first tadpole metamorphosed. Subsequently, the same set of experiments was continued till all the tadpoles either died or completely metamorphosed (Gosner stage 46). The average time taken to attain metamorphosis as well as the average body weight and length at metamorphosis were then recorded.

Genotoxicity assay

The micronucleus (MN) assay was carried out in peripheral erythrocytes in triplicates as described previously (29). Briefly, the tadpoles of *P.maculatus* were randomly segregated into five different groups including both controls and treatments with a total of six tadpoles in each tub. Each group was maintained in silicone coated polypropylene tanks containing 2L of aged water. The treatment groups received one of the following treatments, (a) negative control without any treatment, (b) solvent control DMSO (<0.1%), (c) positive control cyclophosphamide 2 mg/L, (d) PHE 161µg/L (5% of 96h LC_{50} value) and (e) PHE 323 µg/L (10% of 96h LC_{50} value). After 24, 48, 72 and 96-h of exposure, five tadpoles from each group were takeout (n=15 per treatment point) for MN analysis and each of the tadpoles were anesthetized in MS222 and then blood samples were collected by cardiac puncture. Two blood smears were prepared from each tadpole, fixed in cold methanol for 3 min and air-dried. In the following day, each slides were stained in 5% buffered Giemsa for 10 minutes. The slides were observed under light microscope using x1000 magnification under oil immersion and from each tadpole 1000 erythrocytes were analyzed for the presence of MN.

Statistical analysis

Before each statistical analysis, the data sets were analyzed for normal distribution and whenever the assumptions of normality were not met the data were square-root transformed. Probit regression analysis was done for the determination of acute toxic concentrations over the period of 24 to 96-h. To analyze the survival pattern of the tadpoles exposed to different concentrations of PHE, Kaplan Meier product limit estimation was done. Analysis of variation (ANOVA) followed by post hoc (Tukey's) was carried out to compare the means obtained from different life history parameters and MN induction. All the statistical analyses were performed using SPSS 18.0 software package 95% confidence interval and a 'p' value of <0.05 was considered to be statistically significant.

RESULT AND DISCUSSION

Acute toxicity

The LC_{50} values of a chemical refers to its specific toxic potential and provides the lead in deciding on the range of concentrations used to check various sub-lethal effects. The increasing concentrations of PHE caused time and concentration dependent mortality in the tadpoles of *P. maculatus*. Tadpoles treated with the solvent control did not cause any lethal effect during the time frame. The LC_{50} concentrations of PHE with lower and upper bound limits in the tadpoles at 24, 48, 72 and 96-h were found to be 9.67 mg/L (7.99-13.79 mg/L), 6.17 mg/L(5.32-7.16 mg/L), 4.31 mg/L (3.64-4.91 mg/L) and 3.23 mg/L (2.61-3.75 mg/L) respectively at 95% confidence interval (CI) (Fig. 1). The acute toxic concentrations of PHE in many aquatic organisms are reported to be as low as 0.28 µg/L in the planktonic crustacean *Daphnia magna* (32) to 3.2 mg/L in rainbow trout, *Oncorhynchus mykiss* (33). Interestingly, the 96-h LC_{50} value of PHE in *P. maculatus*, obtained in this study is corresponds to the value reported in rainbow trout at similar exposure time duration (33). Aquatic organisms showed unique susceptibility towards contaminants like PAHs. The uniqueness was not only observed within different group of the aquatic animals but also within the same species due to their unique geographical location (26). PHE exposure also induced mortality in both embryo and larvae of red sea bream (*Pagrosomus major*) with 48-h LC_{50} of 1.97 and 1.15 mg/L respectively (34). The estimated 96-h LC_{50} of PHE in the present study is significantly higher than the reported environmental concentrations of PHE. However, the strong negative correlation between the exposure time and the LC_{50} values ($r = -0.968$, $p<0.01$) indicates that exposure to lower concentrations of PHE over longer period of time would cause significant lethal effects to the exposed individuals.

Changes in survival and life history traits

In the present study, the increasing concentrations of PHE exposure effectively decreased the survival percentage of the tadpoles as compared to both the control and vehicle control groups (Fig. 2). Kaplan Meier product limit estimate was observed to be significant ($p<0.001$) suggesting significant lethal effects of PHE in the tadpoles of *P. maculatus*. The survival assay was studied until day 39 as it coincided with the day of first tadpole metamorphosis. All the tadpoles without any treatment survived till day 39 and in the presence of DMSO concentration, only one tadpole failed to survive (Fig. 2). However, the highest phenanthrene concentration (30% of 96-h of LC_{50}) induced 100% mortality of the tadpoles by day 28 of the exposure. Previous studies with PHE showed similar lethal effects in different aquatic test organisms such as meiobenthic copepods *Schizopera knabeni* and *Nitocra lacustris* (20), grass shrimp *Palaemonetes pugio* (35), and in the fishes like *Danio rerio* (14. 15) and *Poecilia vivipara* (16). Likewise, exposure to increasing concentration of PHE increased the rate of mortality in *Eisenia fetida* (19), *Chironomus riparius* (17) and *Chironomus sancticaroli* (18). A known cocktail mixture of naphthalene, PHE and pyrene also caused significant mortality of *Rana temporaria* eggs (21).

Other than the lethal effects, it also could be observed that the tadpoles exposed to increasing concentrations of PHE took longer time to metamorphose as compared with the tadpoles of the control groups (Fig. 3A). All the tadpoles receiving the highest PHE concentrations failed to survive till metamorphosis. The control tadpoles took an average of 45.73±4.35 days to complete their metamorphosis whereas the tadpoles receiving 5%, 10%, and 20% of 96-h LC_{50} took an average metamorphosis time of 51.22±2.75($p<0.001$), 52.63±4.06($p<0.001$), and 57.00±5.32 ($p<0.001$) days respectively (Fig. 3A). The increasing exposure of PHE reduced the body weight (Fig. 3B) and length (Fig. 3C&D) of the tadpoles in a concentration dependent manner. The average body weight of the control and DMSO control froglets were 30.40±4.78 and 29.90±4.54 mg, respectively which significantly decreased ($p<0.001$) to 22.06±4.00 mg in the tadpoles exposed to 20% of 96-h LC_{50} concentration of PHE. Similarly, the body length with respect to SVL, fore and hind limbs were also reduced to a significant level (Fig. 3B). The average SVL of control and vehicle control tadpoles were 6.80±0.48 and 6.89±0.56 mm respectively. However, the tadpoles receiving 5%, 10%, and 20% of PHE had an average SVL of 6.74±0.68, 6.63±0.49, and 6.14±0.36 mm ($p<0.05$) respectively (Fig. 3C). Likewise, the average

length of forelimbs and hind limbs of control tadpoles were found to be 3.97±0.66 mm and 8.30±0.33 mm respectively. On the other hand, the values concerning the length of the fore and hind limbs significantly reduced to 3.29±0.47 ($p<0.05$) and 6.71±0.73 ($p<0.001$) respectively in the tadpoles treated with 20% of 96-h LC_{50} of PHE. Tadpoles receiving vehicle treatment did not show any major change as compared to the control and the values for both fore and hind limbs were recorded as 3.96±0.33 and 8.96±0.50 mm respectively (Fig. 3D). A previous study reported reduced growth of the olive flounder *Paralichthys olivaceus* (36) upon PHE exposure. However, reports regarding the effects of PHE in the growth and development of anuran tadpoles are very rare. Studies with other environmental factors such as predator and chemicals such as cadmium, arsenic on anuran tadpoles were reported to accelerate or delay the metamorphosis process (37, 38, 39). Delaying metamorphosis affects the fitness factor of amphibian tadpoles later in the young adult stages and in some cases the delay of their metamorphosis could in turn affect the survival (40) of the tadpoles since they inhabit the temporary habitat like wetlands, rain-fed ponds which face severe risk of drying up . Any change in metamorphosis time, body length and mass during the developmental process of the tadpoles could act as an indicator of toxicant presence in their environment including PAHs (41). PAHs are known as endocrine disrupting pollutant (42) that can alter hypothalamus–pituitary–thyroid axis and interrupt the normal functioning of the thyroid hormones, hence can disturb regulation of growth, development and metamorphosis process (43).

Genotoxicity

The two sub-lethal concentrations of PHE (5 and 10% of the 96-h LC_{50}) induced MN formation in the peripheral erythrocytes of the tadpoles over the time period of 24 to 96-h in a concentration dependent manner (Fig. 4). The MN frequency in negative control erythrocytes at different time points of 24, 48, 72 and 96-h were 0.20±0.11/‰, 0.27±0.10/‰,0.33±0.12/‰ and 0.33±0.16/‰ respectively. However, the frequency of micronucleated cells increased to 2.20±0.32/‰ in the tadpole erythrocyte exposed to 10% of 96-h PHE LC_{50} concentrations for 96-h of exposure durations. The linear regression analysis between PHE concentration and MN induction was observed to be positively correlated at 24-h ($r = 0.981$, $p<0.01$), 48-h ($r = 0.960$, $p<0.01$), 72-h ($r = 0.984$, $p<0.01$) and 96-h ($r = 0.924$, $p<0.05$) of the exposure (Fig. 5). Micronuclei are small extra-nuclear bodies comprise of broken and/or damaged chromosome fragments (clastogenic effect) or whole chromosomes (aneugenic effect) which are excluded from the daughter nucleus after successive cell division (44, 45). MN is a sensitive and reliable marker of chromosomal loss and breakage brought by different toxicant exposure (46, 47) and acts as a biomarker of genotoxicity in anuran amphibians (29, 31, 39, 48). The findings of the present study are consistent with the findings of the earlier studies with tadpoles of *Rana limnocharis*, *E. cyanophlyctis* and Bullfrog tadpoles with different environmental contaminants such as pesticides and heavy metals (29, 31, 39, 49).

CONCLUSION

From the present study, it can be concluded that the concentrations of PHE along with other potent PAHs are increasing in recent times due to their source multiplications. PHE is regarded as one of the abundant PAH compounds of the aquatic habitat that can cause lethal to sub-lethal effects on the tadpoles of *P. maculatus*. PHE induced significant mortality of the test organism and altered their life history parameters. The tadpoles were extremely sensitive towards the PHE presence in the environment which caused significant increase in the frequency of MN in their erythrocytes indicative of genotoxicity. Moreover, it is evident that the amphibian are experiencing rapid population decline. The present findings suggests that elevated concentration of PHE in the aquatic environment is a significant risk factor and could further contribute towards amphibian population decline. Therefore, the regular monitoring of PHE contamination is a vital requisite for the conservation of amphibian population.

ACKNOWLEDGMENTS

The authors thankfully acknowledge Assam University, Silchar, India, for providing necessary infrastructure and the Department of Biotechnology, Government of India for financial support.

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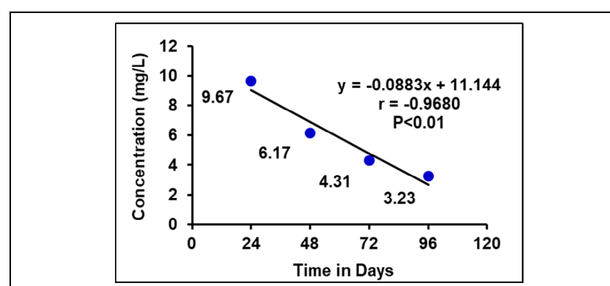

Fig. 1. Linear regression analysis of the LC_{50} concentrations of PHE over the time periods of 24 to 96-h.

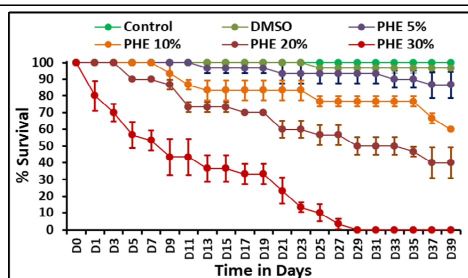

Fig. 2: Survival of *P. maculatus* tadpoles exposed to different concentrations of PHE (mean ± SE) till the first animal metamorphosed (day 39).

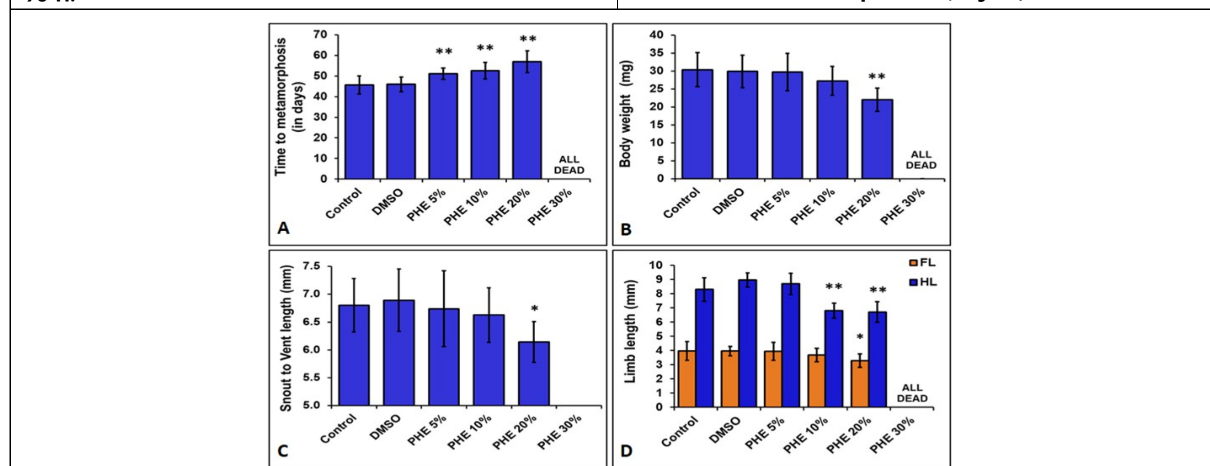

Fig. 3: Histogram showing (A) time to metamorphosis (B) body weight (C) snout to vent length (SVL) (D) lengths of both hind limb (HL) and fore limb (FL) of the tadpoles exposed to control and different PHE treatments at metamorphosis. Values are significantly different from control at p < 0.05 (*) and p<0.001(**).

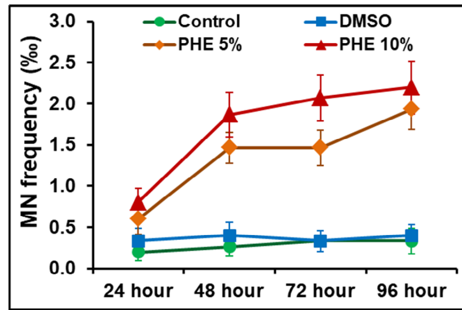

Fig. 4: Graph showing change in micronucleus frequency at different time of exposure in control and PHE (5 and 10% of LC_{50} value) exposed groups. Each point represents mean ± SD (n = 15).

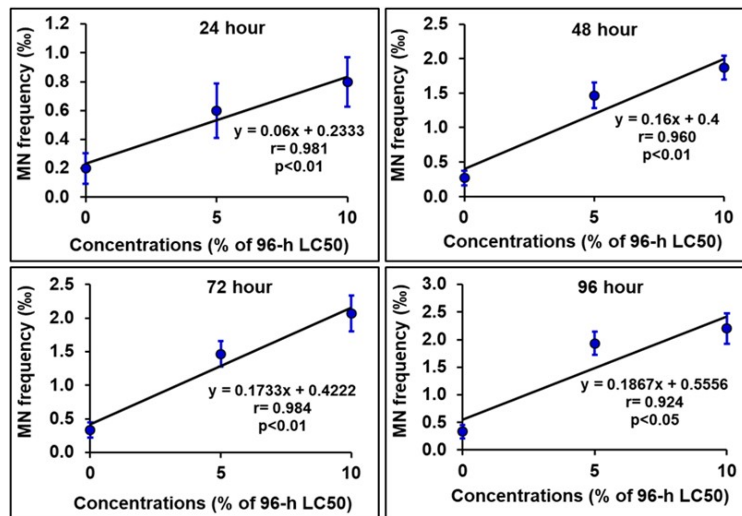

Fig. 5: Graph showing linear regression and correlation coefficient (r) of micronucleated erythrocytes at 24, 48, 72 and 96-h of PHE treatment.

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

A Literature Review Onacne Due to Hormonal Changes and Lifestyle

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Received: 06 Jan 2020 Revised: 09 Feb 2020 Accepted: 11 Mar 2020

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ABSTRACT

Acne is a common skin disorder with more than 10 million cases per year in india. In this literature review article, we will be discussing what is acne, how is it caused, the significant factors involved in the formation of acne in the body, especially the hormonal acne and acne caused due to unhealthy and improper lifestyle that we carry on. Hormonal acne can be caused by fluctuations of hormones from menstruation, menopause, PCOS or polycystic ovary syndrome and increased level of androgen level in the body. Different types of acne are explained in details further distinguishing from each other on their characteristic property. Methodology includes types of acne according to their origin and their causes. An actual survey has been done by taking 40 girls and 10 boys about the age of 19-23 years on the basis of several parameters. In discussion, the factor that is thought to have caused the major impact on the occurrence of acne is further elaborated and various treatments of acne, preventive measures and ways to have a good lifestyle have been discussed.

Keywords: Acne, literature, hormones, parameters, treatments.

INTRODUCTION

We all dream of waking up with a flawless and radiant skin. But it turns into nightmare when we wake up with breakouts of reddish projections called as Acne. Acne can be considered as a major warning sign of imbalance in our body. Pimples or Acnes are a type of skin disease caused due to production of oil from sebaceous glands of our body in high amounts, due to build-up of bacteria, or due to any hormonal change in body, especially in cases of girls. Acne vulgaris is a nearly universal skin disease afflicting 79% to 95% of the adolescent population as in surveyed in

the western societies. A survey of 1066 healthy women and 1089 healthy men aged 18-70 years, performed to determine the prevalence of facial acne, showed that clinical acne was not confined to adolescents. It often causes whiteheads, blackheads or pimples. It usually appears on the face, at the tip of the nose and lesser near it, chest, shoulders and upper portion of back.Acne is also known as acne vulgaris. Acne is most common among teenagers, though it affects people of all ages. The most common factor causing acne is the formation of excess sebum production, follicular epidermal hyperproliferation with subsequent plugging of the follicle, the presence and activity of the commensal bacteria *Propionibacterium, Staphylococcus aureus*and inflammation. The chemical composition of sebum in acne and the androgens are responsible for this hyperkeratinisation. In addition genetics and lifestyle also affects it formation.

Now a days it has been observed that people do not intake proper healthy meals due to their busy schedules which include the teenagers also. With low amount of nutrition and vitamins; and high intake of calorie from the junk foods and soft drinks, the rate of obesity is increasing day by day. Lack of sleep due to usage of electronic devices and other activities which are an add-on depletes their health both physically and mentally. Other factors in causing acne are smoking and stress. The nature of the Skin may be normal, dry or oily. Those with oily skins are often prone to pimples and zit because skin has tiny holes called pores that can become blocked by oil, bacteria, dead skin cells, and dirt. The number of oil glands remains most probably the same throughout the life, whereas their size tends to increase with age. The function of these oil glands is to secrete sebum. Acne can be in form of pustles, papules, whitehead, blackhead, hormonal acne, cystic acne, nodular acne, nodulocystic (acne large nodules greater than 5 mm in diameter, scarring seen), comedones. Comedones are the skin-coloured, small bumps (papules) frequently found on the forehead and chin of those with acne. A papule is a raised area of skin tissue that's less than 1 centimetre around. A papule can have distinct or indistinct borders. It can appear in a variety of shapes, colours, and sizes. It's not a diagnosis or disease. At the age near puberty or above it, there's a strong rise in sebum excretion.

The oil glands are the site for biosynthesis of hormones especially androgen such as testosterone. The active production of Androgen in the oil glands causes proliferation of *Propionibacterium*, which is an anaerobe present within the retained sebum in the pilosebaceous ducts. This organism is said to have contributed for formation of acne on skin. Thus, an increase in sebum excretion is a major cause in the pathophysiology of acne. It had been studied that 8 out of 10 teenagers suffer from acne. We can tell whether an Acne is causing severe affects on us checking the occurrence of scarring, formation of redness and inflammation on the skin, spreading of blemishes over a larger part of face and body. Acne is not life threatening and it's just that it may cause depression and sometimes anxiety on some individuals. Their prolonged presence sometimes lower the confidence of the individual thereby causing emotional distress. The severity of acne can be classified into 4 levels. The first grade do not cause much harm to the skin and cause inflammation only, the second level causes pustles and papules as explained earlier. They show mild effects. The third and fourth grade are severe and cause Deeper inflamed nodules. Factors that worsen the acne are hormones, diet and certain medications which were consumed for some other disease.

Types of Acne

Acne or more commonly known as pimples are of various types. They range from simple to severe forms. Acne can form several types of skin blemish each having a distinct appearance and symptoms. Minor acnes can be treated with home remedies and anti-inflammatory beauty products available in the market. But the severe ones need doctor or dermatologist attention. Acne vulgaris is the medical name for common acne. The most common regions for breakouts are face, shoulder, chest and back. Acne can be categorized depending on whether or not it causes inflammation of the surrounding skin. It is of two types: Non-inflammatory acne and Inflammatory acne

Non-inflammatory acne

It is the simplest form of acne. It doesn't cause swelling and is not very painful. They respond well to over-the-counter treatments. Products with salicylic acid are good for treating non-inflammatory acnes. It acts as natural

exfoliater. It removes dead skin cells that can lead to acnes. Also several home remedies and lifestyle changes help in getting rid of these acnes. It is of two types:

Whiteheads

They are formed when a pore gets clogged by sebum and dead skin cells. The top of the pore is closed. They look like a small, whitish or flesh coloured bump protruding from the skin. Medically they are known as closed comedones. They are difficult to treat because the pores are closed. They typically do not cause scarring

Blackheads

They are formed when the pores are clogged by a combination of sebum and dead skin cells. They are medically known as open comedones. They are different from the whiteheads for the fact that it has open pores, despite the rest of it being clogged. This result in the characteristic black colour of them on the surface of the skin. It's not the dirt that causes the black colour.

Inflammatory Acnes

They are red and swollen in appearance. They are formed either due to sebum or dead skin cells or due to bacterial infection. Bacteria causes infection deep beneath the skin surface which may result in painful acne spots that are hard to get rid of. They are more severe than non-inflammatory acne and cause scarring or pitting. They are of four types:

PAPULES

They are formed under the skin surface. They develop when whiteheads or blackheads cause so much irritation that they damage some of the surrounding skin which leads to inflammation. They are hard, clogged pores that are tender to touch and are generally pink in colour. They are visible on the skin surface. They don't have any visible centre and the pores don't appear to be widened like in whiteheads and blackheads respectively. They may be sensitive to touch. Picking and squeezing of papules leads to scarring.

PUSTULES

They are one more kind of inflamed pimples. They are filled with whitish or yellowish pus with a red base and are formed on breakdown of the pore walls. They are larger, tender bumps with a defined circular center. The pus is formed due to the collection of immune cells and bacterial cells. They typically look like larger and inflamed whiteheads. These bumps come out of the skin and are usually red in colour. They often have white or yellow heads on top. Picking and squeezing leads to scars and dark spots on the skin. Pustules and papules are moderate forms of acne. They may or may not clear with OTC meds and home remedies. They require dermatologist supervision.

NODULEES

They are hard, solid, painful, inflamed lumps located deep within the skin. They are formed when clogged pores damage tissues and cells deep beneath the skin surface. They need dermatologist supervision. The dermatologist generally prescribes the oral medication isotretinoin, which is made from a form of vitamin A. It treats and prevents nodules by decreasing the size of the oil glands in the pores.

CYSTS

They are the most severe types of acne. They are formed by the clogging of pores with bacteria, sebum and dead skin cells. This skin condition mainly affects the face, but also often affects the upper trunk and upper arm. The clogs are formed deep within the skin and deeper than the nodules. They are large, soft, painful, red or white lumps filled with pus. They affect fewer people. Main factors behind the formation of cystic acnes are hormonal disturbances at puberty .It can also occur in older individuals. It has severe affects on facial appearance. Picking or popping can lead to scars. It must be treated under the supervision of a dermatologist.

Some of the other serious conditions related to acne are as follows

Severe Nodulocystic Acne

They are severe forms of inflammatory acnes comprising of both nodular breakouts and cysts together. They are formed due to severe infection in the inflammatory acnes i.e. nodules and cysts. They are larger and deeper than normal pimples. They are very painful. They start off like more mild forms of acne vulgaris and eventually inflame and enlarge to form nodulocystic acne. It is thought to be genetic component. They are not true cysts as there is no lining. They are sometimes called pseudo cysts.In nodular acne you will have large, painful, solid pimples that are embedded deep in the skin. In cystic acne you will have cysts. Cysts are the most serious form of acne breakout deep, painful and filled with pus. In nodulocystic acne you have both types of blemishes-nodules and cyst.As this serious type of acne can cause scarring, it's best to treat it as soon as possible. Over-the-counter acne products are not strong enough to treat nodulocystic acne and therefore the patient must consult a dermatologist on time. Retinoid and antibiotics are good for treating this type of acne. Corticosteroid injections, often simply known as cortisone shots, can be used to quickly heal the bigger and painful blemishes.

Acne Conglobata

It's a form of nodulocystic acne. It is a rare but serious inflammatory skin condition that generally forms on face, chest and back. It causes significant and sometimes disfiguring scaring. It comprises of comedones, nodules, abscesses and draining sinus tracts. It occurs generally between the ages 18 to 30. It persists for a long time and can be seen till 40 years of age. It is generally found to affect men. The real cause of it is unknown. It starts as blackheads around the face, neck, chest, upper arms and buttocks in groups of two or three. Pimples are then formed around these blackheads. These pimples are large and filled with fluids and sensitive to touch. They remain like that for some time and continue to grow and fill with pus and eventually rupture. After the lesions have dried, they are again filled with fluid. After they rupture, several nodules fuse together to form a larger nodule. The lesions remain for a long time and continue to spread outwards forming a scab in the center. When they finally heal, they leave scars which can either be normal acne scars (atrophic) or raised bumps which are caused after a burn or cut (keloidal). The most common treatment for this is isotretinoin. Antibiotics may also be prescribed. Surgery may be necessary in case of large nodules.

Acne Mechanica

They are caused due to heat, friction and pressure against the skin or when skin is not exposed to air. They can develop anywhere on the body or face. The people prone to having acnes are more likely to develop acne mechanica. They vary in appearance from small comedones to papules and pustules. In the early stages, the skin may just feel rough and bumpy without any actual visible acne. Later with time, they progress to form tiny breakouts which eventually may grow to form more obvious, inflamed blemishes. Anything that traps heat for a prolonged period of time, rubs or puts pressure on the skin can lead to acne mechanica. Some examples of such

things are- athletic equipment, pads, helmets, straps from backpacks, bags and purses, hats and headbands, bra straps, tight fitting clothes and undergarments. All these things trap and hold heat and sweat against the skin, causing the hair follicles to get blocked.

Causes of Acne

Acne is a commonly problem seen now a days and is faced generally by teenagers. There are several factors that influence the formation of acne. Some the major causes are excess secretion of oil, clogging of hair follicles, diet, stress, bacteria, hormonal imbalance and lifestyle. Human skin has pores that are connected to sebaceous glands through follicles. A small hair grows through this follicle to the surface of the skin. The gland produces a oily secretion called sebum that helps keep the skin and hair moisturized. Sebum also maintains the flexibility of the skin and protects the skin from bacterial and fungal infections by acting as an barrier. When excess sebum, dead skin cells and dirt are trapped inside the pores, it leads to acne formation. They clump together into a plug and the pimple starts to develop when this plug begins to break down. Sebum production is controlled by hormones- specifically androgens. During puberty, the sebaceous glands enlarge and the hormones become more active and produce more sebum. This is reason for which acne is generally seen during adolescence. During puberty males produce five times more sebum than females. Sebum production starts to decrease by the age of 20 and continues to slow with age. Studies indicate that diet also plays an important role in acne development. Some food items like skim milk, carbohydrate rich food- such as bread, bagels and chips are thought to worsen acne. Chocolate has also shown adverse results in case of acne. A small study of 14 men with acne showed that eating chocolate was related to worsening of symptoms. Several drugs containing corticosteroids, testosterone and lithium have been found to induce acne development. Certain bacteria's also are acne causing agents such as *Propionibacterium*acnes.

Survey

An actual survey has been done in the Ananya hostel located inside the campus of Ramadevi Women's University and around its locality by taking 40 girls for the hostel itself and 10 boys from the locality. All of the individual chosen for the survey were of about 19-23 years. The survey for the girls were on the basis of seven different parameters whereas six different parameters were taken for the surveys in boys. A questionnaire was prepared for the survey. Boys were asked for their skin types, if they get pimple or not, amount of water in a day (in L), junkfood intake in week, sleep activity in hours a day, number of days for workout in a week. The girls were asked the same questions with the addition of another parameter of whether they get periods regularly or not and if anyone is suffering from PCOS (polycystic ovary syndrome). The survey has been represented in the form of tables and bar graphs for easier understanding and visualisation.

Survey on Girls

It has been observed 29 out of 40 girls have pimples on their face and different 29 girls have irregular periods out of which, 6 are suffering from PCOS or polycystic ovary syndrome in which cysts are formed amount the surface of ovaries which make it difficult to release eggs once a month thereby causing irregular periods. 17 out of 40 girls have oily skin where as rest of them have dry skin. 3 girls with oily skin do not have any pimples where as all the girls with oily skin have pimples. It was marked that 1 out of 40 did not eat junkfood at all, 24 out of 40 comsumejunkfood once or twice a week. 8 out of 40 eat junkfood 3-4 times a week and 7 eat 5-7 times a week which means almost daily. Number of girls that workout are very less in number. Hardly 12 out of 40 girls workout in a week. The table above represent the no. of time they workout. The last parameter asked was amount of water consumed in a day (in litres)
It is observed that most of the girls consume very less water in a day.

Survey on Boys

10 boys from the locality were taken into account for survey ranging from 19-23 years. It was marked that out of 10 boys only 3 of them have oily skin where as other have dry skin. But weirdly it was marked that those with oily skin did not had pimples. Thus other parameters were taken into account. Most of them intake junkfood almost everyday which are highly unhealthy. From the above observed survey it has been concluded that boys who intake junkfood everyday have pimples on their face irrespective of their skin type.

DISCUSSION

Hormonalimbalance

Acne is a disorder and is also a warning sign of a major imbalance in our system.Acne is a message from our body that something is wrong inside. This disorder results from the effect of hormones and other substances on the sebaceous glands and hair follicles present in the skin which leads pimples or zits.During puberty period the human body starts producing hormones as androgens or testosterone, increasing in both girls and boys. The androgens cause the enlargement and over stimulation of the sebaceous glands that are found in the hair follicles.The sebum or oil that produces by this glands mixes with those dead skin cells and Bacteria on the surface of the skin and block the pores.Bacteria starts multiplying within the pores which are blocked and cause inflammation which further leads to the lesions that are associated with acne. The most common suffers of acne are the teenagers because of the hormonal shifts that are associated with puberty.

Current figures indicate nearly 85% of people will develop acne at some point between the ages of 12 and 25 years.The skin and sebaceous gland are capable 9f producing by adrenal precursor hormone dehydroepiandrostero (DHEAs). This circulates in the blood stream in high level compared with other hormones. The main androgens that interact with the androgen receptor are testosterone and DHT.Androgen receptor are found in the basal layer of the sebaceous gland and the outer root sheath keratinocytes of the hair follicle. Thee essential role for androgens in stimulating sebum production is supported by several lines of evidence.Systematic administration of testosterone and dehydroepiandrosterone increase the size and secretions of sebaceous glands and cause severe acne.The hormonal changes associated with both the menstrual cycle, pregnancy and menopause also leads to formation of acne in skin. When women are either beginning or ending their useage of birth control the hormonal fluctuations that can occur at this time can cause acne in some women.Most women having a hormonal component to their acne have normal levels of serum androgen because of increased local or peripheral production of androgens.

The skin and sebaceous gland are capable of synthesizing cholesterol denovo from acetate as this cholesterolis utilized in cell membranes in the formation of the epidermal barrier and secreted in sebum, it is used as a substrate for steroid hormone synthesis, in which cholesterol needs to be translocated from the outer to the inner mitochondrial membrane. This process is regulated by the steroidogenic acute regulatory protein. LH/FSH is significantly higher in severe acne in female patients.In all women or children with acne the possibility of hyper androgenic state should be considered, the presence of irregular menses and hirsutism increases the likelihood of finding clinically significant hyperandrogenism.Gynecologic endocrine evaluation may be indicated in women who have acne resistant to conventional therapy, who relapse quickly after a course of Isotretinoin or in whom there is a sudden onset of severe acne.

Causes for hormone a limbalance

Nutrition deficiency and dietary factors

Nutritional deficiency is the major cause of most diseases and dysfunctions. If our body does not have enough energy or building materials to properly eliminate toxins and keep our system balanced, a disease occurs. This disease simply weak body's reaction to the high levels of toxins that are threatening it and manifest a symptom of a disease

such as acne. The natural approach is based on the belief that there are many types of food that can cause acne to appear, food with high levels of toxins as well as acid forming food , refined carbohydrates, dairy products and hydrogenated fats are direct causes for a dysfunctional digestive system which usually fails to evacuate toxic waste, resulting in the elimination of waste through the skin pores.

The difference between the populations was rooted in the eating habits of each population. While Western populations were consuming refined and simple carbohydrates including all sorts of toxic foods, processed and dairy products, the hunters were maintaining a diet consisting of fruits, vegetables, roots, nuts and fish.The high insulin levels in the blood were in fact the direct result of the Western diet producing excessive amounts of insulin, which stimulated the overproduction of sebum in the skin , the same sebum that encourages the growth of bacteria that cause the formation of acne.Fluctuations in blood sugar level cause insulin levels to become elevated thus lowering IGF BP-3, a beneficial hormone that promotes normal skin cell death which prevents the pore from getting blocked. When insulin is elevated, it also increases IGF-1, a hormone that stimulates excessive skin cell growth. Elevated IGF-1 prevents IGFBP-3 from doing its job.Another cause of hormonal irregularity can simply be that body has a certain deficiency in prostaglandins, which are hormones stabilizers that the body produces using a balanced supply of essential fatty acids, without this balance person will likely suffer from a chronic hormone imbalance that leads to acne.

Congested liver, clogged and sluggish bowels

Hormone irregularities can occur if liver is congested with either too many toxins or stones that disrupt it from either deactivating used hormones or expelling them via bowels or both. Hormone irregularities can also occur when bowel and kidneys fail to eliminate deactivated hormones, causing these hormones to be reabsorbed into the blood and becoming active again. This can happen for various reasons, one of which can be that your bowels are also congested with toxins. The bowels could be clogged, filled with mucus that stimulates the growth of parasites and candida. Liver is the main blood filtration organ that neutralizes and eliminates toxins from the body. When the liver is overloaded with toxins and stones , dangerous toxins from bad food, cell waste, parasites, candida and so on are not being expelled properly, they are reabsorbed into blood. These toxins can also be reabsorbed if bowels are clogged or sluggish and cannot handle the blood toxicity overload.

Food allergy

Allergies reaction to food happens when our body identifies a certain food as an invader in system. System is then sent to attack the invader and neutralize it for the purpose of eventually having it removed from body thus causing the allergy symptoms. The body's reaction process results in extra toxins that have to be filtered and expelled through liver, intestines and kidneys, thus putting extra stress on these organs of elimination. This results in the expulsion of these toxins via the secondary channels which are lungs and skin.

Candidaalbicans

Candida is a microorganisms (a vicious yeast) that dwells inside the digestive system and can transform from yeast to a fungus as it seeks the opportunity to propagate. When candida starts to flourish it can negatively affect the colon, bladder, liver and vagina.The most destructive aspect of candida growth results mainly from its waste product that is mycotoxins which can affect the brain, the immune system, joints, muscles, tissues and especially damaging the functionality of the liver. The following are the common symptoms associated with severe candida overgrowth: Recurrent vaginal infections, Recurrent urinary infections, Cramps, menstrual problems, Fatigue, a drained feeling, Depression, Abdominal discomfort, Bloating, belching, gas, Tightness in the chest, Drowsiness and dizziness, Dry skin, psoriasis or rashes, Urinary frequency, Indigestion or heartburn, Sensitivity to milk or wheat, Mouth rashes and

dry mouth, Bad breath even after brushing, Rectal itching, Vagina itching, Insomnia, sleep disturbance, Prostate problems. Recognizing these four or more symptoms then it may indicate that having severe candida overgrowth.

Candida albicans stimulated by antibiotics and refined carbohydrates such as sugar and white flour can grow to a plant-like form, break through the intestinal walls and help the yeast drive through the bloodstream and feed toxins into it.Candida thrives when the blood pH becomes acid. This usually happens when we consume less alkaline foods and more acid foods.When the blood pH becomes more acid, candida overgrows secreting more mycotoxins that put a burden on liver and intestines as well as other organs of elimination.Damaging the liver can have a negative impact on acne. In fact most acne sufferers have an overgrown candida in their system that once eliminated will result in a remarkable improvement on their skin and health condition in general. Controlling candida has proven to be a great influence in significant reducing acne breakouts.

Stress

Although many conventional doctors claim that there is no direct connection between the state of stress or anxiety and acne breakouts it has been tested and proven that stress causes the production of hormones such as cortisol and the weakening of the immune system that directly cause acne to aggravate.In state of stress the body depletes various essential vitamins and minerals such as the vitamins C, potassium, vitamin B and magnesium that are essential for hormonal balance.People infected with acne already suffer from abnormalities in hormone regulations so stress can only aggravate it. Moreover in a state of anxiety the digestive system seems to work more slowly due to lack of blood A state of insufficient blood in the stomach is the result of the body trying to survive by rushing blood from less important organs to more survival dependent organs like muscles.Stress can also put a burden on liver the same as other negative feelings can such as hate , envy and jealousy. This weakens the liver, decreasing its ability to regulate the hormones that have gone out of balance, and it also kills the friendly bacteria in the intestines and makes blood more acidic. The weakened liver and immune system cannot handle the over acidity.

Sleep disorder

The natural way regards insufficient sleep as one of the secondary causes for acne breakouts.When we don't get much sleep, there is an increase in hormone levels which can indirectly leads to acne breakouts. Sleep is a mini-detoxification period needed for the liver to eliminate toxins from body otherwise would be reabsorbed into system to be expelled later through skin.

Relationship between Hormone and Acne

Acne begins at puberty the body starts to produce hormones called androgens. Androgens cause the enlargement and over stimulation of the sebaceous glands in people with acne which leads overproduction of sebum and coupled with a sluggish exfoliation process leads to blocked pores and acne. Sensitivity to these androgens also cause acne during the menopause. It's is important to note that acne is not caused by excess in hormone levels but an abnormal reaction to normal levels of these hormones. The acne disease developing in adulthood more than other people, possible reasons may be diet, lifestyle and more synthetic hormones in environment.

Treatment

Daily intake of vitamin A derivatives for a period of a few months. It is effective treat over 80% of acne patients as it dramatically reduces the production of oil from the glands. However, the treatment requires the patients to take medical tests and examinations due to severe known side effects of the drug.Accutane is a poison that almost completely eliminates the production of sebum by the oil glands. Again the production of sebum oil is only the symptom not the cause of acne. It is a fact that it takes several months for Accutane to become ,acne will get worse

initially and in most cases it will come back as soon as stop taking Accutane.Partial side effects: dry skin, dandruff, headaches, hair loss, liver damage, bleeding from the nose, decreased night vision, birth defects and even arthritis or complete loss of vision. Using cloth or mechanical Tools or chemicals to peel off by scrubbing the external layer of the skin with the help of Salicylic acid and glycolic acid. Oral contraceptives: Oral contraceptives taking hormones to decrease the overproduction of male hormones such as testosterone can lower the production of the acne at best. In extreme cases it can lead to a severe hormonal imbalance which can aggravate acne. Also the body identifies it as another toxin to be eliminated putting more burden on system causing more acne instead of eliminating it.

Using Antibiotics such as Tetracycline or Benzoyl Peroxide over the counters to kill the bacteria harbored in the blocked follicles.It is not only that antibiotics such as Tetracyclines simply do not work in the long run as a treatment for acne bacteria and not the acne disease itself, antibiotics are extremely destructive to overall health as well as acne condition.Over the counter creams and ointments such as benzoyl peroxide are aimed at treating the acne affected areas by killing the bacteria . The problem is that killing the bacteria does not eliminate the problem that caused the excessive production of sebum oil for instance. The treated acne spots may vanish but others will follow.Another problem with this treatment is that some people may be allergic to penicillin or benzoyl peroxide and it may cause extreme rashes , swelling of the face or even result in abnormal breakouts.

Proactive solution: Proactive solution , the 3-product kit (cleanse, toner and lotion for repairing) containing the active ingredient benzoyl peroxide and glycolic acid , do exactly what is expected of an unnatural, extreme Western medical product. It only kills the bacteria by drying the skin and exfoliating the dryness.Proactive solution as well as benzoyl peroxide alone , have helped to reduce the acne formation in some people, but they do not solve the problem, furthermore they cause dryness and irritation.

Prevention for hormonal acne

Self educating to understand the reason why the changes are necessary for both mentally and emotionally. Detoxification diet planning which helps the body to discharge toxic waste. Stress reduction should be planned to daily routine like exercising, meditating, laughing etc.Having sufficient amount of sleep each night.Squeezing and picking the skin to remove the blackheads or whiteheads and this should be done with professionally in a hygienic manner to prevent infection.

Life style that causes acne

Dermatologists say that issues like acne and pimples are often due to simple skincare mistakes and many daily habits that leads to acne. From wrong products to touching face too often can cause acne. Below are some of the factors that affects our lifestyle and their preventive measures.

Phones

We touch our phones over 2000 times a day and bacteria and dirt from our fingers build up on the screen which cause breakouts around chin , cheeks, and mouth. And if have been on phone for over an hour, sweat has built up on screen too which spread of germs onto face. A lots of bacteria gathers on phone screen and the back which when comes in contact with the facial skin can cause acne as well as blemishes.

Using soap

Soap have harsh chemicals and they are not at all meant to be facial cleansers. The skin pH level is a major factor that contributes to skin problem. Ordinary commercial soaps have pH levels between 9 and 11, which increases the skin pH level leading to skin irritable and dryness and eventually lead to acne.

Pillow case

Pillowcase is very common culprit of breakouts as it accumulates bacteria, dirt and oils on the surface of the case that comes from hair and skin. After all skin is rubbing against this fabric for hours at a time while our body is unconscious, it could easily cause a lot of mayhem.

Touching face often

When we often touch our face with unclear fingers, introduces more oil,dirt and germs into skin. The hair care products can also get on hairline or face and block pores. Touching face often is a common cause of breakouts around chin and jawline because this brings bacteria to skin as well as applying pressure to it.

Diet

Dairy can aggravate acne because it can stimulate oil glands and increase clogged pores. Cow's milk contains hormones that can interact with skin as well as influence body's own hormones. Certain foods raise the blood sugar levels very quickly, releasing insulin and this make gland to produce oil and voila-acne. Using organic vegetables, put it in water to be soaked rinse or else in boiling water which kills germs. Junk food should be avoided as much as possible as the name itself suggest that what we intake is only 'Junk'. Eat foods which are low in calorie but high in nutritional value such as foods that are rich in Omega-3 Fatty Acids, Zinc, Probiotics and foods such as spinach, tomatoes, fruits rich in vitamins, Avoid foods which are rich in High Glycemic foods, dairy products, whey protein.

Stress

Stress effects skin by producing more cortisol and stress hormone. Now-a-days people due to work pressure, teenagers due to peer pressure, studies pressure are taking in a lot of stress which is not good for health. Stress causes weight loss, lowers down the energy, menstrual problems which imbalances the hormonal cycle, skin and hair problems such as acne, psoriasis, and eczema.Reducing the hours of sleep and cause insomnia, headache and eventually leads to depression and stress. An individual should sleep for about 6-8 hours for the proper metabolism of the body. Walking at least 30 minutes a day and meditating for a minimum of 5 times a week can help the body to relax, increase blood circulation, increases the release of endorphins, dopamine and serotonin which causes positive reaction on the brain cells thereby making the individual optimistic and stress- free. Laughing, taking up a hobby, trying different relaxation techniques reduces the amount of cortisol in the body thereby decreasing stress.

CONCLUSION

Current treatment for acne are effective but with complex regimens and side effects. There is no complete cure and all available treatments have significant drawbacks, yet no new products are launched or innovated since the last 10 years. "For many years it's been repurposing the same old stuff," says Adam Friedman a dermatologist at the George Washington university school of medicine and health sciences, Washington. The drugs are used extensively for treating acne and the same old drugs are modified and no new products are produced. Current treatments aim at treating any of the one or more of the four stages of acne. In more severe cases a mix n match approach is adopted to prevent formation of new spots and scarring. Although many of the medications work well if used properly, the complex combination treatment regimens required to target different aspects of acne pathophysiology lead to poor adherence, which undermines treatment success," says Steven Feldman, a dermatologist at Wake Forest Baptist Medical Center in North Carolina. Side effects of treatments are common. Benzoyl peroxide causes redness and peeling. oestrogen hormones are unsuitable for boys. The overuse of broad-spectrum antibiotics is thought to lead to

antibiotic resistance and to damage microbiome. Retinoids can cause red, sore skin that blisters and is sensitive to sunlight. The possible side effects of isotretinoin include hyperlipidemia, abnormal liver function tests, loss of night vision, depression and suicidal thoughts.

Isotretinoin is also extremely teratogenic, and so, has to be prescribed and taken with great caution.Some researchers have recently failed clinical trials, notably Xenon's phase II small-molecule inhibitor of sebum production XEN801 and Novan's SB204, an NO-releasing topical gel, which, although more effective than placebo in phase II, failed to meet one of its phase III endpoints. "The biggest hurdle is that clinical trials require both objective and subjective testing," says AnjaKrammer, president of California-based BioPharmX, which is developing the first topical minocycline gel for acne. "The objective test counts the effect a drug has on the number of lesions.

The subjective test, called the Investigator Global Assessment (IGA), relies on physicians to determine the grade improvement in each patient or the severity of those lesions," she explains. "This understandably results in variability because each physician views a patient's progress through his or her unique filter of experience." She says that although a grading scale is provided during investigator training, not all physicians grade exactly the same way. "This creates challenges for developers, several of which have fallen short on their IGA scores." "Endpoints can be very hard to meet sometimes," adds Friedman. "Although patients themselves may be happy, a drug will fail if it hasn't improved enough." Further studies may provide answers that will help us get rid of acne for good.

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Table.1.The sleep activity was taken into account and each of them were asked the hours they sleep in a day

Girls with pimples	29
Girls with irregular periods	29
PCOS patient	06

Table.2. It was observed that out of 40 girls slept for 4-6 hours, 24 of them slept for 6-8 hours and 11 slept for 8-10 hours

Sleep activity in a day (in hours)	No. of girls
4-6 hours	5
6-8 hours	24
8-10 hours	11

Table .3. It was marked that 1 out of 40 did not eat junkfood at all, 24 out of 40 comsumejunkfood once or twice a week. 8 out of 40 eat junkfood 3-4 times a week and 7 eat 5-7 times a week which means almost daily.

Workout (in hours)	No. of girls
1-2 times	4
3-4 times	1
5-7 times	7

Table .4. The last parameter asked was amount of water consumed in a day (in litres)

Amount of water intake in a day (in L)	**No. of girls**
1-2 L	23
3-4 L	6
4-5 L	7
5-6 L	11

Table .5. Junk food intake in a week

Junk food intake in a week	**No. of boys**
0	1
1-2	2
3-4	2
5-7	5

Table .6. The below table shows the sleep activity.

Sleep activity in a day	**No. of boys**
6-8 hours	4
8-10 hours	5
More than 10 hours	1

Table .7. Water intake in a day

Water intake in a day	**No. of boys**
1-2 L	3
2-3 L	1
3-4 L	3
4-5 L	3

Fig.1. BLACKHEADS	Fig.2. PAPULES	Fig.3. PUSTULES
Fig.4. NODULES	Fig.5. CYST ON NOSE	Fig.6. NODULOCYSTIC ACNE
Fig.7. NODUNON	Fig.8.ACNE CONGLUBATA	Fig.9. ACNE CONGLUBATA
Fig. 10.ACNE MECHANICA	Fig.11. Bar graph showing skin types in total of 40 girls.	Fig.12.Bar graph showing amount of junk food
Fig.13.Bar graph showing 10 boys only 3 of them have oily skin where as other have dry skin	Fig. 14.hormones affecting acne both in males and females	

RESEARCH ARTICLE

Field Evaluation of Native Phosphate Solubilizing Bacteria Formulation for Rice (*Oryza sativa* L. Var. IR64) Productivity in Acid Soil

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Received: 09 Jan 2020 Revised: 12 Feb 2020 Accepted: 14 Mar 2020

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ABSTRACT

Phosphorus plays a major role in growth and development of crops and is often found limiting in almost all types of soils. P solubilizing bacteria do the vital processes of mobilization of P from its poorly available sources. In this study, one phospahate solubilizing bacteria *Pseudomonas aeruginosa* strain SRIPs6 has been the isolated and identified. The potency of this bacteria have been evaluated to solubilize P from complexes of Ca-P, Al-P, Fe(II)-P and Fe(III)-P. This strain significantly enhanced soil phosphatase activities, microbial biomass P and plant P concentrations when combinedly applied with SSP. In combination with SSP it solubilized 63 per cent more P compared to pots treated with SSP only. When it was inoculated to rice enhanced the soil available P and phosphatase activity. The 16s rRNA sequence of this strain has been submitted to NCBI and assigned with the gene bank accession number MK764942.1

Keywords: MBP, Phosphatase, PSB, Rice.

INTRODUCTION

Phosphorus is the second most essential macro nutrient after nitrogen required for crop growth (Lal, 2002). Various metabolic processes viz; energy transfer, signal transduction, macro-molecular biosynthesis, photosynthesis, respiration, etc. require P as the key ingredient (Shenoy and Kalagudi, 2005). Mostly soils contain only approximately 1 μmol l^{-1} soluble P, where as the P requirement by the crops is approximately 30 μmol l^{-1} soluble P for optimum productivity (Simpson *et al.*, 2011). Plant roots usually absorb P as dihydrogen orthophosphate ($H_2PO_4^-$) and monohydrogen orthophosphate (HPO_4^{-2}) ion (Panda, 2009). Declined soil reaction (pH) increases the concentration of Fe and Al in soil solution thereby making complexes with aluminum and iron-free oxide sand hydroxides (Fearnside, 1998; Richardson, 2001). Soil acidity thus triggers and increases rate of P fixation as well as immobilization (Fankem *et al.*, 2006). Unlike N, P availability is highly dependent on the type of soil reaction (pH) and no big atmospheric source is there to supplement the P requirement of crops. Again, compared to N and K, total phosphorus level of

soils is low and usually one tenth to one fourth of N and one twelfth of K (Jones and Eva, 2011). Thus, P is also known as the limiting nutrient in almost all types of agricultural soils (Guiñazu *et al.*, 2010). P occurs in both organic and inorganic forms in soil. Foth, (1990) reported that, approximately 70-80% of the total P in cultivated soils is inorganic. In the soil environment, P in the phosphate form (PO_4^{2-}), invariably forms compounds with calcium, aluminium and iron, making it unavailable for crop uptake. P fixation leading to deficiency is characteristics of weathered soils of tropics and subtropics (acid soil) (Hinsinger, 2001). On the other hand P in the organic forms occurs as phospholipids, nucleotides and inositol phosphate (Turner *et al.*, 2002). All these forms of phosphates are unavailable for plant uptake.

Soil microorganisms play the major part in making P available. Soil microorganisms mostly secrete low molecular weight organic acids which perform as chelating agent to solubilize the inorganic fixed P (He *et al.*, 2002). The organic P fraction can be mineralized by different soil enzyme processes (Sarapatka, 2003). Soil phosphatases (acid phosphatase and alkaline phosphatase) found in microorganisms, plant roots and also in extracellular forms in soil (Tabatabai, 1994). P is effectively mobilised by both plant and microbial phosphatases, but microbial phosphatases show greater effectiveness in releasing P (Tarafdar *et al.*, 2001). Hence, manipulation as well as efficient utilization of particular beneficial microorganisms for sustainable approach in agriculture and soil health is the need of the time. This is pertinent to the high-input production systems of the developed world, and also to developing countries where access to mineral fertilizers is restricted. The study revealed the identification and evaluation of potency of one PSB strain to solubilize P in vitro and in pot culture assay with test crop rice. The study also elaborated the dynamics of soil microbial biomass, microbial activity and phosphatase activity in relation to soil reaction, organic carbon, available P and plant P concentrations.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from acid soil areas of five districts viz; Balasore, Cuttack, Khordha, Keonjhar and Mayurbhanj of Odisha. The samples were analyzed for population of heterotrophic bacteria and phosphorous solubilizing bacteria (PSB). Ten P solubilizer (one efficient from each district) was selected for further characterization.

Isolation and screening of native phosphorous solubilizing bacteria

Enumeration of P- solubilizing bacteria from the collected soil samples were done using National Botanical Research Institute's phosphate (NBRIP) growth medium (Nautiyal, 1999) supplemented with tricalcium phosphate (TCP). The diameters of clear zone produced by the isolates were measured using zone scale. NBRIP broth with inorganic phosphates of Calcium, Aluminium, Iron (II) and Iron (III) were prepared and the PSB strains were inoculated. Four replicated broth for each PSB isolates were taken to minimize the error. The broth cultures were incubated at 30 ± 2^0 C till 192 h and then centrifuged at 10,000 rpm for 30 minutes. Water-soluble phosphorus in the supernatant was measured spectrophotometricaly at 660 nm by the chloromolybdic acid method as described by Jackson (1967).

Pot culture experiment

The 10 PSB strains were grown in nutrient broth at 28^0 C and 120 rpm for 72 h. The cultures were grown to achieve optical densities of 0.9 (10^8 to 10^9 CFU ml^{-1}) at 620 nm wavelength. Rice (*Oryza sativa* L. IR64) seeds were surface sterilized with 1 % NaOCl for 5 minutes and then repeatedly (6 times) rinsed with sterile distilled water for 15 – 20 minutes. Sterilized seeds were then placed in glass petridish and coated with bacterial suspension (OD_{620nm} 0.9). Three inoculated seeds were sown per 10 kg of unsterilized soil in earthen pots (Fernández et al. 2007). The

experiment comprised of twelve (12) treatments (T_1-Control, T_2-100% P as SSP, T_3-KED09, T_4-JAG12, T_5-CTC11, T_6-JAJ07, T_7-SRI06, T_8- KED09 + 100% P as SSP, T_9- JAG12 + 100% P as SSP T_{10}- CTC11 + 100% P as SSP, T_{11}- JAJ07 + 100% P as SSP and T_{12}- SRI06 + 100% P as SSP) which were replicated thrice in a statistically randomized block design was conducted. The fertilizer sources N (20 kg/ha) as urea and K_2O (40 kg/ha) as Muriate of Potash (MOP) were given to all the treatments while P_2O_5 @ 40 kg/ha was applied as single super phosphate (SSP) following the treatment schedule. Due care and maintenance were followed till 110 days for growth of plants in the treated pots till maturity and then harvested.

Collection of soil sample from pots

Soil samples were collected from each pot at 40, 75 days after sowing (DAS) and at harvest for soil microbiological analysis.

Soil microbial analysis

Enumeration of total heterotrophic bacteria and P solubilizing bacteria

The soil microbial population was determined by dilution plate technique. 1 g of the soil samples were added to each of ten tubes containing 9 ml of sterile distilled water, serially diluted and spreaded over nutrient Agar and NBRIP (National Botanical Research Institute's Phosphate growth) media for enumeration of total bacteria and phosphorus solubilizing bacteria (PSB) respectively. The plates were incubated at 30°C for 24 hours for bacterial isolation and at 30°C for 48 hrs for PSB. The total no. of bacterial colonies were expressed in terms of log CFU per 1 g dry wt. soil.

Measurement of microbial Biomass Carbon and Phosphorus

Microbial biomass carbon (MBC) was measured by fumigation-extraction [soil: extractant (0.5 M K2SO4) ratio 1:4] method using a conversion factor (Kc) of 0.38 (Vance *et al.* 1987; Hu and Cao, 2007). Microbial biomass phosphorus (MBP) was also measured by fumigation extraction [soil extractant (0.5 M $NaHCO_3$) ratio 1:20] method using a Kp factor of 0.4 (Brookes *et al.*1982).

Phosphatase Activity

Soil phosphatase activity was calculated by colorimetric estimation of the p-nitrophenol released by phosphatase activity when the soil was incubated with buffered disodium p-nitrophenyl phosphate tetrahydrate of pH 6.5 and 11 respectively for acid and alkaline phosphatase at 37° C for 1 h (Tabatabai and Bremner, 1969).

Soil chemical analysis

Soil samples were analyzed for soil chemical parameters viz; soil pH (Jackson, 1967), organic carbon (Page *et al.*, 1982) and available phosphorus by Bray's 1 method (Bray and Kurtz, 1945) as out lined by Page *et al.*, (1982).

P concentration of shoot and kernel

Shoot samples of rice plants were digested in diacid mixture [HNO_3:$HClO_4$ (3:2)]. Phosphorus concentrations were estimated spectrophotometricaly at a wavelength of 470 nm (Page *et al.*, 1982).

Identification of native phosphorus solubilizing bacteria

The efficient PSB isolate were identified by 16S rRNA gene sequencing. 16S region was PCR amplified with 16sF (5′AGAAAGGAGGTGATCCAGCC3′) and 16sR (5′AGAGTTTGATCMTGGCTCAG3′) primers after isolating DNA using PureLink Genomic DNA kit (Invitrogen). Amplicon was electrophoresed in a 1% agarose gel and visualized under UV-VIS gel doc system. The PCR product was then sequenced and the data submitted to NCBI.

Statistical analysis

Data were statistically analyzed by the software R (version 3.2.2) and tested with Duncan's new multiple range test at 5% critical range using the package "agricolae".

RESULTS

Five tentative PSB strains KED09, JAG12, CTC11, JAJ07, SRI06 were analysed for their P solubilization efficiency (PE) in liquid NBRIP medium supplemented with $Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$ and $Fe_3(PO_4)_2$ respectively. Study revealed that isolate SRI06 showed significantly higher PE in the mediums with $Ca_3(PO_4)_2$, $FePO_4$ and $Fe_3(PO_4)_2$ at 48 h of incubation. Continuing the incubation till 192 h, all 10 isolates showed an increasing trend of P solubilization efficiency. SRI06 showed maximum PE in $Ca_3(PO_4)_2$, $FePO_4$ and $Fe_3(PO_4)_2$ compared to other isolates. Results further revealed that, after 8th day of incubation, isolate KED09 recorded least P solubilization efficiency in Ca-P (36.78%), KED09 in Fe(III)-P (8.55%) and JAJ07 in Al-P (2.50%) and Fe (II)-P (5.20%). Among the inorganic P sources, $AlPO_4$ was least preferred substrate for all the isolates.

A pot culture experiment was conducted with rice (*Oryza sativa* L cv. IR64). The soil was sandy loam in texture with sand - 73.5%, silt- 16.75% and clay- 9.00% with acidic (pH – 5.4) reaction. Initial soil organic carbon and available P were 0.46 % and 5.24 mg ha^{-1} respectively. The treatment T_7 (SRI06) recorded highest population of culturable heterotrophic bacteria (7.716 log CFU g^{-1} dry wt. soil) at 40 DAS and 7.934 log CFU g^{-1} dry wt. soil at 75 DAS. In the post harvest soil, the same trend was maintained with highest culturable bacterial population (7.799 log CFU g^{-1} dry wt. soil). Study revealed that heterotrophic bacterial population was significantly increased due to application of PSB strains in combination with P fertilizer (single super phosphate) over sole application of inorganic. Data further revealed that treatment T_{10} (CTC11 + 100% P as SSP) maintained highest colonies of PSB (7.982 and 7.991 log CFU g^{-1} dry wt. soil respectively) at 40 and 75 DAS the population was 7.991 log CFU g^{-1} dry wt. soil and at harvest, T_9 (JAG12 + 100% P as SSP) recorded highest PSB population (7.968 log CFU g^{-1} dry wt. soil) (Table I). PSB population significantly increased due to application of PSB compared to control.

Rhizospheric soil samples were analyzed for soil microbial biomass carbon (MBC) and biomass phosphorus (MBC) at 40, 75 DAS and harvest (Table II). Soil MBC significantly increased due to sole inoculation of five PSB strains (KED09, JAG12, CTC11, JAJ07 and SRI06) as compared to the uninoculated pots at 40 and 75 DAS and harvest, while all five strains (KED09, JAG12, CTC11, JAJ07 and SRI06) in combination with SSP resulted in a significant increase in MBC values over the uninoculated pots. Soil MBP values also increased significantly owing to inoculation of the five strains as sole or in combination over the uninoculated ones at 40, 75 DAS and harvest. In addition, C/P ratio was also calculated for soil samples collected at 40, 75 DAS and harvest (Table II). The results showed a decrease in C/P ratio in all the inoculated pots compared to the uninoculated ones. The pots treated with two (KED09 and JAG12) of the PSB strains combined with P fertilizer (SSP) resulted in the lowest C/P ratio at 40, 75 DAS and harvest.

Acid phosphatase activity significantly increased as a result of sole inoculation of five (KED09, JAG12, CTC11, JAJ07 and SRI06) of the PSB strains over the uninoculated pots at 40 and 75 DAS (Table III) except the strain SRI06 which showed a significant increase even at harvest. When combined with P fertilizer all the five strains significantly

promoted the acid phosphatase activity over the uninoculated treatments at 40, 75 DAS and harvest. However, no significant increase was observed in the alkaline phosphatase enzymes in the pots treated with PSBs only compared to the uninoculated treatment (T_2 – 100% P as SSP). Five (KED09, JAG12, CTC11, JAJ07 and SRI06) of the five strains showed a significantly higher alkaline phosphatase activity when applied in combination with P fertilizer (SSP) at 40, 75 DAS and harvest.

Soil reaction (pH), organic carbon and available P values were recorded at harvest of the plants (Table IV). Soil pH and organic carbon ranged from 5.36 to 5.48 and 0.40 to 0.48 per cent respectively. No great variations were observed with respect to soil pH and organic carbon among the inoculated and uninoculated pots.One set of universal primers were used for determination and identification of the 16S rRNA gene of the isolates. The primer amplified the gene for the five isolates successfully. The size of the 16S rRNA gene product of SRI06 isolate was 1.5 kbp. The 16S rRNA gene sequences were compared with the Genbank database and accession number was received. The bacteria was identified as *Pseudomonas aeruginosa.*

DISCUSSION

Rice rhizosphere harbours so many microbial groups that play the vital roles in performing various soil biochemical processes i.e. mineralization and immobilization of nutrients (N, P, K and C) (Lundberg *et al.*, 2012). There occur specific microorganisms which are the essential part of soil P transformations. Inorganic phosphate in acidic soil occur mostly in the forms of variscite [$Al(OH)_2H_2PO_4$ or $AlPO_4.2H_2O$] and strengite [$Fe(OH)_2H_2PO_4$ or $FePO_4.2H_2O$] (Fearnside, 1998; Richardson, 2001; Bashan *et al.*, 2013) and vivianite [$Fe_3(PO_4)_2.8H_2O$] (Mohsin *et al.*, 1995). In alkaline soils tricalcium phosphates are abundant. These minerals are highly stable and poorly soluble, which makes them unavailable for plants, although soils contain high concentration of total P (Merbach *et al.*, 2010). Consequently, we have taken four representative types of P minerals [$Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$ and $Fe_3(PO_4)_2$] commonly found in acid or alkaline soil.

The study demonstrated the effectiveness of one bacterial strain *Pseudomonas aeruginosa* as P solubilizers in vitro. In this experiment, the ability of five different strains were assessed in the liquid NBRIP medium each containing four types insoluble phosphates [$Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$ and $Fe_3(PO_4)_2$] as the sole source of inorganic P. Among them, $Ca_3(PO_4)_2$ was the best phosphate source for all PSB strains followed by $FePO_4$. $Fe_3(PO_4)_2$ and $AlPO_4$. As revealed in the results all the strains especially *Pseudomonas aeruginosa SRI06* released highest amounts of P in the mediums where $Ca_3(PO_4)_2$, $FePO_4$ and $Fe_3(PO_4)_2$ have been given as the insoluble P sources. Balamurugan *et al.*, (2010) also reported PSB strains solubilizing $AlPO_4$. After 8th day, the P solubilization efficiency increased compared to the data at the 2nd day of incubation.

The bacteria and PSB population showed an increasing trend at 75 DAS but decreased towards harvest. This may be attributed to poor root activity in the harvest soil. Root exudates supply several carbon compounds that attract the heterotrophic soil microbiota (Naher *et al.*, 2008). Markedly, the uninoculated pots showed lower P solubilization compared to inoculated pots. Further, the application of PSB along with the water soluble P fertilizer (single super phosphate) appeared to have increased the population (log CFU per g soil) of PSB as well as heterotrophic bacteria. Panhwar *et al.*, (2012) reported that, addition of inorganic P sources positively affected the bacterial population which in turn enhance its association with the plant roots.

The significant increase in the microbial biomass carbon and phosphorus with addition of inorganic P fertilizer suggest that the applied water soluble P enhanced immediate P availability for crop uptake, which in turn might have increased the activities of root and rhizosphere microorganisms (Attar *et al.*, 2012). Increased microbial activities obviously make the bacteria to transform available P into microbial biomass P (MBP) (Wu *et al.*, 2007). Microbial biomass P is one of the most labile forms of P in soil and plays a vital role in biogeochemical cycling of P in soil. By

taking consideration of microbial biomass C and P, we have calculated the C/P ratio, which showed a decreasing trend in the inoculated pots. In addition, the pots treated combined with PSB and SSP (P fertilizer) C/P ratio declined. Zhang *et al.*, (2014) reported that addition of inorganic P could decrease the C/P ratio in a low P soil. It is evident from the study that, microorganisms compete with plant roots for the orthophosphate and assimilate the P making it temporarily unavailable for the crop. Thus microbial biomass P protects the orthophosphates from binding with cations of Ca, Al and Fe in the soil solution (Olander and Vitousek, 2004).

Soil phosphatases can be classified into two types, acid and alkaline phosphatases basing on the pH. These enzymes mobilize the organically bound P into soluble orthophosphates making them available for both plants and microorganisms (Zhang et al. 2014). Phosphatases are of either plant or microbial origin (Richardson and Simpson, 2011). As mentioned in the result acid and alkaline phosphatase activity increased till the harvest of the crop. Increased activities of phosphatases occur in response to P deficiency in soils (Richardson *et al.*, 2005). The control pots and the pots treated with SSP only were detected with phosphatase activity which may be derived from the root exudates and indigenous microbial activity, as no PSB inoculum was added. Further PSB application enhanced soil phosphatase activity and pots treated with combined application of PSB and SSP favoured phosphatase activity. This may be attributed to higher root and microbial activity (Zhang *et al.*, 2014). Soil available P values were increased in the PSB treated soil. Strains KED09 and JAG12 either sole or in combination with inorganic P (SSP) recorded more soluble P. Soil bacteria solubilize soil inorganic P by secreting low molecular weight organic acids and organic P by producing phosphatases (Rodríguez *et al.*, 2006). More than 40% of culturable bacteria possess the ability to mobilize insoluble and organic P (Jorquera *et al.*, 2008) and release orthophosphates ($H_2PO_4^-$ and HPO_4^{2-}) that can be absorbed by plant roots (Rodriguez and Fraga, 1999). Mobilization of insoluble organic P by soil bacteria can be regulated by addition of inorganic P fertilizer, which can directly affect the activity of soil microbiota and indirectly enhance the plants ability to take orthophosphates from organically fixed P (Zhang *et al.*, 2014). The significant correlation between acid phosphatase and P content of plant proved that, PSB inoculation appeared to be a major factor in the mineralization of organic P which might have improved plant P nutrition.

CONCLUSION

The five PSB strains when combined with SSP (inorganic P fertilizer) increased microbial biomass P and phosphatase activity while simultaneously reduced C/P ratio. All the strains (KED09, JAG12, CTC11, JAJ07 and SRI06) proved to solubilize P in the pot culture assay and improved nutrition of groundnut. However, the strain *Pseudomonas aeruginosa* SRI06 (MK764942.1), performed better in mobilizing soil P and producing phosphatases.

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Table 1. Effect of PSB on microbial population in rice rhizosphere

Treatments	Total heterotrophic Bacteria (log CFU g^{-1} dry wt. soil)			P- Solubilizing Bacteria (log CFU g^{-1} dry wt. soil)		
	40 DAS	75 DAS	Harvest	40 DAS	75 DAS	Harvest
Control	7.613 ± 0.035^{e}	7.580 ± 0.003^{d}	7.567 ± 0.018^{e}	7.581 ± 0.007^{c}	7.592 ± 0.003^{c}	7.501 ± 0.006^{d}
100% P as SSP	7.685 ± 0.033^{bc}	7.754 ± 0.268^{c}	7.746 ± 0.014^{d}	7.592 ± 0.014^{c}	7.661 ± 0.005^{c}	7.621 ± 0.003^{c}
KED09	7.58 ± 0.060^{de}	7.77 ± 0.006^{bc}	7.741 ± 0.007^{d}	7.631 ± 0.009^{bc}	7.832 ± 0.010^{b}	7.798 ± 0.004^{b}
JAG12	7.632 ± 0.008^{cd}	7.791 ± 0.006^{abc}	7.772 ± 0.004^{cd}	7.715 ± 0.539^{abc}	7.812 ± 0.004^{b}	7.777 ± 0.006^{b}
CTC11	7.661 ± 0.060^{bcd}	7.772 ± 0.012^{bc}	7.725 ± 0.002^{d}	7.749 ± 0.006^{abc}	7.793 ± 0.004^{b}	7.793 ± 0.007^{b}
JAJ07	7.68 ± 0.025^{bc}	7.761 ± 0.005^{bc}	7.746 ± 0.006^{d}	7.755 ± 0.005^{abc}	7.816 ± 0.003^{b}	7.784 ± 0.005^{b}
SRI06	7.712 ± 0.007^{b}	7.801 ± 0.003^{abc}	7.797 ± 0.004^{bcd}	7.792 ± 0.004^{abc}	7.805 ± 0.004^{b}	7.805 ± 0.005^{b}
KED09 + 100% P as SSP	7.805 ± 0.006^{a}	7.851 ± 0.002^{abc}	7.838 ± 0.003^{abc}	7.941 ± 0.004^{a}	7.947 ± 0.004^{a}	7.932 ± 0.006^{a}
JAG12 + 100% P as SSP	7.812 ± 0.008^{a}	7.932 ± 0.007^{a}	7.856 ± 0.004^{ab}	7.953 ± 0.007^{a}	7.981 ± 0.004^{a}	7.967 ± 0.004^{a}
CTC11 + 100% P as SSP	7.831 ± 0.005^{a}	7.928 ± 0.004^{a}	7.852 ± 0.004^{ab}	7.981 ± 0.004^{a}	7.992 ± 0.002^{a}	7.962 ± 0.004^{a}
JAG07+ 100% P as SSP	7.856 ± 0.016^{a}	7.915 ± 0.004^{ab}	7.915 ± 0.007^{a}	7.876 ± 0.003^{ab}	7.932 ± 0.004^{a}	7.955 ± 0.003^{a}
SRI06+ 100% P as SSP	7.862 ± 0.009^{a}	7.882 ± 0.007^{abc}	7.868 ± 0.006^{ab}	7.918 ± 0.003^{a}	7.943 ± 0.003^{a}	7.923 ± 0.002^{a}
CV (%)	0.572	1.041	0.551	1.812	0.742	0.812

Tested by Duncan's Multiple Range Test with 5% critical range. Means represented by the same letter are not significantly different. Data given in above are average values of three replicates ± standard error of mean (SEM).

Table 2 Effect of PSB on microbial biomass carbon and phosphorous in rice rhizosphere

Treatments	Microbial biomass carbon (μg C g^{-1} soil)			Microbial biomass phosphorous (μg P g^{-1} soil)		
	40 DAS	75 DAS	Harvest	40 DAS	75 DAS	Harvest
Control	105.356±2.603^{f}	112.560±2.709^{e}	105.652±2.784^{e}	8.522±0.166^{c}	8.962±0.021^{e}	8.562±0.006^{e}
100% P as SSP	118.650±4.305^{e}	121.360±2.952^{d}	120.362±3.466^{d}	9.360±0.131^{c}	10.368±0.109^{d}	10.253±0.158^{d}
KED09	119.522±5.258^{e}	125.328±2.616^{cd}	125.325±2.710^{cd}	11.524±0.102^{b}	12.320±0.036^{c}	12.210±0.613^{c}
JAG12	125.366±4.207^{de}	132.562±5.335^{bc}	133.520±2.902^{bc}	12.360±0.220^{b}	12.352±0.014^{c}	12.208±0.161^{c}
CTC11	135.650±3.482^{c}	138.520±2.885^{b}	137.256±3.155^{bc}	11.256±0.526^{b}	12.382±0.335^{c}	12.310±0.196^{c}
JAJ07	133.560±4.435^{cd}	138.500±5.388^{b}	138.250±3.124^{b}	12.365±0.268^{b}	13.524±0.414^{bc}	13.216±0.114^{bc}
SRI06	138.646±0.984^{bc}	139.860±4.521^{b}	135.622±2.490^{bc}	12.220±0.104^{b}	12.480±0.386^{c}	12.255±0.498^{c}
KED09 + 100% P as SSP	145.682±1.841^{ab}	158.253±5.278^{a}	162.350±3.619^{a}	13.850±0.192^{a}	14.450±0.247^{ab}	14.252±0.267^{ab}
JAG12 + 100% P as SSP	152.653±2.804^{a}	159.365±3.685^{a}	166.422±2.724^{a}	14.256±0.229^{a}	15.655±0.116^{a}	15.286±0.240^{a}
CTC11 + 100% P as SSP	153.365±3.900^{a}	162.422±1.722^{a}	168.352±3.736^{a}	15.360±0.282^{a}	15.685±0.294^{a}	15.322±0.171^{a}
JAG07+ 100% P as SSP	155.360±1.822^{a}	163.520±2.775^{a}	166.522±1.784^{a}	14.235±0.436^{a}	15.852±0.894^{a}	15.226±0.042^{a}
SRI06+ 100% P as SSP	150.380±2.703^{a}	163.560±2.482^{a}	168.354±2.847^{a}	14.250±0.195^{a}	15.689±0.316^{a}	15.302±0.212^{a}
CV (%)	3.916	3.591	4.698	6.834	5.877	6.173

Tested by Duncan's Multiple Range Test with 5% critical range. Means represented by the same letter are not significantly different. Data given in above are average values of three replicates ± standard error of mean (SEM)

Table 3. Effect of PSB on soil enzymes in rice rhizosphere

Treatments	Acid phosphatise (μM PNP g^{-1} soil h^{-1})			Alkaline phosphatise (μM PNP g^{-1} soil h^{-1})		
	40 DAS	75 DAS	Harvest	40 DAS	75 DAS	Harvest
Control	0.086±0.002^{f}	0.097±0.002^{f}	0.102±0.005^{e}	0.006±0.002^{e}	0.013±0.002^{e}	0.016±0.002^{e}
100% P as SSP	0.106±0.009^{e}	0.115±0.003^{e}	0.121±0.003^{d}	0.016±0.004^{d}	0.026±0.004^{cd}	0.026±0.003^{d}
KED09	0.116±0.003^{de}	0.120±0.003^{de}	0.122±0.004^{d}	0.017±0.002^{cd}	0.028±0.002^{bcd}	0.029±0.003^{cd}
JAG12	0.128±0.003^{bcd}	0.133±0.003^{bcd}	0.136±0.003^{bcd}	0.018±0.002^{bcd}	0.028±0.001^{bcd}	0.030±0.002^{cd}
CTC11	0.126±0.004^{cd}	0.135±0.003^{abc}	0.138±0.004^{bc}	0.021±0.002^{abcd}	0.026±0.002^{cd}	0.032±0.003^{bcd}
JAJ07	0.128±0.005^{bcd}	0.130±0.003^{cd}	0.130±0.002^{cd}	0.018±0.002^{bcd}	0.025±0.003^{cd}	0.031±0.003^{bcd}
SRI06	0.126±0.001^{cd}	0.133±0.003^{bcd}	0.135±0.002^{cd}	0.017±0.002^{cd}	0.024±0.003^{d}	0.029±0.001^{cd}
KED09 + 100% P as SSP	0.135±0.002^{abc}	0.138±0.003^{abc}	0.140±0.002^{bc}	0.022±0.002^{abcd}	0.026±0.002^{cd}	0.030±0.003^{cd}
JAG12 + 100% P as SSP	0.148±0.003^{a}	0.148±0.003^{a}	0.158±0.004^{a}	0.026±0.002^{a}	0.036±0.003^{ab}	0.038±0.005^{ab}
CTC11 + 100% P as SSP	0.144±0.002^{ab}	0.146±0.002^{ab}	0.152±0.003^{ab}	0.024±0.002^{ab}	0.038±0.002^{a}	0.042±0.003^{a}
JAG07+ 100% P as SSP	0.138±0.003^{abc}	0.139±.003^{abc}	0.141±0.001^{bc}	0.023±0.002^{abc}	0.033±0.003^{abc}	0.036±0.003^{abc}
SRI06+ 100% P as SSP	0.142±0.002^{abc}	0.145±0.003^{ab}	0.145±0.002^{abc}	0.026±0.002^{a}	0.035±0.001^{ab}	0.037±0.002^{abc}
CV (%)	6.883	5.718	6.399	16.994	16.339	13.519

Tested by Duncan's Multiple Range Test with 5% critical range. Means represented by the same letter are not significantly different. Data given in above are average values of three replicates ± standard error of mean (SEM)

Table .4. Effect of PSB on changes in soil pH and organic carbon in rice rhizosphere

Treatments	pH	Organic carbon (%)	Available P (mg kg^{-1})
Control	5.43 ± 0.030^{abc}	0.40 ± 0.017^{a}	4.16 ± 0.100^{f}
100% P as SSP	5.37 ± 0.056^{bc}	0.41 ± 0.017^{a}	4.97 ± 0.043^{ef}
KED09	5.45 ± 0.030^{abc}	0.40 ± 0.053^{a}	5.89 ± 0.030^{de}
JAG12	5.46 ± 0.052^{ab}	0.45 ± 0.026^{a}	6.88 ± 0.046^{bcd}
CTC11	5.36 ± 0.053^{c}	0.42 ± 0.020^{a}	6.57 ± 0.026^{cd}
JAJ07	5.38 ± 0.053^{bc}	0.41 ± 0.046^{a}	6.13 ± 0.020^{cde}
SRI06	5.41 ± 0.036^{abc}	0.40 ± 0.060^{a}	6.44 ± 0.021^{cd}
KED09 + 100% P as SSP	5.40 ± 0.030^{abc}	0.45 ± 0.046^{a}	6.86 ± 0.030^{bcd}
JAG12 + 100% P as SSP	5.39 ± 0.056^{abc}	0.46 ± 0.052^{a}	8.11 ± 0.026^{a}
CTC11 + 100% P as SSP	5.48 ± 0.017^{a}	0.47 ± 0.046^{a}	8.02 ± 0.026^{ab}
JAG07+ 100% P as SSP	5.40 ± 0.036^{abc}	0.46 ± 0.010^{a}	6.77 ± 0.036^{cd}
SRI06+ 100% P as SSP	5.38 ± 0.020^{bc}	0.48 ± 0.020^{a}	7.20 ± 0.026^{abc}
CV (%)	0.942	8.447	10.266

Tested by Duncan's Multiple Range. Test with 5% critical range. Means represented by the same letter are not significantly different. Data given in above are average values of three replicates ± standard error of mean (SEM).

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effects of P^H on Morphology and Viability of Mosquitoes Aedes Group in CUTM Campus, Bhubaneswar

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Received: 06 Jan 2020 Revised: 11 Feb 2020 Accepted: 14 Mar 2020

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ABSTRACT

Mosquitoes are a highly diverse group of insects are belongs to the order Diptera of phylum Arthropodawhich aregenerally laid their eggs and doing their life cycle in swallow pools,swampy areas, lentic water bodies, rock pools and phytotelmata like areas. There are around 2500 species found almost every continents. And these are basically undergoes 4 stages in their life cycle and most crucial stages are larvae, pupa stage. Most of the developmental changes occur due to the different environmental conditions like temperatures, humidity and water pH.Mosquito larvae are the organic detritus eater from the environment. Like all the insects, the body of mosquito larvae are divided into four main region: head, thorax, abdomen and tail. For theidentification and morphological characterization, eggs were collected from CUTM campus district Khordha, Odisha, India. These egg samples were identified with the help of standard taxonomic keys and different morphological characteristics to confirm the species belongs from *Aedes*group. Then the sample kept under observation for the study and understanding more deeply physiological response due to different climatic changes in their aquatic habitats and viability of the species. The present study provides the important information on the morphological variation in different climatic changes of *Aedes* group of Mosquitoes found in the CUTM campus of khordha, Odisha, which will be useful in acquiring a better understanding of disease causing factors of this flies and planning more appropriate and effective control measures in future.

Keywords: Mosquitoes, pH ,temperature, morphology, viability.

INTRODUCTION

Mosquito is now very common name for biting insects which belongs to the family-Culicidae, suborder-Nematocera, order-Diptera, class-Insecta, phylum-Athropoda. Previously the name for the mosquito in our country was 'gnat'. The change of this name'gnat' took place about 1900, when as a result of Ross's discovery of the mosquito life cycle in

malaria and the importance of these insects to man became realised and knowledge concerning for the first time in more generally. Hurst in 1890 uses the title 'The Life Development and History of a Gnat' for his paper and Giles in 1900 entitled his book 'Handbook of Gnats'. Since 1901 Theobald alongwith all English writerspractically used the name mosquito simply. It is well to remember therefore when looking up old literature that the reference in the index may be to gnats or Culex (Aldrovando. U, 1602).

It is interesting to trace the origin and history of this word mosquito and why and how all of itcome to be used. Clearly the word mosquito is of Spanish or Portuguese origin and it is probably correct to say that, it must have come originally from Spanish or Portuguese America. It is perhaps more probable that its modern use has come from North America. At first find the name, often with variations in spelling, used in accounts of travellers. Such use dates back to the sixteenth century, for Phillips (1583) in Hakluyt's Voyages (1589) is quoted in the Oxford English Dictionary as saying 'We were almost oftentimes annoyed with a kind of flies, the Spaniards call musketas', and a number of other suchexamples of the uses of this word 'mosquito'is given in the dictionary. Humboldt (1819) refers mosquitoes as 'zancudos' and for a smaller kind 'tempraneros'.

The word mosquito he gives as meaning of 'little fly' and 'zancudos' as 'long-legged'. According to Bur-meister (1832) the larva of Anopheles was first describedby Goeze in1775 and later by Lichtenstein in 1800, but the larva of Anopheles was only identified (Fischer, 1812). Now a daysMosquitoes are medically the most significant group of insects according to their important role in the widespread of several human infectious diseases including malaria, dengue fever, encephalitis, yellow fever and filariasis (Weaver and Reisen, 2010). This small flies undergoes basically 3 stages in life after hatching the eggs first one is larval stage then it going to pupa stage after staying in as little as 5 days in larval condition and in 2-3 days of development in pupal condition it became an adult one. The global magnitude of morbidity and mortality caused by arthropod-borne diseases has been a public health emergency of international concern. Early stages of mosquito development are related to aquatic environments, thus understanding the ecological factors involved in the aquatic habitats is essential in order to develop and improve effective strategies of mosquito control (Theovald,1901-1910).

MATERIALS AND METHODS

Study Area

Centurion University of Technology and Management is the first multi-sector, private state university from Odisha, India. Our campus located at Jatni. It was accorded the status of a university in the year of 2010. It spread over 40 acres land in the foothill of Baruneihill, near jatni town the campus is adjacent to National Institute of technology.Samples were collected from the different canals present near the pond of CUTM campus, Odisha, India.

Collection of Samples

Eggs weredirectly collect from surface of the lentic water bodies like shallow pools, marshes, natural containers like rock pools, phytotelmata (water contains cavity of terrestrial plants) artificial container like barrels, buckets, discarded tires etc. by a bristled brush and directly dipping the small containers in the lentic waters.

Preparation of Water Media with Different pH

In this study three pH treatments, pH 4, pH 7, and pH 9.2, were used in each set-up (A and B). Each treatment had three replicates. For the acidic media (pH 4), to acquire the desired pH level, it can use pH capsules. When the desired pH was already achieved, 100 ml of the medium was contained in each container. A total of 300 ml of medium was prepared to fill in the 6 glass containers (at 100 ml each) for pH 4. There were 3 replicates of pH 4

treatment per set-up. For the control treatment (pH 7), an Absolute distilled water was used. A volume of 100 ml of distilled water was contained in each container, and total of 300 ml of distilled water was prepared to fill in the 6 glass containers (at 100 ml each). There were 3 replicates of pH 7 treatment per set-up. The pH level of the distilled water used in the set-up was also checked using a pH meter.

To prepare the alkaline medium, pHcapsules 9was used to produce the water basic. Three pH capsules was added and dissolved into a 300 ml of distilled water. To check whether the desired pH level was already reached, a pH meter was used. A volume of 100 ml of the medium was contained in each glass container. A total of 300 ml of the medium was prepared to fill in the 6 glass containers (at 100 ml each) for pH 9. There were 3 replicates of pH 9.2 treatment per set-up.

Experimental Set-ups in Laboratory

Each pH treatment had three replicates per set-ups. Five newly hatched eggs were placed in each replicates. The larvae were allowed to metamorphosis. Fish pellets of about 10 pieces were placed inside the container to provide the food for the larvae development. The temperature of the set-up is reliant on the heat emitted by halogen lamp in the closed system.

Monitoring the Morphology of Larvae

Daily up to 8 to 10 days observe the larvae set-ups was done to check whether the metamorphosis of the larvae from different instars to pupa occurred. The larvae were checked only before turning the light off. The development of different stages were photo-documented under the microscope and differentiate by direct observing the different parts of the larva and pupa of the species. The number of larvae that survived and those that expired were monitored and recorded daily. The pH of each replicate per treatment was recorded only after the experimental set-ups were terminated. The set-ups were terminated only when the larvae were on their pupa stage. The final pH level of all media was recorded after the termination of the set-ups.

RESULTS AND DISCUSSIONS

Mosquito eggs collected from the surface of the lentic water bodies, shallow pools, phytotelmata were examine to determine the species which is belongs to *Aedes* genus. The egg were laid on the water surface observed that the typical behaviour of the *Aedes* group. The egg were collected and observed under the microscope that the collected eggs had floaters. These floaters are the air floaters and also unpaired, these were the characteristic of an *Aedes*group.The larvae that developed into pupa in the experiment were photo-documented. In describing the morphology of the pupa, the most distinctive feature given focus was its head.

Monitoring of pH

The pH of the all containers were measured and recorded by pH meter. And all the recorded data presented in the below table and graphed. The mean average of all pH treatments slightly decreased at the end of the experiment due to the development and growth of the larvae.

Monitoring of Temperature and Light

The temperatures of the lab set-ups were monitored daily and the temperature was measured before the lights turning on and after turning it off. The temperature basically maintained between 25□ to 35□. Light intensity was

recorded using the light meter and the lights were turned on for 8 hours. The average temperatures of the set-up was initially 26.8□ and final is 34.1□.

Viability

In pH 4 most of the larvae were failed to metamorphose to the second instar. Basically in the term of mortality from the second day, the larvae across all the pH treatments from the experiment gradually decreased. One Way ANOVA was calculate to compare the daily viability of the mosquito larvae across the pH levels in the experiment. The results show that there is a significant difference in the viability of the mosquito larvae at different pH levels in the experiment. The F value is 9.788 and p value is 0.00021. A Post Hoc analysis was conducted and the results show that the viability of the larvae atpH 4 was significantly different from the viability of the larvae at pH 7 and 9 comparatively.

The temperature of the experimental set-up provided by the halogen lamp could also possibly contribute to the high mortality of the larvae, hence affecting its viability. Bayoh and Lindsay (2004) in their study entitled Temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito *Anopheles gambiae*, showed that larval mortality was higher when temperature was high, from 30 to 33□. Couret et al. (2014) reported the same. He found that high mortality of mosquito larvae occurred at 30□; it was lowest at temperature 25□.

CONCLUSIONS

From the end of the day 2 total number of the living larvae starts decreasing eventually according to the tolerance of the pHand temperature. The mortality rate was higher in pH 4 than the other pH levels. Due to the low mortality rate very few number of larvae were able to pupate on 7th and 8th day. The morphology of larva and pupa basically the head part was affected due to the different pH levels pH 4, pH 7, pH 9.

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Table 1.The pH measurements of the experiment.

Experiment(Days)	pH 4	pH 7	pH 9
Day-1	4.34	7.18	9.22
Day-2	4.32	7.15	9.11
Day-3	4.30	7.12	9.03
Day-4	4.24	7.07	8.85
Day-5	4.22	7.01	8.71
Day-6	4.20	6.97	8.63
Day-7	4.16	6.89	8.48
Day-8	4.14	6.82	8.30
Mean	4.24	7.02	8.79
Standard Deviation	0.069282	0.120104	0.299311

Table 2.The temperature measurement of the experiment.

Days	Temperature (Initial) in □	Temperature(Final) in □
Day-1	28.3	34.7
Day-2	27.5	32.1
Day-3	27.1	35.1
Day-4	26.7	33.8
Day-5	25.4	33.5
Day-6	25.1	33.9
Day-7	26.8	34.2
Day-8	27.9	35.5
Mean	26.8	34.1
Standard Deviation	1.241974	0.988685

Table 3.% of daily viability and mortality rate in the experiment.

DAYS	pH 4 viability (%)	pH 4 mortality (%)	pH 7 viability (%)	pH 7 mortality (%)	pH 9 viability (%)	pH 9 mortality (%)
1	0	100	0	100	0	100
2	12	88	32	68	24	76
3	16	84	48	52	36	64
4	24	76	48	52	40	60
5	24	76	56	44	44	56
6	28	72	56	44	44	56
7	28	72	60	40	48	52

Fig 1.Collection of egg sample directly from the swampy canals of CUTM campus.

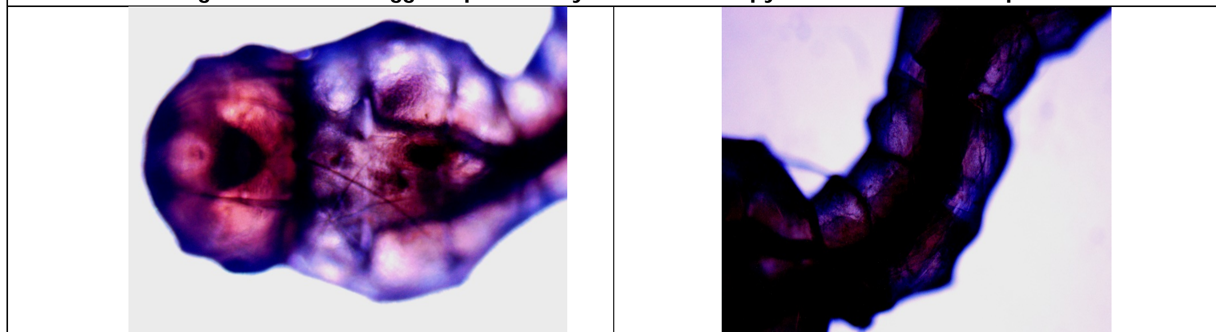

Fig 2.Head and body segments of2nd day of larval stage.

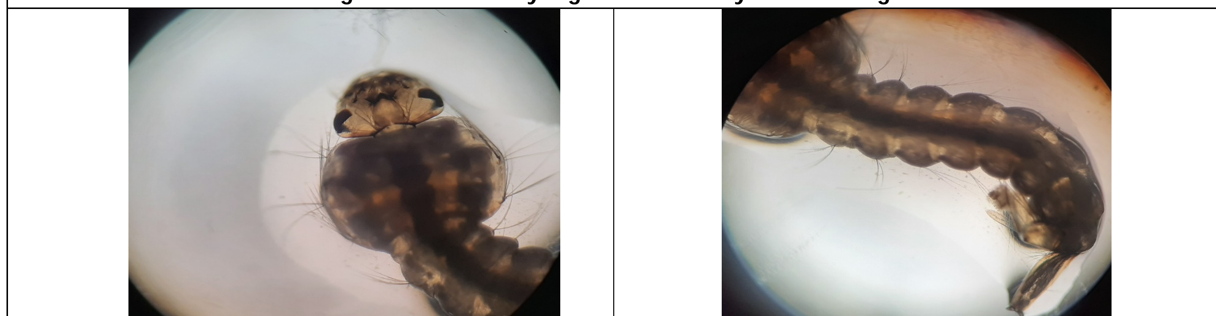

Fig 3. Head and body segments of 3rd day of larval stage

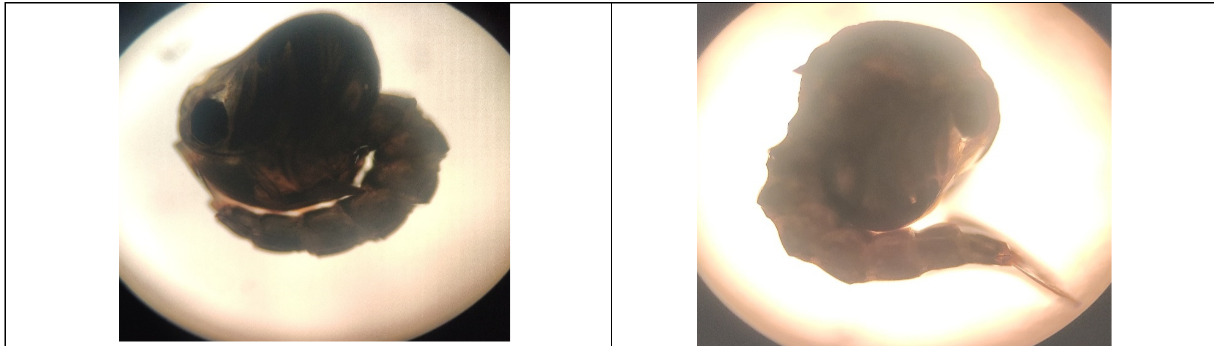

Fig 5.1st day and 2nd day of Pupa condition.

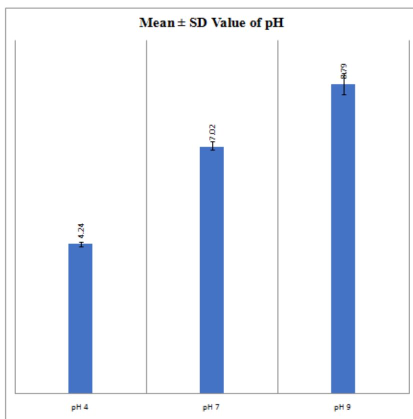

Fig 6.The pH measurements of the experiment.

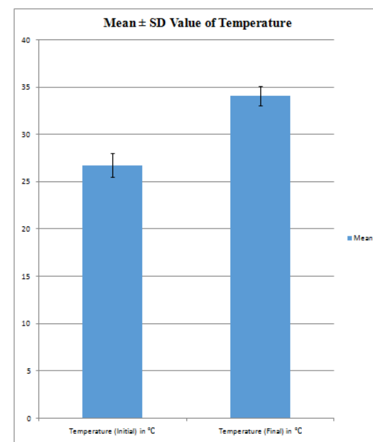

Fig 7. The temperature measurement of the experiment.

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Quantifying Variation of Soil Arthropods in Relation to Edaphic and Climatic Factors in Paddy Field of Cachar, Assam

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Received: 11 Jan 2020 Revised: 13 Feb 2020 Accepted: 16 Mar 2020

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ABSTRACT

Fauna inhabiting in soil plays numerous roles such as the organic matter decomposition, humus formation, and nutrient cycling. Moreover, they are also responsible for altering the soil structure and improving the fertility of the soil. In this experiment, an attempt has been made to assess the extent to which the local microclimatic conditions and edaphic parameters influence the population of soil-dwelling microarthropods. Soil samples were randomly collected on a monthly basis from the study sites over a period of one year (April 2016 to March 2017). A standard protocol was followed in analyzing the soil parameters while meteorological parameters were procured from the nearby tea estates. Results revealed that out of the 15 extracted arthropods groups, Oribatid mites were the major contributors representing almost one third (33.29%) of the population followed by Collembola (32.09%). The population exhibits a trend of fluctuation with a maximum in monsoon season and a minimum in the winter period. Out of the variables (climatic and edaphic) analyzed, only the soil moisture content showed a positive and significant correlation while other parameters revealed a weak positive correlation. These findings provide evidence of the close interaction between the edaphic parameters and the soil-dwelling microarthropod population.

Keywords: CCA, Collembola, Decomposition, Microarthropods, Oribatid.

INTRODUCTION

Soil is one of the most important reservoirs of biodiversity where both the biotic and abiotic components interact closely to maintain the different ecological functions such as the degradation of organic matter and nutrient recycling (1). Besides, it is an important habitation for different groups of mesofauna such as mites, collembola, protura,

nematodes, etc. (2) that performs a major role in various ecosystem services (3). In soil, they are the chief component of the food web (4), and their presence, checks on the distribution, porosity, aeration, and infiltration of soil organic substances (5). The sensitivity of soil-dwelling arthropods towards the changes in the surrounding environment is very rapid (6) and thereby considered them as an excellent bio-indicator of the soil health (7, 8, 9, 10, 11, 12).

In the modern era, due to the choices of advance agricultural practices such as the use of chemical fertilization and pesticides; the soil health is constantly being deteriorating and in the longer run, it affects the population and abundance of soil faunal communities (13). However, the changes in the abundance and diversity of microarthropods might be influenced by several other factors namely soil organic matter (14), moisture content, pH, temperature, etc (15). As that of edaphic factors, meteorological parameters also influence the arthropods population in different agroecosystems (16). In this context, the present study puts forward a simple conceptual approach to shed some light on the value of biodiversity in the soil and their association with soil and climatic factors. Hence, we examine the density of the arthropods and also assess the effects of various meteorological and soil parameters on the soil-inhabiting arthropods in the Cachar district of North-east India.

MATERIALS AND METHODS

The study was carried out in paddy fields located in the Cachar district of Assam which is considered as a part of the biodiversity hotspot of N.E. India. The district covers an area of around 3786 km^2 and lies between 92° 15' E and 93° 15' E longitude and 24° 8' N and 25° 8' N latitude. Subtropical warm humid climate with an average rainfall of 2660 mm (May to September) prevails in the study site. The soil texture of the study area was sandy loam with a mean bulk density of 0.83 g cm^{-3}.

Soil sampling

Soil sampling is one of the important phases for the arthropods extraction and soil analyses purposes. In the study site, soil samples were collected for one year (April 2016-March 2017) at monthly intervals. Samplings were done in the morning hours between 8.00 am-9.00 am from a depth of 0-10 cm. A simple random sampling technique applied to collect the soil sample using a standard soil auger (2.5 cm in diameter). On each sampling, a total of 10 replicates were collected for the extraction purpose whereas 5 replicates were mixed to prepare the composite soil mixture for further physicochemical analyses.

Extraction and identification of soil arthropods

Arthropods were extracted from the collected soil samples by using the modified BerleseTullgren funnel apparatus under 25W of electric bulb. The samples were kept in the apparatus for more than 72 hrs, depending upon the moisture content of the soil (17). All the collected arthropods were kept in vials containing 75% alcohol and later identified with the help of a stereoscopic binocular microscope (10x X 40x).

Physico-chemical analyses of soil

Prior to the analyses, all the soil samples were air-dried and passed through a 2mm sieve except in case of soil moisture and temperature measurement. For the determination of moisture content, the oven-dry method was followed whereas temperature was measured directly by inserting the digital soil thermometer at a depth of 0-10 cm. Other parameters such as organic carbon content were calculated by Walkley and Black's rapid titration method (18) while pH (1:2.5 soil-water suspensions) was measured with the help of digital pH meter (19).

Meteorological data

The monthly climatic data viz., rainfall, atmospheric temperature, and relative humidity were procured from Silcoorie Tocklai Tea Estate during the study period (April 2016-March 2017).

Statistical analyses

Population density of the soil arthropods was calculated in MS Office Excel 2007 while regression analyses were done using the SPSS 13.0 software with significance defined at $p<0.05$. The multivariate analysis, CCA was performed using the CANACO 4.5 to establish the relationship between the arthropods population along with the meteorological and edaphic parameters.

RESULTS AND DISCUSSION

Contribution of the extracted arthropods groups

A total of 15 groups of arthropods were extracted during the sampling period and out of those groups, Oribatid mites were the most dominant ones representing almost one third (33.29%) of the population sampled throughout the year followed by Collembola (32.09%). The dominancy of Oribatid mites among the soil microarthropods was also extensively reported from different ecosystems (20, 21, 22, 23). Their dominancy was linked with the spacious range of feeding habits as they rely on both the living and dead organic materials (24, 25). Moreover, Fujikawa (26) explained that the oribatid mites also have a higher resistant capacity to different climatic conditions. Pie chart reflecting the percent contribution of soil arthropods was depicted in Fig.1.

Monthly variation in the population density

Soil arthropod populations display a perceptible monthly variation and are related mainly to the local precipitation and temperature. The results showed that the population exhibited a monthly fluctuation reaching a peak density in July 2016 ($3.61x100^2$ No./m^2) and August 2016 ($3.40x100^2$ No./m^2), followed by a steady decline to a minimum level in November 2016 ($0.37x100^2$ No./m^2) (Fig. 2). There was a season to season variation in the population density and a remarkable trend of decline in the post-monsoon season. From September onwards the density showed a trend of decrease and finally reached its minimum level in the month of November. Zhu et al (27) reported similar findings of arthropods abundance in the mid of monsoon season. A similar trend of soil arthropod abundance was also followed in the study of Zhimomi et al (28) in a rice ecosystem. The study revealed that the arthropod abundance was recorded maximum during the months of May to September and the minimum was in the months of December and January. Various meteorological factors such as rainfall, relative humidity, and temperature play a prime role in changing their abundance pattern at the soil surface (29). Moreover, Levings and Windsor (30) suggested that the abiotic factors were mainly responsible for changing the seasonal abundance of arthropod populations. The arrival of the monsoon season, in turn, escalating the soil moisture content and exhibited an extensive growth of soil invertebrate population (31). Likewise, the alternation in the precipitation pattern could be responsible for the seasonal variation in soil arthropods community (32).

In this study, an attempt has been made to set up a relationship between the climatic variables with the population density. The mean values of the climatic variables were depicted in Table (1). The arthropod population showed a weak positive correlation with the rainfall ($r=0.36$, $p>0.05$), atmospheric temperature ($r=0.36$, $p>0.05$), and relative humidity ($r=0.21$, $p>0.05$) without any significant effect (Table 2). A similar relation of atmospheric temperature and relative humidity with the soil microarthropods without any significant effect was reported by Ray and Pator (33) in the agricultural field and forest ecosystem, respectively.

Physico-chemical parameters of soil samples and their correlation with microarthropods

The climatic condition and seasonal changes could alter the variables of edaphic factors. For the study site, mean values with standard errors of the soil parameters were presented in Table (3). During the study period, soil moisture content showed a range of variation from 17.97% to 29.13% while soil temperature ranged between 19.8°C-29.1°C. The pH of all the soil samples was acidic and did not show any wide range of variation i.e., 4.68 to 5.15. However, the percentage of organic carbon content varied between 0.24%-1.5%. The study also focused on the effect of these soil parameters on the arthropod population. Here, it revealed that the soil moisture content ($r=0.75$, $p<0.01$) strongly showed a significant positive correlation with the arthropod population while the remaining three variables viz., temperature ($r=0.12$, $p>0.05$), organic carbon ($r=0.27$, $p>0.05$) and pH ($r=0.17$, $p>0.05$) had a positive relationship without showing any significant effect (Table 2). The soil moisture content also exhibited significant correlations with the population in earlier studies (34, 35, 36, 37). It is considered as the foremost factor for the fluctuation of the microarthropod population (38) and their increase in the soil enhances the microarthropod densities (39).

Multivariate analysis

The effect of the different soil and climatic factors on the population has clearly visualized the picture in the CCA (Canonical Correspondence Analysis) diagram. In the CCA ordination diagram, the environmental variables were represented as arrows whereas the arthropod groups marked by triangle shape. The arthropod groups along with its code name depicted in the CCA ordination graph were shown in Table (4).

CCA on the relationship of soil arthropods with the climatic variables

Here in the CCA plot, considering the length of the arrows; atmospheric temperature recognized as the most important factor as compared to the other two variables for the soil-inhabiting arthropods. Axis 1 mainly correlated with the relative humidity whereas axis 2 correlated with the rainfall and atmospheric temperature. The CCA revealed that the Oribatid mites and Hymenoptera were in close association with the relative humidity while the Mesostigmatid mites, Coleoptera, Diptera, and Protura showed correspondence towards atmospheric temperature and rainfall. The result was similar to the findings of Shakir and Ahmed (40) who reported that the Collembola, Acarina, and Hymenoptera showed correspondence towards relative humidity. However, a negative association was observed between microarthropod groups (Collembola, Prostigmatid mites, Diplura) and climatic factors (atmospheric temperature, rainfall) (Fig. 3).

CCA on the relationship of soil arthropods with the edaphic parameters

CCA ordination graph in Fig. 4 illustrating the pattern of relationship of arthropod groups with the soil parameters. The ordination diagram revealed that the soil temperature, pH, and moisture content were associated with the arthropod groups while organic carbon was the least weighted parameter to show correspondence with the population. Here in the graph, axis 1 mainly correlated with the soil temperature whereas axis 2 correlated with the soil pH. Oribatid mites and Diptera were in close association with the soil temperature while Mesostigmatid mites, Hymenoptera and Protura showed correspondence towards soil moisture. A contradictory result of the negative correlation of soil moisture with Hymenoptera was reported by Duyar and Makineci (41). In the CCA diagram, microarthropods groups such as Collembola, Hemiptera, and Diplura were placed opposite to the soil moisture content and showed a negative correlation.

CONCLUSION

In the study sites, various meteorological and edaphic variables prevailed that might control the size of the arthropod population. Here, it revealed that the edaphic parameters specifically the soil moisture content exert a significant effect on the monthly variation of soil arthropods. However, the meteorological parameters being an integral part of the habitat have not exhibited any significant influence on the soil-dwelling arthropods population. Although the moisture plays a key role in the studied sites, the impact of the other parameters cannot be ignored. As the duration of the present study was one year only so it's not convenient to draw a conclusion. Keeping in mind these facts, long-term monitoring is necessary to scrutinize and fully understand the impact of different seasons and agricultural practices; especially the effect of the tillage practices on the soil arthropods density and community structure for the conservation of soil fauna, as well as, for the evaluation of the quality of the soil.

ACKNOWLEDGMENT

The authors thankfully acknowledge the Head, Department of Ecology and Environmental Science, Assam University, Silchar, India for providing the laboratory facilities and also thankful to University Grant Commission, Govt. of India for the Basic Scientific Research fellowship.

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Table 1. Monthly mean value of the climatic variables during the study period (April 2016-March 2017)

Months	Rainfall (mm)	Atmospheric temperature (°C)	Relative humidity (%)
April, 2016	13.04	26.13	84.68
May, 2016	15.96	27.29	84.06
June, 2016	9.62	29.63	89.26
July, 2016	14.65	29.53	88.5
August, 2016	7.85	30.22	88.17
September, 2016	17.06	29.14	87.73
October, 2016	5.16	28.82	86.62
November, 2016	2.27	24.67	86.03
December, 2016	0.35	22.08	83.92
January, 2017	0.00	20.03	80.58
February, 2017	2.15	22.15	75.75
March, 2017	9.51	23.83	89.87

Table 2. Correlation and regression values of different soil and climatic factors in relation to arthropods population in paddy field

Parameters	r value	p value	Regression equation
Moisture	0.75	0.006**	y = 0.2697x - 4.4581
Temperature	0.12	0.733	y = 0.0351x + 0.6957
pH	0.17	0.612	y = 1.2507x - 4.5887
Organic carbon	0.27	0.405	y = 0.7407x + 0.964
Rainfall	0.36	0.253	y = 0.0641x + 1.0816
Atmospheric temperature	0.33	0.299	y = 0.1031x - 1.0892
Relative humidity	0.21	0.515	y = 0.0568x - 3.2475

a) Values are significant at $p < 0.01$ (**)

Table 3. Monthly variations of edaphic variables (mean ± standard error) in paddy field during the study period from April 2016-March 2017

Months	Moisture (%)	Temperature (°C)	pH	Organic carbon (%)
April, 2016	24.42±0.06	25.5±0.12	4.75±0.04	0.92±0.02
May, 2016	22.80±1.53	28.6±0.18	5.05±0.01	0.76±0.01
June, 2016	21.62±0.18	28.8±0.06	4.68±0.06	0.71±0.02
July, 2016	26.02±0.07	29.1±0.17	4.94±0.07	1.01±0.01
August, 2016	29.13±0.04	28.3±0.12	5.02±0.02	1.46±0.04
September, 2016	22.80±0.25	28.2±0.07	5.07±0.02	0.98±0.02
October, 2016	17.97±0.63	28.9±0.12	5.15±0.03	0.24±0.01
November, 2016	19.18±1.11	27.1±0.03	4.83±0.02	1.5±0.03
December, 2016	22.50±0.47	22.3±0.09	5.09±0.04	0.9±0.04
January, 2017	19.90±0.46	19.8±0.03	5.00±0.06	1.08±0.03
February, 2017	21.16±0.32	21.1±0.07	4.98±0.03	0.32±0.04
March, 2017	22.16±0.10	22.4±0.07	4.86±0.05	0.48±0.12

Table 4. List of groups and their code name used in CCA ordination diagram

Sl. No.	Groups	Code name
1	Oribatid mites	Ori
2	Collembola	Col
3	Mesostigmatid mites	Meso
4	Prostigmatid mites	Pro
5	Diptera	Dip
6	Hymenoptera	Hym
7	Diplura	Diplu
8	Protura	Protu
9	Chilopoda	Chilo
10	Diplopoda	Diplo
11	Isopoda	Iso
12	Spider	Spi
13	Hemiptera	Hem
14	Thysanoptera	Thy
15	Coleoptera	Coleo

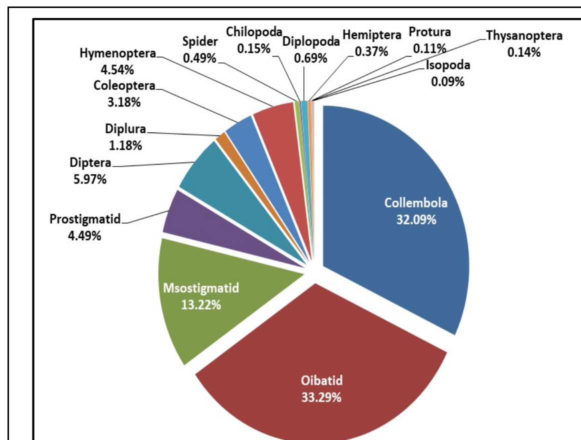

Fig. 1 Percent contribution of the extracted soil arthropod groups in paddy field during the study period from April 2016 to March 2017.

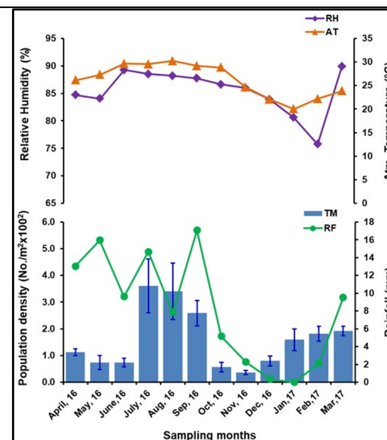

Fig. 2 Monthly variation in the population density of soil arthropods in the study sites (April 2016-March 2017). RF-Rainfall, AT-Atmospheric temperature, RH-Relative humidity

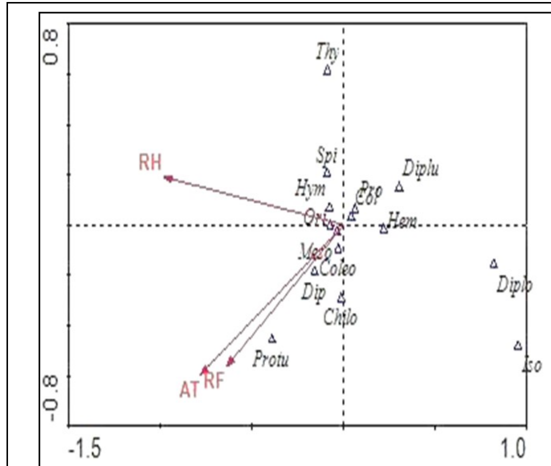

Fig. 3 Canonical correspondence analysis (CCA) between the arthropod groups with the climatic variables in the study sites. RF-Rainfall, AT-Atmospheric temperature, RH-Relative humidity, Ori-Oribatid mites, Col-Collembola, Meso-Mesostigmatid mites, Pro-Prostigmatid mites, Dip-Diptera, Hym-Hymenoptera, Diplu-Diplura, Protu-Protura, Chilo-Chilopoda, Diplo-Diplopoda, Iso-Isopoda, Spi-Spider, Pseu- Pseudoscorpion, Hem-Hemiptera, Thy-Thysanoptera, Coleo-Coleoptera

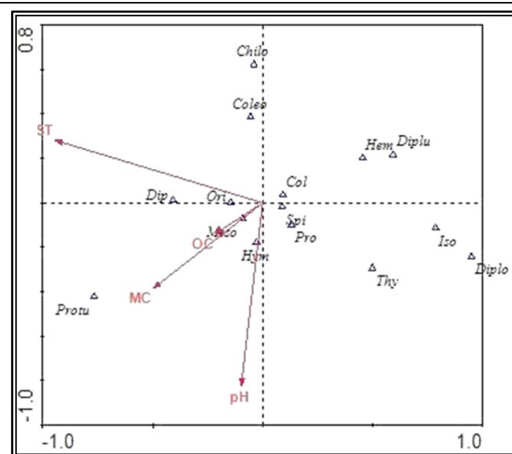

Fig. 4 Canonical correspondence analysis (CCA) biplot ordination graph between the arthropod groups with the edaphic parameters in the paddy field. MC-Moisture content, ST-Soil temperature, OC-Organic carbon, pH-Soil pH, Ori-Oribatid mites, Col-Collembola, Meso-Mesostigmatid mites, Pro-Prostigmatid mites, Dip-Diptera, Hym-Hymenoptera, Diplu-Diplura, Protu-Protura, Chilo-Chilopoda, Diplo-Diplopoda, Iso-Isopoda, Spi-Spider, Pseu-Pseudoscorpion, Hem-Hemiptera, Thy-Thysanoptera, Coleo-Coleoptera

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Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Plant Sources used for Celebrating Hindu Ritual "Makar Sankranti"- The Uttarayan Movement of Sun

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Received: 11 Jan 2020 Revised: 13 Feb 2020 Accepted: 16 Mar 2020

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ABSTRACT

Biodiversity is the most complex feature of our planet that gives vital information to the humanity. Plants play an important role in the human life. Among all the religions, Hinduism is the planet's oldest and original religion as it consists of many cultures. Makar Sankranti is one of the ancient Indian festivals in the Hindu calendar which is dedicated to the deity Surya. According to the Hindu tradition, Hindu people use most of the plant species for worshiping the Gods and Goddess. It has been found that in Makar Sankranti most of the plants and plant parts are used for worshiping. The data about the significance of plant species, use of the plants and plant parts for worshiping were collected through the interaction between the priests and some other knowledge's by the women while worshiping. The plant species with their botanical names, local names, family, habitat, parts used were collected in the written form. A total 24 plants belong to 19 families are recorded during the study.

Key words: Biodiversity, Gods, Hinduism, Makar Sankranti, Plant.

INTRODUCTION

The Hindu is one of the largest and oldest religions on the globe. According to the Hindu tradition, Hindu people use a large number of plants and the plant parts for worshiping to the God and Goddesses (Das et al., 2019). The importance of worshiping God and Goddess has been described in different Vedas. This information is transmitted to generation to generation. The traditional festivals and occasions ceremony literatures regarding Hindu beliefs and worship is insignificant and proper scientific study in this area is very poor (Dalasingh et al., 2018). Makar Sankranti is one of the great festivals in the Hindu religion. Makar Sankranti is a festival dedicated to the deity Surya (Sun). It is

celebrated all over the country with different names like in Tamil Nadu it is celebrated as Pongal, Magh Bihu in Assam, Uttrayan in Gujarat and so on. People donate wheat and sweets on Makar Sankranti and it believed that it brings prosperity and happiness (Anonymous, 2019). Flying kites are seen in the sky, it is done to please the Sun God.

MATERIALS AND METHODS

Study on the documentation of the plants and plant parts are used in Makar Sankranti in different villages, areas and cities carried out during Makar Sankranti. The data were recorded after the significance of the plant species, use of plants and plant parts used for worshiping and those ideas were collected through the interaction between the priests and some other knowledge's by the women while worshiping. The botanical names of plants are documented and identified with the help of "The Flora of Odisha"(Saxena and Brahmam, 1994) and by the help of Department of Botany, Centurion University of Technology and Management, Odisha, India. Finally, the plant specimen was handover to the Department of Botany, Centurion University of Technology and Management, Odisha, India.

RESULT AND DISCUSSION

The total numbers of 24 plant species under genera and families were collected during the study of plants and plant parts used in Makar Sankranti in different areas, cities and villages. The information about the plant species, uses of plants and plant part used in Makar Sankranti for worshiping. The data were collected through the interaction with the specialist (Bhhatla et al., 1984). During the survey 24 plant belong to 19 families were is recorded and collected. The main plant which is used for worshiping is rice i.e. *Oryza sativa*. Details of the recorded 24 plant species in the term of their botanical names, local name, family, habitat, plant parts used and the form of use are given in Table 1.

CONCLUSION

The study of the religious pants and plant parts used in Makar Sankranti demonstrates the importance of plants in day today life of human. The present study helps us to understand how a hindu aboriginal community of Odisha contributing toward the conservation of plants and forest in their own interest in safeguarding their inherent social-cultural and religious activities. Such moments of monitoring and utilizing plant species for the sake of worshipping and socio-social convictions uncovers a solid immensity in the present worry of biodiversity preservation.

ACKNOWLEDGEMENTS

The authors are thankful to villagers, women and priest who have given the information about this work and kind of support. Special thanks to the women, those who were worshiping, Meenakshi Pradhan and her co-members for their valuable help and co-operation during conducting this work.

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Table 1. Lists of the plants and plant parts used in Makar Sankranti for worshiping in Odisha, India

Sl. No	Botanical name	Local name	Family	Habitat	Part used	Uses
1.	*Aegle marmelos* (L.) Corr.	Bela	Rutaceae	Tree	Leaves	Worshipping
2.	*Cynodont dactylon* (L.) Pers.	Duba	Poaceae	Grass	Leaves	Worshipping
3.	*Ziziphus mauritiana* Lam.	Barakoli	Rhamnaceae	Tree	Leaves	Worshipping
4.	*Datura metel* L.	Dutura	Solanaceae	Shrub	Leaves	Worshipping
5.	*Ocimum sanctum* L.	Tulsi	Lamiaceae	Herb	Leaves	Worshipping
6.	*Musa sapientum* L.	Kadali	Musaceae	Tree	Leaves	Worshipping, Prasad
7.	*Mangifera indica* L.	Amba	Anacardiaceae	Tree	Leaves	Worshipping
8.	*Piper betel* L.	Pana	Piperaceae	Climber	Leaves	Worshipping
9.	*Tabernaemontana divaricata* (L.) R.Br. ex Roem. & Schult.	Tagara	Apocyanaceae	Shrub	Flower	Garland
10.	*Tagetes erecta* (L).	Gendu	Asteraceae	Herb	Flower	Garland
11.	*Cascabela thevetia* (L.) Lppold	Kaniyari	Apocyanaceae	Shrub	Flower	Garland
12.	*Hibiscus rosa-sinensis* L.	Mandara	Malvaceae	Shrub	Flower	Garland
13.	*Michelia champaca* L.	Champa	Magnoliaceae	Tree	Flower	Garland
14.	*Nerium oleander L.*	Karvira	Apocynaceae	Small tree	Flower	Garland
15.	*Areca catechu* L.	Gua	Arecaceae	Tree	Fruit	Worshipping
16.	*Santalum album* L.	Chandan	Santalaceae	Tree	Wood	Worshipping
17.	*Oryza sativa* L.	Dhana	Poaceae	Grass	Seed	Worshipping, prasad
18.	*Cucumis sativus* L.	Kakudi	Cucurbitaceae	Climber	Fruit	Prasad
19.	*Pyrus malus* L.	Seo	Rosaceae	Tree	Fruit	Prasad
20.	*Vitis vinifera* L.	Angura	Vitaceae	Climber	Fruit	Prasad
21.	*Psidium guajava L.*	Pijuli	Myrtaceae	Tree	Fruit	Prasad
22.	*Piper nigrum* L.	Golamaricha	Piperaceae	Climber	Fruit	Prasad
23.	*Elettaria cardamomum* L.	Gujurati	Zingiberaceae	Herb	Fruit	Prasad
24.	*Cocos nusifera* L.	Nadia	Arecaceae	Tree	Fruit	Prasad

Fig. 1. Data collection through the survey and interaction with Priests and villegers.

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of Ultrasonic Parameters in Binary Solution of Dextran and Urea at Various Concentration and Temperatures

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Received: 13 Jan 2020 Revised: 15 Feb 2020 Accepted: 17 Mar 2020

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ABSTRACT

In the present investigation ultrasonic speed (U), viscosity (η) and density (ρ) have been estimated at frequency 1 MHz in the solution of urea with dextran in the concentration range 0.1% to 1 % at five different temperatures 303 K, 308 K, 313 K, 318 K and 323 K utilizing ultrasonic interferometer. The measured value of above data have been utilized to calculate the acoustic parameters namely free volume (V_f), internal pressure (πi), attenuation coefficient or absorption coefficient (α), Rao's constant (R) and Wada's constant (W) with a view to investigating the nature and quality of interaction in the binary solution of dextran and 6(M) urea. The molecular interactions like hydrogen bonding, dipole-dipole association, and electrostriction have been analyzed on the basis of these parameters.

Keywords: Dextran. Density, viscosity, and ultrasonic speed.

INTRODUCTION

The measurement of ultrasonic speed with other data like, density and viscosity has been used to explore the different types of molecular interaction, structural changes in pure liquid as well as liquid mixture. The information on the nature, strength and order of molecular interactions can be obtained from the related thermo acoustic parameters such as free volume (V_f), internal pressure (π_i), absorption coefficient (α), Rao's constant (R) and Wada's constant (W) These data are essential for process design and to explore the potential applications in various needs.The study of molecular interaction in urea would be of great interest in potential agricultural applications.

Dextran, a water soluble polymer, has occupied a separate area of investigations by researchers [1-2]. Due to its various applications in pharmaceutical, health care and food industries, it is important to understand the structure and molecular interaction of dextran. It is of interest to study the physico-chemical properties [3] of the polymer dextran in solvent Urea. Ultrasonic method is a versatile nondestructive technique and highly useful for investigation

of various acoustic properties of mixtures at different temperatures [4-5]. In the present investigation, we have studied important ultrasonic parameters like V_f, π_i, α, R and W of dextran solutions at five different concentrations and five different temperatures. The results have been discussed in the light of dextran-ureainteraction and structural change of the dextran and urea in solution. Dextran is the collective term given to a group of bacterial polyglucan composed of chains of D –glucose units connected by alpha – (1 -6) linkages. It is usedwidely in the manufacture of blood plasma expanders [6-7].Urea is an organic compound with chemical formula NH_2 CO NH_2 and widely used in the health care, fertilizers and industrial sector. In view of the importance of dextran and urea, a systematic study of it with urea has been undertaken in the present study.

Experimental Section

Materials andmethods adopted are the same as reported in mypreviouspaper [8].

Theoretical Aspect

The basic parameters U, η, ϱ were measured at various concentration and temperatures. The various acoustical parameters like V_f, π_i, α, R and Wwere calculated from U, η, ϱ value using standard formulae [9].

RESULTS AND DISCUSSION

The experimental values of ϱ, η and U of 6(M) urea with aqueous dextran and calculated acoustic parameters at temperatures ranging from 303 K to 323 K in 5K interval for 1 MHz frequency in various concentration of dextran (Table1-4; Fig.1-12). The perusal of Table 1 and (figure-1 & 2) clearly reveals that the measured parameters viz., density, viscosity and ultrasonic speed increase with increase in concentration of dextran in urea and temperature of the solution. As concentrationof dextran in urea increases, the quantity of particles in the medium builds, making the medium denser, this indications to lesser compressibility and hence sound speed increases. Further, the increase in the quantity of particles simply increases the frictional resistance between the layers of the medium and that likely to increase the η for a given temperature. Moreover, the existing particle–particle frictional resistance expects some interactions [10].

It is seen that $V_f$decreases (figure-4) and $\pi_i$rises (figure-6) withrise in concentration (volume in %) of dextran in urea, indicating the better association of the molecules of dextran in urea. The diminution in $V_f$with rise in concentration focus that the particles organize themselves so that the void space is less accessible demonstrating a reduction of compressibility [11]. When the concentration of dextran rises, $\pi_i$increases (figure.6) andV_f(figure 4) has indicated precisely the opposite pattern with concentration to that of $\pi_i$as expected.The watched increment estimations of $\pi_i$in the framework are because of the nearby association among solute and dissolvable particles. With increased temperature, there is decrease in atomic cooperation as they move away from one another. This diminishes the cohesive force subsequently diminishing the $\pi_i$shown in figure-5. It likewise states to that there is weak interaction between the dextran and ureaparticles at a higher temperature. Therefore $\pi_i$decreases with increase in temperature [12].

Variation in the 'α'is a measure of the spatial rate of reduction in the intensity level of the sound wave. At the pointwhen the concentration of dextran rises, the 'α'builds (figure-8).Further, it diminishes with increment in temperature (figure-7) [13]. Rao's constant and Wada's constant shows an increasingtrend with rise in temperature. Theincreasing trends with temperature suggestthe availability of more number of components in a givenregion thus leading to a close packing of the medium and thereby increasing the strength of interactionsbetween strong dextran and ureainteraction existing in the solution[14-15]. Further, as the concentration increases, progressively mass is accumulated in the components of the mixture rather than size growth.

CONCLUSION

The basic parameter ultrasonic speed, density and viscosity were measured for binary mixture of dextran and 6(M) urea at various concentrations and different temperatures. The various acoustical parameters were calculated from ultrasonic velocity, density and viscosity value using standard formulae. This is a clear indication of intermolecular interactions because of hydrogen bonding among the binary solution of dextran and urea.

ACKNOWLEDGMENTS

The author sincerely thanks Ajay Binay Institute of Technology, Cuttack for their strategic help and encouragement.

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Table.1. Values of 'ϱ'&'η' of dextran with urea solution.

T (K)	Concentration of dextran									
	0.10%		**0.25%**		**0.50%**		**0.75%**		**1%**	
	ρ kg m^{-3}	**η x10^{-3} N s m^{-2}**	**ρ kg m^{-3}**	**ηx10^{-3} N s m^{-2}**	**ρ kg m^{-3}**	**ηx10^{-3} N s m^{-2}**	**ρ kg m^{-3}**	**ηx10^{-3} N s m^{-2}**	**ρ kg m^{-3}**	**ηx10^{-3} N s m^{-2}**
303	1077.26	1.039	1078.00	1.059	1078.75	1.086	1080.04	1.105	1081.00	1.124
308	1075.25	0.965	1076.00	0.990	1076.75	1.036	1078.02	1.054	1079.21	1.073
313	1073.81	0.884	1074.00	0.920	1075.40	0.963	1076.19	0.980	1077.77	0.999
318	1071.38	0.820	1071.75	0.850	1072.57	0.900	1073.00	0.923	1074.54	0.941
323	1067.93	0.750	1068.50	0.783	1069.00	0.834	1069.91	0.851	1071.09	0.868

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Table. 2. Values of 'U' & 'V_f' of dextran with urea solution.

T (K)	U(m/s)					V_f (m^3mol^{-1})				
	0.10%	0.25%	0.50%	0.75%	1%	0.10%	0.25%	0.50%	0.75%	1%
303	1618.50	1622.00	1624.00	1625.00	1627.00	6.940	6.770	6.526	6.365	6.219
308	1623.00	1624.30	1626.00	1627.50	1629.00	7.790	7.503	7.018	6.846	6.678
313	1626.10	1627.00	1627.00	1629.00	1630.00	8.909	8.396	7.846	7.646	7.439
318	1628.20	1629.00	1629.00	1630.00	1631.00	9.985	9.472	8.694	8.373	8.142
323	1631.20	1632.00	1632.00	1633.00	1633.50	11.448	10.740	9.781	9.496	9.215

Table.3. Values of 'π_i' & 'α' of dextran with urea solution

T (K)	π_i (Nm^{-2})					α (npm^{-1})				
	0.10%	0.25%	0.50%	0.75%	1%	0.10%	0.25%	0.50%	0.75%	1%
303	128.85	129.98	131.64	132.84	133.95	9.699	9.833	10.057	10.208	10.344
308	125.87	127.51	130.44	131.63	132.82	8.972	9.186	9.588	9.726	9.869
313	122.21	124.66	127.62	128.78	130.09	8.200	8.524	8.906	9.043	9.190
318	119.34	121.49	125.08	126.69	128.00	7.606	7.872	8.329	8.531	8.674
323	115.57	118.10	121.88	123.15	124.49	6.954	7.248	7.711	7.853	8.001

Table.4. Values of '*R*' & 'W' of dextran with urea solution. .

T (K)	R (10^{-2} $m^3mol^{-1}(m/s)^{-1/3}$)					W (10^{-2}) $m^3mol^{-1}(kg^{-1}ms^2)^{1/7}$				
	0.10%	0.25%	0.50%	0.75%	1%	0.10%	0.25%	0.50%	0.75%	1%
303	108.989	108.993	108.962	108.854	108.802	7.748	7.748	7.746	7.740	7.737
308	109.294	109.247	109.209	109.113	109.027	7.766	7.764	7.761	7.755	7.750
313	109.510	109.511	109.369	109.333	109.195	7.780	7.780	7.771	7.769	7.760
318	109.806	109.786	109.702	109.680	109.545	7.798	7.796	7.791	7.790	7.782
323	110.228	110.187	110.136	110.065	109.954	7.823	7.821	7.818	7.813	7.807

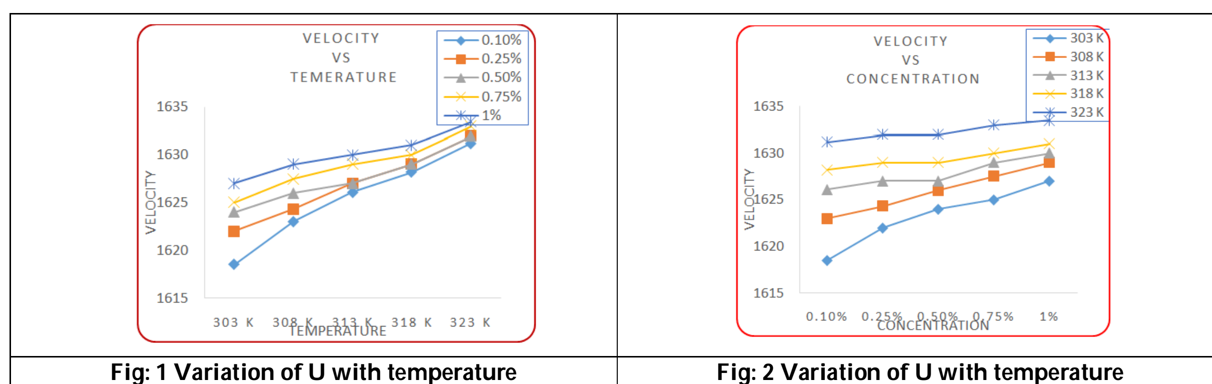

Fig: 1 Variation of U with temperature

Fig: 2 Variation of U with temperature

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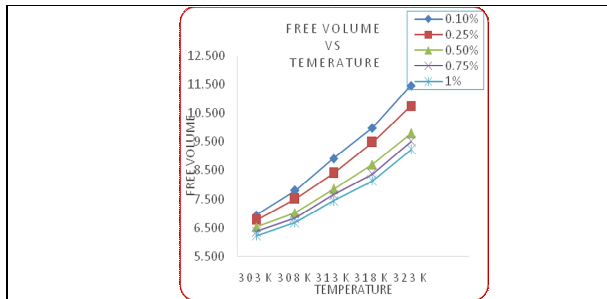

Fig: 3 Variation of V_f with temperature

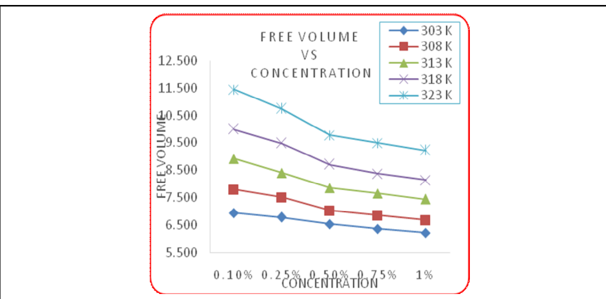

Fig: 4 Variation of V_f with concentrations

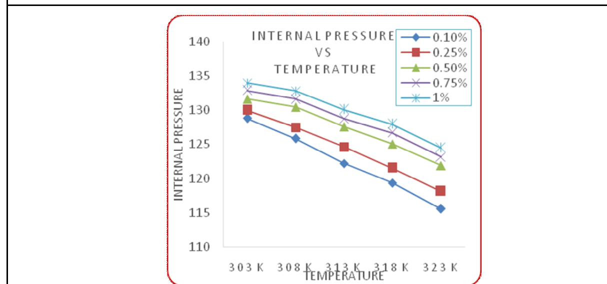

Fig-5 Variation of π_i with temperature

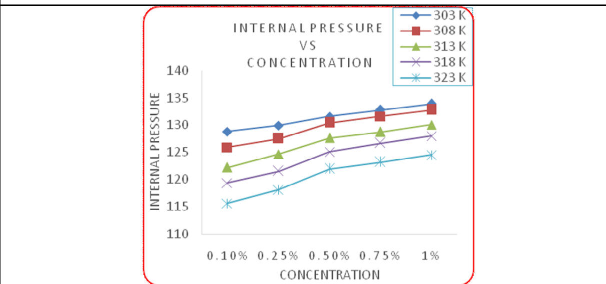

Fig-6 Variation of π_i with concentrations

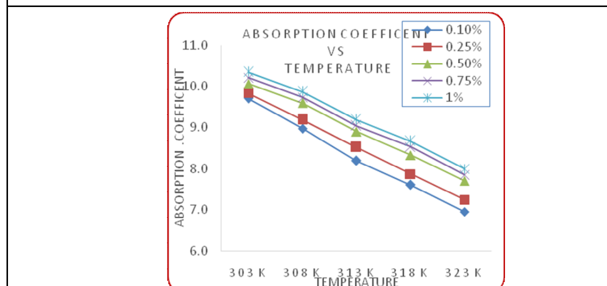

Fig: 7 Variation of α with temperature

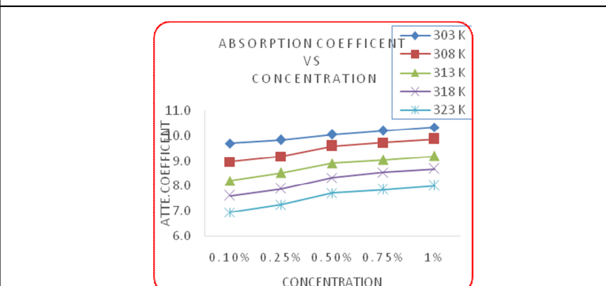

Fig: 8 Variation of α with concentration

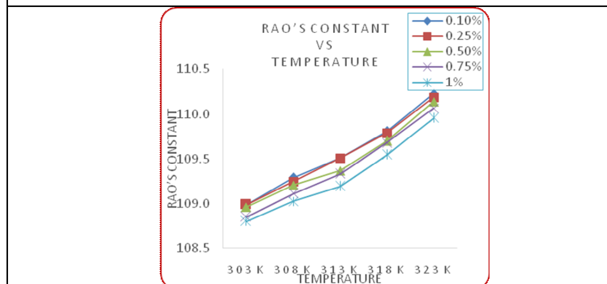

Fig: 9 Variation of R with temperature

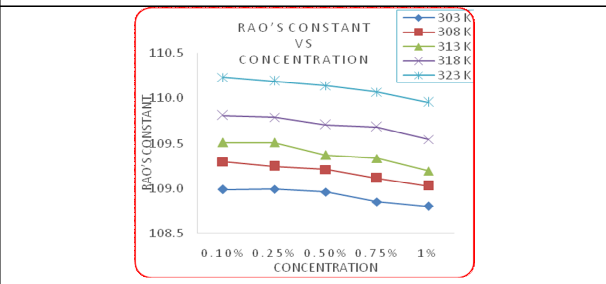

Fig: 10 Variation of R with temperature

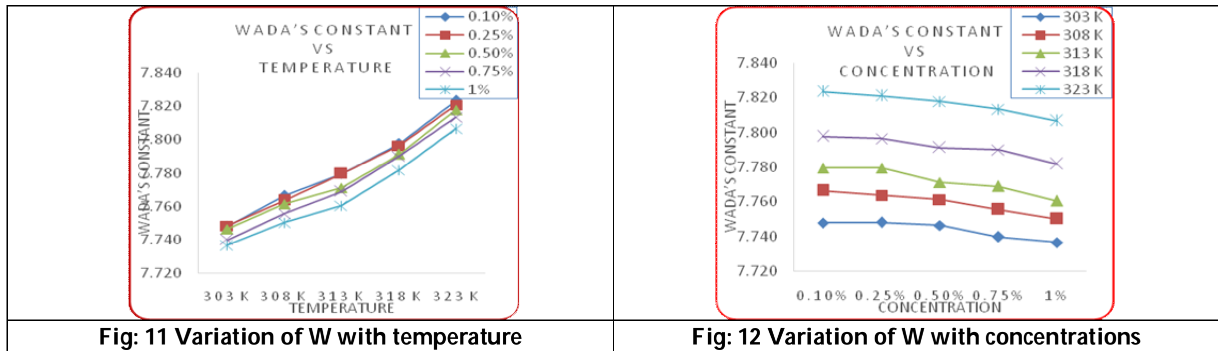

Fig: 11 Variation of W with temperature	Fig: 12 Variation of W with concentrations

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Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Improving of Phosphorus use Efficiency in Acid & Alkaline Soil: a Critical Review Study

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Received: 10 Jan 2020 Revised: 16 Feb 2020 Accepted: 18 Mar 2020

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ABSTRACT

Phosphorus is a secondary essential next to nitrogen, is often the most limiting nutrient for crop and forage production. Major role of phosphorus in a plant is to store and transfer energy produced by photosynthesis for use in development and reproductive processes.Phosphorus presentsin soil as a form of phosphates (PO_4^{3-}), mono-hydrogen phosphate (HPO_4^{2-}), and di-hydrogen phosphate ($H_2PO_4^-$). These anions enthusiastically interconvert, and the predominant species is determined by the pH of the solution or soil. Phosphorus is most available to plants when soil is at pH range between 6.5-7.5. Phosphorus is highly mobile in plants, and when deficient, it may be translocate from old plant tissue to young, actively growing areas. Consequently, early vegetative responses to phosphorus are often observed. As a plant matures, phosphorus is translocating into the fruiting areas of the plant, where high-energy requirements are needed for the formation of seeds and fruit

Keywords: Phosphorus availability, Deficiency, Reclamation.

INTRODUCTION

Phosphorus

Phosphorus is an essential macro-element, which require to photosynthesis, energy transfer and synthesis and breakdown of carbohydrates for plant (Mengel and Kirkby, 1987; Mills and Jones, 1996). The amount of readily available phosphorus is very low compared with the total amount of phosphorus in the soil, less than 20 percent (Lindsay, 1988) unfavorable for agricultural production. According to requirement of crop phosphorus fertilizers

should be applied. Solubility of phosphorus is very low and it is found in soils both in an organic form and an un-organic (mineral) form. There is balance between solid phase phosphorus in soil and soil solution. Plants absorbed phosphorus from soil solution, and maximum amount of soil phosphorus exists in stable chemical complexes, a small amount is available to the plant at any given time.

Availability and fixation in soil:

Soil phosphorus chemistry is different in alkaline and acidic soils. The availability of phosphorus for plant growth is influenced by soil organic matter, pH, and exchangeable and soluble aluminum (Al), iron (Fe), and calcium (Ca) (Ulrich and Schnug, 2013). The availability of Phosphorus in soilmost of the crops soil pH of 6.5 and 7.5. In acidic soils, phosphorus can be dominantly sorbet by Fe/Al ox-ides and hydroxides (Adnan et al., 2003) as well as by clay minerals and form various complexes (Arai and Sparks, 2007). In alkaline soils, phosphorus retention is dominated by precipitation reactions, although phosphorus can also be adsorbed on the surface of Calcium carbonate (Naeem et al., 2013) and clay minerals (Devau et al., 2010) and become unavailable to plants (Arai and Sparks, 2007).

As phosphorus is a very limiting nutrient in both acidic and alkaline soils, continued inputs of high-cost chemical phosphorus fertilizers are required to increase production and maintain plant, more than 90 percent of applied phosphorusremains in the soil as inorganic and organicphosphorus (Nash et al., 2014; Mózner et al., 2012), and this continued input of fertilizer contributes to the deterioration of the environment especially through eutrophication (Song et al., 2017). In contrast, the addition of organic amendments activates Al- phosphorus and Fe- phosphorus in neutral and acidic soils and Ca- phosphorus in alkaline soil (Zhang et al., 2009), causing an increase in the levels of readily available phosphorus for use by plants.

Problems of phosphorus (Acid & alkaline soil)

Acid soils formed because of the results continuous additions of acid-forming fertilizers. Naturally acid soils are usually found within the tropical, by the results of thousands of years excessive weathering of soil minerals. Year-around high temperatures and high rainfall annual precipitation > 600–800 mm leaches all basic cations (such as Na, Ca, Mg, and K) and pH buffering minerals (such as carbonates). Climatic condition give promotes to transform and subsequent leaching of Si from Si-based minerals, leaving acidic iron and aluminum oxides minerals. Saline and sodicsoil is characterized by their electrical conductivity (Ec), soil pH, and exchangeable Na%. When exchangeable Na > 15%, soil aggregates disperse, reducing permeability to water. In saline soils soluble salt concentration restricts the plant growth, though salt tolerance differs with plant species. In sodic soils, excess Na disperses clays and may create nutritional disorders. Generally saline-sodic soils content high both salt and Na.

Types of phosphorus

Soil phosphorus is two typesone organicand another inorganic. Organic phosphorus is found in plant residues, manures and microbial tissues. In soils low organic matter contain about 3% of total phosphorus in the organic form and high-organic-matter content soils contain about 50% or more of their total phosphorus content in the organic form. Inorganic forms of soil phosphorus consist of apatite (the original source of all phosphorus), complexes of iron and aluminum phosphates, and phosphorus absorbed onto clay particles. The solubility of these phosphorus compounds as well as organic phosphorus is extremely low, and only very small amounts of soil phosphorus are in solution at any one time. Most soils contain less than a pound per acre of soluble phosphorus, with some soils containing considerably less.By applicationsatisfactory phosphorus fertilizer and crop management practices, soil solution phosphorus can be replaced quickly enough for optimum crop production.

Phosphorus deficiency

Phosphorus deficiency is harder to diagnose than a deficiency of nitrogen or potassium. Crops usually display no obvious symptoms of phosphorus deficiency aside from a general stunting of the plant during early growth. By the time a visible deficiency is recognized, it's going to be too late to correct in annual crops. Some crops, like corn, tend to point out an abnormal discoloration when phosphorus is deficient. The plants are frequently dark bluish-green in color with leaves and stem pleasant purplish. The degree of purple is influenced by the genetic makeup of the plant, with some hybrids showing much greater discoloration than others. The purplish color is thanks to accumulation of sugars that favors the synthesis of anthocyanin (a purplish-colored pigment), which occurs within the leaves of the plant.

Phosphorus Symptoms

Deficiency symptoms of phosphorus appear on plant stunted growth and leaves are dark purple color, inhibition of flowering and root system improvement. Maximum plants of these symptoms appear when phosphorus concentration in the leaves is below 0.2%. Excess amount of phosphorus is mostly restricts with uptake of other elements, such as iron, manganese and zinc. Over dose of fertilizer with phosphorus is common and farmer apply gratuitously high amounts of phosphorus fertilizers particularly when nitrogen, phosphorus & potassium fertilizers are used.

Phosphorus reclamation

Phosphorus availability in soil is totally depending on soil pH level. If soil is acidic condition then phosphorus react with iron and aluminum, which make complex substance to make phosphorus unavailable and alkaline soil due to presence of excess amount of calcium, magnesium and sodium phosphorus is also become unaccessible.Unavailable phosphorus can be available to plant by application of sufficient amount of lime (calcium hydroxide). That can unlock the phosphorus that was previously unavailable (AdityarupChakravorty 2018). The availability of phosphorus to plants for uptake and utilization is impaired in alkaline and calcareous soil is reduces because of form insoluble calcium phosphate minerals.Adding fertilizer phosphorus at"normal" rates and with conventional methods may not result in optimal yield and crop quality in these soils common in arid and semi-arid regions. Several fertilizer phosphorus management strategies have been found to improve phosphorus nutrition for plants grown in alkaline and calcareous soil.

High amount of phosphorusis requiredfor crops grown in alkaline soil, with increasing of lime content.Concentrated phosphorus fertilizer bandsimprove phosphorus solubility with resulting yield increases, even when applied to crops grown in soil with relativelyhigh soil test phosphorus concentrations. Applying organicallycomplexphosphorus in the formof biosolids or as a mixture of liquid phosphorus and humicsubstancescan alsoenhance phosphorus nutrition and result inyield increases.Application of slowrelease and cationcomplexingspecialty fertilizer phosphorus materials hasalso been shown to effectively increase yields in calcareous soil. In-season applied phosphorus through the irrigation water can deliver phosphorus to plant roots when deficiencies are observed, but the effectiveness and results are less than with phosphorus incorporatedintothe soil. Farmer having the knowledge of proper balance of phosphorus with other nutrients for plant grow which is reduce cost of excess nutrient induceddeficiencies of other nutrients. In somecases, these methods are relatively new and need further refinement with regard to rates, timing, and technique; but all are potential methods for improving phosphorus supply to plants grown in alkaline and calcareous soil (Bryan Hopkins and Jason Ellsworth.2005)

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Phosphorus analysis in soil

Phosphorus test in soil test gives the measurement of availability or supply phosphorus to the soil solution. Phosphorus in soil occurs as orthophosphate in several forms and combination only fraction of total amount present may be available to plant, which is direct relevance in assessing the phosphorus fertility. Phosphorus soil test is actually an index that helps predict the fertilizer requirement of the crop. The recommendations for fertilizer application are determined based on many field tests in many soils and crops. Soil having pH more than 6.5 ,need to follow Olsen method (Olsen et al 1954) and pH less than 6.5 Bray and Kurtz method (Bray and Kurtz 1945).

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Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

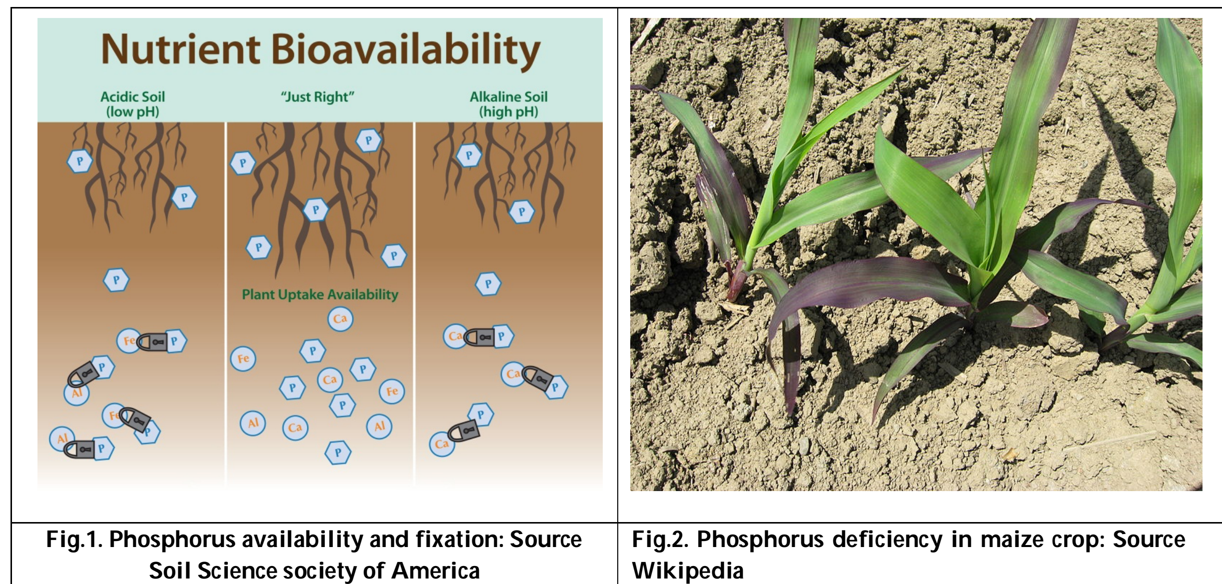

Fig.1. Phosphorus availability and fixation: Source Soil Science society of America	**Fig.2. Phosphorus deficiency in maize crop: Source Wikipedia**

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Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

An Implementation of Enhanced Image Encryption Algorithm by using DRPE-FFT and QR Code for Secure Medical Data in Cloud Storage

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Received: 12 Jan 2020 Revised: 14 Feb 2020 Accepted: 16 Mar 2020

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ABSTRACT

With the development of optical information processing technology, its application is becoming increasingly widespread. The application of optical information processing technology in information security thanks to its high speed, large amount of information and high robustness has become a reference point for research. Image encryption technology is one of the key contents of research in the field of information security. The most representative optical encryption technology is Double Random Phase Encoding (DRPE) based on the 4f optical system. However, there are some shortcomings of DRPE, which are described as follows: The DRPE encryption system is a symmetric system and presents hidden security risks. The encrypted image of the DRPE encryption system is a distributed image of complex value, so it is not easy to record and transmit. The DRPE encryption system requires precise phase alignment of the phase mask, which is complicated and expensive and the system capacity is low. Based on DRPE, this article introduces Arnold's transformation and chaotic system to improve DPRE's nonlinearity and proposes a new type of random double phase. First, the input image is scrambled with the help of Arnold's transformation, and once the image is scrambled, a random double mask is created with the help of the logistics mapping and Chen mapping. Next, get an encrypted image to increase security. After the image has been successfully encrypted, it is converted to a QR code to improve the security of the image file and finally decrypted in the reverse order. Finally, a MATLAB GUI is proposed for encryption and decryption.

Keywords: Optical image encryption, DRPE, FFT, QR Code.

INTRODUCTION

Optical encryption is a typical and effective image encryption method. The essence of optical cryptography is to encrypt the intrinsic information of the image in plain text through optical conversion processes such as interference, diffraction and imaging. During the encryption process, the attributes normally involved include wavelength, focal length, diffraction distance, phase and other attributes. These attributes can be used as multidimensional keys for cryptographic systems. The advantages of optical cryptography are: optical information security technology has the advantages of multi-dimensional, large capacity, high design freedom and high complexity. Refregier proposed the article for the first time in 1995: optical image encryption based on random double coding. This work paved the way for this process through random phase double coding, which has an effective effect on cryptography. Two unrelated random phase models are placed on the input plane and on the Fourier plane to encrypt the image, and then the image on the output plane is extracted as an image of ciphertext.

The specific implementation process is as follows

A lens use the target to convert the plaintext to the frequency domain, process it in the frequency domain first, then convert it to the spatial domain output from another target to get the ciphertext. Two random phase patterns are positioned between the lenses. When the light reaches the posterior focal plane of the lens, it is irradiated to obtain a Fourier spectrum. Figure 1 is a two-dimensional Fourier transform

PROPOSED METHODOLOGY

The image encryption and decryption steps based on the new random double-base encryption are as follows:
Process 1: Shuffle the original image with Arnold's transformation
Process 2: Generate a random mask 1 (RM1) via logical mapping
Process 3: Chen's chaotic system generates a random mask 2 (RM2)
Process 4: Combine Arnold Transform with RM1 and RM2 to get an encrypted image
Process 5: QR code generated by the QRpoint website
Process 6: store the QR code in the cloud store
Process 7: recover the encrypted image using the reverse method of encryption technology.

The proposed algorithm represents in the below:

Arnold Scrambling Image Generation Techniques

This research paper uses the technique of square coincidence division method for Arnold scrambling process. This process done with the help of original image (Ex: M * N) with three forms (M=N;M<N;M>N) as in below flow chart. There are 3 modules are proposed for scrambling the original image

CONCLUSION

This document proposes a new image encryption scheme, which combines DRPE with chaotic logistic mapping. Using multidimensional secret key, the initial conditions for the two logical mappings are derived by providing weights for the bits corresponding to their position in the key. In the new algorithm for the encryption and decryption of images that use Arnold's transformation through the proposed encryption process, three different modules are used to encrypt the pixels of the image and which operation is used for a given pixel is determined by the results of Arnold. Subsequently, using logical mapping and the chen system to create two random masks, the encrypted image is finally converted into a QR code to make the encrypted image more robust than any attack. We

performed statistical analysis, key sensitivity analysis and time complexity analysis to demonstrate the security of the new image encryption program. Finally, we take the comments as a conclusion and we believe that the proposed method can be used for cryptography and real-time image transmission applications.

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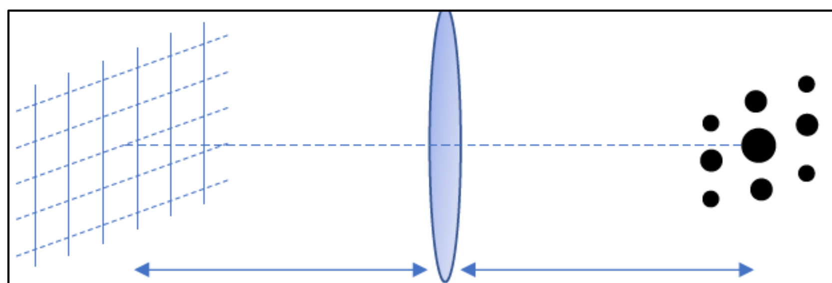

Fig1. Two-dimensional Fourier transform

Convert encrypt image to QR Code
Encryption
Encrypted medical report
QR code of Encrypted Image
DATA OWNER
Original medical report
Medical data Cloud storage
Request file
Decryption
Decode the QR code by password

Fig.2. Proposed System Architecture

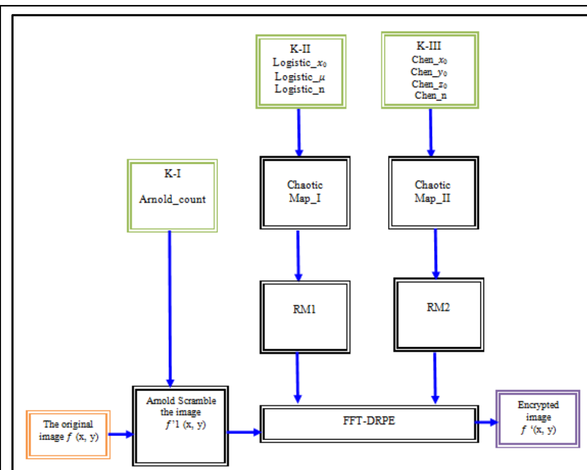

Fig.3. Encryption process

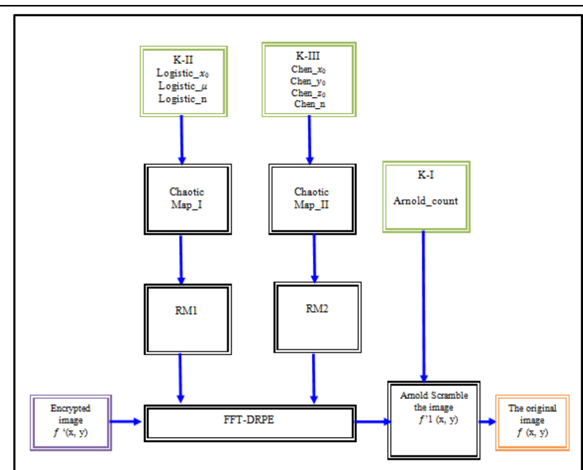

Fig.4.Decryption Process

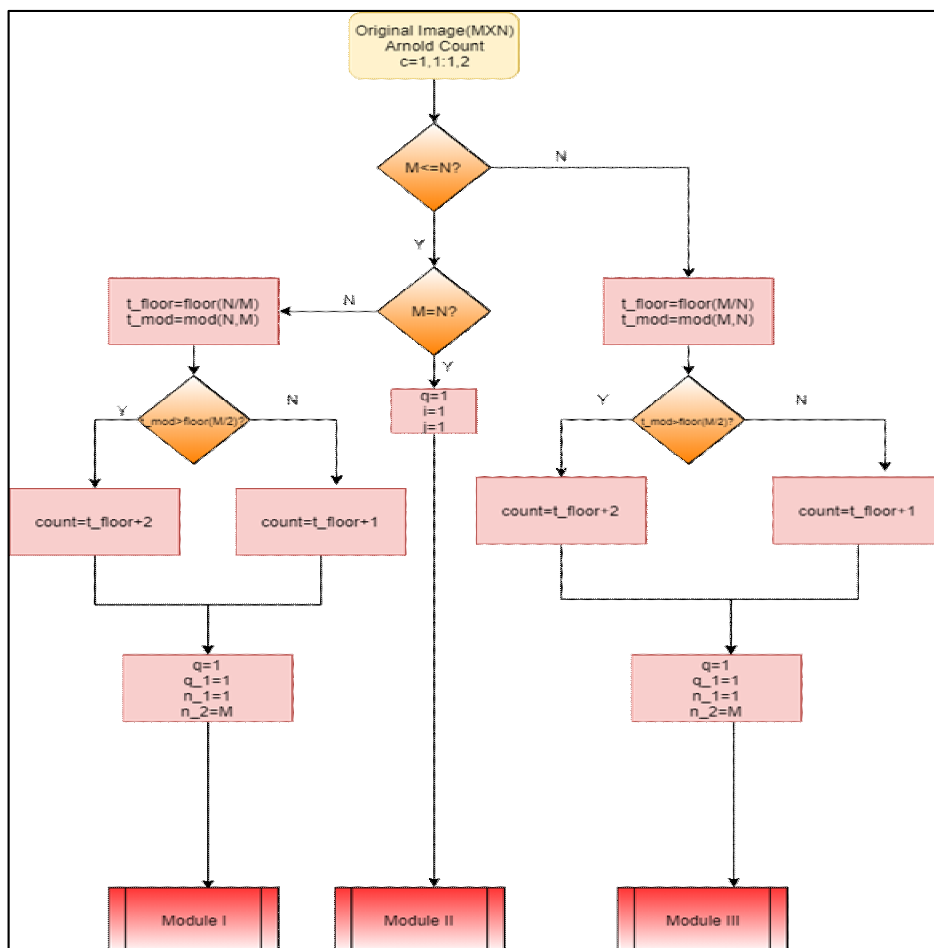

Fig.5. There are 3 modules are proposed for scrambling the original image

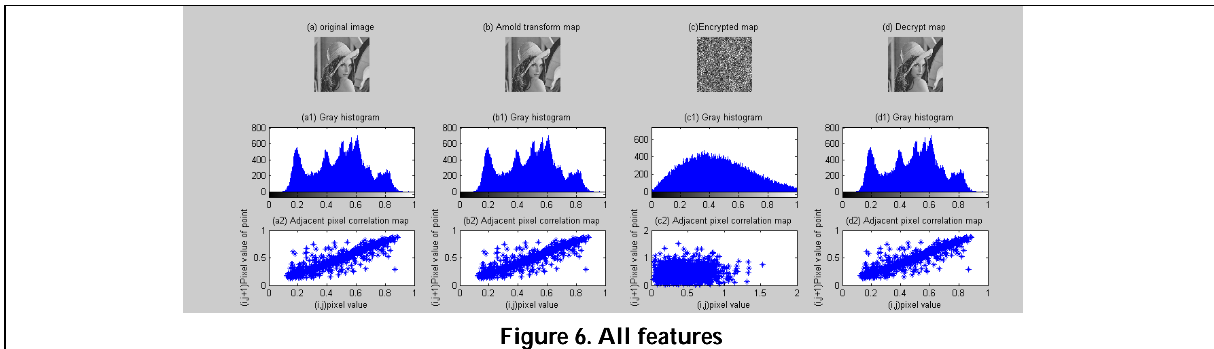

Figure 6. All features

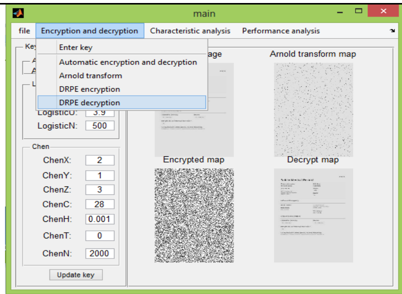

Figure 7. Encryption and decryption process

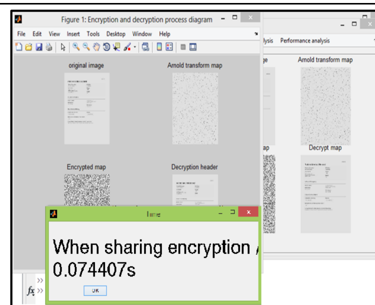

Figure 8. Encryption time

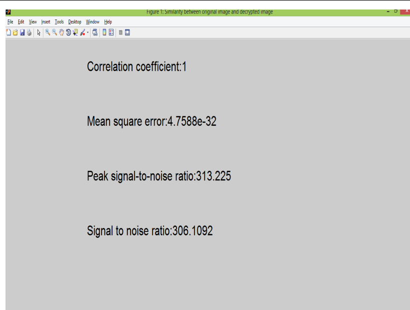

Figure 9. Relationship between original and cipher image

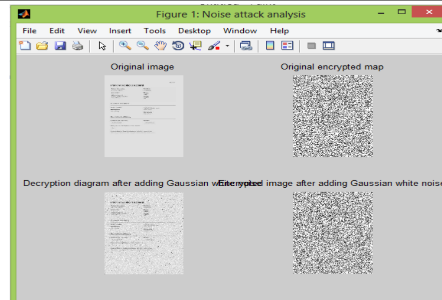

Figure 10. Noise attack analysis

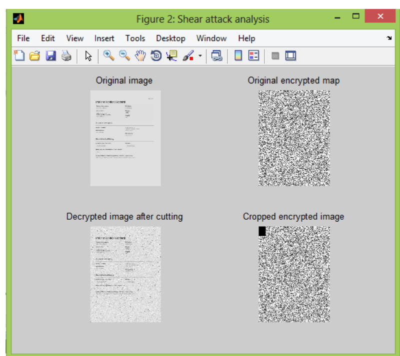

Figure 11. Shear attack analysis

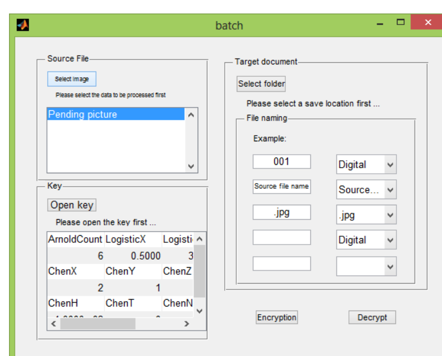

Figure 12. GUI Software

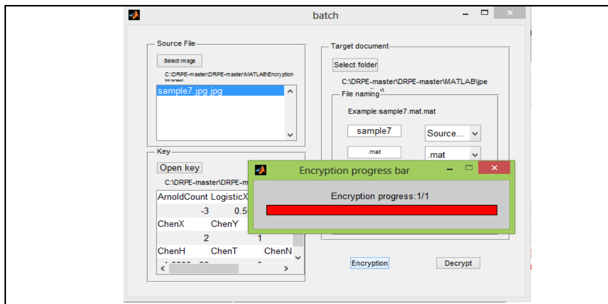

Figure 13. Encryption Progress

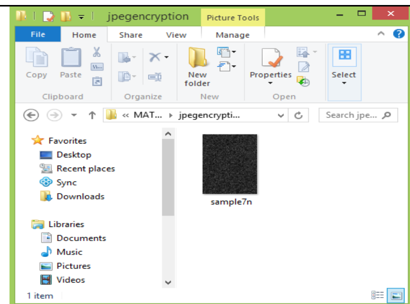

Figure 14. Encrypted image

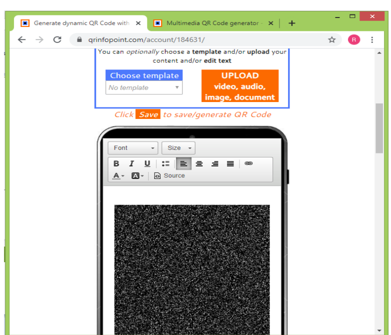

Figure 15. QR Point Website to create QR code

Figure 16. Q Red software for get an image

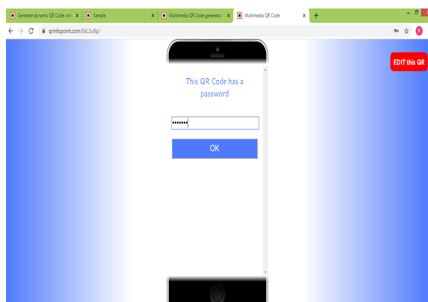

Figure17. Insert Password

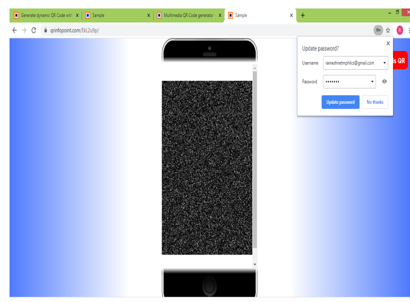

Figure 18. Encrypted image Retrival from QR code

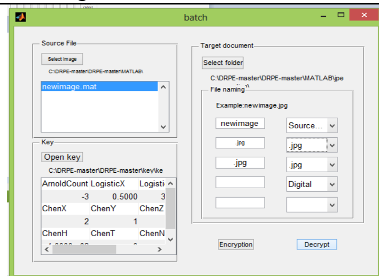

Figure 19. Decryption of Encrypted Image

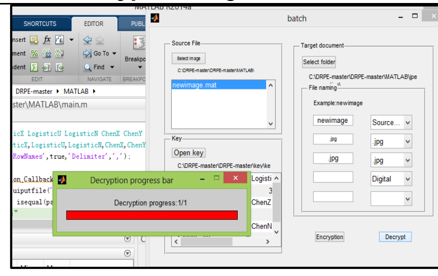

Figure 20. Decryption Progress

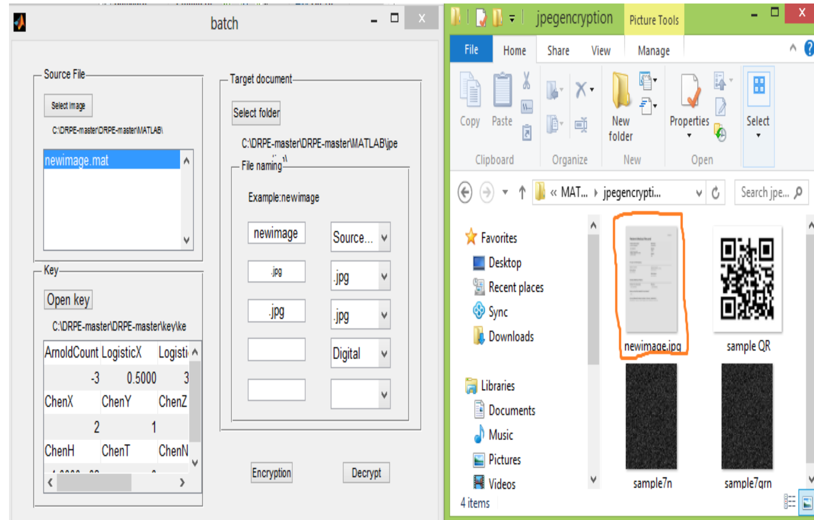

Figure 21. Get Original image

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Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

SHORT COMMUNICATION ARTICLE

Alcohol Dehydrogenase 1 Gene Expression Study in *Saccharomyces cerevisiae* for Bioethanol Production

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Received: 10 Jan 2020 Revised: 12 Feb 2020 Accepted: 16 Mar 2020

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SHORT COMMUNICATION ARTICLE

Combination of bioethanol with fossil fuel in motor vehicle engine reduces green house gases emission and avoids the full dependency on fossil fuel (Kaberger, 2018); thereby using bioethanol it reduces the demand of fossil source which have overbearing cost. On the other hand it is important that modern technologies are required to bring down the expenses of bioethanol production (Gupta andVerma, 2015). In industrial fermentation metal ions have significant impact on conversion of sugar to bioethanol and its yield, cell viability and stress tolerance, extent of foaming and flocculation behavior (Jones and Gadd, 1990), in this regard zinc participate as a co-factor in ADH1 gene plays important role in of carbon metabolism through fermentation and respiration in converting acetaldehyde to bioethanol (de Smidt *et al.*, 2008). Recent studies have focused on zinc which as co-factor regulates gene expression, for its crucial role in modifying transcription factors and biological functions of metalloenzymes (Maret, 2013; Wu *et al.*, 2013). Calcium and zinc ions stimulate flocculation, sugar utilization, acid re-assimilation, butanol production and tolerance at cellular level (Han *et al.*, 2013). *Saccharomyces cerevisiae* is a model microorganism for studying metal transporters and their accumulation in the cytoplasm, the structural simplicity of this organism has made it a potential mode for gene expression analysis (Azhar*et al.*, 2017).

Lignocellulose in bioethanol production involves species with high biomass productivity and cost-effective pre-treatment (Robak and Balcerek, 2018). Therefore, in the present study an attempt was made to up-regulate the expression of ADH1 gene in *Saccharomyces cerevisiae* using calcium and zinc to enhance bioethanol production. In the experiments *Saccharomyces cerevisiae* culture (NFCCI 1248) was obtained from National Fungal Culture Collection of India (NFCCI), Maharashtra, India and sub-cultured in YEPD agar medium, which consists 10g/L yeast extract, 20 g/L peptone, 3g/L ammonium sulphate ($(NH_4)_2SO_4$), 0.5g/L di-potassium hydrogen phosphate (K_2HPO_4) and 50 g/L glucose. Prior to usage, the strain was pre-incubated in orbital shaker (5mL YEPD; 30°C; 120 *rpm*; 12 hours); later, the cells were transferred to 100 mL YEPD and further incubated (30°C; 120 *rpm*; 12 hours). Then the cells were centrifuged at 3000 *rpm* for 10 min and washed twice with sterile distilled water. Cell concentration was adjusted to

$1x10^7$ cells/mL by using McFarland standard Number 1 with YEPD broth. Individual effect of $CaCo_3$ and Zinc was studied according to (Ismail *et al.*, 2014) and the synergistic effect was studied according to (Shabtai *et al.*, 1991) on the expression levels of ADH-1 gene were performed according to modified available protocols of Ismail *et al.*, 2014. In case of individual effect of metal ion the culture was inoculated into fermentation medium and assessed with various concentrations of $CaCO_3$ (2 and 4 g/L) along with control ($CaCO_3$ absent) and incubated at 30°C and 120 *rpm*, at 24 and 48 hours, then the culture was centrifuged at 3000*rpm* for 10 minutes and pellets were taken for the ADH-1 gene expression using RT-PCR., where as in case of individual treatment of Zinc same procedure was followed which was followed for $CaCO_3$. In case of synergetic effect the same procedure was followed but here various concentrations of both $CaCO_3$ (2 and 4 g/L), and $ZnSO_4$ (0.002 and 0.004 g/L) were used along with control ($CaCO_3$ and $ZnSO_4$ absent). RT-PCR was used to evaluate the ADH1 gene expression profile upon induction of different concentrations of Ca and Zn with different time points.

1μg of total RNA was reverse transcribed into first strand cDNA, the reaction volume consisted of 25μl solution (2μl cDNA, 4μl primer, 10μl SYBR Green and 9μl water). Forward primer: CCGCTCACATTCCTCAAGGT, Reverse primer: TGTAACCCATAGCCTTGGCG and ITS-1 (Internal Transcribed Spacer-1; Forward primer: TTTGGGCATTCGAGCAATCG Reverse primer: GCATTTCGCTGCGTTCTTCA) were used as primer sequence for ADH-1. The mRNA expression levels were normalized to the level of housekeeping gene (ITS-1 expression), and the cycle threshold (Ct) value of Calcium and Zinc induced cells, were expressed in terms of fold changes over control setup. In results different concentrations of $CaCo_3$ (2 and 4g/L) increased the expression of ADH-1 gene in fold change by 69.60 and 66.41 at 24- hour time point respectively, further, a fold change of 83.89 and 174.30 was observed at 48- hour time point respectively over the control, similarly different concentrations of $ZnSo_4$ (0.002g/L and 0.004g/L) increased the expression in fold change by 42.18 and 36.09 at 24- hour time point respectively, further, a fold change of 87.26 and 132.67 was observed at 48- hour time point respectively over the control and the synergetic effects of both $CaCo_3$ and $ZnSo_4$ has increased the change by 76.17 and 72.78 at 24- hour time point & 176.56 and 230.78 at 48- hour time point respectively over the control (Figure 1- 4; Table 1 and 2).

Pertaining to above results in discussion, *Saccharomyces cerevisiae* associated in alcohol production by ADH1 gene, Crook *et al.*, (2016) have revealed the knock-down and over expression of different genes to enhance bioethanol production. Metals ions regulate as co-factors for various internal mechanisms, and the ions also protect cells against toxic environment by inducing genes against foreign particles (Bird, 2015). According to Karaoglan *et al.*, (2020) alcohol dehydrogenases contribute to cellular energy metabolism by linking two major metabolic pathways including respiration and fermentation, Kusano *et al.*, (1998) reported that *Saccharomyces cerevisiae* mutants harbouring a single deletion of the ADH1 or of both ADH1/ADH2 exhibited slower glucose level and De Smidt *et al.*, (2013) observed that alcohol dehydrogenase isozymes by characterising its expression in *Saccharomyces cerevisiae* chromosome at molecular level and their phenotypic role by mutants with one functional ADH1, with glucose or ethanol as respective carbon substrates.

Dansky *et al.*, (1966) studied the efficiency of zinc ion in fermentation process where as, low levels of Zn are in effective to activate desulfhydrases path ways as a minimal thres hold is needed for activation; Zinc helps in the fermentation at a maximum level of 0.25 and 0.5 ppm, while optimum level for *Saccharomyces cerevisiae* growth is 1 ppm, hence the growth studies directly correlated with gene expression, so in the present research, maximum fold of ADH1 gene was expressed when the media was supplemented with higher concentration of zinc ions at 48 hours of incubation when compare to lesser concentration. Helin and Slaughter, (1997) reported that in *Saccharomyces cerevisiae* the fermentation rate was decreased at 0.6 ppm of Zn ions and at 0.5 ppm it was enhanced, in our study 8 folds of gene expression was increase at 4g/L of zinc in the medium. Calcium stabilises ADH1 protein in *Saccharomyces cerevisiae* by preventing negative structural configurations such as enzymatic dissociation and unfolding of oxidised state (De Bolle *et al.*,1997), similarly in the present synergetic effect of Ca and Zn have highest folds of ADH1 gene expression in *Saccharomyces cerevisiae* was observed.

Calcium alginate is used in immobilization of *Saccharomyces cerevisiae* in bioethanol production which control the inhibitors in fermentation broth Santos *et al., (*2018), where the results are correlated with the present findings that higher concentration of CaCo3 has shown higher expression of ADH1 gene but continuous ethanol production might induce negative feedback to the ADH1 gene; hence, it is important to selectively remove ethanol by pervaporation membranes (Bai*et al*., 2008). Therefore, very limited studies in the concentration of calcium and zinc in stimulation of gene expression, hence the present work was carried out to know ADH1 gene expression in *Saccharomyces cerevisiae* to produce more ethanol by supplementing different concentrations of calcium and zinc metal ions in separate and in combinatorial doses.

DECLARATIONS

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Authors declare that all generated and analysed data are included in the article.

Competing interests

The authors declare that they have no competing interests.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

KRS designed the research idea, over-all work and wrote the manuscript. TS helped with thecalculations and statistical analysis. STG revised the drafted manuscript and made necessary corrections. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

The authors express gratitude to Government of Karnataka, Bangalore University and Fund for Improvement of Science & Technology (FIST) for providing laboratory facilities.

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Table 1. Effect of Ca and Zn on ADH-1 gene in *Saccharomyces cerevisiae* for ethanol production after 24 hours of incubation

Sl. No.	Concentration	Ct value		Normalized gene expression (Fold change over control)
		House keeping gene	Gene of interest	
01	Zn (0.002 g/L)	24.71	25.85	42.18 ± 0.8
02	Ca (2 g/L)	24.65	25.04	69.60 ± 1.54
03	Control	24.39	30.89	1.00
04	Zn (0.002 g/L) +Ca (2 g/L)	22.84	23.08	76.17 ± 1.8
05	Zn (0.004 g/L)	24.23	25.33	36.09 ± 2.3
06	Ca (4 g/L)	24.28	24.54	66.41 ± 3.2
07	Control	24.00	30.38	1.00
08	Zn (0.004 g/L) + Ca (4 g/L)	22.38	22.58	72.78 ± 2.7

Table 2 .Effect of Ca and Zn on ADH-1 gene in *Saccharomyces cerevisiae* for ethanol production after 48 hours of incubation

Sl. No.	Concentration	Ct value		Normalized gene expression (Fold change over control)
		House keeping Gene	Gene of interest	
01	Control	24.32	30.37	1.00
02	Zn (0.002g/L)	24.04	23.61	87.26 ± 1.8
03	Ca (2g/L)	22.42	22.05	83.89 ± 2.1
04	Zn (0.002g/L) +Ca (2g/L)	24.27	22.82	176.56 ± 3.9
05	Control	24.00	30.14	1.00
06	Zn (0.004g/L)	22.76	21.80	132.67 ± 4.1
07	Ca (4g/L)	24.73	23.38	174.30 ± 5.2
08	Zn (0.004g/L) + Ca (4g/L)	24.31	22.55	230.78 ± 6.5

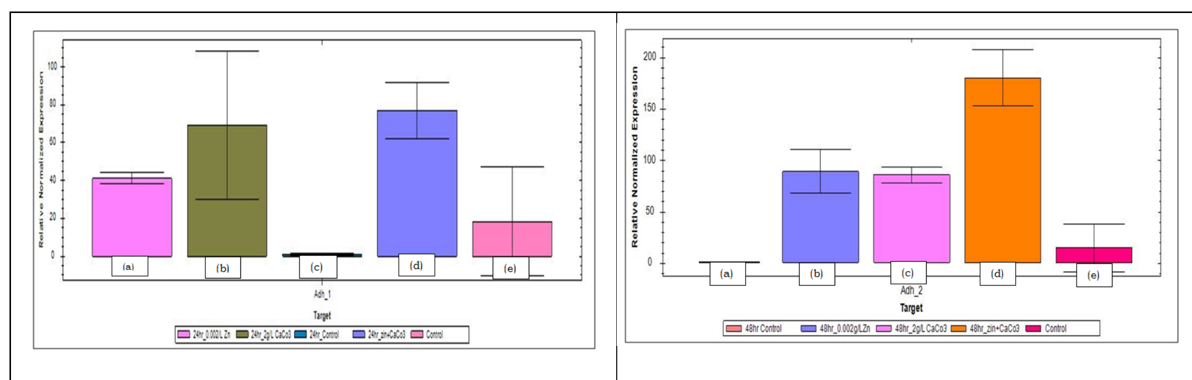

Figure 1 - Effect of Calcium and Zinc in *Saccharomyces cerevisiae* ADH-1 gene expression (24 hour of incubation). (a) 0.002 g/L Zn; (b) 2 g/L Ca; (c) Control (d) 2 g/L Ca + 0.002 g/L Zn; (e) Experimental control

Figure 2 - Effect of Calcium and Zinc in *Saccharomyces cerevisiae*ADH-1 gene expression (48 hour of incubation). (a) Control; (b) 0.002 g/L Zn; (c) 2 g/L Ca; (d) 2 g/L Ca + 0.002 g/L Zn) (e) Experimental control) in 48 hour of incubation

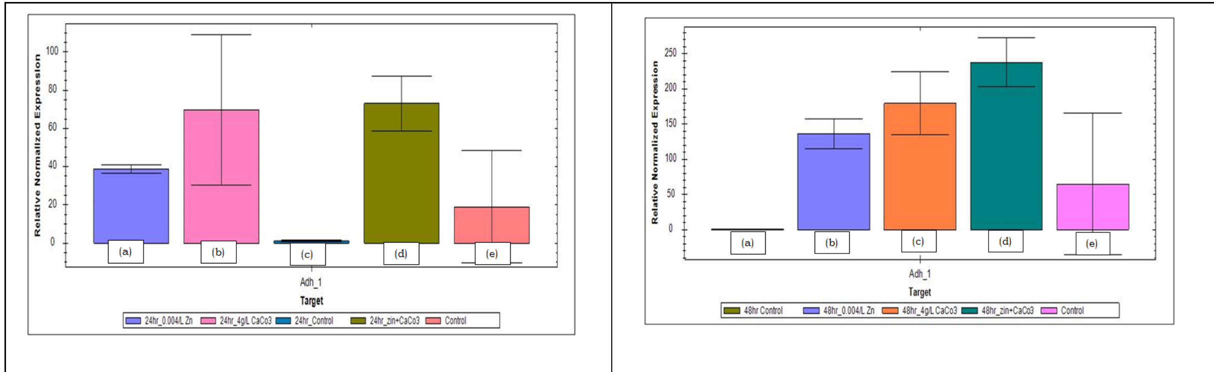

Figure 3 - Effect of Calcium and Zinc in *Saccharomyces cerevisiae* ADH-1 gene expression (24 hour of incubation). (a) Control; (b) 0.004 g/L Zn; (c) 4 g /LCa; (d) 4 g/L Ca + 0.004 g/L Zn; (e) Experimental control	**Figure 4 - Effect of Calcium and Zinc in *Saccharomyces cerevisiae* ADH-1 gene expression (48 hour of incubation). (a) Control; (b) 0.004 g/L Zn; (c) 4 g /LCa; (d) 4 g/L Ca + 0.004 g/L Zn; (e) Experimental control**

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Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Microbial Enzymatic System in the Degradation of Textile Dyes

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Received: 12 Jan 2020 Revised: 14 Feb 2020 Accepted: 16 Mar 2020

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ABSTRACT

The waste produced through the dyes treatment methods and operations includes the inorganic and organic contaminants that cause harm to the biological communities and the biodiversity which directly influence the environment and decline the aesthetic value of water bodies when discharged without treatment. Current physicochemical advancements for color expulsion can't expel all classes of dyes and different innovations are normally consolidated to accomplish attractive decolorization efficiencies. Microbial enzymatic treatment can be more straightforward and considerably more productive than conventional, physical and chemical treatments. This review discusses the capability of enzymatic biodegradation proficiency of synthetic dyes, especially azo dyes and concludes the current research based on the discharging of waste contaminated colours from the wastewater streams after biodegradation by biocatalysts such as azoreductase, laccase, peroxides and tyrosinase. These catalysts reduce the antagonistic effects of dye and make enzymatic wastewater treatment an environmentally sustainable strategy.

Keywords: Decolorization, Azo dyes, Azoreductase, Laccase, Peroxidases, Tyrosinase.

INTRODUCTION

Textile waste effluent is characterized by their high persistent colour, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), suspended solids and alkaline pH (9-10). In textile industries, synthetic dyes are selected instead of natural dyes due to their vast variety of colour and improved properties to dyed materials. Effluent or wastewater from industrial units that fabricate paints, shades and colourful beauty care products accommodate the nature of synthetic dyes. Textile industry effluents accommodate a range of other compounds, which include bleaching and fixing agents, stain removing agents, printing gums, emulsifiers, and heavy metal-containing organometallic compounds [1]. Specific

problems in the textile manufacturing unit include the removal of color from waste effluent and contaminants [2].

The major classes of synthetic dyes have a high extinction coefficient and strong π-π* transitions in UV-visible range that allow us to identify structures as dye chromophore. Azo aromatic dye is among the most prevalent synthetic dye compared to antroquinoid and an indigoid dye, which may have one or more azo (N=N) groups [3]. It is recognized to be recalcitrant, non-biodegradable and persistent to their strength and xenobiotic nature, and therefore is not completely diminished by conventional treatment involving heat, chemicals or activated sludge [4]. Treatment of industrial dye-based effluents is examined to be one of the formidable tasks in the environmental fraternity. Because of the amalgamation with heavy metals on the structural interface and toxic and inhibitory nature, they have comparatively less significance on the surrounding environment.

Textile effluents typically only contain 0.6-0.8 g/l of dye, but the persistence of dyes in the discharged effluent does lead to pollution [5]. Therefore, for the decolorization of such effluent, it is important to search and establish efficient techniques. A broad range of techniques have been developed to treat synthesized dyes from wastewater or effluent, including physical, chemical, and biological procedures to decrease their environmental effect (Figure 1). Physical and chemical strategies are viable for dye removal treatment which requires more vitality and chemicals as compared to biological procedures and sometimes causes contamination into solid or liquid side streams and requires extra treatment. The degradation of textile dyes using biological processes is a favorable, ecofriendly approach and therefore these techniques are the moderator of an inexpensive option for costly physicochemical techniques [6]. These biological techniques are based on microbial enzymes and it can be used for the degradation of synthetic dyes due to their low toxicity [7, 8].

Specificity and a comparative convenience of technology to improve the strength of enzymatic procedures prevail in the removal of dye from effluents, while appropriate dyeing chemicals as well as fibers remain unaffected and can be reused [9]. However, dye compounds demonstrate huge structural variability; they are reduced by few enzymes which are associated with the frequent mechanistic aspects as they all act as catalysts and illustrate relatively vast substrate characteristics. These are degradable catalysts that provide action at low and high concentrations of substrates and activity across a wide range of pH, temperature and alkalinity, has decreased the accumulation of sludge and are both simple and easy to regulate [10]. On account of the catalytic deterioration of dyes, azoreductases, laccases, peroxidases and phenol oxidases (tyrosinases) have the capability, although all of these compounds wavers in their biodegradation abilities in specific perspectives [9]. The azo linkage electron-withdrawing characteristics stop azo dye molecules from being susceptible to oxidative responses. Only reliable azo-reducing enzymes have been discovered to degrade the dyes. The enzymatic catalytic action is adequate and preferred as chemical catalysts because of higher reaction rates, milder reaction action and greater stereospecificity [11].

Microbial Bioremediation of Azo Dyes

Bioremediation is a natural or induced process, which is known as a simpler and cleaner strategy than the traditional approach for the cleanup of polluted systems. The main agents involved in efficient bioremediation processes of pollutants are bacteria, fungi, algae and plants [12]. Environmental conditions permit dye degrading activity of microbes to continue at a faster rate, and their implementation often involves influencing natural parameters to allow them to do so. Only bound species of bacteria and fungi will degrade distinctive toxic pollutants; their numerous strains are known as bioremediation operators, however, solely below laboratory conditions. The restriction of bacterial growth is affected by pH, temperature, salinity, oxygen, moisture, soil structure and nutritional component, poor bioavailability of contaminants, and the existence of other toxic compounds [12]. Table 1 describes the different factors influencing dye decolorization. Maximum

bioremediation mechanism works under aerobic conditions, while the microbial degradation of recalcitrant molecules can occur in anaerobic circumstances [13-15].

Enzyme Action in Dye Degradation

The chemical categories of dyes, azo dyes (-N= N-) are among the biggest classes utilized as a part of the printing and textile handling ventures. The microbial degradation of dyes observed throughout the textile or potentially printing industries is more recalcitrant [10], due to inconsistency in the composition of the effluent, and the basic structural variability of the dye itself. The utilization of enzymes may speak to a good alternative for defeating disadvantage related to the use of microorganisms [32-34]. Enzymatic treatments selectively degrade a lot of the recalcitrant target pollutants, without influencing the other components in the effluent. Catalytic enzymes from both aerobic and anaerobic frameworks have been accounted to be powerful in decolorization of the colours with most of the outcomes anticipated from *Phanaerochaete* white rot fungi as well as Trametes. They secrete laccases and manganese / lignin peroxidases equipped for the azo bond's oxidative free radical cleavage. Enzymes have various aspects that make them progressively reasonable about conventional impetuses. They are impetuses with either narrow (chemo-, locale and stereo-selectivity) or expansive specificity and subsequently connected to a vast scope of various compounds in the blend also. On account of a catalytic abatement of azo dyes azoreductases, laccase, peroxidases and phenol oxidases appear as being the most encouraging enzymes and their numerous industrial applications such as the textile, leather, paper and pulp industry, and so forth [14].

Azoreducates

Azoreductases are synthesized by a numerous trophic group of bacteria which breaks azo bonds (-N=N-) and is responsible for azo dyes degradation and decolorization (electron-deficient xenobiotic compounds) (Table 2). The majority of the azo dyes have high molecular weight and sulfonate groups, rare to migrate across membranes. These properties of dyes do not depend on the dye's intracellular take-up [40]. Azoreductases are often classified as a flavin-dependent or flavin-independent enzyme on the basis of their function and require the electron contributors (NAD(P)H) to degrade azo dyes that produce aromatic amines as derivatives [41, 42]. They are located at the intracellular or extracellular location in bacterial cell membranes and lead to the decoloration of azo dyes in their comparative aromatic amines by catalyzing the azo bond [43]. The bacterial membranes are almost impermeable to flavin-containing cofactors and restrict the transport of equivalents reduction from the cytoplasm to sulfonated azo dyes by flavins. Thus, the reduction of the sulphonated azo dye in bacterial cell membranes with intact cell membranes may be caused by a non-reductive mechanism of the cytoplasmic flavin-dependent azoreductases [12].

In gram-negative bacteria, the components of electron transport must be confined to the outer membrane to have direct contact with either substrate of azo dye or a redox mediator [44]. Hence, a low-weight redox mediator can be used as an electron shuttle with azo dye and a NADH-dependent azo reductase [12, 21]. These components of the mediator are represented by the bacteria in the metabolism of particular substrates or are included outwardly [42]. The aerobic extracellular environment is blocked by the oxygen reduction method as a result of the valuable oxidation of the redox mediator instead of azo dyes [12]. The redox-mediated activities of membrane-bound azoreductase is different in relation to soluble cytoplasmic azoreductases, which are crucial in the removal of non- sulphonated dyes and to enter through the membrane of the cell. The membranous and cytoplasmic azoreductases are therefore two different diverse enzymes [3]. Figure 2 demonstrates a system under anaerobic conditions using entire bacterial cells for redox-mediator-dependent removal of azo dyes. While the complete deterioration of azo dyes is an important chemical redox response in the cell supernatants; the redox mediator relies upon cytoplasmic degrading enzymes to deliver electrons [23, 12]. This redox activity

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of the substance works with direct catalytic action containing azoreductase and dehydrogenase that is combined throughout the cytoplasm and produced within the cell without accumulation [45].

Laccase

Laccases, a cuproprotein small group of enzymes also entitled as 'blue oxidases'. These are extensively studied in their azo dyes degradation and have extraordinary ability in different biotechnological mechanisms [47], because of the absence of necessary cofactors [48], high non-specific oxidation capacity [49], and the utilization of molecular oxygen promptly accessible as an electron acceptor [50]. This enzymatic family catalyzes the electron (4e-) abatement of oxygen into water (at the core of T2-T3 trinuclear Cu) in the presence of accessible oxygen as an electron contributor through successive one-electron take-up from an appropriate diminishing substrate (at the core of T1 mononuclear Cu)/ (Figure 3) [47, 51]. It also induces the oxidation in the presence of oxygen (electron donor) of substitute phenolic and non-phenolic substances [52]. Hence, it leads to a reduction in the production of toxic amines [53, 34]. The most studied laccases are of fungal origin in the literature compared to bacterial laccases, because of the preferences for biotechnological procedures owing to the higher production yields, ease of control, the absence of post-translational modifications and enhancement through the genetic engineering process. However, in the most recent decade, many bacterial laccases have been identified [54, 55]. Laccase-like polyphenol oxidase activity found in prokaryotic laccase was reported in rhizospheric bacteria, *Azospirillum lipoferum* [56]. Polyphenol oxidases (PPO), is a multi-potent PPO which is secreted by *Marinomonas mediterranea* a melanogenic marine bacterium, which have characteristic of both tyrosinase and laccase that oxidizes the substrates [57]. In *Pseudomonas syringae* and *Pedomicrobium* species laccase activity in CopA protein has been reported [58]. By preventing the production of noxious aromatic amines, through a nonspecific free radical mechanism, laccase decolorizes the azo dyes [53]. In 2007, Kalme *et al.,* reported 100% decolorization of different dyes, including Direct Blue 6, Green HE4B and Red HE7B in the presence of purified laccase of *Pseudomonas desmolyticum* NCIM 2112 [59].

Peroxidases

Microbial systems seem, by all accounts, to be more convenient for the treatment of phenols and halogenated phenols present in the wastewater discharge of textile industries. Peroxidases are a ubiquitous catalyst known to successfully catalyze the ample range of substrates that oxidizes phenolic and lignin compounds, to the detriment of (H_2O_2) hydrogen peroxide within the sight of a mediator, and most significantly, anthraquinone and azo based high redox synthetic dyes are highly degraded. Peroxidases can be classified as heme and non-heme peroxidase proteins. Because the prosthetic group heme peroxidases accommodate a protoporphyrin IX (heme), whereas the non-heme peroxidases use metal as a cofactor [60]. Heme peroxidases are divides phylogenetically based on recent classification into two superfamilies and three families, in which peroxidase-catalase and Peroxidase-cyclooxygenase are superfamily as well as di-heme peroxidases, dyP-type peroxidases (DyPPrx) and haloperoxidases (HalPrx) are families [61]. The superfamily peroxidase-cyclooxygenase consists of members, which are from all the domains of life as the previous nomenclature "animal heme-dependent peroxidases" that restricted classification to animal origin peroxidases [62].

In immunology, the superfamily peroxidase-cyclooxygenase would be of clinical significance. The superfamily of peroxidase-catalase could be further subdivided into three categories [63]. Intracellular peroxidases, for example, in the electron transport chain are class I of the peroxidase-catalase superfamily [64]. In Class II, there are extracellular fungal or bacterial peroxidases together with lignin peroxidase (LiP), manganese peroxidase (MnP), and versatile peroxidase (VP), which are identified to lignin reduction and predicted to be crucial within the restraint of lignocellulosic biomass to degraded product [11]. The main ligninolytic-peroxidases were isolated from *P. chrysosporium* and known as lignin peroxidase (LiP) [3, 7] and manganese peroxidase (MnP) [65]. Ligninolytic heme-peroxidases including LiP, MnP, and VP, oxidize precise segments of the lignin

structure [66]. The phenolic structure of lignin was oxidized by MnP and lignin peroxidase (LiP) centers around the non-phenolic compounds; versatile peroxidase (VP) has the oxidation limit for each phenolic and non-phenolic structures. Class III peroxidases includes plant-emitted peroxidase, horseradish peroxidase (HRP), for instance, involves biosynthesis of the cell wall, catabolism of Indole-3-acetic acid and oxidation of noxious components [67, 68].

The synergist oxidation of Mn^{2+} to Mn^{3+} was incorporated by the mechanical action of MnP, which is exceedingly reactive and oxidizes an extensive variety of phenolic substrates including phenolic structures of lignin [69]. With mediator obligations between thiyl or lipid radicals, MnP is equally capable of oxidizing or severing non-phenolic structures [70, 71]. Moreover, natural as well as synthetic lignin and recalcitrant compounds are oxidized and depolymerized by activity of MnP's [72, 34]. The very first reaction that occurs is the oxidation by hydrogen peroxide (H_2O_2) of the resting ferric [Fe (III)] enzyme is production of an intermediate oxo-ferryl compound I. In the next stage, the intermediate oxo-ferryl (lacking of 2e-) is diminished through a substrate, for instance, non-phenolic aromatic substrate (S) that gives compound I an electron (1e-) to form compound II, which is the second intermediate, (lacking 1e-). In the completion step, the diminished substrate molecule requires the resulting addition of a second electron to compound II, thus returning LiP to the resting ferric oxidation state which indicates the oxidation process completed [70].

Tyrosinases

Tyrosinases with various physicochemical properties have been distinguished from different bacterial phyla, for example, In Actinobacteria and Proteobacteria production of tyrosinases are regulated by the environmental burden. Tyrosinases are copper-dependent dioxygen activating enzymes consistently associated with melanin production. Bacterial melanin assumes a defensive part in various ways: it shields DNA from the harms of UV radiation and receptive oxygen species; it can tie toxic heavy metals and collaborate with DNA, potentially backing off the metabolism. Hence, these are crucial for the endurance of the organisms [73]. The o-hydroxylation of monophenols catalyzed by Polyphenol Oxidases (PPOs), for example, tyrosine (phenol particles wherever the benzene ring comprises a solitary hydroxyl substituent) to o-diphenols (phenol atoms comprising two hydroxyl substituents) [74, 75] and catalyzed o-diphenol oxidation to produce o-quinones (Figure 5). The single phenolic ring can be oxidised in the amino acid tyrosine to create the activated o-quinone by the activity of PPOs, and PPOs may also be assigned as tyrosinases. Tyrosinases and catechol oxidases have a place with the group of structurally similar enzymes (PPOs) whose action is restrictive to diphenolic substrates; can overlap with the laccases, which are the structurally diverse enzyme and lacking monophenol hydroxylase reaction and are generally grouped under the determine 'polyphenol oxidase'.'.

Tyrosinases have an activity for phenolic and diphenolic substrates that are continually delivering an activated quinone as a product in the presence of water. Despite this action, they have several biotechnological functions including protein cross-linking, production of novel mixed melanins, production of L-DOPA, phenolic biosensors and dye removal biocatalysis and have been reported in some bacteria including *Bacillus licheniformis, Bacillus natto, Bacillus sphaericus, Streptomyces glaucescens,* and *Streptomyces antibioticus* [74-76].

Genomic Approaches in Bioremediation

Degradation of azo dyes by bacteria generates toxic compounds under both stagnant (anoxic) and anaerobic conditions because of the catalytic process of azoreductase. Produced aromatic end products which result from incomplete degradation (Figure 6) are more toxic than the native dyes [77]. The basic step in azo dyes microbial degradation is the cleavage of electrophilic azo bonds resulting in azo dyes decolorization. By reducing the dye compounds to their intermediates, we solve the problem of visual pollution, but it may create larger and more deleterious problems. Degradation of azo dyes by fungal oxidases don't generate the toxic amines.

Consequently, bacterial and fungal consortium applied for degradation and decolorization of azo dyes; generates metabolites free from noxious amines. Lade *et al.*, (2012), was examined the absence of toxic amines in the product after decolorization of Disperse Rubine GFL dye by a consortium of *Aspergillus ochraceus* NCIM 1146 and *Pseudomonas* sp. SUK1. However, *Pseudomonas* sp. SUK1 showed 0.14 mM concentration of aromatic amines [78]. Therefore, usage of enzymatic processes of consortium for azo dyes decolorization enhances detoxification with an increment of biodegradation rate [78].

But, unlike bacteria, fungi can thrive in moist or humid regions under low pH conditions, which promote organic matter deterioration [79]. On high alkali and pH, fungi are not able to degrade dyes in an effective manner. So it's time to switch to some other methods like proteomics and genomics to solve this problem. Further, utilization of genetic engineering technology or recombinant DNA technology can be used to clone genes of already known derivative enzymes into the single microorganism to improve the decolorization efficiency of synthetic dyes [80]. It is inspired by the exchange of natural genetic material between the microorganisms and produces dye degrading genetically modified (GMM) microorganisms, whose hereditary material becomes transformed. These microorganisms have explored the efficiency for bioremediation of wastewater, groundwater, soil and activated sludge and illustrates the increased ability to degrade dye with the absence of noxious byproducts of a broad range of contaminants [80]. Reduction of noxious environmental pollutants by genetically engineered bacteria using various recombinant DNA technology, pathway alteration, conversion of substrate specificity by *Comamonas testosteroni* VP44 has been reported [81].

Use of rapid-growers as agents for bioremediation of noxious contaminants is the inevitable expansion of undesirable biomass [82]. As a substitute, one of the methods to maximize catalytic capabilities with the least generation of cell mass would be the optimal clean-up agent. In addition, genomic trials are restricted to a few well-characterized bacteria like *B. subtilis, E. coli, and P. putida* [83]. Various bacterial strains ought to be investigated for constructing the transformation of fungal laccases or oxidases genes into the bacterial genome for the improved efficiency of decolorization and detoxification of azo dyes. These genetically engineered strains will be efficient to decolorize azo dye without generating aromatic amines. But the field release of construct GE bacteria in the bioremediation process with an acceptable of environmental inevitability [83].

Endeavors ought to be addressed to inspect the performance of genetically engineered microbes as far as their survival is concerned, the capability of horizontal gene transfer, that may influence the native microbiome inside a diverse natural circumstance. The novel research dependably offers increased fascinating questions pertaining to intriguing inquiries relating to the open concern [83]. In most instances, the design GE bacteria have been constructed for the precise purpose under the lab conditions for bioremediation process, disregarding the field necessity and diverse complex circumstances. There is no proof that the conscious discharge of constructed GE microorganisms for biodegradation has led to a significant antagonistic effect on the natural microbial population. At least the immoderate concept of compromise evaluation has been so controversial and has led to as many exploration efforts that have made a huge contribution to environmental microbiology. However, the endurance of the GE bacteria in complex natural circumstances is even a considerable challenge and requires to be focused on considering recent discoveries [76, 82].

CONCLUSION

Synthetic dyes grant extraordinary shading to textile, leather and wastewater effluents. Dye containing effluents harbors complex and variety of dyes, and when exposed to chemical or light attacks generate toxic compounds that can lead to serious environmental problems. This can be overcome by the use of cost-effective and technically feasible methods such as enzymatic degradation. There are very few known enzymes implicated in the biological process of synthetic dye degradation. The textile industry can significantly profit by the extended

utilization of these enzymes as non-poisonous naturally acceptable compounds. Because of their biological origin, they decrease adverse effect as compared to the physical and chemical method of dye degradation and thus making the enzymatic treatment biologically manageable. Along these lines, the portrayal of enzymes creates a recognizable commitment to the deterioration of synthetic colours and amplifies their biodegradation and biotransformation capabilities. Use of genetic engineering techniques can be further used to increase the biodegradation capabilities of microorganisms by transferring known genes of beneficial enzyme into the fast grower bacterial strain. In this review, we describe the characteristics of azo dyes and their enzymatic processes of biodegradation.

ACKNOWLEDEGMENTS

The author are thankful to all the faculties of School of Life Science for their moral support.

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Abbreviations

DyPPrx- dyP-type peroxidases, HalPrx- haloperoxidases, CcP- cytochrome c peroxidase, PPOs- Polyphenol oxidases

Table 1. Factors affecting decolorization and degradation of synthetic dyes that have been shown below [16].

S. No	Factors	Descriptions	References
1	**Carbon and nitrogen Sources**	Dyes are deficit in carbon and nitrogen sources, and therefore the biodegradation of dyes with no supplement of these sources is incredibly troublesome. Microbial cultures, by and large, need complex organic sources, for example, yeast extract, peptone, or a blend of complex natural sources and sugars for dye decolorization and degradation.	[17]
2	**Dye Concentration**	Prior reports demonstrate that increasing the dye concentration progressively decrease the decolorization rate, likely because of the toxic impact of dyes as to the specific bacteria and/or inadequate concentration of biomass, and in addition blockage of dynamic sites of azoreductase by dye molecules with various structures.	[18, 19]
3	**Dye structure**	Dyes with less complex structures and low molecular weights display higher rates of biodegradation, though the degradation rate is brought down on account of dyes with the substitution of electron withdrawing group, for example, SO_3H, SO_2NH_2 in the para position of phenyl ring, such as the azo bond and high molecular weight dyes.	[20]
4	**Electron Donor**	It has been watched that the use of electron donors, for example, glucose or acetic acid ions, evidently prompts the reductive cleavage of azo bonds. The type and accessibility of electron donors are necessary for accomplishing good colour evacuation in bioreactors operated underneath anaerobic conditions.	[21, 22]
5	**Oxygen and Agitation**	Amid the dye evacuation, if the extra cellular environment is aerobic, the high redox-potential electron acceptor oxygen may prevent the dye reduction process. This is because the electron free from the oxidation of electron donors by the cells are specially used to lessen oxygen rather than the azo dye.	[23, 24]
6	**pH**	pH majorly affects the effectiveness of dye decolorization; the ideal pH for colour evacuation in bacteria is often in the range of 6.0 - 10.0. The tolerance to high pH is crucial for industrial processes using reactive azo dyes that measure typically performed beneath alkaline conditions.	[25, 26]
7	**Redox Mediator**	Redox mediators (RM) can improve numerous reductive mechanisms under anaerobic conditions, along with azo dye evacuation. Flavin-based compounds, for example, flavin adenine dinucleotide (FAD) and flavin adenine mononucleotide (FMN), and quinone-based compounds, for example, anthraquinone-2,6- disulfonate (AQDS), anthraquinone-2-sulfonate (AQS), riboflavin (vitamin B2), cyanocobalamin (vitamin B12) and lawsone (2-hydroxy-1,4-naphthoquinone), have been broadly announced as redox mediators.	[27]
8	**Redox Potential**	The redox potential of the electron contributors and acceptors influences the colour removal process because the rate-controlling steps advance redox equilibrium between the dye and the extracellular diminishing agent. Therefore, the more positive the redox potential, the more promptly the	[28, 29]

		molecule gets reduced. The colour evacuation rate is high when the redox potential of the framework is at its extreme negative and the rate falls as the redox potential of the system increases	
9	**Salt concentration**	Osmotic environment affects the efficiency of the dye degradation. Increasing the salt concentration increase dye degradation effectiveness, after that rate of degradation decreases with further increment in salt concentration, because high salt concentration inhibit the bacterial growth.	[30]
10	**Temperature**	Temperature is an important factor for all measures related to microbial vitality, together with the remediation of water and soil. It has also been recognized that the decolorization effectiveness usually decreases at the higher temperature and this decay could be attributed to the loss of cell viability or denaturation of an azoreductase enzyme.	[31, 18]

Table 2. Different trophic groups of bacteria for dye degradations

Bacteria	Degrading dye(s)	Reference(s)
Citrobacter sp. CK3	Reactive Red 180	[35]
Listeria sp.	Red B5 and Black HFGR	[36]
Bacillus subtilis	Acidblue113	[37]
Klebsiella sp., Salmonella sp., Pseudomonas sp.	Orange 3R	[38]
Enterococcus faecalis strain YZ66	C.I. reactive yellow 145	[39]

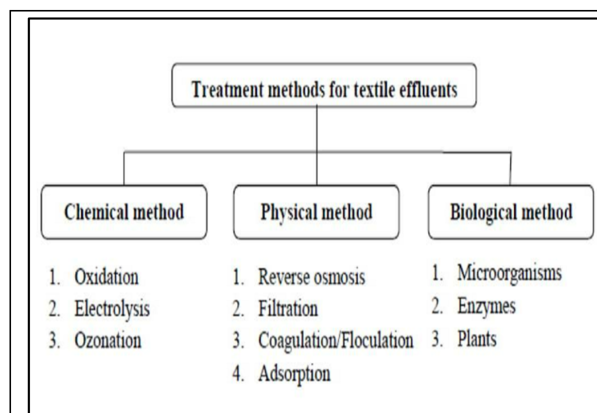

Figure 1. Treatment methods for the removal of dyes from wastewater effluent

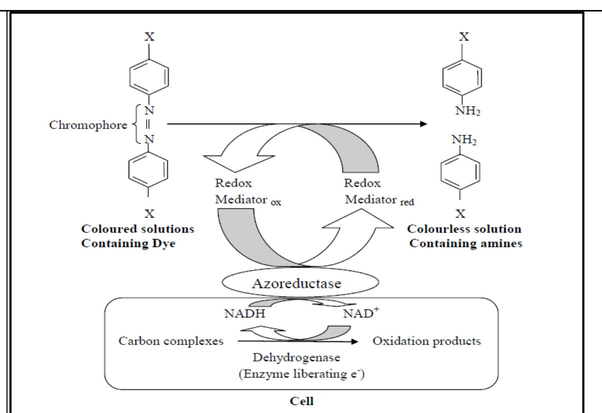

Figure 2. Mechanism for reduction of azo dyes by azoreductase [46]

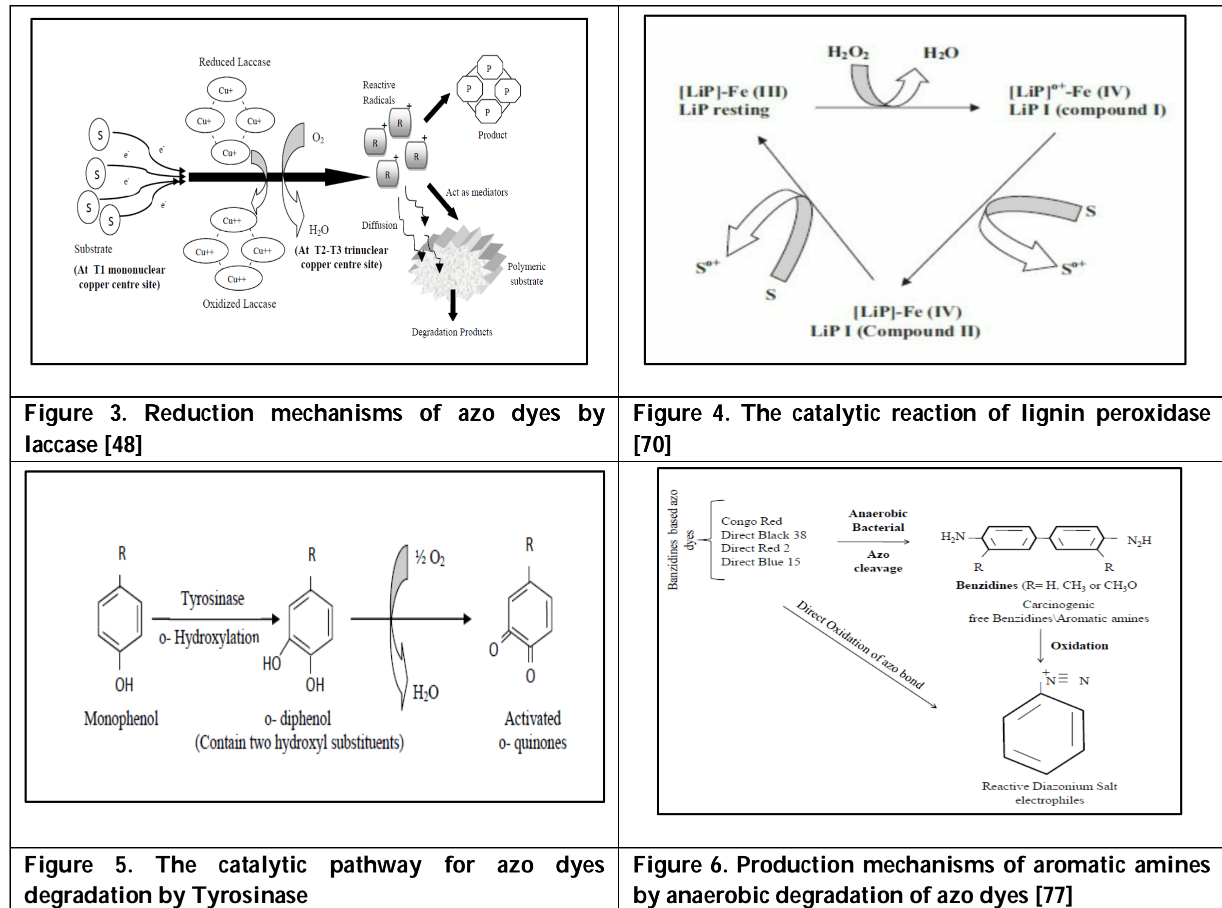

Figure 3. Reduction mechanisms of azo dyes by laccase [48]	Figure 4. The catalytic reaction of lignin peroxidase [70]
Figure 5. The catalytic pathway for azo dyes degradation by Tyrosinase	Figure 6. Production mechanisms of aromatic amines by anaerobic degradation of azo dyes [77]

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.10 / Issue 59 / April / 2020 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Optimization of Resistance Spot Welding Process Parameters using Taguchi Method

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Received: 12 Jan 2020 Revised: 16 Feb 2020 Accepted: 18 Mar 2020

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ABSTRACT

Effect and optimization of welding parameters on the tensile shear strength in the resistance spot welding process has been investigated experimentally and presented in this paper. The experimental studies took place under different conditions of the electrodes, time and current for welding. The welding parameter settings were calculated using L_{27} Orthogonal array system Taguchi experimental design. Through using the Signal-to-Noise (S/N) ratio analysis, the combination of the best welding parameters has been determined. The confirmation test performed clearly indicates that the combination of the correct welding parameters will improve the joint's tensile shear strength. Results obtained from experiment therefore prove the reality of the method used to improve reliable parameters in process welding operation.

Keywords: Resistance Spot Welding, Orthogonal Array, Taguchi Method, ANOVA, Spot Welding.

INTRODUCTION

The process of joining two metal parts by applying pressure is known as resistance spot welding. Through the two work parts heat is created from electrical resistance. In Resistance Spot Welding, two metals are joined by adding pressure in presence of electrical current and it is different from arc welding because no filler metal or fluxes are applied during the welding process to the welding area. Presents study emphasizes on the effect of the process parameters on tensile strength of joint for Stainless steel with the help of Taguchi method. Multi-variables inspired the Taguchi approach which is successful in dealing with responses. This approach significantly decreases number of experiments needed to model the response function against design of the experiment. The main advantage of this is to study how the parameters could interact. The force of welding is given by leg pedal. Electrode is squeezed into the metal part, the correct value of pressure required for the metal part is very critical for obtaining good weld quality

[16]. The amount of heat generated as: $Q = I^2Rt$, where Q = Heat (in joule), I = Current (in ampere) R = Resistance (in ohm), t= Time (in second).

Welding Parameters

Importance of welding parameters can be judged in various ways. Any minor change in one parameter will have effect on other parameters. Welding quality depends on these parameters. Suitable Weld factors will yield stronger joint and enhance qualitative nature various factors of welding are [16]: 1. Force on Electrode 2. Electrode diameter 3. Squeezing duration 4. Welding duration 5. Holding duration 6. Welding current. The cause and effect diagram constructed is specific for the Resistance spot process parameters. Fig.3 represents Ishikawa diagram to identify different process parameters that affects the welding strength.

Weld Material

Experiment conducted on 1.02 mm and 1.22 mm thick stainless steel sheets. The presence of carbon in stainless steel may increase the hardness and brittleness property of welded joints. Strength of joint may be increased by increasing welding current as the thickness of material increases. Specification of specimen: Length = 100mm, Width = 50mm

EXPERIMENTAL METHODOLOGY

Experimental methodology is most important in analyzing the overall process. Twenty seven runs have been carried out during experimentation. Current, welding time and force on electrode are changed as per value of each level given in Table-1. Each setting comprises three responses. Table-2. Represents various parameters taken during experiment.

RESULT ANALYSIS

Overall loss functions and signals to noise ratio

Loss function with consideration of large value for good qualitative output upon which tensile shear strength depends can be expressed as

$$L = k\left[\frac{1}{n}\sum \frac{1}{x^2}\right] \quad \text{.......(1)}$$

$$n = -10 \log_{10} \left[\frac{1}{n}\sum \frac{1}{x^2}\right] \quad \text{.......(2)}$$

Where n = No. of test, yi = Value of ith quality characteristic, Lj = Loss function, j ç = signal to noise ratio. Table 3 represents the experimental result for tensile strength.

Analysis of Variance

ANOVA outcomes for welding outputs are given in statistical manner; F-test gives a conclusion at sure level so as to calculate the significant difference. Greater F-data indicate that change of parameter creates a greater difference on performance.

Analysis for thickness of 1.02mm.

The regression equation is tensile shear strength = 25.7 - 0.540 A + 0.026 B + 0.164

CONCLUSION

The experimental results prove the validity of Taguchi method for improving the welding performance and optimizing the welding parameters in resistance spot welding. Strength of material in tension is found to be optimum as obtained from result. Optimum parameters are obtained for the extreme value of strength in tension by using Signal-to-Noise (S/N) ratio. Higher the thickness of weld specimen proves better result for welding strength and tensile stress.

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Table 1. Welding Parameters

Thickness (mm)	Symbol	Process parameters	Unit	1st Level	2nd Level	3rd Level
1.02	A	Force on Electrode	N	280	290	315
	B	Welding Current	Amp	80	85	95
	C	Welding duration	Cycle	5	10	15
1.22	A	Force on Electrode	N	312	332	345
	B	Welding Current	Amp	90	95	99
	C	Weld Time	Cycle	5	10	15

Table 2. Experimentation Using L27 Orthogonal Array

Experiment	Force on Electrode(Newton)	Welding Current(Ampere)	Weld Time(Cycle)
1	1	1	1
2	1	1	2
3	1	1	3
4	1	2	1
5	1	2	2
6	1	2	3
7	1	3	1
8	1	3	2
9	1	3	3
10	2	1	1
11	2	1	2
12	2	1	3
13	2	2	1
14	2	2	2
15	2	2	3
16	2	3	1
17	2	3	2
18	2	3	3
19	3	1	1
20	3	1	2

21	3	1	3
22	3	2	1
23	3	2	2
24	3	2	3
25	3	3	1
26	3	3	2
27	3	3	3

Table 3- Tensile strength results

Expt. No.	Tensile strength in kN	
	1.02mm	1.22mm
1	25.00	28.3
2	25.67	28.7
3	26.255	29.01
4	26.34	29.254
5	26.24	29.37
6	27.20	30.21
7	27.46	30.57
8	24.89	27.957
9	23.75	26.93
10	25.78	27.29
11	24.34	26.73
12	26.78	28.57
13	23.08	25.83
14	24.25	26.93
15	24.63	27.73
16	23.89	26.837
17	22.67	25.94
18	24.87	28.85
19	24.12	27.57
20	25.34	28.42
21	23.71	27.93
22	22.85	25.89
23	23.45	26.63
24	23.56	27.53
25	27.12	30.81
26	25.54	29.62
27	27.34	30.12

Table 4- Loss Function and S/N Ratio

Expt. No.	S/N Ratio(LB) for 1.02 mm	S/N Ratio (LB) for 1.22 mm
1	27.95	29.03
2	28.18	29.15
3	28.38	29.25

4	28.41	29.32
5	28.37	29.35
6	28.69	29.60
7	28.77	29.70
8	27.92	28.92
9	27.51	28.60
10	28.22	28.71
11	27.72	28.53
12	28.53	29.11
13	27.26	28.24
14	27.69	28.60
15	27.82	28.85
16	27.56	28.57
17	27.10	28.27
18	27.91	29.20
19	27.64	28.80
20	28.07	29.07
21	27.49	28.92
22	27.17	28.26
23	27.40	28.50
24	27.44	28.70
25	28.66	29.77
26	28.14	29.43
27	28.73	29.57

Table 5- ANOVA for S/N ratio [18]

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
A	2	0.37990	0.37990	0.18995	3.92	0.203
B	2	0.09102	0.09102	0.04551	0.94	0.516
C	2	0.87386	0.87386	0.43693	9.02	0.100
Residual Error	2	0.09687	0.09687	0.04844		
Total	8	1.44165				

Table 6. Model Coefficient for SN ratios [17]

Term	Coefficient.	SE Coefficient	T	P
Constant	27.9489	0.07336	380.975	0.000
A 1	0.2839	0.10375	2.737	0.112
A 2	-0.1954	0.10375	-1.883	0.200
B 1	0.0723	0.10375	0.697	0.558
B 2	-0.1422	0.10375	-1.371	0.304

C 1	-0.2723	0.10375	-2.624	0.120
C 2	0.4362	0.10375	4.204	0.052
S = 0.2201		R-Sq = 93.3%		R-Sq(adj) = 73.1%

Table 7. Response Table

Level	A	B	C
1	28.23	28.02	27.68
2	27.75	27.81	28.39
3	27.86	28.02	27.78
Delta	0.48	0.21	0.71
Rank	2	3	1

Table 8. Regression Analysis: tensile shear strength versus A, B, C

Predictor	Coef.	SE Coef.	T	P
Constant	25.739	1.224	21.03	0.000
A	-0.5397	0.3439	-1.57	0.130
B	0.0264	0.3439	0.08	0.939
C	0.1636	0.3439	0.48	0.639

Table 9. Analysis of Variance for SN ratios (www.jmrt.com.br)

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
A	2	3.1768	3.1768	1.5884	4.32	0.188
B	2	0.7903	0.7903	0.3951	1.07	0.482
C	2	7.2901	7.2901	3.6451	9.91	0.092
Residual Error	2	0.7359	0.7359	0.3679		
Total	8	11.9931				

Table 10. Model Coefficients for SN ratios

Term	Coef.	SE Coef.	T	P
Constant	25.0391	0.2022	123.836	0.000
A 1	0.8215	0.2859	2.873	0.103
A 2	-0.5635	0.2859	-1.971	0.188
B 1	0.1826	0.2859	0.639	0.588
B 2	-0.4180	0.2859	-1.462	0.281
C 1	-0.7930	0.2859	-2.773	0.109
C 2	1.2587	0.2859	4.402	0.048
S = 0.6066		R-Sq = 93.9%		R-Sq(adj) = 75.5%

Table no .11- Response Table

Level	A	B	C
1	29.21	28.96	28.78
2	28.67	28.83	29.27
3	29.01	29.11	28.85
Delta	0.54	0.27	0.49
Rank	1	3	2

Table no.12- Regression Analysis: tensile shear strength versus A, B, C

Predictor	Coef.	SE Coef.	T	P
Constant	27.963	1.240	22.55	0.000
A	-0.3212	0.3485	-0.92	0.366
B	0.2841	0.3485	0.82	0.423
C	0.1211	0.3485	0.35	0.731

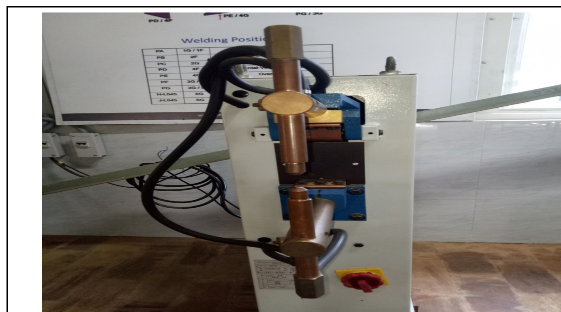

Figure 1 Resistance Spot Welding

Figure 2 Resistance Welding Machine Circuits

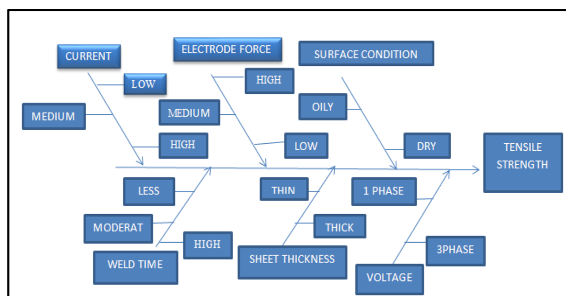

Figure 3. Ishikawa Cause and effect diagram

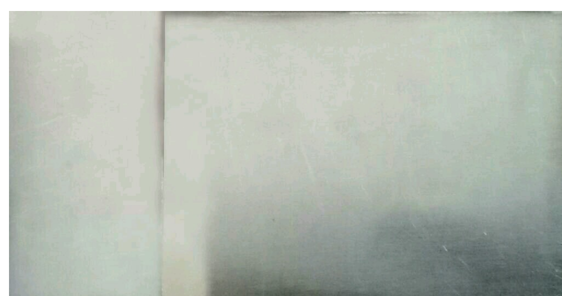

Fig 4. Work piece before welding

Fig 5. Workpiece set up

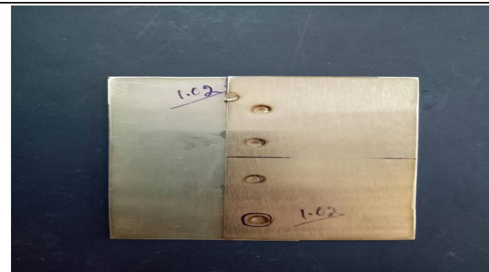

Fig 6. Workpiece after Spot

Fig 7. Workpiece after Spot Welding of Thickness

Fig. 8. Tensile stress check of the workpiece

Fig. 9. Welded workpiece specimen of 1.02 thickness

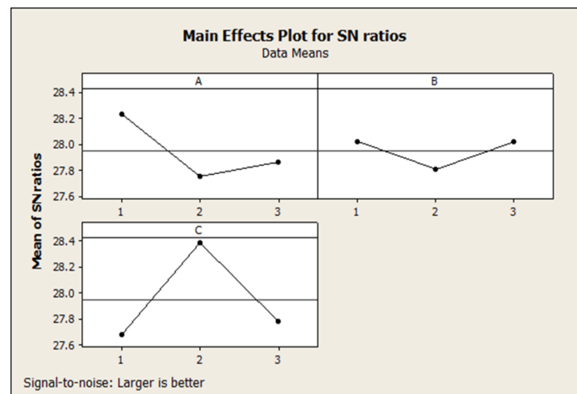

Fig. 10. Main effect plot for SN ratio

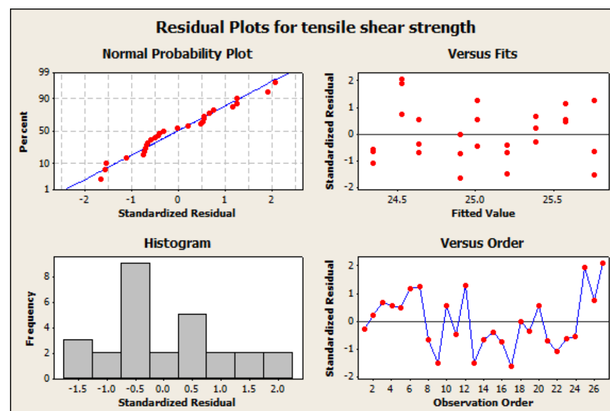

Fig 11. Regression analysis residual plots

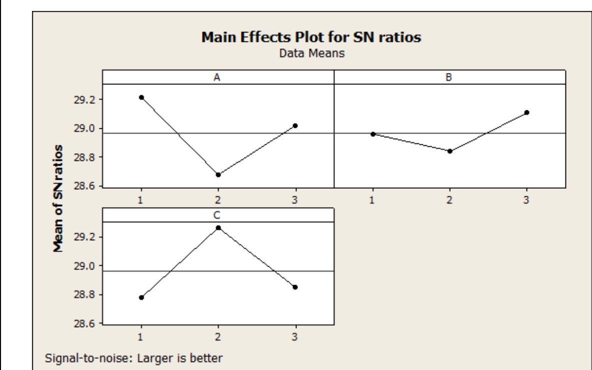

Fig 12. Main effect plot for SN ratio

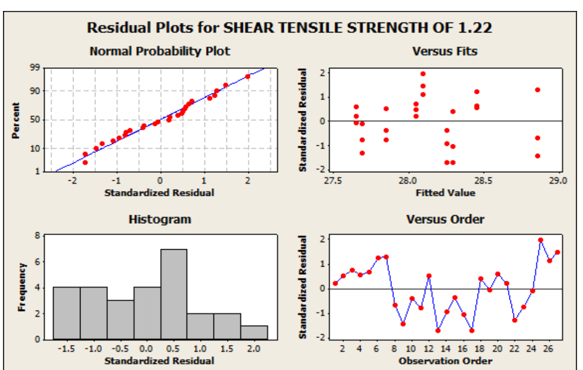

Fig.13 Regression analysis residual plots