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Review Article

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The use of microbial inoculants in clean farming systems, present situation and future prospective

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ABSTRACT

Chemical farming depends predominantly on the exhaustive claim of a variety of agrochemicals mainly mineral fertilizers and pesticides that deteriorated health and environmental ecosystems. On the other hand, clean farming systems applies novel agricultural biotechnology basically microbial inoculants to remediate polluted ecosystems, enhance plant growth, and suppress plant disease as well as reducing agrochemical uses and conserving biodiversity. Realizing the potential of beneficial microorganisms, they are now urgently needed to combat environmental and health adverse impacts of agrochemicals. This review focuses on the use of beneficial microbial inoculants to sustain farming systems.

Keywords: Bioremediation, Biofertilization, Biocontrol control, PGP microbes, sustainable farming

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INTRODUCTION

Since 1930 the worldwide food famine enforced many countries to validate chemical farming systems that acclaimed to accomplish vast paces towards evoking agricultural production. However, it heightened antagonism and disordered symmetry between nutrient elements, shifted pH, disfigured biomass, broadened pollutants in soil and aqueous ecosystems. These obstacles led to a severe unfriendly environmental aftermath, as well as unsustainable farming [1].

To tackle this doomed situation, a novel clean farming system was synchronized depending on microbial decontamination of farming ecosystems, composting of organic residues, organic manuring, microbial fertilization and microbial control of pests. Despite being in the decades of biological credibility, several constraints are still surrounding the notoriety of clean farming systems. The most substantial one is conceptual, others, however, might be technical and/or economic. Intonations, in the present paper, are being guided to a better perceive of the use of microbial inoculants in clean farming systems, through presenting both theoretical concepts and practical approaches.

MICROBIAL INOCULUMS IN FARMING

Although many microorganisms have been demonstrated to stimulate plant growth and harvests on bench scale and greenhouse trails, results have been poorly repeatable in field applications [2] creating a barrier to commercialization and widespread use [3]. Experience confirmed that indigenous strains of rhizobacteria, isolated from the rhizosphere of the intended plant are better adapted to the local soil ecosystem and have high competitive power and potential as inoculants in clean farming systems[4]. The careful selection of microbial inoculants reduces the risk of environmental damage and potentially human health; they are safer to apply; their activity should be more targeted; they are effective in small intensities; they are able to vastly proliferated given appropriate conditions besides being able to survive for more than one season, they could be applied solely or in a mixture [5]. When microorganisms are used together with chemical fertilizers, it would be necessary to define the most effective ratio between inoculum size and rate of fertilizers application. Management strategies combining pesticides or herbicides application and bio-inoculants must be tested for resistance of the microbial inoculant to the agrochemicals and for optimal methods of co-application.

In addition to the ecological considerations, there are also the economic and manufacturing factors that need to be taken into account with regards to the commercialization of microbial inoculants. The mass production of microorganisms could be technically challenging and expensive; products need to be formulated to have long shelf life (transport and storage), which might be problematic in particular with gram-negative bacteria that do not form spores. Further, registration procedure could be expensive and time consuming besides their application must be both simple and compatible with agronomic practices and equipment [6], [7]**.** Recent R&D focused on PGP microorganisms has led to a prototype commercialized now in the markets [8].

Data given in the (Table 1) summarize FAO-BioDeC that confirm that R&D research on microbial inoculants is now at an early stage of development in Africa and Asia, (with the exception of China and India), while Latin American countries are already using advanced techniques on a more routine basis and some of the results obtained are already being tested. It should be borne in mind that many production and post-production processes already in use by farmers have a concrete microbiological basis using techniques developed as indigenous or local knowledge of farmers and consumers [9].

Table (1) Number of initiatives to develop and use microbial techniques

Technique	Region																													
	Africa					Asia					Eastern Europe					Latin America					Near East					Total				
	C	T	E	U	N	C	T	E	U	N	C	T	E	U	N	C	T	E	U	N	C	T	E	U	N	C	T	E	U	N
Biocontrol	-	-	11	-	7	-	-	3	-	3	-	2	6	-	2	-	9	2	-	2	-	-	2	-	2	-	11	24	-	16
Biofertilizers	1	-	7	6	10	-	-	10	3	6	-	4	1	-	4	-	3	3	-	3	-	-	1	-	1	1	7	22	9	24
Fermentation	-	-	4	-	5	-	-	7	-	2	10	1	1	-	4	1	3	7	-	5	-	-	-	-	-	11	4	19	-	16
Other	-	-	-	-	-	-	-	-	3	2	-	3	3	-	2	-	2	5	-	4	-	-	-	-	-	-	5	8	3	9
Total	1	-	22	6	12	-	-	20	6	7	10	10	11	-	5	1	17	17	-	6	-	-	3	-	2	12	27	73	12	32

**http://www.fao.org/docrep/008/y5800e/y5800e05.htm*

C: technology used on a routine basis and products available on the market; T: results being tested; E: number of activities at experimental level (including laboratory or glasshouse activities); U: activities in unknown phase; N: number of countries involved. (Totals of this column have been calculated taking into account that sometimes more than one activity is being carried out by the same country, although that country will only be counted once).

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Data in Table (1) shows also that reported research on biological control agents is only at the bench scale in Africa, with emphasis on few application on cowpea in Ghana and Kenya, sorghum in Ethiopia, Kenya and Zimbabwe, banana in South Africa, Uganda and Zimbabwe, cassava in Malawi and sugar cane in South Africa. In Eastern Europe, both Georgia and the Republic of Moldova have trials underway on use of *Trichoderma sp* as a bio-control agent. Research on *Metarhiziumanisopliae, Entomophora sp*. is undertaken in Georgia and on pathogenic bacteria, fungi and viruses in the Republic of Moldova. In Asia, use of *Steinernem athailandensis* is established and laboratory research is underway on Bt and NPV for use as bio-pesticides in Thailand. In Latin America, Guatemala is testing botanical extracts and *Bacillus subtilis* on melons and tomato, *B. subtilis* on pea, entomopathogenic fungi on sugar cane and pasture, and Bt/NPV on ornamental, horticultural and other crops. Chile is researching bio-control of scab in apple. In the Near East, Egypt and Morocco are researching bio-control of *Fusarium* in date palm [10].

Bioremediation

Bio-remediation is the use of a process or technology to a mixture of pollutants to procure a satisfactory clean ecosystem. There are three principals that form the basis of all-remedial technologies, physical separation, physic-chemical and/or biological reaction [11]. Microbiologists have conceived the expressiveness of the microbial world to perform an urgent mode in the devastation of pollutants in farming ecosystems, and several researchers have attempted to develop means of using microbes and microbial enzymes for detoxification of soil and aquatic ecosystems. However, the area of biotechnology entailing bacterial enzymes instead of whole cells for pollution control has some auspicious practices if certain limitations could be overwhelmed. Diverse microorganisms are fitted for degrading pesticides or carcinogenic hydrocarbons into nontoxic carbon dioxide and water. No doubt, microorganisms proved to be conventional and contemporary substantial origins of bioremediation technologies. Environmental practices are carried out with microbial biomass residing single or multiple strains acting with highly disparate microbial communities. Lack of perceiving of microbial interplay that coordinates their activities constitutes a major impediment to judicious optimization of a broad range of existing, and the development of new, inventive environmental biotechnological practices [12]. . The focus has now reassigned to pick bacteria with transport enhancing properties. Electrostatic and hydrophobic surface properties are auspicious, while motility customarily limits the transport over long distances although it pretends to embellish the spreading of the bacteria at micro-scale [13].

The microorganisms act against the pollutants only when they had access to a variety of materials, compounds to help them generate energy and nutrients to build more cells. In a few cases the natural conditions at the polluted ecosystem provide all the essential materials in large enough quantities that microbial remediation could occur without human intervention, a process called intrinsic microbial remediation [14]. A critical factor in deciding whether microbial remediation is the appropriate cleanup remedy for a soil is whether the pollutants are susceptible to biodegradation by the indigenous soil microorganisms or by zymogenic microorganisms that would be successfully added to the soil. Although existing microorganisms could detoxify a vast array of pollutants, some are more easily degraded than others. The suitability of a soil for microbial remediation depends not only on the pollutant's biodegradability but also on the soil characteristics. A scheme that works optimally under specific conditions might be inappropriate for *in situ* microbial remediation.

Bioreactors technique represents highly controlled methods of treating polluted soils. Because temperature, pH, nutrient levels, and agitation could be controlled in batch or continuously fed reactors, microbial activity, and thus pollutant degradation, could be optimized. The use of microorganisms in remediating soil inorganic pollutants showed several advantages over inorganic treatments in terms of its simplicity, high yield of pollutant extraction, lower chemical consumption, and minimum reduction in nutrients [15]. A microbial mediated soil extract was as effective as nitric and hydrochloric acids [16]. The most widely used microorganisms in pollutant remediation are *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* [17]. . However, in microbial leaching with *A. ferrooxidans* and *A. thiooxidans*, the reaction time to leach pollutants from soils to a recommended level was found to be very long (8–32 days) and the initial pH should be maintained to 2.5 to 4.0.

Elemental sulfur is oxidized to H_2SO_4 by sulfur-oxidizing microorganisms, which might decrease soil pH [18]. The rate of sulfur oxidation in soils had been related to a wide number of factors such as temperature, moisture, sulfur particle size, aeration and microbial biomass. The acidification of soil with elemental S is a common agronomic practice, which could be used to mobilize pollutants cations in soil. The greatest decrease in pH (0.5 units) appeared after 21 days of incubation, [19] in all treatments (3.14, 4.71 and 6.28g sulfur kg^{-1} soil). After that, a steady state was reached for all S doses. There were increasing and widespread interests in the maintenance of soil quality and remediation strategies for management of soils polluted with organic pollutants [20]. They added that there is also a growing body of evidence that *arbuscular mycorrhizal* (AM) fungi could exert protective effects on the host plants under conditions of soil pollution. They reviewed some recent work and highlighted some new data which indicate that the alleviation of pollutants phytotoxcity, particularly zinc toxicity, by *arbuscular mycorrhiza* might occur by both direct and indirect mechanisms. Bending pollutants in *mycorrhizal* structure and their immobilization in the mycorrhizoshere might contribute to the direct effects. Indirect effects might include the mycorrhizal contribution to balanced plant mineral nutrition, especially P nutrition, leading to increased plant growth and enhanced pollutant tolerance. A series of analysis on soils collected were from different soils with regard to pH, EC, organic matter, $SO4^{-2}$, $NO3^{-1}$ and exchangeable cations [21]. Microbial community identified in the soil samples was different from reference soil. Vesicular arbuscular *mycorrhizae* (VAM) population associated with *Zea mays* contained 3 ecotypes of VAM, viz., *Glomus mossea*, *Glomus spp*., and *Acualospora spp*. Out of 34 bacterial strains isolated and characterized, Dominant genera were *Bacillus, Micrococcus* and *Listeria.* Bioremediation is an integrated management of a polluted ecosystem where different organisms are employed to catalyze the natural processes that decontaminate the environment [14]. They also showed that Arbuscular mycorrhizal fungi are soil microorganisms that establish mutual symbiosis with the majority of higher plants, providing direct links between fungi and roots. Polluted soils are often nutrient poor or sometimes nutrient deficient, due to the loss of beneficial microorganisms [22]. However, such soils could be made nutrient rich by applying pollutant tolerant microorganisms, especially the plant growth promoting rhizobacteria, which would provide not only the essential nutrients to the plants growing in the polluted sites but would also play a major role in detoxifying pollutants. It is most convenient to divide decontamination techniques into three major categories, first, electro kinetic methods, second inorganic methods such as solidification, precipitation, or ion exchange, and third, biological methods, which use plants or microorganism to remove pollutants. In the past few years, innovative approaches such as passive treatment technologies for soil pollutants had been developed [23]. Once accumulated in the soil, pollutants inversely affect the microbial compositions, including plant growth promoting rhizobacteria (PGPR) in the rhizosphere, and their metabolic activities. In addition, the elevated concentration of pollutants in soils and their uptake by plants adversely affect the growth, symbiosis and consequently the yields of crops by disrupting the physiological process, such as, photosynthesis, or by inactivating the respiration, protein synthesis and carbohydrate metabolism.

Microbial transformation of organic pollutants normally occurs because microorganisms are able to use certain organic pollutants for their own growth and reproduction. Organic pollutants serve two purposes for the microorganisms, they provide a source of carbon, which is one of the basic building blocks of new cell constituents, and they provide electrons, which the microorganisms could extract to obtain energy.

Microorganisms gain energy by catalyzing energy-producing inorganic reactions that involve breaking inorganic bonds and transferring electrons away from the pollutant. This type of inorganic reaction is called an oxidation/reduction reaction, where the organic pollutant is oxidized. The pollutant is called the electron donor, while the electron recipient is called the electron acceptor. The energy gained from these electron transfers is then "invested," along with some electrons and carbon from the pollutant, to produce more cells. Both electron donor and acceptor are essential for cell growth and are commonly called the primary substrates. White-rot fungus, *phaneorochaete chrysosporium*, could bind to, and in some instances, mineralize a wide array of organic pollutants**,** including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and the predominant conventional explosives TNT, RDX, and HMX. The lignin-degrading, or wood-rotting, enzymes produced by white-rot fungus had been reported to be the key to its degradation capabilities. Two different treatment configurations had been tested for white-rot fungus, bioreactor and *in situ* systems.

During bioremediation, microorganisms utilize inorganic pollutants in the soil as an energy source and, through oxidation-reduction reactions, metabolize the target pollutant into useable energy for microorganisms. By-products (metabolites) released back into the environment are typically in a less toxic form than the parent pollutants. For example, hydrocarbons could be degraded by microorganisms in the presence of oxygen through aerobic respiration. The hydrocarbon loses electrons and is oxidized while oxygen gains electrons and is reduced. The result is formation of carbon dioxide and water [24]. When oxygen is limited in supply or absent, as in saturated or anaerobic soils or lake sediment, anaerobic (without oxygen) respiration prevails. Generally, inorganic compounds such as nitrate, sulfate, ferric iron, manganese, or carbon dioxide serve as terminal electron acceptors to facilitate biodegradation.

Three primary ingredients for bioremediation are, presence of a pollutant, an electron acceptor, and presence of microorganisms that are capable of degrading the specific pollutant. Generally, a pollutant is more easily and quickly degraded if it is a naturally occurring compound in the environment, or inorganically similar to a naturally occurring compound, because microorganisms capable of its biodegradation are more likely to have evolved. Hydrocarbons are naturally occurring inorganic; therefore, microorganisms which are capable of attenuating or degrading hydrocarbons exist in the environment. Development of biodegradation technologies of synthetic inorganic such DDT is dependent on outcomes of research that searches for natural or genetically improved strains of microorganisms to degrade such pollutants into less toxic forms. There is an increasing and widespread interest in the maintenance of soil quality and remediation strategies for management of soils polluted with organic pollutants [25]. Their research focused on the mechanisms involved and raised the prospect of utilizing the mutualistic association in soil re-vegetation programmes. Effects of cultivation of rice (*Oryza sativa L.)* and PAH-degrading bacteria (*Acinetobacter sp.*) were separately studied separately, and in combination, on the dissipation of spiked phenanthrene and pyrene (0, 50+50, 100+100, 200+200 mg kg−1) in waterlogged soil using pot trials [26]. The population of introduced PAH-degrading bacteria remained at 105 CFU g^{-1} dry soils after 20 days of treatment with *Acinetobacter sp*. only, but increased to 106 when planted with rice simultaneously. Shoot and root biomass of rice when grown alone was adversely affected by spiked PAHs, but significantly increased by 2–55% and 8–409%, respectively, when inoculated with *Acinetobacter sp*. Phenanthrene and pyrene concentrations in roots ranged from 1–27 and 20–98 mg kg^{-1}, respectively, while their concentrations in shoots were generally lower than 0.2 mg kg^{-1}. The dissipation of phenanthrene was mainly due to abiotic loss as 70–78% phenanthrene was lost from the control soil at the end of 80 days, while removal of 86–87% phenanthrene had been achieved after 40 days in the treatment co-cultivated with *Acinetobacter sp*. and rice. Compared with the control where only 6–15% of pyrene was removed from soil, a much higher dissipation of pyrene (43–62%) was attained for the treatments co-cultivated with *Acinetobacter sp.* and rice at the end of 80 days. Their results demonstrated that co-cultivation of rice and PAH-degrading bacteria might had a great potential to accelerate the bioremediation process of PAH-polluted soil under waterlogged conditions. Pollutants could adsorb to soil particles, rendering some pollutants unavailable to microorganisms for biodegradation [27]. Thus, in some circumstances, bioavailability of pollutants depends not only on the nature of the pollutant but also on soil type. Hydrophobic pollutants, like hydrocarbons, had low solubility in water and tend to adsorb strongly in soil with high organic matter content. In such cases, surfactants are utilized as part of the bioremediation process to increase solubility and mobility of these pollutants.

Organic manuring

The organic matter content in most Egyptian soils do not exceed 2% and is less than in newly reclaimed sandy soils. The benefits of organic matter in ameliorating soil conditions are evident. At the time being most of the Egyptian farmers do not add organic manures, and few farmers add 20 m3 with sowing. The very low quality of Egyptian farmyard manure enriched soil salinity level and infect the soil with plant pathogens. Under theses condition compost is a marvelous solution within clean farming systems. It is worthy to mention that residues left after harvesting exceed 50 million tons annually besides huge amounts of organic wastes generated from other sources. The residues are an excellent raw material for composting. On the other hand the rate and way of adding organic manures should be justified. The chemical changes that took place during composting of the four agricultural residues were followed up for four months [1]. Biological oxidation of organic matter during the composting course led to a persistent decrease in the organic carbon contents in the different composted materials, yet at varied rates. Such decreases exhibit the efficiency of biomass in decomposing the raw materials. Bio-fortification of the agricultural residues with cellulose decomposing microorganisms led to a higher rate of organic matter oxidation. The percentage losses in organic carbon of the sheath of peanut pods after 120 days composting period were higher in bio-fortified treatments associated with either chemical or organic accelerator. Nevertheless, the differences between the influences of the two accelerators were slight. The final organic carbon contents were 72 and 71% of their initial values in bio-fortified composts receiving respectively either chemical or organic accelerator, while it was 83% in control compost (potting soil).

Whole management of organic residues performs a crucial role in clean farming systems. They are a precious renewable resource, which completes the nutrient elements cycle, improves biological, chemical and physical properties of soil, and is indispensable as a source of energy for the soil ecosystem. Great accentuation should be exercised on composting, storing, handling and spreading of organic fertilizers in agriculture fields. Concern should be stretched to recycle all types of organic waste, particularly, farmyard manure, composted, sewage sludge and city refuse.

In Egypt, the amount of generated agricultural organic residues is roughly estimated at more than 27 million tons/ year, 60 % of which is used as fuel, while most of the rest as animal fodder in rural areas. Farmyard manure generation is estimated at 187.6 million metric tons/year, 113 million tons of which is used as fuel, the surplus as organic fertilizer. The sum of sewage sludge for all Egypt surpasses ten million tons/year.

Municipal solid waste is generated at the rate of 37,000 tons/ day containing nearly 60 % organic fraction. These quantities, besides others, are fair enough to fulfill the requirements of clean farming in immense areas [28],

The first major integral part of clean farming is fortification of the rhizosphere with bio-fertilizers associated with profound organic fertilizer. The effect of single and multi bio-fertilization associated with different rates of chemical fertilization and plant cultivators were trailed in several soil types under both pot and field conditions at the National Research Centre (Egypt). The two criteria followed were; plant dry weight and nutrient elements uptake. Both parameters were found to be merit, as they echo the resulting aftermath of fortification in furnishing a better rhizosphere for plant growth [29].

Bio-fertilizers

Long ago, single-strain bio-fertilizers as rhizobial inoculants had been used in legume fields. Nowadays, emphasis on multi-strain bio-fertilizers has already been tidied. Bio-fertilizers are biological preparations embodying, essentially, sufficient densities of potent strains of microorganisms, having a tangible beneficial role in fitting a proper rhizosphere for plant growth [30]. The concept of bio-fertilization, within the frame of clean farming systems, was developed to reduce the use of agro-chemicals thus conserving the environment, and subsidies agricultural sustainability. However, it is worthy to tell that bio-fertilizers, at the time being, do not exclusively replace agro-chemicals, but markedly diminish their rates of application [1]. The most important features of bio-fertilizers in relation to plant growth are symbiotic and asymbiotic nitrogen fixation, mobilizing plant nutrient elements and/or secreting plant growth promoting principles bio-controlling soil-borne diseases.

The institution of a mother culture collection, biodiversity gene bank, accommodating strains of the diversified aforementioned physiological groups of soil microorganisms, is a mandatory for manufacturing all types of bio-fertilizers (Table 1).

Different strains from rhizosphere soil of field and horticultural crops should be isolated, purified and their potency estimated. The most active strains are selected, on the basis of their efficiency and acclimatization in rhizosphere, and preserved for use in preparing various combinations of Multi-strain bio-fertilizers. The mother culture should always be renewed and supplemented with fresh potent strains.

Table (1) Bio-fertilizers could be grouped in different ways based on their nature and function

http://agritech.tnau.ac.in/org_farm/orgfarm_biofertilizertechnology.html

S. No.	Groups	Examples
N_2 fixing Bio-fertilizers		
1.	Free-living	*Azotobacter, Beijerinkia, Clostridium, Klebsiella, Anabaena, Nostoc*
2.	Symbiotic	*Rhizobium, Frankia, Anabaena azollae*
3.	Associative Symbiotic	*Azospirillum*
P Dissolving Bio-fertilizers		
1.	Bacteria	*Bacillus megaterium* var. *phosphaticum, Bacillus subtilis, Bacillus circulans, Pseudomonas striata*
2.	Fungi	*Penicilliumsp, Aspergillusawamori*
P Mobilizing Bio-fertilizers		
1.	Arbuscular mycorrhiza	*Glomussp.,Gigasporasp.,Acaulospora sp., Scutellospora sp.* & *Sclerocystis sp.*
2.	Ectomycorrhiza	*Laccaria sp., Pisolithus sp., Boletus sp., Amanita sp.*
3.	Ericoid mycorrhizae	*Pezizellaericae*
4.	Orchid mycorrhiza	*Rhizoctonia solani*
Bio-fertilizers for Micro nutrients		
1.	Silicate and Zinc solubilizers	*Bacillus* sp.
Plant Growth Promoting Rhizobacteria		
1.	Pseudomonas	*Pseudomonas fluorescens*

Bio-fertilizer production also deals with the preparation of suited carrier, e.g., peat moss competent to forward the desired strains to the rhizosphere in an efficient intensity. Bio-fertilizers are practiced as seed coats or seedling dressers just anterior to sowing. Before adding bio-fertilizers, a sticky ingredient like gum Arabic is bestowed to make the seeds or seedlings surface-adhesive. Bio-fertilized seeds or seedlings ought to be sown within few hours after bio-fertilization in a moist soil.

Results evinced a retentive conspicuous trend of increase in the dry matter and nutrient elements uptake. Such findings indicate a positive retort to bio-fertilizers, irrespective of the enormity of the increases attended to. In many experiments the effects of bio-fertilization have been notably wavering. In all likelihood, such diversity depends upon the types and mastery of microorganisms that were included into each bio-fertilizer. The dry matter increased by 259 % in wheat, 112%in corn, 234%in barley, 112%in squash and 119%in tomatoes, over control [1].

Research on symbiotic nitrogen fixation by rhizobia legume associations is the most intensively studied of all the nitrogen-fixing symbiosis. They affirmed to be a seriously competent mechanism skilled of furnishing nearly all of the N demands of the accommodating host plant. Biological nitrogen fixation has been stunt spaciously in agricultural practices and its enormous dissemination, through bio-fertilization, could be succor in rehabilitating mistreated lands to productivity [30]. Successful effective nodulation with rhizobia would make the host plant independent of soil nitrogen [31}. Averages ranging from 49 up to 224 kg/N2/ha annually were found to be fixed in pulses and from 62 up to 897 kg/N2/ha in green forage and shade trees [1].

Combined bio-fertilization of *Leucaena leucocephata* with *Azospirillium brasilense* and *Rhizobia* enhanced nitrogenease activity of Rhizobia to 181 mole C_2H_4/g dry nodule/hr [32] Novel outcomes argue that Rhizobia could associate intimately with various Gramineae. Trials were carried out to identify those associations that occur naturally to exploit those which could enhance grain yields, especially rice, with less dependence on N-inputs. Natural associations of rhizobia and rice would likely occur where rice and legumes are successfully rotated, and if so, this type of associations should prove inoculant strains that already competitively occupy colonization niches on rice under field conditions [33]. Bio-fertilizers enhanced rice growth via N fixation, stimulation of nutrient acquisition and mineralization of organic forms of nutrients. In a field trial it was confirmed that the use of *Azolla*, *Azospirillium* and mycorhizal-rice associations increased the efficient use of the low dose of N fertilizers applied under flooded conditions [34].

Studies on the metabolic processes in actinorhizal root nodules facilitate comparisons with those in leguminous nodules. There exists much excellent description on the infection and nodulation process of actinohizea, but the experimental analyses are still at their infancy juvenility [35].

The accessible extensive data on associative nitrogen fixation exhibit the positive contribution that these microorganisms make towards crop growth through nitrogen input and secreting plant growth promoting substance [36]. Bio-fertilization of millet with *Azospirillium* caused increases in the plant dry weight and in the number of root hairs and lateral roots. Supplying a combination of indole acetic acid, gibberellins and kinetin [37]. could mimic these effects. Some large effects on plant growth, arising from the use of mixed inocula of *Azospirillium*, *Azotobacter*, *Bacillus* or mycorrhizae were reported [38].

Bio-fertilization with diazotrophs was only fortuitous with strains superior to indigenous diazotrophs. It is of crucial importance that introduced strains should acclimatize themselves promptly in soil to represent the population majority. In a pot experiment with tomato plants, multi bio-fertilization with *Azotobacter, Azospirillium* and *mycorrhyzae* with one third the usual rate of chemical fertilizers increased the dry weight 245%over control. Moreover, there was a significant increase in root biomass reaching between 200±600% [39] .

In greenhouse experiments, sorghum was more responsive to inoculation with *Azospirillium* than rice plant. Maximum benefits were obtained when the N fertilizer level was reduced to 30 kg/acre in combination with A. lipoferum, giving virtuosity in yields and yield components [40] .

In a field trial with wheat, under sprinkler irrigation, 15N isotope was practiced to appraise N fixation using associative diazotrophs. Results implied that wheat positively responded to bio-fertilization and fertilization. Irrespective of bio-fertilization, maximum increases in straw, grain, N yields and N recovery were obtained when compost was applied at the rate of 32 ton/ha [41]. **The** application of mineral N and bio-nitrogen fertilizers increased the height, head diameter, and seed yield, oil and mineral contents in sunflower plants. Results indicated that organic fertilizers increased the efficiency of mineral and bio-fertilizers [42] .

In pot experiments, single, dual and triple bio-fertilization of rice with *Azospirillium blasilense* Cd ATCC 29710, *Azospirillium lipoferum* ATCC 29731 or *Klebsiella pneumonia* ATCC 13883, exo-polysaccarides-overproducing *Klebsiella pneumonia* Misr 81, elucidated highly significant growth improvement.

Root length and root surface area were also significantly increased. Triple bio-fertilization increased the mineral content in rice plants. Under field conditions, dual bio-fertilization with *Azospirillium* and exopolysaccarides-overproducing *Klebsiella* had more promotive efficacy on growth of lowland rice than *Azospirillium* or *Klebsiella* [43].

Response of wheat plants was significantly favorites by individual bio-fertilization of seeds with *Bacillus polymyxa*, *Azospirillium brasilense* and *Azospirillium lipoferum* and two yeasts (*Saccharomyces cervisiae* and *Candida utilis*). Mixed bio-fertilization with these microorganisms in a field trial led to an increase in grain and N% [44].

It is quite consistent in the results of several experiments that although phosphate-dissolving microorganisms are present in the rhizosphere of the test plants, yet inoculation of seed and/or soil had overbearing effects. Several papers give the impression that bio-fertilization with phosphate-dissolving microorganisms might be correspondent to a treatment with a chemical phosphatic fertilizer, the increase in dry weight of inoculated plants ranged from 5.1±18.0 % with grain crops, sugar beet, potato, tomato, cucumber and soybean [45]

Often, the use of bio-fertilizers mends soil fertility and ameliorates its biological activity. Under clean farming, squash yields increased from 4.88 to 5.45 ton/acre and corn grains by 6.19% [46, 47].

Biological control

The intensive use of pesticides at a large scale was noticed worldwide during the last decades. Insecticides, herbicides and fungicides are employed in chemical farming to control pests and to increase crop harvests. Control of pests with synthetic chemicals led to quite a lot of adversative impacts on environment and health. A momentous injury on non-target biodiversity was evidently pragmatic. Pesticides also have destructive impacts on humans and domestic animals [1].

Biological control of plant pathogens had been auspiciously endured within clean agriculture. Bio-pesticides might execute directly via antagonism, or indirectly by affording stress or other circumstances eager for lethal expression of the pathogen. Bio-pesticides affect only the targeted pest and have minor consequences on either environment or human health, almost zero in most cases. A careful gleam on literature displays that disparate bio-pesticides, e.g., Mycorrhyzae, *streptomycetes, Enterobacter, Verticillium, Pseudomonas, Agrobacterium, Bacillus, Aspergillus, Trichoderma.,* are legalized to be competent in controlling destined soil-borne diseases.

Distinct from bio-pesticides, the much broadly applied bio-chemicals in clean farming, for pests control, are mineral oil, sulfur, stone meals, some plant extracts, hormones, pheromones (sex-attracting compounds) and/or enzymes.

Other methods include adhesive sheets and solarization. They function in a deviant way from chemical pesticides and are constantly less toxic to non targeted organisms. Biochemicals control certain critical functions of the pests by disputing their mating patterns, stunting their growth or repelling them. They do not inevitably kill or destroy the targeted pests. Generally, they are secure and tend to be more environmentally compatible.

The breakdown level of organic matter in soil precisely affects the composition of bacterial taxa as well as the population and activities of added bio-control agents. Competition, antibiosis, parasitism and systemic-induced resistance are all touched. Composts could contribute as a perfect food base for bio-control agents and pose an opportunity to introduce and establish specific bio-control agents into rhizosphere soil, which in turn usher to sustain biological control based on the activities of microbial communities [48].

Whenever pesticides are practiced, abundant bulk inevitably gets to the soil where they might be adsorbed, chemically bound, volatilized, leached into the ground and/ or surface water, taken up by plants or biodegraded, so leading to pensive environmental exacerbating uncertainty as well as to unsustainable agriculture. For the motive of sustainability, farming management should take the chemical time bomb into deem and weigh its jeopardy to soil over time [49].

In recent years, the prospects of bestowing bio-pesticides in clean farming have attained a new momentum as an outcome of growing environmental and health security. When bio-agents are used in a convenient successiveness for a proper duration, they reduce venture to pesticides handlers, contamination of food crops, and overthrow of non targeted organisms and pollution of the environment.

Only few indicative examples from the vast literature will be presented. For several decades, formulations of *Bacillus thuringiensis* have been seen as an ideal mean of bio-controlling pests in agriculture. This microbial bio-pesticide has no toxicity to non targeted pests, environmentally safe, immune to pesticide resistance phenomenon, merges into whole pest control methods, and could be mass produced at low cost [50] .

A wettable powder formulation of *Bacillus thuringiensis* (Dipel 2X) was used as baits for bio-controlling greasy cutworm *Agrotis ypsilon* larvae attacking peas and lentils. When Dipel 2X was applied at the rate of 250 g/acr, it was almost as effective as the chemical insecticide hostathion at the rate of 1.5 liter/fed [39]. Field applications carried out in

Taiwan concerted that *Bacillus thuringiensis* was more effective than conventional chemical pesticides in cruciferous vegetable [51].

On the other hand, a great debate about the safety of BT was raised during the last years in many scientific meetings. Some scientists are convinced that Bt, particularly in transgenic organisms, might have certain adverse health and environmental consequences.

Trichoderma harzianum affirmed to be qualified in controlling potato blights, damping-off disease in rye grass, cotton [52] and sunflower [53]. Cultural filtrates of *Trichoderma harzianum* were bestowed to soil for bio-controlling root knot nematodes. Fungal filtrates abridged the number of galls on tomato roots as well as the population of Meloidogyne incognita in soil [54].

Enhanced decomposition for soil pollutants by composts has been endorsed by various workers [55, 56]. Farmers might inoculate their composts with the targeted decomposing micro-organisms. Arrest of the soil-borne disease *Phytophthora cinnamoni, Rhizoctonium solani, Fusarium oxysporium* and *Pythium aphanidermatum*, after the application of composted organic fertilizers to many crops has been reported.

In growth chamber experiments, compost considerably suppressed damping-off of cucumber caused by *Pythium irregulare* and *Pythium myriotylum* [57]. Spent mushroom compost or straw mulch raised marketable potato tuber weight by means of abridging the effects of potato early dying disease caused by *Verticillium dahliae* and/or *Pratylenchus pentrans* [58]. The antagonistic bio-agents *Bacillus subtilus, Tricoderma harzianum, Tricoderma koningii, Penicillium citrinum* and *Penicillium oxalicum* isolated from composted swine fertilizer, in a potting medium suppressed *Rhizoctonia solani, Phytophthera capsici* and *Pythium aphanidermatum* [59]. In greenhouse experiments *Rhizoctonium solani* was suppressed in potting medium mended with compost made from organic household wastes using cucumber as a sensitive herbaceous test plant [60].

Bio-pesticides: Microbial plant pathogens found in the soil ecosystem are all not so friendly to plants, they cause disease or damage the plant. Bio-pesticides, a contraction of 'biological pesticides', include several types of pest management intervention: through predatory, parasitic, or chemical relationships. The term has been associated historically with biological control - and the manipulation of living organisms that are usually applied in a manner similar to chemical pesticides, but achieve pest management in an environmentally friendly way. With all pest management products, but especially microbial agents, effective control requires appropriate formulation and application [61, [62]. Bio-pesticides for use against crop diseases have already established themselves on a variety of crops. For example, bio-pesticides already play an important role in controlling downy mildew diseases. Their benefits include: a 0-Day Pre-Harvest Interval, the ability to use under moderate to severe disease pressure, and the ability to use as a tank mix or in a rotational program with other registered fungicides. Because some market studies estimate that as much as 20% of global fungicide sales are directed at downy mildew diseases, the integration of bio-fungicides into grape production has substantial benefits in terms of extending the useful life of other fungicides, especially those in the reduced-risk category.

Bio-Nematodes: In the past 20 years three developments had been occurred which have had significant effects on the prospects and opportunities for the biological control of plant-parasitic nematodes. Several nematicides have been withdrawn from the market because of health and environmental problems associated with their production and use [63]. The past 20 years have seen a significant increase in the number of scientists involved in research on the biological control of nematodes. Surveys and empirical tests are being replaced by quantitative experimentation and basic research on the modes of action, host specificity and epidemiology of selected organisms. Such basic information is essential for a realistic appraisal of the impact of molecular biology on the improvement of microbial agents and monitoring the spread and survival of released organisms, and for the development of rational strategies for control [64].

Bio-herbicides: A bio-herbicide is a biologically based control agent for weeds. Among the three major types of pesticides (agricultural pest-control agents) herbicides are used to control weeds or undesirable plants without the environmental hazards posed by synthetic herbicides. The microbes possess invasive genes that could attack the defense genes of the weeds, thereby killing it.The benefit of using bio-herbicides is that it could survive in the environment long enough for the next growing season where there will be more weeds to infect. It is cheaper than synthetic pesticides thus could essentially reduce farming expenses if managed properly. Further, it is not harmful to the environment compared to conventional herbicides and will not affect non-target organisms. Under greenhouse condition, *Polyogonmon speliensis, Phalaris paradox and Convolvulus* seedlings dry weight were reduced by (40, 46.4 and 32.6 %) for *Pseudomonas syringae st.2*, (25, 43.2 and 21.4%) for *P. syringae st.1* and (31, 31.2 and 16.1%) for *Colletotrichum sp*. respectively, compared to control [65]. They added that the phytotoxcity varied according to microorganism and weed species where *Pseudomonas spp.* metabolites were more phytotoxic than *Colletotrichum sp*. Metabolites, and *Phalarisparadoxa* was more sensitive to microbes and their phytotoxins than other weed tested. Also, biological control with foliar pathogens has more potential for broad-leaved weeds than for grassy weeds [66]. There are10 bio-herbicides(herbicides pathogens) have been registered or sanctioned for use as bio-herbicides since the early 1980s are given in (Table 2) [67].

Table (2) A list of some pathogens registered and/or worldwide approved as bio-herbicides

Pathogen	Weed	Commercial Product name
Alternaria destruens E.G., Simmons	*Cuscuta*spp.	Smolder
Chondrostereum purpureum (Pers.:Fr.) Pouzar	Broad-leaved trees	Chontrol
		MycoTech
		BioChon
Colletotrichumg loeosporioides	*Malvapusilla* Sm.	BioMal
Colletotrichum gloeosporioides (Penz.) Penz. &Sacc. In Penz. f.sp. aeschynomene	*Aeschynomenevirginica* (L.) B.S.P.	Collego
Cylindrobasidium leave (Pers.:Fr.) Chamuris	*Acacia* spp.	Stumpout
Phytophthorapal mivora (E.J. Butler) E.J. Butle	*Morreniaodorata* (Hook. &Arn.) Lindl.	DeVine
Puccinia canaliculata (Schwein.) Legerh.	*Cyperusesculentus* L.	Dr. BioSedge
Pucciniathlaspeos C. Schub.	*Isatistinctoria* L.	Dyer's woad rust strain Woad 006489
Xanthomonas campestris Migulap var. *poae*	*Poaannua* L	Camperico

Bio-insecticide: Biotechnology could also help in developing alternative controls to synthetic insecticides to fight against insect pests [68]. Formulas for coatings on the seed (inoculants) which carry these beneficial organisms could be developed to protect the plant during the critical seedling stage. Many different products have been registered and approved for managing diseases in organically-grown crops in the USA. Active ingredients include a microorganism that acts as a biocontrol agent, directly affecting the pathogen (e.g. Contans), or produces a compound during fermentation that provides control (e.g. Sonata). Some products suppress disease by inducing the plant to activate its defense mechanisms (e.g. Companion, Regalia). There is a long list of crops and diseases on the labels for many organic fungicides, thus growers now have a lot to choose from[69], [70].[71].

Transgenic Organisms

The declaration of the 1st prosperous transgenic bacteria by application of r-DNA technique in 1971 was caught with a tumult from scientists, environmentalists and religious fanatics that doomsday has become abutting. Twentyfive years later, the sparse was based more on anxiety of the unknowns. Thereafter, humanity has gathered immense profits from advances in modern biotechnology with r-DNA technique. Fear was dissipated in a large part because of better knowledge on how genomes perform and how they could be subdued. Naturally, those processions have also widespread the periphery of technological impacts on health and environment.

Nowadays, perhaps the largest field in agricultural biotechnology is the use of genetic manipulation to develop improved transgenic organisms. This is an area of accelerated advances and the primary focus of corporate and academic investments and media attention. However, we should not let the concentrated efforts on plant genetics overshadow the proven and promised scientific and commercial value of plant biotechnology using microorganisms to improve clean farming systems [72]. In the earlier days of genetic engineering, from a viewpoint of clean farming, it was the question of escaping from transgenic material from laboratories, today it is the question of deliberate release during the use of such material into the environment on an enormous scale. Tens of millions of acres were planted to transgenic crops since 1971, and the trend is fast expanding. The change in scale alone professes newborn questions of bio-safety. There is a bargain that the precautionary principle applies here, when an activity boosts warning of harm to human health or environment [73]. Modern agricultural biotechnology and its applications in the field of genetically modified organisms furnish new and forcible devices for intensifying the development of more and better food. In spite of all the charity of biotechnology, it is eminently important to nourish efforts to develop and apply safety assessment criteria to warrant the wholesomeness and safety of food supply. Risk assessment should be undertaken in order to recognize the thinkable inauspicious effects of genetically modified organisms. Risk management mechanisms, measures and strategies should commensurate with the results of risk assessment.

In Agenda 21, governments undertook to think about international collaboration on safety in biotechnology. The commitments encompass sharing experience, capacity building and international agreements on principals for safety.

Biosafety issues could be tackled at different levels ranging from voluntary code of conduct to guidelines to restrictive regulations. A momentous component is the right of the purchaser to know, through befitting labeling, the origin of the product used.

Application of Microbial Inoculants

Successful application of bio-control strategies requires more knowledge-intensive management. Various methods for application of bio-control agents include: application directly to the infection court at a high population level to swamp the pathogen, application at one place in which bio-control microorganisms are applied at one place (each crop year) but at lower populations which then multiply and spread to other plant parts and give protection against pathogens and one time or occasional application that maintain pathogen populations below threshold levels [74].

Commercial use and application of biological disease control have been slow mainly due to their variable performances under different environmental conditions in the field. To overcome this problem and in order to take the bio-control technology to the field and improve the commercialization of bio-control, it is important to develop new formulations of bio-control microorganisms with higher degree of stability and survival. Majority of bio-control products are applied against seed borne and soil borne fungal pathogens, including the causal agents of seed rot, damping-off and root rot diseases [75], [76], These products are mostly used as seed treatment and have been effective in protecting several major crops such as wheat, rice, corn, sugar beet and cotton against fungal pathogens [77], [78]. However, in some cases, biocontrol microorganisms have also been tested as spray application on foliar diseases, including powdery mildew, downy mildew, blights and leaf spots [79].

A few post harvest fungal diseases have also been controlled by the use of antagonistic fungi and bacteria [80]. Bio-control microorganisms are also being used as the form of composts in some plants [81]. Research data and observations in nurseries have shown that addition of composted organic matter to potting mixes results in suppression of soil borne diseases.

A significant improvement have been made in different aspects of biological control of fungal plant diseases, but this area still need much more development and investigations to solve the existing problems. In order to have more effective biological control strategies in the future, it is critical to carry out more research studies on some less developed aspects of bio-control, including development of novel formulations, understanding the impact of environmental factors on bio-control agents, mass production of bio-control microorganisms and the use of biotechnology and nano-technology in improvement of bio-control mechanisms and strategies [82], [83],[84].

Future outlooks of bio-control of plant diseases is bright and promising and with the growing demand for bio-control products among the growers, it is possible to use the biological control as an effective strategy to manage plant diseases, increase yield, protect the environment and biological resources and approach a sustainable agricultural system.

CONCLUSION

In most developing countries, flourishing agricultural production is more deliberate than sustainability, natural resources management or even environmental quality. Development and sustainability, however, should not be incompatible, as they represent the way through which agricultural growth is secure [1]. Lack of agricultural development would lead to environmental worsening problems, particularly in low potential areas, where accretion and increased production are crucial to help restore the fragile natural resources base. Sustainability would never be achieved farewell as agricultural practices continue beyond the carrying capacity of the farming ecosystem through the exaggerated abuse of agricultural chemicals. The rapid growth of agricultural productivity in chemical farming systems is shrinking off. Moreover, environmental torrent from agricultural activities jeopardizes agricultural growth in several countries. Problems associated with the wealthy agricultural production in the developed world and underproduction in developing countries necessitates a widely accepted assessment of the present status of agriculture. It is time to install new farming systems committed to following environmental and sustainable approaches, and producing healthy food free from agrochemical residues. Ecologically oriented farming routines are being developed within the frame of the recent achievements in environmental biotechnology, the most important of which is the clean farming system which is increasingly acknowledged as a potential solution to copious problems overlaying present world agriculture. It is a farming system, which aims at evading the routine use of agricultural chemicals and reducing their rates of application. Clean farming systems directly give rise to four environmental biotechnologies, i.e., recycling of composted organic waste, fortifying the rhizosphere soil with bio-fertilizers, encouraging the use of bio-pesticides in agricultural practices and bioremediation of polluted agro-ecosystems. [1].

The need for a continuous supply of food has led conventional agriculture to be strongly dependent on chemicals. The increasing concern of consumers and government on food safety has led growers to explore new environmentally friendly methods to replace, or at least supplement, the current chemical-based practices. The use of bio-pesticides has emerged as a promising alternative to chemical pesticides.

Plant growth promotion is a complex phenomenon rarely attributable to a single mechanism as most PGP microbes influence plant growth and productivity through multiple mechanisms, and in some cases their PGP effect may only occur through interactions with other microbes. Despite the challenges, a growing variety of microorganisms with properties that could be exploited in plant growth promotion are being discovered and tested under field conditions, with the number of successful cases increasing. The direct benefits of such research are both financial, from reductions in the use of chemical fertilizers and pesticides, and productive through improved crop yield, while indirect benefits include reduced toxin accumulation in agricultural soils and reduced environmental pollution with agricultural runoff. Microbiological quality is by far the most common food safety aspect safeguarded by filtration. Additionally, where ingredients are aseptically dosed into a sterile environment, sterile liquid filters are selected to provide microbiological removal where heating would otherwise destroy heat sensitive ingredients. In certain dry powdered products, proper filtration of the air that comes into contact with these powders during manufacturing could reduce or eliminate unwanted microbes, which could later thrive in reconstituted form.

Success is often associated with a combination of inoculants possessing complementary beneficial traits, e.g. bio-fertilizers that increase nutrient availability in the proximity of the roots together with bio-control agents that enhances the root system and assists the plant to control of plant diseases.

As far as clean farming is carefully thought about, it is one thing to identify an organism and show it could do something beneficial, but it is quite another to put it into user and environmentally friendly formulations which are stable and efficacious. They must be also befitting in registration and could endure the stringency of commercial dispersal and handling. Most farmers are restrained in the deliberations overwhelmed with chemical farming and it is time for them to start thinking about biological territory. In reach of the 21st century, an overview on the results profited from clean farming clearly shows that the potential is gigantic. It is validated, as far as clean farming is performing, to lay ample rhythm on information dissemination, prescribed extension sites, training, executing R&D programs, instituting gene banks and prescribing quality control measures [1]. Economic harness could mimic a much more momentous capacity in the forecast of clean farming, especially since it substantiates the main prevalent tendency of environmental expediency.

One important subsist denotes to the foresight that more faith in economic implements should provoke agricultural chemicals to take preventative rather than restorative measures.

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