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RESEARCH ARTICLE

REVIEW ON ADVANCEMENT OF BIOFILM BIOFERTILIZER AND ITS IMPACT ON STRESS RESISTANT PLANTS

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Abstract

Nutrients from plants are essential for the cultivation of crops and the production of healthy food to feed the world's growing population. The impacts of global warming threaten nearly every economic sector, including agriculture. In order to maintain ecological equilibrium, microorganisms play an important role in the natural carbon, nitrogen, sulphur, and phosphorus cycles. Microbial biofilms are an intriguing issue because of their implications for ecology, economy, and human health. Advances in biochemical and molecular approaches have helped us gain a better grasp on how biofilms form and evolve. Growers are starting to take notice of biofilms because of their vast potential in agricultural productivity, security, and enhancement. Biofilms not only increase plant productivity, but they also play a critical role in the colonization of surfaces including soil, plant roots, and plant shoots. Utilizing bio-fertilizers allows plants to better absorb nutrients, thrive, and resist environmental stresses such as drought and pests. Potential biological fertilizers would be essential to the productivity and sustainability of soil, as well as to environmental conservation, because they are both environmentally beneficial and economical inputs for farmers.

Introduction:-

Agriculture, like most other industries, is vulnerable to the effects of a warming planet. Plants and animals in an ecosystem are always interacting with one another, either mutually beneficially or competitively. Maintaining a stable and well-balanced ecosystem is crucial so long as all of its interactions continue to function normally. Soil microbial diversity has a major effect on ecosystem function stability [1] and insects play a crucial role in forest structure and diversity. Especially in arid regions, global warming has a negative impact on soil fertility. Soil organic matter will decrease as a result of higher soil temperature since its breakdown will proceed more quickly [6]. Soil infertility is the primary factor limiting agricultural output in developing nations; say Khosro and Yousef [3], especially for smallholder farmers. Therefore, in large swaths of the world that need the basics of good agricultural practise, preserving soil quality can assist to alleviate problems associated with land degradation, decreasing soil fertility, and rapidly diminishing productivity [11].

Due to tremendous advancements since the 12th century, agriculture is currently widelypractised all over the world. Food and Agricultural Developmentreportsthat in India, the agricultural and related industry employs 54.6% of the labour force and generates 17.8% of the country's GDP in 2019-2020. Twenty percent of the African population

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relies on agriculture as their main source of income, and in many parts of Nigeria, particularly the rural regions, people rely only on agriculture as their means of survival.

Globally, farmers have likely employed fertilisers to increase nutrient availability. There are two basic categories of fertilisers. Both chemical and biological fertilisers (a and b). To meet the demands of a rising human population, conventional agriculture relies heavily on the use of chemical fertilisers and pesticides. Chemical fertilisers provide consistently consistent amounts of nitrogen, phosphorous, and potassium since they are produced in factories. Overusing chemical fertilisers (CF) reduces agricultural yields by weakening plant roots and increasing susceptibility to hazardous diseases due to a lack of microbial diversity in the rhizosphere [11]. This has a domino effect on the soil and plant communities, leading to a breakdown of their connections [4]. Because of eutrophication, it also contributed to air and ground water contamination. Soil acidification is hastened using chemical fertilisers and pesticides; however Chun-Li et al. warn that this practise also threatens ground water supplies. [7] Biofertilizer has been identified as an alternative to chemical fertiliser for increasing soil fertility and crop yield in sustainable farming. Biological fertilisers have the potential to be both environmentally benign and cost effective for farmers, making them an important input for ensuring the long-term viability of soil production. They would also serve to protect the natural world [3]. Beneficial bacteria and fungi, such as nitrogen-fixing bacteria and arbuscular mycorrhiza fungi (AMF), also known as plant growth-promoting rhizobacteria, are increased in the soil after biofertilizer has been applied (PGPR). Unfortunately, farmers have been slow to embrace biofertilizer. When compared to traditional biological fertiliser, biofilm biofertilizer is superior in its capacity to boost crop yields and its resistance to environmental challenges, predators, and antagonists [12].

O'Toole et al. [2] describe microbial biofilm as populations of one or more microbial species adhered to an abiotic or biotic surface by means of an extracellular polymeric material that is both sticky and extracellular (EPS).

Algae, bacteria, or other sorts of cells can form organised communities inside a self-produced polymer matrix and connect to a nonliving or living surface. Biofilms are populations of microorganisms that have become well-organized and adhere to man-made surfaces. Biofilms, as defined by Seneviratne et al. [4], are heterogeneous colonies of a wide variety of microorganisms that form on or around plant roots. The communities that form in biofilms can either be harmful/pathogenic or helpful [33]. Root-bound beneficial biofilms on some crops may aid in nutrient cycling, plant growth promotion chemical production (such IAA), and pest and disease biocontrol [50]. It is possible to cultivate these biofilms in a laboratory dish. This review will discuss the role of biofilm biofertilizers in sustainable agriculture in an effort to meet the needs of agriculturists and plant biologists whose work focuses on developing safe and effective ways to improve soil quality by feeding and maintaining the beneficial and natural flora of microorganisms. In addition, it details the most up-to-date findings in agricultural management, which show the benefits of using biofilm biofertilizers in terms of enhanced nutrient profiles, plant growth and production, and resilience to stress.

Effect of various elements on soil fertility

The soil is the medium in which most microorganisms and plants thrive. the topsoil, the subsoil, and the parent material are its separate levels. To grow healthy plants, you need soil that has the right balance of minerals, air, water, living organisms, and inorganic and organic materials, as well as a neutral or slightly alkaline pH. Half of a soil's volume is made up of minerals, and these minerals typically consist of 93% silica, 8% aluminium oxide, 4% calcium oxide, 4% potassium oxide, 3% sodium oxide, and trace quantities of chlorine, molybdenum, zinc, copper, boron, phosphorus, and sulphur, among many others [14]. Nitrogen

One of the most important elements for plant growth is nitrogen. The formation of RNA and DNA base pairs, protein phosphate groups (such as the hemp group of chlorophyll), hormones like cytokines, metal uptake, transport in xylem and phloem, osmoregulation (such as in lettuce and spinach), alkaloids, and microbiochemicals like mescaline and quinine are just a few of its many roles.[13]. Fertilizer, biological nitrogen fixation, rainfall and thunder, and the decomposition of organic matter all provide nitrogen to the soil in the form of nitrates, ammonium, and rarely urea [46]. If a plant is lacking in nitrogen, it will show symptoms like as stunted development and pale green or yellow leaves, as stated by Barak [13]. One of the most severe signs of insufficiency is necrosis that forms a "v" pattern at the tip of older leaves.

Potassium

Water absorption, root development, transpiration, and stomatal control are all areas where potassium has a role, making it a key factor in the water economy and crop growth. It promotes cold tolerance and resistance to bacteria and fungi in some plants, which is important because: [5] The cell walls of cereal straw get thicker as a result of the increased production of high molecular carbohydrates. It aids in photosynthesis, protein synthesis, and fruit quality; it catalyses the actions of various enzymes; it is required for the proper functioning of guard cells; and it stimulates the production and accumulation in plants of certain vitamins. (1,13). And those are potassium's roles in the body. Potassium ions, which are absorbed by plants, are insoluble in water.

Phosphorus

Phosphorus is required in higher concentrations than nitrogen and potassium, but lower concentrations than both. The development and size of nodules, as well as the overall productivity of legumes, are all boosted by this treatment. Phospholipids in the membrane increased both the quantity and quality of the crop [36]. Seeds can't grow without it. Phosphates, which are introduced to the soil in the form of fertilisers and super phosphates, are the primary type of phosphorus taken by plants. Phosphorus is phloem mobile, hence the effects of a deficit can be seen everywhere in the plant, as stated by Barak (13). In extreme circumstances, the leaves of grassy plants like maize can become red. This, according to Scalenghe and coworkers. Inorganic phosphorus makes up the vast bulk of the element, and too much of it is hazardous since plants can't utilise it. The symptoms of phosphorus overdose in plants are premature plant maturity and poor crop production.

Biofilm biofertilizer and its impact on Agriculture

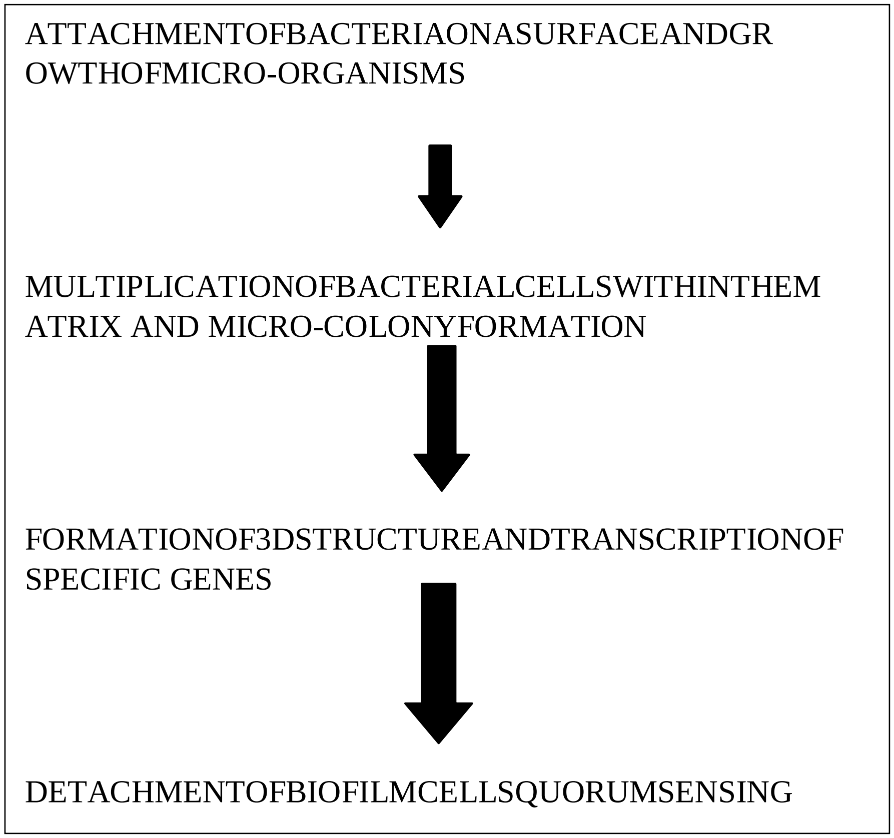
“Populations of microorganisms concentrated at a solid-liquid interface and surrounded by anextracellular polymeric substance matrix”. Strongly connected communities of microbes on a surface, formed by the secretion of an extracellular polymer matrix. This area is either completely covered by water or is situated in a very humid setting. Theymay contain->

1. Live/deadcells
2. Protein
3. Sugars/Polysaccharides
4. Metabolites
5. Quorum sensing signaling molecules

Biofilm Researchers at the National Institute for Fundamental Studies created the biofertilizer and were granted a patent for it. Biofilms are communities of microorganisms that form on or near a surface or other interaction. [15] Syntrophic food uptake and protection from environmental challenges (such as UV radiation, desiccation, acidity, alkalinity, and osmotic shock) are only a few of the benefits that biofilm development delivers to its microbial partners. [22,23]. Persisters, subpopulations of resistant phenotypes inside the biofilm, contribute to antibiotic resistance as well. [24]

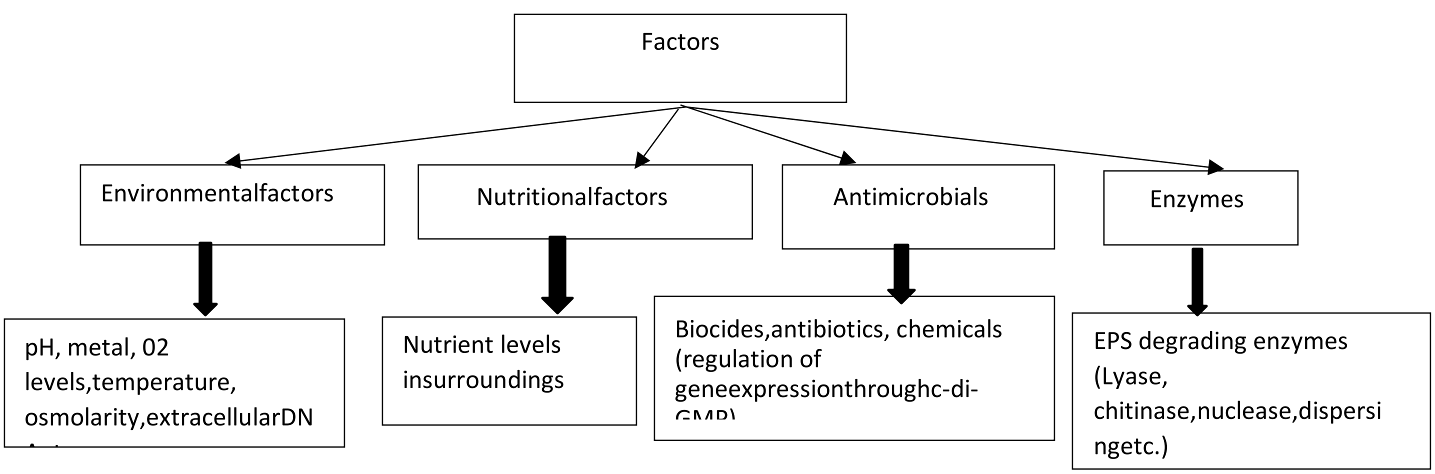
Biofilmformation

It is common practise to choose antibiotic-resistant microorganisms for biofilm development [24]. There are four steps involved in making biofilm bio-fertilizer [25]. Micro-colony creation begins with bacterial adhering to the substrate, leading to rapid proliferation of the micro-organisms. Now, EPS, which includes carbohydrates, proteins, nucleic acids, and lipids, is produced as a consequence of transcription of certain genes from the interacting bacterial cells. The process of attachment might trigger the production of extracellular matrix. With increasing EPS thickness, the biofilm becomes anaerobic, and polysaccharide-cutting enzymes eliminate any newly created daughter cells [26]. To prevent biofilm development and toxic intestinal flora, bacteria often cease EPS synthesis and detach into the environment via "quorum-sensing," which facilitates communication between intra- as well as inter-species.

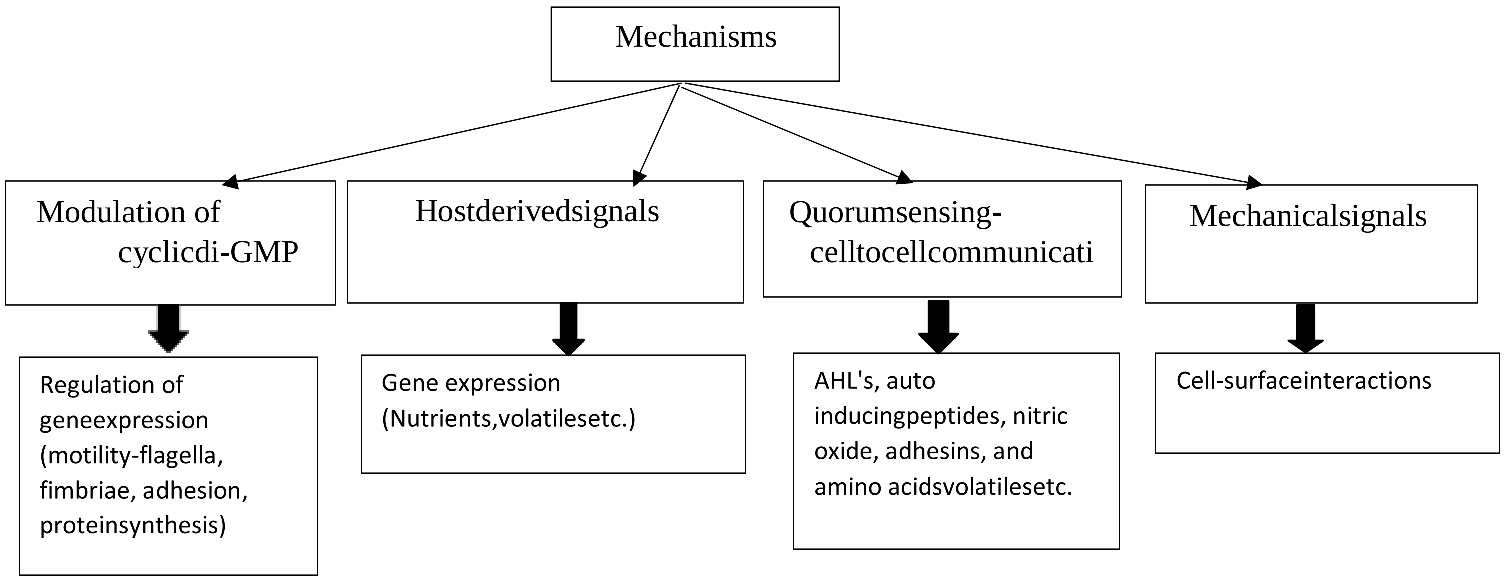


Preparation of Biofilm Biofertilizer

To convert biofilms into bio-fertilizers, it's important to think about things like the bacteria' development profiles, the different sorts of organisms and their optimal environments, and the inoculum's formulation. There are six procedures involved in making biofilm fertiliser. Actionable microbes, target bacteria, propagation technique, carrier material, phenotypic testing, and exhaustive testing are all on the list [11]. The following characteristics are crucial for a suitable carrier material, as stated by Somasegaran and Springer [21]. It needs to be cheap and readily available, with enough of stock on hand. Sterilization using gamma irradiation or autoclaving should be straightforward. It needs to be non-toxic to the plants and microorganisms it is applied to, as well as easy to process and free of chemicals that produce lumps. It needs to absorb lots of water. Having a capability to retain water of at least 50% is essential. The material ought to stick to seeds well. It must be able to act as a pH buffer effectively [31]. Lots of organic material must be present.



One of the PGPR species, *Azospirillum*, may release auxins, ethylene, and gibberellins. Plants are home to a wide variety of bacteria, some of which have the ability to promote the synthesis of phytohormones. Inoculation of lodge pole pine roots with *Paenibacilluspolymyxa*, for instance, increased IAA levels in the pine roots. IAA was found to be produced by *Rhizobium and Bacillus* at a range of pH values, temperatures, and in the presence of agricultural waste. Etherene, in contrast to most other phytohormones, inhibits plant development. [22]. A mixture of JH 7, JK 7, and JK 17 isolates were utilised to dissolve potassium.



Effects of Beneficial Microorganisms as a part of Biofilms Biofertilizer

Common nonmycorrhizal soil fungi (e.g., *Penicillium spp.*), and the rhizobia creating the biofilms, were evaluated for their effects on non-leguminous crops [8]. In this investigation, researchers focused on N2-fixing bacteria and P-solubilizing fungi. Because the bacteria colonised and connected to fungal mycelia, the resulting biofilms are referred to as fungal-bacterial biofilms (FBB), and when the bacterium is a Rhizobium species, they are referred to as fungal-rhizobial biofilms (FRB). The FRB interaction was found to physiologically fix N2. Soybean (*Glycine max)* was modulated by the *Bradyrhizobiumelkanii*SEMIA 5019 rhizobial strain, which has a high N2-fixing capability. Furthermore, it was discovered that in mixed cultures of different bacteria without biofilm formation, pH did not correlate significantly with the production of indoleacetic acid-like substances (IAAS) [9]. It was determined that the high acidity of the biofilms generated was due to the elevated generation of IAAS. In most cases, a high acidity is necessary to combat pathogens. Using biofilm inocula, beneficial bacteria supplied to plants for biocontrol of diseases may be successfully established in the host plants. For example, a Pleurotusostreatus-*Pseudomonas fluorescens* biofilm (FBB) increased endophytic colonisation of tomato (*Lycopersicon lycopersicum*) by *P. fluorescens*, a bio controlling agent, by more than 1,000 percent compared to inoculation with *P. fluorescens* alone under in vitro conditions [10].

Cyanobacteria as biofilms biofertilizer

Cyanobacteria are a significant category of photosynthetic prokaryotes. An very large fraction of the world's atmospheric CO2 and N2 are fixed by this process, making it an essential environmental factor [37]. Even though all cyanobacteria are photoautotrophs, many of them are also facultative heterotrophs. Therefore, you may find them in the dark sections of a plant as well as the bright areas, including the roots, stems, leaves, and thalli. Most plant hosts include bryophytes, cycads, the angiosperm Gunnera, the water fern Azolla, and fungi (to form lichens).[38]

Rhizobacteria as biofilms biofertilizer

Colonization of the rhizosphere by plant growth-promoting rhizobacteria (PGPR) has a beneficial effect on the host plants and is thus commonly utilized in agriculture. [16, 17] Rhizobacteria are beneficial to plants because of their ability to build biofilms, which is contingent on microbial colonization of plant roots. [12,16]. *Bacillus spp.* and other common biocontrol agents can inhibit soilborne diseases and promote plant development. [18]. *Bacillus velezensis* as well as *Bacillus subtilis*, both of which are gram-positive bacteria favorable to plants, are widelyutilised in the biofertilizer industry. *Bacillus velezensis* SQR9, formerly known as *Bacillus amyloliquefaciens* SQR9, is a plant growth-promoting rhizobacterial (PGPR) strain with strong root colonisation ability that is utilised commercially as a biocontrol bacterium. [19,20]

Endophyticandepiphyticbacteriaasbiofilmbiofertilizer

Endophytic and epiphytic microorganisms were separated from two soybean varieties (Foscarin and Cristalina). The isolates were identified by analysing their partial 16S rDNA sequences; most of the bacteria belonged to the families Pseudomonaceae, Burkholderiaceae, and Enterobacteriaceae [53]. The potential of the isolates for enhancing plant development was evaluated by measuring their ability to produce indoleacetic acid (IAA) and to dissolve the mineral phosphate. Even though 34% of endophytic bacteria generated IAA, 52% of them were able to solubilize mineral phosphate. Sixty percent of endophytic isolates and sixty-nine percent of epiphytic isolates were able to generate IAA, solubilize mineral phosphate, and fix nitrogen in vitro [30].

Biofilmproducingmicroorganisms

Sticky microbes, soil particles, and plant roots are ideal for biofilm development. Antibiotic-resistant gram-positive and gram-negative bacteria are employed with fungus and other microorganisms. A wide variety of fungal structures, including spores and hyphae, can be used as a conduit for communication between different bacterial strains searching for a food supply. The colonized plant root can act as a biofilm home for these relationships.

Table 1:- Beneficial bacterial strain and its consequences.

Bacterialstrain	**Host mycorrhizal species**	**Consequences**	**Reference**
Pseudomonassp.	*Rhizophagus irregulars*	Phosphorussolubilisation	Ordonezetal.(2016)
"*Bacillus sp, Bacillusthuringiensis,Paenibacillus rhizosphaerae*"	*Gigasporamargarita*	Causes more ethylene to be produced, promotes hyphal growth, and slows the development of pathogenic fungi.	CruzandIshii (2012)
"*Bacilluspabuli*"	*Glomusclarum*	Promotes fungal development, spore germination, as well as root colonization.	XavierandGermida(2003)
"*Bradyrhizobialstrain*"	*Penicilliumspp*	N2-fixing	Jayasinghearachchi (2004)
"*Pseudomonas fluorescens*"	*Laccariabicolor*	Stimulate the host's development	NoirotGros et (2018)

Table 1:-Shows the correlation between the bacterial strain and host mycorrhizal species and its consequences.

Importantmicroorganismsforagriculturethatform biofilms

Numerous bacterial and fungal species are found in soil microflora, and these organisms are sometimes referred to as plant growth-promoting microorganisms because of their role in fostering plant development (PGPM). It's not uncommon to see an uneven distribution of these species in soil. The rhizosphere, or the region around the root zone, has a significantly more concentrated PGPM dispersion than the remainder of the soil [57]. The synthesis of several phytohormones such cytokinins, gibberellins, indoleacetic acid, and ethylene by PGPM such as *Azotobacter spp., Rhizobium spp., Pantoeaagglomerans, Rhodospirillum rubrum, Pseudomonas fluorescens, Pseudomonas putida, Lactobacillus, Trichoderma* [60,61]

Table2:- Beneficial bacterial strain used in biofilm and its host-plant.

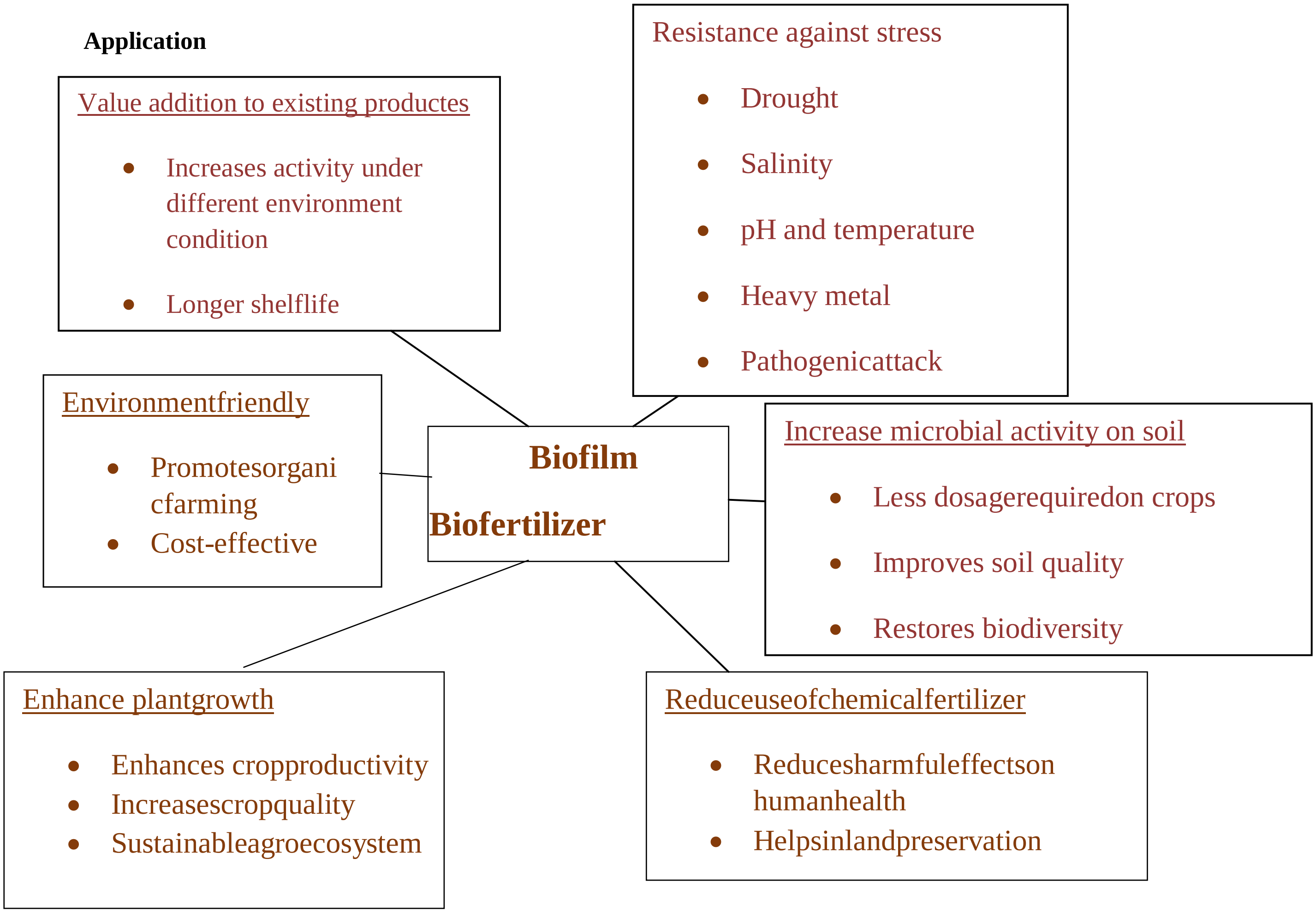
Bacterial strain used inbiofilm	Host plant	Consequences	Reference
Pseudomonassp.	Rice	Phenanthrene bioremediation	Zhou andGao(2019)
"Azospirillumbrasilense"	Wheat	Nitrogenfixation	Souza *et al*.(2014)
"Paenibacilluspolymyxa"	Arabidopssa ndPeanut	Safety against environmental and infectious hazards. Biological management of crown rot	Timmusk*etal*.(2005)
"Pseudomonas putida and Bacillusamyloliquefaciens"	Chickpea	The plant's growth-promoting properties are amplified through synergistic growth.	Kumar *et al*.(2016)
"Azospirillumbrasilense"	Grass	Fixed nitrogen and the element iron (Fe) are both necessary for plant development.	A.B. Housh(2021)
"Bradyrhizobiumelkanii(SEMIA 5019) Penicilliumspp"	Soybean	N2-fixingsymbiosiswithsoybeanu ndergreenhouse conditions.	Jayasinghearach chi(2004)
"Bacillus cereus(commercialstrain)"	Arabid opsis	Biocontrol	Bais*etal*. (2004)
"Bacillusamyloliquefaciens,Bacil luspolymyxa"	Tomato	Biocontrol	Nihorimbere *et al*.(2012)
"Azospirillumbrasilense(rhizos phereofsorghum)"	Sorghum	Boosts Nitric Oxideandindole-3-aceticacid	Kouletal.(2015)
"Proteussp., Pseudomonasp.,Ensifermeliloti(metal-pollutedsoil)"	Alfalfa	Soil decontamination from metals	Raklami*et al*.(2019)

Table 2 shows the correlation between the bacterial strain and host plant and its consequences

Advantages

1. Improves soil health and microbial interaction, leading to a higher rice harvest.
2. Maize, vegetables, and plantation crops are only few examples of non-leguminous crops that might benefit from this method.
3. The incidence of red onion basal rot disease is reduced in the Alfisol, Entisol, and Vertisol zones as a result of the increased availability of phosphorus.
4. Increases nutrient density and organic matter in soil (N, P and K).
5. It lessens nitrogen loss through leaching and boosts dry matter buildup in plants.
6. Exoenzymes, signal molecules, and ion channels all play a role in the efficient absorption of liquid organic materials.
7. The microbial components of the biofilm community interact with one another, sharing genes and metabolic processes.
8. Success in rapidly filtering out waste water's organics, pathogens/parasites, and suspended particles.
9. Ability to clean marine environments that have been polluted by oil and petroproducts.
10. Hormones such indole acetic acid are produced by FBBs, which can aid in plant development (IAA)

Application



Limitation of Biofilm Biofertilizer

1. Reason: Not enough of a certain strain is now available to make it a viable option. No matching transport company was available.
2. Farmers' general lack of knowledge
3. Staffing shortages and a lack of experience
4. Natural restraints
5. You should never put bio-fertilizers in direct sunlight.
6. Bio-fertilizers should be kept at a constant temperature that is neither cold nor hot, between 0oC and 35oC.
7. Used solution should not be stored for more than a day.

Future aspects

Due to farmers' unregulated overapplication of chemical fertilisers during intensive agricultural practises, soils have built up an insufficient amount of nutrients (especially Phosphorus), which has resulted in withering off the soil. It is for this reason that scientists are devoting a great deal of time and effort to finding ways to make agricultural plants thrive with less reliance on traditional inorganic fertilisers. Various experts, such as economists, plant breeders, plant pathologists, nutritionists, and soil microbiologists, will need to work together on short-, medium-, and long-term research initiatives.

Conclusion:-

This demonstrates the promise of BFBFs in cutting CF use in half, helping to ameliorate the environmental damage caused by CF's widespread use. Biofertilizers aid plant development and growth through a variety of processes, including as biological nitrogen fixation (BNF), nutrient mineralization and solubilization, plant hormone production, pest and disease control, and stress tolerance. Because of their ability to engage in a wide variety of chemically and metabolically regulated processes when in bio-film mode, the bacteria in BFBFs are able to sustain the biological system. With rising health and environmental concerns, together with the requirement to expand global food production to satisfy the demands of a growing worldwide population, it is essential to identify a long-term replacement for chemical inputs, in particular CF. In this sense, BFBFs are not only very helpful for sustainable agriculture, but also offer many benefits for environmental protection.

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