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Agricultural Science

Research and Reviews

Volume III

Editors

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COVID 19: Impact and Response Volume V

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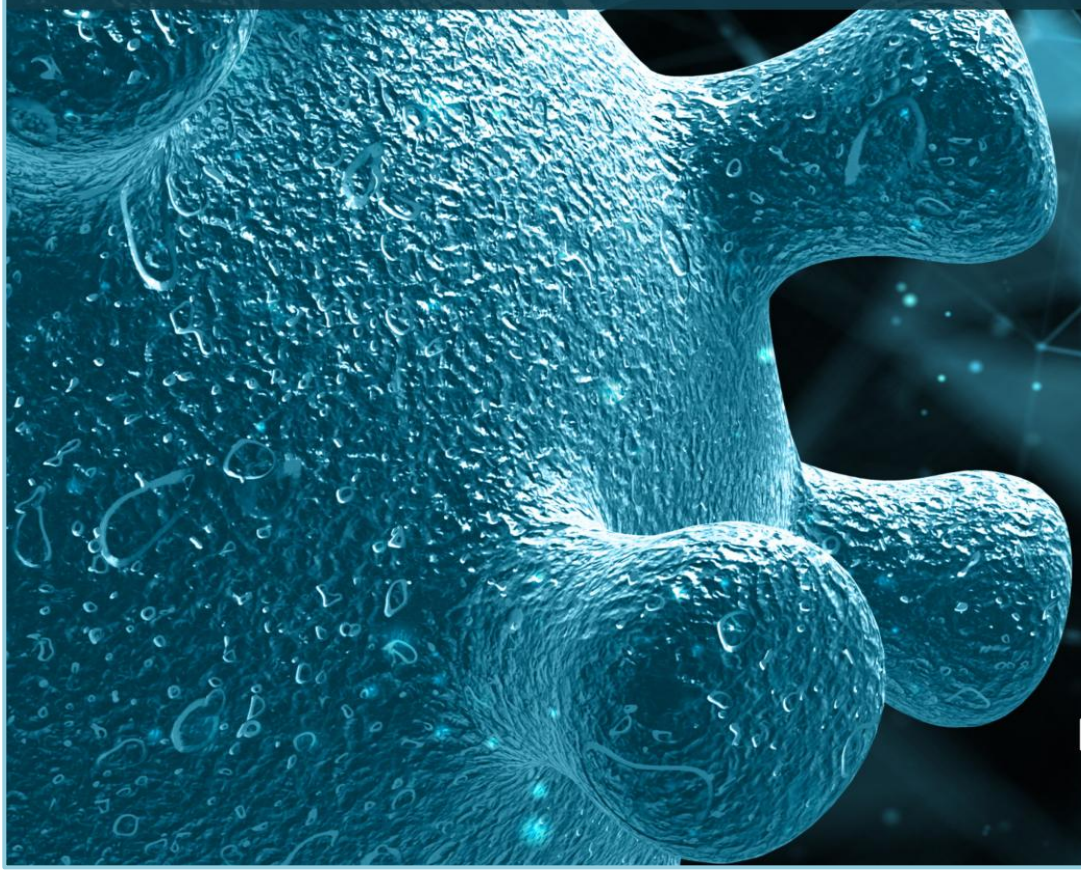

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Advances in Animal Science

Volume I

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MECHANISM OF SURVIVING IN EXTREMES

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Some living organisms enter in low or no physical and physiological activity in order to survive the extreme climatic condition. It is genetically coded when to enter such phase depending upon the signal from environment and the body's biological rhythm**.** Viruses, bacteria, protozoan also use this method for the survival. Viruses remain metabolically inactive for many years, bordering the death -life thin line,viruses such as poxviruses and picornaviruses after entering the host can become latent for long periods of time or even indefinitely until they are externally activated. Every organism has to get fair chance of survival (planet belongs to all), as it is survival of fittest.we intake antibiotic, vaccinesto renderthem unfit for survival, but they have found theway of survival undergoing mutation,having small amount of genome makes it quite easily possible.In higher animals, when the environment becomes too hostile for an animal, they have to find a way to cope. Some animals migrate out of the area, while others enter an inactive state, which they stay in until conditions suit them better. If this inactive state lasts for a long time during the winter, it is called hibernation, but if it is in a hot climate, it is called aestivation

Mechanism in virus and bacteria

Virus latency (or viral latency) is the ability of a pathogenic virus to lay dormant (latent) within a cell, denoted as the lysogenic part of the viral life cycle (Villarreal, 2005). The herpesviruses that infect humans characteristically establish a latent infection that may be reactivated later. The consequences of reactivation range from asymptomatic shedding to severe disseminated infection.Latent herpes simplex virus is known to reside in neurons, Epstein-Barr virus is also known to persist in a non-replicating state as extrachromosomal DNA in B lymphocytes (the immune cells of system) to cause "immortalization" of the infected cell; persistence of the viral genome in epithelial cells may also result in malignant transformation, such as nasopharyngeal carcinoma (Jordan *et al.,* 1984). More serious ramifications of a latent infection could be the possibility of transforming the cell andthereby forcing the cell into uncontrolled cell division. This is a result of the random insertion of the viral genome into the host's own gene and expression of host cellular growth factors for the benefit of the virus.

Thelatency in reservoir of HIV may be the inability of antiretroviral treatment to cure HIV infection (Finzi *et al.,* 1997; Chun and Fauci, 1999; Persaud *et al.,* 2000; Blankson *et al.,* 2002). Viruses show three types of latencies, Episomal latency, Proviral latency, maintaining latency. In the Episomal latency type, viral genes are stabilized, floating in the cytoplasm or nucleus as distinct objects. In the proviral; provirus is a virus genome that is integrated into the DNA of a host cell. The virus gets advantages of proviral latencyas (automatic) host cell division results in replication of the virus's genes and the fact that it is nearly impossible to remove an integrated provirus from an infected cell without killing the cell (Marcello, 2006). Maintaining latency; both proviral and Episomal latency may require maintenance for continued infection and fidelity of viral genes.Thus, viruses have survived from ancient times, many of them have been endogenous inhuman, we have 4-5 percent genome of some viruses included in as human genome.

Many bacteria can survive in the adverse conditions such as extreme temperatures, desiccation, and antibiotics by forming endospores, cysts, or states of reduced metabolic activity lacking specialized cellular structures (Sussman, and Douthit, 1973). Cytoplasmic fluidity and dynamics in bacterial cell dramatically change as cells shift between metabolically active and dormant states in response to fluctuating environment. During dormancy, when such metabolic activities are put on hold, the cytoplasm behaves like a solid glass, 'freezing' subcellular structures in place and perhaps protecting them, while allowing small molecules like metabolites to move freely through the cell, which may be helpful in cells transitioning out of dormancy (Parry, 2014). The species of Gram-positive genera Bacillus, Clostridium, Desulfotomaculum, Sporolactobacillus, and Sarcina form endospores on confronting the adverse environmental conditions, like lack of water or depletion of essential nutrients, and so forth.we are still unable to control tuberculosis as tuberculosis bacillus can remain as spore for hundreds of years and infect the host.Besides endospores, some bacteria develop exospores (e.g., Methylosinus trichosporium) or undergo encystment to form cysts (e.g., the species of genera Methylocystis and Azotobacter). Many species of Azotobacter can withstand drying of the soil for significantly long times without undergoing any structural or physiological change (world encylopedia).

Mecanism in higher animals

Dormancy

Dormancy is the stopping of physical activity by organism also minimizes metabolic activity thereby help an organism to conserve energy. Dormancy is said to be closely associated with environmental conditions. In ‘Predictive’ dormancy the animal enters dormant stage as it

receives presignals from environment stating the arrival of adverse condition, like the decrease in photoperiod, temperature, scarcity of water. Whereas in the 'Consequential'dormancy organisms enter a dormant phase after adverse conditions have arisen, this is a sudden phase where organism unprepared, it can occur due to unpredictable climate changes, this can cause high rate of mortality, but at times it can be beneficial as organism can use maximum resources for survival.Dormancy has become an essential part of the life cycle, allowing an organism to pass through critical environmental stages in its life cycle with a minimal impact on the organism itself. There are different types of dormancytorpor, hibernation, brumation, aestivation and diapause

Torpor

Torpor is a state of lowered body temperature and metabolic activity assumed by many animals in response to adverse environmental conditions, especially cold and heat. The torpid state may last overnight, as in temperate-zone hummingbirds and some insects and reptiles; or it may last for months, in the case of true hibernation

Birds typically do not hibernate, instead utilize torpor. An exceptional bird known as the Poorwill does hibernate (Jaeger, 1948). In 13 bird families known to contain heterothermic species, the common poorwill (Phalaenoptilus nuttallii) is the only species that ostensibly hibernates. Poorwills selected winter roosts that were open to the south or southwest, facilitating passive solar warming in the late afternoon. The findings showed that during winter poorwills exhibit physiological patterns and active rewarming similar to hibernating mammals (Christopher *et al.*, 2019). Many experts believe that the processes of daily torpor and hibernation form a continuum. These are nocturnal members of the nightjar family are the only bird species known to go into a torpor, a similar state to hibernation, during which the animals can bring their body temperature down to 41 degrees.

On nearing of the sun set, hummingbirds increase their feeding in order to prepare themselves for their nighttime sleep. They go into a deep sleep similar to a bear going into hibernation for the winter, but they do it every night this called as daily torpor, torpor (pronounced TOR-per) and it is a state where the bird slows down all of its body functions for the night. The heart rate, body temperature, decreases, they hand upside down. The duration of their torpor varies from 5 to 10 hours. The researchers noted that the longer the birds remained in torpor, the lower their loss of body mass. They fed on stored fat to keep just warm enough to stay alive. This ability makes a hummingbird a "heterotherm," meaning they can switch between being both warm-blooded and cold-blooded (Bob Yirka, 2020) Raccoons and raccoon dogs, at least in parts of their range, also enter torpor for several weeks. Short-term torpor of several days

or even daily torpor is much more widespread also among larger mammals – both American and European badgers enter daily or short-term torpors, with body temperatures of about 28 °C. Daily or short-term torpor, in general, reduces body temperatures to 10–30 °C; metabolic rates are reduced to values of about 30%. Most mammal species entering daily torpor are small and nocturnal such as small marsupials (dasyurids, petaurids, and didelphis), mouse lemurs, hedgehogs, tenrecs, shrews, or bat (Encyclopedia of Ecology, 2008). The extended torpor is hibernation.

Hibernation

Hibernating mammals are good models for investigating the relationship between physiology, behavior, and environment, as hibernation patterns are important determinants of survival (Turbill *et al.,* 2011). Hibernation may be predictive or consequential.This is an energy-saving mode and is characterized by many physiological changes, mainly decreased body temperature, decreased heart rate (by as much as 95 percent), and lower metabolic rate.Animals that hibernate include bats, ground squirrels, other rodents, mouse, the European Hedgehog and other insectivores, monotremes, marsupials and lemurs. The animals turn off its thermostat, the most famous hibernator is bear the temperate species of genus Ursus, experiments conducted on brown bears showed that entry in the den for hibernation was controlled by climate warming climate could result in later den entry.There studies suggested that Scandinavian brown bears terminated their hibernation due to physiological cues (Singh *et al.,* 2016). Although many studies have shown that den entry and exit are related to food availability, but climate change also appears to be an important factor affecting the timing of the life events of the brown bear. In present scenario of climate change there is reduction in hibernation time of black bears, lead to have implications for human–bear conflicts (Pigeon *et al.,* 2016; Krofel *et al.,* 2017). In the colder, northern parts of Alaska, bears hibernate about 7 months of the year. Bears in the warmer, coastal regions of this area hibernate for 2-5 months. Bears have developed unique adaptive strategies in order to survive for so long without food and water (Stenvinkel *et al.,* 2013). They utilse the body fat, especially the brown fat for the maintenance of the body physiology. They also almost donot defecate or urinate, bears have unique ability to recycle urea (aminoacids are recycled again by the body to build up proteins) during hibernation, so also they form anal plug called ‘tappen’or‘Rectal Plug’ to stop defecation and prevent entry of insects during hibernation, however is found that Bears will go through a lot of posture changes where they wake periodically to shift around.It is thought this helps prevent pressure sores from developing. Bears also shift positions to better conserve heat. Bears body temperature only drop

3-5ºC compared to other animals, bears body temperatures drop 32ºC or more. During hibernation a Black bear's heart can drop from 40-50 to 8 beats per minute.The hibernating animals have ability to heal wounds which is a survival advantage in hibernating species, decreased peripheral blood flow during hypothermia and immobilization may impede delivery of oxygen and nutrients to the wound site. Bears in zoos donot hibernate as food is available, though they will slow down and sleep more than usual. Ethically more zoos are allowing their bears hibernate during winterit helps the bears stay leaner and healthier.

Bears mate in the spring, but the fertilized eggs do not immediately begin developing into bear embryo. Instead, the fertilized eggs suspend development until the fall (Embryonic diapause). It is believed that sometime around the beginning of hibernation the eggs implant into the uterine wall. It's counter-intuitive – just as the mother bear stops eating and begins to slow down for hibernation, the eggs within her begin growing into embryos.The cub is born after just a few months of development in the mother's womb. The cubs eyes are closed and can do little movements attaching to the nipple. The tiny bear cubs are just a fraction of one percent the mother bear's weight (Alaska Fish & Wildlife News June 2007 Riley Woodford). Females of some bear species, including American black bears, will enter hibernation for the duration of their pregnancy. Nearly all pregnant Asiatic black bears hibernate as well. The mothers will greatly increase their body mass before hibernating and gestation will occur while they are dormant. The offspring are born either during or shortly after the period of hibernation. The health and weight of the offspring is a direct reflection of how much weight the mother could amass before hibernating.

Marmots hibernate for up to eight months.Four months they are awake having breeding, raising pupsand preparing for the next hibernation. During hibernation they take only 2-3 breaths a minute and their heartbeat slows down from their normal 120 beats to 3-4 beats a minute.

Fat-Tailed Dwarf Lemurs (the noctural animals and highly endangered species) of Madagascarthe coldest time of the year, Lemurs finds a tree and settle there for about seven months until the rains return in November and food is available again, they utilize the fat.

Hedgehogs are some of the deepest hibernators, Hedgehogs are immobile during hibernation they are very vulnerable to climate and disturbance hence, the need for protective hibernacula.

Dormice (*Glis glis*) family Gliridae, French word "dormir," which means to sleep. They can hibernate for more than 11 months at a time, more dormice die in hibernation than at any other time. Dormice that live in temperate climates go through long periods of hibernation lasting six months or more. They make their nests along the forest floor, hidden by logs and piles

of leaves. While analyzing rodent fossils, scientists (funded by the Swiss National Science Foundation) have come up with a novel hypothesis: hibernation was a survival strategy 34 million years ago. Some rodent fossils suggest that hibernation was an established strategy 2.6 million years ago—their incisors show seasonal interruptions in growth. It is thought that this ability to hibernate emerged and spread in response to the difficult survival conditions at the time, which corresponds to the beginning of the Quaternary ice age—the current geological period (Swiss National Science Foundation, 2021). Ancestors of Dormice may have hibernated as early as 34 million years ago.

The Alaskan (Arctic Circle) ground squirrel curls into furry little balls within burrows deep underground, as if dead. The squirrel is as cold as ice — literally. Its body temperature is -2° Celsius (28° Fahrenheit). Its heart beats only once every 15 seconds. Its breathing stops for minutes at a time (Bethany Brookshiresns@sciencenews.org). "These small hibernators can survive having their brains essentially turned off at these low temperatures,"

In hibernating animals male arouse first, they are shown to begin with process of spermatogenesis, making the territory, female arouse latter.

Brumation

In the wild, reptiles are presented with both internal and external cues that it is time to brumate. Herpetologists have classified these cues into two main categories. The first are endogenous cues, which originate within the animal. Theories regarding endogenous cues suggest that some reptiles (but certainly not all) undergo hormonal changes as well as shifts in neurotransmitter levels and amino acid concentrations. These factors are directly affected by circadian rhythms and the environment, making these biological cues little more than a secondary function of natural climatic changes. It is still unknown fact whether internal changes occur spontaneously and trigger brumation or if the animal begins to brumate and then these physiological changes occur (Reptilian Brumation by Jonathan Rheins)

Winter dormancy in reptiles, which is also called brumation, is akin to hibernation in mammals. Instead of experiencing long, sustained periods of inactivity, brumating reptiles occasionally to drink water; however, they may go without food for several months.in cooler winters we in tropical areas also do not find wall lizard (geckos)movements at night.Reptiles can tolerate colder temperatures much better than they can tolerate higher ones. For this reason, during hot weather they must seek refuge underground or in cool, shady places, where they remain physiologically active. Desert reptiles, in particular, exhibit such temperature responses daily. Brumation is the appropriate term used for the period of dormancy that most commonly

occurs in reptiles in the colder months. During brumation; many animals will stop eating, bury them and may or may not intermittently wake to drink. This process is commonly practiced by temperate species (most box turtles species) and is necessary for reproductive health (Boyer and Donal, 2006), brumation typically occurs between the months of October to April in north hemisphere. Brumation behaviour is induced by a reduction in environmental temperatures (by 5-15°C dependingon species) and daylight hours.During brumation, snakes migrate into warmer places such as dens, burrows made by other animals where they will bury themselves or in tree stumps, caves, and deep caverns in the wild. Survival is their main goal during brumation. In the cities and suburbs, snakes crawl into spaces like basements, boiler rooms, garages, woodpiles, open pipes, barns, sheds, storage spaces, or even car engines to keep warm.sub-tropical animals, as well as those found near the equator, often do not undergo what herpetoculturists call a "true brumation."Reproductive Connotations cooler temperatures trigger the production of sperm in males, and prepares females for ovulation in spring, however, if the choice of hibernaculum is poor, they donot emerge in spring, reptile like the hibernators brumate to heal the injury.The length of time a reptile brumate is extremely variable (Reptilian Brumation By Jonathan Rhein).

Aestivation

The phenomenon of laying inactivedue to the summer heat is aestivation. Invertebrate and vertebrate animals are known to enter this state to avoid damage from high temperatures and the risk of desiccation. Both terrestrial and aquatic animals undergo aestivation (Pinder etal1992),by creating burrows. The aestivator depends on burrow buffered soil micronutrients then the upper strata nutrients. Among invertebrates (e.g., earthworms and insects) aestivation usually involves an inactive stage with a water-resistant covering. For example, aestivating earthworms, form a mucus cocoon to resist desiccation, many insect pupae are remarkably resistant to water loss. Amongst vertebrates, fishes, amphibians, and reptiles enter a similar aestivation state. Fishes and amphibians often form a cocoon of dried mucus (e.g., African lungfishes), African and Asian clariid catfishes have long been reputed to survive habitat desiccation by remaining dormant under dry mud or sand, in the manner of the lungfish Protopterids. (Bruton, 1979) or shed epidermal layers (e.g., some desert frogs) to resist epidermal water loss; the cocoon covers the entire body surface except for the nostrils. Reptiles have a relatively water-impermeable epidermisas the skin is has scales, warts and do not need to form a cocoon to reduce evaporative water loss. Aestivating ectotherms typically have an intrinsic metabolic depression for energy conservation (Encyclopedia of Ecology, 2008). The fossil record suggests that aestivation may have evolved several hundred million years ago.

Diapause

Diapauseis an endocrine-mediated metabolic and developmental arrest induced by changes in abiotic cues that indicate the onset of adverse environmental conditions (Yevgeniya *et al.,* 2013). Some species undergo diapause as early embryos; while others enter diapause as pharate first instar larvae ("hatch-ready" larvae) within the eggshell.Diapause in the adult stage is characterized by a halt in reproduction. Adult diapause is especially common in the Coleoptera, but is also well seen among the Lepidoptera, Diptera, Hymenoptera, Hemiptera, Homopteran, Orthoptera and Neuropteran (Denlinger, 2000). Embryonic diapause is defined as the temporary suspension of development of the embryo and occurs in more than 130 mammalian species including the mouse and rat as well as a wide range of species of bears, seals, bats, and marsupials (Fenelon *et al.,* 2014). Embryonic diapause occurs at the blastocyst stage for variable lengths of time.

Hibernation, torpor, and sleep are integral to energy homeostasis.One function of sleep is to restore brain energy homeostasis, while the primary function of hibernation and torpor is to restore or protect somatic energy homeostasis (Drew and Jinka, 2021). Adenosine is unique amongst the putative mediators of torpor because it affects sleep.Adenosine is molecule in the body made from a sugar bound to a nitrogen-based material. It helps the body transport energy in molecules such as ATP. It also promotes sleep in people, In Hindu mythology Kumbhakarn brother of Ravan (a character) in Ramayana, well known to sleeps for 6 months (hypersomnia) probably adenosine must have played role in the sleeping nature of Kumbhakarn. To break sleep the coffee (Caffeine) like substance plays role to block adenosine receptors.Caffeine, an adenosine antagonist, is the most widely used mood-altering drug in the world. Caffeine is rapidly absorbed and distributed throughout the body with peak plasma concentrations typically reached 30–45 min after ingestion. The average half-life of caffeine is 4–6 h. Homeothermslike birds and mammals, are nearly to death in the hibernation, torpor, this is amazing phenomenon, human beings will face cardiac arrest if temperature of body drops to 32 degree or below (hypothermia).

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Volume II

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Dr. Med Ram Verma

Dr. Satyendra Nath

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PREFACE

We are delighted to publish our book entitled ***"Agricultural Science: Research and Reviews Volume II"****. This book is the compilation of esteemed articles of acknowledged experts in the fields of basic and applied agricultural science.*

The Indian as well as world population is ever increasing. Hence, it is imperative to boost up agriculture production. This problem can be turned into opportunity by developing skilled manpower to utilize the available resources for food security. Agricultural research can meet this challenge. New technologies have to be evolved and taken from lab to land for sustained yield. The present book on agriculture is to serve as a source of information covering maximum aspects, which can help understand the topics with eagerness to study further research. 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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CO-EVOLUTION OF PATHOGENS AND PLANTS

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The term coevolution (the influence of closely associated species on each other in their evolution) is used to describe cases where two (or more) species reciprocally affect each other's evolution. Coevolution is likely to happen when different species have close ecological interactions with one another. These ecological relationships include: Predator/prey and parasite/host, Competitive species, Mutualistic species.Pathogens are able to infect a host and, as a result of infection, they causedamage to this host. Woolhouse *et al.* (2002) pointed to three conditions that are required forhost-pathogen coevolution: (1) reciprocal effects of the relevant traits ofthe interaction (e.g., defense and pathogenicity) on the fitness of the twospecies (Le., pathogens and hosts), (2) dependence of the outcome of the host-pathogen interaction on the combinations of host and pathogengenotypes involved, and (3) genetic variation in the relevant host andpathogen traits.Terrestrial plants evolution occurred in the presence of various pathogens.Coevolution is defined as the process of reciprocal adaptation and counter-adaptation between ecologically interacting species (Janzen, 1980). The sophisticated and complex association between plants and pathogens, including bacteria and fungi, have existed since the early stages of life on Earth.The evolution of terrestrial plants from the aquatic environment brought a plethora of challenges.

The evolution of plants has been accompanied by the evolution of beneficial and non-beneficialpathogenic microbes and others, all of which play critical roles in modern plant physiology and developmentAn expanding body of fossil evidence shows the interactions among early terrestrial communities included Virus, bacteria, fungi, algae, lichens, and bryophytes—the ecosystem servicesprovided by these organisms include the weathering of parent rock material, soil formation, stabilization of sediments, and the productivity of ecosystems (Elbert *et al.*, 2012; Edwards *et al.*, 2015)

Pathogenic microbes establish complex and diverse intimate relationships with plant hosts to obtain nutrients required for microbial growth and development, thus causing plant infection and disease (Mendes *et al.,* 2013) Pathogens that cause plant diseases are mainly

microorganisms divided into biotrophs and necrotrophs, which rely on living or dead plant tissue.Over the course of evolution, plants have developed robust immune systemswhich confer the ability to resist pathogen infection. These systems include harnessing beneficial bacteria that can contribute to plant defenses against pathogenic microbes. Plants face a wide variety of organisms that range from being extremely pathogenic to being highly beneficial. Major pathogenic organisms include viruses, bacteria, fungi, nematodes and insect pests that have evolved quite distinct and with specialized strategies for attacking plants.

The virus infection in plant can be symbiotic, increasing survival chances, virus infection may be beneficialfor plants, as shown by an increase of tolerance to abiotic stress in virusinfected plants as compared with uninfected controls (Xu *et al.,* 2008), or by a decreased herbivory on tymovirus-infected Kennedia rubicunda (commonly known as the dusky coral pea, is a species of flowering plant in the family Fabaceae, endemic to Australia) in Australia (Gibbs, 1980). Thus, it is obvious that the effects of virus infection on plant fitness in natural ecosystems may vary largely according tothe specific virus-host interaction, or on other hand viral infection can be destructive it may reduce the competitive ability of the infected plants, a phenomenon (apparent competition) that may also occuramong genotypes of the same species (Pagan *et al.,* 2009). Virus infectionhas also been shown to increase mortality and to reduce fecundity in wildcabbage in southern England (Maskell *et al.,* 1999), and to reduce lifespanof wild pepper in its natural habitat in México (unpublished results)

Bacterial pathogens infect a wide variety of evolutionary distinct hosts, including both lower and higher eukaryotes. In all of these cases, the pathogen must have the ability to recognize, become associated with, exploit the nutrient reserves of, and combat the defense responses of its specific host. To accomplish these tasks, pathogens use an extensive arsenal of virulence-related factors.Plant pathogenic bacteria cause many different kinds of symptoms that include galls and overgrowths, wilts, leaf spots, specks and blights, soft rots, as well as scabs and cankers. In contrast to viruses, which are inside host cells, walled bacteria grow in the spaces between cells and do not invade them. Pathogenic bacteria cause many serious diseases of vegetables. They do not penetrate directly into plant tissue but need to enter through wounds or natural plant openings. Wounds can result from damage by insects, other pathogens, and tools during operations such as pruning and picking. Bacteria and there spores can survive in the soil and crop debris, and in seeds and other plant parts. Weeds can act as reservoirs for bacterial diseases. Bacteria spread in infected seed, propagating material and crop residues, through water splash and wind-driven rain, and on contaminated equipment and workers' hands. Overhead

irrigation favors the spread of bacterial diseases.Bacterial canker (*Clavibacter michiganensis pv. michiganensis*). Seedlings may die and older plants may wilt and die eventually. Older plants have leaves that turn yellow and wilt only on one side Cankers on stems and fruit. Tissue inside stems becomes discolored bacterial soft rot (*Pseudomonas spp.*, Erwinia spp.). Wet, slimy, soft rot that affects any part of vegetable crops including heads, curds, edible roots, stems and leavesand may have a disagreeable odor.Bacterial blight (*Pseudomonas syringae* - various strains) Beet – irregular, round leaf spots with a grey center surrounded by a purple margin. Spring onions/shallots – pale yellow to light-brown lesions with a water-soaked appearance around the margins; outer leaves wither and die and youngest leaf turns lemon to light-green. Leeks – brown streaking on the shank (*Pseudomonas syringae pv*. tomato). Small dark spots surrounded by a yellow halo on leaves; dark raised specks on fruit.Epidemiological studies carried out in the 1970s suggested that clinical isolates of *Pseudomonas aeruginosa* might be capable of causing disease in plants.Bacteriophages, the viruses of bacteria, have received increased research interest in recent years as a realistic environmentally friendly means of controlling bacterial diseases. Their use presents a viable control measure for a number of destructive bacterial crop diseases, with some phage-based products already becoming available on the market.

The other positive side of this coevolution is some plants engage in symbiosis with bacteria called rhizobia that "fix" nitrogen from the atmosphere, making it available to the plant. Rhizobia enable legumes like soybeans and alfalfa to grow without nitrogen fertilizer.Some legume seeds, such as soybeans and peanuts, contain high levels of protein and are among the most important agricultural sources of protein in the world, also acacia species of savannah is using bacteria for nitrogen fixation, no wonder they are surviving in harsh climate of savannah.Soil bacteria, collectively called rhizobia, symbiotically interact with legume roots to form specialized structures called nodules in which nitrogen fixation takes place. This process entails the reduction of atmospheric nitrogen to ammonia by means of the enzyme nitrogenase. Therefore, using rhizobia is a natural and environmentally-friendly way to fertilize plants.

A nutrient depletion zone can develop when there is rapid soil solution uptake, low nutrient concentration, low diffusion rate, or low soil moisture. These conditions are very common; therefore, most plants rely on fungi to facilitate the uptake of minerals from the soil. Mycorrhizae, known as root fungi, form symbiotic associations with plant roots. In these associations, the fungi are actually integrated into the physical structure of the root. The fungi colonize the living root tissue during active plant growth. Mycorrhization, the plant obtains phosphate and other minerals, such as zinc and copper, from the soil. The fungus obtains

nutrients, such as sugars, from the plant root. Mycorrhizae help increase the surface area of the plant root system because hyphae, which are narrow, can spread beyond the nutrient depletion zone. Hyphae are long extensions of the fungus, which can grow into small soil pores that allow access to phosphorus otherwise unavailable to the plant. Mycorrhizae function as a physical barrier to pathogens. They also provide an induction of generalized host defense mechanisms, which sometimes involves the production of antibiotic compounds by the fungi. Fungi have also been found to have a protective role for plants rooted in soils with high metal concentrations, such as acidic and contaminated soils.There are two types of mycorrhizae: ectomycorrhiza and endomycorrhiza. Ectomycorrhiza form an extensive dense sheath around the roots, called a mantle. Hyphae from the fungi extend from the mantle into the soil, which increases the surface area for water and mineral absorption. This type of mycorrhizae is found in forest trees, especially conifers, birches, and oaks. Endomycorrhiza, also called arbuscular mycorrhizae, do not form a dense sheath over the root. Instead, the fungal mycelium is embedded within the root tissue. Endomycorrhiza are found in the roots of more than 80 percent of terrestrial plants.

Pests are programmed to recognize and rapidly respond to patterns of host cues.The phytophagous insects that exist today and the plants they feed on are the product of a coevolutionary process that has been ongoing for 400 million years (Labandeira, 2013). Studies of fossil plant–insect associations suggest that insects have been feeding on plants for 400 million years.Phytophagous insects due to their role as pests in agricultural ecosystem have at large and negative impact on food security for humanity and related species (Bruce, 2010). They not only damage agricultural crops but also medicinal and aromatic plants. The grubs of the three species of scarab beetles that damaged rose plants in Assam attacked the root system of the plant. The rose plant has high economic importance in making rose water, aromatic compounds, cosmetics, medicines. The adult beetles were positively phototactic which came out at night and fed on the leaves of rose plant by making some holes and the severe infestation led to complete plant defoliation. Even the plant like Neem was attacked by *Parasa hilaris* (Suresh, 1992). There are 13 pests that attacked neem in southern Tamil Nadu. Tulsi, *Ocimum sanctum* were seriously attacked by sucking insect pests like lace wing *Cochlochila bullita* (Stal.) (Verma, 2006) and Aphids which are very common and kill the plants. Twelve species of aphids were found to cause considerable damage to medicinal and aromatic plants in Chikkamagaluru district, Karnataka. 26 species phytophagous arthropods were reported on ashwagandha. This is major threat to Ayurveda medicine manufacturer, learning from the above literature, the coevolution so strong that, they are going along together for ages.

Coevolution entails the rise of new alleles, by mutation or migration, and the fixing in the population (Woolhouse *et al.,* 2002). Two models have described the dynamics of the coevolution process. The first, the Red Queen hypothesis, is synthetized as 'running as fast as you can to stay in the same place'. It posits that for a given species adaptation increases the fitness against another interacting species, but at the same time such adaptation of the first necessarily causes a decline in fitness of the second species (Rausher 2001; Woolhouse *et al.,* 2002; Paterson *et al.,* 2010). Red Queen metaphor became central in the description of continuous race in the process of evolutionary adaptation to prevent extinction (Antonovics *et al.,* 2011; Jensen *et al.,* 2012; Nemri *et al.,* 2012). Such coevolutionary interactions give rise to continual natural selection for adaptation and counteradaptation in interacting species.The second evolutionary hypothesis of coevolution is known as the 'Arms Race model', here the Coevolutionary dynamics are described as a continuous escalation of defenses and counter-defenses gained with new genetic traits that can be fixed in the population through a slow process. In such a model, genetic improvements are accumulated in both populations. The natural selection of new genetic traits is a biological phenomenon.

Plants also have had to defend themselves against insect attack. Being rooted to the ground they are unable to run away from attacking herbivores. They have evolved a wide range of sophisticated defense systems to protect their tissues (De Moraes *et al.,* 2001; Kessler and Baldwin, 2001; Ballare, 2011). These include toxic or anti-feedant secondary metabolites that represent a major barrier to herbivory (Harborne, 1993; Mithoefer and Boland, 2012), and physical defenses such as lignin (Franceschi *et al.,* 2005). These provide direct defense via toxic, anti-nutritive or repellent effects on herbivores. The capsaicin from chills, caffeine from coffee, tannin from tea, gossypol of cotton plant, but human is clever pest like other animals for plant he thrives and relishes on these products and he knows to use them, thus started cultivation of this plants in large scale which is positive side because which the plant species flourishedthis can be marked as coevolution.

Plant defenses can be classified into resistance against herbivore, tolerance to herbivore, phenological escape from herbivore and overcompensation (Agrawal 2000). Classic example of coevolution is of *Acacia nigrescens* (nick name Devil thorn) and Giraffe, Giraffes have a fondness for the tree that is unrivalled by any other because it leaves (umbrella canopy) are at great height from ground, and this fondness results in a fascinating 'to and fro' relationship between fauna and flora. Over time, the acacia tree has developed several clever defense mechanisms to prevent giraffes from munching on them. The giraffe's tongue is about 45cm in length and highly prehensile. This allows the animal to successfully negotiate the bigger thorns

and pull the leaves from the branch. But the acacia trees have the developed a further defense – the release of tannins, thereby acacia trees within 50 yards react to the release of the tannin by their neighbour and start by emitting their own. Thus, making Giraffe to stay away from excessive eating, but at the some instances this plant needs Giraffe during flowering season, as they bring pollination of flowers, As giraffes move from treetop to treetop, pollen gets stuck on their heads and necks, and is transferred between trees, aiding in pollination, but they also eat lot of flowers, this sets classy example of coevolution.

Similar are insect pollinators having coevolution with plants, the grand example is honey bees despite human being using the pesticide they are stillsurviving, the reason is the phenomenon of haplodiploidy, queen mates with several drones who are sons of different queens who are healthy biotypes withstanding the chemical (pesticide) attacks on them. As the workers and queen as diploid developed by fertilization of ova of queen the sperm from drone , whereas the drone are haploid, with set of chromosome from mother. During her mating flights, early in life, she will mate with many drones. Since these drones have various genetic traits, they offer a genetic diversity that will serve the queen and her offspring well. Her mating flights, across a few days, will result in collecting sperm from 10-25 drones. She will store this sperm in her spermatheca for many years. This protects the population from undergoing devastation due to any calamity. If all the honey bees die the pollinating plants will perish in no time. Flowers too love the presence of honey bees, thus attract them by vibrant colors and the scent.

A case study of coevolution: squirrels, birds, and the pinecones, where squirrels are the main seed predator, trees should have stronger defenses against squirrel predation, and where birds are the main seed predator; trees should have stronger defenses against bird predation. This turns out to be true. Where there are squirrels, the pinecones are heavier with fewer seeds, but have thinner scales, like the pinecone on the left. Where there are only birdcrossbills, pinecones are lighter with more seeds, but have thick scales, If the crossbills have evolved in response to the pine trees, there is geographic differences in birds: where the pinecones have thick scales, birds should have deeper, less curved bills than where the pinecones have thin scales,So we have evidence that the trees have adapted to the birds (and the squirrels) and that the birds have adapted to the trees. It is easy to see why this is called a coevolutionary arms race: it seems possible for the evolutionary "one-upping" to go on and on even thicker-scaled pinecones are favored by natural selection, which causes deeper-billed birds to be favored, which causes even thicker-scaled pinecones to be favored (Benkman, 2010).

Many fruit-eating birds, especially in tropical rain forests are coevolving with the plants whose fruits they eat. The birds get nourishment, and in the process the plants get their digestion-resistant seeds dispersed by regurgitation or along with the birds' droppings. Many characteristics of the plants have evolved to facilitate dispersal and the behavior and diets of the birds have responded to those changes. In particular, the plants have evolved conspicuously colored, relatively odorless fleshy fruits to attract the avian dispersers of their seeds. They are coevolving in response to the finely honed visual systems of the birds; plant species coevolving with color-blind mammalian seed-dispersers have, in contrast, dull-colored but smelly fruits. The bird-dispersed plants often have evolved fruits with giant seeds covered by a thin, highly nutritious layer of flesh. This forces the bird to swallow the fruit whole, since it is difficult or impossible just to nip off the flesh. In response, birds that are specialized frugivores (that is, that do not take other kinds of food) have evolved both bills with wide gapes (so they can swallow the fruit whole) and digestive tracts that can rapidly dissolve the flesh from the large impervious seed, which then can be regurgitated (Ehrlich *et al.,* 1988.).

There many seeds from plant which require wash of animal digestive enzyme on them for germination process, the extinction of ‘Dodo’ bird, an endemic sapotaceous tree Calvaria major found on the island of Mauritius is nearly extinct because its seeds apparently required passage through the digestive tract of the now-extinct dodo (*Raphus cucullatus)* to overcome persistent seed coat dormancy caused by a specially thickened endocarp.Coevolution is complex web , yet beautiful creation of nature it has to sustain for benefitting of flora and fauna.

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PREFACE

The new respiratory pandemic disease i.e. COVID-19 has caused disruptions in the lives and customs of people with significant impact on the economies of nations. The outbreak of the disease is a global health emergency and of international interest. This global health challenge leads to the infection, morbidity and mortality of many people.

In the weeks since the World Health Organization manifest the corona virus (COVID – 19) episode a worldwide unstipulated wellbeing crisis, the COVID-19 pandemic has influenced 212 nations and forfeit increasingly than 400,000 lives. Still today there is no successful remedy to lockup the spreading of this infection. The pandemic is developing prior disparities, uncovering vulnerabilities in social, political and financial frameworks which are thusly intensifying the effects of the pandemic.

Governments of various nations adopted restrictive measures involving both within the countries and at international borders as effective response to the corona virus pandemic. These measures includes confinements of workers and order to work from home, banning of social and religious gatherings, closure of market places, closure of workplaces including airports, building or creation of testing and isolation centers, quarantining/isolation of suspected persons, self-imposed isolations, and the use of face masks whether surgical or cloth type in situations where there is a cogent reason to defy the restriction.

Academic communities were not left out as institutions of learning were requested to close in many countries since it is very easy to spread the virus among students and youths in tertiary institutions where socialization is an essential part of their lives.

To address the various issues related with the COVID – 19 we have published the present book. The interdisciplinary approach of the book will make the book useful and informative to the students, teachers, researchers, scientists and policy makers in India and abroad.

We thank all contributors, publishers and all our well-wishers for their blessings, without which this book would not have come into existence.

- Editorial Team

COVID 19: Impact and Response Volume IV

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TRACKING THE CORONA VIRUS AND OUT BREAK

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A study has reported that many viruses that exist today have very ancient evolutionary histories dated back to the first vertebrate or even the first prokaryote that existed, there are still many millions more viruses still to be discovered. The word ***krimi*** is used in Vedas more than 5000 years ago for different macroscopic and microscopic creatures. Two types of ***krimi*** viz. *Drishta* (Visible/Macroscopic) and *Adrishta/ aatisukshma* (Invisible / Microscopic) were described in Vedas. There are Hymns which describe *krimi* and diseases caused by them. Over 60% of Atharva Veda is devoted to Ayurveda, charak samhita describing the ways to combat epidemic. Various sages like Āngirasas, Sāmbu, Jamadagni, Kaṇva and Kaśyapa were well known for their expertise in discovering and recognizing new herbs for remedial purposes. The Charak Samhita's explains, *Sleshma Krimi* after settling in the respiratory system creates cough, severe congestion, and breathlessness and sometimes leads to death. Every description of disease and the medicines is written in texts of different religions but we are unable to decipher it.

Viruses are named based on their genetic structure to facilitate the development of diagnostic tests, vaccines and medicines. Virologists and the wider scientific community do this work, so viruses are named by the "International Committee on Taxonomy of Viruses" (ICTV). Scientists first identified a human corona virus in 1965, it caused a common cold. Later that decade, researchers found a group of similar human and animal viruses and named them after their crown-like appearance. Corona viruses are a big family of different viruses. Some of them cause the common cold in people and infect animals, including bats, camels, and cattle. SARS-CoV-2, the new corona virus that causes COVID-19, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It was first isolated from three people with pneumonia connected to the cluster of acute respiratory illness cases in Wuhan. All structural features of the novel SARS-CoV-2 virus particle occur in related coronaviruses in nature.

Seven corona viruses can infect humans. Studies have suggested that bats are the natural reservoir of a range of corona viruses (CoVs), and that Rhinolophid bats harbour viruses closely related to the severe acute respiratory syndrome (SARS) CoV, which caused an outbreak of respiratory illness in humans during 2002–2003 (Jie Cui, Naijian Han 2007). SARS emerged in

southern China in 2002 and quickly spread to 28 other countries. More than 8,000 people were infected by July 2003, of which 774 died. A small outbreak in 2004 showed only four cases. This corona virus causes fever, headache, and respiratory problems such as cough and shortness of breath. Studies in species other than bats have examined host-virus phylogeny and identified coevolutionary relationships (Lukashov and Goudsmit, 2001). There is also study of Host-pathogen divergence and host shifts in the recent evolutionary history of these viruses and their hosts. Other studies have demonstrated that the relationship between viral phylogeny and geographic location and identification of host's viral phylogeography (Holmes *et al.,* 2004) can yield information on the origin of emerging zoonoses (Chen *et al.*, 2006).

MERS started in Saudi Arabia in 2012,(Middle East respiratory syndrome coronavirus, or MERS-CoV) nearly 2,500 cases reported who live in or travel to the Middle East. This corona virus is less contagious than its SARS cousin but is more deadly, killing 858 people. It has the same respiratory symptoms, but can also cause kidney failure.

Learning through above literature this virus's family already existed and have harmed human species. There is host shift phenomenon. After reading the literature one can see the years of outbreak 2002, 2012, 2019-20 almost after gap of a decade their sudden upsurge, this shows the cyclic pattern.

The controversy is whether it was a part of the research work in Wuhan lab or had come from wet market of Wuhan.The investigations are at international level. The infection was quick spreading because the virus can spread from an infected person's mouth or nose in small liquid particles when they cough, sneeze, speak, sing or breathe. These particles range from larger respiratory droplets to smaller aerosols. People get infected by breathing in the virus if they are near someone who has COVID-19, or by touching a contaminated, (through fomite), surface and then your eyes, nose or mouth. The virus spreads more easily indoors , in crowded settings, poor ventilated area. A recent review of the survival of human corona viruses on surfaces found large variability, ranging from 2 hours to 9 days.The survival time depends on a number of factors, including the type of surface, temperature, relative humidity and specific strain of the virus.Laboratory study showed that the coronavirus SARS-CoV-2 can persist on plastic and stainless steel for days (Van Doramalen *et al.,* 2020). Reports stated that Worker's spray disinfectant on a street in Shijiazhuang, China, in January 2020 (China News).

Goldman, a microbiologist at Rutgers New Jersey Medical School in Newark, took closer look at the evidence around fomites and found that there was little to support the idea that SARS-CoV-2 passes from one person to another through contaminated surfaces. The Lancet Infectious

Diseases in July, states that surfaces presented relatively little risk of transmitting the virus 2 (Goldman, 2020). Similar conclusions were drawn by other they seem to hold true, because during lock down from March 2020 to August 2020, with limited period of marketing essential commodities people hardly wore gloves the currency transaction was by hands, touching mobile screen, other goods. People did extensively use hand sanitisers.

In fact, the US Centres for Disease Control and Prevention (CDC) clarified its guidance about surface transmission in month of May, stating that this route is "not thought to be the main way the virus spreads". It now states that transmission through surfaces is "not thought to be a common way that COVID-19 spreads". The focus on fomites rather than aerosols emerged at the very beginning of the coronavirus outbreak because of what people knew about other infectious diseases. In hospitals, pathogens such as methicillin-resistant Staphylococcus aureus, respiratory syncytial virus and norovirus can cling to bed rails or hitch a ride from one person to the next on a doctor's stethoscope. So as soon as people started falling ill from the coronavirus, researchers began swabbing hospital rooms and quarantine facilities for places the virus could be lurking, seemed to be everywhere.

In medical facilities, personal items such as reading glasses and water bottles tested positive for traces of viral RNA the main way that researchers identify viral contamination. Bed rails and air vents. In quarantined households, wash basins and showers harboured the RNA, and in restaurants, wooden chopsticks were found to be contaminated. An early study suggested that contamination could linger for weeks. Seventeen days after the Diamond Princess cruise ship was vacated, scientists found viral RNA on surfaces in cabins of the 712 passengers and crew members who tested positive for COVID-19.Surface transmission, although possible, is not thought to be a significant risk (Morb *et al.,* 2020).

How did the virus create pandemic situation? The mystery remains unsolved till date, Was it planned by some country as biological means of warfare, or to create economic collapse, whatever may be reason it actually broke down bones of even superpower nation. There is huge loss of human life and aftermath disaster. There are theories of virus from Wuhan virology centre, the theory is not yet proved. China being strong communist dictator ruled country nothing can be found out; they keep everything in tight secret.World Scientists donot have enough evidence about the origins of SARS-CoV-2 to rule out the lab-leak hypothesis, or to prove the alternative that the virus has a natural origin. Scientists found SARS-CoV-2's closest known relative, RATG13, in a horseshoe bat *(rhinolophus affinis*). SARS-CoV-2's closest relative still has not been found in an animal. Another suggests it is no coincidence that COVID-19 was first detected in Wuhan, where a top lab studying coronaviruses, the WIV, is located, lab-leak

proponents contend that the virus contains unusual features and genetic sequences signalling that it was engineered by humans.

Several researchers have looked into whether features of SARS-CoV-2 signal that it was bioengineered. One of the first teams to do so, led by Kristian Andersen, a virologist at Scripps Research in La Jolla, California, determined that this was "improbable" for a few reasons, including a lack of signatures of genetic manipulation. The virus's furin cleavage site a feature that helps it to enter cells is evidence of engineering, because SARS-CoV-2 has these sites but its closest relative's do not. The furin cleavage site is important because it is in the virus's spike protein, and cleavage of the protein at that site is necessary for the virus to infect cells. Another feature of SARS-CoV-2 that has drawn attention is a combination of nucleotides that underlie a segment of the furin cleavage site: CGG (these encode the amino acid arginine). A Medium article that speculates on a lab origin for SARS-CoV-2 quotes David Baltimore, a Nobel laureate and professor emeritus at the California Institute of Technology in Pasadena, as saying that virus's donot usually have that particular code for arginine, but humans often do a "smoking gun", hinting that researcher might have tampered with SARS-CoV-2's genome.

Virologist Andersen however has ruled out that, in SARS-CoV-2, about 3% of the nucleotides encoding arginine are CGG, further points out that around 5% of those encoding arginine in the virus that caused the original SARS epidemic are CGG, too. Baltimore agreed with - Andersen could be correct that evolution produced SARS-CoV-2, but adds that "there are other possibilities and they need careful consideration. The highly transmissible variant of SARS-CoV-2 first reported in India (B.1.617.2, or Delta) has mutations in the nucleotides encoding its furin cleavage site that appear to make the virus better at infecting cells (Peacock *et al.,* 2021).

During the WHO-led investigations origins probe earlier this year, WIV researchers told investigators that they cultured only three coronaviruses at the lab, and none were closely related to SARS-CoV-2. when researchers want to study or genetically alter viruses, they need to keep them (or synthetic mimics of them) alive, by finding the appropriate live animal cells for the viruses to inhabit in the lab, which can be a challenge.

Did coronavirus emerge from Bat food products- Since ancient times, people all over the world have eaten bats . Bats have also been used in traditional medicine. Bat meat consumption is most common in parts of Asia and the Pacific Islands. It is been confirmed that the outbreak stemmed from a wholesale meat market in Wuhan. The virus was found in the area where live animals were kept, according to a 2020 (from the journal Microbiology Australia). The outbreak

clearly began epidemiologically at the Wuhan market, and a number of environmental samples from around the live animal section of the market were subsequently found to be positive for SARS-CoV-2(World Health Organization (2020) Novel coronavirus (2019-nCoV) (Situation Report February, 2020; Chinese Centre for Disease Control and Prevention, 2020). China's CDC detects a large number of new coronaviruses in the South China seafood market in Wuhan. (chinacdc.cn). Bats are the reservoir hosts of a number of additional novel coronaviruses, particularly Chinese horseshoe bats, and a number of these novel coronaviruses can efficiently use multiple orthologs of the SARS receptor, human ACE2, and replicate efficiently in primary human airway cells and achieve in vitro titres equivalent to epidemic strains of SARS-CoV (Menachery *et al.*, 2015)

Whatever may be the reason of out break of covid-19 -BUT

The economy slashed down at various sectors in all the countries. There was upsurge or rise in new industries like mask manufacturing companies, PPE kit, sanitizer manufacturer, oxygen suppliers, ventilator manufacturers, syringes manufacturing , pharma industry sector gained a lot ,the paramedical staff demand increased like the CTscan, pet scan, X-ray, also medical staff was at toes.Thus this outbreak gave rise to new industries. India is using different fashioned mask irrelevant of its capacity to save from viral infection. This new upcoming trend in fashion industry of wearing matching masks as per the costume and moreover it is flourishing. Disinfectant factories worked around the clock to keep up with heavy demand. Thus the governments, companies and individuals continue to invest vast amounts of time and money in deep-cleaning efforts. By the end of 2020, global sales of surface disinfectant totalled US$4.5 billion.

The loss of human life is huge, the cities, towns, village looked dead in the entire lock down. It was difficult to carry on with the life being caged in house like animals in zoos. There was upsurge in domestic violence cases all over the world. Keeping self in locked conditioned for months was creating unsound mental health, thuscreating awar situation.Collapse in education sector till date, extensive use of online media, mobile phone, laptops. There is rise in the sale of the electronic gadgets like mobile, PC, head phone etc., tourism and hotel industry collapsed, till date they are unable to recop. Huge loss in rural sector industry of small venders, and manufacturers. Outdoor activity stopped, public relation, physical meeting collapsed. Since physical meeting have collapsed difficult to get understand body language, gesture which human psychology. The children born in lock down were exposed to only parents, now when see many people around they are confused, unsecured and cry aloud seen the crowd.

Positive side of this maasive impact is that people realised importance of healthy life style, doing exercise, yoga, consuming homemade food, new recipes invented, they started realizing importance of relationship, youth became aware of house hold cores.

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PREFACE

We are delighted to publish our book entitled ***"Advances in Animal Science Volume II"****. This book is the compilation of esteemed articles of acknowledged experts in the fields of basic and applied animal science.*

This book is published in the hopes of sharing the excitement found in the study of animal science. Animal 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, lndia 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.

- Editors

Advances in Animal Science Volume II

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UNDERSTANDING GENETIC

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Genetic is the study of transmission of hereditary characters from parents to offspring's, the physical, morphological characters (traits) observed in organisms called as phenotype. The traits are coded on genes present on DNA segments, whereas RNA fragments in lower prokaryotes called as genotypes. Different researchers have given definition for the genetics. Genetics is a field of science that includes the study of inheritance and genetic variations by investigating the DNA, genes, genome, chromosome and other components of it. The phenomenon of inheritance was first explained by Gregor Johann Mendel during the late 18's. As per Mendel's finding, "the traits inherited from parents to their offspring's." Some traits are physical while some are biological. What he called a trait is now known as genes; genes pass down to consecutive generations. The phenotypes of an individual maybe different then the genotype, expression of phenotypic characters depend upon the expression of dominant and recessive genes present in the individual. There are various branches of genetic-Human genetics, Clinical genetics, Population genetics, Molecular genetics, Cytogenetics, Preimplantation genetics, Plant genetics, Microbial genetics, archaeogenetic are some areas.

Human Genetic:

Gotra system of Hinduism to prevent inbreeding

Hereditary characters are traits expressed in the family from great ancestors, in Hinduism it called as *Gotra system ("the word gotra denotes the progeny (of a sage) beginning with the son's son). The marriages from same gotra are prohibited as they have same (mythological) ancestry*, People within the gotra are regarded as siblings and marrying such a person can lead to higher chances for the child to get genetically transferred diseases or weak genes or accumulation of recessive genes. Reason behind this practice (not marrying in same gotra) is the "Y" Chromosome which is expected to be common among all male in same gotra. So, the woman too carries similar X Chromosome and if married, their offspring may be born with birth defects, in almost all Hindu families, marriage within the same gotra is not practiced, thus to prevent inbreeding and completely eliminate all recessive defective genes from the human DNA. The ancestry is tracked from great sages, it is basically lineage of "Y" chromosome, because it is

standalone chromosome which does not have crossing over, gene exchange, thus passes the genes from father to son to grandson to great grandson and so on (so paternal lineage is considered). Gotra system also protects the Y chromosome, also diluting the frequency of defective genes or eliminating them. The gotras are named as Kashyap, Gautam, Shandilya, Angirasa, Atri, Bhrigu, Vasishta, Kutsa, Bharadwaj are some of the names. Typically, genes from the mother and father are shuffled or "cross over" to produce a genetic combination unique to each offspring. But the Y chromosome does not undergo crossing over, and, as a result, its genes tend to degenerate, while repetitive DNA sequences accumulate.Y chromosomes are highly dynamic and have mechanisms to acquire and maintain genes," says Amanda Larracuente, an assistant professor of biology at Rochester.

With a 30% difference between humans and chimpanzees, the Y chromosome is one of the fastest-evolving parts of the human genome (Wade, 2010). The Y chromosome was identified as a sex-determining chromosome by Nettie Stevens at Bryn Mawr College in 1905 during a study of the mealworm (class Insecta) *Tenebrio molitor*. Stevens proposed that chromosomes always existed in pairs and that the Y chromosome was the pair of the X chromosome discovered in 1890 by Hermann Henking.Stevens named the chromosome "Y" simply to follow on fromHenking's "X" alphabetically derived (Bainbridge, 2003; Schwartz, 2009).

The idea that the Y chromosome was named after its similarity in appearance to the letter "Y" is mistaken. All chromosomes normally appear as an amorphous blob under the microscope and only take on a well-defined shape during mitosis. This shape is vaguely X-shaped for all chromosomes. It is entirely coincidental that the Y chromosome, during mitosis, has two very short branches which can look merged under the microscope and appear as the descender of a Y-shape.

The genes on the Y chromosome cannot undergo genetic recombination, the "shuffling" of genes that occurs in each generation which helps to eliminate damaging gene mutations. Deprived of the benefits of recombination, Y chromosomal genes degenerate over time and are eventually lost from the genome. Thus few functional gene are present on y chromosome like, TDS testis determining factor, sex-determining region SRY, hypertrichosis, Y-chromosome-linked diseases are rare.

Animal behaviour to prevent inbreeding

Even the animals do not allow inbreeding between brothers and sister. In elephants the male calf is distanced away from the matriarchal herd ones he attains sexual maturity. The entire

heard is of female and males are solitary. Same is the case with the Lions pride. This means it's genetically coded to follow this phenomenon which is passed over from millions of years. This behavior brings genetic variations and saves the herd from deleterious effects, thus the survival of species is benefitted. This is subject of evolutionary genetics. Better progeny is reproduced from out breeding, also called as out breeding vigor, this brings new variations have better chances of survival, also diluting the recessive genes ,which is observed in nature, whereas, inbreeding has deleterious effects with expression of recessive and weak genes (alleles from same ancestors), in breeding also creates depression termed as 'inbreeding depression'.

Human intervened in breeding of some domesticated species.People were improving plant crops and domesticated animals by selecting desirable traits from individuals for inbreeding, selective breeding. The example milk gene trait in cattle, muscle trait for beef production, egg laying in white leghorns, fast development of broiler chicken (white leghorn variety bred for meat), which is doneby using biological skills, sheep are careful selected to produce more wool, in vitro, in vivo fertilization. Systematic inbreeding and maintenance of inbred strains of laboratory mice and rats is of great importance for biomedical research. The inbreeding guarantees a consistent and uniform animal model for experimental purposes and enables genetic studies in congenic and knock-out animals. Inbreeding is generally deleterious, even in flowering plants. Inbreeding is generally deleterious, even in flowering plants. Since inbreeding raises the risk that bad copies of a gene will be expressed, inbred progeny suffers from reduced viability.

Care taken by lower organisms to save the progeny

Several organisms perform binary fission. Bacteria, for instance, use it as a way to reproduce. Bacterial fission entails chromosomal replication, chromosomal segregation, and cell splitting**.** The protozoans like amoeba, paramecium, euglena etc., undergo asexual reproduction as well as sexual reproduction. When conditions are optimal and favorable, they use asexual mode of reproduction called binary fission it can be called as cloning. The word asexual describes a reproduction that occurs without involving sex cells (gametes). Instead, the somatic cells undergo an asexual process that will produce a clone of the parent. So that large number of progenies is produced, latter in unfavorable conditions some of them can survive. These organisms do undergo sexual reproduction, Woodruff (1907 published in 1929) claim of keeping paramecium healthy for 22,000 generations without conjugation (sexual reproduction). If binary fission continues repeatedly for a longer period of time, Paramecium loses its vigor andare physiologically depressed, reduces in size, ceases to multiply, degenerates in the organization, and eventually die, but the clone can be rejuvenated to regain its former vigor by nuclear

arrangement, this is brought about by conjugation. Thus, conjugation is essential for continued binary fission.In species of *Paramecium tetraurelia*, the asexual line of clonally aging Paramecia loses vitality and expires after about 200 fissions if the cells fail to undergo autogamy or conjugation. Experiments by Smith-Sonneborn, Holmes and Holmes and Gilley and Blackburn demonstrated that, during clonal aging, DNA damage increases dramatically. When clonally aged P. *tetraurelia* are stimulated to undergo meiosis in association with either conjugation or automixis, the genetic descendants are rejuvenated, and are able to have many more mitotic binary fission divisions.

Therefore the primitive way asexual reproduction advanced to sexual reproduction, causing the genetic material to reshuffle by mechanism of crossing over in gametogenesis in higher organisms, so that they get fair chance of survival.

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Volume II

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Volume II

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PREFACE

Nanotechnology is one of the most promising technologies of the twenty-first century. Nanotechnology is described as the design, development, and implementation of materials and technologies on the nanoscale with the smallest functional components (1 to 100 nm). Nanotechnology covers a wide range of issues, from standard device physics and chemistry extensions to entirely new techniques based on molecular self-assembly, from developing new Nano size materials to investigating whether we can directly alter matter at the atomic scale level.

Nanotechnology can be used in a variety of fields, including medical, agriculture, and environmental protection. Many diseases for which there are presently no treatments may be treated in the future as a result of nanotechnology. The use of nanotechnology in medical therapy needs a careful examination of its risks and potential side effects. Even scientists who oppose the use of nanotechnology agree that advancement in the field should continue since it offers enormous benefits, but more testing is needed to ensure its safety in people. Nano medicine may play a key role in the treatment of human and plant disorders, as well as the enhancement of normal human and plant physiology and systems, in the future.

Nanoscience and nanotechnology are the study and application of extremely small objects, with applications in chemistry, biology, physics, materials science, and engineering, among other fields. Nanotechnology is being used in a range of energy-related applications, including increasing the efficiency and cost-effectiveness of solar panels, producing new types of batteries, boosting fuel production efficiency through better catalysis, and building better lighting systems. Nano science and nanotechnology applications in engineering connect academic research in Nano science and nanotechnology to industry and everyday life. As a result, a diverse range of nanomaterials, nano devices, and nano systems have been developed and deployed for human benefit in a number of technical applications.

Nanoscience and Nanotechnology in Engineering is based on the authors' numerous lectures and courses given all over the world. Nanotechnology has also helped to design more efficient and long-lasting materials, such as self-cleaning and self-repairing concrete and windows. Coatings based on nanotechnology can help with fire protection, corrosion resistance, insulation, and a range of other applications. All scientists, academicians, researchers, and students working in the fields of chemistry, biology, physics, materials science, and engineering, among other fields, will find this book quite valuable.

This book with valuable book chapters from eminent scientists, academicians, and researchers will surely be a part of utmost information for the coming new research taken by the researchers in the field of chemistry, biology, physics, materials science, and engineering, among other subjects.

ABOUT THE BOOK

As scientists endeavour to comprehend the mechanisms of natural and biomolecular computing, Nano scale science and computing is becoming a key research subject. The architecture and design of molecular self-assembly, nanostructures, and molecular devices, as well as understanding and harnessing the computational processes of biomolecules in nature, are all topics in this discipline.

This book provides a unique and authoritative view of contemporary Nano scale science, engineering, and computing research. The book is appropriate for academic and industrial scientists and engineers working in Nano scale science, particularly those interested in molecular level computing.

Nano science and nanotechnology are the study and application of extremely small objects, and they can be applied in chemistry, biology, physics, materials science, and engineering, among other subjects. Nanotechnology is being employed in a variety of energy-related applications, including improving the efficiency and cost-effectiveness of solar panels, developing new types of batteries, improving the efficiency of fuel production through better catalysis, and developing better lighting systems. Engineering's application of Nano science and nanotechnology connects academic research in Nano science and nanotechnology to industry and everyday life. As a result, a wide range of nanomaterial's, Nano devices, and Nano systems for a variety of technical applications have been produced and deployed for human benefit. Nano science and Nanotechnology in Engineering is based on the many lectures and courses presented around the world by its authors. Nanotechnology has also aided in the development of more efficient and long-lasting materials, such as self-cleaning and self-repairing concrete and windows. Nanotechnology-based coatings assist in increasing fire resistance, corrosion resistance, insulation, and a variety of other uses. This book is very useful to all scientists, academicians, researchers and students in the field of chemistry, biology, physics, materials science, and engineering, among other subjects.

This book with valuable book chapters from eminent scientists, academicians, and researchers will surely be a part of utmost information for the coming new research taken by the researchers in the field of chemistry, biology, physics, materials science, and engineering, among other subjects.

Dr. Bassa Satyannarayana
Editor

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Chapter
2

ROLE OF NANOTECHNOLOGY IN *LANTANA CAMARA* RESEARCH

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ABSTRACT

Lantana camara contains several bioactive molecules having antimicrobial, fungicidal and insecticidal properties and acts as alternatives in some therapeutic markets. Due to demand supply gap it is essential to extract the bioactive substances from natural sources. It is biomass source from nature that occurs in tropical and subtropical region of world. It shows significant pharmacological and therapeutic properties dueto richness in content of important secondary metabolites that have different biological activities. However, considering trace level of few of these bioactive molecules, it is advisable to implement nanotechnology approach in research and development of these compounds. Nanoparticles are unique subset of the broad field of nanotechnology. Being sparingly soluble and labile biological active substance, it has great potential in the promising drug delivery system. Due to biocompatible and biodegradable properties, it interacts with target cell and improves the function of cell. A well-known versatile drug Lantadene / penta cyclic triterpenoids isolated from Lantana camara. Hence, special attention was given to importance of Nanotechnological workflows with different perspectives into research of this biochemically significant plant species.

KEYWORDS: Anti-microbial, Therapeutic properties, Lantana camara, Nanotechnology.

INTRODUCTION

The source of various novel bioorganic compounds is waste lands. A waste land weed ; Lantana camara play vital role as anti-motility, anti-ulcerogenic anti-microbial activity, allelopathic activity, anthelmintic, anticancer, antifungal, cytotoxicity, nematocidal, insecticidal, analgesic, anti-inflammatory, antimalarial, haemorrhoidal activity, antipyretic, larvicidal, anti-tumour activity etc. The plant Lantana camara derived pharmaceutical active compounds namely pentacyclic tripene have complex structure making chemical synthesis, an economically competitive option. Yet, the derived compound and its potential need to get investigated for its additional potential.

Lantana camara is rich source of natural product. The natural products contains active and bioactive molecules that are heterocyclic in nature. The *Lantana camara* exhibits various organic compounds extracted with organic and aqua solvents. The diversified processes for extraction of organic molecule depend on the physical properties such as its solubility and polar or non-polar bonds.

Reflecting on the last decade of biosensor development, one can apparently perceive the impact of nanotechnology in this research area. Antiquity, Ag-based antiseptics [Ratyakshiand Chauhan; 2009] Ag salts showed antibacterial [Khan etal; 2011]. Due to its pharmaceutical, stabilizing and reducing and

catalytic activity, the plant used for the fabrications of nanoparticles to advances significance in the filed of chemistry. Goswami-Giri and Ingawale; 2012 exhibited AgNP catalyses the chemical reactions of natural product including Pentacyclic triterpenoids having antitumor activity. Reduction of metal into the particle deviates the appearance of particles. Therefore, the potential for chemical reactivity of reaction extensively used by researcher for drug delivery (Narayanan and Pal; 2008, Hodges; 2011, ShuangToh, etal; 2013, Solomon etal; 2007). Considering above benefits, there is needed to develop bioactive silver nanoparticle having application as a bacterial inhibitor for the potential confirmation in chemical reactivity for urinary tract infection. Nano-technological approaches in the *Lantana camara* evaluation had pronounced attention as an alternative medicine.

SYNTHESIS OF SILVER NANO PARTICLES (AGNP)

METHODS

IDENTIFICATION OF LANTANA CAMARA PLANT

Lantana camara was identified and collected from VPM's B.N Bandodkar College, (Autonomous) Thane (MS)-India campus. Segregated all part were air dried, powdered and store until used.

EXTRACTION OF BIOACTIVE COMPOUND FROM LANTANA CAMARA L.

Hundred gram of powder of Lantana leaf, stem powder, flowers and ripened black fruits were treated separately with 500 ml organic solvent/methanol and refluxed it for 3 hours to observed respective extract. The extract was concentrated under vacuum at 13-14 mm/Hg at 58^{o}C.The obtained residue suspended in water, after filtration the residue was treated with methanol-water (1:7) followed by ethyl acetate (2 X 25cm3) and washed with n-butanol ((2 X 25cm3). The residue of lantana plants part loaded over silica gel column (60–120 mesh) using chloroform-methanol (9:1) as eluting solvent. The extract was re-chromatographed using n-Hexane and acetone. The concentred extract was suspended in water and then consequently extracted with ethyl acetate and n- butanol. The ethyl acetate fraction is loaded on a silica gel column using a mixture of CHCl3-Methanol with increasing solvent polarity as effluent for the segregation of layers. Neutral layer examines with n-Hexane and acetone to yield active compound.

The similar process was carried out using aqueous reflexion under vacuum at 14 mm/Hg at 84^{o}C to become concentrated residue. The residues obtained with methanol and aqueous media of all plants part were qualitatively analysed by TLC, HPLC UV, IR analysis, TEM.

PREPARATION OF SILVER NANO PARTICLE (AGNP) OF BIOACTIVE MOLECULE

The above characterized pentacyclic compound was used for the preparation of nanoparticles. Analytical pure grade Silver nitrate and tri-sodium citrate were used as starting materials without further purification. The silver nanoparticles of lantana camara were prepared by using chemical reduction method. 100M $AgNO_3$ (90cm3) was heated to 80-90^{o}C with 10 cm3 of 1 % trisodium citrate by gentle stirring on magnetic stirrer. In this reaction solution, 10cm3 (100µl) bioactive Pentacyclic triterpenoids isolated from *Lantana camara* (leaves) was added dropwise under vigorously, stirring. The obtained Silver nano particles were analysed by UV-Visible spectrophotometry and evaluated its role as an antibacterial and antifungal using amala fruit fungus.

The fungus isolated from Amala fruit were utilised in the method. Silver nanoparticles of bioactive molecule (10 mgs) were used to urge concentration ranging from 25 to100 ppm by mixing methanol and distilled water. The experiment was carried out using hot malt agar broth under sterilised condition. The agar poured into Petri plate which was solidified into transparent solid. The disc test method was used to check antifungal and antibacterial activity. Three an inoculated plate was used for the evaluation by

using various drug disc along with the nanoparticles by streak plate method. The disc plates dried at 35-37 ^{0}C incubated for 48 hours. One-disc plate was kept as controlled (containing malt agar). This control plate was kept as control without Silver nano particles. A fungus mycelium taken from the 48 hours old fungus culture were placed in disc containing silver nano particles and incubated at 35^{0}C. Observe the anti-fungal effect and % of inhibition of silver nano particles on the radial growth of fungus after three days. The Aggregate of lantadene nanoparticle was kept for room temperature for its atmospheric nature study. Serially up to four month sits nature was observed.

SILVER NANOPARTICLE OF PENTACYCLIC COMPOUND LANTADENE

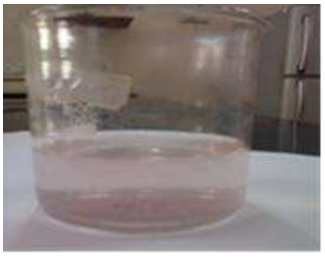
The silver colloid particles were formed immediately after addition of reacting materials. Vigorously shaking with magnetic stirrer along with cooling the colourless solution changed to white, indicates the formation of AgNp of lantadene whereas without Lantadene it showed pink to violet (figure 1).The particle having reaction mechanism of reaction is as follows –

$4Ag+ + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag + C_6H_5O_7H_3 + 3Na+ + H+ + O_2\uparrow$

Also, Lantadene + Silver nanoparticles = Aggregation (figure2)

FOR THE SILVER NANOPARTICLES OF LANTADENE SAMPLE

UV spectrum was carried out of isolated pentacyclic compound and its nanoparticles at Shimadzu-1800 spectrophotometer. Silver nano particles of pentacyclic compound/Lantadene is hydrophobic in nature due to these particles were dissolved in methanol and water. Purity and quality of solvents were strictly maintained. Correction line of methanol solvent was determined, after the standardization of the system. Sample solution of silver nano particles was subjected for the determination of UV-Visible spectra along with the determination of UV maxima. Colloidal particles have a +ve or –ve electrostatic charge. Due to presence of electrical fields particle dispersion is more and the particles travel in oppositely charged directions. Hence its migration due to scattering light measured by Doppler shift method totally depends on electrophoresis mobility. Silver particle in suspension exhibited zeta potential because all solid, liquid & gases states and colloidal states have great impact on Van der Waals attractive (VA) and electrical double layer repulsive (VR) forces that exist between particles and colloidal system become stable due to energy barrier. The extract acts as reducing and stabilizing agent hence can be used in particles. Zeta potential is a very excellent index of the magnitude of the interface between colloidal particles and dimensions of zeta potential are normally used to evaluate the firmness of colloidal systems. It depends on pH and naturally occurring material aqua material. This information may be useful in the field of pharmaceuticals, agrochemicals, pigments, dyestuffs, foods and explosives. Conformation of same molecule in different solvents showed different stability and consequently its bioavailability gave knowledge to the chemotherapy of life. Crystals from acetone were treated with boiling methanol and then cooled at 4^{o}C.



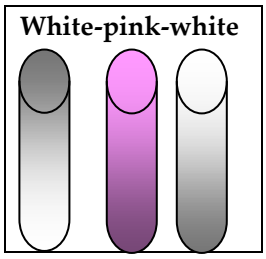


Fig. 1: Changes in color of nano particle a. Colorless b. Pink c. White

Fig. 2: AgNp-lantadene aggregated compound (10mgs)

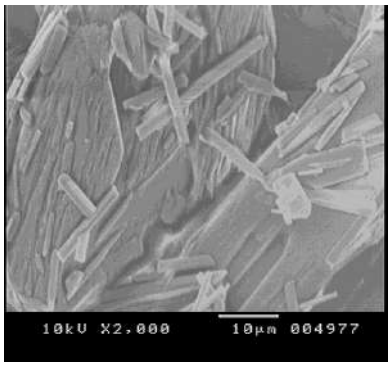


Fig. 3:(a)SEM of pure Lantadene

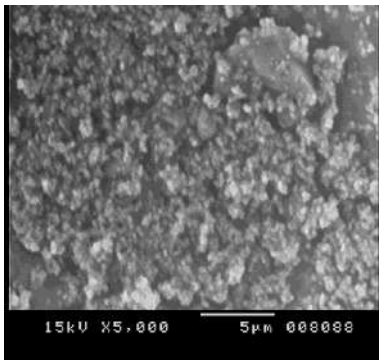


Fig. 3:(b) SEM of Nanoparticles (5 µM)

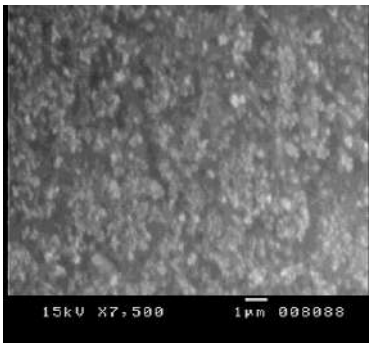


Fig. 3:(c) SEM of Ag Nanoparticle (1 µM)

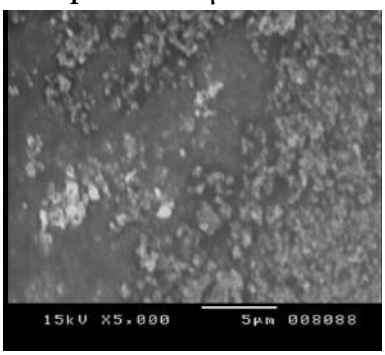


Fig. 3:(d) SEM of Ag Nanoparticles (5 µM)

Fig. 3: SEM of silver nano particles

Silver nano particles with 100 ppm cause reduced the colonies number. Pentacyclic compound/ Lantadene AgNp by exhibiting excellent antifungal activity. The effect of AgNp treatment was evaluated by measuring the number of fungi colonies. Black, brown, green and yellow colored fungi detected on the plates. By varying (25ppm, 50ppm and 100ppm) concentration of Silver nano particles of Lantadene there was not considerable reduction of fungal growth observed. The incubation period by comparing number of different colored fungus growth showed the actual inhibition of growth of fungus. As in all three concentrations there was much growth of fungus observed towards the periphery as compared to the center of the plate. The efficiency of silver nano particles treatment was evaluated by measuring the fungi colonies diameter.

RESULT AND DISCUSSION

The literature review on *Lantana camara* has been dealing with the various aspects of bioactive molecules and its approach towards nano-techniques. The biophysical, biochemical methods are used for the characterization with the search for new microorganisms providing higher control rates. The purity of bioactive compounds and its nanoparticles effective of fungus inhibition. The structural elucidation of the bioactive molecules and its potential inhibition explore in the field of medical application which verifies the pivotal role of Nano technological processes.

Organic molecule in plant material is depending on solubility, polarity, extractions of a specific bioactive compound in the nature and extraction medium. The extract was carried out by conventional, novel

methods and the yield of triterpenoid/tripene was compared in rotation with time. Assortment of effective organic solvent for natural product is very much important for the maximum recovery of product. Antibacterial screening with the help *Lantana Camara* pentacyclic compound and its fabricated silver nanoparticles. The extracts *Lantana camara* parts exhibits UV range from 270 nm to 330nm. UV spectrum of Lantana Camara Ag-Np's were exhibited between 270-350nm. Various colour on TLC was monitored having Rf values are 0.82, 0.86, 0.46 for Lantana leaf, stem powder, flowers and ripened black fruits respectively.

The FTIR spectra of leaves crude bioactive compound belongs to O-H and C-H with stretching modes by exhibiting at 3465 cm^{-1}, 2700-3100 cm^{-1}1457 $cm^{-1}$and at 1302 -1396 cm^{-}1. The involvement of function group of Lantana Camara leaf extract in the reduction and capping process of nanoparticles was well displayed in FTIR. Lantana camara leaves having anti-filarial activity as well as metabolites isolated from leaves possess antitumor, anti-thrombin, anti-nociceptive and antipyretic activity. The bioactive molecules observed in lantana various atoms with 270 normal modes of vibration.

Antimicrobial activity of *lantana* and its Silver nanoparticles also possessing antifungal activity. Antibacterial effect was evaluated with B. Subtilis, S. aureus, S. typhi and E.coli. TEM analysis of extract disclosed spherical and crystalline shaped nanoparticles along with antifungal activity. The consequence of nanoparticles X-ray diffraction pointed peak at 38 representing the crystalline nature.

Contribution of phenol, amino groups in Lantana may have important role in toxicity. During processing, ethyl acetate does not sort out entire extract of tripene in methanol–water mixture. Hence, Ethyl acetate, methanol used in reaction along with hexane to observe maximum yield. Quantifications of it were completed with qualitative test-Salkowski Test, Liebermann Burchard test, and colorimetric method. By surface morphology was evaluated to characterize coating surface with high resolution and by recording of X-ray spectra. Figures 3 indicate that there are no significant changes in the surface morphology. Nevertheless, it exhibited fluffy and rod-shaped uniform crystals which also confirmed with X- ray diffraction.

Silver nanoparticals were synthesized from fruit extract using spectroscopy. Secondary metabolites, various acids indicate the interaction between silver nanoparticles is present in fruit extract of Lantana Camara Linn. Oleanolic acid was isolated from root.P. Rama Devi 2015 also evaluated antimicrobial active Silver nanoparticle synthesis from Lantana Camara seed extract. The process used in biosynthesis process of antibacterial silver nanoparticles using Lantana camara seed extract are conjugated with organic bioactive molecules .The particles and its physical properties depend on the various dyeing material and its surface area ratio.

Lantana nanoparticles have articulated substantial improvements owed to wide range of applications in the field of biomedical, sensors, antimicrobials, bio-insecticides, catalysts, electronics, optical fibers, agricultural, bio labelling, Bioremediation, their role in health care system, diagnosis in drug delivery. In the modulation of the size and shape of the nanoparticles product is nontoxic and to protect the environment. It also opens up the application in wood chemistry, industrial waste absorbent and harmful chemicals to useful with traditional medicinal properties. The nanoparticles are economy source and driver for its application in the field of medicine.

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PREFACE

Analytical chemistry has a vital role in every stage of life. It is the science of obtaining, processing, and communicating information about the composition and structure of matter. In other words, it is the art and science of determining what matter consists of and measuring the same. By applying analytical chemistry, we have gained insight into the origin and evolution of the universe and life on our planet. Through these insights, we can improve the material characteristics of natural resources and industrial materials to benefit humankind. Today we cannot think of even a single product of commercial use which has not been tested using analytical techniques before clearance from consumption.

There are two essential aims of analytical chemistry – the first is to attain analytical information of the highest quality with the lowest possible uncertainty, and the second is to solve analytical problems derived from biochemical details of different areas. This way, analytical chemistry plays an enormous role in various fields such as drug manufacturing, process control, medical diagnostics, environmental monitoring, food production, and forensic surveys. In our modern world, without analytical chemistry, we would not be able to make any important decisions such as soil remediation, limiting values for environmental pollution, choosing the correct dosage of medicines and food for patients, etc.

Analytical chemistry is also focused on improvements in experimental design, chemometrics, and the creation of new measurement tools. It has broad applications in medicine, science, and engineering. It is poised to make more significant contributions to improving life and understanding new materials. It will lead the way to developing new materials with desired features and detection at levels that could not be imagined before. Analytical chemistry has evolved dramatically over the past few decades from the traditional notion held for centuries to that of a modern, active discipline of chemistry. It produces quality (bio) chemical information of global and partial type from natural and artificial objects and systems to solve analytical problems derived from information needs. Analytical chemistry is an information discipline and is highly essential to modern society.

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Chapter
10

CHROMATOGRAPHY: A VERSATILE TECHNIQUE FOR SEPARATION

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INTRODUCTION

Chemical analysis is the concern with the identification and estimation of a component in a given substance to evaluate the scientific problem. To identify unknown compounds/ elements, proportions/amount is the major concern with using new, easy, and quick analytical methods and improving existing works. Ancient time quantitative approach in chemical research and normal experimental is the key role in the development of science. All branches of chemistry including biological, environmental, pharmaceutical, medicinal, petroleum chemistry depend on analytical techniques such as chromatographic and spectroscopic techniques. Hence, in present chapter discussed separation techniques- different types of chromatography procedures, advantages, and their application.

Chromatography is the biophysical technique based on a principle where molecules in a mixture applied onto a solid surface or liquid phase are separated from each other by running with the mobile phase. Chromatography is regarded as a method of separation in which the separation of solute occurs between stationary and mobile phases. This separation of solute occurs between the stationary and mobile phases. This separation technique becomes universal and has been extended in chemistry, biology, medicine, and pharmaceutical industry in the manufacturing of pure chemicals and bioscience for the separation of biomolecules. The name chromatography means color, writing. In chromatography method, it involves the following steps.

1. Adsorption of substance on the stationary phase.
2. Separation of the absorbed substance by the mobile phase.
3. The recovery of the separated substances is called elution.

4. Quantitative and quantitative analysis of the eluted substance.

Types of Chromatography and its Stationary and Mobile Phase

Sr. No.	Chromatography	Stationary Phase	Mobile Phase
1.	Column Chromatography	Solid	-liquid
2.	Partition chromatography	liquid	liquid
	e.g. Paper chromatography	liquid	liquid
3.	Adsorption chromatography		
	e.g. Thin layer Chromatography (TLC)	Solid	liquid
	High-Pressure Thin layer Chromatography (HPTLC)	Solid	liquid
	High-Pressure Liquid Chromatography (HPLC)	non-polar	moderately polar
4.	Gas-liquid chromatography	liquid	Gas
	Gas solid Chromatography	Solid	Gas
5.	Iron Exchange Chromatography	Solid	Gas
	Iron Exchange Chromatography	Solid	liquid
6.	Gel-permeation (molecular sieve) chromatography	A stagnant liquid in porous bead	liquid
7.	Affinity chromatography	Solid	Lysate/ liquid

The chapter deals with Column chromatography, Partition chromatography Paper chromatography, Adsorption Chromatography- a)Thin-layer chromatography (TLC),b) High-pressure Thin-layer chromatography (HPTLC) and c) High Pressure Liquid chromatography, Gas Chromatography, and Ion Exchange Chromatography.

COLUMN CHROMATOGRAPHY

The technique in which the stationary phase is alumina or silica gel and the mobile phase is either gas or liquid is known as adsorption chromatography. These techniques are used for Identification of the non-identified two substances, in determining the concentration of products, contaminants in commercial products, in separation and Purification of technical products, etc.

The principle of chromatography is based on differential adsorption of substances by the adsorbent.

Factors Affecting Column Efficiency: Following are some of the important factors after column efficiency.

1. Dimension of the column

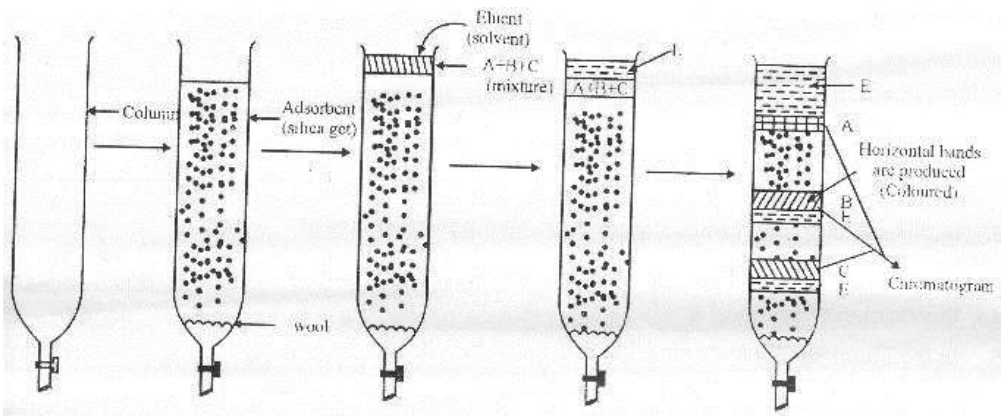
Column efficiency has been improved by increasing the length/width ratio of the column for column preparative separations sample/column packing ratios from 1:20 to 1: 100 in adsorption and from 1:50 to 1:500 in partition chromatography. Recently length/ width ratios of 10: 1 to 100: 1 are more satisfactory.

2. Particle size of column packing

Particle size plays an important part in chromatography is improved by decreasing the particle size of the adsorbent. This is probably due to the rapid decrease in flow rates. The recommended particle size used for both adsorption and partition is 100-200 mesh range.

3. Pore diameter of column packing:

Polar adsorbents have been found to have a pore diameter of 20 A^0 according to keen a decrease in average pore diameter from 70-20 A^0.



Elution is a chemical process that involves removing a material's ions by ion exchange with another material. Eluent is a solvent/mobile phase that passes through the column. In liquid chromatography, the eluent is liquid while in Gas chromatography eluent is a gas carrier.

PARTITION CHROMATOGRAPHY

Paper Chromatography

In partition chromatography, the substances are distributed between the stationary liquid/stationary phase and the moving liquid/mobile phase. The component of the mixture to be separate traveled at different rates and appeared as spots at different points on method. Originally paper chromatography was used to separate a mixture of organic substances such as dye and amino acids, steroids, vitamins, Pesticides, Pigments but now this method has been used to separate cation and anion of inorganic substances as well.

Procedure

1. A drop of the test solution is applied to the bottom of a filter paper. After drying the spot, filter paper is placed in a suitable solvent in such a way that the edge of filter paper is deep into a solvent called *developing solvent*.
2. As soon as the filter paper gets the liquid through its capillary axis and reaches to spot of the test solution, the various substances are moved system at various speeds. When

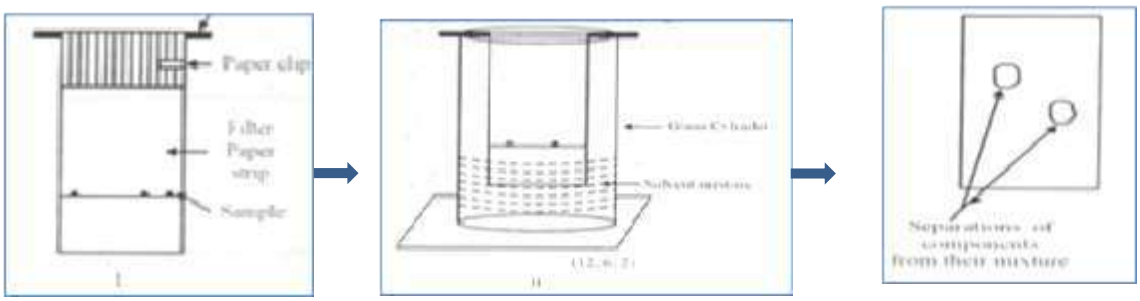
the solvent has moved to a suitable height the paper is dried and various spots are visualized by the suitable reagent called *visualizing reagent*. The movement of the substance relative to a solvent is expressed in terms of Retention Factor/ Retardation Factor (RF values).

$$\text{R.F.} = \frac{distance\ traveled\ by\ solute\ from\ original\ line}{distance\ traveled\ by\ solvent\ from\ the\ original\ line}$$

Types of paper chromatography: There are 5 types of paper chromatography

1. **Descending, b) Ascending, c) Ascending-descending, d) Circular and e) Two-dimensional**

When the development of the paper is done by allowing the solvent to travel up the paper. It is known as *Ascending technique while as* the solvent travels down the paper it is known as **the** '*Descending technique'*. Both ascending and descending techniques have been employed for the separation of organic and inorganic substances. The advantage of the descending technique is that the development can be continued even though the solvent runs off at the other end of the paper.



1. **Ascending-descending** - This is the hybrid of both of the above techniques. The upper part of ascending chromatography can be folded over a rod to allow the paper to become descending after crossing the rod.
2. **Circular chromatography** –The sample is applied at the center of circular paper after marking with a pencil. After drying, kept it in a Petri dish by wick of the paper dipped in the solvent. As solvent rises through the wick, components get separated into concentric rings.
3. **Two-dimensional**- on square or rectangular filter paper the sample is applied to one of the corners and development is performed at a right angle to the direction of the first run.

Limitation of paper chromatography

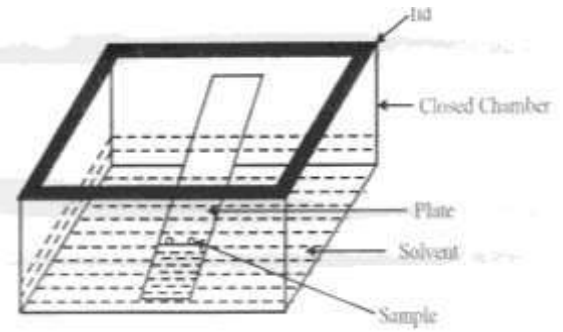
1. A large quantity of sample and Corrosive material cannot be applied on paper chromatography

2. Complex mixtures cannot be separated

ADSORPTION CHROMATOGRAPHY

Thin Layer Chromatography: TLC was first introduced in 1938. It is used for the isolation of non-volatile mixtures. It's also called as TLC is also made by other names such as-

1. Surface chromatography
2. Column chromatography



ADVANTAGE OF TLC OVER OTHER CHROMATOGRAPHY TECHNIQUES

TLC is superior to column and paper chromatography. The main advantage is given below-

1. TLC required simple equipment and methodology.
2. It required less analysis time for development for drying and Ultra-choice of stationary phase.
3. It requires low solvents can be analyzed numbers of samples.
4. Complex mixtures easily separated based on polarity
5. Required very small quantity of samples
6. **Easy recovery:** It is possible to remove the powder coating of plates by scraping a knife.
7. **Easy equalization of the paper** component. Detection of a component under U.V. light is easier than paper chromate.
8. **Chemically inert stationary phase** the greatest advantage of this technique is very strong corrosive reagents such as concentrated $H_2\ SO_4$ can also be used.

APPLICATION OF TLC

Due to its simplicity, sharpness, high sensitivity in separation, and easy recovery, TLC has found increasing application in all branches of chemistry and its allied branches. The application of TLC in organic chemistry is as follows.

1. It is used for checking the purity of organic, inorganic, and biological samples

2. To observe the purified product and also measurement cycles in the purification process
3. Estimation of reactions/progress in reactions/purification method and for the identity of separated organic compounds such as amino acids, proteins, alkaloids, phospholipids antibiotics, acids, alcohols, ethers, amine, etc.

LIMITATIONS OF TLC

1. It is used only for small preparative work but researchers have obtained high resolution by using TLC while using film techniques.
2. Humidity and temperature can affect the result
3. Compounds run streak rather than spot
4. A limited amount of material can be isolated

PROCEDURE FOR TLC

1. TLC plate is be prepared by using silica as a stationary phase with fine and uniform.
2. Mark the line by pencil to the bottom of the plate and upload the sample to be separated
3. A suitable solvent or solvent mixture which is pure and particulate-free can be used as a mobile phase. Sample loaded TLC plate is placed into TLC chamber in such a way that the loaded sample should be well above the solvent system/mobile phase and Cover the chamber with lid
4. After the development of the spot remove the plate and observed the spot under the UV chamber
5. Mark the spot and calculate RF values of spots.

HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

The advanced version of TLC is HPTLC, working on the adsorption-based principle for separation. HPTLC can be used as an alternative technique for high-performance liquid chromatography (HPLC) and gas chromatography (GC). HPTLC is also called flat-bed chromatography or planar chromatography.

APPLICATION OF HPTLC

1. It is cost-effective, easy to maintain and Multiple analyses can be done simultaneously
2. No risk of contamination and have a wide range of stationary phases
3. The method is sensitive, rapid, reproducible, precise Required less solvent for separation

DISADVANTAGE

1. Limited samples can be tested on a plate

2. Short separation of bed
3. Silica also detected in the test

PROCEDURE

1. *Sample preparation-* For HPTLC 0.1µl concentration of sample used for transferring the sample solutions to the thin layer qualitative work.

2. *Preparation of chromatographic layers***-** Layers can be performed on a sheet of glass, plastics, and or aluminum foil which is coated with a thin layer of adsorbent materials/stationary phase. Generally, silica gel, alumina oxide, calcium phosphate are used as a coating material for better plating some binders like gypsum, calcium sulphate, starch are added to absorbent gypsum is most used.

1. The various methods of preparing layers such as Pouring, dipping, spraying, and spreading. After preparing slurry in water or solvent, the plate dipped into slurry, remove and dry it well. The slurry is spread uniformly on the glass surface. After setting the layer of adsorbent activate the plate by keeping it in an oven at 100 ^{o}C for 1 hr.

2. *Washing and conditioning-* Methanol and also in combination with ethyl acetate wash the plate. Plates should be handled only at the upper age to avoid contamination. For quantitative analysis and reproducible results plated need to equilibrate.

3. *Sample application-* For a sampling of the standard sample, a capillary tube or micropipette can be used for spotting. The spot can be placed 2cm above the base of the plate. The plate should be kept into the

4. *Selection of mobile phase-* It depends on the stationary phase used in the system and chemical properties of solvents such as Diethyl ether, methylene chloride, and chloroform combined individually or together with hexane. The Choice of solvent is an important decision. In practice generally benzene, chloroform, acetone, benzene-methanol, chloroform-Methanol

5. *Chromatographic development*: Assembling of chromatography T.L.C. plate is placed in the development chamber with angle 45^{0} it is imp that development chamber is perfectly saturated with solvent paper.

6. *Detection of spots:* The lower edge of the plate is deep into the closed developing chamber. Due to capillary action, the samples run up to desired distance .depending upon the vapor pressure in the chamber and composition of components, the stationary phase absorbs molecules from the gas phase, and migration of components of the mobile phase is separated. Chamber saturation is more important for the detection of spots

7. *Documentation:* the developed plate may be digitally documented under UV and white light

HIGH-PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

HPLC is a non-destructive method used for the simultaneous analysis method. The separation of sample ingredients/ analytes between the mobile phase and stationary phase. The distribution of analytes depends on chemical structure, intermolecular interaction between molecules, and packing material in the column. Different common packing materials are for normal phase, reversed phase, size exclusion, ion exchange, affinity chiral, or hydrophilic interaction for the separation in HPLC. Change in composition of mobile phase in HPLC the system is known as gradient elusion system.

HPLC is mainly divided into two types.

1. Normal phase HPLC and

2. Reversed-phase HPLC.

PRINCIPLE

Resolving power of chromatography column increases with increase in column length. There is development in adsorption partition, exchange affinity chromatography resulted in faster resolution hence HPLC is the most popular, powerful, and versatile form of chromatography.

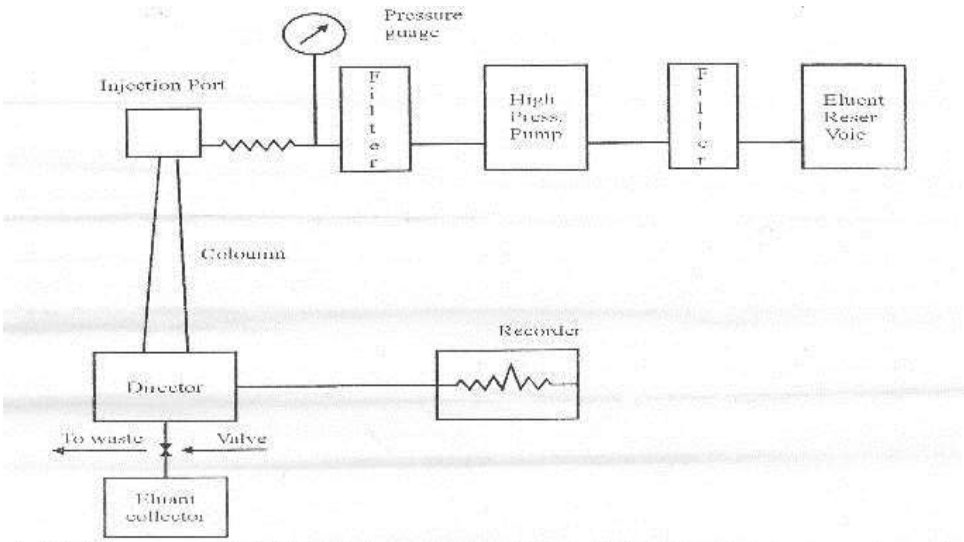
EQUIPMENT

Fluent reservoir, de-gasing solvents, Gradient controller and mixing unit, filter, Hinge press. Pump, filter, pressure gauge injection port (Column), director, recorder, eluent collector.

This is a schematic representation of high performance eluent-

1. From the solvent reservoir achieves a constant flow of solvent at 100 psi.
2. The flow gets smooth with the dumper.
3. It is possible to measure the inlet press of column with manometer after leaving the column the sample studs high pressure. The sample is injected through a spectrum in an injection hole either directly or to the column or on a small plug, employing an appropriate valve. The fluent form a channel section through the injection pole. Degassing is used to prevent gas bubbles in the pump detector. The solvent must be passed through the column at high pressure while as stationary phase particles are smaller (5-10μ) resistant to the flow of solvent hence high pressure is recommended. A flow rate of 0.1to 10 ml/min is recommended. The outlet which leads directly onto the column is highly impure solvents, Urine, whole blood which has preferably been detected by high resolving power. The separated analytes are recorded by the system in the form of peaks. Total all peaks are known as a chromatogram. All peaks given the information about the analytes such as shape, the intensity of the peaks, time required to

appear for peaks. The area of peaks that depend on the concentration of analytes is known as Gaussian bell-shaped curve. Delay time, retention time, peak width, and Trailing factor /peak symmetry



ADVANTAGES

1. It is simple, rapid, reproducible, Repeatable, and sensitive methods
2. It exhibits accuracy, precision and its stationary phase is chemically inert.
3. Less amount of mobile phase is required in developing chamber.
4. Techniques are important for the validation of the product, quality control studies.
5. It is used for both analytical and preparative purposes.
6. HPLC is used in easy to fractionate and purify

APPLICATIONS OF HPLC

1. It is used to detect, identify, quantify and purify all chemical and biological molecules.
2. HPLC is widely used for the separation of Polar components such as vitamins. Steroids, polyphenols, Peptides. The separation of some highly polar compounds such as amino acids can be separated economically by this method.

GAS CHROMATOGRAPHY

It is quite similar to column chromatography except that gas is used as a mobile phase instead of a liquid. Gas solid chromatography (GSC) and Gas liquid chromate (GCL) is encountered. The main advantage of gas chromatography are as follows-

1. A complex mixture can be resolved easily.

2. It gives good precision and accuracy.
3. The analysis is completed in a short time.
4. The cost of the instrument is relatively low.
5. Its life is generally long for the operation of gas chromatography. It doesn't require. A highly skilled person for calculation.

PRINCIPLES OF GAS CHROMATOGRAPHY

When the gas of vapors comes in contact with the adsorbent certain amount of it gets adsorbed on solid. In the system Helium, argon, or nitrogen are used as carrier gas. The component separated inside the column and detector measure the number of components. The separation techniques are capable of separating complex mixtures based on physical constant, polarity, and vapor pressure.

PROCEDURE

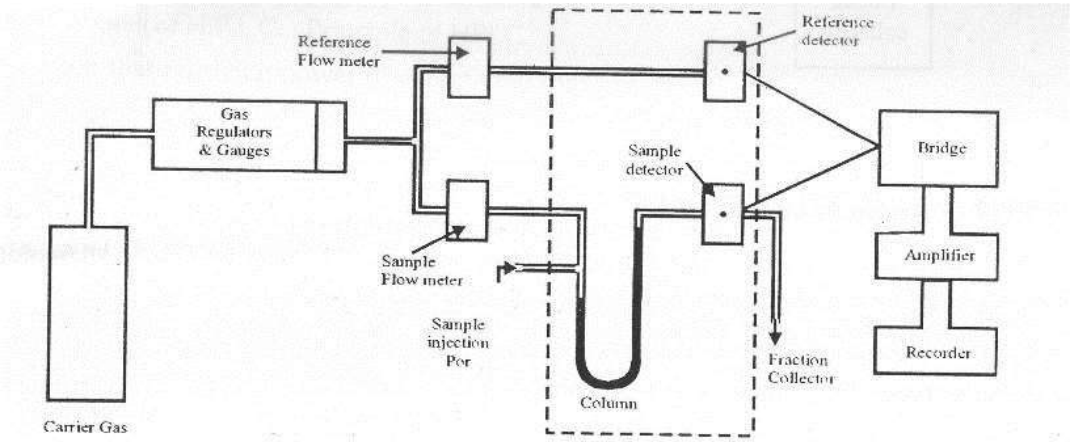
Its operation is similar principle of column chromatography where samples are dissolved in the mobile phase and passed through the stationary phase.

There are 4 steps in the analysis

1. *Sample collection*- sample is introduced into the stream of gas. A gas sample is collected and then it is introduced into an inert gas stream called a carrier gas
2. *Sample injection*- here it's in hot conditions which allows the solvents and compounds to evaporate (liquid sample need to be evaporated before injecting into the carrier)
3. *Sample separation*- samples moved through the packed column, with the stationary nonvolatile phase. Samples interacted with the stationary phase very less
4. *Sample detection*- Samples quantified collected through the detector.

APPLICATIONS

1. The detection of steroid drugs used by international sports competitions.
2. Hazards pollutants such as CO2, formaldehyde, benzene can be monitored by G.C.
3. An analysis of food products like milk, sugar, butane, and added colors and salts G.C.cab be easily identified.
4. It is also used in drug analysis, identification of plastic, paints, and synthetic polymers, and various environmental studies

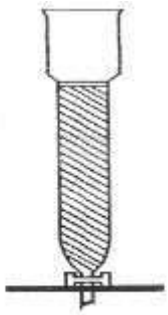


ION EXCHANGE CHROMATOGRAPHY

PRINCIPLE

The principle feature underlying this form of chromatography is the attraction between oppositely charged particles. Ion exchange separations are mainly carried out in a columns pack with an Ion exchanger. There are two types of ion exchangers, namely caution and anion exchanges. Cation exchanges possess negatively changed groups which will attract negatively charged molecules.

The quality of an ion exchange resin is determined by its capacity which in turn depends upon the total number of ion active groups per unit weight of the material. Greater the number of ions the greater the capacity of the resin for the exchange process. The efficiency of the resin has been found to depend upon the degree of cross-linking and the higher the efficiency of the resin.



Univalent anions, the capacity has been found to decrease	$1^{-}>NO_3^{-}1^{-}>Br^{-}>CN^{-}>C1^{-}>OH^{-}>F^{-}$
Univalent cation the capacity has been found to decrease	$H^{-}>cs^{+}1^{-}>Rb^{+}>NH_4^{+}>Na^{+}>Li^{+}$

A glass column fitted with glass wool or ordinary burette with resin provides a large surface area for contact between the solution and the resin.

APPLICATIONS OF ION EXCHANGERS

Ion exchange is used mostly in inorganic chemistry.

1. Separation of amino acids and sugars from food, actinides
2. Purification of organic compounds
3. Separation of contaminations from water and for analysis of pollutions

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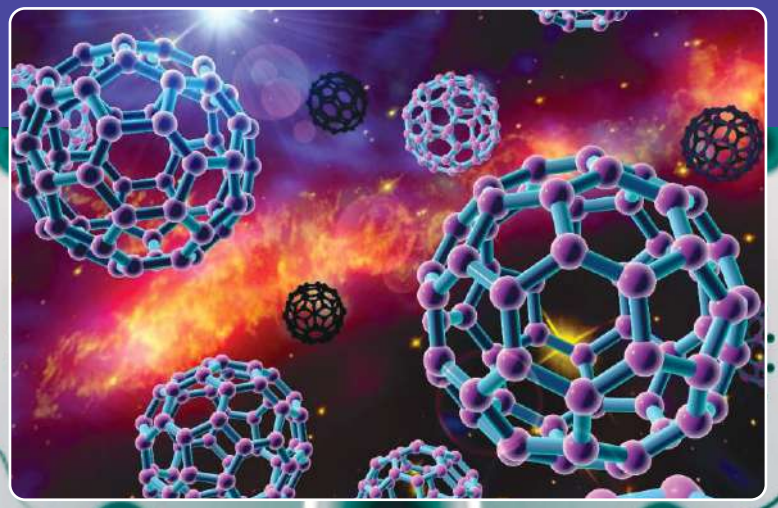
Volume - 1



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ADVANCES IN
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Volume - 1

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Preface

Nanotechnology achieved considerable importance when researchers realized that the size of the material is a key factor that influences the properties of a substance. In the recent past, several conventional methods have been used for nanomaterials synthesis. Nanomaterials are synthesized by top-down and bottom-up approach depending upon the starting materials used for its synthesis. Various greener and non-greener methods are used for the nanomaterial synthesis. Nanomaterials have wide applications in cosmetics, food products, clothing, household appliances, fuel cell catalyst, disease treatment, and renewable energies. Nanomaterials are also being applied to a variety of industrial processes including construction materials, sensors, nanorods, graphene, water filtration, and wastewater treatment. This book provides some new research and developments in nanomaterials synthesis and applications. It presents the basics of the synthesis and fabrication of nanomaterials and some important characterization tools.

This book consists seven chapters. First chapter gives brief idea about nanomaterial characterization tools. Third chapter gives brief explanation on dendrimers and their application in drug delivery. Chapter four and seven explains history of nanomaterials. These chapters also present development of different methods of synthesis and applications. Chapter five covers synthesis of nano sulfated metal oxides and their application as catalyst for multicomponent reaction. Chapter second and sixth presents recent advancements in greener synthesis of nanomaterials and their application for the organic transformation.

I am sure that this book will be useful for undergraduate, post graduate and Ph.D. Scholars, researchers, and faculties of Chemistry, Physics, Material science, Botany, Biotechnology, life science, Microbiology, Biochemistry and pharmacy. I am thankful to all contributors for their valuable work. I appreciate the efforts of integrated publication for bringing out the book. I am also thankful to Principal, Dr. B. B. Sharma, Dr. Ajit Kengar, Dean HR, KET's V.G. Vaze College (Autonomous), Mumbai for their encouragement and support in the task of publishing this book.

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Chapter - 5
Nano Sulfated Metal Oxides Catalyzed Multicomponent Reactions

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Chapter - 5

Nano Sulfated Metal Oxides Catalyzed Multicomponent Reactions

Sandeep S. Kahandal

Abstract

The development of efficient and cost-effective green practices would be required to ensure the availability of a clean environment for the welfare of living things. It is necessary to convert "non-green" protocols to "green" protocols for the long-term sustainability of the environment. Surface modified metal oxides and mixed metal oxides are constantly used due to their extensive applications across a wide variety of chemical sciences. One of the most promising alternatives for functional group conversions has been found as a solid acid. Sulfated metal oxides and sulfated mixed metal oxides catalysts have been extensively explored for a range of industrially important chemical processes due to their excellent thermal and chemical stability and a large number of acid sites. Preparation techniques, precipitating agent, surface modification source, calcination temperature, retreatment, and storage all affect catalytic activity. These sulfated catalysts have good catalytic activity in multicomponent reactions. The current study focuses on the utilization of sulfated metal oxides in the synthesis of multicomponent reactions, which are majorly employed as pharmaceutical intermediates and for a variety of medical purposes.

Keywords: nanomaterials, sulfated zirconia, multicomponent reactions, green chemistry

1. Introduction: Surface modified nano metal oxides and multicomponent reactions (MCRs)

Surface-treated metal oxides (sulfated zirconia) have introduced a lot of alternatives for their use as a catalyst in different acid-catalyzed organic synthesis and functional group transformations. The catalytic properties of surface-treated metal oxides are altered by preparation procedures, sulfating agent, and calcination temperature. Solid acids are employed in various areas of the chemical sector. The basic interpretation of acid-induced or acid

catalyzed reactions covers a wide variety of issues, from large-scale industrial hydrocarbon chemical processes to enzyme-controlled processes in living cells. Several researchers have attempted to modify sulfated zirconia such that the catalyst is more robust and stable under harsh reaction conditions. One method of addressing the problem and making sulfated zirconia more sustainable is to produce nanocrystalline-sized sulfated zirconia [1]. Nano-sized sulfated zirconia as a solid acid catalyst has improved catalytic performance and selectivity [2] for the several organic functional group transformations [3] including multicomponent reactions (MCR).

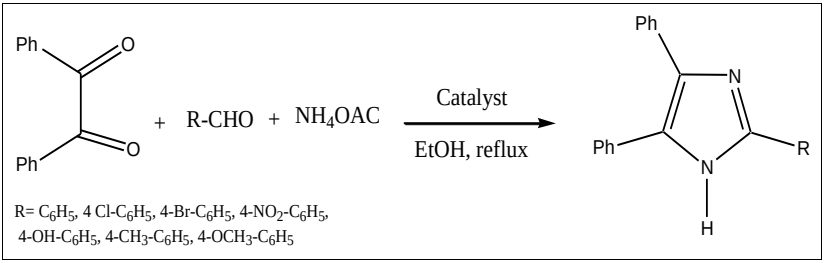
2. Applications of nano sulfated zirconia to multicomponent reactions

Multicomponent Reactions (MCR) are processes in which more than two starting components combine to form a compound that contributes almost all or most atoms to the final product. To build a product, a multicomponent (MCR) reaction employs a series of fundamental chemical processes. Multicomponent reactions (MCRs) have gained a lot of interest from the standpoint of optimal synthesis because of their uniformity, productivity, ease of execution, and usually high yield of products [4]. Furthermore, the initial components are either readily available commercially or simple to prepare [5]. The rejuvenation of multicomponent reactions has been fuelled by great progress in multicomponent reactions involving Passerini, Ugi and Mannich reactions over the previous decade [6]. The main emphasis of the present investigation is on the recent applications and catalytic activity studies of nanocrystalline sulfated zirconia for industrially and pharmaceutically important multicomponent reactions (MCR) [7].

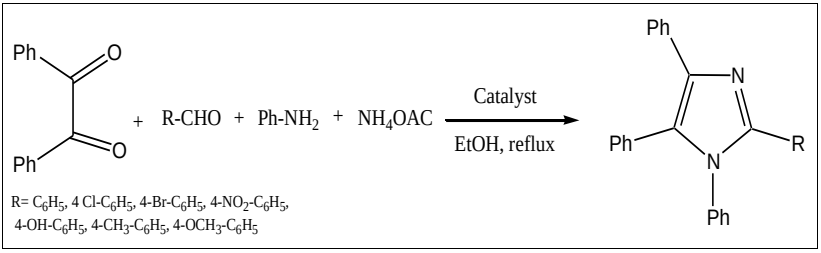
2.1 Synthesis of 2,4,5-trisubstituted and 1,2,4,5-tetrasubstituted imidazoles

Imidazoles are a group of nitrogen-containing heterocyclic compounds that are now receiving a lot of attention owing to their wide variety of uses and the fact that they have a lot of pharmacological characteristics and play essential roles in biological processes [8]. The imidazole pharmacophore's promise in a wide variety of applications may be ascribed to its hydrogen bond donor-acceptor capabilities as well as its strong affinity for metals. Many of the those substituted compounds are used as p38MAP kinase inhibitors, fungicides, herbicides, plant growth regulators, antibiotics, antitumor, insecticides, medicinal agents [9-15] also alkylated imidazoliums compounds are significant for showing numerous applications in ionic liquids [16].

The synthesis of 2,4,5-trisubstituted imidazoles is accomplished by a three-component reaction between aldehydes, 1,2-dicarbonyls and ammonium acetate, as nitrogen source compared using nano-crystalline sulfated zirconia with clays and zeolite in ethanol (Scheme 1) at reflux temperature. Among all the catalysts, the best catalyst for the multi component reaction was sulfated zirconia. Additionally, the same set of protocol was further extended for the one-pot synthesis of 1,2,4,5-tetrasubstituted imidazoles (Scheme 2) with good yields, 90-110 min. reaction time and milder reaction conditions, easy workup, and simple purification of products. The catalysts could be recycled up to 3 successive consecutive runs, without a decline in catalytic activity [17].



Scheme 1: Nano-sized sulfated zirconia catalyzed synthesis of 2,4,5-trisubstituted imidazoles



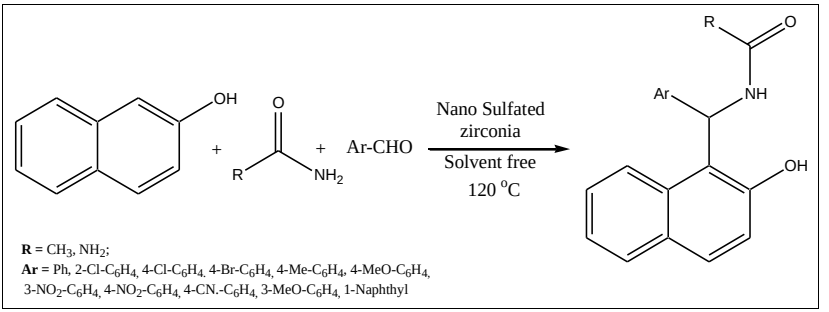
Scheme 2: Nano sulfated zirconia catalyzed synthesis of 1,2,4,5-tetrasubstituted imidazoles

2.2 Synthesis of amidoalkyl naphthols

The amidoalkyl naphthol frameworks are valuable intermediates that may readily be transformed into bioactive compounds by amide hydrolysis. 1-Pyrrolidinylmethyl-2-naphthol hydrochloride (TPY-*β*) showed a very promising result in the lowering of blood pressure and heart rate in anesthetized rats [18]. The ionic mechanism of TPY-*β* mediated circulatory activity suggests that these inhibitory effects of TPY-*β* are directly inhibiting cardiac and vascular smooth cells [19]. TPY-*β* compound with potassium and

calcium channel antagonistic properties may exhibit novel antiarrhythmic agent [20].

A one pot of multicomponent technique for the synthesis of amidoalkyl naphthols under solvent-free conditions was utilized to analyse the reactions from aromatic aldehydes, 2-naphthol, and amide/urea using nanosulfated zirconia as a catalyst (Scheme 3). In the substrate scope, aromatic aldehydes with electro-donating or electron-withdrawing groups provide good yields (81-94%) nevertheless aromatic aldehydes with electron-withdrawing groups react faster than electron-donating groups [21]. The formation of the tetragonal crystalline phase, maximum specific surface area, and the average pore size, etc. properties of metal oxide have been remarkably enhanced by the introduction of anionic sulfated species [22].



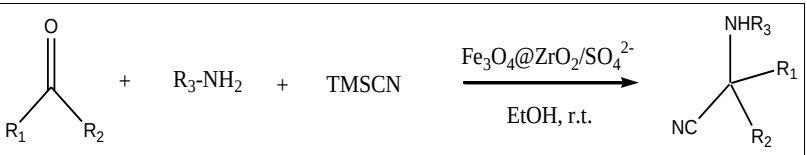
Scheme 3: Multicomponent synthesis of amidoalkyl naphthols using nano sulfated zirconia

2.3 Synthesis of α-aminonitriles

The synthesis of α-aminonitriles was the first multicomponent reaction (MCR) explored and developed by Strecker in 1850 [23]. These compounds are also useful intermediates in the synthesis of α-amino acids, 1,2-diamines, nitrogen-containing heterocyclics such as imidazoles and thiadiazoles [24], as well as other medicinally important compounds such as Saframycin A [25]. The combination of an amino and a nitrile group enables such nitriles ideal as reactants for the synthesis of a number of compounds having one or two functional groups [26].

The traditional Strecker reaction and their several other modifications for the synthesis of α-aminonitriles were reported in the literature using several cyanide sources. Among the cyanide sources used for the synthesis of α-aminonitriles, trimethylsilyl cyanide (TMSCN) is an alternate cyanide ion source, for nucleophilic addition procedures in the presence of Bronsted or Lewis acids. Ghafuri *et al*., investigated $Fe_3O_4@ZrO_2/SO_4^{2-}$ catalyzed

one-pot multicomponent Strecker reaction of aldehydes/ ketones and amines with TMSCN to give α-aminonitriles (Scheme 4). The separation of the catalysts has been achieved by mounting nano sulfated zirconia on Fe_3O_4 magnetic nanoparticles [27]. The XRD pattern study reveals that the peak position of the magnetite crystalline structure is essentially maintained during the synthesis of $Fe_3O_4@ZrO_2/SO_4^{2-}$. The synthesized catalyst and magnetite core provide an average particle size of about 30 and 25 nm respectively.



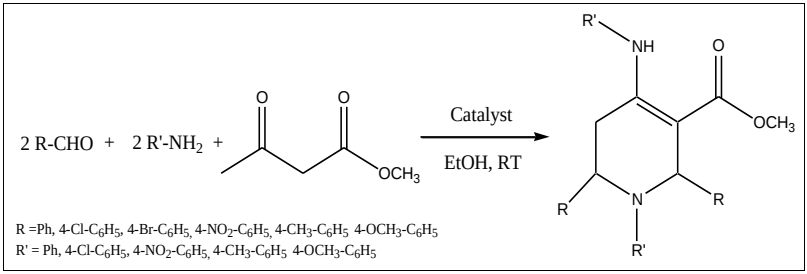
R_1 = 4-Cl-C_6H_4-, C_6H_5-,2-Cl-C_6H_4-, 2,4-Cl-C_6H_4-,2,6-Cl-C_6H_4-, 4-NO_2-C_6H_4-, 3-NO_2-C_6H_4-, 4-Me-C_6H_4-4-MeO-C_6H_4-, 4-OH-C_6H_4-, 3-OH-C_6H_4-, C_8H_7-, C_4H_3O-, C_4H_3S-, $C_{10}H_7$-, CHO-C_6H_4-, $(Me)_2NH$-C_6H_4-; **R_2** = H-, Me-, C_6H_5-; **R_3** = C_6H_5-, CH_3-C_6H_5-, C_6H_5-CH_2-,4-Br-C_6H_4-, $(Me)_2CH$-C_6H_4-, C_6H_5-CO-; **R_1, R_2** = cyclohxyl

Scheme 4: The Strecker reaction catalyzed $Fe_3O_4@ZrO_2/SO_4^{2-}$ using TMSCN

2.4 Synthesis of functionalized piperidines

Piperidine and its derivatives as a major structural unit found in a variety of natural substances, biologically active compounds, and organic fine chemicals [28]. Most of the piperidine compounds find their applications in the pharmaceutical industry [29], many of the structural piperidines act as anti-hypertensive [30] anti-bacterial [31] anti-convulsant, anti-inflammatory [32], and antimalarial activities [33].

Teimouri *et al.* explored the synthesis of functionalized piperidine by reacting aromatic aldehyde, aniline, and methyl acetoacetate using various nano-structured catalysts. All nano form of Zinc oxide, sulfated zirconia, γ-alumina and ZSM-5 zeolite catalysts investigated with diverse electron-donating and electron-withdrawing substituents on aldehydes and amines provided moderate to excellent yields of functionalized piperidines (Scheme 5). In general, electron-releasing substituents on anilines react faster as compared to electron-withdrawing substitutes on anilines. Among all the discussed catalysts in the investigation, sulfated zirconia was found to be the best choice in terms of superior catalytic activity [34]. In comparison with reported methods and catalysts, discussed in terms of yields, workup procedure, catalyst loading, and reaction conditions, sulfated zirconia was found to be the best catalysts for the synthesis of piperidines.

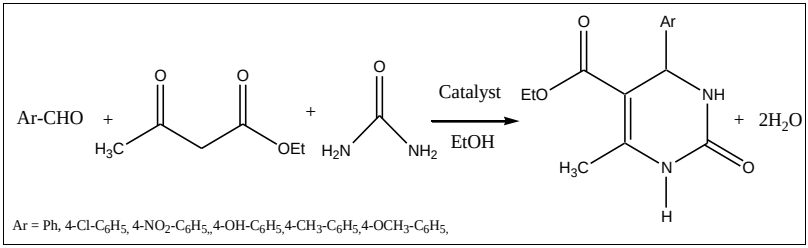


Scheme 5: Synthesis of functionalized piperidines using solid acid catalysts

2.5 Synthesis of dihydropyrimidones

Dihydropyrimidones and related structures have shown biological properties as calcium channel blockers [35] drugs to treat high blood pressure [36] α_{1a}-antagonists [37] and neuropeptide Y antagonists [38]. These compounds with considerable efficacy against HIV in AIDS treatment [39] also have been classified as a broad range of biologically active compounds [40].

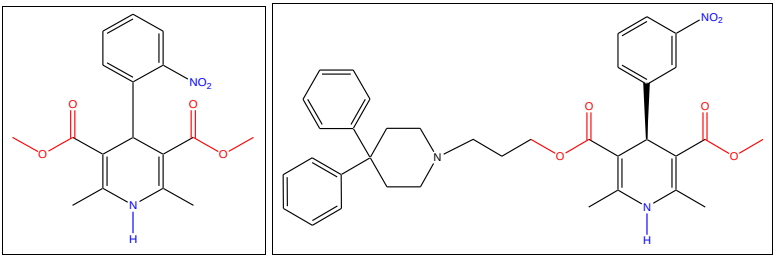
In 1891, Pietro Biginelli investigated a multi-component reaction between aldehyde, ethyl acetoacetate and urea which provides pyrimidines.[41] In recent years, reactions involving an aldehyde, β -keto ester, and urea using various Lewis and Bronsted acids have been reported as an alternative approach to the synthesis of dihydropyrimidone derivatives [42]. Teimouri *et al*., described nano solid acid-catalyzed synthesis of dihydropyrimidones derivatives using β-keto esters, aldehyde, and urea (Scheme 6). It also investigated the yield of dihydropyrimidones using various nano solid acids provide the order of, nano sulfated zirconia (10 mg) > nano -ZMS-5 (10 mg) > nano-γ-alumina (10 mg) = nano ZnO (10 mol%). Further, it also compared the earlier reports with nano sulfated zirconia in terms of reaction conditions and yield of the dihydropyrimidone derivatives [43]. It is concluded from the comparison of several catalytic activity studies, sulfated zirconia nano form was found to be the best candidate for the study of dihydropyrimidone derivatives.



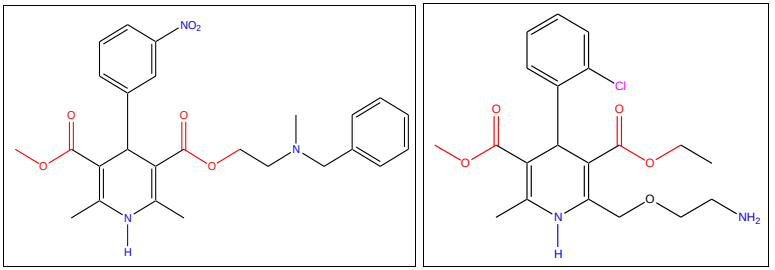
Scheme 6: Synthesis of dihydropyrimidone derivatives using nano solid acids

2.6 Synthesis of 1,4-dihydropyridines

In the realm of medicines and pharmaceuticals, dihydropyridines and their analogues are an important class of compounds [44]. There are number of compounds used in the treatment of high blood pressure based on dihydropyridines (Figure 1) [45]. A variety of diverse biologically active molecules, for instance, anticonvulsant, antidiabetic, anxiety, antidepressing, anticancer, analgesic, sedative, vasodilator, bronchodilators, hypnotic and anti-inflammatory properties, are common dihyddropyridine heterocyclic rings [46]. Additionally, 1,4-dihydropyridine products have a range of biological potency such as suppression of HIV protease [47].



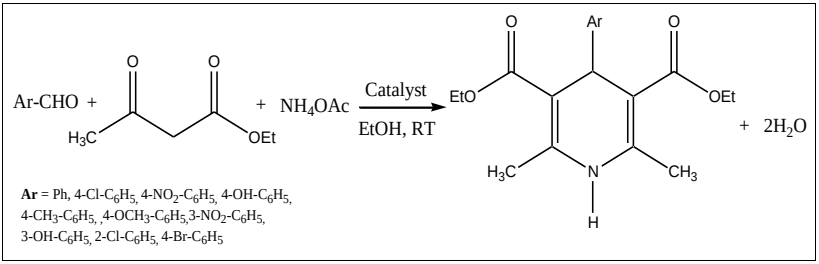
a) Nifedipine b) Niguldipine



c) Nicardipine d) Amlodipine

Fig 1: 1,4 dihydropyridine used in the treatment of hypertension

Teimouri *et al*. studied the multicomponent reaction between aldehydes with compounds of *β*-dicarbonyl and ammonium acetate catalyzed by nanostructured solid acids in ethanol at room temperature (Scheme 7). The catalytic activities of various solid acids were investigated at room temperature conditions for the study of 1,4-dihydropyridines, yields of nano-sulfated zirconia (10 mg, 92%) > nano-ZnO (10 mol%, 79%) > nano-γ-alumina (10 mg, 80%) and nano-ZSM-5 (10 mg, 85%). Further substrate scope was also explored with electron-rich groups attached to aldehydes providing better yields as compared to electron-deficient groups attached to the aldehydes [48].



Scheme 7: Nano solid acid catalyzed synthesis of 1,4-dihydropyridines

3. Conclusions and future opportunities

Sulfated zirconia in the nanocrystalline form of solid acids has demonstrated strong catalytic activities with a range of chemical processes such as multicomponent, etherification, esterification, substitution, elimination, and several industrially important organic transformations. The number of acid sites and higher surface area as compared to bulk catalysts makes the nano sulfated zirconia the best choice for many multicomponent reactions such as synthesis of imidazole, amidoalkyl naphthols, α-aminonitriles, functionalized piperidines, dihydropyrimidones, 1,4-dihydropyridines, etc. These Nanocatalyst have ample opportunities to substitute for many industrial processes which generally use environmentally harmful liquid Bronsted and Lewis acids. Many researchers have reported extensive discussions about the catalytic activity of bulk sulfated zirconia and associated catalysts in recent decades.

For other industrially relevant multicomponent reactions, there is a significant need to examine these nanomaterial systems. The surface characteristics of these nano-catalysts are early signs of improvement of the surface Bronsted and Lewis acidity by surface modification with anions. It would be possible to tailor the catalysts for environmentally benign chemical processes. Finally, several multicomponent reactions of industrial importance should be investigated. Many multicomponent reactions are not yet thoroughly investigated through the use of sulfated zirconia, which is extremely important for sustainable development in green chemistry.

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Vidya Prasarak Mandal's B. N. Bandodkar College of Science (Autonomous)

NAAC Reaccredited 'A' Grade, College with Potential for Excellence (CPE), Best College Award, ISO 9001: 2015 Certified, Recipient of RBNQA Commendation Certificate From IMC, Recipient Of Jagar Janivancha Awards of Govt. Of Maharashtra, Supported Under Fist O Level (Min. Of Sci. & Tech.) & DBT Star College Scheme, BEQET Special Citation Award

Jnanadweepa, Chendani Bunder Road, Thane (W) 400 601. Maharashtra

in association with

Mycological Society of India, Mumbai unit

Dr. V. V. Bedekar
Chief Patron

Dr. Moses Kolet
Principal, Convener

Dr. Kalpita Mulye and Dr. Jayashree Pawar
Organizing Secretaries

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THE LESSER-KNOWN OPPORTUNISTIC FACET OF FUNGAL GENUS CHAETOMIUM: A BRIEF REVIEW

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ABSTRACT

Fungi are omnipresent organisms in nature. While some cause allergies, others may be pathogens, a huge majority exhibit neutral behavior in their coexistence with humans and some also have valuable positive applications. Fungal diseases usually are difficult to cure, requiring perfect diagnosis and prolonged treatments. The levels of mycosis have appropriately been identified however this important topic, affecting mankind, is apparently and largely ignored in academic curricula. A matter of concern is those saprophytic fungi, not featuring in the usual groups of pathogenic fungi, and considered as 'not dangerous' for human beings. are increasingly found in the most unlikely locations, inside vital organs. The predominantly saprophytic genus *Chaetomium* is one such fungus, species of which have been isolated from unnatural and most unlikely locations, namely, from inside vital human organs and cutaneous infections indicating opportunistic behaviour. Reports of such case studies have increased in last 30 years and have generated concern and interest leading to this review study.

Introduction: Fungi are omnipresent organisms in nature. Most of them exhibit neutral behavior in their coexistence with human beings while sharing the planet while some have positive applications for wellbeing of mankind. Some fungi are also known to negatively affect human beings by way of causing allergies and mild discomfort. Yet others are known offenders causing invasive infections and mycosis of a more serious nature. Most fungal diseases and disorders are very difficult to cure, requiring perfect diagnosis and prolonged treatments. Besides the trauma, some disorders originating from fungi cause social inferiority complexes, social discomfort and negatively affect social interactions of the affected. Some infections such as those affecting finger and toe nails are also largely ignored unless severe repercussions arise in rare cases. The levels of mycosis have appropriately been identified from superficial to deep mycosis however this important topic, affecting all mankind, is apparently and largely ignored in academic curricula. Fungi causing mycosis are identified and grouped into functional groups such as dermatophytic fungi, keratinophilic fungi or simply disease-causing fungi. Most of such organisms are well documented in case studies.

A matter of concern is those saprophytic fungi, not featuring in the above groups, and considered as 'not dangerous' for human beings are increasingly being found inside vital organs, reports of which are rising in the last 30 years, raising doubts over whether entry and presence of fungi turning opportunists and ending up in form of invasive infections inside the organ can prove fatal to the individual. Such questions largely go unanswered and reasons are attributed to

compromised immunity of the patient along with some other predisposing factor or medical condition of the individual. The predominantly saprophytic genus *Chaetomium* is one such fungus, species of which have been isolated from unnatural and most unlikely locations, namely, from inside vital human organs, leading to questions on safety and life preservation. Reports of such case studies have increased in last 3 decades, sparking need for concern and have generated interest leading to this review study.

The Genus Chaetomium: The genus *Chaetomium* (Family Chaetomiaceae) was named so after its ascomatal hairs resembling plumes of helmet (Ames, 1961) used by soldiers in olden times. *Chaetomium globosum* Kunze was the first species described and this genus now has around 100 species according to current estimates; all of them predominantly saprophytic. The genus is a known member of the highly consistent group of cellulose degrading fungi and has widespread applications in disposal of agricultural residues and municipal solid wastes.

Species of *Chaetomium* are commonly found as saprophytes in soil, plant debris, decaying plant matter, dung, cellulosic substrates and organic wastes, residues and deteriorating cellulosic materials and articles of human origin and usage. Its ascospores are a common occurrence in air samples. *Chaetomium*, apart from its role as an indoor allergen, and few rare human pathogenic attributes of *C. atrobrunneum* Ames, was largely considered 'not dangerous' from human point of view; however in spite of its saprophytic nature, rare instances of pathogenicity, external as well as internal organ infections due to probable opportunistic pathogenic nature of this genus, even resulting in fatalities, have been reported in the last 30 years, mostly attributed to immunocompromised nature of patients and presence of predisposing factors. This study was taken up with this aim to shed light on invasive mycosis, the *Chaetomium* connection and this rare and untold story of genus *Chaetomium*.

Materials and Methods: Case studies on pathogenicity reports of genus *Chaetomium* were reviewed in order to determine the opportunistic pathogenic roles of its various species as probable causal organisms of invasive infections in external parts of the human body as well as internal organs. Selected indicative case studies from PubMed and NCBI literature sources were taken into consideration for the study, and limited to the ascomycetous genus *Chaetomium*.

Results and Discussion: The various instances of species of the saprophytic genus *Chaetomium* behaving as opportunistic pathogens and causing fatal infections to internal human organs are depicted in Table 1. The genus *Chaetomium* is fairly common in the air samples of indoor environments and a potential allergen (Apetrei et al., 2009; Salo et al, 2020). Some of its species are implicated in invasive infections and cutaneous infections suggesting opportunistic nature. Huang *et al*. (2018) indicated *C. globosum, C. atrobrunneum, C. strumarium* and *C. perlucidum* in invasive and superficial infections. Schulze *et al*. (1997) and Lesire *et al*. (1999) suggested that fungal invasion especially that of Chaetomium occurs only in presence of predisposing factors and is a consequence of

some other serious medical condition; which was observed in all cases reviewed during this study. Guppy *et al.* (1998) indicated *Chaetomium atrobrunnnium* also to be responsible for cerebral abscesses, which were till then assumed to be solely caused by Aspergilli. Thomas *et al*. (1999) hinted that the rapid development of infection in the brain is suggestive of the brain tissue providing favourable environment for growth and proliferation of the opportunistic invasive fungus. Barron *et al*. (2003) added *Chaetomium perlucidum* to the list of invasive species of genus *Chaetomium* and documented ability of this organism to spread beyond the central nervous system.

Yu *et al.* (2006) isolated *C. globosum* from necrotic tissues on the face and established its connection with painful erythema. *C. brasiliense* and *C. globosum* were reported from the ear canal and nail infection respectively (Hubka *et al*., 2011). *C. strumarium* (Reddy *et al*., 2017) and *Chaetomium* sp (Jayaraman *et al*., 2011) were reported to be associated with corneal infections. *Chaetomium* sp. was indicated in peritonitis (Issa *et al*., 2013).

Tap *et al*. (2015) reported *C. globosum* to cause cutaneous infection. This first described species of *Chaetomium* was reported from different parts of the world as the causal organism of onychomycosis (Stiller *et al*., 1992; Aspiroz *et al*., 2007; Latha *et al*., 2010; Hubka *et al*., 2011; Kim *et al*., 2013; Shi *et al*., 2016).

C. atrobrunneum was linked with causing pneumonia (Wang *et al*., 2016) and black grain eumycetoma (Madura foot) (Mhmoud *et al*., 2019). Earlier, Yeghen *et al.* (1996) linked *C. globosum* with pneumonia while Capoor *et al.* (2016) associated this species with invasive pulmonary infection stating *Chaetomium* has hidden clinical potential for causing invasive infections. Most of the cases cited were fatal while some reported cure.

Conclusion: It is apparent that emerging opportunistic fungal infections are rising and opportunistic fungi such as species of *Chaetomium* are being detected from most unlikely places such as inside human organs albeit in presence of predisposing factors, risk factors and compounded by immunocompromised status of patients. This is matter of great concern considering the rising pollution, predisposing factors and rising numbers of immunocompromised patients; and the fatal nature of such invasive infections.

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Table 1: Indicative instances of *Chaetomium* causing invasive infections in Human beings

No.	Species	Isolated from	Reported by and Year
1	*C. globosum*	Brain	Anandi et al., 1989
2	*C. strumarium,* *C. atrobrunneum*	Brain	Abbott et al., 1995
3	*C. globosum*	Lungs	Yeghen et al., 1996; Paterson et al., 2005; apoor et al 2016
4	*Chaetomium* sp.	Sinus	Aru et al., 1997
5	*C. homopilatum*	Trachea	Schulze et al, 1997
6	*C. atrobrunneum*	Brain	Guppy et al., 1998; Thomas et al., 1999
7	*C. perlucidum*	Brain, Heart, Lungs, Spleen	Barron et al., 2003
8	*C. globosum*	Lymph nodes	Teixeira et al., 2003
9	*C. globosum*	Necrotic tissue on Face	Yu et al., 2006
10	*C. atrobrunneum* *Chaetomium sp.*	Cerebro spinal fluid Lungs	Al-Aidaroos et al., 2007
11	*C. atrobrunneum*	Retina	Tabbara et al., 2010
12	*C. brasiliense* *C. globosum*	Ear canal Nail infection	Hubka et al., 2011
13	*Chaetomium* sp.	Cornea	Jayaraman et al., 2011
14	*Chaetomium* sp.	Abdomen, peritoneum	Issa et al., 2013
15	*C. globosum*	Cutaneous infection on foot	Tap et al., 2015
16	*C. globosum*	Toe nails	Stiller et al., 1992; Shi et al., 2016
17	*C. atrobrunneum*	Trachea	Wang et al. 2016
18	*C. strumarium*	Cornea	Reddy et al., 2017
19	*C. atrobrunneum*	Eumycetoma	Mhmoud et al., 2019
20	*C. strumarium*	Brain, CSF	Del Castillo et al., 2021
21	*C. globosum*	infection on legs	Cronin et al., 2021

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ISOLATION AND SCREENING OF POTENTIAL CELLULOLYTIC FUNGAL FLORA FROM DETERIORATINGWOODEN PANELS

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ABSTRACT

Introduction: Cellulose is the most over abundant natural substance on earth, found largely in plants. Several cellulosic primarily based objects have found their way into human needs and became articles essentially. Fungi inflicting deterioration of wood and wood objects; additionally referred to as wood-destroying fungi will breakdown cellulose by their cellulolytic enzymes, into simple sugars. Cellulase group of enzymes even have vital applications within the management of municipal solid wastes, agricultural residues and all organic residues and also additionally give organic matter in a readily usable form, for boosting soil fertility and productivity.

Materials and Methods: Isolation of fungal organisms from the wood samples was done by serial dilution method (Pramer and Schmidt, 1966). Cellulolytic activity of the isolates was determined by loss in weight of filter paper method (Fergus, 1969). The isolated fungi were identified using standard literature.

Result and conclusion: In the present work, deteriorating wooden panels were screened for the existence of cellulolytic fungi. Throughout the study the usually encountered fungal genera were *Aspergillus, Chaetomium* and *Penicillium*. Determination of the loss in weight of filter paper was evaluated for determining the cellulolytic activity of fungal organisms, throughout that5 isolates revealed promising cellulolytic activity.

Keywords: biodeterioration, wood samples, cellulolytic activity, cellulose degrading fungi.

INTRODUCTION

Plant biomass is the most overabundant carbon source on Earth and thus plays an important role in ecology and therefore the world carbon cycle. Fungi are extremely adequate and efficient degraders of plant biomass. Fungi play a vital role in the worldwide carbon cycle because of their ability to utilize plant biomass (polysaccharides) as carbon supply. Degradation of cellulose in nature by fungal organisms could be a well-studied phenomenon. Amongst the numerous subdivisions of fungi could be a comparatively little however extremely consistent group of cellulose degrading fungi, particularly the cellulolytic species. Over one hundred cellulolytic fungi are reported and this variety is increasing with advances in research analysis. Besides cellulolytic fungi, the cellulose utilizing population of microorganisms includes aerobic and anaerobic mesophilic bacterium, thermophilic and alkaliphilic bacterium, Actinomycetes and few protozoa. Among the foremost studied wood and cellulose degrader generaare *Chaetomium, Coriolous, Phanerochaete, Poria, Serpula, Aspergillus, Fusarium, Penicillium* and *Trichoderma*

[Viikari and Ragauskas, 2009] whereas *Clostridium*, *Cellulomonas*, *Thermomonospora*, *Trichoderma*, and *Aspergillus* are amongst the extensively studied producers of enzyme cellulase [Kuhad et al., 2011; Sukumaran et al., 2005].

Cellulose is the main polymeric component part of the plant cell wall. Cellulose is the most overabundant polysaccharide on Earth, and a vital natural resource. Its chemical composition consists of D-glucose residues joined by β-1,4-glycosidic bonds to make a linear polymeric compound chains of over 10,000 glucose residues. Cellulose contains both highly crystalline regions where individual chains are linked to each other and less-ordered amorphous regions. Though with the chemicals simple, the intermolecular bonding pattern might result in a very complex morphology [Hon, 1994]. Ascomycetous and Basidiomycetous fungi are the most potent degraders of this chemical compound as a result; several species grow on dead wood or litter, in environments rich in cellulose. Fungal cellulolytic systems differ from those of bacteria while the differences between individual taxonomic groups are less pronounced [Lynd et al., 2002].Cellulase because of its numerous and large relevance has been utilized in various industrial processes [Ekperigin, 2007; Chakraborty et al., 2000; Vaithanomsat et al., 2009] as well as in agricultural and plant waste management [Mswaka and Magan, 1998; Lu et al, 2004]. With the help of cellulolytic systems, cellulose can be converted to glucose which can be a multi-utility product, during a low-cost and biologically favorable method [Gupta et al., 2012]. The search for economical microorganisms which might manufacture all the 3 styles of cellulases that may facilitate the breakdown of cellulose to glucose is of dominant importance[Maki et al., 2009].

Considering the importance and applications of fungal cellulases, this study was designed to isolate and characterize economical and efficient cellulose degrading fungi from deteriorating wooden panels to put a base for agricultural application of cellulase producing fungi.

MATERIALS AND METHODS

Isolation of fungal organisms

Portions of wooden panels showing few signs of deterioration were collected from Thane and Bhiwandi in a western part of Maharashtra state. The samples once collected were stored in sterilized polythene bags at room temperature for more process. Deteriorated parts of the sample were scraped and serial dilution method [Pramer and Schidmt, 1966] was used for isolation of cellulolytic fungi on selective media CzepekDox Agar (CZA) for isolation of pure cultures of fungal organisms from the samples. Suspensions from the samples were diluted up to 10^5 and 1 ml each of the respective dilutions was plated on nutrient medium (CzapexDox Agar, CzapexDox Agar with cellulose and PDA) in separate petri plates. The plates were incubated 37° C room temperature for expression of fungal growth. Antibiotic like Streptomycin (50 mgl^{-1}) was supplemented to suppress bacterial growth and contamination. The isolated fungi were identified using standard literature and the standard system of fungal classification[Ames, 1969; Ellis, 1971; Pitt,

1979; Tzean et al., 1990].

Loss in weight of filter paper

The experiment was totally based on the method described by Fergus [Fergus, 1969]. Isolates were fully grown on Czapek Dox Broth [Difco Manual, 1969] on Whatman No. 1paper (circular discs, with diameter90mm) as the sole source of carbon, at pH 5.2 before autoclaving. Mean dry weight of the filter paper was recorded. The experiment was conducted in petri plates of 90mm diameter. Every petri plate contained one filter paper disc of known weight and 1 ml of CzapekDox Broth without sucrose. A skinny uniform mat of surgical cotton was placed on every petri plate below the filter paper for retention of wetness. The experiment was conducted with twelve replicates i.e. triplicates were maintained for the observance of the results at 7 day intervals up to 28days. The same set maintained in identical conditions functioned as the controlset. All plates were autoclaved at 15 lbs psi pressure for 20 minutes. The inoculum was prepared in the form of suspension of spores from 15-day old cultures of isolates fully grown on CzapekDox Agar medium by adding 10ml of sterile water. The mixture was shaken well using a vortex mixer to obtain a uniform spore suspension (10^5 spores/ml). 0.5ml of the suspension was added to each plate as inoculum. In case of the control set an equal quantity of sterile distilled water was used instead of suspension. The plates were incubated at room temperature (average mean temperature 28° C) for 7, 14, 21 and 28 days respectively.

At the end of the respective incubation periods the filter paper discs were oven dried at 80° C, allowed to cool down to ambient temperature in a desiccator and then weighed to the closest mg on an electronic weighing balance instrument. The difference in weight of every filter paper disc was calculated by comparing it with the original dry weight and additionally by considering the mean difference in weight shown by the control set. The net loss in weight was attributed to cellulose degradation. The results were recorded in terms of loss in weight further as a sign of deterioration on filter paper discs. The percentage loss in weight caused by every isolate was calculated by using the formula [Ghewande, 1977]

$$\% \text{ loss in weight} = \frac{\text{Difference in weight}}{\text{Initial Weight}} \times 100$$

RESULTS AND DISCUSSION

Table 1: Screening of isolated fungal organisms for their cellulolytic activity in terms of loss in weight of filter paper

No.	Organism	% loss in weight during period of incubation			
		7 Days	14 Days	21 Days	28 Days
1	*Aspergillusniger*	2.6	8.0	11.6	15.5
2	*Aspergillusterreus*	2.1	7.6	10.1	11.8
3	*Penicillium* sp.1	3.3	6.9	8.3	11.4
4	*Aspergillus* sp.1	5.6	9.3	13.7	15.3
5	*Chaetomium* sp. 1	6.3	11.5	14.3	16.9

6	*Penicillium* sp.2	3.1	7.3	11.6	16.4
7	*Penicillium* sp. 3	3.5	4.6	6.9	10.5
8	*Chaetomium* sp.2	2.7	5.5	7.9	11.5
9	*Aspergillus* sp.2	2.5	5.9	8.3	11.5
10	*Penicillium* sp. 4	3.5	13.6	16.3	18.3
Control	Control set	1.66 (+)	1.66 (+)	1.66 (+)	1.66 (+)

Mean initial weight of filter paper 853 mg +gain in weight 10 mg (1.66%) in control set.

A total of 10 isolates were obtained from the samples collected from wooden panels. Three completely different genera of fungi were encountered within the study on deteriorating wooden panels. Table 1 reveals progressive report of percentage of loss in weight of filter paper in the succeeding weeks. Five isolates, namely, *Chaetomium* sp.1, *Aspergillus niger*, *Aspergillus* sp.1, *Penicillium* sp. 2 and *Penicillium* sp.4 revealed superior activity in terms of percent loss in weight of filter paper.

Determination of the loss in weight of filter paper is one amongst tactic method commonly reported in order to evaluate the cellulolytic activity of fungal organisms, whereby the loss in weight is alleged to correspond to the amount of cellulose degraded, that successively correlate with the cellulolytic activity of the individual organism. Results obtained are in agreement with those of similar studies carried out [Lintang et al., 2021].

CONCLUSION

Fungal organisms isolated from deteriorating wood panels were individually tested for their cellulolytic capability in terms of percent loss in weight of filter paper. The efficient organisms can further be utilized for degradation of other forms of organic cellulosic waste which can be converted into compost which will benefit organic farming and help in restoration of soil fertility.

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STUDY OF SELECT ISOLATES FROM VETIVER ROOT MICROBIOTA AS POTENTIAL IMMOBILIZED BIOFERTILIZER

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ABSTRACT

Introduction: Rhizosphere, colonizes PGPR that promote plant growth and development. *Vetiver (Vetiveria zizanoides)* is a fast growing graminaceous plant native to India. It is known for its role in soil erosion control, phytoremediation, production of essential oils and tolerance to extreme climatic variations.
Materials & Methods: Plant growth-promoting rhizobacteria (PGPR) associated with *Vetiveria zizanoides,* were characterized and growth-promoting properties of selected isolates in free and immobilised form on *Vigna radiata* (moong) seeds was investigated. 16SrRNA gene analysis of the isolates were carried out.
Results & Conclusion: The endophytic isolate E1 and rhizospheric isolate R1 were capable of producing Indole Acetic Acid (IAA) and ammonia along with fixing nitrogen and solubilizing phosphate. Molecular identification by 16SrRNA gene analysis speculated isolate R1 and E1 to be 'yet uncultured bacteria'. Efficiency of E1 and R1 as potential biofertilizer on growth of *Vigna radiata* was evaluated in free suspension and immobilised (Calcium alginate and Polyvinyl alcohol) systems maintained in controlled environment (*in vitro* tube setup) and open pot setup. Comparable results were obtained with suspension system in pot and tube setup. Alginate immobilized cultures were able to promote plant growth in pot system when compared with *in vitro* system indicating involvement of environmental factors in determining final effect of isolates on promoting plant growth. However, PVA system were unable to germinate Moong seeds possibly because of reduction in cell viability due to exposure to boric acid during immobilization.

Keywords: PGPR, Vetiver, Immobilization

INTRODUCTION

Meeting the ever-increasing food demand is a serious concern in developing countries. In view of the well-known harms to environment and humans by the chemical fertilizer, use of biofertilizer is an important strategy in safe organic farming.

'Rhizosphere', 'the soil influenced by roots', colonizes PGPR that promote plant growth and development, by nutrient acquisition and assimilation (nitrogen, phosphorus, potassium, other essential minerals), phytohormone modulation, suppression of plant pathogens, combating abiotic stress (Pereira S. *et al*, 2020). Use of PGPR as an active ingredient of biofertilizers is an eco-friendly way to increase soil fertility and crop productivity.

Endophytes, the microorganisms found within plant tissues, are subset of the root microbiome that can be beneficial or non-

beneficial to the host plant. Many endophytic bacteria, however, have been shown to have PGP properties. Though many 'yet-unculturable' endophytes have been known, they have not been isolated in pure culture to facilitate their use in various applications (Gaiero et al., 2013).

The PGPR inoculant formulation can be either in free or immobilised form. Alginate and PVA polymers were selected as entrapping agents for present study. Alginate cross linked with $CaCl_2$ is most commonly used to immobilise microorganisms due to its low cost, non-toxic and biodegradable properties. Immobilisation of the bacteria in alginate beads leads to slow release of microbes for colonization of roots (Bashan Y., 2002). PVA cross linked with boric acid is used to prepare elastic beads with high strength and durability. Addition of calcium alginate helps to eliminate the agglomerization of PVA (Zhan J. *et al.*, 2013).

Vetiver (Vetiveria zizanoides) is a fast growing graminaceous plant native to India. It is known for its role in soil erosion control, phytoremediation, production of essential oils and tolerance to extreme climatic variations (Truong *et al*, 2000). At least few of the unique physiological characteristics of this plant are attributed to the microbiome associated with the plant rhizosphere (Chen et al., 2020). In our previous study (Pawar *et al.,* 2017), we have reported the isolation of endophytic bacterium isolate E1, and rhizospheric bacterium isolate R1 from roots of *Vetiver* using 'plant based dilute cultivation media'. These isolates were also shown to exhibit nitrogen fixation and phosphate solubilization activity. Present study aimed at molecular identification of these cultures, further study of the plant growth promoting activities of these isolates, along with determination of their efficiency as free-living cultures and immobilized biofertilizer.

MATERIALS & METHODS

Production of IAA:

IAA produced by the isolates E1 and R1 was determined using the colorimetric method described by Gordon & Weber (1951).

Ammonia production:

The isolates were inoculated in 1:100 dilute nutrient broth and incubated at 27±2°C for one week. 1ml of culture was added to 0.5ml of Nessler's reagent (Agbodjato *et al.*, 2015). The development of brown to yellow colour indicated ammonia production. Positive control with NH_4Cl and negative control with distilled water were maintained.

Molecular Identification and Phylogenetic Analysis of isolate E1 and R1:

Isolate E1 and R1 culture pellets were outsourced for molecular identification to Chromous Biotech Pvt. Ltd., Bengaluru, Karnataka, India.

For molecular identification, genomic DNA was extracted from the isolates followed by 16S rRNA gene amplification by Polymerase Chain Reaction (PCR) using consensus primers.

Amplification of bacterial 16S rRNA genes: The PCR mix contained the following: DNA, 1 µl; dNTPs 2.5mM each, 4µl; 10X Taq DNA polymerase Assay Buffer, 10 µl; Taq DNA polymerase, 3.0U/µl, 1µl; 16S rRNA gene

primers forward [5'–AGHGTBTGHTCMTGNCTCAS–3'] and reverse [5'–TRCGGYTMCCTTGTWHCGACTH – 3'], 400ng each; PCR grade D/W to make volume to 100 µl. All PCR reagents were of Chromous make. Amplification conditions were as follows: initial denaturation, 95°C, 5 min; 35 cycles (denaturation, 94°C, 30 sec; annealing, 50°C, 30 sec; extension, 72°C, 1.30 min); final extension, 72°C, 7 min. The PCR product of 1.5 kb was separated by agarose gel electrophoresis along with molecular weight marker.

Amplicon sequencing: The 1.5 kb 16S rRNA gene amplification product was sequenced bi-directionally by Sangers method. Sanger's sequencing was carried out in ABI 3500 Genetic Analyzer according to the BigDye Terminator v.3.1 Cycle Sequencing Kit protocol (Applied Biosystems, United States). The reaction mixture contained the following: Ready Reaction Premix, 4 µl; primer, 2 µl; Template,100 ng/ µl, 1µl; Milli Q Water, 3µl. Reaction conditions: 25 cycles (96°C, 5 mins; 96°C, 30 sec; 50°C for 30 sec; 60°C, 1.30 min).

Phylogenetic analysis: The 16S rRNA gene sequences were analysed by nucleotide BLAST and 'Seqmatch' tool of the Ribosomal Database Project. The sequences have been deposited in the ENA (European Nucleotide Archive) sequence repository (accession numbers LT984792.1 and LT984793.1). Phylogenetic analyses were conducted using the MEGA7 software package (Kumar et al. 2016). Sequences were aligned with closest BLASTn matches using the CLUSTALW algorithm. Molecular phylogenetic analyses were inferred by using the Maximum Likelihood (ML) method. Genetic distance between homologous sequences was calculated using the Tamura 3- parameter nucleotide substitution model. Both trees were rooted with the outgroup *Deinococcus radiodurans* strain DSM 20539 (NR 026401.1) and *Clostridium perfringens* strain ATCC 13124 (NR 121607.2).

Immobilization of the cultures: Both the isolates were inoculated in 1: 100 dilute nutrient broth and incubated at 27±2°C for one week. They were, individually and in consortium, were immobilized with the following two immobilizing systems: (i) 4% sodium alginate and chilled 6% calcium chloride (Schoebitz M. *et al.*, 2013) (ii) 8% Polyvinyl alcohol (PVA) + 1% sodium alginate and chilled 5% boric acid + 6% calcium chloride (Zhan J. *et al.*, 2013). The immobilized beads were stored overnight at 4°C and washed with sterile distilled water. Control beads were made with sterile distilled water.

***In vitro* and Pot system:**
Moong seeds were washed under tap water for 5 minutes followed by washing with diluted detergent Tween 20 for 20 minutes. Seeds were washed with distilled water 3 times; surface sterilized with 70% ethanol for 30 seconds, washed with sterile distilled water 3 times. They were transferred to 0.1% mercuric chloride with intermittent shaking for 4 minutes, washed with sterile distilled water 3 times and dried on sterile filter paper. Surface sterilized seeds were then transferred to sterile soil in tube. Following systems were maintained to evaluate the efficacy of immobilized isolate with suspension of the isolate:

(A) Suspension system:

- Control for suspension system (C): Soil fed with only water.
- Sus (E), Sus(R), Sus(E+R): Soil inoculated with free cell suspension of E1, R1, E1+R1 respectively.

(B) Immobilized PVA system:

- PVA (C): Soil with PVA beads without culture
- PVA (E), PVA (R), PVA (E+R): Soil with PVA immobilized E1, R1, E1 + R1 respectively.

(C) Immobilized Calcium alginate system:

- Alg (C): Soil with calcium alginate beads without culture
- Alg (E), Alg(R), Alg (E+R): Soil with calcium alginate immobilized E1, R1, E1+R1 respectively.

Each system maintained in duplicates was supplied with 5ml sterile distilled water on Day 1 to maintain sufficient moisture level in soil. After 7 days of incubation, seedlings were taken out of each of the system and its root length, shoot length was measured. Vigour index for each of the system was calculated.

$$Vigor\ index = \frac{Germination\ percentange * mean\ of\ seedling\ length\ (root + shoot)}{100}$$

For evaluation of efficacy of free suspension and immobilized endophytic and rhizospheric isolate as PGPR on *Vigna radiata* in natural environment, same systems as above were maintained in plastic bags containing non-sterile soil. Three seeds of *Vigna radiata* were sown in each of the plastic bag and watered daily. Root length and shoot length was measured and vigour index was calculated.

Statistical analysis:

The *in vitro* seed germination data obtained was subjected to Kruskal Wallis in SPSS software whereas data of pot system was analysed using ANOVA in SPSS software. The difference among various treatments means were compared using least significance difference (LSD) at 5% probability level.

RESULTS AND DISCUSSION:

In spite of several reports demonstrating the application of microbial products as biofertilizers, the full potential of several beneficial rhizobacteria as biofertilizers remains largely unexplored. Isolating efficient PGP strains that are part of different soil and root microbiomes, in formulations with longer shelf-life, would certainly play major role in this area.

Different PGP activities of isolate R1 and E1 were determined.

IAA production:

A wide range of processes in plant development are known to be regulated by exogenous IAA in which a low amount of IAA can stimulate primary root elongation, whereas high IAA levels decrease primary root length, increase root hair formation, and

stimulate the formation of lateral roots, giving plants greater access to soil nutrients. IAA produced by isolate E and R was found to be 4.75 and 3.625 µg/ml respectively after 48 hours of incubation.

Ammonia production:

Both isolate E1 and R1 showed change in colour to yellowish brown with Nessler's reagent indicating their ability to produce ammonia, affirming their nitrogen fixation ability.

Molecular Identification of the cultures:

BLASTn analysis of 16S rRNA gene of isolate R1 showed sequence similarity to 16S rRNA gene sequence of *Acinetobacter pittii* strain L3/3, a PGPR isolated from rice rhizosphere; as well as to that of uncultured bacteria. SeqMatch RDP (Sab score 1.000) tool supported these results, showing similarity to *Acinetobacter* as well as uncultured bacteria. Phylogenetic analysis in MEGA7 software, however, revealed its closest match to bacterium strain BS0171 and bacterium strain WG90917, both 'yet uncultured bacteria'.

BLASTn analysis of 16S rRNA gene of isolate E1 showed sequence similarity to uncultured bacterial 16S rRNA gene clones. RDP analysis confirmed its similarity to 'yet uncultured bacteria' (Sab score 1.000). Phylogenetic analysis in MEGA7 software also showed closest match to *Chryseobacterium geocarposphaerae* strain pgl4, isolated from rhizosphere soil of *Magnolia officinalis*.

Considering all these results, we speculate that isolate R1 and E1 might be 'yet uncultured bacteria' from rhizosphere and root tissue of *Vetiveria zizanioides* respectively. Further genetic analysis of the isolates needs to be performed to confirm the same.

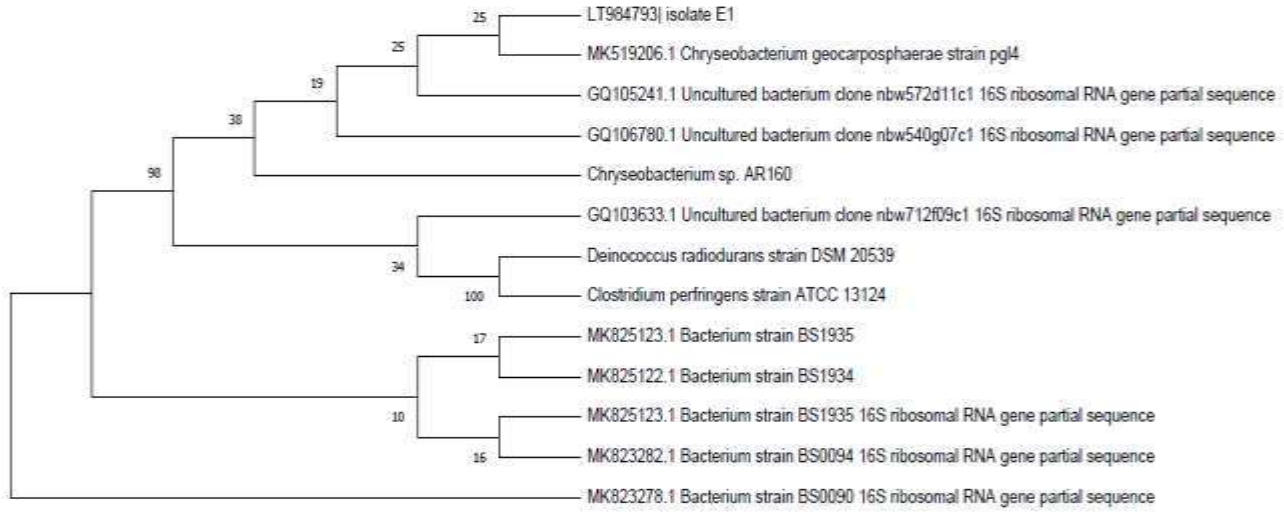

Fig.1 Phylogenetic tree of isolate E1

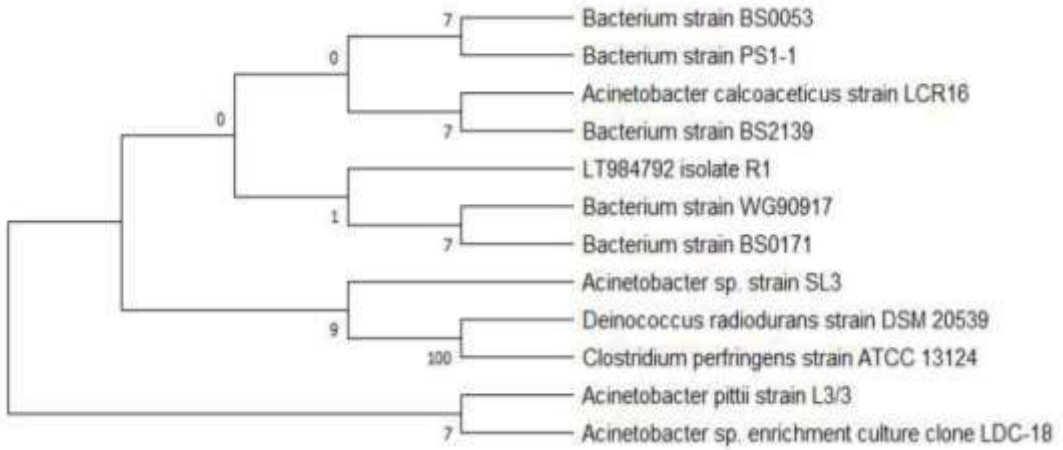

Fig.2 Phylogenetic tree of isolate R1

***In vitro* and Pot system:**

Sus (C) was found to give better vigour index than suspension E, R and E+R using *in vitro* setup (Kruskal Wallis, $p \leq 0.05$). Similarly, Sus (C) promoted plant growth significantly compared to other suspension systems in pot system setup. Hence, results obtained with E1, R1 and consortia of E1 and R1 were less promising in both the suspension systems. It might be because the isolates would be involved in enhancing the plant growth at later developmental stages and not in breaking seed dormancy.

However, unlike *in vitro* setup, Sus (E+R) showed significant effect in inhibiting plant growth compared to Sus (C) and Sus (E) (ANOVA, SPSS, $p \leq 0.05$) in pot system setup. This result might be attributed to the combined inhibitory effect of environmental factors plus consortium of endophytic and rhizospheric isolate on the growth of moong in open pot system. Similar effect in suspension system of *in vitro* setup was not seen probably because of controlled parameters.

Moong seeds were unable to germinate in PVA system in both *in vitro* and pot system setup. During immobilization, bacterial cells might be exposed to boric acid reducing cell viability. Sodium sulphate might be used as an inducer in crosslinking of PVA beads which can reduce the severe impact of boric acid on the cell viability (Takai T. *et al*, 2011).

In the *in vitro* setup, alginate immobilized R and E+R showed no significant difference in promoting growth of moong when compared with the control. Alginate immobilized E did not show any growth in *in vitro* system (Kruskal Wallis, $p \leq 0.05$). This might be because of inability of endophytic isolate to promote plant growth under controlled environmental conditions of *in vitro* setup (Kloepper *et al.,* 1980).

In pot system setup, calcium alginate immobilized E, R and E+R showed significant effect in promoting plant growth when compared with Alg (C) (ANOVA, SPSS, $p \leq 0.05$) indicating that the treatment might be enhanced by environmental conditions (soil characteristics and weather)

of pot system. Vigour indices also were found to be in parallel with the statistical analysis for both the systems.

System	(C)	Sus (E)	Sus (R)	Sus (E+R)	Alg (C)	Alg (E)	Alg (R)	Alg (E+R)	PVA (C)	PVA (E)	PVA (R)	PVA (E+R)
Vigour index (*in vitro* system)	228	137	134.5	224	146.5	0	57.25	75	0	0	0	0
Vigour index (Pot system)	254.3	249.3	230.3	159.7	23.7	183.3	167.7	165.7	0	0	0	0

Fig.3 Vigour indices of *in vitro* and pot system

Symbiotic bacteria are known to be present in the intercellular spaces of the host plant and may form mutualistic interactions with their hosts and penetrate plant cells (Vejan et al., 2016). Since isolate E1 was obtained as an endophyte from the roots of *Vetiveria zizanioides*, it's possible that it is highly specific in forming association and showing PGP activities with this particular plant only, and hence lower PGP activity was observed when *Vigna radiata* was used as the test system. Also, the isolates were obtained from *Vetiver*; a monocot plant whereas tested for efficacy in *Vigna radiata*; a dicot plant.

Different chemicals and signalling molecules in the form of root exudates released by the plant are known to elicit the interaction between plant root and soil microorganisms. Also, single change in plant genotype might alter the rhizosphere microbiome (Berendsen

R., *et al*, 2012). Thus, possible specificity of root exudates to deter or attract microorganisms might be an important factor responsible for the poor vigour indices.

Although PGPR are effective at promoting plant growth and development, few bacterial species may inhibit growth (Vejan et al., 2016). Hence, to peruse the plant growth promoting ability of the isolates, the experiments need to be repeated with *Vetiver* as test system.

CONCLUSION

Present study highlights the varied efficiency of probable yet uncultured bacteria isolated from *Vetiveria zizanoides*, on the growth of *Vigna radiata*. The results indicate that the efficiency of the microbiota as PGPR might depend on soil characteristics and plant species along with the form in which they are applied. Better understanding in context of host specificity, plant–microbial interactions, study based on randomized block design, prolonged observation of effect of isolate on plant growth and detailed characterization of yet uncultured bacteria is required.

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ISOLATION OF NOVEL PGPR FROM VERMIWASH MICROBIOTA

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ABSTRACT:

Introduction: Vermiwash is leachate collected from vermicompost pile. It is a rich source of vital nutrients that promote plant growth. A vast brigade of microbes flourishing in vermiwash contributes greatly to its nourishing potential. Regular farming practices involve use of diluted vermiwash and rarely its neat preparation. It is noted that Plant growth promotion capacities of vermiwash vary with respect to its concentration and plant species to which it is applied.
Materials & methods: Limited literature is available on exploring the possibility of exclusive microbial community thriving at different concentrations of vermiwash that might be contributing in rendering growth promoting attributes. Present study explores different 'Culturing' approaches for isolation of novel Plant Growth Promoting Rhizobacteria (PGPR) and for analyzing exclusive bacterial flora of vermiwash at different concentrations and their individual nourishing potential. Different concentrations of vermiwash viz. 10%, 20%, 50% and neat sample were used and subjected to culturomics approaches namely- media based variation and sample- based variation. Select microbial isolates thriving exclusively at specific vermiwash concentrations were considered for molecular analysis using 16s rRNA gene sequencing studies.
Results and Conclusion: The results revealed, four of the six isolates to be probable novel PGPR. All the isolates could fix atmospheric nitrogen, four isolates could produce auxin IAA and one could also solubilize phosphate. The use of dilute media simulating natural environment was found to be effective approach for cultivation of 'yet uncultivated' bacteria. Vermiwash concentration seemed to be an important factor determining the prevailing microbiome.

***Keywords*:** vermiwash, microbiome, media-based variation, sample-based variation, 16S rRNA gene analysis

INTRODUCTION:

Uncontrolled applications of chemical fertilizers for maximizing yield outputs have worsened soil health during the era of green revolution, so much so that one may term this era as era of "greed evolution". Besides posing severe pollution and health hazards to community, the same has also devastated agro-economy of the country. Thus, emphasizing on increasing use of effective biofertilizers as alternate or complementary measure is a priority. Organic compost, humus, vermicompost and vermiwash are proven bio fertilizers which were and are still reliable ones among most frequently applied biofertilizers.

Contribution of earthworms in conventional farming is incomparable. In natural habitats, earthworms help degrade organic waste material to form Humus and manage nutrient flux through

'vermicomposting'. Besides humus, it generates another valuable liquid manure namely vermiwash. Vermiwash is a liquid that can be collected after the passage of water through an active vermicompost pile. It is a collate of excretory products, mucus secretion of earthworms along with micronutrients from the soil organic molecules. Earthworms indirectly influence the dynamics of soil chemical processes and affect the activity of the soil micro-flora. Vermiwash as well, harbor a vast brigade of microbes that contributes greatly to its nourishing potential. Number of studies reported plant growth promoting effect of vermiwash used at lower concentration. Efficacy of dilute vermiwash when used as foliar spray and liquid manure has also been substantiated. (Mohammad H., 2014; Gopal M., 2010; Mujeera, F., and Malathy, S. 2014.) In present study, we aim to isolate novel Plant Growth Promoting Rhizobacteria from Vermiwash microbiota, hypothesizing that specific concentrations of vermiwash would have unique microbial communities. These novel PGPR isolates can be further explored as potential biofertilizers.

MATERIALS AND METHODS:

Collection of vermiwash:

To active vermicompost pile (earthworm species*: Eisenia fetida*), water was evenly sprinkled and allowed to seep through the vermicompost bed overnight. The dark brown colored, seepage drained from the compost bed was collected in the collector which was then filtered through Whatmann Filter paper No. 1 and stored in sterilized glass bottle. The sample was kept in refrigerator till use.

Preparation of Vermiwash samples

Vermiwash samples were diluted using sterile Distilled water to get 10%, 20%, 50% concentrations and one sample used for undiluted or Neat. The samples were incubated at room temperature overnight before they are used as samples for culturing the microflora.

Media based variation:

To study the influence of concentration of media components and presence of known and unknown growth factors on microbial recovery three media formulations were used.

A) Use of General-purpose medium:

Conventional nutrient agar medium was prepared mixing 1.3g dehydrated Nutrient Broth powder (Himedia laboratories) and 3g Agar Agar powder in 100mL distilled water. The media was sterilized and then used for making the culture plates by pouring 20 ml molten medium in individual plate.

B) Use of 1:100 diluted medium containing 10% vermiwash:

To make dilute medium simulating natural environment 0.013g dehydrated Nutrient Broth powder (Himedia laboratories) was mixed with 90mL Distilled water and 10mL vermiwash. The medium was autoclaved at 121°C for 15 min. before making plates.

C) Use of Gradient plate technique:

Concentration gradient of vermiwash microenvironment was established using basal agar which was overlayed with vermiwash medium.

Basal medium: 0.013 g Nutrient Broth + 100 mL Distilled Water + 3 g Agar agar

Overlayed medium: 0.013 g Nutrient Broth+ 100 mL Vermiwash + 3 g Agar agar

The vermiwash present in top layer would diffuse vertically to form smooth gradient of vermiwash concentration.

Sample Preparation

After overnight incubation at room temperature the samples viz. 10%, 20%, 50% dilute samples and Neat sample (i.e. undiluted vermiwash) were used for streaking general purpose media , 1:100 diluted nutrient media plates and for spreading the gradient media plates. All the plates were incubated at room temperature for three weeks and observed daily for colonies and micro colonies.

Analysis of Plant Growth Promoting Ability:

Nitrogen Fixation: All the isolates were screened for nitrogen fixation capacity by culturing them on Ashby's Mannitol Agar.

Phosphate Solubilization: Pikovaskayas's Agar (Himedia laboratories) was prepared and used for culturing and screening phosphate solubilization capacities. The isolates revealing zone of clearance were considered as phosphate solubilizers.

Indole Acetic Acid production (Plant Growth hormone): IAA assay

IAA produced by the isolates was determined quantitatively using colorimetric method described by Gordon and Weber (1951). The isolates were grown in nutrient broth containing 0.1g/L of L-tryptophan at 27± 2°C for 48 hours. After incubation, culture was centrifuged at 8000 rpm for 10 minutes. 1mL of the supernatant was added to 2mL of Salkowaski's reagent (0.5M $FeCl_2$ and 35% H_2SO_4) and incubated at room temperature for 25 minutes. The pink coloration developed was measured colorimetrically at 530nm.

Gram staining and basic biochemical profiling:

The selected isolates were Gram stained by following the standard protocol. Standard biochemical tests IMViC, catalase and oxidase were performed.

16s rRNA gene analysis:

The isolates were selected on the basis of their exclusive presence and growth promoting features and were maintained on dilute nutrient medium. After incubation period they were subjected to 16s rRNA gene analysis.

Molecular Identification and Phylogenetic Analysis of Cultures:

Cloning based on 16S rRNA gene identification technique was employed for molecular identification of the cultures. The 16s rRNA gene sequences were further analyzed by nucleotide BLAST and SeqMatch tool of Ribosomal Database Project. The sequences have been deposited in the ENA (European Nucleotide Archive) sequence repository. Phylogenetic analyses were conducted using the MEGA7 software package (Kumar et al.,2016)[1]. Sequences were aligned with closest BLASTn matches using the Clustal W algorithm. Molecular phylogenetic analyses were inferred by using the Maximum Likelihood (ML) method. Genetic distance between homologues

sequences was calculated using Tamura 3-parameter nucleotide substitution model.

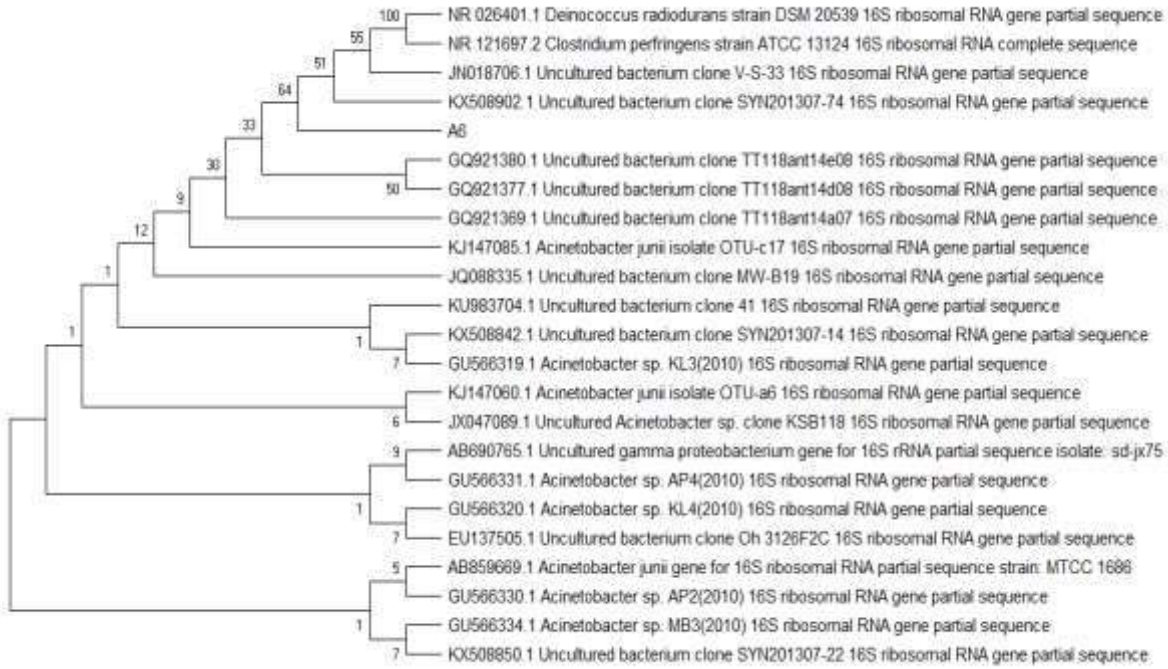
Observation table:

Isolate/ Accession number	Colony Characteristics	Gram Nature	BLASTn	RDP	Phylogenetic Analysis
A6 LR596009	Concentric colony, fluorescent	Gram negative	Uncultured /*Acinetobacter* (100%)*	*Acinetobacter* / uncultured (1.000)#	Uncultured bacterium
A8 LR596011	Pink, circular, fluorescent, centre raised	Gram negative, bacilli, capsule like structure	*Methylobacterium aqaticum* (99%)	Uncultured *Alpha proteobacterium* (1.000)	Uncultured bacterium
B1 LR596010	Agar embedded, slightly raised, irregular	-	Uncultured / *Pseudomonas* (100%)	*Pseudomonas* / Uncultured (1.000)	*Pseudomonas stutzeri*
B4 LR596012	Small colony, raised, irregular	Gram positive, Diplococci in cluster	Uncultured / *Acinetobacter* (99%)	*Acinetobacter* / Uncultured (1.000)	Uncultured bacterium
C3 LR596013	Faded, concentric, slightly irregular, fluorescent, flat	Gram negative, coccobacilli / rods (might be mixed)	Uncultured bacterium / *Rheinheimera sp*. (99%)	Uncultured bacterium / *Rheinheimera sp*. (0.986)	Uncultured bacterium
D2 LR596789	Fluorescent, diffused growth, irregular, Flat (Pseudomonas like)	Gram negative, rods	*Pseudomonas* (99%)	*Pseudomonas* (0.983)	*Pseudomonas sp.*

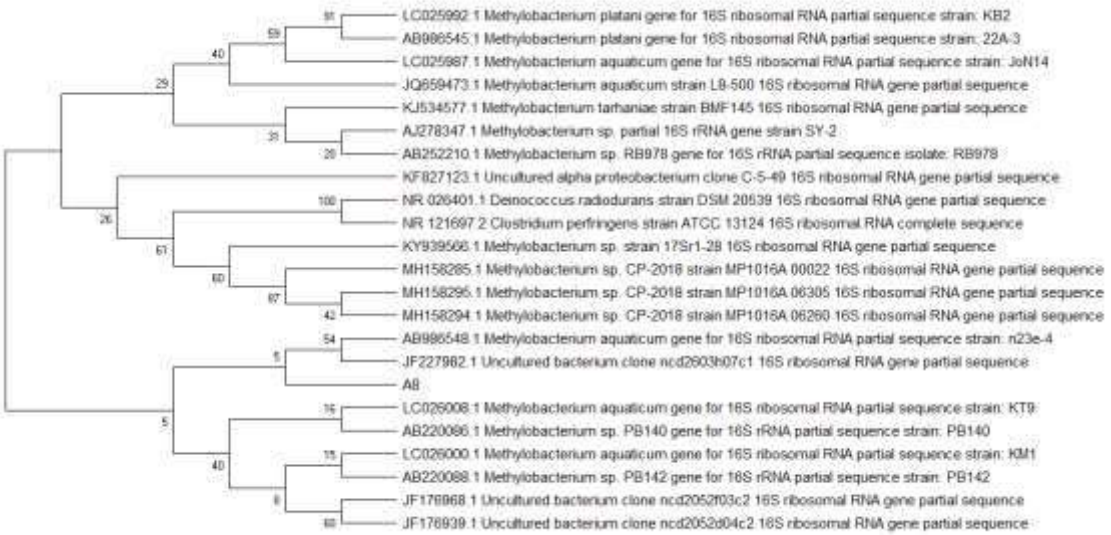
[* - Score, # - S_ab]

Phylogenetic trees:

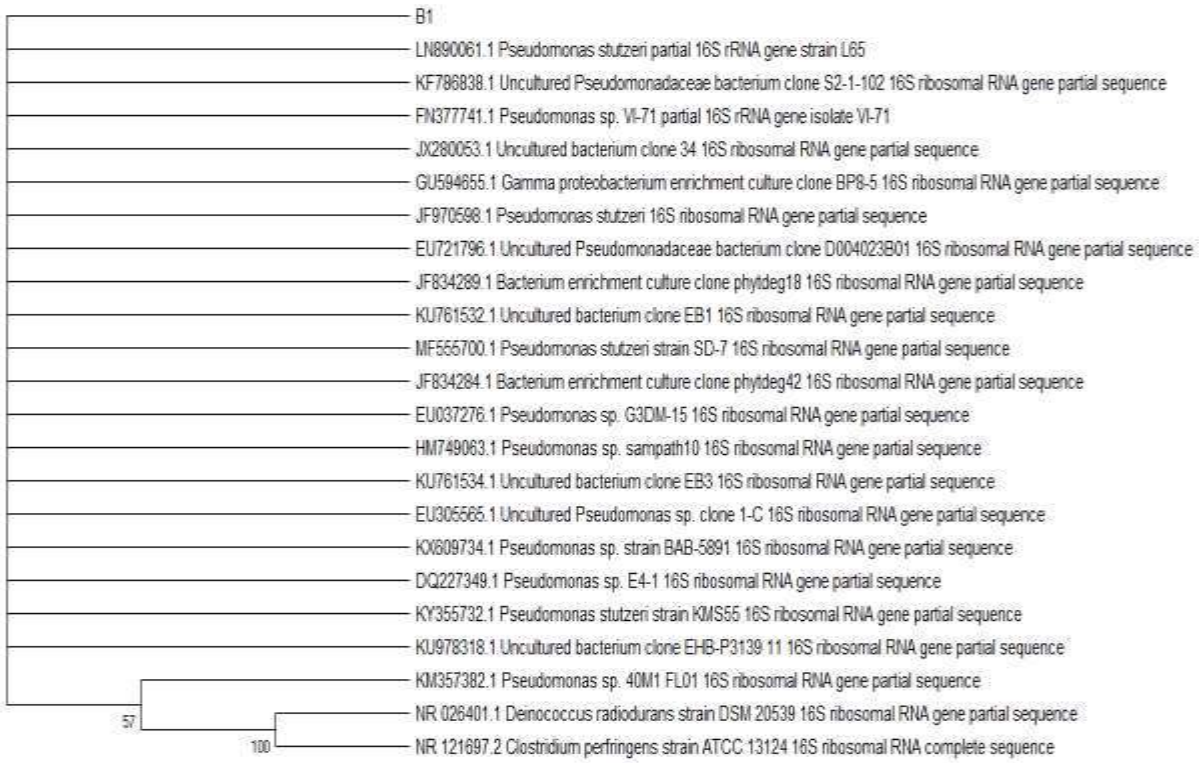
Isolate A6:



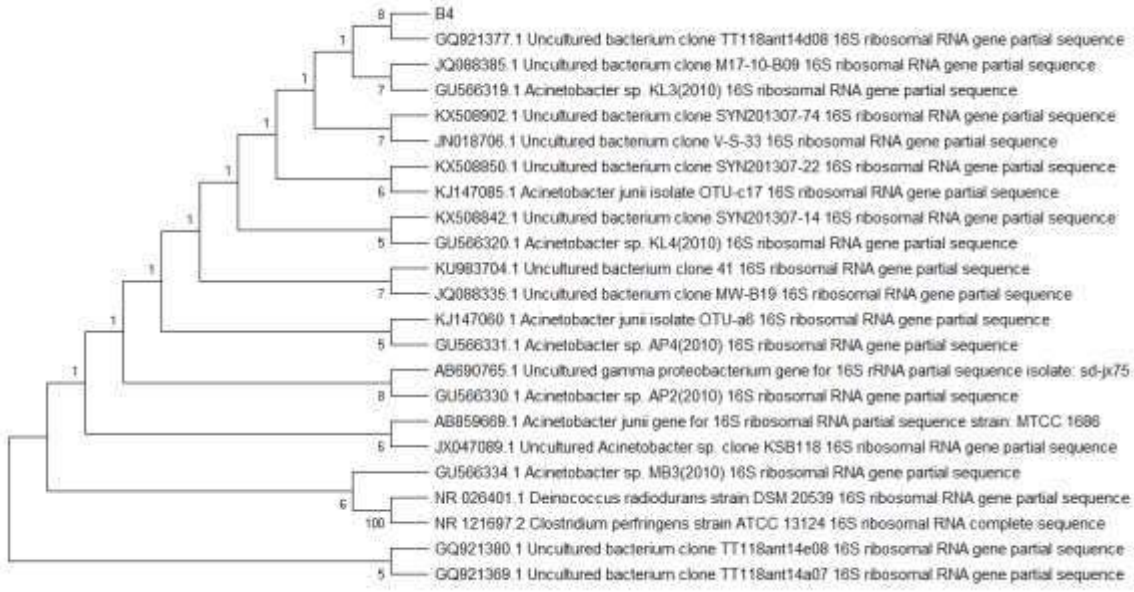
Isolate A8:



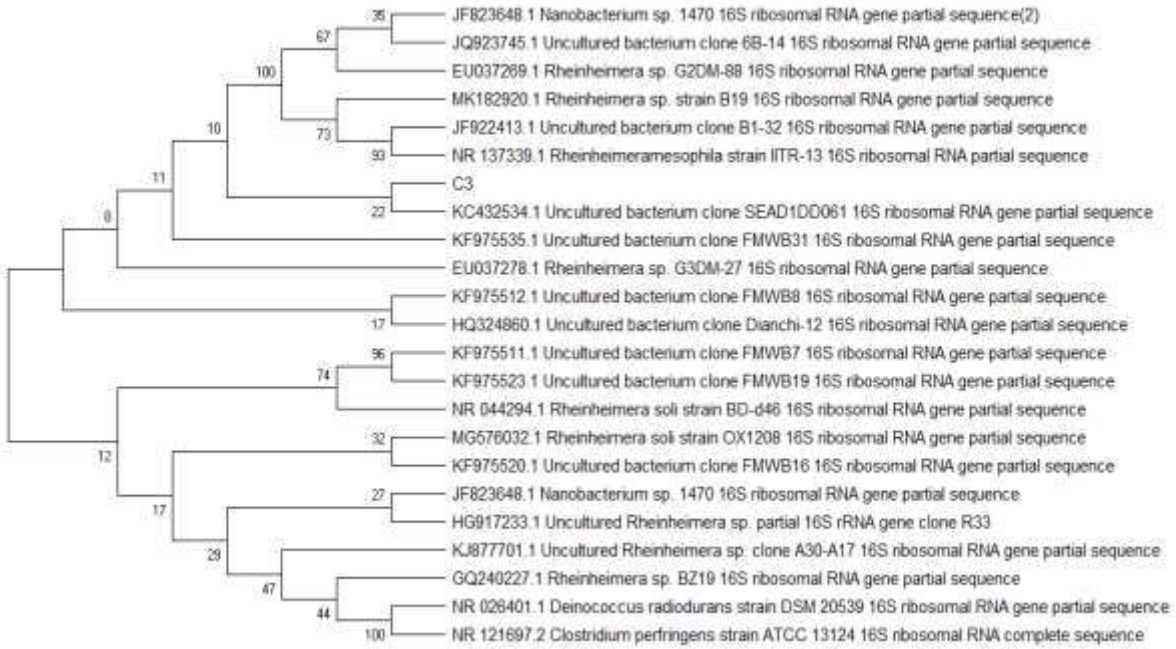
Isolate B1:



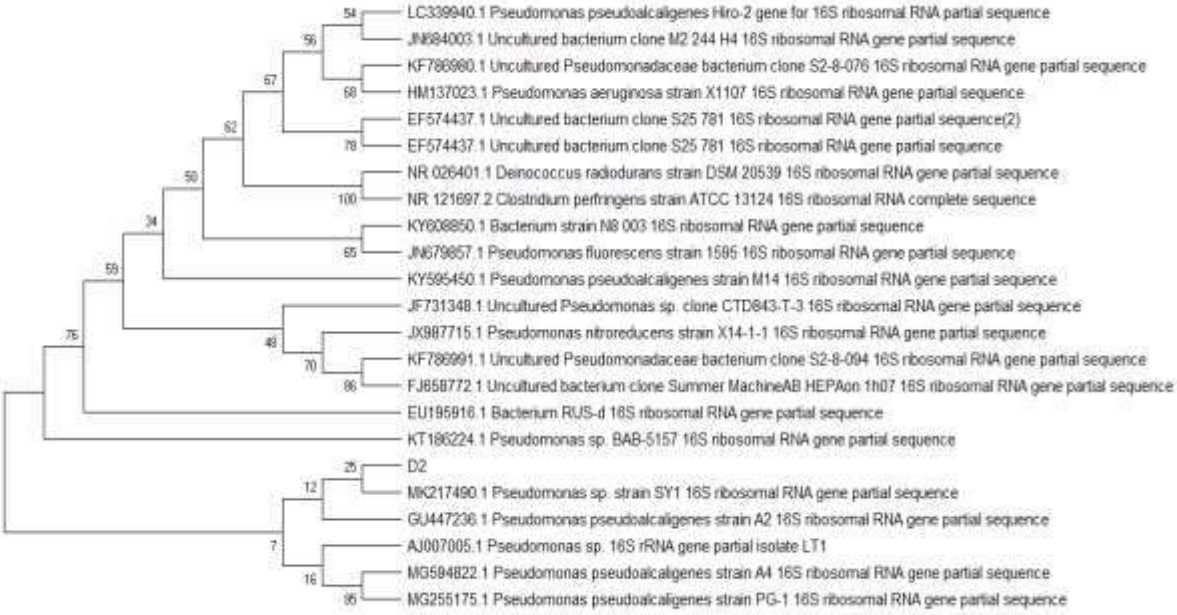
Isolate B4:



Isolate C3:



Isolate D2:



DISCUSSION:

Vermiwash is one of the most popular organic fertilizers that are being used effectively in the organic farming practices. Studies have reported different modes of preparation of vermiwash and its varied application as well. Numerous studies have reported Its efficacy as a soil additive and foliar spray for various crops and even for ornamental flowers. (Chattopadhyay A. (2015), Murali G. et al (2010)

In present study, we attempted to compare, the microbial recovery on general purpose media and that on dilute medium simulating natural environment was drastically different. General purpose medium revealed many of *Pseudomonas sp.* dominating. In contrast, the dilute medium showed enormous microbial diversity in form of vast number of micro-colonies that were observed to be slow growers.

It is observed that classical microbiological strategy involves study of microbes in isolation however in reality, the microbes tend to work closely and not in isolation. This is identified as one of the major hurdles, why few species resist to get recovered during their culturing *in vitro*.

All four dilutions viz. 10 %, 20 %, 50 % and neat or 100% samples when streaked on general purpose medium and dilute medium showed microbial diversity which was noted in 10%, 20% and 50% whereas, the neat samples showed dominant presence of *Pseudomonas spp.*

Reason of this observation is probably the chance that these microbial communities perform Cell-Cell Communication, and the environment has a low nutrient concentration where oligophiles can flourish.

Also, we used gradient plate technique where we created the gradient of concentration of vermiwash on which an attempt was made to check if specific types of organisms get cultured at specific concentration of vermiwash. When vermiwash samples were spread on such gradient medium preferential growth trends were noted, through microcolonies appearing at lower concentrations of vermiwash and prominent presence of *Pseudomonas sp.*

Gradient plate technique is conventionally being used to study antibiotic resistance. Apart from this, the technique has been used to study the effect of variable environmental factors on the growth of particular organism [Panagou E. *et al* (2005)] ; to screen a potential strain for biomineralisation of contaminants[Gajendiran A. *et al* (2017)] [Bhalerao T. *et al* (2007)]. We have used Gradient Plate technique to isolate novel PGPR from Vermiwash. As per our literature study, prior to us only Webster N. *et al* (2000) has used this technique to isolate previously uncultured bacteria associated with a marine sponge. Thus, our approach of isolating novel bacteria using Gradient plate technique is somewhat a less explored application of this technique.

Depending on exclusive presence and striking morphological features 6 isolates were selected for further analysis.

Using nucleotide BLAST and SeqMatch tool of Ribosomal Database Project, 16s rRNA gene sequences were analysed and compared with their microbial and cultural morphologies for final phylogenetic analysis.