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Frontiers in Life Science

Volume II

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PREFACE

Life Sciences have always been a fundamental area of science. The exponential increase in the quantity of scientific information and the rate, at which new discoveries are made, require very elaborate, interdisciplinary and up-to-date information and their understanding. Enhanced understanding of biological phenomenon incorporated with interdisciplinary approaches has resulted in major breakthrough products for betterment of society. To keep the view in mind we are delighted to publish our book entitled ***"Frontiers in Life Science Volume II"****. This book is the compilation of esteemed articles of acknowledged experts in the fields of basic and applied life science.*

This book is published in the hopes of sharing the new research and findings in the field of life science subjects. Life science can help us unlock the mysteries of our universe, but beyond that, conquering it can be personally satisfying. We developed this digital book with the goal of helping people achieve that feeling of accomplishment.

The articles in the book have been contributed by eminent scientists, academicians. Our special thanks and appreciation goes to experts and research workers whose contributions have enriched this book. We thank our publisher Bhumi Publishing, India for taking pains in bringing out the book.

Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

- Editorial Team

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CONTENTS

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HABITAT, THREATS AND FACTORS AFFECTING HOUSE SPARROW (*PASSER DOMESTICUS*) SURVIVAL IN BARMER DISTRICT, RAJASTHAN, INDIA

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Abstract:

The house sparrow is present everywhere and inhabited in almost all types of environment like rural area, urban areas, forests, woodland and cultivated land. Presence and population of these birds has been declined drastically in urban and as well as rural areas due to lack of appropriate nesting sites, roosting and feeding habitat. Predation by domestic cats, feral dogs and snakes, excessive use of insecticides and pesticides, food scarcity, nestling mortality, environmental pollution, electromagnetic radiations and avian diseases are responsible for dramatically declining house sparrow population at global level. Cutting of large and old trees reduced availability of cavity and hole in stems has also reduced nesting habitat of house sparrow in urban areas. Immense road trafficking and industrial areas has increased the risk of collision of birds with fast moving vehicles which has also contributed to increased mortality rate in house sparrow's particularly in urban area. Initiatives related to sparrow saving action plan (SSAP) at different levels like town, village, colonies and city can be useful for conservation of house sparrow population and habitat, similarly organization of awareness program among public and community level may also help in saving the current population of sparrows and further increase in sparrow population in near future while providing suitable nesting sites and feeding opportunities to them.

Keywords: House sparrow, habitat, decline, threat, nesting, population, conservation.

Introduction:

Birds are an essential and important part of our ecosystem and food webs. House sparrows presence is an important sign of good quality habitat. These birds are very susceptible to change in the environmental conditions. They act as a bio indicator species for healthy urban ecosystems and indirectly represent status of human health in its surroundings. They perform vital functional role in food chain and food web of terrestrial ecosystem.

House sparrow is closely related and inhabited near human dominated landscape, cultivated areas, forest and urban areas and provides large and different kinds of value in ecosystem like recreational value, economical and aesthetic value (Ghosh *et al.,* 2010). Presence of house sparrow near human dominated habitat can be seen in various places such as gardens, rural areas, colonies, suburban areas, parks, agricultural land, feedlots and granaries, which are well studied at global level by different researchers (Louther and Cink, 1992; Monika, 2005; Sharma, 2009; Balaji, 2014; Bavia *et al.,* 2014 and Kamath *et al.,* 2014;). At global level house sparrow population decline was recorded to be highest in London (60%), Glasgow (99%) and Hamburg (77%) and is categorized as threatened under red list species (Crick *et al.,* 2002; Prowse, 2002 and Smith, 2005). Natural predator like hawk, eagle, black kite and owl hunts and feed upon house sparrows. Domestic cats are significant predator and are also responsible for dramatic decline of house sparrow population worldwide (Churcher and Lawton, 1987). House sparrow populations were also adversely affected by epidemic avian diseases (Menegaux, 1919-1921 and Stenhouse1928) considering the fact that such type's diseases and declination in population are limited in particular or specific areas.

Increasing anthropogenic activities have been modified and converted the natural habitats into urban areas which had accelerated the habitat alteration rate and vanishing of natural vegetation (Marzluff *et al.,* 2001; 2008). Urbanization exposes animals to potentially critical survival factors adversely such as human generated disturbances, pollutants, artificial lightening, and scarcity of food which are also responsible for biodiversity decline (Shochat *et al.,* 2006). The House sparrow is primarily a seed eater bird found in almost all habitats and mostly seed availability occurs in cultivated area of rural landscape, but they also show wide variety of food preferences and choices like grains, seeds, insects, spider, nectar, caterpillars, human made food like wheat and *bajara* breads and digestible materials from cow dung (Yahanghi *et al.,* 2010). Food shortage can also affect individual fitness and survival rate of house sparrow species which results into breeding failure due to poor development and malnutrition (Newton, 1998). Extension of industrialization, civilization and human settlement leads to lack of foraging site, feeding habitat and nesting places in cities which has largely contributed in declination of house

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sparrow population (Cram *et al.,* 1995; Rao, 2000; Summer & Smith, 2003; Robinson *et al.,* 2005; Pineda *et al.,* 2013). In the present chapter we discuss about habitat, behavioural biology, threats and conservation issues related to house sparrow's in Barmer district of Rajasthan, India from January, 2016 to December, 2020.

Materials and Methods:

The present study was carried out in different microhabitat of Barmer district of Rajasthan, India from January, 2016 to December, 2020. The geographic location of Barmer district is located between 24.58' to 26, 32'N latitudes and 70, 05' to 72, 52'E longitudes. The average temperature is high and show large fluctuations in various seasons and as well as day and night. In summer season temperature reaches up to 46°C to 51°C and in winter it drops to 0° C and annual precipitations is very low approximately about 277mm. Scheduled visual observations were done with the help of binoculars (Nikon 8x40)and accordingly photographs were clicked with the help of Nikon P900 and P1000 cameras. Data were collected from various habitats of house sparrows like- cultivated land, urban area, rural area, grasslands and forest area. Observation hours were set in early morning hours from 6.00am to 9.00 am and in evening hours from 4.00pm to 7.00pm when house sparrows were comparatively more active as compared to rest of the hours of the day.

Result and Discussion:

Distribution and population

House sparrow's (*Passer domesticus*) are widely distributed in all types of habitat throughout Indian subcontinent and other parts of the world (Blair, 1999). In context of Indian subcontinent they are generally found in all states of India and as an introduced population in Andaman Island (Ali and Ripley, 1987). During survey maximum house sparrow populations were recorded in rural area as compared to urban landscape out of different study areas. Cultivated lands of rural areas provide suitable habitat, nesting material (Figure 1, 2, 3) and more availability of food material as compared to urban areas.

Morphology and systematic position

House sparrow belongs to the Passerine family and is a small, stock song bird with chunky bill, short legs, length approximately 14-16 cm, weight 26-32 gm and having 19-25 cm open wing span. House sparrow are monogamous pairing for long time during breeding season, sexual dimorphism is distinct and well developed and male and female can be easily identified

and differentiated by necked eye (Anderson, 2006)(Figure 4,5). While males have a grey crown, a chestnut back and neck, wings having black tip, white checks and grey rump and tail, the females are relatively uniform pale and light grey coloured with dark spikelet wings. Male is heavier and larger in size as compared to the female house sparrow's (The House Sparrow- *Passer domestics*, 2007).

Taxonomic position

Kingdom- Animalia

Phylum- Chordata

Class- Aves

Order- Passeriformes

Family- Passeridae

Genus- *Passer*

Species- *domesticus*

Habitat and feeding behaviour

The house sparrows are unique wild birds and closely associated with nearby human settlements like houses, agricultural land and both rural areas as well as urban areas (Crick *et al.*, 2002). House sparrow usually feed upon Oats, Wheat (Figure 6), *Bajara* or *Bajari* (Figure 7), Corn, Sorghum and other major sources of seeds and on other seasonal and perennial herbs such as grasses (Graminae), rushes (Juncidae) and goose foot (Chenopodium).They also feed upon fruits and beery like Pillu (*Salvodora persica*) (Figure 8), Ber (*Ziziphus mauritiana*) and other edible fruits and seeds. House sparrow prefers more insects in diet during breeding season which plays a potential role in early development of nestling and enhance breeding success rate in these birds (Figure 9). House sparrow commonly feed upon honey bees, wasps, ant, componotus, aphids (Aphidoiea), spiders (Arachnida), beetles (Coleoptera), weevils (Curculionidae), grasshopper (Orthoptera) and caterpillars of butterflies and moths (Lepidoptera) (Wilson *et al.*, 1999). In summer season house sparrow eat insect and feed them to their nestling. House sparrow catches insects in air by sudden jumping and flying on them during more active feeding hours of early morning and evening time and also feeding with other birds (Figure 10).

Nesting and breeding behavior

House sparrow build their nest on various nesting sites for example high on walls of house, roof spaces, trees and hole of trees, other safe and undisturbed locations of cemented house, cottage in both urban and rural habitat (Figure 11,12). Mostly they construct their nests in roof spaces of hut, cattle shades, wooden fencing, mud houses and tree cavities in rural areas (Figure 13, 14). Sometime unexpected nests were also observed at electrical transformers and

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electric meter boxes, safe and undisturbed terminal of various pipes and in between bricks and stones (Figure 15, 16). House sparrows are monogamous and pairs in breeding season and in small colonies while feeding in flock and sometimes individually (Figure 17). They breed in the months of September to October in central India, March to June in Northern India and throughout the year in southern India. House sparrows generally laid four to five eggs and minimum clutch size is two eggs. Eggs are bluish white or greenish white with grey or brown spots. The incubation period of house sparrow ranges from 10 to 12 days.

Threats and conservation problems

House sparrow population rapidly declined in urban area and rural area due to lack of suitable breeding or nesting sites, food scarcity, invasion of exotic species plant and animal species, establishment of cemented houses and buildings and various petrochemical and other industries. At present house sparrow population and abundance had dramatically declined in metropolitan cities like Bangalore, Mumbai, Hyderabad and New Delhi (Rajasheker and Venkatesha, 2008). Another reason for their population declination includes excessive use of hazardous and noxious compounds such as insecticides and pesticides used in cultivation of crops and gardens. These chemicals are responsible for breeding failure and mortality of sparrows and other birds. House sparrow population also declined due to competition with other species for food, roosting and nesting places and excessive predation by domestic cats and sometime feral dogs in urban areas (Figure 18, 19). Many studies had been conducted on status and trends of house sparrow population declination in India (Summers and Smith, 2003). Also sparrow and other bird population had declined due to environmental stress badly affected by various types of pollutions (Baker *et al.,* 2005 and Shaw, 2009). Reduction in arthropod diversity might also be responsible for population declination of insectivorous birds. Because insects perform crucial role as a food material during developmental stages of breeding season (Peach *et al.,* 2008). In urban area sparrow populations also declined due to scarcity of food material and inter specific and intra specific competitions with other birds for nesting and roosting places. House sparrow generally compete for food with red vented bulbul, yellow vented bulbul, feral pigeons, babblers, robins and other frugivorous and insectivorous birds.

Factor responsible for nestling and adult mortality

Nestling and adult house sparrows died due to attacking of predators such as crow, *shikra*, black kite, hawks, eagles and owls. Sometime nestling and adult died and injured due to collision with plastic threads and anthropogenic materials in urban areas. House sparrow population also declined due to low rate of hatchling and nestling success in urban and rural areas. Inter specific and intra specific competition for nesting places is responsible for breeding

failure among birds. Several toxic compounds are now continuously increasing in natural environment like pesticides, insecticides, heavy metal contamination in food chain, industrial and sewerage waste, combustion and emission of fuels or gases are creating unfavorable habitat for biotic life including human beings. Some of these toxic chemical and pollutants adversely influence and are responsible for population decline of birds at global level including house sparrows (Newton, 1998). Multiple factors are responsible for the cause of the decline of house sparrow population in urban, semi urban, industrial area and rural areas. Contaminated water and chemically treated grains caused poisoning and mass mortality of house sparrows and other granivore birds. The stress from growing human activities such as expansion of civilization, construction of cemented houses and buildings, establishment of various industries, habitat fragments and alteration, deforestation, excessive use of insecticides and pesticides, mobile tower radiations and spreading of invasion species, over abundance and congested spaces affected house sparrow population and survival rate adversely.

Effects of urbanization and environmental factors

Expansion of urbanization becomes limiting factor and causes negative impact on growth of nestling, body size and feather quality of house sparrow (Meillere *et al.*, 2017) and hence poor growth and development. Electromagnetic radiations also affected house sparrow fitness and survival among urban habitats in a declining state. Modern designs of house and building infrastructure do not leave any space or cervices even outside the building which has reduced the nesting sites of house sparrows and other small birds (Figure 20). Environmental and climatic conditions like seasonal factors such as annual precipitation, temperature, humidity, wind velocity, time of dawn and dusk has also influenced the population density of house sparrows. High wind velocity also responsible for breeding failure due destruction of nests and fallen of eggs on ground (Figure 21).

Conservation recommendations for saving house sparrows:

Artificial and wooden boxes for nests are useful in conserving house sparrow population by providing suitable habitat for nesting and breeding success particularly in urban areas. Enhancement and establishment of huts with the help of tree branches and grass material are also helpful for enhancing sparrow population. Similarly mud houses also hold large number of nests of house sparrows. Encourage low level of pesticide and insecticide use in cultivated land, gardens and parks also reduces bird's mortality rate. Public awareness and conservation program organized in schools, colleges and villages are also helpful for better conservation measures for house sparrows in study area. Further scientific and genetic studies at population level and regional level are required in determining factors and cause of rapid declination of this bird species *Passer domesticus* in current scenario.

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Figure 1: Male house sparrow collect nesting material bark of *Azadirachta indica*

Figure 2: Male house sparrows collecting nesting material from huts (*Leptadenia pyrotechnica* herbs)

Figure 3: Male and female house sparrow collect nesting material from *Crotalaria burhia* plant

Figure 4: female nestling of House sparrow

Figure 5: Morphological difference between male and female house sparrow

Figure 6: Female house sparrow feeding on wheat bread (Indian bread or rotti)

Figure 7: Male house sparrow feed upon grains of *Pennisetum glaucum*in rural area

Figure 8: Female house sparrow feeding fruit of *Salvodora persica*

Figure 9: Male house sparrow capture caterpillar

Figure 10: House sparrow communally forage with seven sister babblers

Figure 11: House sparrow nesting on *Prosopis cineraria*

Figure 12: House sparrow nesting in urban area at cemented teen shade

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Figure 13: House sparrow nesting in huts of rural area

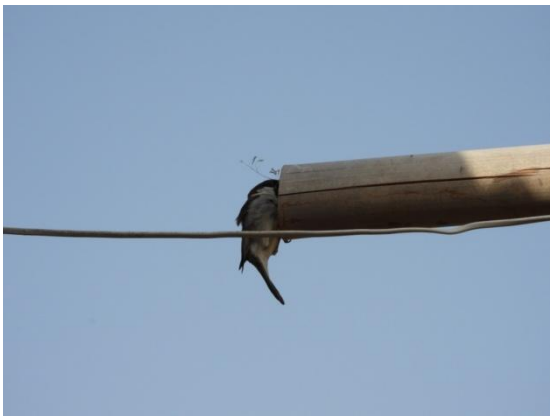

Figure 14: House sparrow construct nest inside stem of *Bambusa vulgaris*

Figure 15: House sparrow nesting inside electric transformer

Figure 16: House sparrow nesting inside electric meter box

Figure 17: Female house sparrow forage and feeding near cattle shade

Figure 18: Domestic cat movement around sparrows' habitat

Figure 19: Domestic cat movement in houses of urban areas

Figure 20: Lack of safe nesting site house sparrows egg fallen on ground and broken

Figure 21: Due to high wind velocity sparrow nest scattered and eggs fallen on ground

Conclusion:

The house sparrow is gregarious at all season and live in flock or groups and feed upon grains, seeds, insects, fruits, berry, insects, kitchen food and flower buds in rural and urban habitat. Abundant population of domestic cats and feral dogs has also reduced the house sparrow population including adults and nestling. Lack of suitable nesting sites for house sparrows and other small birds with other factors may be responsible to shift this bird species towards extinction in near future. House sparrow population has majorly declined due to extension of civilization areas at the cost of degradation of natural environment and lack of nesting places at all the levels. Organization of house sparrow conservation and saving programs among community, villages, farmers, might be helpful for saving this beautiful neighbor of humans, the house sparrows from extinction.

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THE EFFECT OF ENDOSULFAN ON OXYGEN CONSUMPTION OF FRESH WATER FEMALE CRAB *BARYTELPHUSA GUERINI*

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Abstract:

Respiration is a process during which the organisms obtain oxygen from external medium and use it for the purpose of energy release during oxidative metabolism. As such the process of respiration in animals is studied by determining the oxygen consumption. Existence of living organisms depends on the ability of their cells to incorporate a number of simple compounds and transform them into more complex molecules required for cellular structure and function. The energy required for these synthetic reactions must be obtained from the same substrates by suitable oxidation reactions coupled to the generation of high energy phosphate esters. The entire process ultimately depends on the availability of molecular oxygen in cellular environment. Oxygen is made available to the tissues and in turn to cells by respiration. The present study deals with oxygen consumption, when experimental animal fresh water female crab *Barytelphusa guerini* is exposed in the pesticide like Endosulfan. It shows variable changes in respiratory physiology which discussed by statistically and graphically.

Keywords: Endosulfan, Oxygen consumption, Female Crab, *Barytelphusa guerini*.

Introduction:

The chlorinated hydrocarbons are basically organic compounds that have been chlorinated with several atoms of chlorine per molecule. These insecticides have very low solubility in water but are readily soluble in fats. These compounds are chemically stable and show considerable persistence upon introduction into physical environment. Since these compounds are stable and persistent, they are referred to as "hard pesticides" (Abbot *et al.*, 1966). Some examples of these pesticides are DDT, Lindane, Heptachlor, Mirex, Chlordane,

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Aldrin, Methoxychlor, Dieldrin, endosulfan, Toxaphene etc. These compounds exhibit biological magnification in the food chain (Macek, 1969).

Organochlorine pesticide action was said to be neurotoxin. They were found to create energy crisis by inhibiting Mg2+ ATP ase and aerobic segmental enzymes of the brain (Philip, 1984). The effects of various organochlorine compounds on different animals (like rat, fish, chicken and mice) and concluded that these compounds disrupt ATP dependent active transport. ATP activity is perhaps disrupted by the uncoupling of oxidative phosphorylation, have studied (Rangaswamy, 1984).

It has been reported that respiratory distress is one of the common symptoms manifested during organochlorine insecticide toxicity (Sathyaprasad, 1983). It has reported that the rate of decrease in oxygen consumption of fish increase with increase in the concentration of pesticides in the Biosystem.

BHC produced a significant increase in haemolymph sugar, protein; ICDH, SDH and LDH in the crab, *Ozitelphusa senex senex* (Basha *et al.,* 1984) have observed inhibition of oxidative metabolism in *Tilapia mossambica* during sublithal exposure to Lindane. Glycogenolysis and Gluconeogenesis were found to be elevated in the carp, *Cyprinus carpio*, exposed to Aldrin, Dieldrin and Endrin (Gluth and Hanke, 1985).

Material and Method:

Oxygen consumption was determined by Wrinkler method (Welsh and Smith, 1953). The freshwater female crab *Brytelphusa guerini*was used for experimentation. The animals were collected form their natural habitat and maintained in the laboratory before experimentation. The proper selection of animals were made for present investigation and procedure was followed for the exposure of animal with pesticide pollutant i.e. Endosulfan.

The apparatus used in this experiment was similar as described by Saroja. The apparatus mainly consisted of a reservoir (R) and respiratory chamber (RC). A 500ml wide mouthed bottle was used as respiratory chamber. The size of the bottle was such that it was not too big for the enclosed crab to give considerable difference in oxygen content between initial and final sample. The chamber was coated with black paint to avoid the activity due to light.

Before starting the experiment crab was left in running tap water for about 10 minute to facilitate them to reach a state of normality from a state of excitement; if any. After this equilibration period, one crab was kept in respiratory chamber without causing any damage to the animal and initial sample was collected immediately as described above. Then the crab was

allowed to respire for one hour. Immediately after one hour final sample was collected. The amount of dissolved oxygen in this sample was determined by the standard Winkler's method, as given by.The total oxygen consumption and rate of oxygen consumption was calculated by considering the wet weight of animals. The values for total oxygen consumption are expressed as ml. (c.c.) of O2 animals / hr and for the rate of O2 consumption are expressed as ml. (c.c.) of 02 / gm / hr wet wt. of the animal.

Result and Observation:

Effect of Endosulfan on Total Oxygen Consumption and Rate of Oxygen Consumption in Freshwater Female Crab *Barytelphusa guerini*

Effect of Endosulfan causes changes in total oxygen consumption. Total oxygen consumption expressed in ml/lit., is the average of six observation ± S.D.

Table 1: Effect of Endosulfan on total oxygen consumption in female crab *Barytelphusa guerini*

Sr. No.	Duration of Exposure	Control	Experimental
1	24	1.28 ± 0.072	0.74 ± 0.076***
2	48	1.23 ± 0.069	1.78 ± 0.032**
3	72	1.19 ± 0.072	1.35 ± 0.057***
4	96	1.33 ± 0.072	0.70 ± 0.057***

Note: 1) Values expressed as Beats/min. of animals. 2) Each value is mean of six observations ± S.D.
3) Value are significant at * = P<0.05, ** = P < 0.01, ***=P < 0.001 & NS – Not significant

Effect of Endosulfan causes changes in rate of oxygen consumption. Rate of oxygen consumption expressed in ml/hr/gm wt. /lit., is the average of six observation ± S.D.

Table 2: Effect of Endosulfan on rate oxygen consumption in female crab *Barytelphusa guerini*

Sr. No.	Duration of Exposure	Control	Experimental
1	24	0.035 ± 0.0019	0.023 ± 0.0024**
2	48	0.033 ± 0.0018	0.053 ± 0.0009**
3	72	0.030 ± 0.0018	0.041 ± 0.0017*
4	96	0.032 ± 0.0017	0.021 ± 0.0017**

Note: 1) Values expressed as Beats/min. of animals. 2) Each value is mean of six observations ± S.D.
3) Value are significant at * = P<0.05, ** = P < 0.01, ***=P < 0.001 & NS – Not significant

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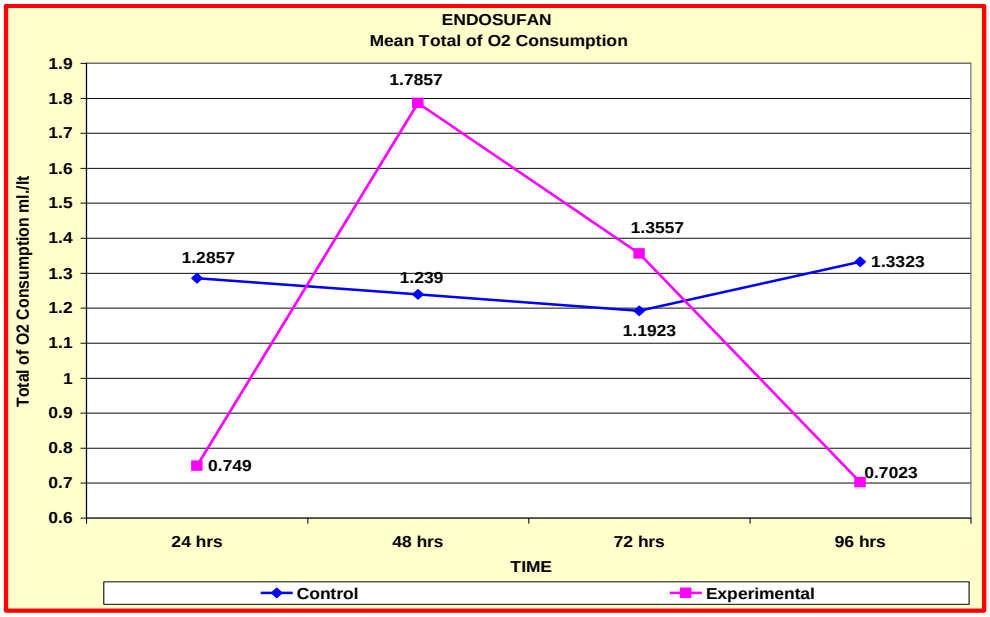

Figure 1: Effect of Endosulfan on Total Oxygen consumption in *Barytelphusaguerini*

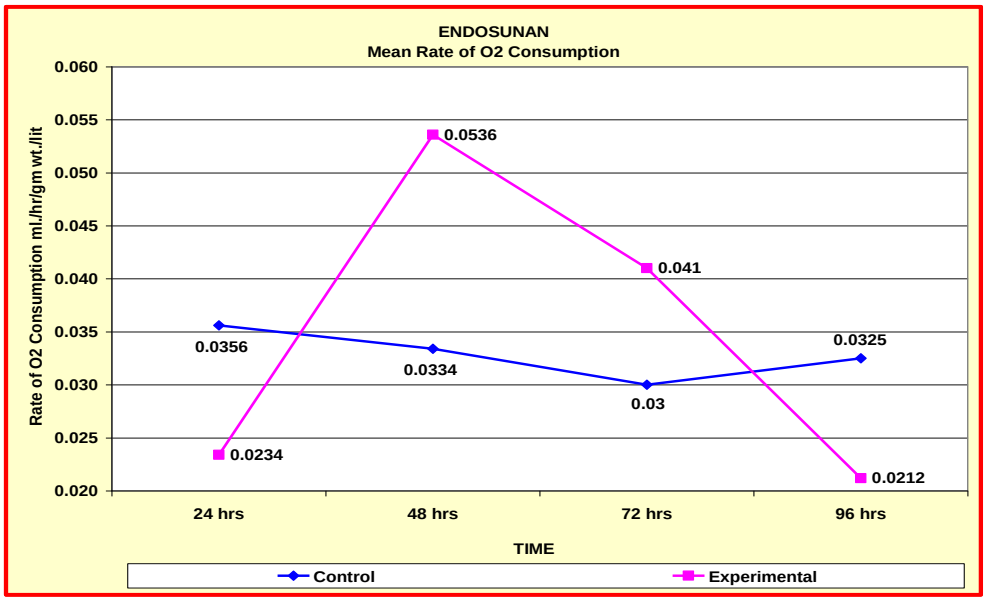

Figure 2: Effect of Endosulfan on Rate of Oxygen consumption in *Barytelphusa guerini*

Result:

- The freshwater female crab *Barytelphusa guerini* showed variation in the Rate of oxygen consumption and Total oxygen consumption when exposed in endosulfan.
- In the present investigation it was showed that the oxygen consumption in the animal exposed to Endosulfanwas decreased upto 96 hours. The Endosulfan exposed animals

showed initially decreasing at 24 hours while increased rate of respiration at 48 hours and then gradually decreased in oxygen consumption upto 96 hours as compared with control.

- **Effect of Endosulfan:**

  The oxygen consumption was studied in the present investigation with the effect of Endosulfan. It shows a sudden increase & deeply decreasing graph. It exhibits a decreasing trend of respiration upto 96 hours as compared to control. As shown in Table (1 and 2) and Figure (1 and 2).

Discussion:

In present investigation female crab *Barytelphusa guerini* exposed to sublethal concentration of endosulfan for a successive intervals of 24, 48, 72 and 96 hours.

The change in rate of oxygen consumption is a good index of the metabolic capacity of an organism to face environmental stresses. In this experiment, it was observed that the rate of oxygen consumption was increased initially up to 24 hours and then there was decline in oxygen consumption rate in sublethal level compared to control. Increase in rate of oxygen consumption indicate the mobilization of metabolic reserve and hence an increased demand for oxygen in response to pesticidal stress. The freshwater female crab.*Barytelphusa guerini* exhibits decrease in the rate of oxygen consumption on exposure sublethal concentrations of above pollutants. This decline form of graphical representation indicates that a long term exposure upto 96 hours in the pollutants causes decrease in osmotic work of the animals at cellular level resulting in reduced oxygen consumption. At specific periods of exposure, animal shows slight elevation in the rate of oxygen consumption with different inorganic pollutants.

In present experiment the significant increase in the oxygen consumption was observed. This result may be due to the pollutants after prolonged time of exposure. Similar observation made by Gangshettiwar (1986) on freshwater prawn *Macrobranchium lamerri* after exposure to phenol.

It is evident from the obtained results that the interpretation of influence changes in respiration is some what complicated by the fact that such as alteration differ from toxicant to toxicant, from species to species and from one experimental condition to another. Thus pollution act as physiological stress as do the altered environmental parameters, Newell (1973), some workers has pointed out the changes in normal respiration is due to its intimate contact with polluted water which cause. Some pathological changes and decrease the oxygen diffusing capacity of the gills. They also observed massive mucous secretion occurring and this heavy

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mucous secretion and coagulation may cause breathing distress to the animal due to precipitation of mucous over the gills.

Several authors have reported decrease in animal oxygen consumption of different freshwater species exposed to different insecticides. Reddy and Gomathy (1977) reported decrease in animal oxygen consumption of *Mystus vittatus* treated with sublethal concentration of endosulfan. Subhadra Devi (1985) observed a significant decrease in animal oxygen consumption of *Oziotelphusa senex senex* exposed to sublethal and lethal concentrations of endosulfan.

Conclusion:

- All organisms require oxygen for their metabolic activates. Generally metabolism of an organism is measured by estimating oxygen consumption. The rate of oxygen consumption of an intact animal and its tissues reflects the respective metabolic rates and hence energy output. It also provides information on the ability of the organism to extract oxygen from the environment.

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OYSTER MUSHROOM: CULTIVATION, BIOACTIVE SIGNIFICANCE AND COMMERCIAL STATUS

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Abstract:

Mushrooms have been included in the category of tasty and nutritional food, by different societies worldwide. Mushroom is the choice of many gourmet persons due to its delicious taste and health benefits. Mushrooms seem to be an important cultural heritage as they have been used from the time immemorial. The knowledge of its edible species and medicinal properties has been transmitted from one generation to another**.** Their diversity, habitats, and properties have been reported in folk literature. Thus there is a relationship between mushrooms and man from the ancient time. Supply of a sufficient protein content as body-building material is necessary in a healthy diet which can be fulfilled by the inclusion of mushrooms in diet since they are nutritive with good quantity of vital proteins, vitamins and minerals. Hence mushrooms have been recommended for obese persons and diabetes patients because of low caloric value. Mushrooms exhibit health-promoting properties viz. antimicrobial, antioxidant and immunomodulating activities. These afore-mentioned properties have recognized in some countries, especially in China and now China is the top leader in the production of cultivated mushrooms. *Lentinula* is the first major genus, contributing in the world's cultivated mushrooms, whereas *Pleurotus* is a close second, with its five or six cultivated species. Research interest in mushroom is increasing day by day as many bioactive compounds and chemical constituents have been reported from mushrooms. In this review chapter, we have focused on cultivation, bioactive significance and commercial status of oyster and some other mushrooms.

Introduction:

Mushroom cultivation is the gaining prominence worldwide because of a natural source of dietary food protein. Mushroom cultivation is unique in the sense that it is the most efficient and economically valuable technology for conservation of ligno-cellulosic material into a great quality of protein containing food. Mushroom cultivation is a side business of many farmers (Maher, 1991). Of the many species, oyster mushroom is the most suitable for cultivation by utilizing various agro wastes without composting. Oyster mushroom is generally known as the wood fungus and in India commonly known as Dhingri. Oyster mushroom is scientifically known as *Pleurotus* sp. Oyster mushroom was first described scientifically in 1975 by a Dutch naturist Nikolaus Joseph Freiherr von Jacquin (1721-1817) and named *Agaricus ostreatus*. Later on, these species of oyster mushrooms were included in the genus *Pleurotus* by a German mycologist Paul Kummer in 1871 (Stamets 1993). This species has been classified as kingdom-fungi, phylum-basidiomycota, class-agaricomycetes, order-agaricales, family-pleurotaceae and genus-*Pleurotus*. There are about thirty eight species described under genus *Pleurotus* from different part of the world, out of which 25 species are under cultivation. Some commercially important oyster mushrooms predominantly include *Pleurotus ostreatus*, *Pleurotus eryngii*, *Pleurotus flabellatus*, *Pleurotus sajor-caju*, *Pleurotus eous*, *Pleurotus florida*, and *Pleurotus cornicorpae. Pleurotus sajor-caju* is commonly called grey oyster. *Pleurotus florida* is popularly called white oyster. *Pleurotus eous* is generally known as pink oyster (Smith *et al.,* 1993; Raman *et al.,* 2020). The external appearance of oyster mushrooms have been characterized by the presence of a spatula shaped cap called pileus. This is fleshy part. Fruiting body of oyster mushroom has a stalk, which may be short, long, lateral or central. This stalk is called stipe. An interesting and attractive feature of oyster mushroom is presence of long ridges and furrows underneath the pileus which is called gills or lamellae. The gills bear the germinating-spores which help in reproduction of oyster mushroom. These spores are characterized as smooth and cylindrical which can germinate on any type of mycological media within incubation period of 48 to 96 h. The mycelium color of *Pleurotus florida*, an oyster species, is purely white (Chitra *et al.,* 2018). Evolutionary connection among species in the genus *Pleurotus* is still not clear and many taxonomic issues remain controversial. The genus *Pleurotus* is highly diverse since its many species have been characterized and identified. They show a general life cycle of basidiomycetes (Stamets and Chilton, 1983). Taxonomy and cultivation methods of *Pleurotus* species have been described by Oloke and Adebayo (2015).

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Cultivation and growth parameters:

Flank (1917), cultivated the oyster, *Pleurotus ostreatus,* simply on experimental basis on tree stumps and wood logs in Germany. Then, cultivation of diverse varieties of oyster mushroom was initiated in India. Mushrooms can be cultivated under different culture techniques viz. log culture, mound (bed) culture, column culture, bag culture, rack culture and tray culture (Stamets and Chilton, 1983). The procedure for oyster mushroom cultivation can be divided into following four steps viz. preparation of spawns, substrate preparation, spawning of substrate and crop management. Spawns can be purchased from agro companies or can be prepared from pre-existing pure culture of mushroom. Large volumes of rice and wheat straw are produced as agricultural by-products that can be used as substrates for mushroom cultivation. Substrates need to be sterilized by chemical method or moist heat sterilization technique prior to use. Oyster mushroom can grow at moderate temperature ranging from 20 to 30 ^{o}C and humidity 60-80% up to 6 to 8 months of monsoon and winter in a year. It can also be cultivated in summer months by providing the extra humidity required for its growth. In an average, about 600 kg fresh oyster mushrooms can be yielded by using 100 kg of wheat and paddy straw in 2 months (Uddin *et al.,* 2011).

Bioactive significance:

The health-promoting properties of mushrooms have long been recognized in some countries, especially in China. Application of modern analytical techniques has identified various mushroom-derived compounds, polysaccharides and tri-terpenoids for example, which exhibit a wide range of medicinal properties including immuno-enhancing, anti-tumor, antiviral and hypocholesterolemic activities (Wasser 2010). It has been proved that mushroom nutriceuticals help to boost immunity. Mushrooms produce many bioactive proteins and peptides, primarily including lectins, fungal immunomodulatory proteins, ribosome inactivating proteins, antimicrobial/antifungal proteins, ribonucleases and laccases (Xu *et al.,* 2011). Metabolic diversity of mushrooms is integral to bioremediation and biocontrol functions (Petre, 2016).

Oyster mushrooms are a good source of vitamin C and B complex. Niacin content is very high in oyster mushroom. Crude protein content of oyster mushroom is lower than animal meats but higher than milk which is an animal product. *Pleurotus* species have been recognized as edible mushroom with dual functions to humans; both as food and medicine. It contains mineral salts which are helpful to human body (Adebayo and Oloke, 2017). Mushroom is a good source of folic acid which can fulfill its daily requirement in our body. Inclusion of mushrooms in the diet

of people suffering by hypertension, obesity and diabetes has given promising results. Moreover, dietary supplement of mushroom has given satisfactory results in patients suffering by acidity and constipation problems. Pleurotin is an aromatic compound found in *Pleurotus griseus* which exhibited antibiotic properties (Naraian *et al.,* 2016). *Pleurotus eryngii* is known as king oyster mushroom (KOM) and popular for its rigid structure, delicious taste, savory flavor and nutrient content. Oyster mushroom waste (OMW) is a by-product which can be used as an additive in poultry nutrition (Hassan *et al.,* 2020). Oyster mushrooms are used in traditional Chinese medicines to stimulate both innate and adaptive immunity. Most studies in solid culture aim at fruit bodies production. The submerged culture of the genus *Pleurotus* has also been studied by several authors with the most varied objectives including the production of liquid inoculums, extra-cellular enzymes, flavoring agents, β-glucosidases, antimicrobials, vitamins, and extracellular polysaccharides (EPS). Traditionally, extracts from *Pleurotus* species have been reported to be used in treating some ailments (Martin, 1992; Garzllo *et al.,* 1994; Adebayo and Oloke, 2017; Bellettini *et al.,* 2019).

Antitumor activity:

Mushrooms produced bioactive compound1,6-branched1,3β-glucans which have been reported to inhibit tumor growth by activating immune compatible cells and cytokine production (Hetland *et al.,* 2011**,** Roupas *et al.,* 2012)**.** Water soluble extract from *Pleurotus ostreatus* showed significant effects against prostate cancer PC-3 cells. Hot water extracts of *Pleurotus ostreatus* also suppressed proliferation of MCF-7 human breast cancer cells (Martin and Brophy, 2010).

Antimicrobial activity:

Bioactive substances of several *Pleurotus* sp. have showed antibacterial, antifungal, antiviral, and antimicrobial activities. Many studies have demonstrated the antimicrobial efficacy of extracts obtained from oyster mushrooms. Secondary metabolites, protein-polysaccharide compounds, some phenols and phytochemicals, free fatty acids and their derivatives may have the role in producing antimicrobial effects (Bala *et al.,* 2012). These constituents of mushroom make them suitable candidates in drug discoveries.

Antioxidant activity:

Antioxidant compounds help to prevent from oxidative damage of cells and tissues. Stress on the body due to aging, obesity, and detrimental lifestyle choices is another serious

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health issue, which often takes the form of oxidative damage to tissues. The requirements for natural sources of antioxidant foods have been identified in edible mushrooms (Khan *et al.*, 2010). A whole range of edible mushrooms (wild and cultivated) were reported to possess antioxidant activity and broadly recited as *Agaricus bisporus, Agaricus brasiliensis, Agrocybe aegerita, Auricularia auricular, Auricularia cornea, Auricularia polytricha, Auricularia mesenterica, Auricularia fuscosuccinea, Agrocybe cylindracea, Amanita rubescens, Agaricus arvensis, Armillariella mellea, Agaricus silvicola, Agaricus silvaticus, Agaricus romagnesii, Antrodia camphorate, Boletus edulis, Boletus badius, Cantharellus lutescens, Cantharellus clavatus, Cantharellus cibarius, Cordyceps sinensis, Calvatia gigantea, Cerrena unicolor, Coprinus comatus, Dictophora indusiata, Flammulina velutipes (white), Flammulina velutipes (yellow), Inonotus obliquus, Ganoderma lucidum, Ganoderma tsugae, Grifola frondosa, Ganoderma applanatum, Geastrum arenarius, Geastrum saccatum, Ganoderma atrum, Hericium erinaceus, Hericium coralloides, Hydnum repandum, Hygrophorus agathosmus, Hypsizigus marmoreus, Hypholoma fasciculare, Helvella crispa, Lepista nuda, Lentinus edodes, Lactarius sanguifluus, Lentinus squarrosulus, Lactarius deliciosus, Lentius sajor-caju, Leucopaxillus giganteus, Lactarius piperatus, Laetiporus sulphureus, Lycoperdon molle, Lycoperdon perlatum, Lactarius piperatus, Morchella esculenta, Morchella conica, Macrolepiota procera, Morchella angusticeps, Macrolepiota procera, Pleurotus ostreatus, Pleurotus eryngii, Pleurotus citrinopileatus, Pleurotus djamor, Pleurotus sajor-caju, Pleurotus cystidiosus, Pleurotus australis, Pleurotus tuber-regium, Phellinus linteus, Phellinus rimosus, Phellinus merrillii, Polyporus squamosus, Picoa juniperi, Pleurotus florida, Pleurotus pulmonarius, Paecilomyces japonica, Piptoporus betulinus, Russula brevipes, Russula cyanoxantha, Russula delica, Ramaria botrytis, Russula vinosa, Sparassis crispa, Suillus bellini, Suillus luteus, Suillus granulatus, Sarcodon imbricatus, Schizophyllum commune, Sparassis crispa, Suillus bellini, Suillus luteus, Suillus granulatus, Sarcodon imbricatus, Schizophyllum commune, Tricholoma acerbum, Tricholoma equestre, Tricholoma giganteum, Tricholomopsis rutilans, Termitomyces microcarpus, Termitomyces schimperi, Termitomyces mummiformis, Termitomyces tylerance, Termitomyces heimii, Termitomyces albuminosus, Termitomyces robustus, Terfezia claveryi, Tremella fuciformis, Trametes (Coriolus) versicolor, Trametes orientalis, Verpa conica* and *Volvariella volvacea* (Kozarski *et al.*, 2015).

Mushroom immunomodulators:

Immunomodulators are also known as biological response modifiers, immunoaugmentors, or immunorestoratives. In any healthy organism, the immune system produces a wide range of immunomodulators to maintain homeostasis within the body. Lectins, terpenoids, proteins, and polysaccharides are some classes of immunomodulators, classified on the basis of their chemical nature; whereas in clinical practice they are classified into immunosuppressants, immunostimulants, and immunoadjuvants. Top ten mushrooms with immunomodulatory activity are *Agaricus subrufescens*, *Cordyceps sinensis*, *Ganoderma lucidum*, *Grifola frondosa*, *Hericium erinaceus*, *Inonotus obliquus*, *Lentinula edodes*, *Pleurotus ostreatus*, *Poria cocos* and *Trametes versicolor* (Enshasy and Hatti-Kaul 2013).

Nutritional value:

Many researchers have assessed nutritional value of dietary mushrooms and found to be satisfactory to keep us healthy.

(a) Vitamins composition

Edible mushrooms have been reported to be a good source for several vitamins. Vitamin B3 (nicotinic acid), B5 (pantothenic acid), B2 (riboflavin), B1 (thiamine), B6 (pyridoxine), B7 (biotin) and B9 (folic acid) are found abundantly in *Pleurotus citrinopileatus*. Maximum amount of vitamins were reported from *Pleurotus ostreatus* as vitamin E (7.23 mg/g), vitamin A (0.363 mg/g) and vitamin C (0.363 mg/g) (Adebayo and Oloke, 2017).

(b) Fatty acid composition

Fatty acids are structurally characterized as straight-chain-mono-unsaturated and polyunsaturated with branched chain building blocks of dietary fats and oils. The essential fatty acids viz. linoleic and linolenic acids are two long chain fatty acids that are fundamental to human diets. Total fat or lipid content production varied from one species of *Pleurotus* to the other. Linoleic acid (19.1%) was found in *Pleurotus ostreatus*. Palmitic acid (18.4%) was found in *Pleurotus pulmonarius.* Linoleic acid (13.5%) was found in *Pleurotus sajor-caju.* Several other fatty acids produced by these organisms are pentadecanoic acid, stearic acid, oleic acid, methyl hexadecanoate, ethyl hexadecanoate, methyl 8,11-octadecadienoate and ethyl linoleate (Kaur *et al.*, 2014; Sande *et al.*, 2019).

(c) Protein, carbohydrate, vitamin and mineral composition

Lee-Hoone *et al.* (2020) reported nutrient content in oyster mushroom as protein (3.31g), ash (1.01 g), carbohydrates (6.09 g), dietary fibre (2.3 g), calcium (3 mg), copper (0.24 mg), iron (1.33 mg), magnesium (18 mg), manganese (0.11 mg), phosphorus (120 mg), potassium (420

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mg), selenium (2.6 µg), sodium (18 mg), zink (0.77 mg), thiamin (0.125 mg), riboflavin (0.35 mg), niacin (4.96 mg), and pantothenic acid (1.29 mg) per 100 g edible portion (Ho *et al.,* 2020, Raman *et al.,* 2020).

The nutritional attributes of oyster mushroom stated above hold tremendous promise in complementing the human diet. Consumption of these mushroom products could be nutritionally and medicinally beneficial to human.

Commercial and economical scenario:

Mushrooms impact on human welfare in many ways. In the world, more than 60 species are now cultivated on a commercial scale, and this figure is increasing every year as more species are domesticated. One important facet of mushroom biotechnology is focused on mushroom products obtained by fermentation or extraction from fruiting bodies, fungal mycelium, or spent culture liquor. It is this sector of the mushroom industry, currently estimated to be worth in excess of 20 billion US dollars annually (Grimm and Wosten, 2018).

Lentinula is the first major genus, contributing in the world's cultivated mushrooms, whereas *Pleurotus* is a close second, with its five or six cultivated species. China is the main producer of edible mushrooms contributing 85% of the total world production. In India, the domestic demand of oyster mushroom is very low. Large export demand of mushroom can be fulfilled only if a linkage is developed between manufacturer, cooperatives and exporters. About 25 countries of Asia, Europe and America cultivate oyster mushroom at large scale. At present India is producing approximately 10,000 tons of oyster mushroom by the year. It is popularly grown in the states of Orissa, Karnataka, Maharashtra, Andhra Pradesh, Madhya Pradesh, and West Bengal and in the North-Eastern States of Meghalaya, Tripura Manipur, Mizoram and Assam (Royse *et al.,* 2017).

Conclusions:

Several species of oyster mushroom are grown at a large and small scale in many countries. Oyster mushrooms have notable place in nutraceutical science, which are rich nutritionally, with medicinal values, especially as antioxidant, antimicrobial, anticancer, immunomodulators. Furthermore, high content of proteins, amino acids, vitamins, minerals and low content of fat and sugar in oyster mushroom make them suitable to include in diet. The current nutrient deficiency and health problems all over the world may be brought under control

by regular consumption of *Pleurotus.* The consumption of edible mushrooms will help to maintain longevity and life quality.

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COMPARATIVE EVALUATION OF NUTRIENTS OF RAW AND COOKED SAMPLES OF *RHEUM PALAMATUM*- A POTENT MEDICINAL PLANT

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Abstract:

Like all leafy vegetables, rhubarb is good source of precious nutrients like vitamins, minerals and health benefiting anti-oxidants. Rhubarb is commonly known as Chinese medicinal plant. Vitamin K plays very significant role in Alzheimer`s disease. Copper and iron content in Rhubarb which are helpful in blood circulation and is used in cancer prevention. Rhubarb contains only 21 calories, and low in fat and cholesterol hence it is used in cardiovascular diseases. Rhubarb extract significantly inhibits ADA (Adenosine Deaminase) enzyme activity both in cancerous and non-cancerous gastric and colon tissue. In the present investigation the extract collected from young stem raw as well as cooked was analyzed for nutrient composition such as inorganic phosphorus, copper, vitamin –C, calcium, magnesium and zinc. Inorganic phosphorus and copper were determined by spectrophotometry and was found to be 82% and 85.3% loss on cooking respectively, while copper content has not been detected by others. Vitamin– C was determined by redox titration and was found to be 99.1% loss, calcium, magnesium and zinc were determined by complexometric titration and was found to be 25%, 33.3% and 66.25% loss on cooking respectively. Hence it is better to use raw rhubarb in salad form than in cooked form.

Keywords: Rhubarb, Nutrients, Titration, Minerals, Cooked vegetable.

Introduction:

Rhubarb (*Rheum palmatum*) is a perennial vegetable that grows in most of the United States. Rhubarb is a species of plant in the family polygonaceae (Cai, 2004). It produces large

leaves that are triangular with long fleshy edible stalks and small flowers grouped in large white to red inflorescence. Rhubarb is thick and branching with a brown exterior and a yellow interior. The medicinally used rhubarb part is root /rhizome. Garden rhubarb typically grows to about three feet and has reddish to purple stems. It contains similar active ingredients and has medicinal value (Shou-zhong, 1997). Rhubarb is most often used to make jellies, jam, cakes, muffins and other desserts. It can also be used in savory dishes and is good as a sauce to serve with meats and fish. One characteristic consistent with all rhubarb is the toxicity of the leaves and roots. Rhubarb is a hardy as a weed (Locock *et al.,* 1997). It is a very beautiful garden plant, with huge extravagant, lush green leaves and brown or red stalks. But the leaves contain high amount of oxalic acid, a toxic and potentially deadly poisons (Bradley, 1992). Rhubarb is an ancient plant as well. Chinese rhubarb has been traced back to 270 BC (Li *et al.,* 2018; Tsai *et al.,* 2013). The deeper the red, the more flavorful the stalks are likely to be medium size, stalks are generally tenderer than large ones, which may be stringy (Castleman, 1991). The freshly harvested stalks can be kept in the refrigerator, unwashed and wrapped tightly in plastic for up to three weeks. Rhubarb requires the addition of sugar to combat its extreme tartness (Lee *et al.,* 2017).

Rhubarb is one of the lowest caloric vegetable on the market, and as such, it is often recommended for people who are struggling to lose weight, but still want to remain healthy as 100 g of rhubarb contains only 21 calories. Rhubarb is extremely low in fat and cholesterol, the vegetable poses no threat to cardiovascular health (Cao *et al.,* 2017) and it can actually increase the levels of good cholesterol due to the presence of dietary fiber, which is known to scrape excess cholesterol from the walls of blood vessels and arteries (Chen and Wang, 2017). The trace amounts of copper and iron found in rhubarb are enough to stimulate the production of new red blood cells, increasing the total RBC count in the body and increasing oxygenation of essential areas of the body thereby improving their function and boosting the overall metabolism of the body. Our digestive system plays a huge part in digestive system healthy and regulated (Chen *et al.,* 2009). The high amount of dietary fiber found in rhubarb can help guaranteed a healthy digestive system by bulking up stool and making sure that bowel movements are smooth and regular (Commission, 2015). Rhubarb has traditionally been used as a cure for constipation, but it was only recently discovered why it had such a powerful effect. By easing constipation and other digestive issues, rhubarb prevent a wide range of more serious gastrointestinal disorders, including bloating, cramping, and even colorectal cancer (Lin *et al.,* 2009). The most prominent vitamin in rhubarb is actually vitamin k and plays a significant role in brain and neuronal health (Lu *et al.,* 2010; Chiou *et al.,* 2012), it can prevent the oxidation of brain cells and stimulates

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cognitive activity, thereby helping to delay or even prevent the onset of Alzheimer's disease (Liu *et al.,* 2010; Liu *et al.,* 2013; Kwon *et al.,*. 2014; Yoon *et al.,*. 2016). Along with its role in protecting the brain from neural degeneration, vitamin k also promotes osteotrophic activity meaning that it stimulates bone growth and repair. Combined with the rich amount of calcium and other minerals found in rhubarb, the vegetable as a whole is a major player in bone protection.

The antioxidants in rhubarb ensure that free radicals don't cause heart disease and a wide range of other dangerous health condition (Chu *et al.,* 2018). Modern pharmacological studies indicated that these anthraquinones possess a wide spectrum of pharmacological properties, such as anti-inflammatory (Ma *et al.,* 2014), antioxidant, antitumor, and antivirus (Zhang *et al.,* 2014). Antioxidants have been widely studied in recent years due to their ability to neutralize free radicals throughout the body. Rhubarb is a good source of beta carotene and other polyphenolic compound like lutein and zeaxanthin which act in similar way to vitamin A, protecting the skin and eyes from the effects of free radicals (Xun Li *et al.,* 2019). A decent amount of antioxidants in our diet can help delay premature aging, cataracts, macular degeneration and wrinkles. Rhubarb anthraquinones which play a neuroprotective (Dong *et al.,* 2016) role in CIS are mainly emodin, chrysophanol, and rhein or different rhubarb anthraquinones act together (Shu-Chun Hsu and Jing-Gung Chung, 2012; Sun and Liu, 2015). The main effective ingredients of rhubarb are anthraquinone derivatives including emodin, aloe-emodin, chrysophanol, rhein (Zhou *et al.,* 2015), physcion and danthron (Huang *et al.,* 2007). In addition to this, aqueous extract from rhubarb plant inhibits Adenosine deaminase activity in cancerous and non cancerous human gastric and colon tissues (Zhi-Kun *et al.,* 2012; Li and Fan, 2010; Durak *et al.,* 2015). Investigation of possible effects of rhubarb extract on adenosine (ADA) deaminase activity (Xiang *et al.,* 2020) in cancerous & non-cancerous human gastric & colon *tissue's* has been made to obtain information about possible mechanism of anticancer action of rhubarb on cancerous and non-cancerous human gastric and colon tissues, tissues removed from patients by surgical operation were used in the studies. As it has many health benefits and medicinal value the present investigation has been undertaken to explore the health benefits of raw as well as cooked rhubarb sample. There are mainly two varieties red petioles (leaf stalks) includes sub-varieties such as Canada Red (long, thick stalks, extra sweet), Cherry Red (rich red inside and out), Crimson Red (tall, plump petioles), Mac Donald (tender skin; brilliant red), Valentine (petioles 22 by 1-1/2 inches, good flavor), Ruby and Green Petioles (leaf stalks) with sub variety

Victoria (shaded with red). In this study Mac Donald variety was used as it was available in the local market.

Materials and Method:

Chemicals:

General chemicals and solvents used were of analytical grade obtained from RANKEM. They are potassium dihydrogen phosphate and ammonium molybdate, copper sulphate, FAS, thiocyanate, 1, 10-phenanthrolein, acetic acid, sodium acetate, Ce (IV) sulphate, Zinc Sulphate, EDTA, ammonium chloride etc.

Collection of the extract from Rhubarb:

One Kilogram of fresh green ***Rheum palmatum*** **(Rhubarb) Mac Donald** variety was obtained from the super market. The leaves were cut off and 0.5 kg of young stem of raw rhubarb was grinded from which 250 mL of the extract was obtained through filtration and centrifugation. Similarly 250 mL of extract was obtained from 0.5 kg by steam cooked for 10 mins. The collected extract was stored in freezer and then the frozen extract was used for further analysis.

Determination of inorganic phosphorus by spectrophotometry:

Phosphorus is one of the major nutrients required for better growth. It participates in the synthesis of important organic compound such as phospholipids, ATP, Phosphoproteins, nucleic acids and other. Here the phosphorus can be determined by spectrophotometer, which is based on Beer – Lambert`s law. It forms the complex in the form of inorganic phosphorus molybdate by reacting with ammonium vanadate and ammonium molybdate (Mendham *et al.,* 2006). The absorbance of the complex was measured at 460 nm. Where the absorbed color is blue and the transmitted color is bright yellow. Into a series of 25 mL volumetric flask 0.5, 0.1, 1.5, 2.0, 2.5 and 3.0 mL standard phosphate solution was added through micro burette. To each of the flask (including test solution) 2 mL of ammonium vanadate solution, 2 mL of 2M HNO_3 solution was added to produce bright yellow color of phosphovanadate molybdate complex. These solutions were allowed to stand for about 15 minutes. Each of these solutions was diluted up to the mark and shaken well for uniform concentration with distilled water. The absorbance of these solutions was measured at 480 nm using UV-Visible single beam spectrophotometer. A calibration graph of absorbance versus the concentration of standards was plotted and the concentration of inorganic phosphorus in the form of phosphate in the sample extract was calculated.

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Determination of Copper by Spectrophotometry:

Intensity of light decreases exponentially when the concentration increases arithmetically. Solution of $CuSO_4$ salt gives blue color with ammonia and intensity of blue color varies with the concentration of Cu^{2+} ion. When monochromatic light is focused on a homogeneous medium, it is partly reflected, partly absorbed and partly transmitted. Using spectrophotometer absorbance was measured for different concentration of Cu^{2+} ion in standard solutions and sample extract of raw and cooked rhubarb. A graph of optical density versus concentration gives straight line passing through origin from which concentration of copper in the extract samples can be determined. About 5 mL of 0.1 M solution of copper sulfate was pipetted into 100 mL volumetric flask and made up to the mark with distilled water. Add 1.25, 2.5, 3.75, 5.0, 6.25 and 7.5 mL of the solution was run down into 6 different 25 mL standard flask. About 5mL of liquor ammonia was added to each flask to get cuprous ammonium sulfate solution and made up to the mark with distilled water. Similar procedure was carried out with the unknown amount of extract sample. The absorbance was measured at 480 nm. A calibration graph was constructed by plotting the absorbance versus concentration of copper sulfate from which concentration of the copper in the extract samples was calculated.

Determination of Vitamin – C by redox titration:

Vitamin – C can be estimated by titrating against cerium (IV) using ferroin as an indicator. Being a strong oxidizing agent Ce (IV) oxidizes vitamin – C in acid medium to its dehydro form. Ten mL of 0.01 M FAS was pipetted into a clean 100 mL volumetric flask, 2 mL 2M H_2SO_4 was added and titrated against Ce (IV) solution taken in the burette using ferroin as an indicator. Titration carried out till the color changes from orange red to pale green. Titration was repeated to get concordant value. Titer values were recorded and concentration of Ce (IV) solution was calculated. Two mL of the latex extract was pipetted into 10 mL volumetric flask, 2 mL H_2SO_4 was added and titrated against standard Ce (IV) solution, taken in the burette till the color changes from red to pale green by using ferroin as an indicator. Titration was repeated to get concordant value and amount of vitamin – C present in latex extract was calculated.

Determination of Calcium and Magnesium by Complexometric titration:

Ethylene diamine tetra acetic acid is a hexadentate ligand. It has six potential sites for binding with metal ion. In strongly basic solution (pH 10) all the HCOOH groups are deprotonated and forms 1:1 complex with a variety of multivalent metal ions like Mg^{2+}, Ba^{2+} and Ca^{2+} etc. The indicator used was EBT (Eriochrome Black-T). In the pH range 7-11 the indicator is blue in color. In the pH range below 5.5 the indicator is red in color. To the metal indicator

complex which is wine red in color, if EDTA is added will displace the indicator from the metal indicator complex and forms metal EDTA complex, just after the end point. On the contrary Patton reeder's indicator is used to determine the calcium alone in the presence of Magnesium in the raw and cooked sample. About 5 mL of the extract sample both raw and cooked were pipetted into a clean conical flask. Diluted with 10 ml of distilled water and 3 ml of ammonia-ammonium chloride buffer was added to maintain pH 10, followed by 3 drops of Eriochrome Black T indicator. Solution turns wine red colour. The solution was titrated with the standard EDTA solution until the colour changes to blue. Similarly, for calcium alone, Patton Reeder indicator used, pH maintained at 11 by 8 M KOH. The amount of Mg and Ca present in the extract sample calculated using the relation - 1 mL of 1 M EDTA = 24 mg of Mg and 1 mL of 1 M EDTA = 40 mg of Ca.

Determination of Zinc by Complexometric titration:

About 5 mL of the extract sample was taken and pipette out into a clean conical flask. Diluted with 10 mL of distilled water and 3 drops of xylenol orange indicator was added. A spatula of powdered hexamine added with agitation to the resulting yellow solution until it acquires an intense red color. The solution was titrated with the standard EDTA solution until the color changes sharply from red to yellow. The amount of zinc present in the extract sample calculated using the relation 1mL of 1M EDTA = 65.38 mg of zinc.

Results and Discussion:

Rhubarb commonly known as Chinese medicinal plant is traditionally used against tooth ache in china. Vitamin-K promotes Osteotrophic activity meaning that it stimulates bone growth and the repair. Combined with rich amount of calcium and other minerals found in rhubarb, the vegetable as a whole is a major player in "Bone protection. Rhubarb is a good source of "Beta Carotene" and other polyphenol compounds like Lutein and Zia xanthine which acts in a similar way to Vitamin-A, protecting the skin and eyes from the effects of free radicals. These phenolic compounds have been connected to preventing oral and the lung cancer. The most important vitamin present in rhubarb is Vitamin –K, plays a very significant role in brain and neuronal health. It prevents the oxidation of brain cells and stimulates cognitive activity (Komatsu *et al.,* 2006), thereby helping to delay or even prevent the onset of Alzheimer's disease. Rhubarb is extremely low in fat and cholesterol, the vegetables poses no threats to cardiovascular health.

In the present study the 1kg of rhubarb young stem was collected from market, from that 0.5 kg of rhubarb young raw stem was grinded and extract was collected. Another 0.5 kg of rhubarb stem was boiled in pressure cooker for about 10 mins, and cooled and grinded, the

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extract was collected. Both extracts were analyzed for phosphorous, copper, vitamin –C, zinc, calcium and magnesium in raw as well as cooked sample. Inorganic Phosphorus was determined by spectrophotometry and it values were shown in the table 1. It was found that raw rhubarb contain 8.8 mg/100 g rhubarb and cooked contain 1.6 mg/100 g rhubarb, whereas 82% of inorganic phosphorus has been reduced in cooked extract of rhubarb.

Copper was analysed by spectrophotometry by using copper sulphate and liquor ammonia as a reagent. Its graphical representation is shown in the fig-1. From this analysis it was found that raw rhubarb contains copper 0.68 mg/100 g of rhubarb and cooked rhubarb contains 0.1 mg/100 g rhubarb and it was found to be 85.3% loss, where as its copper content has not been detected by others.

Table 1: Determination of Inorganic phosphorus using spectrophotometry

Sr. No.	Volume of phosphate solution in mL	Concentration of phosphate in ppm	Reagent 2M HNO_3	Reagent Ammonium vanadate solution	Absorbance at 480 nm
1	0	0	2 mL	2 mL	0
2	0.5	20			0.04
3	1.0	40			0.078
4	1.5	60			0.12
5	2.0	80			0.17
6	2.5	100			0.23
7	Raw sample	-			0.228
8	Cooked Sample	-			0.204

The vitamin -C content was determined by redox titration. It was found that 100 g of raw rhubarb contain a 10.8 mg, whereas 100 g cooked rhubarb contain 0.01 mg of vitamin – C it was found the while cooking the amount of vitamin – C has drastically reduced, about 99 % loss has been reported indicating highly thermo labile.

The calcium content was determined by complexometric titration. Raw rhubarb of 100 g contains a 121 mg of calcium and cooked rhubarb contains a 91 mg whereas 25% of calcium has been reduced while cooking the young stem of rhubarb.

The magnesium was also determined by complexometric titration. Raw rhubarb contains a 12.6 mg of magnesium and cooked rhubarb contains a 8.4 mg per 100 g of rhubarb indicating 33% of magnesium has been reduced drastically on cooking.

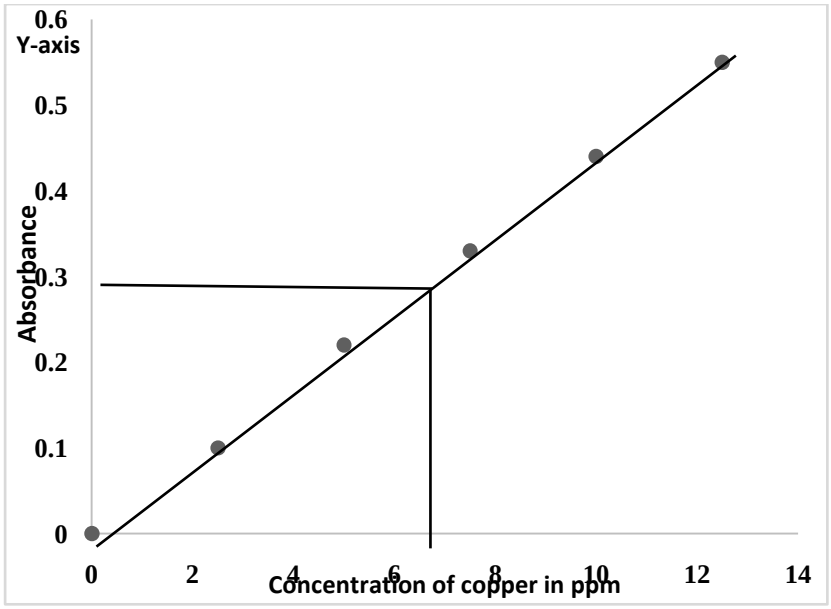

Figure 1: Calibration curve for the determination of copper in the Rhubarb extract

Zinc was determined by complexometric titration by using xylenol orange as an indicator, raw rhubarb contain 0.16 mg of zinc/100 g of rhubarb and cooked rhubarb contain 0.054 mg/100 g of rhubarb. Hence 66.25 % of zinc has been lost while cooking. Similar results have been reported earlier for the other nutrients such as proteins, manganese, fluoride, iron (II), and iron (III). In cooked sample protein, manganese and fluoride has been lost about 40 %, but iron (II) and iron (III) has been lost about 70 % (Jamuna, 2021).

Table 2: Comparing the value of Raw Rhubarb and Cooked Rhubarb

Sr. No.	Nutrients	Raw sample/ 100 g in mg	Cooked/ 100 g in mg	% of loss on cooking
1	Inorganic phosphorus	8.8	1.6	82
2	Copper	0.68	0.10	85.3
3	Vitamin - C	10.8	0.01	99
4	Calcium	121	91	25
5	Magnesium	12.6	8.4	33.3
6	Zinc	0.16	0.054	66.25

Summary and Conclusion:

In the present investigation the extract was collected from 0.5 kg of young stem of Rhubarb *(Rheum palmatum).* Extract collected from young stem raw as well as cooked was

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analyzed for nutrients such as inorganic phosphorus, copper, vitamin –C, calcium, magnesium and zinc. Except for calcium and magnesium which were lost below 35 %, all the other nutrients analysed were lost above 60 % indicating they are thermo labile. The above analysis indicates that cooked sample has lost some % of nutrients. Hence raw sample can be used in the form of salads, since some of the nutrients are thermo labile.

Rhubarb contains only 21 calories in 100 g of rhubarb. It is rich in precious nutrients like calcium and magnesium. It is also a very rich source of many vital phyto-nutrients, vitamins, minerals and health benefiting anti-oxidants. Hence the above investigation was carried in order to explore the importance of nutritional composition of rhubarb and also to exhibit that there is some percentage of nutrient loss in cooked sample, so it is better to use raw rhubarb in salad form than cooked rhubarb.

List of abbreviations:

ADA – Adenosine Deaminases Activity
ATP – Adenosine Tri Phosphate
CIS – Central Ischemic Stroke
FAS – Ferrous Ammonium Sulphate
EDTA – Ethylene Diamine Tetra Acetate
EBT – Eriochrome Black T
RBC – Red Blood Cells

Chemicals Molecular Formulae:

KOH – Potassium hydroxide
HNO_3 – Nitric acid
HCl – Hydrochloric acid
H_2SO_4 – Sulphuric acid
Ce (IV) – Cerium IV sulphate
$CuSO_4$ – Copper sulphate
$ZnSO_4.7H_2O$ – Zincsulphate heptahydrate

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PLANTS BASED ETHNO-MEDICINE IN THE LAMBANI COMMUNITIES IN THE VICINITY OF VIJAYAPURA DISTRICT OF KARNATAKA

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Abstract:

Ethno botany is the study of plants and its applications on the inhabitants of an area and deals with folklare concepts of classification by habitat and usage or some other parameters. Ethnobotanical surveys are important steps in the identification, selection and development of therapy from medicinal plants which further serve as a base for development of new compounds with active principles for phytochemical, pharmacological and clinical research. Now days, the whole world is relying on plant-based health remedies for safe and effective cure of dangerous human ailments. In this present content, we have focused on the ethno botanical knowledge and culture of the Lambani ethnic group from Vijayapura district of Karnataka. Lambani community people basically stay in remote area and these communities still rely only on their traditional medicinal practices for their health care needs. We selected a total of 23 villages with Lambani community for the study in Vijayapura district. Ethnomedicinal information on medicinal plants was recorded through interviews, discussions and field observation of traditional healers and practitioners with knowledgeable people of selected study area using a specialized questionnaire. In this study, 25 folk herbal practitioners aged between 25 - 75 years were consulted to gather the information for analysis on the study area. Most of the respondents (70%) received only primary and high school education. While, a few (10%) received pre-university education; 5% were degree holders and 10% were illiterate individuals. Majority of the traditional healers acquired the therapeutic knowledge from their ancestors. In the present study, we have documented around 42 medicinal plant species from 28 families were used for the treatment of human and livestock ailments. Plants belonging to Lamiaceaea, Fabaceae, Menispermaceae, Amaranthaceae,

Apiaceae, Apocynaceae families were used for medicinal purposes. Majority of the traditional healers acquired the healing knowledge from their ancestors. *Leucas aspera* cure the ear for removing wax and cure pain, *Mentha arvensis* were for cured body pain or relief, *Ocimum tenuiflorum* and *Ocimum sanctum* were cure cold and cough, *Clerodendrum inerme* species were relive asthama and snake bite disorder and *Coleus amboinicus* species were cure bronchitis, asthma and cough followed by other species were cure stomach disorders, skin disease, diabetes, cutting wounds, piles, blood purify, urinal genital disorder, menstrual cycle disorder, cure wounds, skin glow and control hair fall, dysentery, diarrhea, fever, edema, stimulant to the uterus, improve the menstrual discharge, malarial fever etc. Most of the healers in the study area preferred single plant species for the preparation of juice (35%) followed by paste (23%) and decoction (15%). However, in case of poly-herbal formulation, juice (4%) was dominant over paste (8%), chewing raw leaves (13%) and latex (2%). This study provides primary and evidence based information about the phytochemical potential of plants and their curative properties used in the traditional folklare medicine in the Vijayapura district of Karnataka.

Introduction:

India is one of the mega biodiversity countries in the world and also ethno medicinal knowledge of herbal healers. Medicinal plants are an alternative source of ayurvedic drugs and play a vital role in the remote area health care systems throughout the world. Major worldwide populations are dependent on medicinal plants because of their effective remedy, cultural knowledge in local areas, and lack of modern health care facilities (Martins, 2014). Ethnobotany has grown enormously as vast scientific knowledge branch of science, which provides vast medicinal knowledge to pharmaceutical industry. Various communities use over 50 percent of the plant species of any ecosystem as medicine and in general over 7500 species are utilized in primary health care by various tribes. The people who live in and around forest area utilize plants for their basic needs like food and medicine in the form of non timber forest products. The knowledge of plant parts to cure diseases and disorders is inherited to these people from generation to generations. 'Traditional medicinal knowledge' is declining rapidly, mainly due to the attraction of folk or tribal people towards the modern allopathic medicine and unhealthy believes of the peoples. The knowledge of the community may be lost over a period of time (Naik *et al.,* 2012; Yuan *et al.,* 2016). Ethno botany was originally based on quantitative methods such as inventories of plants and their uses, with major focus on the economic importance studies by national and international organization have shown that for 75-90 % of the rural population of

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the world, the local herbalists alone attend to their medicinal problems (Walikhan and Khatoon, 2008).

Today, a large number of pharmaceutical industries depend on the ethno-medicinal plants as source of useful drugs to treat chronic diseases including cancer, jaundice, paralysis, diabetes, and cardiac and respiratory problems (Kumar *et al.,* 2021). Interestingly, many modern medicines have been developed based on the 'traditional medicinal knowledge'. A considerable number of researchers have documented ethnomedico-botanical information from many parts of India (Shah and Gopal, 1985; Bhandary *et al.,* 1996; Huidrom, 1997; Parinitha *et al.,* 2005; Mishra *et al.,* 2006; Purkayastha and Nath, 2006; Pattanaik and Reddy, 2008; Shivanna *et al.,* 2008; Pesek *et al.,* 2008; Rajakumar and Shivanna, 2009; Rajakumar and Shivanna, 2010; Vinayaka and Krishnamurthy, 2012). These investigators collected the primary information from tribes, ethnic, folk/local and rural communities. The ethnomedico-botanical knowledge of indigenous-local, ethnic, and rural communities of India have been documented from parts of India (Debbarma *et al.,* 2017) and certain parts of Karnataka (Kshirsagar and Singh, 2001; Kattimani *et al.,* 2001; Bhandary and Chandrashekar, 2002; Prashantkumar and Vidyasagar 2006; Upadhya *et al.,* 2010; Kadanakuppe and Bhat, 2013; Kumar, 2018), but no complete documentation of ethnomedical knowledge for Vijayapura district of Karnataka is available because of insufficient and poor survey of local practitioners and many of people those studied ethnobotanical documentation mainly focused on Western Ghats of South India only (Parinitha *et al.,* 2004). Hence, an attempt has been made to collect and document their inventory of indigenous herbal medicine knowledge record in this study area. The main objective of this ethno botanical investigation is the identification and documentation of various plants and how they are used in the traditional ethno medicinal uses and culture of the Lambani (Banjara) ethnic group from Vijayapura district of Karnataka. Lambani peoples basically stayed in remote areas and this community still relies only on their traditional medicines for their health care needs. However recently, young people of this community have started depending on the allopathic medicine rather than on their own traditional system of medicine.

Materials and Methodology:

Study area:

Vijayapura district is located in the Northern part and also in the semi-arid climatic region of Karnataka. The district is bounded by Solapur district to the North and Sangli district to the North-west, Belgaum district on the West, Bagalkot district to the South, Gulbarga district to

the East and Koppal district to the South-east. Vijayapura district is spread over an area of 10,498 sq km and lies between 16° 49' 39.1620" N latitude and between 75° 43' 31.1772" E longitude at a mean elevation of 592.23 m (Fig 1). Vijayapura formally called as Bijapur has a very interesting history with many dynasties ruling the district for many centuries. The monuments in Vijayapura speak about the history that the city faced from different rulers. The history of Vijayapura goes back to Palaeolithic age. In summer session especially in April and May it is too hot temperature ranges from 40^0C to 42^0C and the average annual rainfall for the whole district is 552.8 mm in rainy days. The total livestock population of the district is 326, 360 according to 2011 census. The study area is known for the major portion of the district consists of this kind of soil which has a great moisture-holding capacity. Second one is "red soil" (masari/maddibhoomi), which is generally poor, good for irrigation and horticulture. Krishna River, which is the most important river of the district, it flows about 125 miles in the district. A dam is built across the river at Almatti and Bhima river flows in northern part of district for about 20 miles. It overflows in the rainy season and spreads over a wider area, which is thereby rendered extremely fertile land. In central part of district Doni river flows some area of irrigated land total five rivers flows in this district. Agriculture plays a major role in the economy of this district and which is good for the crops like jawar, wheat, pulses, sunflower, groundnut, sugarcane, and cotton and fruit crops like grapes, lemon and pomegranate the main crops provide sources for irrigation. Natural vegetation occurred in this study area comprising of semi-arid forest shaving a variety of medicinal plants. In the present study, ethno-medicinal surveys were carried out in the taluka places including Vijayapura, Basavana Bagewadi, Sindagi, Indi, Muddebihal, Nidagundi, Talikote, Babaleshwar, Tikota, Chadchan, Devara Hipparagi, Kolhar surrounding interviewed to Lambani peoples in Vijayapura district. These communities have their own language and food habits, dress code, historical culture and socio-religious myths.

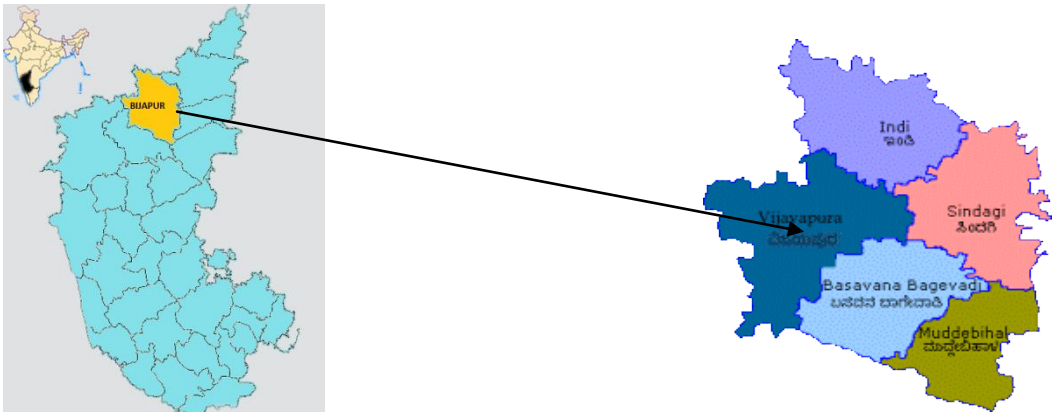

Figure 1: Map showing study area Vijayapura and its taluka places

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About the Lambani communities of Vijayapura district:

In the present study, we have focused on local herbalists, healers; farmers and village headman of the Lambani communities found in different "thandas" were interviewed for responses regarding traditional knowledge and their uses. In Karnataka, the Lambani community is also refered as Banjara, Lambadi, Lamani, Gor/Gour Banjara, Gour Maati, Naik and Sugali. These community women wear colorful and beautiful costumes like phetiya (as Ghagra) and Kancholi (as top) and have tattoos on their hands. These inhabitants of this community live in remote places of villages and they rely mainly on surrounding bioresources for their food, shelter and medical care etc. The study area belonged to different Jath/ gothras of lambani communities such are list below.

The gothras of Rathod/Bhukya and their 27 padaas or variants, Pawar and their 12 padas or variants, Chavan/Chauhan and their 6 padas or variants, Vadithya/Jadhav and their 52 padas or variants , Banoth and their 15 padas or variants. Villages of most gothras are majorly situated in the remote areas; usually people of certain gothras are experts of ethno medicinal treatement of various diseases. The knowledge about ethno-medicinal plants is specialized by the Rathod and their padaas Meghavath, Rajaavath and Ramaavath and Gothras of Chuhaan and their Padas Korra and Mood and Gothras of Pawar and their Vislaavath were experts to ethnobotanical 'nativaidyas' or country medicines in study area.

Data collection:

Frequent field surveys were conducted at 23 randomly selected villages in Vijayapura district during the period from April 2019 to March 2021. Ethnomedicinal information on medicinal plants was recorded through interviews discussion and field observation of traditional healers and practitioners with knowledgeable people of selected study area using a specialized questionnaire. Among them 25 folk herbal practitioners aged between 25 and 75 years were consulted to gather information in the study area, which included 20 were male and 05 female respondents under the age group herbal healers have 15-25 years of experience in the treatment of different diseases. An ex-post facto research design was adopted to assess the ethno-medicines used in the different places of Vijayapura district in Karnataka. The responses included details about the botanical and common name of the plant prescribed for medicine purpose, part of the plant used, mode of preparation (decoction, paste, powder or juice), dosage and duration were confirmed through discussions with respondents who practiced the use of the documented plants for medicinal purposes.

Figure 2: Pictorial view of traditional healers with mode of medicine preparation

Result and Discussion:

The present study areas of some villages possess interesting characteristics related to life in these remote areas were dependency on agricultural field plants and local wild plants for making various food products and therapeutic herbal medicine. In rural areas many plant parts are used for medicinal purposes although they may have other potential utility values. Such ethnic medicine purpose their potential health benefits and their health risks associated with such remedies. Some of these knowledge have been documented and codified or studied scientifically. Most of the ethno medicine practitioners were avoids the transfer of their traditional herbal knowledge to others. Because they believed that the medicinal practices by a layman on remedies of the plant species would not lead to recovery or cure the disease to the other people.

The present study documented the ethno medico botanical uses of 46 plants belonging to 28 families (Table-1) that have been gathered as primary information and documented from local herbal practitioners in the present study. Majority of the plant species belongs to the family Lamiaceae (13%), other families such as Papilionaceae, Caesalpiniaceae, Menispermaceae, Amaranthaceae, Apiaceae, Apocynaceae were represents by 7%, families like Asteraceae and Myrtaceae are represented 4% and remaining all families were present in 2% respectively. Total 42 plant species were projected for their use as medicinal plants, among them 26 plants are commonly collected from wild habitats such as scrubby forest area, road side, gomalas and river canal border only and remaining 16 plant species were cultivated and collected from agricultural land and house premises.

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Table 1: Showing List of plants used by the Lambani community for ethnomedicinal purposes with their formulations

Sl. No.	Botanical Name	Family name	Local name	Ailment	Mode of uses
01	*Abrus precatorius* L.	Fabaceae	Gulaganji	fevers, coughs and colds	A cup of tea is made from the leaves used every morning
02	*Acacia ferruginea* L.	Mimosaceae	Banni mara	itching, leucoderma, ulcers	Fruit juice is taking orally every day up to one week
03	*Achyranthes aspera* L.	Amaranthaceae	Uttarani	menstrual cycle disorder	Root juice with 250 ml water taken orally twice a day for 5 days
04	*Aloe vera* (L.)	Asphodelaceae	Lolesera	Skin glow and control hair fall	*Aloe vera*gell is directly apply on face and hair
05	*Amaranthus caudatus* L.	Amaranthaceae	Rajagiri soppu	Enrich of protein and energy	whole plant are used as edible and are frequently used as a source of food in India
06	*Amaranthus spinosus* L.	*Amaranthaceae*	Mulluarivae sappu	urinal genital disorder	Leaves along with masala ingredients, salt making food eating with rooti
07	*Argemone Mexicana* L.	Papaveraceae	Dhatthoora	Pain killer and cough	Leaves paste applied on effected part of body and the seeds decoction drunk to relieve cough
08	*Azadirachta indica* A. Juss.	Meliaceae	Bevu	Wounds and cleaning tooth	Leaves paste is applied on effected parts and twig is used for making tooth brash
09	*Azima tetracantha* L.	*Salvadoraceae*	Uppi mullu	poison vomiting	Crushed leaves with Neem leaves paste mixing with water taken orally to poison dunked person
10	*Basella alba* L.	Basellaceae	Basalae	Dysentery	Leaf juice with half spoon salt mix with boil water is taken orally for 2 days

11	*Caesalpinia bunducella* L.	Caesalpiniaceae.	Gajagadgida	Stimulant to the uterus, improve the menstrual discharge, malarial fever. relieving intestinal worms	The fruit nut and root bark extract has been used twice every day up to 15 days
12	*Calotropis procera* (Aiton) W.T.Aiton	Apocynaceae	Beli yakka	Cutting wounds	Plant latex is applied on affected parts
13	*Cassia auriculata* Linn.	Fabaceae	Tavarkegida	Diabetes	Chewing Bark every day along with bettle leaves
14	*Cassia fistula* Linn	Fabaceae	Kakkegida	skin diseases	Flashy fruit paste is applied on face every morning
15	*Centella asiatica* (L.) Urban	Apiaceaea	Ondelaga	Stomach disorders	Leaves paste taken orally for 5 days
16	*Clerodendrum inerme* (L.)	Verbinaceae	Kaadu mallige	asthama and snake bite	Leaves and flower extracts with 250 ml water is taken orally for weakly two times and root paste is applied on effected parts for cure snake bite
17	*Cocculus hirsutus* (L.) Diels	Menispermaceae	Gowri huvu	Preparation of chair	Stem is used in making basket chairs
18	*Coleus amboinicus* Lour.	Lamiaceae	Doddapatre	Cough	Plant extract is taken orally for 6 days
19	*Coleus amboinicus* Lour.	Lamiaceae	Dodda patre	Cure bronchitis, asthma,	Leaves extract drinking orally twice a day upto 15 days
20	*Coriandrum sativum* L.	Apiaceaea	Kotambari sappu	Increase iron content in blood	Seeds are used for preparation of decoction taking orally twice a day
21	*Cynodon dactylon* (L.) Pers.	Poaceae	*Cynodon*	blood bleeding	Root decoction 3-4 drops is taken orally in nose for 2 days

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22	*Datura metel* L.	Solanaceae	Daturi	headaches	Dry leaves inhalation trice a day up to 12 days
23	*Eucalyptus globulus* Labill.	Myrtaceae	Neelagiri	Fragrance and insecticide property	Plant oil is used for fragrance and oil is used to control insecticide property on dead body
24	*Ficus religiosa* L.	Moraceae	Arali mara	vomiting, & cough	Bark extract is taken orally for 6 days
25	*Lawsonia inermis* L.	Lythraceae	Gorenty	Blood purify	Leaves decoction is taken orally twice a day for 10 days
26	*Limonia acidissima* L.	Rutaceae	Belada mara	Increase blood cell	fruit paste with cow milk is drunk daily two times for 20 days
27	*Mentha arvensis* L.	Lamiaceae	Pudina	Body pain	Plant leaves juice is taken orally for 2 days and leaves paste applied on external affected parts
28	*Michelia champaca* L.	Magnoliaceae	Sampige gida	Oral cancer & gonorrhea disease	Flowers paste are used orally to animal
29	*Nelumbo nucifera* Gaertn.	Nelumbonaceae	Tavare huvu	skin burn	leaves paste is applied on external affected parts till cure skin burning
30	*Nerium indicum* L.	Apocynaceae	Kanagilae	piles	Root juice with one hand full *lawsonia intermis*, one onion grinding with water taken orally for 15 days
31	*Ocimum sanctum* L.	Lamiaceae	Tulasi	Cold and cough	Raw leaves chewing every day up to 12 days
32	*Ocimum tenuiflorum* L.	Lamiaceae	Tulasi	Cold & cough	Leaves juice is taken orally for 5 days
33	*Oroxylum indicum* (L.) Vent.	Bignoniaceae	Aralu	Diarrhoea, Fever, Oedema	Flowers and fruit were eaten with rice.
34	*Peucedanum graveolens* (L.) Hiern	Apiaceaea	Sabbasige sappu	wounds	Plant paste with turmeric powder is applied on affected area
35	*Piper beetle* L.	Piperaceae	Veelyadaele	tooth ache	Leaves along with 3 clove, 2 pieces of garlic paste applied externally on effected tooth till cure

36	*Santalum album* Linn.	Santalaceae	Gandada mara	Insecticide property	Sandal wood oil is used on dead body for control germs and insect
37	*Sauropus androgynus* L.(Merr.)	Phyllanthaceae	Chakramuni	Weight loss and improvement of macronutrients	The leaves extracts with 250 ml water taken daily twice times
38	*Syzygium cumini* (Linn.) Skeels	Myrtaceae	Nerale	Cure dysentery	Bark extract decoction is used twice a day upto one week
39	*Tagetes erecta* L.	Asteraceae	Chendi huvu	blood purify	Flower juice drunken for one weak
40	*Tinospora cordifolia* (Thunb.) Miers	Menispermaceae	Amrutha balli	diabetes	Leaves juice is taken orally with water for daily morning
41	*Tinospora corifolia* (Thunb.) Miers	Menispermaceae	Amruth balli	Cureing chicken gunya disease	Stem boiling with water up 10 minute then using plant decoction every morning
42	*Tridax procumbens* L.	Asteraceae	Gejje tikke	Skin disease	Leaf paste is applied on sun burning skin up to 30 days
43	*Trigonella foenum-graecum* L.	Fabaceae	Mentte sappu	Increase immunity	Along with food, *Trigonella* leaves are chewing raw is used for increasing body immunity
44	*Vigna sinensis* (L.) Endl.	Fabaceae	Halasandi	For strength and energy	Leaves is used as cooking food for gaining multi vitamin and protein
45	*Vinca rosea* L.	Apocynaceae	Sada mallige	diabetes	Stem decoction is taken orally twice a day
46	*Ziziphus mauritiana* Lam.	Rhamnaceae	Badara	Cure Piles,	Fruit juice drinking every day up to 30 days

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In some cases, ethnic people suggest single plants species are uses specific ailments and some cases to added different ethno botanical ingredients were crushing, powdering and boiling plant specimen to make a various ailments, some of the medicinal plants leaves and fruits directly recommended for chewing in raw to cure various diseases such as, the family Lamiaceae some species like *Leucas aspera* were used to cure the ear for removing wax and cure pain, *Mentha arvensis* were used used to cure body pain or relief, *Ocimum tenuiflorum* and *Ocimum sanctum* species were used cure cold and cough, *Clerodendrum inerme* species were used relive asthama and snake bite disorder and *Coleus amboinicus* species were used to cure bronchitis, asthma and cough. Similarly other plant species remedies were included by the practitioners for their use in cure of various diseases like stomach disorders, skin disease, diabetes, cutting wounds, piles, blood purify, urinal genital disorder, menstrual cycle disorder, enrich of protein and energy, cure wounds, increase iron content in blood, skin glow and control hair fall, dysentery, diarrhea, fever, edema, stimulant to the uterus, improve the menstrual discharge, malarial fever, relieving intestinal worms, increase immunity, for strength and energy, fevers, skin diseases, cough, body pain, preparation of chair, blood purify, curing chicken pox disease, Insecticide property, fragrance and insecticide property, wounds and cleaning tooth, cure skin burn, tooth ache, blood bleeding control, pain killer, weight loss and improvement of macronutrients, Increase blood cell, cure piles, poison vomiting, cure headaches, cure vomiting (Table-1).

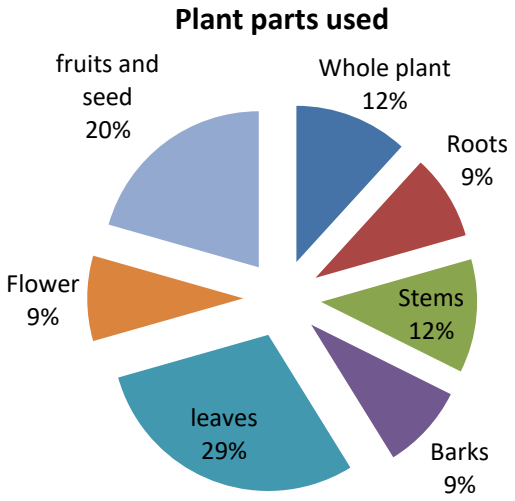

Figure 3: Pi chart showing Plant parts used for drug formulation

Most of the healers in the study area preferred single plant species for the preparation of juice (35%) followed by paste (23%) and decoction (15%). However, in case of poly-herbal formulation, juice (4%) was dominant over paste (8%), chewing raw leaves (13%) and latex (2%) were used (Fig.3). The percentages of different plant parts used for the preparation of medicine (Fig.4) Whole plants (11%) were found to be the most frequently used plant parts in the preparation of medicine followed by roots (8%), stems (11%), bark (9%), Leaves (29%), flowers (9%) and fruits and seeds (20%) and background literature on ethnobotanical studies confirmed that leaves are the major portion of the plant used in the treatment of diseases. Ethno botanical survey is of crucial importance in finding some miraculous medicines for curing various human and veterinary diseases. Hence ethno botanical survey is of crucial importance in finding some miraculous medicines for curing nearly 32 human diseases. The list of folk medicinal plants from the northern region of Karnataka and their utilization will provide primary data for further studies aimed at conservation, cultivation, traditional medicine and economic welfare of rural and tribal population of the region.

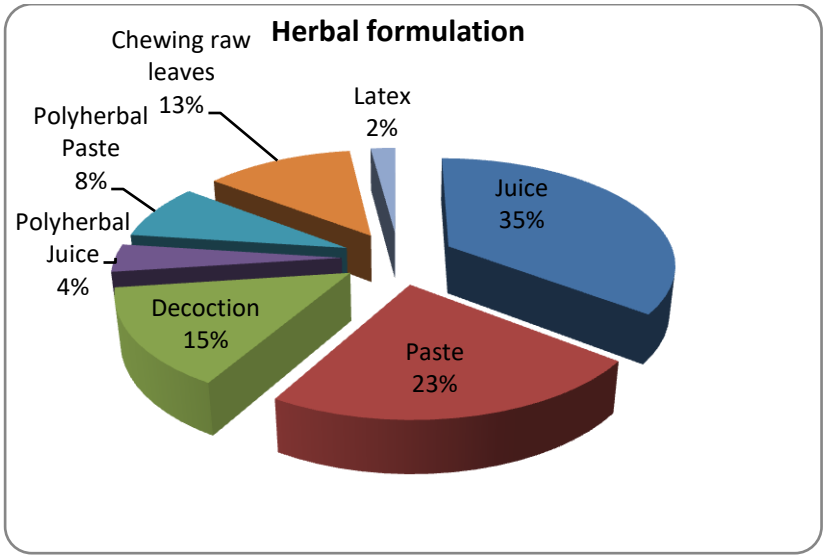

Figure 4: Pi chart showing Preferred Herbal formulation in the study area

The preparation of herbal medicine is varies from one place to other place and differs to usage of ethno medicine in allied disease. For example in study area Vijayapura district ethnic people uses *Tridax procumbens* species used to cure skin disease, the same species used in Shimoga district tribal peoples to cure toothache (Shivanna and Rajkumar, 2010) similarly *Vinca rosea*, *Tinospora cordifolia* and *Cassia auriculata* species were used to cure diabetes, the *Tinospora cordifolia* plant is used as reduce sugar level in blood (Mishra *et al.*, 2019)., *Calotropis procera* species are used cure cut wounds, same plants in Hosanagara taluk is used to

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cure Epilepsy (Shivanna and Rajkumar, 2011), *Nerium indicum* were used to cure piles (Chetwani *et al.,* 2017). *Nerium indicum* also act as antibacterial potential, *Amaranthus spinosus* for curing urinal genital disorder, in this plant studied (Rahman *et al.,* 2013) by earlier workers for the cure Asthama and *Achyranthes aspera* species roots extract can form abortion. This same plant is used in study area for curing menstrual cycle disorder, *Coriandrum sativum* to enrich iron content in blood in Indonesia this plants used to cure gastrointestinal disorders (Jadid *et al.,* 2020), *Aloe vera* and *Cassia fistula* to enhance skin glow and cure skin disease. Mishra *et al.,* (2019) discussed same plants used body balancing level of glucose content. *Coleus amboinicus* is used to cure throat infection (Somashekar *et al.,* 2010) but in the present study it was documented as used to cough. *Azadirachta indica* for curing wounds and cleaning tooth, *Syzygium cumini for cure dysentery, Piper betle* is used as cure toothache, in Indonesia same plants used to cure urogenital and gynecological problems (Jadid *et al.,* 2020). The present study, a list of folk medicinal plants from the northern region of Karnataka and their utilization will provide primary data for further studies aimed at conservation, cultivation; need to explore traditional medicine and economic welfare of rural and tribal communities of the region. Further survey and research focusing the on the medicinal plants documented in this study might give information regarding the hunt for bioactive compounds and the discovery of new drugs to fight diseases in an effective manner.

Conclusion:

This ethno botanical work is first recorded in study areas of Lambani communities in different region of Vijayapura district of Karnataka. A total 42 plant species belong to 28 families were used cured 32 human diseases. The present study focused on ethno medicinal uses and their potential health benefits in rural areas. Investigators noticed that younger generation they do not believe in their ancestral traditional medicine and have developed dependency on allopathic medicine for faster relief of disease. Some pharmaceutical companies' approaches to ethnic people for documentation on utilization of a number of medicinal plants species used to treat aliments. Hence the younger researchers focus on to collect this information and develop a database of medicinal plants for future research and potential development of new drugs.

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IN VITRO SCREENING OF ANTIFUNGAL BACTERIA AGAINST PHYTOPATHOGENIC FUNGI OF PUMPKIN (*CUCURBITA MAXIMA*)

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Abstract:

Pumpkin is a nutritious vegetable cultivated and consumed worldwide for its nutritional as well as medicinal values. It is also used in the production of many commercial products. Like all other vegetable crops pumpkin is also susceptible for many phytopathogens especially fungi. Though these plant pathogenic fungi can be managed by the application of synthetic fungicides, their extensive usage leads to many adverse effects. Thus presently interest is towards the usage of biocontrol strategies for the management of phytopathogens. Owing to this in the present study nine MTCC bacterial cultures reported to have antifungal activity were screened against three pathogenic fungi infecting pumpkin such as *Alternaria* sp., *Drechslera* sp., and *Fusarium* sp., isolated from infected pumpkin plant explants. The screening was done by dual culture method. Two bacterial isolates namely *Enterobacter aerogenes* (MTCC 8558) and *Pseudomonas aeruginosa* (MTCC 7904) exhibited comparatively better antifungal activity against all the fungi tested such as *Alternaria* sp., *Drechslera* sp., and *Fusarium* sp. Therefore these two bacterial strains could be further studied for the development of biopesticide formulations that could be used for effective and eco-friendly management of these fungi.

Keywords: Pumpkin, fungal diseases, phytopathogenic fungi, antifungal bacteria, dual culture, biocontrol

Introduction:

Cucurbita maxima (pumpkin) is a popular type of hard squash that belongs to the family Cucurbitaceae and the genus Cucurbita. In India pumpkin is commonly known as '*Kashiphal*' or '*kaddu*' and in kannada it is called as "*Kumbala kayi*" (Ahamed *et al*., 2019). Pumpkin plants are hardy creepers or soil surface runners, but able to climb where there are supports. Pumpkins are cultivated for their ripe fruit with the seeds in the central cavity and the yellow or orange flesh being eaten (Ravani and Joshi, 2014).

Pumpkin is one of the important vegetable crop because of the nutritional and medicinal values. Pumpkin is a good source of vitamin C (ascorbic acid), minerals, carotenes (α and β), various dietary fibers and phenolic mixtures. Seeds of pumpkin are also rich in protein, low in phytic acids and trypsin inhibitor and high in sulfur-containing amino acids. In addition to high levels of Zn, P, K, Se, Mn and Cu the seeds of the pumpkin also contain Mg and Fe and has high amount of fatty acids content like palmitic, stearic, oleic and linoleic acids. The oil of pumpkin seeds improves the nutrition of human diets having high unsaturation and tocopherol content (Ahmad and Khan, 2019). Pumpkin contains an important antioxidant, beta-carotene, which is converted to vitamin A in the body (Ravani and Joshi, 2014).

Due to the medicinal properties of pumpkin the whole part of the vegetable can be used for human consumption. Pumpkin also finds its application in the production of commercial products such as jam, jelly, marmalades, puree, sauces, chutney, pickle, halwa, curry, soups, stews, cookies and weaning mix, pies and beverages. Worldwide production of pumpkin, squashes, and gourds is 24.62 million metric tons from an area of 5,10,0000 hectares and in India, the total production is 49,00,000 tons from an area of 45,000 hectares (Ahamed *et al*., 2019).

As with all crops, pumpkins are also subject to abiotic and biotic diseases that can limit crop quality and yield. Abiotic diseases are caused by environmental conditions such as soil nutrient or pH imbalances, soil moisture extremes and chemical injuries (herbicides and other toxic chemicals). Biotic diseases are caused by plant pathogens that include fungi, bacteria, viruses and nematodes. Plant pathogens can directly attack the fruit, rendering it unmarketable. In addition, biotic diseases indirectly reduce yields by killing plants prior to harvest or by causing defoliation, which can reduce fruit size and quality as well as exposing fruit to sun scald (Damicone and Brandenberger, 2019).

Fungi are the major pathogens causing diseases in pumpkin. Some of the important fungal disease of pumpkin are leaf blight caused by *Alternaria cucumerina;* leaf spot caused by *Alternaria alternata, Cercospora citrullina*, and *Drechslera rostrata*; crown and foot rot caused by *Fusarium solani*; Gummy stem blight incited by *Didymella bryoniae*; wilt caused by *Verticillium dahlia*, etc. (Anonymous, 2021).

Fungal diseases of pumpkin are spreading at an alarming rate and needs to be managed properly at the earliest to minimize the yield loss as well as loss of quality of the fruit caused by these diseases. The use of fungicides is the regular approach for the management of phytopathogenic fungi but this is not eco-friendly, as its usage can cause many problems like adverse effects on beneficial soil microorganisms (Srinivasulu and Ortiz, 2017) and many plant

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pathogens may develop resistance to synthetic fungicides with continuous exposure (Possiede *et al*. 2009). Extensive usage of synthetic fungicides can also result in residual toxicity and pollution and are shown to have carcinogenic and teratogenic attributes on humans and animals (Kalemba and Kunicka, 2003). Therefore an alternative method for the effective management of these phytopathogens is required. Hence to develop an eco-friendly biocontrol management strategy against the phytopathogenic fungi of pumpkin, the present study was undertaken with the objectives of isolation of plant pathogenic fungi from diseased pumpkin plant explants and screening of MTCC bacterial cultures for antifungal activity by dual culture against these isolated phytopathogenic fungi.

Materials and Methods:

Isolation of phytopathogenic fungi from infected pumpkin plant explants:

The technique used for isolation of pathogens was tissue isolation procedure as described by Dhingra and Sinclair (1995). The infected pumpkin leaves and fruit samples were collected from the field and transported to the lab in clean polythene covers. The collected infected leaves and fruit samples were first washed in running tap water to remove dirt and other foreign materials. Affected portion of the pumpkin leaves and fruits were cut into small bits (4-5 mm). These bits were surface sterilized with sodium hypochlorite and then washed five times with sterile distilled water. Finally the disinfected bits were transferred with the help of a sterilized inoculating needle to the Petri plates (4 bits per plate) containing sterilized solidified potato dextrose agar (PDA, Himedia) amended with 100 ppm of chloramphenicol (20 ml per plate). All these operations were carried out inside aseptic inoculation chamber. The inoculated plates were incubated for 7 days at room temperature (RT) with 12 h photoperiod and observed for the growth of pathogens from the plated plant tissues. The incubation was continued for 15 days to allow sporulation. The spores were observed microscopically. Identification of the isolated pathogens was done with the help of standard manuals based on cultural characteristics and sporulation.

All the isolates were sub-cultured on to fresh PDA media plates by disc inoculation method and the inoculated plates were incubated at RT for 7 days. The plates were preserved in a refrigerator at 5°C until used and renewed once in 30 days.

Screening of bacteria for antifungal activity by dual culture:

Nine bacterial cultures obtained from MTCC (Microbial Type Culture Collection, IMTECH, Chandigarh, India) such as *Bacillus amyloliquefaciens* (MTCC 10439), *Bacillus*

cereus (MTCC 9017), *Bacillus subtilis* (MTCC 8142), *Enterobacter aerogenes* (MTCC 8558), *Erwinia* sp. (MTCC 2760), *Pseudomonas aeruginosa* (MTCC 7904), *Pseudomonas fluorescens* (MTCC 9768), *Pseudomonas marginalis* (MTCC 2758), and *Pseudomonas monteilii* (MTCC 9796), were screened for their antifungal activity. These nine bacterial cultures were sub-cultured on nutrient agar (NA) slants.

Screening of antifungal activity of bacteria was done by dual culture method (Girish and Bhavya, 2018). 5.0 mm discs, obtained from 7-day-old pure cultures, of the isolated phytopathogenic fungi *Alternaria* sp., *Drechslera* sp., and *Fusarium* sp., were inoculated at the center of the solidified PDA medium taken in a 90 mm Petri plates, separately. Each bacterial culture was then streaked above and below the fungal disc with sterilized inoculation loop at a distance of 2.0 cm, separately. The inoculated Petri plates were then incubated at RT for 10 days. After incubation the plates were observed for the presence of zone of inhibition or reduction in the mycelial growth (in comparison to control plates) indicating the antifungal activity of bacteria.

Results:

Isolation of phytopathogenic fungi from infected pumpkin plant explants:

Phytopathogenic fungi infecting pumpkin such as *Alternaria* sp., *Drechslera* sp., and *Fusarium* sp., were isolated from diseased pumpkin plant explants such as leaves and fruits (Figure 1). They were identified based on cultural characteristics and sporulation (Figures 2 - 4).

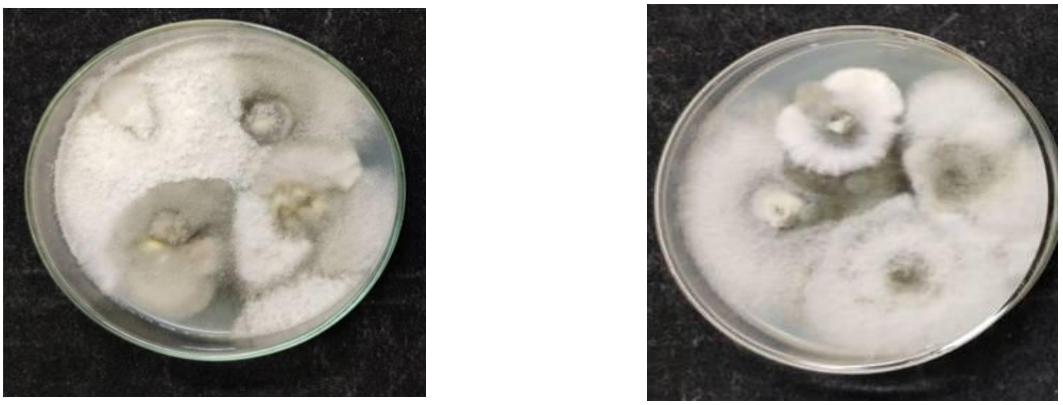

Figure 1: Fungal growth from infected pumpkin plant explants (fruits and leaves) on PDA plates after 7 days of incubation

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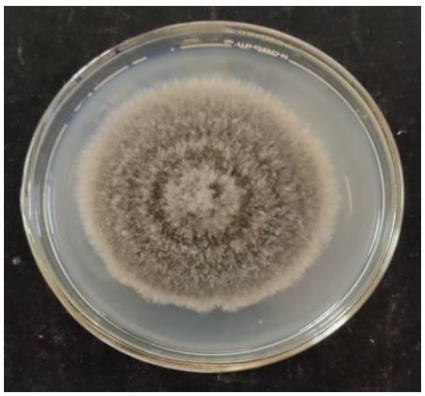
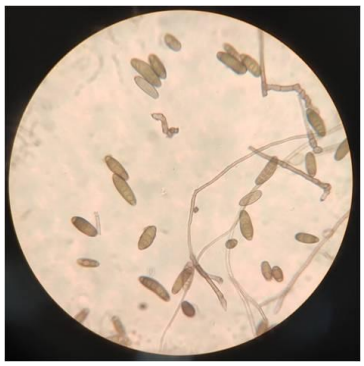


Figure 2: (A) Pure culture of *Alternaria* sp., on PDA plate (10-days-old)

(B) Conidia of *Alternaria* sp.

Figure 3: (A) Pure culture of *Drechslera* sp., on PDA plate (10-days-old)



(B) Conidia of *Drechslera* sp.

Figure 4: (A) Pure culture of *Fusarium* sp., on PDA plate (10-days-old)

(B) Macroconidia of *Fusarium* sp.

Screening of bacteria for antifungal activity against *Alternaria* sp., by dual culture method:

Among the nine different bacterial cultures screened, bacteria such as *Enterobacter aerogenes* (MTCC 8558), *Erwinia* sp. (MTCC 2760), and *Pseudomonas aeruginosa* (MTCC 7904), exhibited comparatively more effective antifungal activity against the fungi *Alternaria* sp., showing the zone of inhibition and reduced mycelial growth in comparison to control after 10 days of incubation by dual culture method.

Pseudomonas marginalis (MTCC 2758), *Pseudomonas monteilii* (MTCC 9796), *Pseudomonas fluorescens* (MTCC 9768), and *Bacillus subtilis* (MTCC 8142) suppressed the growth of *Alternaria* sp., whereas *Bacillus amyloliquefaciens* (MTCC 10439) and *Bacillus cereus* (MTCC 9017) did not show any antifungal activity (Figures 5 and 6).

Screening of bacteria for antifungal activity against *Drechslera* sp., by dual culture method:

All the nine different bacterial cultures screened, exhibited effective antifungal activity against the fungi *Drechslera* sp., showing the zone of inhibition and reduced mycelial growth in comparison to control after 10 days of incubation by dual culture method.

Comparatively *Pseudomonas marginalis* (MTCC 2758), *Pseudomonas monteilii* (MTCC 9796) and *Pseudomonas fluorescens* (MTCC 9768) showed less antifungal activity against *Drechslera* sp. (Figures 7 and 8).

Screening of bacteria for antifungal activity against *Fusarium* sp., by dual culture method:

Out of the nine different bacterial cultures screened, bacteria such as *Enterobacter aerogenes* (MTCC 8558), *Pseudomonas aeruginosa* (MTCC 7904) and *Pseudomonas marginalis* (MTCC 2758), exhibited comparatively better antifungal activity against *Fusarium* sp., showing the zone of inhibition and reduced mycelial growth in comparison to control after 10 days of incubation by dual culture method.

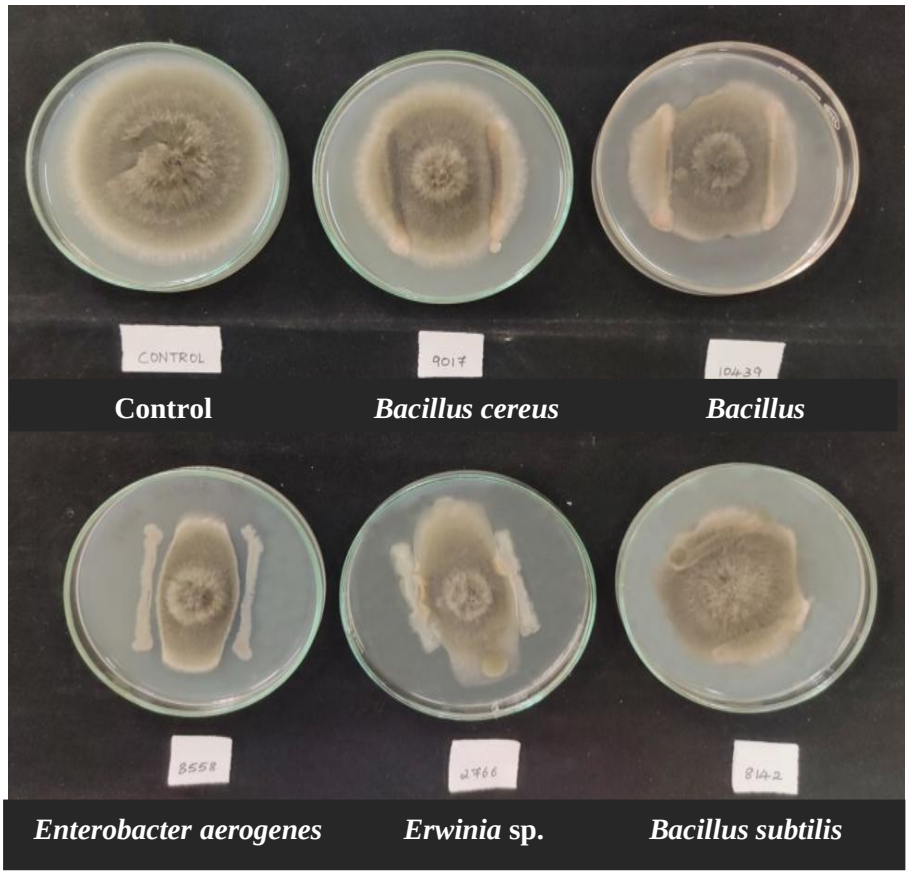


Figure 5: Antifungal activity of bacteria against *Alternaria* sp.

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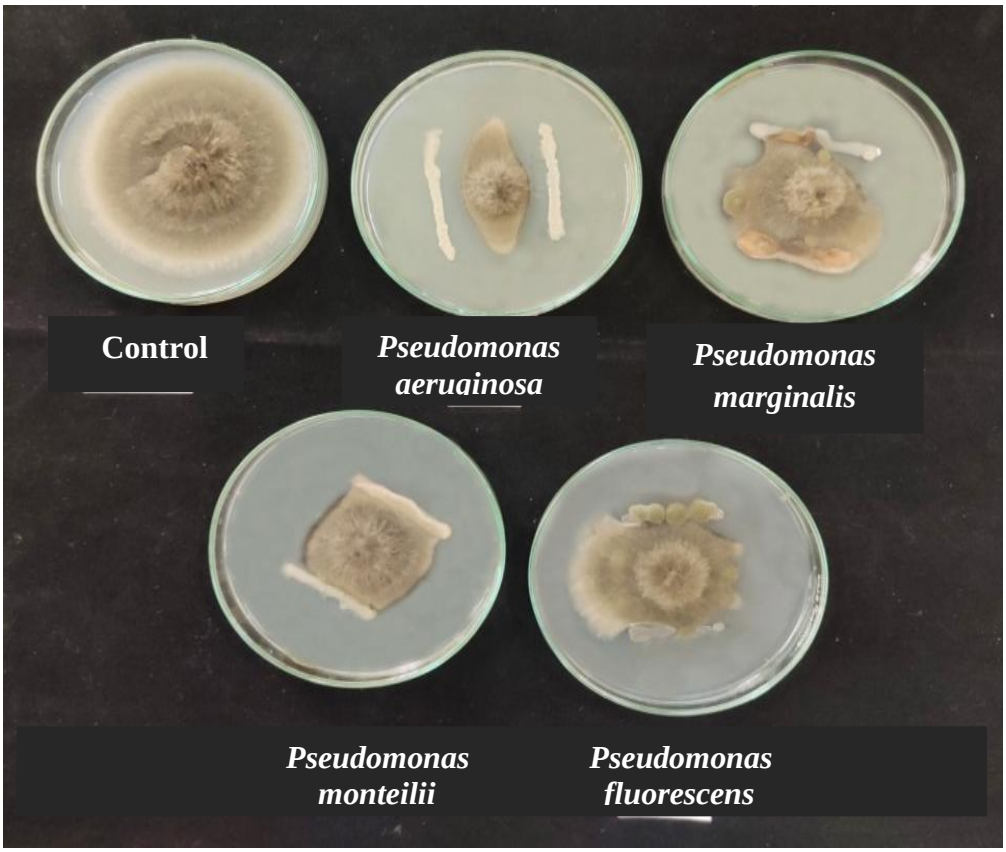

Figure 6: Antifungal activity of bacteria against *Alternaria* sp.

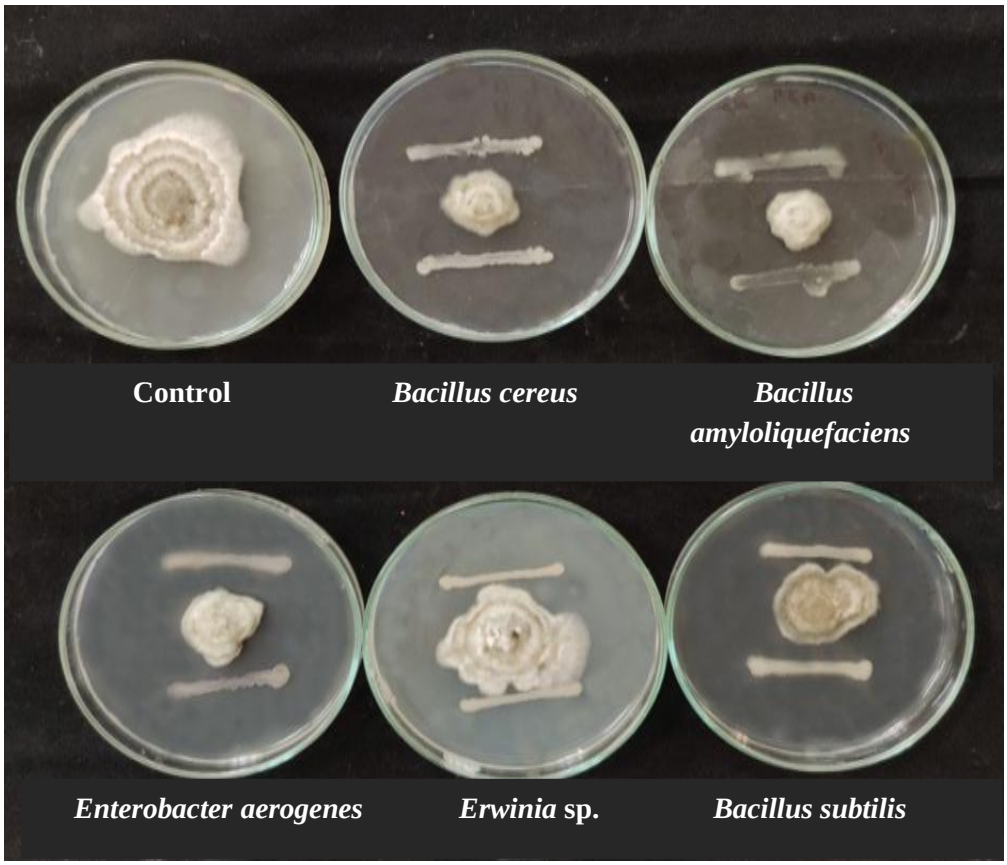

Figure 7: Antifungal activity of bacteria against *Drechslera* sp.

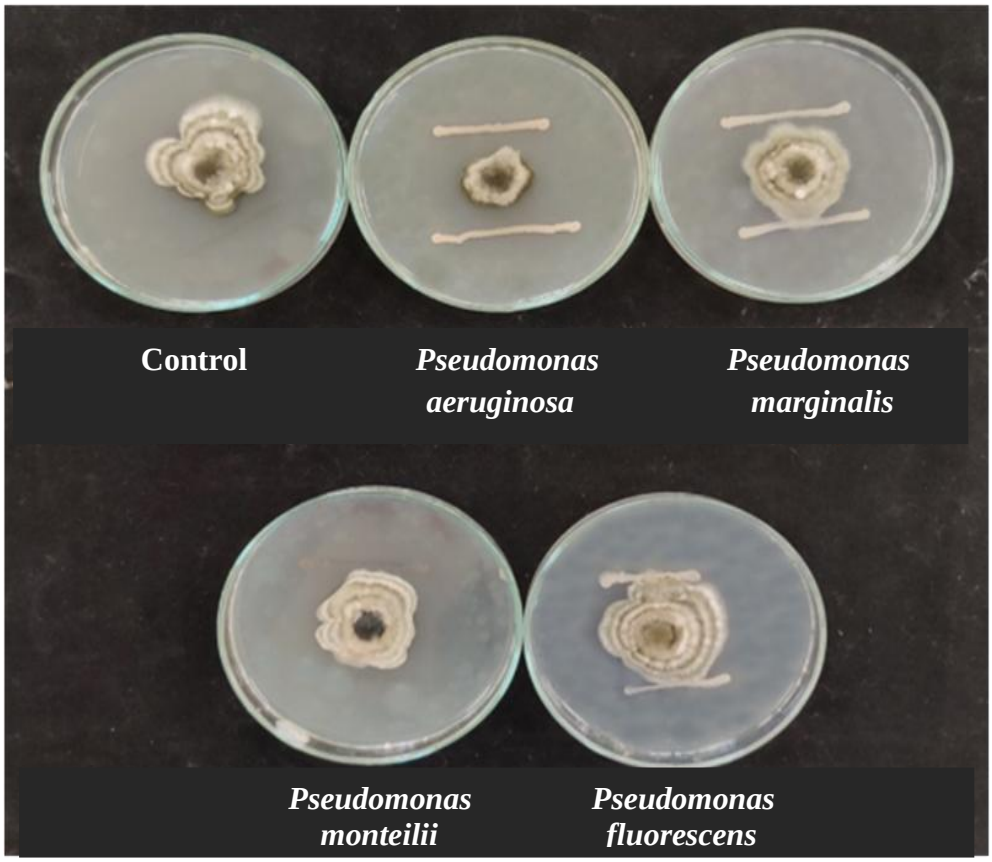

Figure 8: Antifungal activity of bacteria against *Drechslera* sp.

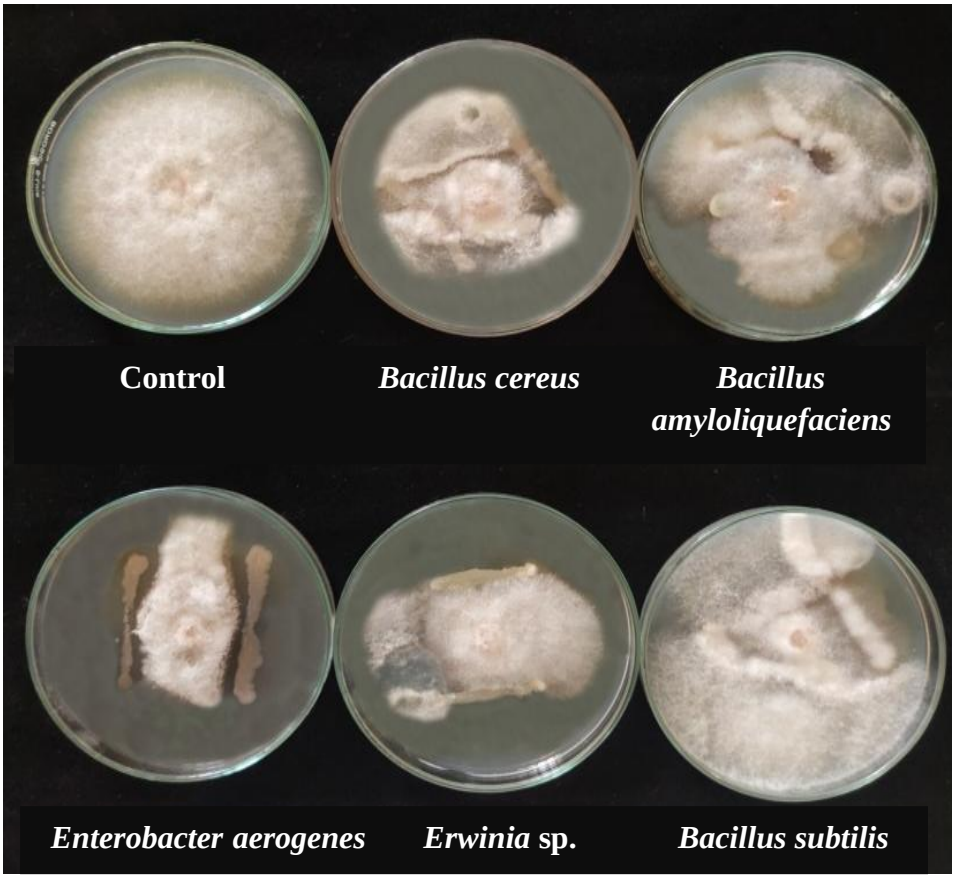

Figure 9: Antifungal activity of bacteria against *Fusarium* sp.

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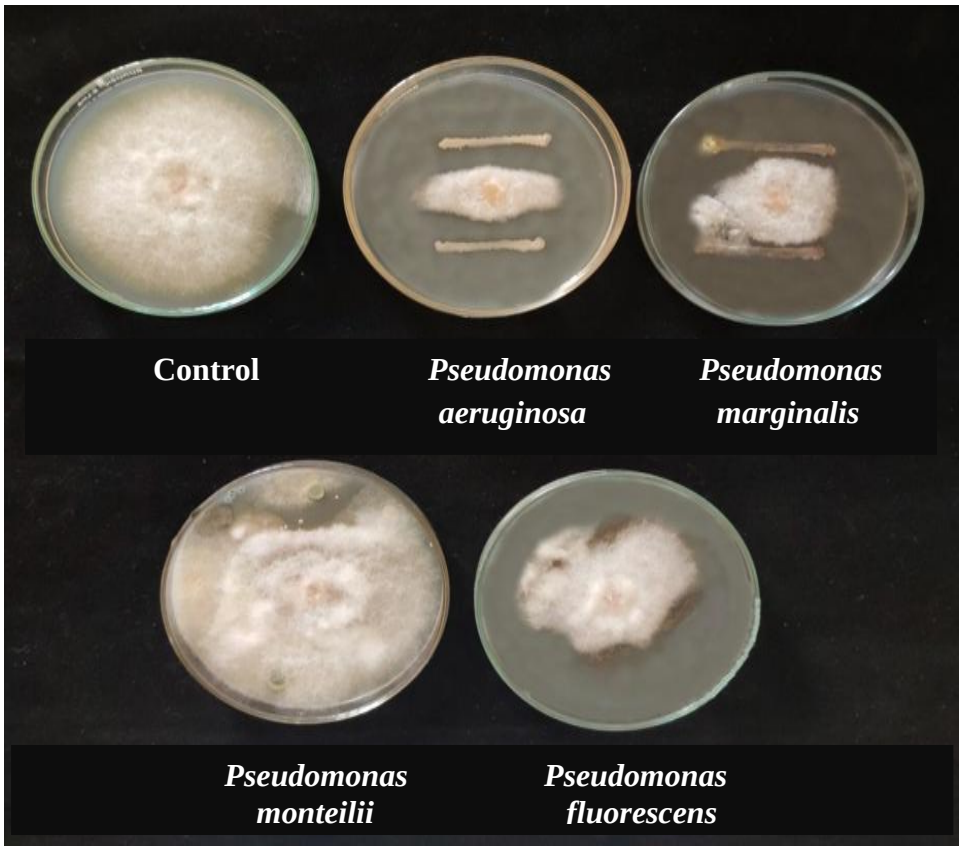

Figure 10: Antifungal activity of bacteria against *Fusarium* sp.

Bacillus cereus (MTCC 9017), *Erwinia* sp. (MTCC 2760), and *Pseudomonas fluorescens* (MTCC 9768) suppressed the growth of *Fusarium* sp., while *Bacillus amyloliquefaciens* (MTCC 10439), *Bacillus subtilis* (MTCC 8142) and *Pseudomonas monteilii* (MTCC 9796) did not show any antifungal activity (Figures 9 and 10).

Enterobacter aerogenes (MTCC 8558) and *Pseudomonas aeruginosa* (MTCC 7904) exhibited effective antifungal activity against all the fungi tested such as *Alternaria* sp., *Drechslera* sp., and *Fusarium* sp., and could be further studied for the development of eco-friendly management strategy against these fungi.

Discussion:

The major reason for carrying out the analysis of antifungal activity of bacteria extensively and intensively in recent years is the concern on human health. Chemical drugs, synthetic fungicides and preservatives are considered to be responsible for many carcinogenic and teratogenic attributes, as well as residual toxicity (Moreira *et al.*, 2005). The chemical additives and synthetic products are viewed in a suspicious manner by consumers and thereby the demand for the natural products has been intensified (Kalemba and Kunicka, 2003). Most of

the natural products are socially acceptable as they protect the people, livestock and food from diseases, pests and spoilage without any harm to environment and health.

The use of many synthetic fungicides has been cautioned due to their polluting effects, non-biodegradability and residual toxicities. Most of these fungicides have become a popular target of conservationists and are treated to be one of the most vital man-made pollutants (Khoshoo, 1980). Further many plant pathogens can develop resistance to synthetic fungicides with continuous exposure (Brent, 1995). This has led to finding eco-friendly alternative approaches for management of plant diseases (Girish *et al*., 2009).

Softer biological measures for the control of plant diseases are gaining popularity in recent years. Biocontrol agents are considered as better alternatives with different mechanisms of action than chemical pesticides. Search is on for the development of plant disease control agents, which are non-toxic, biodegradable and ecofriendly. Bacterial cultures show antifungal activity against a wide range of phytopathogenic fungi. A lot of researchers have documented the antifungal activity of bacterial cultures against different fungal species (Shoda, 2000).

The present study evaluated the effect of nine bacterial cultures obtained from MTCC on phytopathogenic fungi of important commercial crop pumpkin such as *Alternaria* sp., *Drechslera* sp., and *Fusarium* sp. Although *Erwinia* sp. (MTCC 2760), *Pseudomonas marginalis* (MTCC 2758), *Pseudomonas fluorescens* (MTCC 9768) inhibited the growth of fungi, *Enterobacter aerogenes* (MTCC 8558) and *Pseudomonas aeruginosa* (MTCC 7904) proved to be more potent in inhibiting the mycelial growth of tested fungi. Comparatively bacteria were more potent against *Drechslera* sp., while least potent against *Fusarium* sp. Screening was done by dual culture method. This method has been regularly employed by researchers for initial screening of antifungal activity of bacteria (Girish *et al*., 2009; Mahmoud *et al*., 2016; Girish and Bhavya, 2018). The results of present study indicated that bacterial cultures tested possess significant antifungal activity and could be exploited as an ideal treatment for pumpkin plant disease management.

Similar results of antifungal activity by *Enterobacter* spp., and *Pseudomonas aeruginosa* have been reported by many workers. *Enterobacter agglomerans* IC1270 exhibited significant antifungal activity against *Rhizoctonia solani* causing root rot in cotton (Chernin *et al*., 1995). *Enterobacter asburiae* completely inhibited the growth of *Aspergillus flavus* and also the production of aflatoxin by it (Gong *et al*., 2019). *P. aeruginosa* PGPR2 was reported to be very effective against charcoal rot disease causing *Macrophomina phaseolina* and other phytopathogenic fungi such as *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., and *Rhizoctonia* sp.

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(Hameed *et al*., 2013). *P.* aeruginosa showed significant antifungal activity against *Fusarium oxysporum* causing root rot when screened using dual culture assay (Mahmoud *et al*., 2016).

Mechanisms of biocontrol of plant pathogens by bacteria *in vitro* are the result of mainly antibiosis (Kohl *et al*., 2019). The antifungal activity observed in the present studies may also be attributed mainly to antibiosis. *Enterobacter agglomerans* produced pyrrolnitrin having broad spectrum antagonistic activity against fungi (Chernin *et al*., 1996). *Pseudomonas aeruginosa* was reported to produce pyocyanin active against *F. oxysporum* (Mahmoud *et al*., 2016). Bacteria can also cause degradation and digestion of cell wall components resulting in hyphal perforation, loss of structural integrity of the mycelium, along with degradation of conidia (Harish Kumar *et al*., 2017). This is a preliminary investigation to know the potential of these bacterial cultures against phytopathogenic fungi. Further study is warranted to know the actual mechanism of antifungal activity against the phytopathogenic fungi.

However, in the field these bacteria may also exhibit, in addition, other modes of antagonism reported such as predation or parasitism, induced resistance of the host plant, and direct competition for space and limited resources (Kohl *et al*., 2019), and understanding of this requires further research.

Conclusion:

The results of the present study indicates that out of the nine MTCC bacterial cultures studied, two bacteria namely *Enterobacter aerogenes* (MTCC 8558) and *Pseudomonas aeruginosa* (MTCC 7904) possess significant antifungal activity against all the three tested plant pathogenic fungi and could be exploited as biopesticides for the effective and eco-friendly management of pumpkin phytopathogens such as *Alternaria* sp., *Drechslera* sp., and *Fusarium* sp. However this is a preliminary investigation and requires further studies to have the precise knowledge of active fractions and their activity in green house and field conditions, so that effective biopesticide formulations of these two bacterial strains could be developed.

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AUTOMATED DRIP IRRIGATION SYSTEM FOR SUSTAINABLE CROP PRODUCTION

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Abstract:

Nowadays agriculture uses 85% of water for irrigation purposes only and this percentage may increase due to increased population growth and food demands. The most significant environmental factor affecting growth, production and quality of crops is water stress. The modern and updated irrigation techniques introduce automated irrigation using differentequipment to provide water and nutrients to plants as soon as they need it. Automation is a sort of innovation, where the manual interventions are substituted by an automated system to carry out all the operations. It is relatively new but gaining popularity day-by-day. Various types of automated irrigation systems are available such as time-based system, volume based system open loop system, closed loop system, real time feedback system, computer-based irrigation control systems, sensor controlled micro irrigation etc. The use of automation in drip irrigation system can help to overcome the problem of water wastage especially in areas where water is scarce. The automatic drip irrigation scheduling can be a better substitute for manual drip irrigation operation and it also enhances the water use efficiency and quality of the produce. As the farm holdings are not large enough in India, appropriate low cost automatic irrigation is suitable to farmers.

Keywords: Automated drip irrigation, Water stress, Water use efficiency

Introduction:

Agriculture is the prime backbone of Indian Economy, but in India most of the irrigation systems are still operated manually. In the changing climate scenario, the water resource has become very scarce and also being unscientifically used in the farming fields.The available traditional methods of irrigation are drip irrigation, ditch irrigation, sprinkler system. Nowadays automated and semi-automated technologies been installed for irrigating the field which has

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replaced the traditional agricultural mechanism (Phuntsho *et al.*, 2011). Automation is the godsend of farming and a sort of innovation, in which the manual interventions are replaced by an automated system to execute all the operations (Anjaly and Hakkim, 2017). In developed countries a large area has been covered under automated micro irrigation system (Edordu and Sacks, 2006). It is relatively new but getting popularity day-by-day. Now, in India predisposition towards automization of drip or micro irrigation is attaining momentum due to:

- Automation gets rid of manual operation to open or close valves, particularly in intensive irrigation process.
- Possibility to change recurrence of irrigation and fertigation process and furthermore to optimize these processes.
- Adoption of advanced cropping systems and new technologies, especially intensive cultivation which are complex and difficult to operate manually.
- Utilization of water from various sources and increased water use efficiency.
- System can be operated at night; thus the day time can be used for other agricultural activities.
- Pump starts and stops precisely when required, thus optimizing energy requirements.

Micro-irrigation:

The term micro-irrigation relates to irrigation technologies utlizing water emitters with tiny apertures whichconvey water at low flow rates, less than 200 L h^{-1}. In the last decade, the utilization of micro-sprinklers has been stretched out to the irrigation of vegetables and field crops and in mobile center pivots and linear-move laterals. Micro emitters are commonly made from rigid plastic materials and are much smaller and less expensive than conventional sprinklers. Spoke-type static deflectors emit a number of streams that spray out from the emitter. These deflectors are less sensitive to windy conditions and the emitter is reliable because there are no moving parts. In the vibrating deflector type, the water is discharged from a circular orifice and strikes a deflector that scatters the water around. This type of emitter is simple and reliable. Micro-irrigation with foggers is used frequently in greenhouses for increasing the relative humidity and decreasing the temperature of the ambient air (Abdul *et al.*, 2016). They are operated intermittently in pulses by an automatic controller. Bridge type micro-sprinklers offerenhanced support to the rotating spinner, but the vertical part of the bridge creates a dry area behind the vertical support.

Drip irrigation:

Drip irrigation is used for the most precise water application related to crop water requirement and root system development. Drip irrigation uses a lower pressure than sprinkler

irrigation and can be conveniently integrated with different levels of automatic control. Thus, it is very well suited to fertigation Boopathy *et al*., 2014). Drip irrigation is independent of wind conditions and can be applied at any time of the day. Weed development is restricted because there is only partial wetting of the soil surface. A voiding the wetting of crop leaves decreases infection and spread of leaf diseases and leaf scorch. Drip irrigation sends an even, deep supply of water directly to the root zone of the plant without waste or run off (Erdem *et al*., 2010). It utilizesaround half less water than conventional systems, spares plantstress brought about by the wet-dry cycles of overhead watering and minimizes erosion, soil compaction, leaf burn, mold and fungal diseases (Galande, 2012).

Scope of automation in drip irrigation / microirrigation:

1 The scope of automation has become a new development in micro irrigation sector.
2 In India, the pressurized irrigation has created a great awareness among the farmers.
3 From government side several initiatives have been taken for popularization.

Various types of automation system:

1. Time based system:

In time based system, time is the basis of irrigation. Time of operation is calculated according to volume of water required and the average flow rate of water. The duration of individual valves has to be fed in the controller along with system start time, also the controller clock is to be set with the current day and time. As the start time of programme knocked bythe clock of the controller, it starts conveying signals to the first automatic valve of the programme sequence, at the same time the pump also gets started. As soon as first valve duration is over, the controller either stops or switches on to next valve. Whenever the operation of last valve will over, controller will stop sending signals to valves and pump. The same process is repeated at next run time.

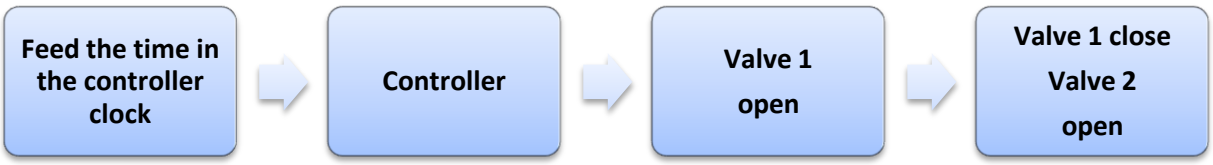

Figure 1: Flowchart of time based system

2. Volume based system:

In volume based system, water can be applied in different field segments through automatic volume controlled metering valves. Automation using volume based systems are of two types.

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In first type of system, automatic metering valve with pulse output furnishes one pulse after completion of one dial in the automatic metering valve. Consequently, it can count the volume of water passed through by counting the number of pulses received by the controller. After providing required volume of water through first valve, it closes down and controller switches on the next valve subsequently.

In second type of system, there is no requirement of any controller. Automatic metering valves are placed near the field of each segment. In series all automatic metering valves are interconnected with the help of control tube. Components like T-connector, shuttle valve and a 3-way relay are also installed along the circuitfor automatic closing and opening of the metering valves with the help of water pressure signal. In the time of sequential operation only one automatic metering valve remains open. After closing the first valve, the next valve in the series opens. Shut down of the irrigation pump can be made automatic after closure of the last valve in series by connecting the spare end of the last valve T-connector to a microswitch with control tube. Micro-switch is connected to the pump motor starter's magnetic coil. When the last automatic metering valve closes, it transmits pressure signal to the micro-switch by means of pressure which in turn activates a pressure switch and terminates the motor starter circuit resulting in automatic shutdown of irrigation pump.

The major advantage of volume based irrigation system over time-based system is assurance for delivering the preset amount of water irrespective of continuous availability of electricity, but time based system is comparatively cheaper and hence gaining more popularity than the volume based system.

3. Open loop systems

In an open loop system, the operator decides the amountan time of application of water. This information is programmed into the controller and the water is applied according to the desired schedule. Open loop control systems uses either the irrigation duration or a specified applied volume for control purposes. Open loop control systems are cheaper and readily available. The drawback of open loop systems is that they do not respond automatically to changing conditions in the environment. In addition, they may require frequent resetting in order to achieve high irrigation efficiency.

4. Closed loop systems

This system requires feedback from one or more sensors. The operator develops a general control strategy. After defining general strategy, the control system takes over and makes decision regarding time and amount of irrigation. Irrigation decisions are made and actions are

carried out based on data from sensors. In this type of system, the feedback and control of the system work continuously. Closed loop controllers require data acquisition of environmental parameters such as soil moisture, temperature, radiation, wind-speed as well as system parameters like pressure, flow, etc.

5. Real time feedback system

Real time feedback is based on actual dynamic demand of the plant itself, plant root zone effectively reflecting all environmental factors acting upon the plant. It operates within controlled parameters and the plant itself determines the required amount of irrigation. Various sensors such astensiometers, relative humidity sensors, rain sensors, temperature sensors etc. control the irrigation scheduling. These sensors provide feedback to the controller to control its operation.

6. Computer-based irrigation control systems

A computer-based control system consists of a hardware and software that works as a supervisor for managing irrigation and other related practices such as fertigation and maintenance. Generally, the computer-based control systems that are used for managing micro irrigation systems can be divided into two categories:

A. Interactive systems, B. Fully automatic systems.

Closed control loops are used in these systems which include:

- Monitoring the state variables that are pressure, flow within the system.
- Comparison of state variables with their desirable state.
- Decision regarding actions are necessary to change the state of the system.
- These functions requires a combination of hardware and software that must be implemented for each specific application.

7. Interactive Systems:

Interactive systems requires a microcomputer, either a standard personal computer (PC) or a specially designed unit. The information is transferred into a central unit either directly from sensors in the pipeline or from intermediate units which collect the data from a number of sensors and then process and store them temporarily for further transfer to the central computer. These systems have features that allow the operator to send commands back to different control units of the irrigation system. The field devices such as valves, regulators, pumpsare fitted with electrically operated servo-devices which allow actuation of the pumps, closing and opening of valves, and adjusting pilot valves of flow regulators. This type of system enables the operator to control the flow from the central computer by controlling flow parameters such as pressure and

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flow rate, according to specific needs at the given time, and to receive immediate feedback on the response of the system.

8. Automatic Systems:

In fully automated systems the human factor is not required and it is replaced by a computer specifically programmed in order to react appropriately to any changes in the parameters monitored by sensors. The automatic functions are activated by feedback from field units and corrections in the flow parameters by control of devices in the irrigation system until the desired performance level is achieved. Automatic systems can also perform auxiliary functions like stopping irrigation in case of rain, injecting acid to control pH, sounding alarms, etc. Most of the control systems include protection in emergencies such as loss of the handled liquid due to pipe burst.

Intelligent IoT Based Irrigation System:

The automated irrigation system consists of two units are control unit and sensing unit. The control unit consists of microcontroller which controls the execution of operation and the sensing unit consists of different sensors such as DHT11 sensor, ARDUINO board and soil moisture sensor. The DHT11 sensor is used to measure the temperature and humidity of a root zone of plants in dry and wet conditions. For remote monitoring, GPRS based systems are employed on wireless sensor networks for monitoring the temperature and humidity data continuously or periodically (Hui *et al.*, 2008). A threshold can be set such that the microcontroller unit sends an SMS to the mobile phone of an owner. Threshold must be chosen in the range of 40 to 30 degrees centigrade, because the most of the values fall on those ranges during normal and wet conditions (Rajendranath and Hency, 2015). The microcontroller sends the alert SMS and also send the particular exceed value of a temperature or humidity or soil moisture value to the owner in consecutive SMS. This kind of system proved that the use of water can be diminished and hence water will not be wasted and reduces the human resources (Shekhar *et al*., 2017). The overall system can be powered up using solar cells to maintain the system in low cost (Rajendranath and Hency, 2015). This type of system can be extended by using WSN nodes for transmit data and also using data base systems to store the data at the field. In Future, an Intelligent IoT based Automated Irrigation system can be extended not just for irrigating the field with water but also for deciding on spraying appropriate chemicals for proper growth of crop.

Development of automation in drip irrigation system

Agarwal *et al.* (2014) developed a soil moisture sensor based on the fact that water is not pure water which is non-conductor, but it is impure which is slightly conductor. Water sensor is nothing but a series of very close printed circuit boards (PCB) tracks. In normal mode these tracks do not conduct, but when some water falls on these tracks start conducting slightly, some positive voltage is available at the base of transistor So negative positive negative (NPN) transistor is on and NPN transistor provide a negative voltage as a pulse to the microcontroller. The output voltage of a sensor is amplified by an operational amplifier, and is provided to the base of transistor. The moisture sensitivity adjusting the gain of an operational amplifier by variable resistor (VR).

Chandrasekhar and Chakravarthi (2013) developed an automatic drip irrigation system using low cost sensors and simple circuitry. Irrigation system uses valves to start and stop irrigation. These valves are automated by using controllers and solenoids. The humidity sensors are constructed using aluminium sheets and housed in easily available materials. The aim is to use the easily available material to construct sensors involving low cost. Five relays are controlled by the microcontroller through the high current driver IC, ULN2003. Four relays help in controlling four solenoid valves in order to control the flow of water to four different parts of the field. One relay is used to shut-off the main motor which is used to pump the water to the field.

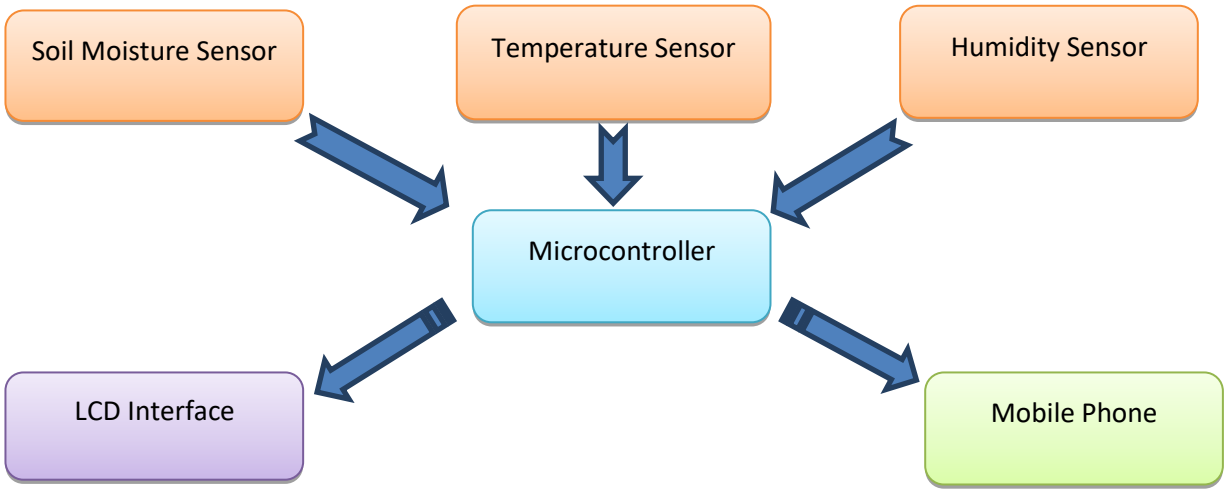

Figure 2: Block diagram implementation of an automated irrigation system

Ingale and Kasat (2012) prepared a circuit which is cheap and reliable to develop an automated irrigation system. The system provides with several benefits and can operate with less manpower. The system supplies water only when the humidity in the soil goes below the

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reference. Due to the direct transfer of water to the roots, water is not wasted and it also helps in maintaining the moisture to soil ratio at the root zone constant to some extent. Thus the system is efficient and compatible to thechanging environment. Also the system saves the water and improves the growth of plants.

Hameed and Agarwala (2009) proposed a novel approach to determine the efficient water requirement of agricultural fields for farming in a most scientific and cost effective manner and to design a ultra-low cost moisture sensor using computer and thereby to manage the water resources more appropriately in agricultural farms for the plants irrigation and its results reveals that the capacitance response characteristics make it possible to maintain soil moisture at the desired level as per the crop requirement and its withstanding capacity from its field capacity level. Due to plant uptake, evapotranspiration, atmospheric temperature, the soil moisture continues to vary and most of the crops can give a better yield at up to 50% decrease in soil moisture content from its field capacity. So, in this experiment an effort has been made to use a sensor to estimate the PWP and FC and a comparative study is made using tensiometerconcurrently and its being concluded that it may improve a new insight to irrigation automation and will be a boon to unskilled farmers to optimize their crop yield as well as saving water, electricity apart from reducing greenhouse gases emission from agricultural fields which takes place up on full saturation.

Cepuder and Nolz (2007) stated that water is a limited resource, mainly in intensively used agricultural areas in Austria, where groundwater is used as drinking water as well as for irrigation purposes. In order to guarantee a sustainable use of irrigation water, soil water measurement devices can be used to optimise irrigation, which means that controlling the soil water content in the entire root system may prevent water stress due to water deficiency on the one hand, and over wetting on the other hand. Furthermore, losses of nutrients due to leaching can be avoided. Sensors in various depths measure the plant water uptake in the root zone under standard irrigation practices on different sites and soils, respectively.

Use of automated irrigation system for different crops

Anand and his co-workers investigated the effect of automation irrigation system for maize in polybags, they collected data on plants of equal height and diameter continuously for 8 days. From Table 1, we can conclude that there is a considerable faster growth was observed with automation irrigation (34.70 cm) compared with without automation (34.20 cm) in maize.

Table 1: Effect of automated irrigation system on maize (Anand *et al.*, 2018)

Day	With Automation		Without Automation	
	Plant Height (cm)	**Diameter (cm)**	**Plant Height (cm)**	**Diameter (cm)**
1	34.00	1.75	34.00	1.75
2	34.20	1.76	34.00	1.75
3	34.20	1.76	34.00	1.75
4	34.50	1.77	34.10	1.76
5	34.50	1.78	34.10	1.76
6	34.50	1.78	34.10	1.76
7	34.60	1.78	34.10	1.76
8	34.70	1.79	34.20	1.77

In this automated irrigation system wheat crop was grown under two different water regimes, 80% and 40% by using humidity sensors. Fig. 3 and 4 show that there was no significant difference between plant height in automated and manual irrigation but there was a significant difference in total dry weights both at 80% and 40% of field capacity.

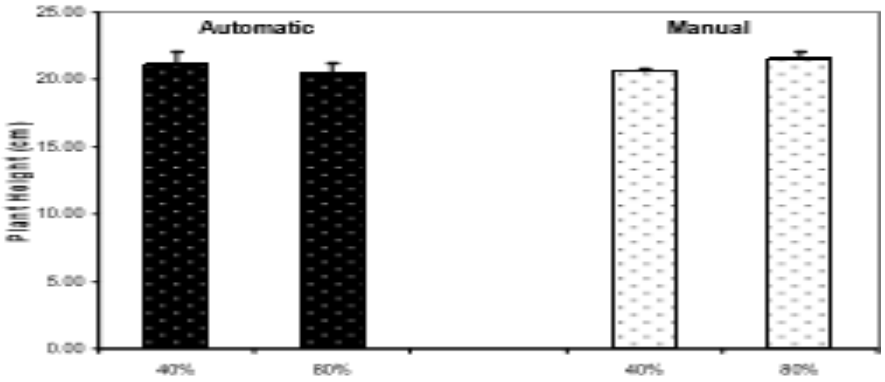

Figure 3: Effect of automated and manual irrigation on plant height (Boutraa *et al*., 2011)

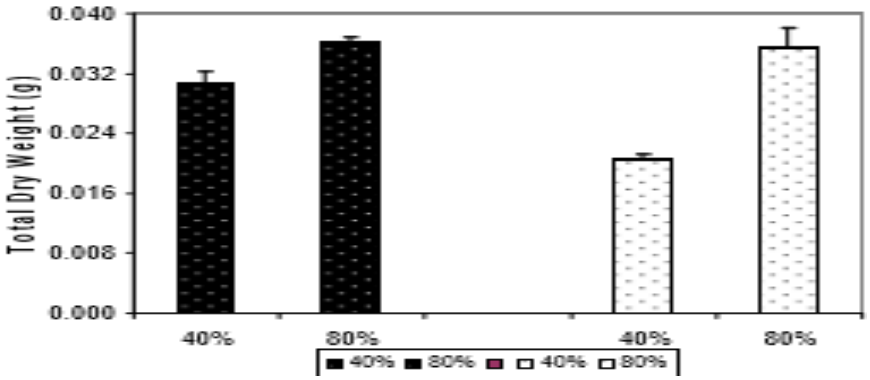

Figure 4: Effect of automated and manual irrigation on total dry weight (Boutraa *et al*., 2011)

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As a result of this two-year field studied for wheat crop under arid region, it was concluded that the IIS method under sprinkler irrigation offered significant advantage for seasons, its easiness application and more water saving (Table 2). The ICS treatment resulting in greater yield than IIS could be attributed to variation of amount of water added to the two treatments. Therefore, the IIS irrigation method would be recommended due to its easiness application and more water saving. The IIS technique conserved irrigation water by 26% more than that provided by control system ICS.

Table 2: Comparison of yield components and efficiencies using sprinkler irrigation systems for both intelligent irrigation (IIS) and control (ICS) (Ghobari *et al*., 2013)

Character	2009 -2010 Season		t- sign	2010 - 2011 Season		t- sign
	IIS	ICS		IIS	ICS	
Grain yield (GY)	5.07	6.10	**	5.98	7.56	**
Biological yield (BY)	13.35	16.02	**	15.87	18.60	**
Harvest index (HI)	0.38	0.38	ns	0.40	0.38	ns
1000 Kernel weight (KW)	39.12	47.68	**	42.42	48.66	**
Plant height (PH)	49.50	66.25	**	80.80	85.0	ns
Spike length (SPL)	9.50	10.00	**	9.50	10.88	**
WUE (kg m^{-3})	1.27	1.13	**	1.64	1.47	**
IWUE (kg m^{-3})	1.12	1.06	**	1.37	1.21	**

*,** t is significant at 0.05 and 0.01, respectively
ns = Not Significant

Lailhacar *et al.* (2008) noticed that the modern technologies could able to improve irrigation efficiency of turfgrass, encouraging water conservation and decreasing environmental impacts. The objectives of thier research was to compute irrigation water use and to assess turf quality differences between (1) Time-based scheduling with and without a rain sensor (RS); (2) A time-based schedule compared to a soil moisture sensor (SMS) based irrigation system; and (3) Different commercially available SMS systems. SMS based treatments comprised of irrigating one, two, or seven days a week, each with four different commercial SMS brands. Time-based treatments with or without RS and a non-irrigated treatment were also executed. Remarkable differences in turfgrass quality among treatments were not detected due to the sustained wet weather conditions during the testing periods. The treatment with the rain sensor resulted in 34% less water applied than without the rain sensor (2-WORS) treatment. Most of SMS brands recorded irrigation water savings as compared to 2-WORS, ranging from 69 to 92% for three of four SMSs tested, depending on the irrigation frequency. Therefore, SMS systems represent a promising technology because of the water savings that they can achieve during application.

Conclusion:

The automation of the irrigation process is important for three main reasons: scarcity of water, timely irrigation and maximum crop profit. Automated irrigation system permits cultivation in places with water scarcity thereby improving sustainability as well as maintains optimum soil moisture levels required for crop. This reduces the human Automated errors in maintain soil moisture and nutrients at optimum levels, there by increases yield with less cost of production as cost on workforce will be cut down. Automated system components require minimum maintenance**.** Reductions in water use range as high as 70% compared to farmer practices with no negative impact on crop yields. Automatic irrigation systems presently available are costly and are not adopted by most of the Indian farmers. To apply simple electronic circuit principles in irrigation an attempt has been made to develop low cost automated drip irrigation based on soil moisture.

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STUDIES ON SOME PROTOZOAN PARASITES OF INDIAN GOAT *CAPRA INDICA*

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Abstract:

Goats and sheep are the important source of animal protein. They are primarily useful for meat and wool production. They also produce a considerable amount of manure, which is of special importance in those areas where cattle are of lesser importance. Parasites infections of goats are major factor responsible for economic losses through reduction in productivity and increased mortality. Goats harbor a variety of gastrointestinal parasites that affect the growth as well as production of animal.

Keyword: Protozaon parasites, *Capra indica*.

Introduction:

Most of the protozoans are highly pathogenic to their vertebrate hosts and hence are of concern to veterinary and human medicine. In subkingdom protozoa coccidia is included in phylum Apicomplexa. The phylum Apicomplexa was established to include protozoans that possess a certain combination of structures collectively known as the apical complex which is distinguishable by electron microscopy.Coccidia are microscopic, spore-forming, obligate, and intracellular parasites, they must live and reproduce within an animal cell, belonging to the class Sporozoea according to revisedclassification.

Coccidian parasites infect the intestinal tracts of animals, and are largest group of protozoa. *Eimeria* is a genus of apicomplexan parasites that includes various species responsible for the disease coccidiosis. These species cause pathological damage and mortality in poultry, cattle, sheep, goat, pig, rabbit and other animals. The genus *Eimeria* (Schneider, 1975) with more than 1400 species described to date, is the largest genus, and may be the most specious genus of all animal genera. Coccidia have a complex life cycle and other unusual characteristics which have led to investigations by increasing number ofbiologists.

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Coccidiosis:

In India studies on Coccidiosis have been relatively scanty. Mandal (1975) in review on Fifty years progress in the taxonomy of coccidian (Protozoa: Sporozoa) from India, estimated that about 200 species of coccida spread over seventeen genera were described till then this information is thus very negligible. Considering the very large size of our country and the endless variety of animal fauna that exists here is very mearge. The necessity for basic taxonomic studies is also reflected in the statement of Levine (1973) that *Eimeria*had been described only from 1.2% of the world's chordates and 5.7% of the world's mammals. He also estimated that if all chordates were examined perhaps atleast 34000 species of *Eimeria* might be found and that 3,500 of them would be from mammals. In India the studies on the coccidia of invertebrates have been carried out mostly in west Bengal and Andhra Pradesh.

The few contributions on the coccidia of fishes, Amphibia and Reptiles are also from west Bengal. While a contributions on the coccidia of birds have come from Bengal and Uttar Pradesh. The mammalian coccidia study was done from the veterinary laboratories at Bombay, Calcutta, Mathura, and Izatnagar. During the last ten years more and more workers have been attracted to this area and many useful contributions have been made from the different parts of the Northern India. Comprehensive studies on the incidence of coccidia have been made by workers from the protozoology laboratory of the Dr. Babasaheb Ambedkar Marathwada University, about 40 years ago. During this period six major contributions have been made on the coccidian fauna of the Marathwada region.

The first contribution covered the systematics of avian coccidia. The second deals with the prevalence and systematics of the coccidia in goats of Parbhani district, besides a taxonomic survey of Coccidia of rats and Squirrels.

The third contribution made an extensive analysis of the prevalence and systematics of the sheep and goats of this region. In this series fourth attempt covers a comparative study of the Coccidia of Goats and Sheep in the Marathwada region, beside a taxonomical account of the coccidia of rabbits in this area. Fifth research in this series deals with the prevalence of incidence and morphological study of the coccidia of buffaloes and goats. Sixth deals with study of coccidiosis in chicken in Aurangabad District. Seventh in this series is comparative study of species composition of coccidia in sheep and goats in Beed district (M.S). The present author worked on studies on some endoparasites of *Capra indica* (Indian goats).

Table 1: The comparative prevalence of coccidia in Indian goats in different regions

Sr. No.	Authors	Number of Positive samples/ total samples	Prevalence percentage	Area
1.	Shah and Joshi (1963)	115/300	38.33	Madhya Pradesh
2.	Singh (1964)	214		Mathura (U.P)
3.	Jha and Subramanian (1966)	152/243	62.55	Uttar Pradesh
4.	Jha (1966)	36/50	72.00	West Bengal
5.	Kshirsagar and Krishnamurthy(1976)	237/649	36.51	Parbhani (M.S.)
6.	Kshirsagar (1976)	307/1639	18.73	Parbhani (M.S.)
7.	Banerjee and Pradhan (1980)	77/152	50.65	Calcutta
8.	Deb et.al. (1980)	219/500	43.8	Ranchi (Bihar)
9	Nikam (1983)	155/575	26.95	Aurangabad (M.S.)
10.	Jadhav (2002)	82/297	27.60	Marathwada
11.	More (2011)	528/2636	20.03	Beed (M.S)
12	**Present Author**	**246/1135**	**21.67**	**Malegaon**

Conclusion:

The several workers has been recorded the prevalence of coccidia from the different parts of the world, including India. Rao and Hiregaudar (1954), Ray (1961) and Bhatia and pande (1970) recorded the prevalence but did not clearly mention about the host that whether the material collected from goats or sheep. Some other workers in India studied the prevalence in goats such as Shah and Joshi (1963), Singh (1964), Jha and Subramanian (1966), Jha (1966) Sivanarayana and Venkataratnam (1969), Krishnamurthy and Kshirsagar (1976), Kshirsagar (1976), Banerjee and pradhan (1980), Deb, Sinha, Ansari and Sahai (1980), Nikam (1983) , Jadhav (2002) and More B.V (2011) as shown in table **1.**

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CHEMICAL ANALYSIS OF SUMMER HONEYS COLLECTED FROM *APIS DORSATA* HIVES OF CHIMUR TAHSIL OF CHANDRAPUR DISTRICT OF MAHARASHTRA STATE (INDIA)

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Abstract:

The present investigation was undertaken to determine the chemical analysis of 5 summer honey samples (CHN-CHI-ALL, CHN-CHI-TAL-N, CHN-CHI-JAM, CHN-CHI-TUK, CHN-CHI-BOR) collected from forest area of Chimur Tahsil of Chandrapur District of Maharashtra State (India). These samples were analysed for several parameters such as moisture, total reducing sugar, Levulose or Fructose, Dextrose or Glucose, L/D ratio, Sucrose, Acidity. This type of chemical analysis favours the utilization of the honey for good quality in this area.

Keywords: Chemical Analysis, Summer Honey, Chimur Tahsil.

Introduction:

Honey is a natural product rich in carbohydrates, extracted from nectar by bees. Honey has been used as nutritious food and traditional medicine by all civilizations. The quality of honey depends on many physiological factors such as climate and soil. Sugar, protein, moisture, vitamins, minerals, enzymes, polyphenols, and flavonoids (Al-Manary et al., 2002) Due to this unique and complex nature, honey has been shown to be useful for treating burns, wounds, and skin ulcers Antioxidants, external eye disease (Balasubramanyam, 2011). In addition, honey is a very valuable ingredient in spices, beverages, sauces and candies. In fact, many studies on the physical, chemical and melisopalinological parameters of honey have been published around the world. (Adenken *et al.*, 2010; Anklam, 1998; Cherian *et al.*, 2011; Borkar Laxmikant and Mate Devendra, 2014; Downey *et al.*, 2005; Ramnath nad Shivaramm, 2012, Terrab *et al.*, 2002;

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Xesus *et al.,* 2010). The scientific literature indicates that there is no information on the chemical properties of Chimur Tahsil honey from the Chandrapur region of Maharashtra, India. The purpose of this study is to study some of the chemical parameters of honey from different areas of Chandrapur Chimur Tahsil district, such as moisture, total sugar, L- or fructose, dextrose or glucose, L-/dextrose, sucrose, acid and microscopy analysis. Maharashtra, India

Material and Methods:

Chemical analysis of the honeys are carried out by using Indian Standard Specification, IS: 4941 (1974) and IS: 8464 (1977). The percentage of Total Reducing Sugar, (Levulose or Fructose + Dextrose or Glucose), Levulose, Dextrose, Sucrose, Acidity, Moisture and L/D ratio were estimated.

Results and Discussion:

Table 1: Chemical Analysis of honey samples obtained from Chimur Tahsil of Chandrapur District

			Parameter						
Sr. No.	**Location of Parameter**	**Date of Collection**	**Moisture %**	**Total Reducing Sugar %**	**Levulose or Fructose %**	**Dextrose or Glucose %**	**L/D Ratio**	**Sucrose %**	**Acidity %**
1	CHN-CHI-ALL	13/05/2013	19.5	66.648	36.648	20.970	1.368	1.059	0.4541
2	CHN-CHI-TAL-N	18/05/2013	18	68.101	38.613	29.488	1.497	3.088	0.3427
3	CHN-CHI-JAM	19/05/2013	19	71.352	37.813	33.539	1.286	1.656	0.2829
4	CHN-CHI-TUK	21/05/2013	19	70.285	36.295	33.990	1.185	1.565	0.174
5	CHN-CHI-BOR	23/05/2013	19.2	68.715	34.05	34.665	1.089	1.401	0.3105

The chemical properties of the 5 summer squeezed honey samples (Viz. CHN-CHI-ALL, CHN-CHI-TAL-N, CHN-CHI-JAM, CHN-CHI-TUK, CHN-CHI-BOR) were collected during the period 13 May, 2013 to 23 May, 2013 form Allizanza, Talodhi Naik, Jamani, Tukum and Borgaon respectively from Chimur Tahsil of Chandrapur District of Mahartashtra State are reported in table.

In this study, the moisture content of the sample is between 18-19. The temperature rises and the humidity is low, while the temperature drops and the humidity is high. The increase in

the moisture content of honey also indicates that it is forged. It is an important part of the system that protects honey from microbial attack.

Sugar:

Honey is mainly composed of glucose and fructose. The actual fructose to glucose ratio in a given honey depends largely on the source of the nectar. All samples contain more fructose than glucose. This shows that Chimur honey is less prone to particle formation. The fructose content of honey is higher than that of glucose. Honey with a high fructose to glucose ratio stays liquid longer.The fructose/glucose ratio affects the taste of honey because fructose is much sweeter than glucose.

Acidity:

The acidity of honey samples ranges from 0.2829 to 0.4541. You can point out that yeast fermented honey sugar.

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STUDY ON SOCIO-ECONOMIC STATUS OF FISH FARMERS IN CHIKKAMAGALURU DISTRICT, KARNATAKA-INDIA

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Abstract:

Fish farming practices also hold promises for many small farmers and potential significant benefit for strengthening the socio-economic status of the people those who are directly or indirectly connected with exploitation, production and processing of fish. The present study is an attempt to understand the socio-economic condition of fish farmers in the Chikkamagaluru district, Karnataka. A total of 10 fish farmers from each selected 10 villages of the Chikkmagaluru district, were interviewed to collect the information with a pre-tested structural questionnaire. Socio-economicstatus namely, family size, age, gender, marital Status, Education, Type of occupation, Experience, Income, Utilization of supplementary fish feed and Involvement in fish culture were collected and calculated on percentage basis. It was observed that the highest number of fish farmer's age were above 20 up to 60 years (75%). It was found that 35% of farmers were belonging to the medium sized family. It was found that 35% of farmers were belonging to the medium sized family. Thus it can be concluded that fish farming is a profitable business that can help the fish farmers to uplift their livelihood as well as economic status.

Keywords: Socio –economic status, fish farming, family size, marital status.

Introduction:

India is the second largest producer of fish next to China. In India, aquaculture constitutes about 5% of the global fish production and 3% of the global fish trade. India is one of the major fish producing countries in the world hiring over seven million people in fishing and associated industries and contributing 60 crores annual1y to national income and mainly generating employment for rural people (Gadage, 2005). Fisheries sector also supports canneries and processing establishments, gears and equipment manufacturers, boat yards, refrigeration and ice

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making plants and transport services in addition to those working in State fisheries Department, Fisheries Corporation and other Government based fisheries institutions (Bias, 2018). Fisheries development in India has made considerable progress over the successive Five Year Plan periods.

Fish protein is easily digestible and has proved growth promoting value for human consumption. The fish protein comprises all the ten essential amino acids, minerals, vitamins and fat. With increase in human population, shortage of human dietary protein occurs, thus this shortage can be fulfilled by fish protein, mainly in developing countries, where protein shortage is serious. But, this goal may be difficult due to the high cost of good quality fish meals which costs about 40-60% of total operating costs in intensive aquaculture enterprises.In present-day economic, environmental and social styles are pushing farmer to look for viable options to accomplish the nutritional requirement for the growing population.

A fisheries sector have significant role in the national economy through improved food supply, employment and income, and contributes 1.5% to total GDP and 4.3% to the GDP from agriculture. Mainly, Karnataka has rich and varied inland water resources in the forms of ponds, tanks, reservoirs, rivers and lakes which offer great potential for freshwater aquaculture development. The state ranks tenth in the country in terms of inland fish productionAnd contribute about 3.3% to the Indian inland fish production. Both fish and seed production in the state have been rising gradually in the recent year.

Fish farming practices also hold promises for many small farmers and potential significant benefit for strengthening the socio-economic status of the people those who are directly or indirectly connected with exploitation, production and processing of fish.

Study of socio-economic status of fish farmers is important because of the fact that on the one hand it has influence on the farming practice implemented by the farmer and on the other hand it is the outcome of the farming practices and performance. In the earlier year, fish practice was totally dependent on the captured fishes but now culture fish practice has also started. Study on the economics and technical bottlenecks of fish culture on the regional basis is highly necessary. The present study is an attempt to understand the socio-economic condition of fish farmers in the Chikkamagaluru district, Karnataka, India. This understanding is important to trigger the policy measures in the right direction.

Material and Methods:

Study area:

Chikamagaluru is situated in the Malenadu region of Karnataka in the Deccan plateau in the foothills of the Western Ghats. Chikamagaluru is located between 13.33°N latitude and 75.77°E longitude. It is situated at an elevation of 1,090 metres (3,580 ft.) above mean sea level.The Yagachi River provides water resource to the district. Chikamagaluru generally has a moderate to cool climate.The district receives normal average rainfall of 1762mm and temperature varies from 11-20°C during winter to 25-32°C during summer.30% of the district measures with forests and Agriculture are the economic backbone of the Chikamagaluru district with coffee cultivation farming the major part of it.

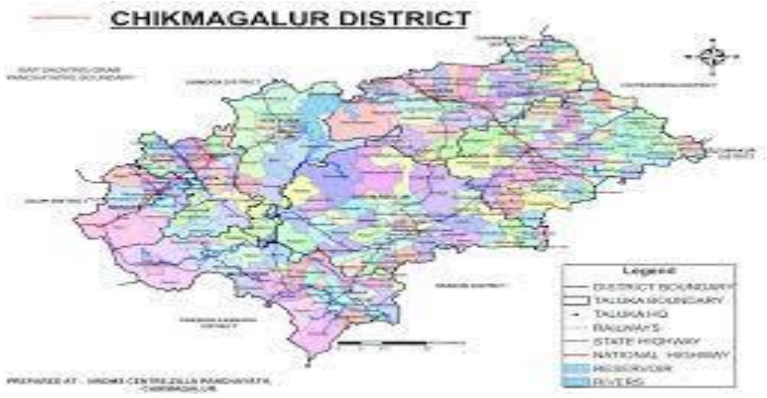

Plate 1: Map of study area

Sampling procedure and sample size:

A total of 10 fish farmers from each selected 10 villages of the Chikkmagaluru district, Karnataka were interviewed to collect the information with a pre-tested structural questionnaire. Following factors viz., related to their socio-economic status namely, family size, age, gender, marital Status, Education, Type of occupation, Experience, Income, Utilization of supplementary fish feed and Involvement in fish culture were collected and calculated on percentage basis (Perm Kumar *et al*., 2015).

Result and Discussion:

Socio-economic indicators are closely interconnected. In the present study some socio-economic indicator are summarized and explained in the following paragraphs (Table.1).

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Table 1: Socioeconomic profile of fish farmers in Chikkamagaluru district, Karnataka

Sr. No.	Variable	Percentage
1.	**Family size:**	
	1. Small size (<4 members)	-
	2. Medium size (4-6members)	62.5
	3. Large size (>6members)	37.5
2.	**Age:**	
	1. Below 20	-
	2. Above 20 [up to 60]	75
	3. Above 60	25
3.	**Gender:**	
	1. Male	100
	2. Female	-
4.	**Marital status:**	
	1. Single	25
	2. Married	75
5.	**Education:**	
	1. Primary (1th -5th)	12.5
	2. Secondary (5^{th} -10^{th})	37.5
	3. Higher Secondary (10^{th}-PU)	38
	4. Above	12
6.	**Type occupation:** (Fish rearing)	
	1. Part time	100
	2. Full time	-
7.	**Experience in fish rearing :**	
	1. Less than 5years	37
	2. 5-10 years	25
	3. More than 10 years	37.5
8.	**Income category:**	
	1. High income (>36000)	25
	2. Middle income (12-36000)	75
	3. Low income (>10000)	-
9.	**Type of fish culture:**	
	1. Monoculture	-
	2. Integrated	100
10.	**Utilization of supplementary fish feeds :**	
	1. User	60
	2. Non-User	40
11.	**Involvement in fish culture:**	
	1. Regular	62.5
	2. Occasional	37.5
	3. No involvement	-

Family size:

Family size is significance socio-economic indicator as it reflects the income, food consumption and socio-economic status of the families (Hussain *et al*., 2009). The present study was categorized in three family size groups viz., small family (<4 members), medium family (4-6 members) and large family (>6 members). Majority (62%) of farmers belong to medium family (Sen and Roy, 2015). (Table 1) which is followed by small family and large family (38%) (Fig.1)

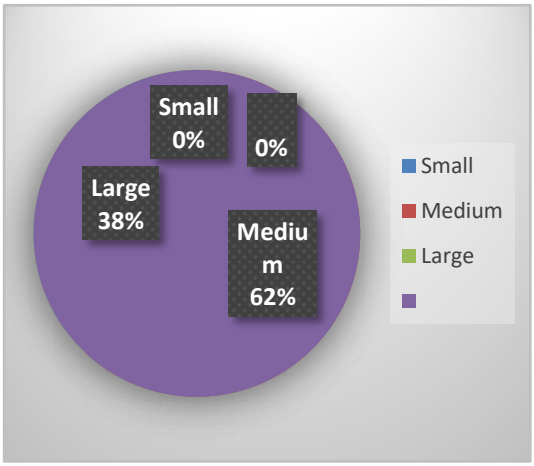

Figure 1: Family size

Age structure:

Understanding of age structure of fish farmers play a major role in calculating potential productive human resources.In the present study, the average age of fish farmers was found to be 46years ranging from a minimum of 20years to a maximum of 65 years. Majority of fish farmers (75%) are middle age group between 20 to 60 years which is followed by old age group (above 60) registering 25% of the total farmers (Fig.2).

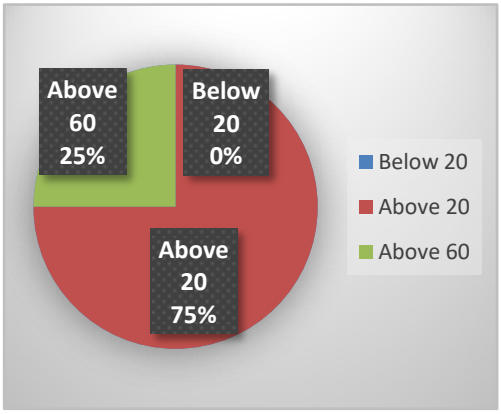

Figure 2: Age structure

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Gender:

In spite of the efforts of fisheries agencies to encourage for fish farming and farming communities, gender inequity is seen.The present study represents that there were no women involved in fish farming in the selected area. Only men involved in fisheries activity i.e. 100% of the total data collected (Fig.3).

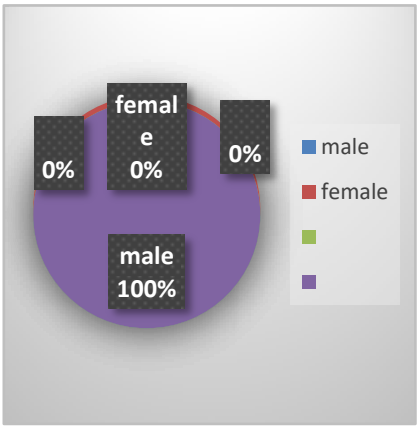

Figure 3: Gender data

Marital Status:

Fishery activities are energy demanding jobs, so the farmers should be physically strong.In the present study, majority (75%) of the farmers interviewed in this study were married and the remaining 25% were unmarried (Fig.4).

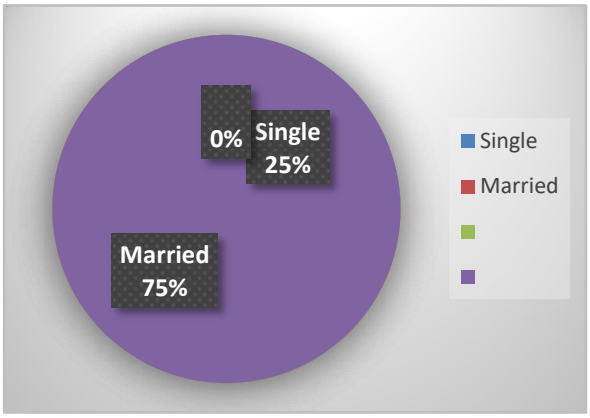

Figure 4: Marital status

Education:

Education and literacy are considered to be good indicators of development in a society.Fishing activity requires knowledge, skill developments, production technology and marketing practices (Sen and Roy, 2015). In the present study, maximum number of individuals were literates. Some of them have studied up to primary levels (around 12.5%) (Table 1). Hardly a few individuals have studied up to secondary (5^{th} – 10^{th}) (37%).The maximum number of fish

farmers associated with the categories studied above 10th (38%)and 12% of them were degree holders. However, this study shows that the majority of farmers have secondary and higher secondary education. This study represents that the young generation are stepping towards higher education despite fish culture activities and other agricultural activities(Fig.5).

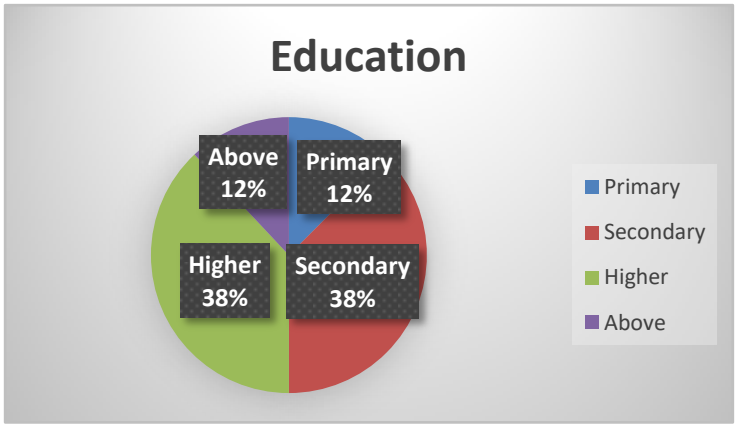

Figure 5: Education

Occupation:

The standard of living and earning of fish farmers depends on their occupation (Goswami, *et al*. 2002).Main stream occupation respondents in the present study was agriculture and associated activities. In the present study, data shows 100% of the farmers were involved in both fish farming as well as agriculture practice. In the study area, the majorities of the fish farmers were involved in agricultural farming as a principal occupation (38%), followed by business including small trading and shop keeping (26%). Only 6% of the pond fish farmers were involved in fish farming as their principal occupation. 36% of the respondents stated that, they were involved infish farming as their secondary occupation to secure their livelihood (Fig.6). In the study, only primary occupation was insufficient to provide adequate means of livelihood.

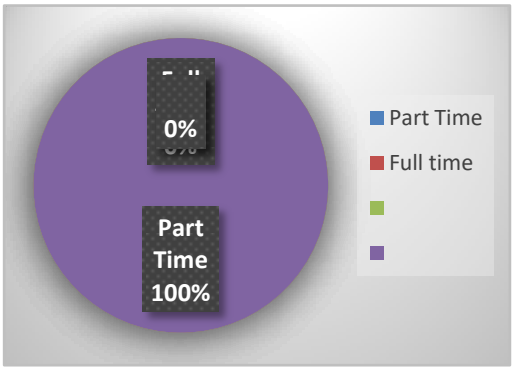

Figure 6: Occupation

Experience:

Experience is most important indicator in the fisheries sector. It helps in improvement of traditional knowledge and techniques to getting good output.In the present study, 37.5% of

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people experience of less than 5 years 25% of 5-10 years and 37.5 % of people were experienced more than 10 years in the fishing activities (Fig.7).

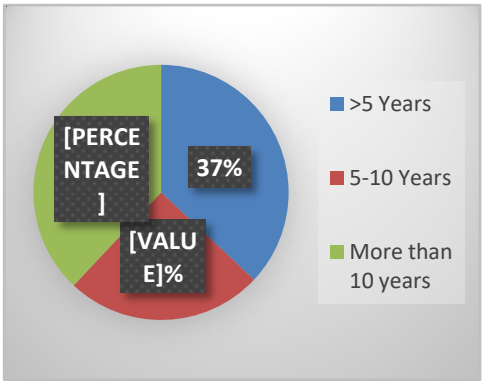

Figure 7: Experience

Income:

Income determines standard of living. It plays a major role in socio-economic status of the state and country. In the present study, 25% of people were having highincome [>36000] and 75% of people were having middle income (12-36000) (Fig.8).

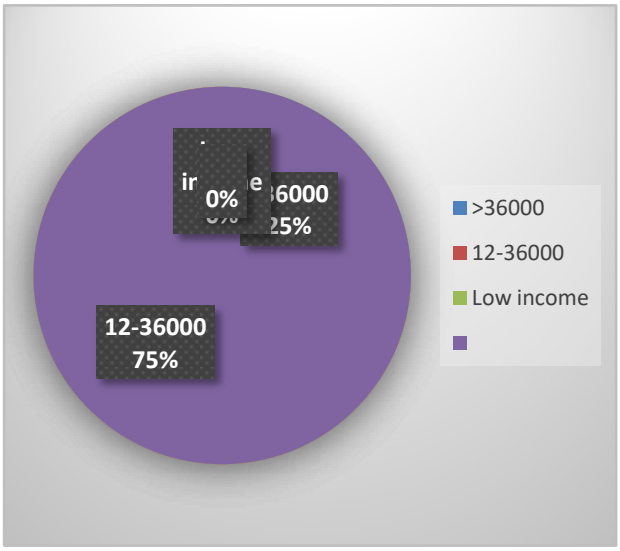

Figure 8: Income

Fish species cultured:

Cultivation of fishes in confined geographical areas with utmost care to get maximum yield. Catla (*Catla catla*), rohu (*Labeo rohita*) and mrigala (*Cirrhinus mrigala*) and jalebi fish (*Tilapia*) were cultivated in Polyculture system (100%). Mainly, the fish farmers were raring Indian major carp (IMC) namely *Catla catla, Labeo rohita* and *Cirrhinus mriagala* integrated with one or two species of Exotic major carp (EMC) such as *Cyprinus carpio* (Common carp). This study revealed that the common carp were found to be maximum cultured species this may

be due to consumer preference, faster growth rate, and low mortality, disease resistant and easy to culture with low input.

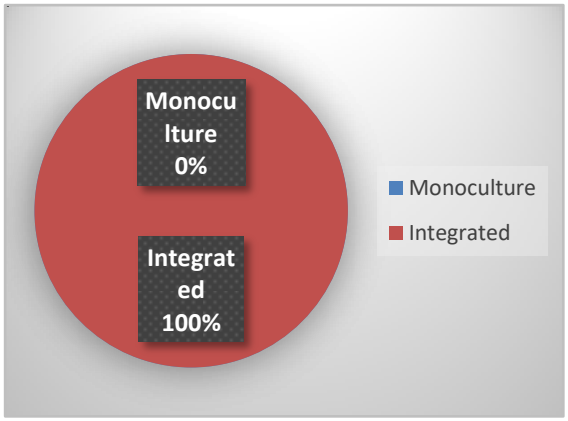

Figure 9: Type of fish culture

Utilization fish feed:

The fish farmers were using different types of organic andInorganic fertilizers toenrich the natural planktons. However, It was found that 60% of the farmers applied supplementary and homemade feed prepared with rice-bran and ground nut oil cake and about 40% of the farmers were entirely dependent on natural food (Fig.10).

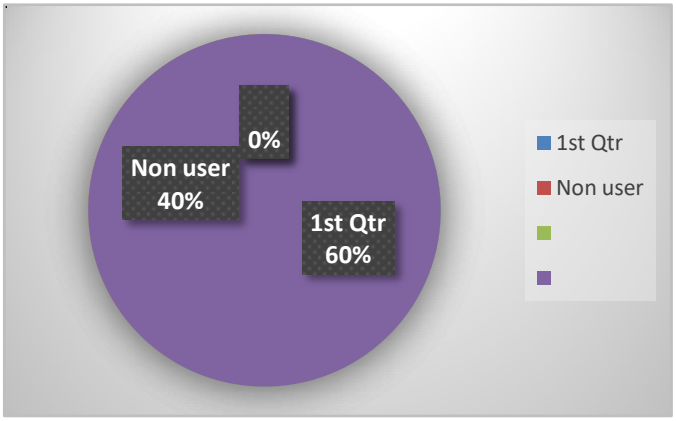

Figure 10: Utilization

Involvement in fish culture:

The involvement of fish farmer in fisheries activity was divided into three different groups such as regular, occasionally, and no involvement. In the present studymost of them were regularly involved in Fish rearing activity (62.5%) and 37.5% fish farmers were occasionally involved fishing activity (Fig.11).

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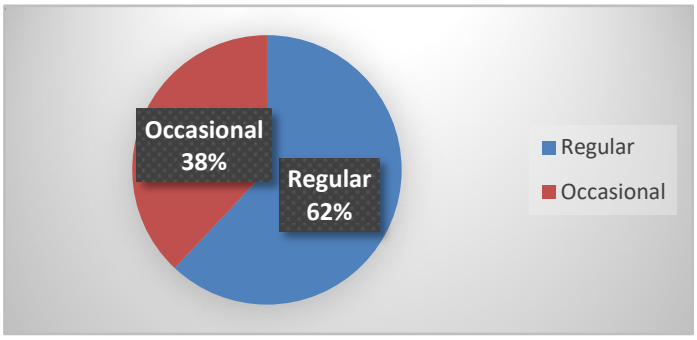

Figure 11: Involvement

Source of information on aquaculture, fish seeds and diseases treatment:

Majority of the fish farmers of Chikkmagalurudistrict, Karnataka learnt aquaculture practices through day-to-day experience and traditional knowledge.Information gathers from the government extension workers, educational institutes and other farmer friends, neighbours.

Livelihood Outcomes:

Livelihood outcomes of fish farming and related activities were positive and most of the people had increased their income. In the present study, 94% of the fish farmers had improved their socio-economic conditions through fish farming. Only 6% of the farmers had not improved their socio-economic conditions due to poor knowledge on fish farming, high price of fish feed, poor marketing facilities and lack of money.

Conclusion:

The study is needs to know the socio-economic status of the farmer's status. In the present study, data were collected by 10 fish farmers of different villages of Chikkamagaluru district. Data were collected through the field survey and interview. Before collecting data, a questionnaire was prepared.From the results of present study, it can be concluded that fish culture has broad socio-economic benefits for the fish farmers. It was observed that the highest number of fish farmer's age were above 20 up to 60 years (75%).It was found that 35% of farmers werebelonging to the medium sized family. In the study area farmers were more commonly cultivating Catla (*Catla catla*), rohu (*Labeo rohita*) and mrigala (*Cirrhinus mrigala*), Jalebi fish (Tilapia).This study shows that the majority of farmers have secondary and higher secondary education. Government should support to increase their income level in general and fish culture in particular. This will attract new generations in scientific fish farming which is a need of the time for the fisheries sector togrow further and faster and to contribute towards overall economic development of the state.

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ACUPUNCTURE THERAPY: A FASCINATING APPROACH FOR PALLIATE HEALTH

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Introduction:

As the name suggests, Acupuncture Therapy is an alternative therapeutic treatment in this the specific unique techniques or healing force is used to treat illness. It works on the belief of vitalism. This chapter mainly emphasizes on the history of acupuncture therapy, basic principles of acupuncture therapy, treatment methods used in this therapy and its applications.

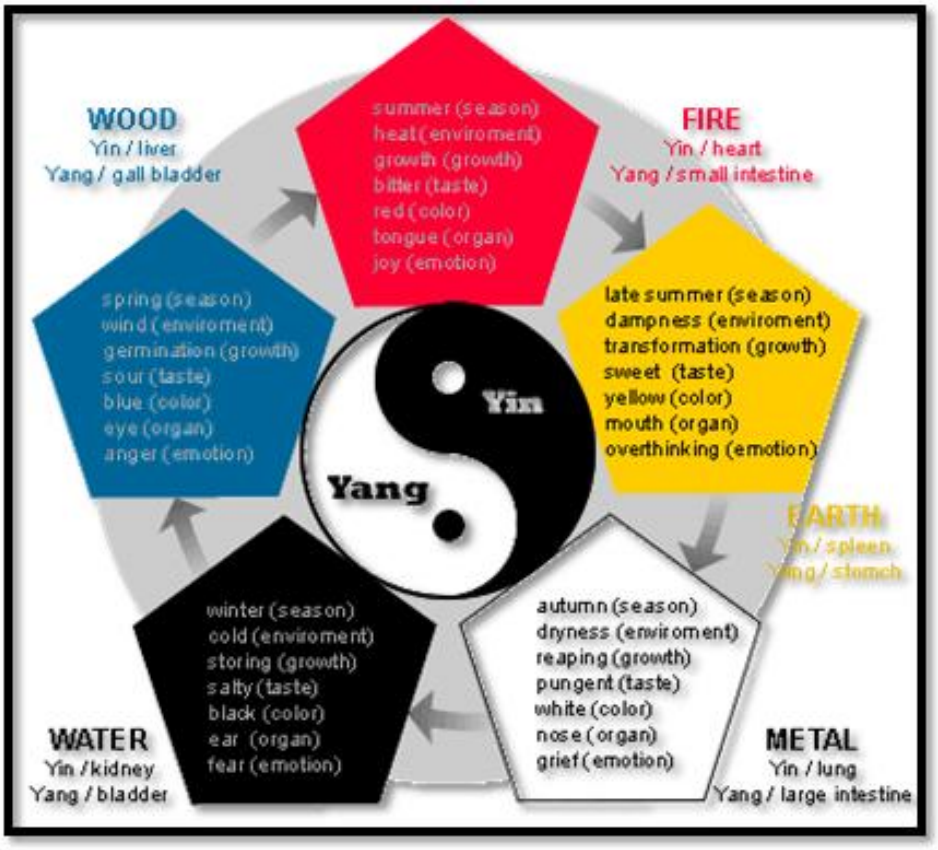
Acupuncture therapy is an fundamentally portion of an primitive Chinese framework of pharmaceutical that has been utilized for more than 2500 years a long time to treat infections and relieve pain (Grigory *et al.*, 2005).

"De qi" is the equivalent word of conventional needle therapy; this can e utilized to depict the association between needle therapy and the body's energy pathways. The "de" component of the word compound implies "obtain," and the "qi" component is "vital energy" (Kaptchuk, 2000). Customarily, other terms have been utilized for the same marvel counting "qizhi", which indicates" arrival" of qi. Needle therapy is the incitement of particular acupoints along the skin of the body including different strategies such as the application of needling, moxibustion, electric needle therapy (electro-acupuncture), laser needle therapy (photo-acupuncture), miniaturized scale framework needle therapy such as ear (auricular), confront, hand and scalp needle therapy, and acupressure.

Acupuncture is based on following principle:

- **Yin-Yang Theory:** Yin-Yang's theory is a concept based on a philosophy that is two opposite aspects of nature that are interrelated with each other. Yin- Yang theory developed in the Chinese they, side that the entire space is regulate by two opposing forces. All matters which are present in nature can classify by Yin and Yang. The manner of Yin philosophy fuscous on the feminine, latent and passive principle; while the Yang describe

the masculine, active and positive. Principle of the two competing universal powers by which creative energy is split and the fusion of which in physical matter produces the unimaginable universe.



Sr. No.	Nature	Yin	Yang
1	Colour	Dark	Light
2	Temperature	Cold	Warm
3	Time	Night	Day
4	Humidity	Wet	Dry
5	Texture	Soft	Hard
6	Gender	Female	Male
7	Weather	Winter	Summer
8	Position	Down	Up
9	Movement	Still	Move
10	Action	Passive	Active

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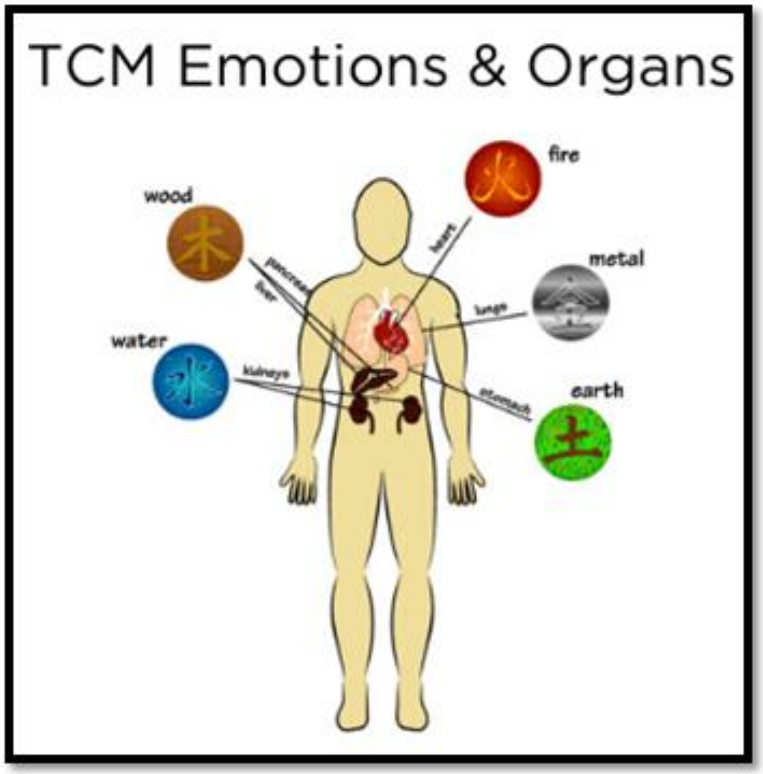
Four examinations observe according to the traditional Chinese medicine system:

Sr. No.	Traditional Chinese Medicine four Examination	Yin	Yang
1	**Inspection**	Dim	Bright
2	**Listening and Smelling**	Low and weak voice, silent and reticent	High and strong voice, talkative and agitated
3	**Inquiry**	Cold , not thirst, diarrhea	Hot, thirst, constipation
4	**Palpation**	Deep and slow, thread, forceless pulse	Floating, full, slippery, forceful pulse

➢ **Five Phases Theory:** Five phase's theory/Five Elements Theory is another basic theory in TCM. By comparing their structures, properties and behavior, the ancient Chinese divided material objects or phenomena into five groups, based on observations. The theory of the Five Phases posits wood, fire, earth, metal and water as the fundamental elements of the material world and these elements are continually moving and evolving in relation to each other.



Sr. No.	Human body	Five Phases				
		Wood	**Fire**	**Earth**	**Metal**	**Water**
1	**Zang Organ**	Liver	Heart	Spleen	Lung	Kidney
2	**Fu Organ**	Gallbladder	Small intestine	Stomach	Large intestine	Urinary bladder
3	**Orifice**	Eye	Tongue	Mouth	Nose	Ear
4	**Tissue**	Tendon	Vessel	Muscle	Skin & hair	Bone
5	**Secretion**	Tear	Sweat	Serous saliva	Nasal discharge	Mucous saliva
6	**Manifestation**	Nail	Face	Lip	Body hair	Hair
7	**Emotion**	Anger	Joy	Pensiveness	Grief	Fear
8	**Voice**	Shout	Laugh	Sing	Cry	Groan
9	**Action**	Gripping	Restlessness	Vomiting	Coughing	Shivering

- **Theory of Qi, Blood, Fluid and Humor:** Qi, Blood, Fluid, and Humor theory in TCM is used to explain the human body's growth, transportation, distribution, physiological function, pathological change, and reciprocal relationships of Qi, Blood, Fluids, and Humors. In TCM, Qi refers to the purified nutrient that flows in the body and to its functional activities. Blood is a kind of nutritionally rich red liquid that circulates inside the blood vessels, which has the functions of nourishing and hydrating the entire body. Fluid and humor refer to all types of normal body fluid, excluding blood, also known as body fluids. They are the essential materials that form the human body and that regulate the activities of life. Though the three have different properties, shapes and functions, they are physiologically dependent on each other and pathologically influence each other.
- **Visceral Manifestation Theory:** The internal organs and associated terminologies in TCM differ greatly from those of Western medicine. e The organs are divided into 'Zang' or 'viscera' (i.e., Lung, heart, liver, spleen, and kidney), where Essence and Qi are developed and stored, and 'Fu' or 'bowel' (i.e., gallbladder, stomach, large intestine, small intestine, urinary bladder and triple energizers) where food is provided,
- **Meridian, Collateral and Acupoint:** Meridian and collateral systems serve as conduits facilitating QI and blood circulation, bowel contact, viscera, extremities, superficial organs and tissues, creating the body an organic whole. It consists principally of the twelve meridians/channels, the eight extra meridians and the fifteen collateral vessels.

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Acupoints are subdivided into three categories:

Acupoints are present along each meridian for application of acupuncture and moxibustion.

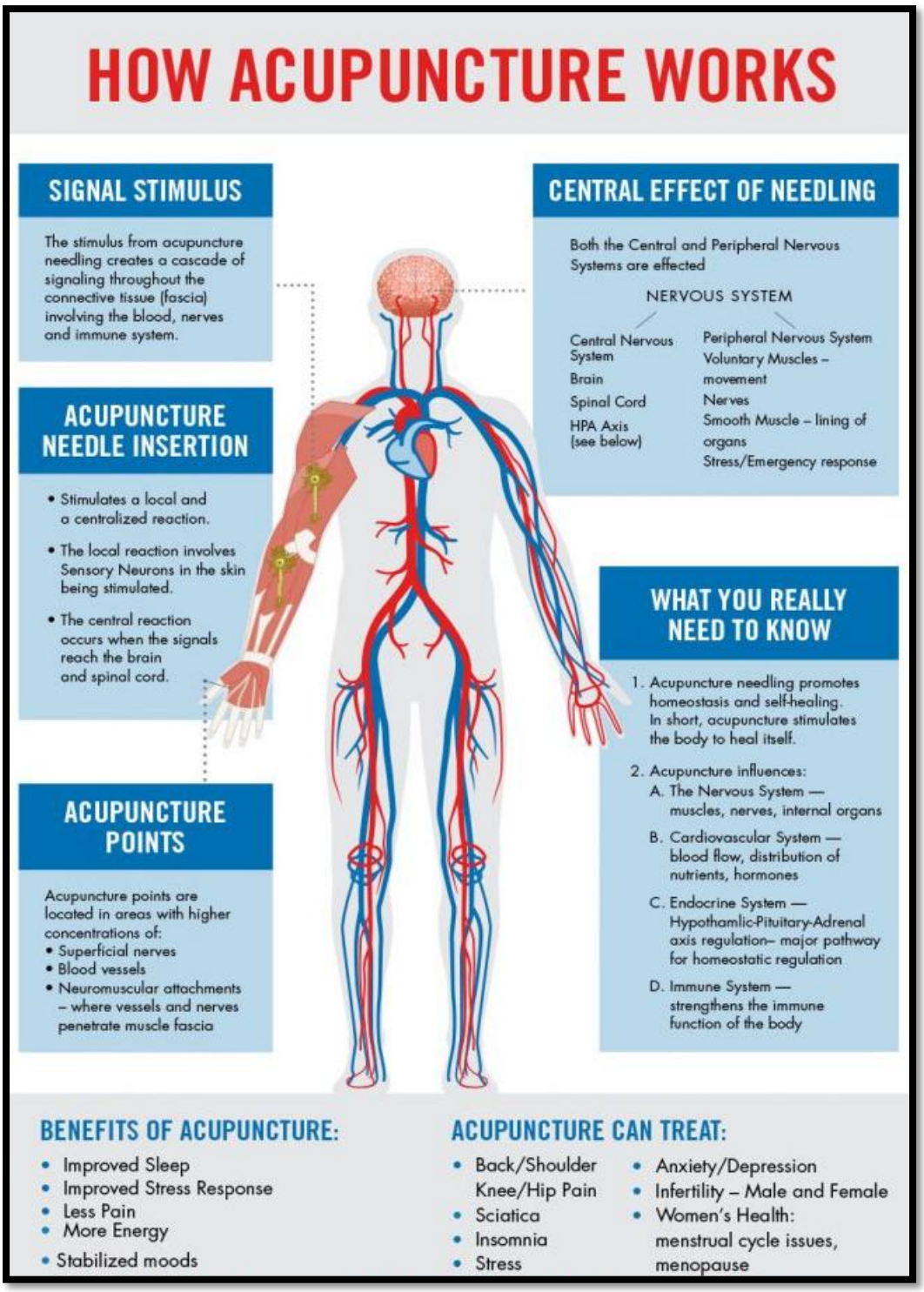
1. **Acupoints of 14 meridians:** total of 361 acupoints which make up most of all points present on human body.
2. **Extra point (Nonmeridian):** Acupoints which are useful in treatment plan as they have been discovered during practice. They have exact positions but aren't Fourteen meridians situated on.
3. **Ouch point (Ashi):** The location of which in some Diseases defined in some diseases by tenderness, responsiveness, or other pathogenic responses. We do not have any clear locations or name which is "where there is a painful spot, there is an acupuncture point". (Virginia and Wong, 2008-2014).

Diagnosis in Acupuncture Therapy:

In TCM, disease is generally perceived as a disharmony (or imbalance) in the roles or interactions of yin, yang, qi, xuA, zA ng fc, meridians etc. and/or the human body's relationship with the environment. Practitioners may analyze items like the color and form of the tongue, the relative intensity of the pulse points, the odor of the breath, the performance of the breath to evaluate the pattern is at hand (Maciocia, 1995; 2005; Shen, 1996; Qiu *et al.*, 1997).

Applications of Acupuncture Therapy:

1. **Osteoarthritis:** acupuncture to provide clinically significant relief from knee osteoarthritis pain and a larger improvement in function (Selfe *et al.*, 2008). Childbirth: In childbirth, acupuncture analgesia is useful for relieving labour pain and can significantly reduce the duration of labor (Sheng and Fan, 2010).
2. **Infections:** It has been confirmed that acupuncture is beneficial in the treatment of acute bacillary dysentery Acupuncture may be helpful in the treatment of pertussis (whooping cough), cough relief and heal (Marwick, 1997).
3. **Respiratory disorders:** Acupuncture is often used in treating respiratory disorders. Allergic rhinitis is among the major indications. In controlled studies, it has been shown that acupuncture is more effective than antihistamine in bronchial asthma acupuncture is effective (Lau and Jones, 2008).



4. **Digestive disorders:** Epigastric pain is a common symptom in diseases of the stomach, including peptic ulcer, acute and chronic gastritis, and gastric spasm. Acupuncture provides satisfactory relief of epigastric pain (Klein, 1988).
5. **Pain:** Acupuncture seems to have it can be prescribed for good analgesic and antispasmodic activity on the biliary tract. Acupuncture may serve as a complementary or alternative therapeutic measure (Helms, 1995).

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6. **Urinary disorder:** Acupuncture shows satisfactory result in treatment of urinary stone. Urinary retention (Philp *et al.,* 1988). Treatment of defective ejaculation (no ejaculation during intercourse) (Emmons and Otto, 2005).
7. **Cardiovascular disorders:** Acupuncture is effective in the management of primary hypotension and early hypertension (Wood *et al.,* 2003).
8. **Psychiatric disorders and mental disturbances:** Taking acupuncture in psychological disorders is rising (Boyd *et al.,* 2001).
9. **Skin diseases:** Acupuncture worked considerably better than vitamins C and E, Using as therapy for acne vulgaris (Lewas, 1992).
10. **Gynaecological and obstetric disorders:** Acupuncture was deemed successful in treating anovular infertility in females. Primary dysmenorrhoea prevents miscarriage, morning sicknessstimulates Milk production during conception which may be used to combat emotional lactation deficits or stress (Zhu and Zhu, 1995).

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MUCORMYCOSIS (BLACK FUNGUS): INTRODUCTION, PATHOPHYSIOLOGY AND ITS IMPACT ON PEOPLE OF INDIAN SUBCONTINENT

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Abstract:

Mucormycosis or black fungus infection is a rare and serious opportunistic infection which is caused by molds of family Mucormycetes. Black fungus infection is non-communicable infection that occurs mostly in immunocompromised patients, patients with high iron load (and using deferoxamine) and in patients with uncontrolled diabetes mellitus.This disease primarily affects nasal sinuses, orbits, brain, lungs and stomach. If untreated hematogenous dissemination occurs and patient dies within 2 weeks.

Treatment includes treatment of underlying cause (diabetes mellitus, metabolic acidosis and reduction in dose of immunosuppressant medication), IV therapy with liposomal amphotericin B and surgery of necrotic tissue.

Indian subcontinent is contributing 71% to the global pool of black fungus infection. This disease is prominently affecting patients who have uncontrolled diabetes mellitus, CoVID-19 recovered patients who were given unjustified dose of steroids, and those who have abused zinc metal (as tablets) during home isolation. So far Nepal and Bangladesh have also reported 1 case each, Russia also has unreported load of black fungus infection. Is another pandemic knocking the door of world in the form of black fungus infection? Is the world ready?

Keywords: Mucormycosis, black fungus infection, immunosuppressant, amphotericin-B, uncontrolled diabetes mellitus, pandemic.

Black Fungus Infection: an introduction

Mucormycosis or black fungus disease is a rare (but sometimes serious) fungal infection caused by *Mucor, Saksenaea, Absidia and Apophysomyces* (all are members of family of molds called Mucormycetes)

Mucormycosis or black fungus infection was previously called as Zygomycosis but today it is well established that Zygomycosis is a broad term which includes Mucormycosis and Entomophthoramycosis (a topical infection of para-nasal sinus or subcutaneous tissue mediated by *Basidobolus* and *Conidiobolus*) [1, 2]

Predisposing factors:

Black fungus can as such cause no harm in any immune-competent person but certain conditions make body prone to infection by the abovementioned opportunistic infectious agent(s). Some of them are:

- Profound neutropenia or pancytopenia (due to bone marrow insufficiency or under influence of bone marrow suppressing agents).
- Corticosteroids use
- Iron overload (specifically if patient is receiving deferoxamine therapy)
- Breakdown of cutaneous barriers (due to burns/surgical wounds and trauma)
- Uncontrolled Diabetes Mellitus and metabolic acidosis. [3, 4]

Diagnosis:

Although MRI and CT scan are most useful in determining rate and extent of infection prior to surgery yet microscopic techniques are also indispensible. There are two prominent features of Mucormycosis: ischemic/hemorrhagic necrosis and invasion of vasculature by aseptate hyphae of uneven diameter (6-15 micron) which can be confirmed well by using the aforementioned techniques. Tabulated below are the techniques and their use. [5-7]

Table 1: Techniques used in diagnosis of mucormycosis

Sr. No.	Testing/diagnostic technique	Useful for
1	Computerized Tomography	Detection of bony erosions.
2	Magnetic Resonance Imaging	Infection of frontal lobe and carotid artery in siphon.
3	Biopsy and histologic section (in KOH)* *Methanamine-silver dye may be used as staining agent	Lesions of lungs and craniofacial structures
4	Wet smear of crushed tissue	Rapid diagnosis of black fungus infection
5.	Magnetic Resonance Angiography (MRA; gadolinium enhanced)	Invasion or obstruction of carotid siphon

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Pathology:

It is well established that black fungus is an opportunistic infection. It happens only when immune system of a person is compromised (idiopathic/iatrogenic/infectious/metabolic disorder). Black fungus infection affects three primary locations: nasal sinus (which may lead to orbital and craniofacial infection), lungs and stomach. In some cases, skin is also affected but such cases are highly rare. If untreated, hematogenous dissemination may occur and chances of survival decreases (life cannot be expected beyond 2 week). [8-9]

Tabulated below are the location and attribution for black fungus infection.

Table 2: location of infection and its probable causes in mucormycosis

Sr. No.	Location of infection	Reason
1	Mucormycosis in para nasal sinuses and nose (which may lead to orbital and craniofacial infection).	Poorly controlled diabetes mellitus
2	Mucormycosis of lungs (accompanied with nose)	Occurs in patient who has undergone organ transplant or who has received long term deferoxamine therapy or in patient who has hematologic malignancy.
3	Gastrointestinal Mucormycosis	Uremia, severe malnutrition, diarrheal disease

Clinical Manifestation:

Clinical manifestation depends on the site which is infected by black fungus.

- **Mucormycosis affecting nasal and para-nasal sinus:**

This infection projects a different clinical picture. It is often characterized by dull pain in sinuses, nasal congestion, and bloody nasal discharge. Patient may have low grade pyrexia. Delineated area of necrosis (respecting midline) occurs in hard palate. Facial skin near to para-nasal sinus becomes red, purple and black.

This infection may then rise to upper levels and may involve eyes and brain. When eyes are affected; clinical features includes double vision, obtundation. Along with this unilateral chemosis, unilateral proptosis and reduction of unilateral generalized ocular motion is also seen. During orbital invasion, blood may clot in sinus (Cavernous Sinus Thrombosis).

Once brain starts getting infected, clouding of sensorium may be attributed to diabetic acidosis. Coma occurs due to invasion offrontal lobe by pathogen.[10]

- **Pulmonary Mucormycosis:**

The prominent feature of pulmonary mucormycosis is severe pyrexia and pneumonia. Cavities are formed in infected area. Along with this hemoptysis can occur from cavities so formed. [11]

- **Gastrointestinal Mucormycosis:**

This kind of infection is expressed by an individual as multiple gastric ulcers which are prone to perforation. [12]

- **Cutaneous Mucormycosis:**

This kind of infection is highly rare and occurs mainly in burn eschars, at the site of minor trauma in immune-compromised patient, in low weight neonates and below occlusive dressings. [13]

Treatment of Mucormycosis:

There are three strategies for treatment of black fungus infection. First one is regulation of underlying cause which could be diabetes mellitus (by using appropriate hypoglycemic agents) or by controlling bone marrow suppression (which requires dose reduction of immunosuppressant or other causative agent) Second one includes extensive debriment of craniofacial lesions and Orbital exenteration (if required). Third strategy includes treatment with IV amphotericin B (Liposomal formulation, dose: 5mg/kg for 10-12 weeks). There is very high mortality rate in patients receiving deferoxamine and those with pulmonary, gastrointestinal and disseminated mucormycosis. [14-16]

Impact of mucormycosis on people of Indian subcontinent:

CoVID-19 pandemic undoubtedly left entire globe in destitute. With more and more number of cases being reported, it has claimed 3,718,849 lives till now (as on 04-JUN-2021). [17] But does this end here? Answer is no. Another challenge which has started claiming lives of people (especially in India) is black fungus infection. Indian subcontinent has started to suffer so much because of this infection that some reports have started identifying black fungus disease as Pandemic within the pandemic. [18]

As on 06thJUN-2021, India reported 8848 infection cases and 219 deaths due to black fungus disease [19]. Same has now been declared as epidemic in Tamil Nadu, Gujarat, Odisha,

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Bihar, Uttarakhand, Rajasthan, Telangana and Chandigarh. Such a high mortality in this infection is attributed to the fact that most of the Indians either used drugs irrationally (self-medication or overdose) especially of zinc along with excessive use of stream. Not only this, according to one report, irrational and unjustified use of steroids in patients by doctors is also one of the factors why black fungus infection is happening in persons with CoVID-19 history.[20, 21]Black fungus infection has also started to affect neighbor countries. Nepal also reported its first clinical case of black fungus disease on 04th JUN-2021. [22]

According to a report, each patient requires approximately 50-60 doses of amphotericin-B. Profiteers did not leave this opportunity as well. Injections of liposomal Amphotericin-B were hoarded and black marketed. States of India started facing shortage of liposomal amphotericin-B injections. Government took cognizance of this problem and just like remdesivir; amphotericin-B was also supposed to be supplied centrally and by state government directly to the hospitals. Its export was also restricted. Use of oral posaconazole medications was also suggested if IV therapy of black fungus infection is not possible.

Some countries like Bangladesh and Egypt have responded well to some Indian state's (like Rajasthan) global Expression of Interest (EOI) request for amphotericin-B [23-26]

Having mentioned all these things a valid question now arises here; is another pandemic also knocking the door of this globe in the form of black fungus disease? Is the world ready?

Conclusion:

Although no person to person transfer has been established for black fungus infection yet we need to exercise utmost caution. People must maintain a high level of hygiene and must avoid residing in damp places.

People must try to.control their sugar intake as uncontrolled diabetes mellitus has highest attribution in the cases of mucormycosis. There is a need that government must also ramp up production of amphotericin B since many have died in India due to lack of therapeutic agent on account of either delay in supply or acute shortage

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PALYNOLOGY IN FORENSIC SCIENCE

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Introduction:

Forensic science is a word derived from 'forensis' having latin origin. Forensic science deals with associating people, places and things involved in criminal acts. It is science which aids in criminal and civil cases[1]. Forensic science is divided into sub-branches like Forensic Biology, Forensic Chemistry, Forensic Physics, Cyber forensics etc.

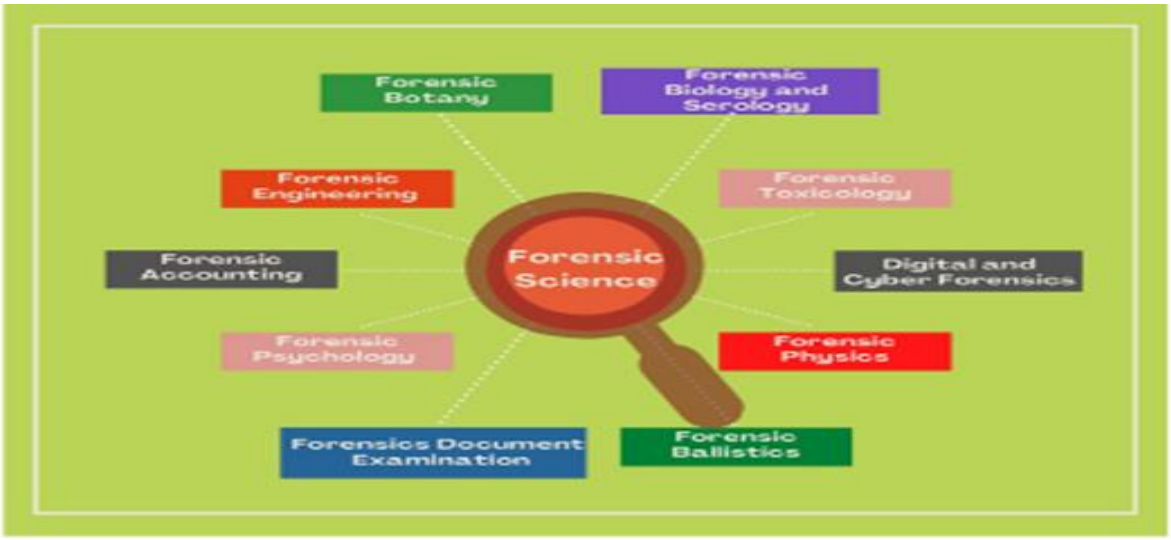

Figure 1: Branches of forensic science

Forensic biology is the branch of forensic science that deals with application of biological evidences to catch the criminals. There is other sub-discipline which comes under the Forensic biology like Serology, limnology, Forensic entomology, Forensic anthropology, Forensic Palynology etc.

Forensic expert deals withdifferent forms of evidences that assist investigators to solve the crimes.Most often encountered evidencesare in the form of trace evidences. Trace evidence is

the microscopic evidence left behind by two objects or people coming in contact. Trace evidence is ruled by Locard's principle of exchange which states that “Every contact leaves a trace”[2]. Different trace evidences involve Hair samples, fibers or fabrics, grass, soil particles, skin remnants, etc.

One such crucial evidence which can provide an important link in investigation is pollen grains.Pollen grains are the coarse powder containing micro-gametophyte of the seed, plants that produce the male gametes (sperm cell)[3].Forensic Palynology, a subdivision of forensic biology that mainly deals with the application of pollen and spores in criminal investigations. Experts in the field are called palynologists.

Pollen grains vary in size from 5µm-20µm. Pollen is in distinct shapes for instance, circular, triangular, elliptical etc. Some may have variation even in the basic shape. When observed with naked eyes it seems like the dust of yellow powder but even the colour varies from white, crème to yellow from species to species. Pollen grains have hard coats i.e., exine made up of “sporopollenin” that protect the gametophyte during the process of their movements from stamen to pistil of flowering plants or from male cone to female coniferous plants. The inner layer of pollen is called intine, made up of cellulose and pectin degrades faster as fossilization takes place[4].

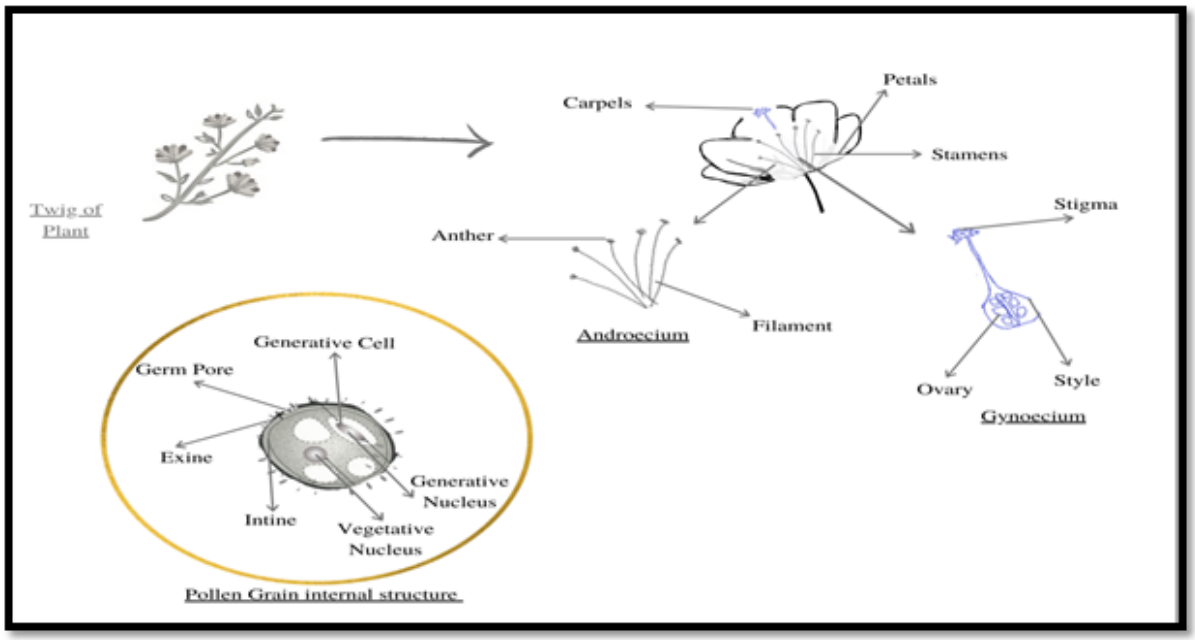

Figure 2: Schematic representation of Androecium, Gynoecium and Pollen grain

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Pollination takes place commonly by these modes:

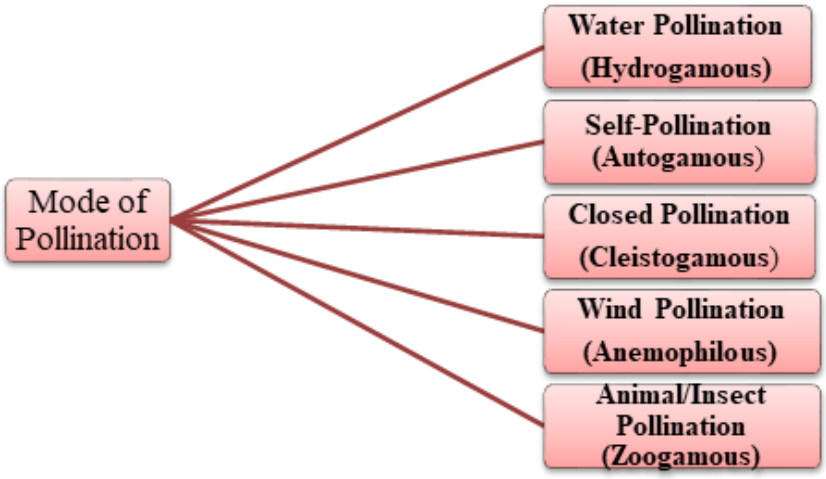


Figure 3: Different Modes of Pollination [4]

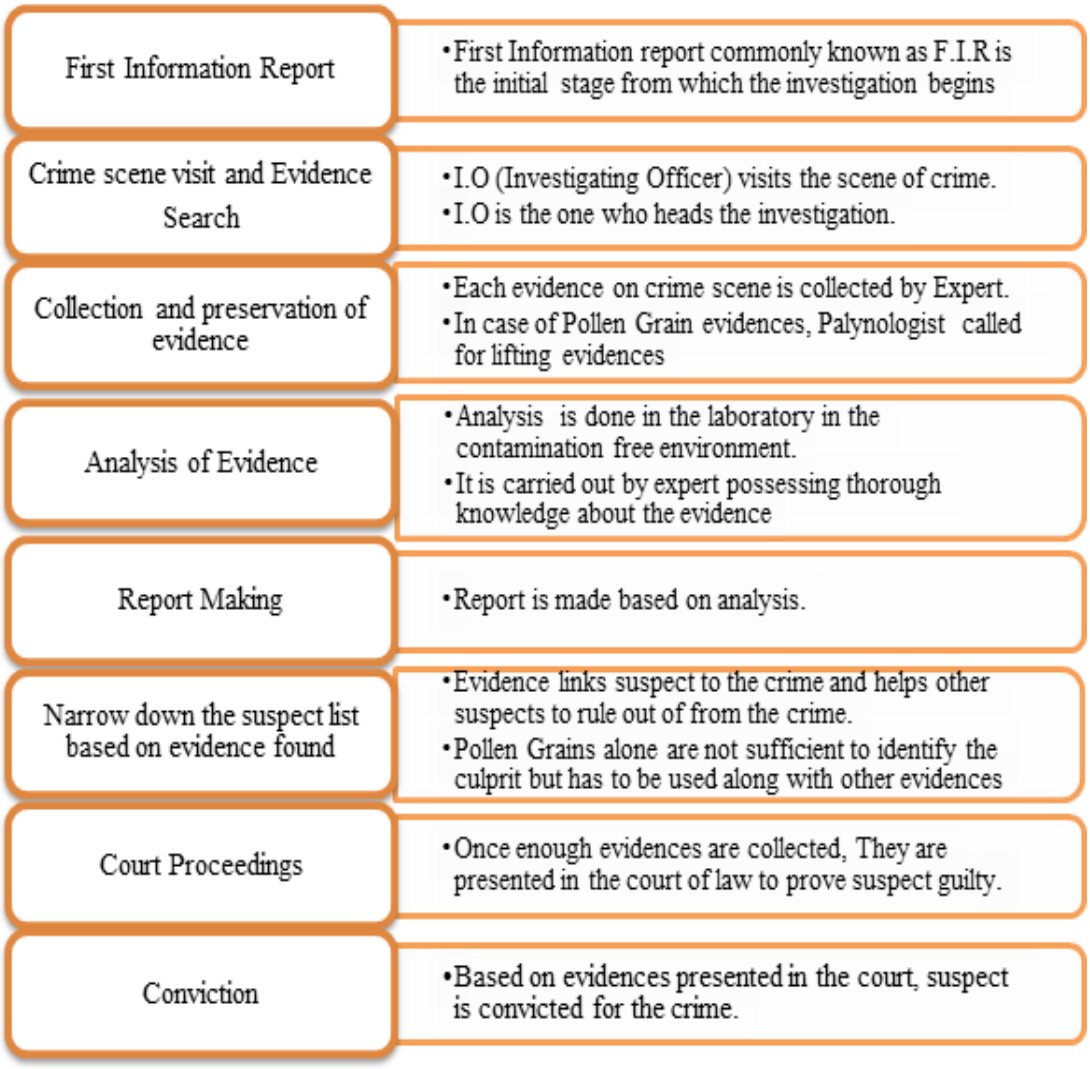
Palynology has an extremely important role to play in palaeontology, archaeology, and forensic science. Pollen grains are used as a tool to reconstruct the past environmental activities. Exine possessed by pollen grain can withstand environmental degradation for thousands of years. Palynologists can learn about the flora that existed in the past. Studying the Pollen grains, Archaeologists can tell how humans in the past interacted with their environment [5].

Being microscopic, found on nearly every surface and objects, suspects will be carrying them unknowingly on their clothing, hairs, footwear's, etc. Pollen grains are unique to each species of plant and geographic location[5]. Pollen grains are extremely resistant to environmental degradation and can remain unaffected for years[6]. Thus, pollen is considered as important trace evidence and can help to link a suspect to a crime scene.

Table 1: Entire Process from filing of report to Conviction of Accused[7][8]

Forgery	Assaults	Robbery	Rapes
Genocide	Terrorism	Arson	Hit and run crimes
Withdrawal of artifacts from historic or archaeological sites	poaching of animals including fish	pollution of the environment	Homicide

Sample collection:



Pollen can solve the cases such as

Forensic Significance:

Pollen is a form of trace evidence that is present in small but measurable amounts. The forensic significance of pollen analysis is support to prove or disprove a linkage between people and objects with places or with other people.

- **Pollen grains can tell us about the geographical area from where it belongs.**

Pollen grains can help us to trace the geographical location which is most often the scene of offence or sometimes the origin of victim, suspect or any object whose presence is unsure at that place.

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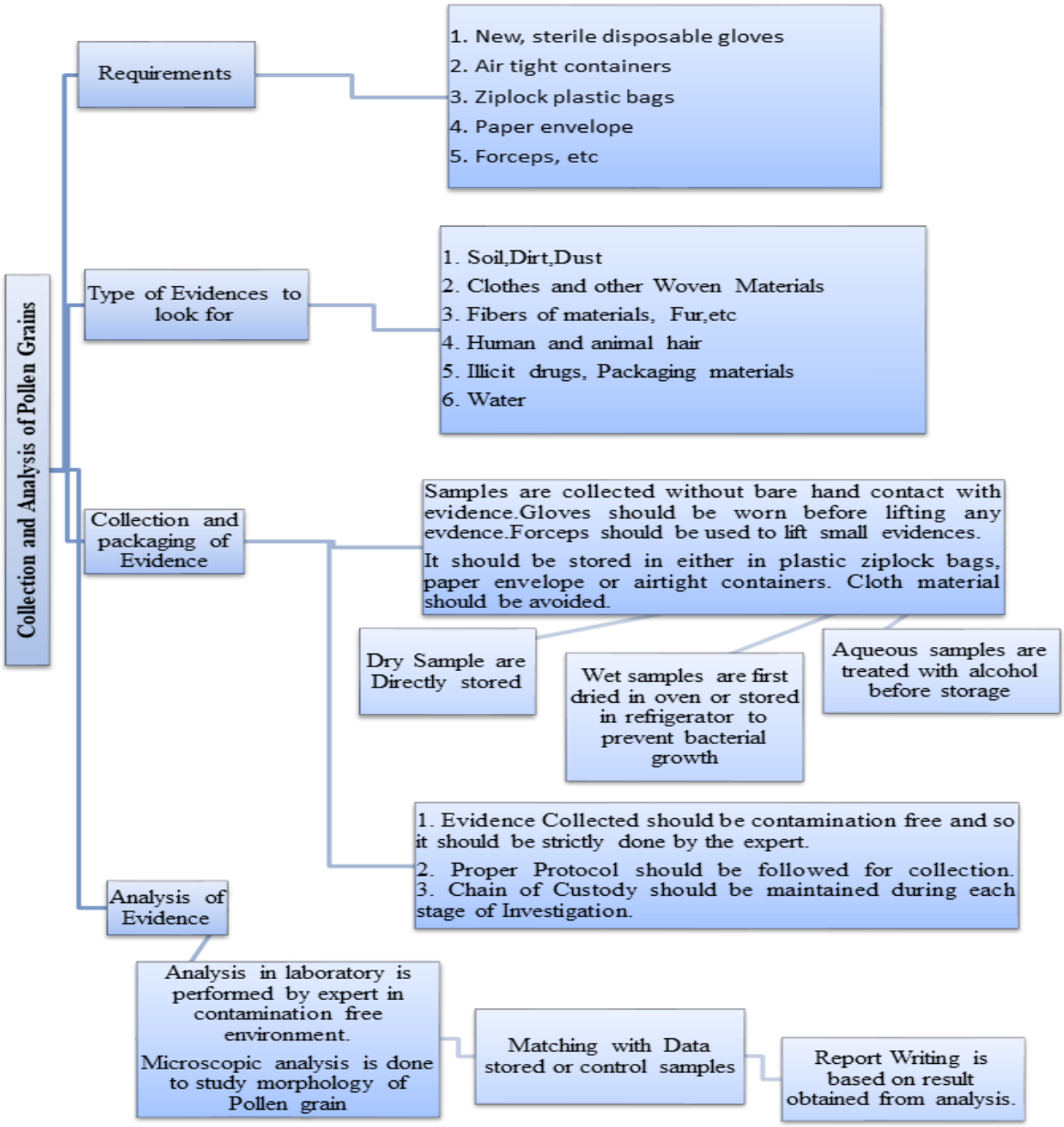

Figure 4: Process of Evidence collection and Analysis[9]

In 1979, a case in which the body of a young girl was found in the maize field in Rochester, New York [10]. All her belongings were missing which would have helped to identify. Also her fingerprints were not in the record as she was too young for her fingerprint data to be in record. It was about 3 decades from then since her identity was not established even after a lot of investigative efforts. But then in 2006, Pollen analysis was done ofher clothes. The pollen extracted from her pockets suggested the region which was about 2000 miles away to the west from where her body was recovered as they belonged to an Australian pine which was not common in the local area. Also, it was said by investigating palynologist that it was impracticable that this pollen has travelled thousands of miles to the site of offence.During

investigation in 1979, Officers assumed that the girl belonged to the local area and had restricted their investigation.The case remained unsolved as investigators were unable to prove the identity of the victim as there was no missing complaint lodged in any station house.

This case suggests that if the evidence is preserved properly without any contamination, it can be used for analysis even after years of crime and still give significant lead in the case.

- **It can determine the geographic source of fruit, drugs, and imported goods.**

Pollen grains assist to determine the source of transported goods. It helps to understand the locality of particular fruit or drug or any goods that investigators come across. There have been several cases which got lead through pollen analysis. One such case took place in 1990[11]. Aerial surveillance helped police to locate a large field of marijuana grown illegally. A day after the police noticed a well-dressed person coming out of the bushes a few kilometersapart from the place. Upon Searching officers acquired a small soil testing kit and few uprooted plants. On questioning the man told that he was there to get some new plants for planting in his yard. After analysing the soil in the testing kit and from plants uprooted, police found that it belonged to the same place where illegal planting of marijuana was done. When taken into custody, the man confessed being the owner of those plants.

Other cases in which there was drug trafficking and illegal poaching and its transportation from one place to another were also given insight through pollen analysis[12].

- **Determine the travel history of items.**

Location of travelling of person can be determined by analysis of the suspects belonging. Pollen grains found on the belongings can be used to understand the probable location that he would have travelled.

In one caseman robbed an entire store and tried to escape. But the police officers chased him. He managed to abandon his motorbike and ran into the woods. The Next day one man came to the police station to lodge a complaint that his motorbike was missing. Police officials were in suspicion that he is the same guy that robbed the store and escaped yesterday as his personality was matching the previous day man. Upon enquiry he denied to be that person. When his belongings were analysed, it was found that his boots contained the mud which was present on the same hilly woods and also got the same pollen flora.This confirmed that the person was gone in the woods and later the suspect confessed his crime[13].

- **Confirms victim's locality.**

So often it becomes difficult to identify the victim as there is no solid clue for their identification. In such cases Pollen analysis gives at least some lead.

Recent case in whicha corpse was found with hardly any belongings. Her hands and feet were cut so that no identification can be done from fingerprints or footprints. It became difficult

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to trace her location from where she belonged. There were no blood traces present which confirmed that it was a secondary scene of crime.

On hard try later a palaeontologist came into the process. Upon analysing local pollen flora and the Pollen grains from victims hairs and nasal hair it was found that there was significant difference in the variety of both samples. When the pollen grains from the victim's body were matched with the Available data for pollen, it matched with the area which was about 150 miles away from the place where the body was recovered.Later on investigation was done in that area which gave lead in the case. The case is still under trial[11].

- **Associate a suspect to the scene of a crime and discovery site.**

Pollens being unique and different for each place can provide a link in the investigation. Suspects can be identified or ruled out from suspicion by examining traces that can give hints as to whether they took part in the crime[14].

A case came to an end after about 28 years in 2012. In 1985 Christopher Laverackfrom Humberside, UK went missing from his Uncle Melvyn Read'splace. Two days later the body was found folded in the carpet which was weighted along with brick. Childs Uncle was the prime suspect in the case. On investigation, Police learned about the paedophilic behaviour of Uncle Read. Although there were not many evidences against Melvyn Read to prove him guilty for the murder of Christopher Laverack.In 2002 he was arrested for number of sex offences against children. In 2008 Melvyn Read died in custody due to cancer. Laterpalaeontologist was involved in the case, Pollen analysis was done from the clothes worn by child on the day of his murder. This pollen was the same when compared with the soil of Uncle Read's garden. Also the Brick found near a body supposed to be from Read's Garden. These helped to conclude Uncle Read as murderer of the Christopher Laverack in 2012[11].

- **Relate an item left at the crime scene or discovery scene to a suspect.**

Any material left behind by the criminal can be useful to track down his locality. This can be well understood from the case in which a Van was stolen. It was used for a bank robbery in Oahu, Hawaii. Robbers abandoned this car a few miles away from the crime scene. This car was found by the investigators. Upon pollen analysis of air filters, Palynologist found pollen grains not only from the region of bank but also from the mountainous region of Koolaumountains. These mountains lie at the central part of Oahu Island. Investigators took this as supportive evidence for geolocation information. Upon enquiry in this region of Koolau mountain, a clerk of one store recognised the Van and also told investigators that he saw this van a few miles away from the store near a cabin on his way back home. Investigators caught Robbers who were hiding in the same cabin and were waiting for the chance to escape from the island[15].

The van abandoned provided information as to the possible location of the robbers.

- **Helps narrow down the suspect size.**

When there are many suspects involved in an investigation in a particular crime, It becomes a tedious job for the investigator to handle the case and come to a conclusion. In such cases Pollen analysis helps investigators to a greater extent to confirm the presence of a particular suspect.

A casein which a body of the farmer hanged was found in the outhouse. At first glance it appeared to be suicide. But there was not solid reason found for his suicide as he was not depressed or worried neither any suicide note was found. On Enquiry, Police found five suspects who can be directly benefitted from victim's death. However, each suspect has his own reason to deny the charges against them. On pollen analysis of the rope used to hang the victim it was found that the pollen belonged to the farm where mostly vegetables are grown. The outhouse where the dead body was found was a maize field. One suspect from five had a small vegetable farm, from which he used to earn a living. Although this evidence was not sufficient for the conviction of the accused, it helped to narrow down the number of suspects to one. On thorough investigation Police officers were able to collect enough evidences against suspect to be held responsible for farmer's murder[16].

- **It can be helpful to find the season of crime.**

As some plants are seasonal, pollens produced by them are also dominant in the same season. For example, in India spring is the dominant season for pollen as most of the plants and trees bloom during spring. Careful analysis and thorough understanding of the Pollen Populations and their seasonal variation in particular areas can prove very important in the Investigation.

In the year 1994 Magdeburg, Germany, 32 skeletal remains of young males were found during excavation. Examination of the body revealed that they all were headshot and then buried. But the question arises, who was responsible for the same? Few traces of clothes and personal belongings couldn't give conclusive evidence about who was the mass murderer. There were two assumptions made. Either they were partisans who were killed by Nazi Gestapo in late winter or early spring of 1945 or possibly they were Russian soldiers killed by Soviet secret police who killed soldiers because they refused to kill local German citizens during riots against Soviet secret police in late summer of 1953. When the nasal cavity of a few skulls were rinsed and examined, the Palynologist found pollen grains from trees which belonged to summer pollinators. This pollen was possibly inhaled before death. Thus this confirmed that the pollen grains found in the nasal cavity were dominant in June and July month and thus they were the soldiers who were shot by soviet secret police[17].

Summary:

Forensic palynology is one of the vital branches in forensic science and it is in its infancy. This field can help to apprehend the criminals within a time frame and it will be helpful in reducing the burden of forensic science professionals as well as the judicial system.

However, since last decade the use of palynological evidence has increased and the value of these evidences is recognised at large. There are many cases solved till date in which palynological evidence has provided a foremost breakthrough in the case[18]. Few landmark cases such as Baby Doe case[12], Cocaine smuggling case in New York[19], etc.

Though there are some countries in which the use of palynology is less as evidence[20]. Some major research needs to be done in this area like the Pollen database, which should be authentic and officially hosted by investigating agencies thus can be withheld in the court of law.

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BASIC BIOLOGY OF INFLAMMATORY BOWEL DISEASE

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Abstract:

Autoimmune diseases are the conditions where the body's immune system act against the body's tissues. Inflammatory bowel disease highly prevalent in developed countries like the United States of America is an example of autoimmune disease. Inflammation of the small and large intestine is the major symptom of inflammatory Bowel Disease which is classified as Ulcerative colitis and Crohn's disease. Various studies link the association of microflora with the pathogenesis of IBD. The ability of such microbes to hide from the host immune system is required. And the breaching of such a hiding mechanism might lead to IBD. The unbalancing between the beneficiary and harmful microbes is another reason for the same. This article is an attempt to discuss the possible therapeutic strategies to overcome IBD and also the need of including probiotics as a suitable approach.

Keywords: Inflammation, Auto-immunity, Probiotics, Therapeutics, Micro-flora

Introduction:

The immune system detects and eliminates any foreign disease-causing agent from our body and protects us from infections. But what happened when due to some mistakes it recognize self-body parts as foreign? It starts attacking its healthy cells, tissues, and organs. This situation is known as autoimmune disease, which leads to life-threatening conditions. Around 80 such autoimmune diseases are known for example type 1 diabetes, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, celiac disease, Graves' disease, inflammatory bowel disease (IBD), psoriasis. Various types of interaction between genetic and environmental factors might be the reason for this. Apart from this, Gender, race, and ethnicity behaviors are also associated with the emergence of autoimmune disease. Inflammatory bowel disease (IBD) is the inflammation of the small and large intestine and is classified as Ulcerative colitis (UC; affects the colon) and Crohn's disease (CD; affects the whole intestinal wall but mainly to the ileum) (Dalal *et al.,* 2014). The major affected nation due to IBD is the United States where 1-1.6

million people are having IBD (most prevalent to the younger population of 15-30 yrs) (IBD). The major symptoms include Abdominal cramping, fever, fatigue, weight loss, sweats, diarrhea, constipation, growth retardation, and abnormal bowel movement. The disease is not limited to the digestive tract inflammation, but it can give rise to arthritis, thromboembolism, cardiovascular-, pulmonary- & neurological diseases. It has a long history of family-run and can affect the whole family started from one member.

Microbial flora and IBD:

As we know that 10-100 fold more microbes are present in our gut as compared to our mammalian cells. 1,800 genera with 15,000 - 36,000 bacterial species are found in the intestine of one healthy person (Peterson *et al.,* 2008). The entry of these microbes into the gut is controlled by the epithelial layers (Goto *et al.,* 2012). The balance between the microbial flora and immune system is required to prohibit the disease conditions. But the unbalancing leads to the inflammation associated with the IBD as the name suggests. The molecular patterns like PAMPs (pathogen-associated molecular patterns) for example the toll-like receptors (TLRs) present on the surface of microbes are recognized by the immune cells at the beginning of the immune response. Some metabolites from microbes are also shown to induce inflammatory responses. It was also observed that the penetration of the bowel wall by microbes provoked the immune responses (Becker et al. 2015) as in the case of Ulcerative colitis (UC) and Crohn's disease (CD) (CAMPIERI *et al.,* 2001; Sheil *et al.,* 2007; Verna *et al.,* 2010). The correlation of bacterial load with inflammation rate in IBD is well accepted. As higher the load, the higher is the inflammation. The Experimental mice inoculated with the bacteria got IBD is the proof of it (Becker *et al.,* 2015). The use of antibiotics leads to amelioration of IBD suggests the involvement of microflora in IBD. The identification of >160 SNPs related to hosting responses against the microflora is also proving the role of the same in IBD pathogenesis (Dalal *et al.,* 2014).

It was shown that decrease in the ratio of beneficial bacteria: harmful bacteria results in the occurrence of Crohn's disease (http://www.sciencemag.org/news/2014/03/crohns-disease-marked-dramatic-changes-gut-bacteria). In both UC and CD, a load of beneficiary bacteria like Firmicutes and Bacteroidetes decreased. Not only the bacteria but the involvement of fungal flora in the pathogenesis of IBD especially UC was demonstrated. Change in the concentration of *Salmonella, Mycobacteria, Shigella, Yersinia, Clostridium difficile,* and *Bacteroides vulgates* are linked with IBD progression (Steidler, 2001). *E. coli* also has a significant role in IBD as its load was found to be decreased in UC patients and increased in CD patients (Mylonaki *et al.,* 2005).

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It was assumed that the change in Gut microbiota activates the immune responses. The lack of any type of intestinal inflammation due to immune activation in germ-free (GF) rodents vis-a-vis low density of T cells supports this narration (Smith *et al.,* 2007). *Enterobacteriaceae*, *Bacteroides*, and *Bifidobacterium* can elicit the T-cell response in the gut of individuals (Duchmann *et al.,* 1999). The T cell responses are not just elicited by the pathogens, but also by the microbes which are breaking the homeostasis (Larmonier, Shehab, Ghishan *et al.,* 2015). Apart from hiding from the effector T cells, these microbes use another strategy to protect themselves in the form of initiation of regulatory T cell responses (Larmonier *et al.,* 2015). These findings suggest the involvement of microflora in the pathogenesis of IBD.

Possible treatments for IBD:

There is no perfect treatment is available for IBD, instead, symptomatic approaches are being used to control the inflammation. Aminosalicylates like Mesalazine, Sulfasalazine, Olsalazine, Balsalazide, Prednisone, and Corticosteroids like Budesonide, Dexamethasone, Hydrocortisone, Methylprednisolone, and Prednisolone are known to regulate the inflammatory responses (Sales-Campos *et al.,* 2015; Williams *et al.,* 2011). Aminosalicylates activate the PPARγ, Inhibit the chemotaxis of the macrophage and also block the NF-κB, STAT & AP-1 signaling. Corticosteroids are the down-regulator of NF-κB singling. They also interfere with the production of cytokines, immune cell recruitment at the site of infection and also inhibit the expression of adhesion molecules. Metronidazole and Ciprofloxacin antibiotics are also useful to control the bacterial load. These antibiotics also suppress the immune system in the intestine (Underhill *et al.,* 2008). Azathioprine and 6-mercaptopurine suppress the immune systems by blocking the proliferation of lymphocytes and inducing apoptosis of activated T cells (Sales-Campos *et al.,* 2015). Anti-TNF like Infliximab, Adalimumab, Certolizumab pegol, Golimumab, and Anti-α4-integrin like Natalizumab, Vedolizumab is also useful to prevent the inflammatory responses by blocking the activity of TNF-α and migration of immune cells. But these types of treatment of costly and many side effects are also making the process challenging. Diarrhea, nausea, vomiting, headache, abdominal pain, hepatic abnormalities, arthralgia, myalgia, Osteoporosis, metabolic syndrome, cardiovascular disease, infections, osteonecrosis, cataract, numbness, tingling, muscle pain, weakness, and tendon rupture are the side effects observed in the patients going under the above treatments. Down-regulation of immune system might be lead to other infections, skin cancer, liver disorders, or autoimmunity.

The possible adverse effects of the said therapeutics prompted the investigators to explore the medicinal potentials of probiotics. Lactobacillus species, Bifidobacterium species, E. Coli Nissle, and yeast *Saccharomyces boulardii* are known to inhibit the harmful bacteria from

intestine linings, blocking inflammation, and facilitate the normal flora (Bibiloni *et al.,* 2005; Fedorak *et al.,* 2004).

Conclusion and future perspective:

The development of suitable therapeutics for IBD is the demand of time and for this, it is mandatory to understand the association of microflora with IBD pathogenesis. Therapeutics including antibiotics, drugs, and immune modulators are useful for the symptomatic or timely management of the diseases. The majority of these agents are active through the down-regulation of immune systems and this might be lead to other health risks. As we know that the weakening of the immune system is linked to many infections and co-infections by different pathogens can add to this risk. Therefore, the discovery of safer natural treatments is desirable. And, hence the exploration of the therapeutic potential of probiotics against IBD is under investigation.

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IMPACT OF CLIMATE CHANGE ON BIODIVERSITY IN INDIA: REVIEW

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Abstract:

A changing climate has a constant impact on biodiversity. Conditions vary at different rates around the globe, causing biological relationships to reorganize. Climate change and, as a result, biodiversity loss pose a threat to humanity's survival. The anthropological system is under peril from the loss of biodiversity that has been occurring over the planet. India's biodiversity is diverse, and in recent decades, it has been threatened by climate change, which has been hastened by human activities from different sources of pollution. Climate change has emerged as the decade's most pressing environmental issue. Much attention is being paid to lowering carbon emissions and greenhouse gas emissions from the industrial, energy, and transportation sectors by reducing fuel usage and increasing the use of renewable/green energy sources. Climate change is becoming one of the most serious threats to biodiversity, putting more strain on genetic resources, species, and populations. The review shows that climate change is a severe environmental threat that jeopardizes efforts to achieve long-term development. Climate change mitigation strategies include biodiversity protection and sustainable development. Despite the fact that adequate efforts have been made around the world to address environmental concerns, the negative effects of climate change are still growing, and the global rate of biodiversity loss is ongoing.

Keywords: Environmental threat, Biodiversity, Climate change, Renewable sources.

Introduction:

Significant and long-term changes in a region's climate are referred to as climate change. These changes can take decades or millions of years to occur. Climate change has an impact on entire ecosystems, as well as the plants and animals that dwell within them. All living animals have had to adapt, shift, or die out as the environment has changed over Earth's history. Ecosystems and species can evolve together when these changes occur gradually. A gradual

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transition also allows organisms to adapt to changing conditions. However, when change occurs rapidly, as it does now, the ability of species to adapt or relocate—assuming a suitable area exists—is a major worry. The global drive for modernization has resulted in excessive expansion in industrialization, urbanization, and transportation, resulting in the loss of the natural balance through climate change. Greenhouse effect, global warming, ozone depletion, and epidemics are some of the key impacts of climate change, all of which have direct or indirect effects on nature's biological resources and life-sustaining systems. Climate change is causing a great deal of worry around the world. This problem is being caused by over use of nature, which is affecting the weather pattern. Global warming is caused by rising levels of greenhouse gases and deforestation, which alters weather patterns, wind patterns, and higher atmospheric circulation (Pandey, 2007). Humans have modified ecosystems faster and widely in the last 50 years than at any other time in human history. "If current warming rates are maintained, Himalayan glaciers could decay at very rapid rates, shrinking from the current 500,000 square kilometres to 100,000 square kilometres by the 2030s," according to a recent summary of the conclusions of Working Group II of the Intergovernmental Panel on Climate Change. India is likewise not immune to the effects of climate change. Shrinkage of glaciers, decreased water flow in perennial rivers, prolonged rain failure during the monsoon season, heavy and un-occasional rain in coastal areas, shorter winters and longer summer seasons are some of the most significant examples of climate change described under Indian perspective (Thomas, 2007).

Biodiversity of India:

India is known around the world for its great past and diverse flora and fauna. India is home to over 45,000 plant species and 65,000 animal species, according to estimates. There are 15,000 species of flowering plants, with several hundred (5000-7500) being endemic. More than 50,000 insect species, 4,000 mollusk species, 6,500 other vertebrates, 2,546 fish species, 197 amphibians, 408 reptiles, 1224 birds, and 350 mammalian species are found in various settings. As a result of its rich flora and fauna, the country has been added to the list of mega biodiversity hotspots around the world.(: (Myers *et al.,* 2000). The Western Ghats and the Eastern Himalayas are India's mega biodiversity hotspots.

Climate change and its impact on Biodiversity:

Ecosystems that are healthy and have a diverse range of species are essential for life on our planet. Several species' habitats are being impacted by climate change, forcing them to adapt

or relocate to locations with better conditions. Small variations in average temperatures can have a big impact on ecosystems**.**

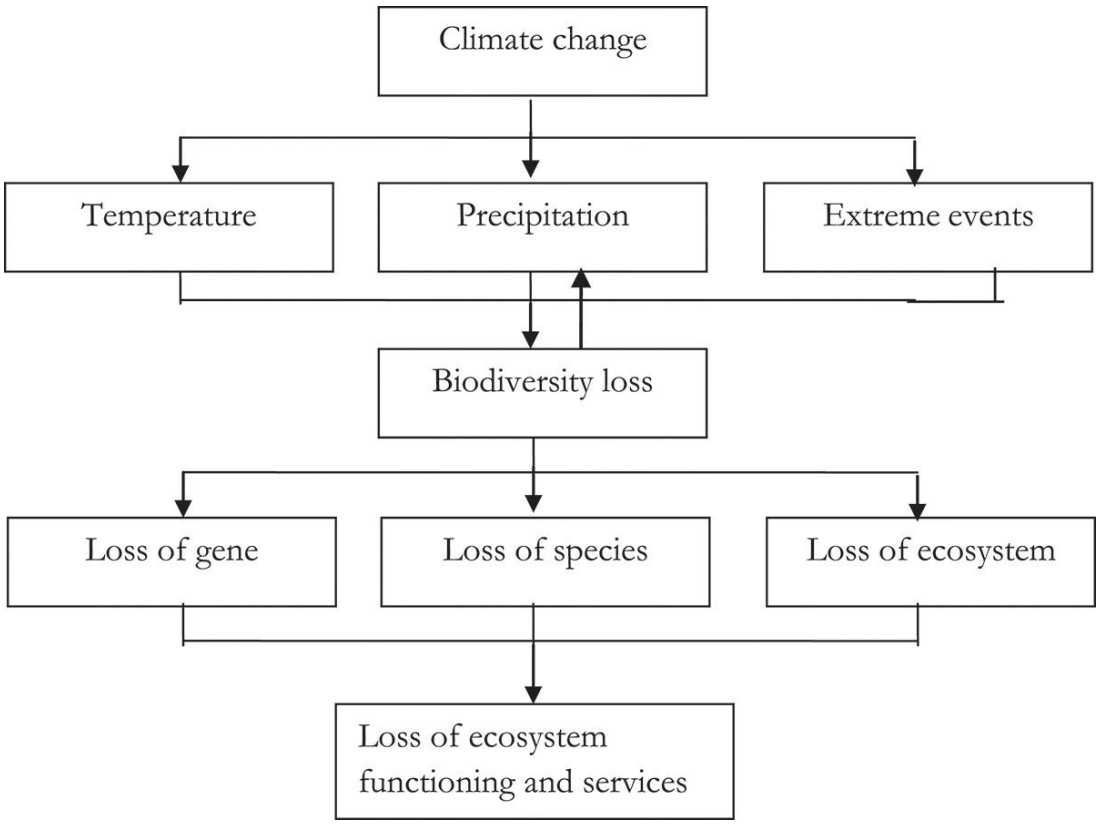

Figure 1: Link between climate change and its impacts on biodiversity and ecosystem services, and the impact of biodiversity loss on climate change

Climate change has been highlighted as a significant threat to biodiversity, which are expected to have negative consequences for biodiversity Climate variability in terms of precipitation and temperature are two crucial characteristics that are predicted to have a direct and major impact on India's biodiversity. According to Thuiller (2007), every 10°C increase in temperature shifts the occurrence zone of certain specialist species by 160 metres vertically and 160 kilometres horizontally. Although the effects of climate change on India's natural resources by region is still being researched. According to Sukumar (1995), indigenous mammals such as the Nilgiri Thar are in risk of extinction. The impact of climate change on biodiversity is expected to increase in magnitude and prevalence as CO2 levels and temperatures continue to rise, and extreme conditions, such as heat and storms, increase in frequency and intensity, according to a study by the Intergovernmental Panel on Climate Change (IPCC), a United Nations Scientific Consortium (IPCC, 2007). Biodiversity is crucial in mitigating and responding to the effects of climate change. Forests and peat areas, for example, trap carbon in their flora and soil, providing climate-regulating functions around the world. Globally, greenhouse gas

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emissions have been observed to constitute a hazard to biodiversity. The IPCC predicted in 2007 that global surface temperatures will rise by 1.8 to 40 degrees Celsius by the end of the century, and that a rise of 1.5 to 2.50 degrees Celsius would endanger the extinction of 20 to 30 percent of the world's plant and animal species. Climate change, according to Bates *et al.,* (2008), may have a negative influence on over 5,000 plant species due to the lack of suitable habitats. According to Gitay et al. (2002), the global mean surface temperature has risen by 0.6°C in the last 100 years. According to the IPCC and Anon (2007), global average temperature will rise by 0.1- 0.30 degrees Celsius a decade. The rise in CO2 concentration from 280 parts per million (ppm) in 1750 to 379 ppm in 2005 to around 395 ppm at present, according to IPCC (2007), indicates that humans are playing a major role in raising global atmospheric CO2 levels. According to the IPCC assessments, human activities have had a significant impact on the worldwide water cycle via influencing the global carbon cycle (NASA, 2010).

Climate has a crucial impact in people's lives and livelihoods in socioeconomic development. During the period 1901–2012, the climate warmed by 0.890°C (0.69–1.080°C), owing primarily to anthropogenic activities. According to IMD (2012), increasing temperature trends on the order of 0.60°C have been seen over the last 112 years. According to Goswami et al. (2006), there has been an increase in heavy rainfall events and a decrease in low and medium rainfall events across India. Extreme weather events such as hurricanes, as well as climate change, will have an impact on coastal development, water supply, energy, agriculture, and health, to name a few. Climate change causes abiotic environments to shift, affecting biological systems and processes. The rate, amount, and nature of climate change, as well as ecological sensitivity and adaptation capability to environmental change, all influence biological responses. It has also been stated that a combination of these factors has an impact on biodiversity at all levels. According to Campbell (2009), climate change may hasten the destruction of wetlands and freshwater ecosystems such as lakes, marshes, and rivers. Water quality may degrade as a result of rising temperatures, which will have significant consequences for aquatic life, including the extinction of some species. The character, quantity, and rate of changes experienced by a species or system (exposure), the degree to which they are influenced by or sensitive to those changes (sensitivity), and the ability to accept impacts with little disturbance determine biodiversity's susceptibility to climate change. Due to uncertainty in climate change estimates for the coming decades and limitations in our knowledge of biological and ecological responses to these changes, each of these components is difficult to quantify (Glick *et al.,* 2011).

Anthropogenic stressors such as land use change, non-native invasive species, exploitation, pollution, and illness all have an impact on biodiversity. Other stresses are presently or will be the principal drivers of biodiversity loss in many cases. Overall, climate change's effects are expected to become more prominent and dominating in the future decades, interacting synergistically with other stressors to increase biodiversity's vulnerability. Despite the fact that the overall impact on biodiversity is projected to be detrimental. As a result, an urgent goal is to conduct a full census and catalogue of the country's biological richness before it is lost forever. This will allow for the creation of effective biodiversity management plans as well as the determination of whether biodiversity changes are caused by environmental deterioration caused by other factors or by climate change. The authors used a contemporary information system to consolidate information from many corners of the literature, while also inserting their own opinions. Climate change is having and will continue to have widespread and varied consequences on all components of biodiversity, according to the results of this review. The author attempted to highlight the existing and future implications of climate change on biodiversity, as well as significant vulnerabilities, dangers, and potential risk-reduction techniques. Many species are shifting their geographical ranges, distributions, and phenologies at faster rates than previously assumed as a result of climate change; however, these rates are not uniform among species. According to mounting evidence, range shifts and new climates will result in new community assemblages, species connections, and interactions that have never existed before. In reaction to climate change, differences in how organisms adapt decide which species or populations will prosper (winners) and which will decrease and possibly become extinct (losers). Developing climate change adaptation methods and limiting biodiversity loss in the future decades would require identifying highly vulnerable species and understanding why they are fragile. Effective management and conservation decisions require consideration of uncertain future estimates as well as historic conditions when species shift in location and time as a result of climate change. Climate change is influencing biodiversity in a variety of ways, and the effects are predicted to worsen in the coming century; hence, an assessment of biodiversity risks and exposures is required to assist decision-making and where and how to best deploy scarce resources.

Management of climate change:

Climate change is a worldwide issue, and India may have a dual role to play in addressing it as a responsible member of the international community and in its own self-interest. Mitigation and adaptation are two aspects of the reaction to global warming. Controlling the

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mechanisms that cause climate change in the region is one of the mitigation techniques. Reduced greenhouse gas emissions can help to mitigate the effects of climate change such as warming, glacier melting, river flooding, and habitat loss. In the region, aforestation should be carried out. Producers and consumers may be enticed to invest considerably in products, technology, and processes that release fewer greenhouse gases as a result of policy tools. Global greenhouse gas emissions will continue to rise in the coming decades and beyond without new mitigation initiatives. To achieve a stabilization of greenhouse gas concentrations in the atmosphere, rapid global investments and deployment of mitigation technology, as well as research into new energy sources, will be required. Floods, extreme weather events, and coastal erosion are some of the effects of climate change that can be mitigated through improved physical infrastructure. Changes in temperature and water availability can be mitigated by switching to different crops, seeds, or agricultural practices. Adaptation efforts can be aided by education, training, and rural extension services. Improved weather and flood predictions, as well as improved communications, can help with evacuation, relief, and recovery. As a developing country, India's primary focus must be on adaptability. Tourism is another key activity in the region, as there are numerous tourist attractions. As a result, habitat disturbance may occur as a result of mass heat, dhabas, restaurants, transportation, and sports activities. Climate change necessitates adaptive forest design and management practices.

Climate change considerations must be factored into long-term forest planning and policy development. The Forest Departments' traditional Working Plan approach to forest management, which is insufficient even in the absence of climate changes, may need to be upgraded and made dynamic to account for climate impacts. India has a wide range of tropical and subtropical forest habitats that are susceptible to a variety of socioeconomic stresses. The complex forest ecosystems will be put under even more stress as a result of climate change.

To make a meaningful assessment of the effects of climate change on forest ecosystems and biodiversity, modeling that incorporates socioeconomic and land-use change pressures as well as expected climate change parameters is required. Furthermore, models, as well as input data on vegetation parameters, soil and water characteristics, climate variables, and socioeconomic aspects, constrain climate impact and vulnerability assessment studies. Enhanced crop and grazing land management (e.g., improved agronomic methods, nutrient utilization, and tillage and residue management), restoration of organic soils drained for crop production, and restoration of degraded lands are some of the other choices. Mitigation strategies are needed to minimize global greenhouse gas emissions with the goal of stabilizing atmospheric

concentrations at a level where a sustainable dynamic equilibrium between climate, ecosystems, and human society may be maintained. However, because of the inertia of both the climate system and our energy infrastructure, greenhouse gases that have accumulated in the atmosphere since the preindustrial era will continue to have an impact on global climate for a long time. Adaptive solutions are required to improve the coping skills of valuable ecosystems, vulnerable communities, and exposed infrastructures, given the existing exposure of many communities and assets to weather extremes. Changes in consumption patterns can aid in the fight against climate change. Certain fossil fuel alternatives should be discovered and invented as mitigation methods to reduce greenhouse gas emissions. Governments can use a number of policy mechanisms to incentivize mitigation activity, including legislation, taxation, tradable permit schemes, subsidies, and voluntary agreements.

Conclusion:

Climate change has emerged as the decade's most important environmental issue. Reduce carbon and greenhouse gas emissions from energy, industrial, and transportation sources by reducing the quality and quantity of fuel used, implementing improved and advanced technologies, increasing public awareness of the environment in which they live, and raising awareness of the importance of biodiversity and climate change. Promoting suitable and effective coordination among biodiversity and climate change initiatives in India through the incorporation of eco-friendly environmental policies and the integration of biodiversity and climate change into national plans and programmes. Developing policy and guidelines for biodiversity and climate change in order to reduce local communities' vulnerability to climate change impacts and increase their flexibility in dealing with the effects of climate change. Since it is vital to know the demands because they are dependent on a specific ecosystem, public participation is required to integrate ecosystem conservation and rural development. Determine which sectors of the country are particularly vulnerable to climate change, such as water resources, agriculture, health, coastal areas, and forests. Encourage research into the development of methodologies for tracing changes in glaciers, river flows, and biodiversity as a result of climate change. Examine the need for national and local adaptation to future climate change impacts, as well as the scope for incorporating the findings of such assessments into relevant programmes, such as watershed management, coastal zone planning and regulation, agricultural technologies and practices, forestry management, and health programmes. Coastal management plans, as well as infrastructure planning and construction standards, should explicitly reflect the susceptibility of coastal areas and its biodiversity to climate change and sea

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level rise. Identify the most significant information gaps that impede the national ability to develop and implement climate change adaptation policies for species, ecological systems, and functions. Improve the country's climate modeling capability to acquire a better understanding of the effects of climate change on biodiversity at the national and local levels. Decision-makers are increasingly realizing that biodiversity is not an afterthought in human affairs, but rather the essential core of our existence.

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REVIEW ON ROLE OF PHOSPHATE SOLUBILIING BACTERIA IN SUSTANINABLE AGRICULTURE

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Abstract:

Compared to other major nutrient, phosphorus is least mobile and available for plant in most soil. Although P is abundant in soils, in both organic and inorganic forms, its availability is restricted because it is most often in insoluble form.The average soil phosphorus content is approximately 0.05%(w/w) but due to low solubility and its fixation in soil, 0.1% of the total phosphorus is available to plant.An adequate supply of phosphorus in the early stages of plant development is essential for laying down the primordia of plant reproductive parts.it helps in seed formation and early maturation of crops like cereals and legumes.Poor availability or lack of phosphorus (P)clearly reduces plant size and growth.Phosphorus is about 0.2 - 0.8% of the plant dry weight.To satisfy crop nutritional requirements, P is usually added to soil as chemical P fertilizer, however synthesis of chemical P fertilizer is extremely energy intensive processes, and has long term effect on the environment in terms of eutrophication, soil fertility depletion, carbon footprint.furthermore, plants can use only a small amount of this P since 75–90% of added P is precipitated by metal–cation complexes, and dissolves quickly in soil.Such environmental concerns have concerns motivate the search for sustainable way of P nutrition of crops.In this regard phosphate-solubilizing microorganisms (PSM) have been seen as best eco-friendly and increase phosphate uptake by the plant and crop yield.This review is focused on the role of 'P' and 'PSB' in plant nutrition and sustainable agriculture.

Introduction:

As the worlds human population grows, so will the demands on agriculture for future food supplies is a major challenge facing the agrarian community. In order to meet this challenge, a great deal of effort focusing on the entire soil biological system and agro ecosystem

as a whole is needed to understand better the complex processes and interaction governing the stability of agricultural land.At the present time,food is not (usually) in short supply (conversely, lack of time delivery of food where needed) to some extent due to high-input farming,resulting in this green revolution.The green revolution has been one of the most extremely successful human activities resulting in global food security and, consequently, transformed some of the developing countries,like India, from being food-shortages to having a food surplus.However, consistent and worrying human population growth is at risk again worlds food security.There is an urgent need for second green revolution to increase food production by almost 50% in the 20 years to keep up with population pressures (Vasil, 1998; Leisinger, 1999).

Chemical fertilizers, e.g., formed water-soluble phosphatic (WSP) fertilizers (superphosphates) have played aimportant role in the green revolution and it is generally recommended to eliminate phosphorus deficiencies. Most developing countries, however, import these fertilizers, which are often in limited supplies and resources represent a major expense for poor farmers. In addition, the intensity of agricultural production in these countries requires not only to increase crop yields but also to improve the phosphatic condition of soil to prevent further degradation of soil, furthermore, the use of chemical fertilizers has reached the theoretical maximum use and there will be no further increase in crop production (Ahmed, 1995). However, the traditional farming is becoming clearer day by day, practice production base, healthy plants can not sustain soil system, for long time;so, to the increase crop production,agronomists rely heavily on chemical fertilizers.

Phosphorus (P), a major nutrient, is required for various metabolic processes such as energy transfer, signal transduction, macro-molecular biosynthesis photosynthesis and respiration by plants (Fernández *et al.,* 2007). Phosphorus is present in the soils both in organic and inorganic forms. Of these, P is an important reservoir is, with organic matter found in humus and other organic matter, including decaying plant, animal, and microbial tissues, with approximately 20-80% of the total soil phosphate being stable (Richardson *et al.,*1994). Phosphorus in labiled organic compounds can be gradually mineralized as inorganic P or it can be immobilized as part of the soil organic matter (Mckenzie *et al.,* 1986). The process of mineralization or immobilization is carried out by microorganisms and has a profound effect on soil moisture and temperature.Soil inorganic phosphorus, however, is controlled mainly by concentration of solution ph and cation and in most soils maximum phosphorus availability is found between ph 5.5 to 7.In this ph range, as a result, the most efficient use of P occurs in neutral and calcareous soils and P is determined by the hydrous oxides of Fe, Al, and Mn while between pH 6 to 8.5, P is fixed by silicate minerals and Ca. Thus, the majority of P is unavailable

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for uptake by plants due to its rapid rate of fixation/complex formation with other elements of soils (Goldstein *et al.,* 1986).In the united states, for example, an average of 29% P is added to manure and compost (Sharpley *et al.,* 2006). Therefore , P fertilizer are applied to the soil to meet the P demand of the growing plant.Phosphate fertilizers are expensive because the efficiency of added phosphate fertilizers is about 10% lower (Werft Van Der *et al.,*1996). This has led to the search for environmentally friendly and economically feasible alternative strategies to improve crop yield in low or phosphate deficient soil.In this context, organisms with phosphate solubilizing activity are called phosphate solubilizing microorganisms (PSM), PSM can provide viable alternatives to chemical phosphate fertilizers. Of the different PSMs inhabiting rhizosphere, PS bacteria [PSB] are consider as promising bio fertilizers because they can supply plant with P from sources otherwise poorly available.Although PSBs are commonly found in soils, their formation and performance are severely affected by environmental factors, especially under stressful conditions.However, the beneficial effect of the inoculation with PSB (Poonguzhali *et al.,* 2008, Chen *et al.,*2008) , alone or using other rhizosphericmicrobes have been reported (Zaid *et al.,* 2006; Vikram *et al.,* 2008).

Importance of phosphorous in plant growth:

Phosphorus is considered to be one of the most essential macro elements required for plant growth and development. Phosphorus nutrition is related to many of the main function of plant including root development, stalk and stalk strengthening, flower and seed preparation, crop maturity and quality in production, determination of nitrogen in legumes and strengthening of seedlings from disease.Phosphorus is an part of many essential plants as a catalyst in the conversion of numerous key biochemical reactions in compositional compounds and plants. Phosphorus is especially renowned for its role in converting solar energy into useful plant compounds.Phosphorus is an important component of DNA, the genetic “memory unit” of all living things. Also it is component of RNA, the compound that reads the DNA genetic code to build proteins and other compounds essential for plant structure, seed yielding and genetic transfer. The structures of both DNA and RNA are linkedtogether by phosphorus bond.Phosphorus is an important component of ATP, the “energy unit” of plant .ATP forms during photosynthesis ‘has phosphorus in its structure, and processes from the beginning of seedling growth through to the formation of grain and maturity.Phosphorus can be found naturally in soil solution in a variety of forms.They can be broadly classified as insoluble in inorganic phosphorus and insoluble organic phosphorus.However, due to low solubility and

fixation in soil, only a small fraction of phosphorus is available in the soil solution (1 ppm or 0.1%), which is readily available to plants.

Phosphate solubilizing bacteria:

Different types of microorganisms have the ability to dissolve phosphate.These include bacteria, fungi, actinomycetes, and algae and are collectively called as Phosphate solubilizing microorganisms.Bacteria are more effective in Phosphate solubilization than fungi.Phosphate solubilizing bacteria belong to the PGPR. PSB are ubiquitous and vary in size and population in different soil.Their population in the soil depending on the chemical and physical properties, organic matter and P content of the soil. PSB found in majority of soil.Their population was generally low in arid and semi-arid region, possibly due to low organic matter levels and high temperatures. The PSB population was higher in soils under mild and moist climates than in dry ones. The larger population of PSB is present in agricultural and rangeland soils.Among the whole soil microbial population, phosphate soluble bacteria are 1 to 50%, while phosphorus soluble fungi have only 0.1 to 0.5 % phosphate soluble capacity.Evidence of natural phosphate formation dissolving microorganisms date back to 1903. Phosphate soluble bacteria have the ability to dissolve phosphate and can convert inorganic phosphate compound to make them soluble in soil and make them available for plant absorption. These bacteria in the presence of labile carbon serve as a sink for P by rapidly immobilizing it even in low P soils. Subsequently,PSB become a source of P to plant upon its release from their cells.Dissolution of insoluble phosphate in rhizospheres is the most common method of increasing the availability of nutrients to the bacteria that cause plant growth. The PSB may release several organic acids including oxalic, citric, butyric, malonic, lactic, succinic, malic, gluconic, acetic, glyconic, fumaric, adipic, and 2-ketogluconic acid (Rose, 1957). Salstorm in 1903 first demonstrated the microbial solubilization of inorganic phosphates by incubating TCP (Tricalcium phosphate) with bacteria from milk and soil infusions as mentioned by Deepshika *et al.* (2014) and Gerretson in 1948 demonstrated the microbial activity in the rhizosphere could dissolve the sparingly soluble inorganic P and increase plant growth, mentioned the same (Deepshik *et al.*, 2014).

Different bacterial genera and within genera different bacterial species have been reported to have ability to solubilizing P. Strains from bacterial genera Bacillus megaterium, Pseudomonas, Bacillus, and Enterobacter (Whitelaw, 2000), Rhizobium, B. polymyxa, B. sircalmous, B. circulans, B. subtilis, Pseudomonas striata, and Enterobacter are the most powerful P solubilizers (Subbarao, 1988).

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Table 1: Organic acids involved in P solubilization and produced by PS bacteria

Bacterial communities	Organic acids produced	References
Bacillus, Rhodococcus, Arthrobacter, Serratia and one Chryseobacterium, Delftia, Gordonia, Phyllobacterium, Arthrobacter ureafaciens, Phyllobacterium myrsinacearum, Rhodococcus erythropolis and Delftia sp.	Citric acid, gluconic acid, lactic acid, succinic acid, propionic acid	[12]
Enterobacter intermedium	2-ketogluconic acid	[30]
Bacillus amyloliquefaciens, B. licheniformis, B. atrophaeus, Penibacillus macerans, Vibrio proteolyticus, Xanthobacter agilis, Enterobacter aerogenes, E. taylorae, E. asburiae, Kluyvera cryocrescens, Pseudomonas aerogenes, Chryseomonas luteola	Lactic acid, itaconic acid, isovaleric acid, isobutyric acid, acetic acid	[31]
Pseudomonas cepacia	Gluconic acid, 2 ketogluconic acid	[32]
Bacillus polymyxa, B. licheniformis, Bacillus spp.	Oxalic acid, citric acid	[18]
Pseudomonas striata	Malic acid, glyoxalic acid, succinic acid, fumaric acid, tartaric acid, a-ketobutyric acid	[33]
Arthrobacter sp.	Oxalic acid, malonic acid	[34]
Bacillus firmus	2-ketogluconic acid, succinic acid	[34]
Micrococcus spp.	Oxalic acid	[34]

Acetobacter sp. (Joseph and Jisha, 2009), Acetobacterdiazotropicus (Maheshkumar *et al.*, 1999), (Rodriguez and Fraga, 1999), Agrobacterium sp. and Alcaligenes sp., Corynebacterium sp. (Gupta *et al.*, 1998), Azotobacterchroococcum (Kumar and Narula, 1999), Burkholderia sp., Gluconacetobacter sp., Enterobacter sp. (Chung *et al.*, 2005; Kim *et al.*, 2003), Flavobacterium sp. Micrococcus sp. (Goldstein, 2001), Pseudomonas, Bacillus, Rhizobium, Micrococcus, Flavobacterium, Achromobacter, Erwinia, Acinetobacter sp. and Agrobacterium (Rodriguez and Fraga, 1999) are among the frequently reported PSB.Of all the PSB, rhizobia has a double

advantage; they provide N in addition to phosphate dissolution and can improve the growth of legumes with other PGPR or mycorrhizaal fungi.

These PSB are ubiquitous but can vary in mineral phosphate solubilization capacity from soil to soil or from one production system to another production system and greatly affected by the nutritional status of soil and environmental factors. However, the metal, temperature, and concentration of C and N sources greatly affect the PS potential of these bacteria in vitro condition (Zaidi *et al.,* 1999). These organisms are usually isolated from rhizosphere and non-rhizosphere soil, rhizoplanes, phyllospheres, RP deposit area soils, and even in stressed soil by serial plate dilution method or enrichment culture technique. Since 1948, when Gerretson suggested that the phosphate which is present in soil, microbe could hardly soluble available form and play an important role in providing phosphate to plant, a number of methods and medium, such as (Pikovskaya *et al.,* 1948), bromophenol blue dye method (Gupta *et al.,* 1994) and National Botanical Research Institute P (NBRIP) medium (Nautiyal *et al.*,1999) have been proposed. Bacterial strains with phosphate solubilizing function are found due to the formation of clear halo (a sign of solubilization) around the colonies (fig 2). Due to inconsistency and variations in PS activity, the bacterial cultures are frequently sub-culture to test the persistence of PS potential. Once selected efficient PSB, the release of P by PS bacteria is quantitatively assayed and the potential P solubilizers are then mass produced and tested under pot/field environments with different samples.

Mechanism of Phosphate solubilization by PSB

While reviewing phosphate chemistry in soil, sims and pierzensky (2005)identified the major processes in the phosphate cycle that affect the soil solution P concentration as 1.dissolution-precipitation (mineral equilibria), 2. Sorption-desorption (interaction between P in solution and soil solid surface, and 3. Mineralization–immobilization (biologically mediated conversions of P between inorganic and organic form). The main phosphate merger processes employed by soil microorganisms include: (1) release of complexing or mineral dissolving compounds e.g. organic acid anions, siderophores, protons, hydroxyl ions, CO2, (2) liberation of extracellular enzymes (biochemical P mineralization) and (3) the release of P during substrate degradation (biological P mineralization) (McGill and Cole 1981). Therefore, microorganisms play an important role in the three major components of the soil phosphate cycle (i.e. dissolution-precipitation,sorption-desorption, and mineralization-immobilization,).In addition to these microorganisms,the presence of labile C act as a sick for phosphate, making it rapidly competitive even in low phosphate soil; so phosphate solubilizing microbes become a source of phosphate for plants after they are excreted from their cells.Release of P immobilized by PSB

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primarily occurs when cells die due to changes in environmental condition, starvation or predation.Environmentalchanges,such as drying-rewetting or freezing-thawing,can result in so called flush-events, a sudden increase in available P in the solution due to an unusually high proportion of microbial cell lysis. Grierson *et al.* (1998) found that about 30–45% of microbial P (0.8–1 mg kg-1) was released in a sandy spodosol in an initial flush after drying–rewetting cycles within the first 24 hour.

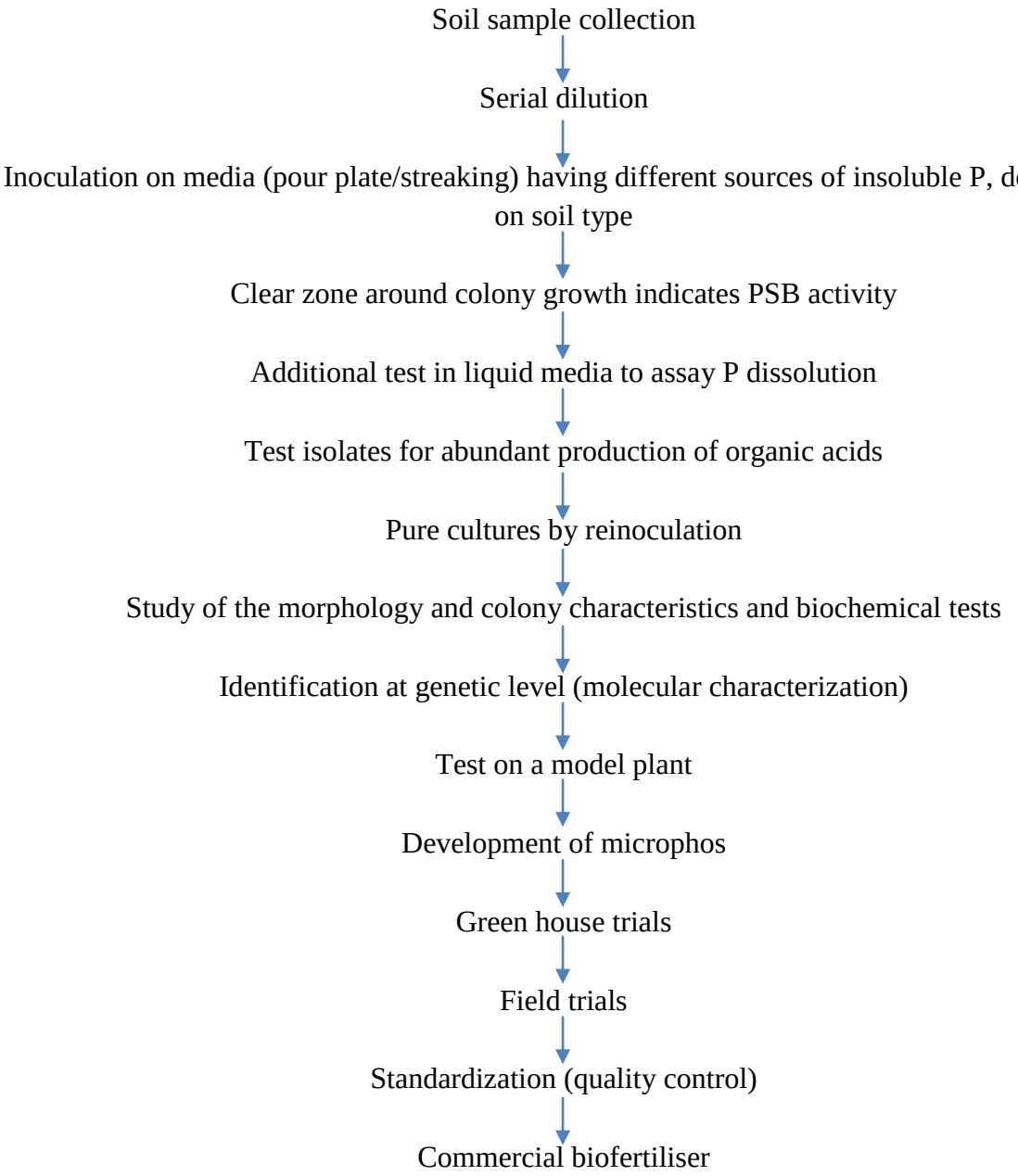

Figure 1: Protocol for isolation and development of effective inoculants of PSM based biofertiliser

Figure 2: Solubilization of tri-calcium phosphate on Pikovskaya plates by species of (A) Pseudomonas, (B) Serratia, (C) Bacillus

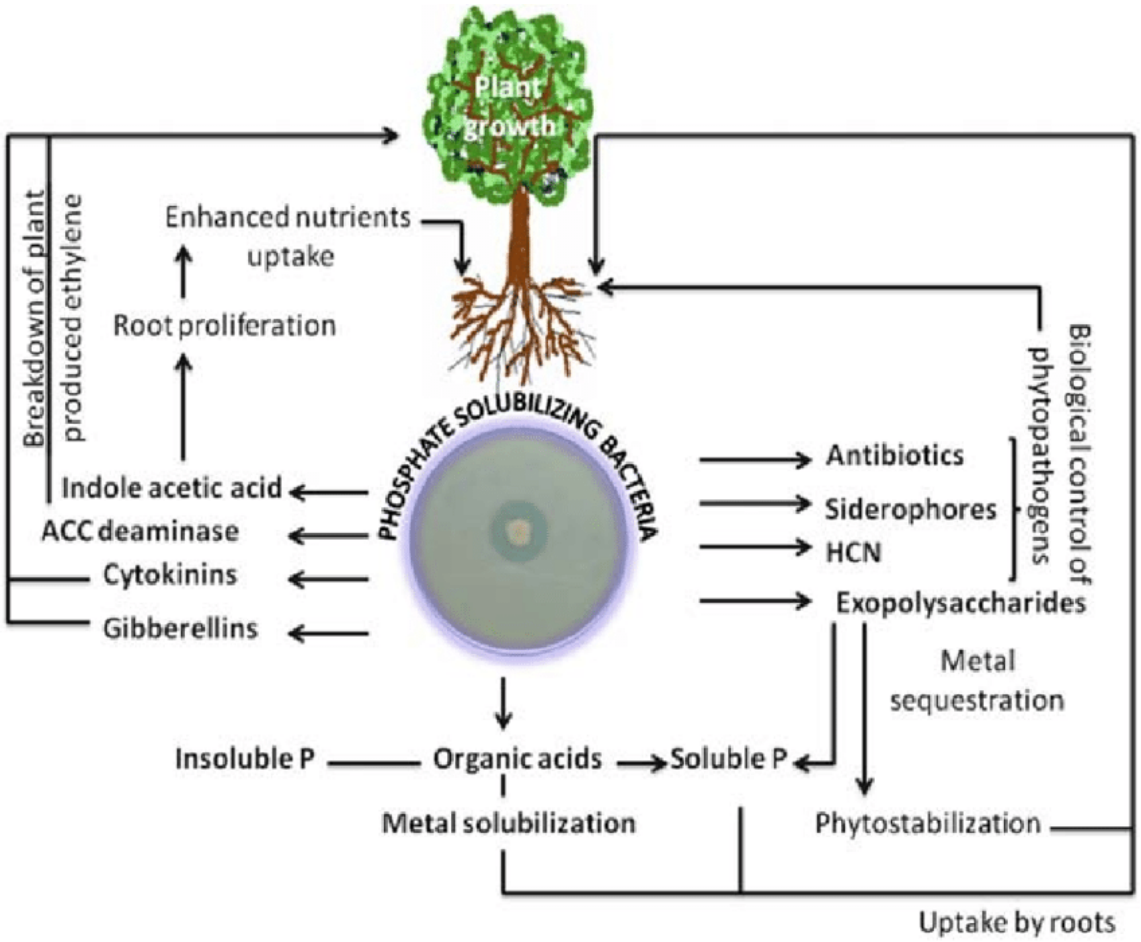

Figure 3: Mechanism of soil P solubilization/mineralization and immobilization by PSB

Inorganic P solubilizing:

P-solubilizing Bacteriasolubilise inorganic P mainly by organic acid production, either by: (i) lowering the pH, or (ii) by enhancing chelation of the cations bound to P (iii) by

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competing with P for adsorption sites on the soil (iv) by forming soluble complexes with metal ions associated with insoluble P (Ca, Al, Fe) and thus P is released. The lowering in pH of the medium suggests the release of organic acids by the P-solubilizing microorganisms (Whitelaw 2000; Maliha *et al.,* 2004) via the direct oxidation pathway that occurs on the outer face of the cytoplasmic membrane (Zaidi *et al.,* 2009). These acid are products of microbial metabolism, mainly by oxidative respiration or by fermentation of organic carbon sources, or such organic acid can either dissolve mineral phosphate directly as result of anion exchange of P by acid anion or can chelate Fe, Al and Ca ion associated with P (Omar, 1998).Monovalent anion phosphate H2PO4 is soluble form of inorganic phosphate that usually occurs at low PH. However as the ph of the soil environment increases the divalent and trivalent form of Pi (HPO4 -2 and HPO4 -3 respectively) occur. Thus, PSB strain is synthesis and discharge of organic acid into the surrounding environment acidify the cell and their surrounding environment which causes the P ions to be released from P minerals by H+ substitution for cation that binds phosphate.Major acids secreted by PSB dissolution of insoluble P contains oxalic acid, citricacid (Kim *et al.,* 1997), gluconic acid (Di-Simine *et al.,* 1998; Bar-Yosef *et al.,* 1999), tartaric acid, aspartic acid (Venkateswarlu *et al.,* 1984). Evidence from an abiotic study using, P solubilize by HCL and gluconic acid indicated that chelation of Al3+ by gluconic acid could be a factor in the solubilization of colloidal Al phosphate (Whitelaw *et al.,* 1999). Organic acid produced by PSB can detect by high efficiency liquid chromatography and enzymatic methods. (Parks *et al.,* 1990; Whitelaw 2000) However, acidification does not appear to be the only mechanism for dissolving, as in some cases the ability to lower the Ph does not match the ability to dissolve mineral phosphate (SubbaRao, 1982). No organic acid were found in culture filters and therefore, the authors concluded that acidification was probably not the major mechanism of dissolution as ph never came below 5. The action of phosphate dissolution was attributed to both chelation and reduction processes. However, organic acid has been suggested as the main mechanism of P solubilization, solubilization of P by inorganic acid (HCL) has been reported, although HCL could dissolve at low ph acid from hydroxyapatite than citric acid or oxalic acid at the same ph. (Kim *et al.,* 1997). Bacteria of the species Nitrosomonas and Thiobacillus can also dissolve phosphate compounds by producing nitric and sulphuric acid (Azam and Memon 1996).According to sink theory, PSB remove and assimilate phosphate from the liquid and therefore, activate the indirect decomposition of calcium phosphate compounds by continuously removing P from the liquid culture medium.For example,the P content in the biomass of Pseudomonas sp. And P. Aurantiogriseum was similar to that seen in non-P-solubilizing

microorganisms (Illmer *et al.,* 1995) this can be explained by the fact the content of phosphate in the biomass of organisms is constantly related to the organic decomposition of P containing organic substrates (Dighton and Boddy, 1989). The other mechanism is the production of H2S, which reacts with ferric phosphate to form ferrous sulphate released in association with P. (Swaby and Sperber, 1958). Parks *et al.,* (1990) proposed the H+ excretion originating from NH4 + assimilation is the alternative mechanisms of P solubilization.An HPLC analysis of the culture solution of Pseudomonas sp., in contrast to the expectation, did not detect any organic acid while solubilization occurred (Illmer and Schinner, 1995). They also reported that the most probable reason for solubilization without acid production is the release of protons accompanying respiration or NH4 + assimilation. Krishnaraj *et al.* (1998) has proposed a model explaining the importance of protons outside the cell responsible for P solubilization.Here the direct role of organic or inorganic acid is denied. For some microbes, NH4+driven proton release appears to be the only mechanism to promote phosphate solubilization.According to a study by Pseudomonas fluorescence,the nature of the C supply (e.g. NH4+ vs. NO3-) had the greatest effect on proton release (Park *et al.,* 2009). Acidification of the rhizosphere of cactus seedlings (giant cardon, Pachycereuspringlei) after inoculation with the plant growth promoting bacterium *Azospirillum brasilense*, in the presence or absence of ammonium and nitrate,was studied and assumed to be so the effect of inoculation with this PGPB on plant growth, combining nitrogen with nutrients may affect one or more things due to metabolic pathways of plants that increase proton flow from the roots and released organic acid,leading to rhizosphere acidification (Carrillo *et al.,* 2002). This suggests that for different species, different mechanisms are responsible for proton release, depending only on the presence of NH4+. Goldstein (1995) suggested that extracellular oxidation via direct oxidation pathway may play an important role in soils in which calcium phosphates provide a significant pool of unavailable mineral phosphorus.

Organic P solubilization:

Organic P solubilizationis also known as mineralization of organic phosphorus. Soil organic mineralization plays an essential role in the phosphorus cycling of phosphorus cycling of phosphate farming systems.Organic phosphate can be 4-90% of the total phosphate soil. Such P can be released from organic compounds in soil by enzyme:

- Non-specific acid phosphatases (NSAPs) which dephosphorylate phosphor-ester or phosphoanhydride bonds of organic matter.In a variety of the phosphatase enzyme class released by PSB, phosphomonosterase (often simply called phosphatases) are most abundant and best studied (Nannipieri *et al.,* 2011)**.** Based on their ph optima,these enzymes are divided into acid and alkaline phosphomonoesterases and can be produed

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both by PSB depending on external conditions (Kim *et al.,* 1998; Jorquera *et al.,* 2008). Usually, acid phosphatases predominate in acid soils, so alkaline phosphatases are more abundant in neutral and alkaline soils (Eivazi and Tabatabai 1977; Juma and Tabatabai 1977, 1998; Renella *et al.,* 2006). Although plant roots produce acid phosphatases but rarely produce large amounts of alkaline phosphatases, this suggests that this is a potential niche for PSB (Juma and Tabatabai 1998; Criquet *et al.,* 2004). It is also difficult to distinguish between root and PSB produced phosphatases (Richardson *et al.,* 2009a, b) but some evidence suggests that phosphatases of microbial origin are more abundant in organic phosphate compounds than those derived from plant roots (Tarafdar *et al.,* 2001).

- Phytases, which in particular cause release of P from phytate degradation. Basicallyphytate is the main source of inositol and the main stored form of P in plant seeds and pollen and is a major component of 0rganic phosphate in the soil. (Richardson, 1994)
- Phosphonatases and C–P Lyases, the phosphonates degrading enzymes that perform C–P cleavage in organophosphonates (Rodriguez *et al.,* 2006).

Role of siderophores in P solubilization:

Siderophores are complex agents that have a high iron attraction and form almost all microorganisms in response to iron deficiency.In iron limiting condition, siderophore act as solubilizing agent for iron from minerals or organic compounds. There are approximately 500 known siderophore, most of which are used by all microbs and plants some of them being exclusively used by the microbial species and strains that produce them (Crowley 2007). Given the predominance of mineral dissolution on ligand exchange by organic acid anion as a phosphate solubilizing mechanism, the potential role of siderophores in increasing phosphate availability needs to be clarified.

Role of EPS in P solubilization:

The role of polysaccharides in the microbial mediation of phosphorus was recently evaluated by Yi *et al.* (2008). Microbial exopolysaccharides (EPS) are polymers consisting primarily of carbohydrates in which certain bacteria and fungi are excreted on the outside of their cell walls. Their structure and composition are very different; they can be homo or heteropolysaccharides and can also contain a lot of organic and inorganic substances (Sutherland 2001). Four bacterial strains of, Arthrobacter sp. (ArHy-505), Enterobacter sp. (EnHy-401), Enterobacter sp. (EnHy-402), Azotobacter sp. (AzHy-510) had the ability to dissolve TCP (Tri

calcium phosphate), (Yi *et al.* 2008) and was used to evaluate the role of exopolysaccharide (EPS) in the solubilization of P. These phosphate solubilizing bacteria produced significant amount of EPS and showed a strong potential for phosphate solubilization.

Phosphate solubilizing bacteria in sustainable agriculture:

Energy crisis, environmental hazards, and depleting soil fertility etc. are the factor which is responsible for increasing the risk to sustainable agriculture. Therefore the use of bio fertilizers in agriculture has proven to be environmentally friendly, productive and accessible (Sheraz *et al.*, 2010). PSB are a major contributor to plant nutrition in agriculture. The use of PSM is currently considered as a concrete strategy to improve the productivity of the soil under crop production. PSB have been used as bio fertilizers since the 1950 (Krasilinikov, 1957). PGPR and PSB collectively can reduce the use of phosphate fertilizer by 50% without significantly reducing the yield of crop (Jilani *et al.*, 2007). Seed inoculation with PSB is a promising technique that eliminates phosphate deficiency (Qureshi *et al.*, 2012). inoculation with PSB such as *Pseudomonas, Bacillus, Rhizobium, Micrococcus, Flavobacterium, Achromobacter, Erwinia,* and *Agrobacterium* have been reported to increase the curtainPS ensuring high crop yields (Rodriguez and Fraga, 1999). Bacillus and Paenibacillus have been reported to be potential PGPRs in sustainable agriculture and are major sources of broad spectrum peptides antibiotic that are active against various microbial and nematode pathogens (Venkadasamy *et al.*, 2010). Ankit *et al.* referred to Bacillus as the PGPR in crop ecosystem (Ankit *et al.*, 2011).Diriba *et al.*, phosphate in the presence of two phosphate sources, hydroxyapatite and tricalcium phosphate, the solubility of different strain of rhizobacteria associated with *Coffea arabica* L. is evaluated. Total 395 is isolated tested, more than 72% *Pseudomonas erwinia* and *P. chlororaphis* were mainly able to strongly dissolve phosphate sources, and the analysis of HPCL showed several organic acid, with 2-ketogluconic acid dominating (Diriba Muleta *et al.*, 2013). Isolates *Bacillus* spp. (67%) and *Burcholderia* spp. (58.5%) were shown in the corn rhizosheresolubilisation of aluminium, phosphate (AlPO4), and tricalcium phosphate (Ca3 (PO4)2) (Oliveira *et al.*, 2009). Studies on Pseudomonas and Trichodermam have shown positive IAA (indole-3-acetic acid) production, and antagonistic action against *Fusarium oxysporum* and *Rhizoctonia solani* to increase growth and disease consumption (Jay *et al.*, 2014). Experiment on the effect of Pseudomonas letiola on mobilization of P and growth of Ligol (young apple trees) grown in a pot, significantly increased the total shoot length and solubilised insoluble P compounds (Ewa *et al.*, 2013). *Rhodococcus* sp. EC35, *Pseudomonas* sp. EAV and *Arthrobacter nicotinovorans* EAPAA when inoculated with *Zea mays* and grown in P deficient soils amended with tricalcium phosphate enhanced the plant growth (Sofia and Paula, 2014). PSB can be considered as broad

spectrum biofertilizer. PSB like Bacillus *megaterium, Bacillus circulans, Bacillus subtilis* and *Pseudomonas straita* are effective biofertilizers. *Burkholderia cepacia* in the rhizosphere of maize showed tricalcium phosphate solubility and promoted the growth of both healthy and *Helminthosporium maydis* infected maize plant, indicating that the isolate was a good candidate to be applied as a biofertilizer (Ke Zhao *et al.,* 2014). *Pseudomonas sp., Serratia marcescens* and *Bacillus cereus* were known to reduce the bacterial wilt caused by *Ralstonia solanacearum* in tomato proved to be effective biocontrol agents (Henok and Kerstin, 2013). In a pot experiment *Pseudomonas striata* when combined with endophytic fungi *Piriformospora indica* increased the dry plant biomass in chick peasand increased the population of P. striata in the rhizosphere (Kamlesh *et al.*, 2010). *Pseudomonas chlororaphis, Bacillus cereus,* and *Pseudomonas fluorescens* inoculated with walnut seedlings improved height, shoot and root dry weight, and increasing P and nitrogen (N) uptake of the seedlings (Xuan *et al.*, 2011). Evaluation of the effect of PSB, with differentiation of, *Bacillus* M-13 and without varying amounts of phosphorus on growth and production of sunflower in agricultural condition gives the highest yield of sun flower seeds (Zehra, 2010). *Bacillus* sp. enhanced the growth and yield of cotton (Qureshi *et al.,* 2012). In addition to the above phosphate solubilising microorganisms facilitate the growth and development of plant in the presence of various stresses. *Pseudomonas* sp. enhanced plant growth in salt stress (Shimaila *et al.,* 2014) and *Pseudomonas putida* (Pandey *et al.,* 2006), *Pseudomonas corrugate* (Trivedi and Sa, 2008), *Mycobacterium* sp. (Egamberdiyeva and Hoflich (2003) are showed to be cold tolerant. *Pseudomonas* sp. (Sandhya *et al.,* 2010), Arthrobacter sp., *Bacillus* sp. (Banerjee *et al.,* 2010) showed tolerant towards drought stress and *Bacillus* and *Hallo Bacillus* towards salinity stress (Dhanushkodi *et al.,* 2013).

Conclusion:

In agricultural practices, chemical phosphate fertilizers are applied to eliminate phosphate deficiency. However, excessive and harmful use of these fertilizers poses a serious threat to microbial diversity, soil microbial community structure, soil fertility and consequently crop productivity in various agricultural systems around the world. Phosphate and Phosphatic fertilizers are expensive in most soil around the world, so the focus is on the use of soil microorganisms rich in phosphate solubilization capacity, which cannot be utilized to deter phosphate from sources not available in the soil. The use of such phosphate solubilizing microorganisms along with bacteria opens up new horizons in terms of better plant production and protects agro-ecosytem from the dangers of agrochemicals. Protecting the soil environment

by using PSB can be a big win for crops grown in decomposing soils. Furthermore the molecular engineering of PSB has provided new insights for the cultivation of crop in P deficient soil.

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