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Review Article

Biofertilizer as a Novel Tool for Enhancing Soil Fertility and Crop Productivity: A Review

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ABSTRACT

Keywords

Biofertilizer, Soil fertility, Crop productivity

To enhance crop productivity and to feed the people without adversely affecting the environmental quality is a challenge to agro-technology. Green revolution, while bringing in self sufficiency and security on food front, resulted in some undesirable side effects. Crop production became highly capital and energy intensive enterprise, and a source of soil and water pollution. Such consequences raised question of sustainability, i.e., maintaining production at higher level without compromising with environmental quality, exploiting the natural resources in present mainly land, a precious natural resource without lowering their potential for the future. Increasing use of chemical fertilizers, their high costs, low efficiency, deterioration of soil structure and pollution problems requires management approach. The fertilizer production in our country is less than the required amount. In order to fill this gap, alternative sources of nutrients have to be looked for such as biofertilizers and organic wastes. They can be used as an alternate source to meet the nutrient requirement of crops and to bridge the future gaps.

Introduction

In recent years the exorbitant cost of nitrogen fertilizers and environmental awareness on use of chemical fertilizers catalyzed a search for an alternative to boost per unit area agricultural production. Considering the negative and hazardous effects of chemical fertilizers, biofertilizers can be safer alternative to chemical inputs and will help in reducing the rate of ecological disturbance to a great extent. A biofertilizer is a substance containing living microorganisms which when applied to seeds, plants, or soil, colonizes the rhizosphere or the interior of the plants and promotes plant growth by increasing nutrient supply to the host plant (Vessey, 2003; Bardi and Malusa, 2012; Malusa and Vassilev 2014). Biofertilizers can add up to 30- 300 kg N/ha by fixation under optimum conditions and solubilize immobilized 30 to 50 kg P_2 O_5 /ha. They liberate growth promoting substances and vitamins and help to maintain soil fertility. Biofertilizers suppress the incidence of pathogens in control plant diseases increasing crop field by 10 to 35%. Biological nitrogen fixation contributes 69% of global nitrogen fixation. Legume-rhizobia symbiosis is the most promising because it supplies about 80 to 90% of the total N requirement of legume. Residual N from symbiosis benefits the subsequent cereal crop. Biofertilizers are cost effective eco friendly in nature and their prolonged use enhances fertility status of the soil (Mahdi *et al.,* 2010; Singh *et al.,* 2011). They are the cheapest source of plant nutrients and are very significant suppliers of micronutrients, organic matter, secrete growth hormones and help in counteracting negative impact of chemical fertilizers (Gaur, 2010).

History of biofertilizer

Agricultural practices aim to enhance crop yield. Chemical fertilizers are being used for increasing productivity. It leads to bad effect on the soil health through affecting its biodiversity by changing the chemical composition, microbial flora and ecosystem (Wall *et al.,* 2015). In early nineteenth-century chemical fertilizer industries started producing synthetic fertilizers and pesticides consisting of phosphorous (P), potassium (K) and nitrogen (N) to increase crop production and protecting the plant from various diseases (Belay *et al.*, 2002; Meng *et al.*, 2013). Studies showed that long-term continuous application of chemicals led to soil acidification and reduced soil quality which ultimately affects human health and creates environmental instability (Geisseler and Scow, 2014). Hence there is an increasing need to have alternative sustainable agricultural practices to enhance crop productivity. In this approach microbes play a major role in maintaining agricultural sustainability by maintaining diversity of ecosystems and improving soil health. (McDaniel *et al.,* 2014; Altieri 1999). Bacteria possessing the traits which is advantageous for the plant in growth and disease protection are termed as plant growth-promoting bacteria (PGPB) (Mantelin and Touraine 2004; Bashan 1998; Bashan and de-Bashan 2005). Bioformulations were in agricultural practice in the history where discovery of Bassi in 1835 showed *Beauveria bassiana* infection in silkworm (Brownbridge *et al.,* 2012). This discovery led to identification of role of microbes in disease protection. The discovery of Bt (*Bacillus thuringiensis*) toxin gave more strength to the idea of researchers to think more about microbes as an alternative for chemicals (Sayyed *et al.*, 2003). Later most of the bacteria were reported for their plant growth-promoting and biocontrol activity. Many studies suggested the successful application of various bioformulations in controlling the disease and enhancing plant growth (Glick and Bashan 1997). The commercialization of PGPR started in the late eighteenth century, and it gained popularity over the time with successful use as bioinoculants. The application of PGPB in sustainable agriculture is the need of the hour (Brockwell and Bottomley 1995; Vessey 2003). Mechanism of action of these microbial inoculants varies and is specific to host and region. Based on their expressive traits, numerous numbers of biofertilizers with various types of formulation came into existence. Recent studies on agriculture revealed that microbiome activities in soil and sustainable agriculture are interconnected to each other (Fig. 1; Table 1 and 2).

Symbiotic N_2 fixers

Leguminous crops fix atmospheric N through symbiotic association with *Rhizobium* which harbors either in root or stem nodules. *Rhizobium* is N fixing bacterium which is rod shaped, motile, have one polar or sub polar flagellum and are gram negative. Successful *Rhizobium* legume symbiosis depends on the entry of micro symbiont (Rhizobia) into the macro symbiotic (legume) which take place in a series of events. Infection and nodule formation as host specific phenomena. This establishment requires a complex interplay between the host and symbiont that results into the formation of nodules wherein *Rhizobia* colonize as intracellular symbiont (Allito *et al.,* 2015). *Rhizobium,* *Bradyrhizobium, Sinorhizobium, Azorhizobium* and *Mesorhizobium* are collectively called as Rhizobia. Host bacterium symbiosis may be determined by the binding of host legume lectin (glycoprotein) to characteristics carbohydrate receptor on the *Rhizobium* cell surface. Lectins have recognition functions. Binding occurs only between compatible partners. When binding has occurred, the tip of the root hair bends and bacteria penetrates and grow in the form of an infection tube. Normal diploid cells are destroyed and in tetraploid cells rhizobia multiply. As growth hormones are produced and then the root epidermal cells undergo multiplication. The cells along the neighbouring diploid cells are stimulated to divide rapidly to produce a nodule. The rapidly dividing bacteria grow in deformed cells called as bacteroides having more than 10 times the volume of rhizobia. The bacteroids singly or in groups are surrounded by peribacteroid membrane. The tissue containing the bacteroids is red because of leghaemoglobin. The nodules turn green during ageing due to breakdown of leghaemoglobin to green bile pigments. When the nodules die, stationary phase rhizobia are released and can multiply by using degradation products of nodules as substrate. All the nitrogen fixing microorganism have enzyme known as nitrogenase which have the ability to reduce nitrogen to ammonia with the help of energy in the form of ATP. Nitrogenase enzyme have two components one containing Mo-Fe protein and other Fe protein. Among strains of *Rhizobium*, *Azorhizobium* is a stem nodule-forming symbiotic bacteria that form stem nodules and fixes atmospheric nitrogen (Gourion *et al.*, 2015). They also produce significant amount of indole acetic acid (IAA) that promote plant growth. *Bradyrhizobium* is not only an efficient nitrogen fixer and when the strain is inoculated in mucuna seeds, but also it enhances total organic carbon, nitrogen, phosphorus, and potassium contents in the soil. Thus, it boosts up plant growth, soil microbial population, plant biomass as well as reduces weed population (Youssef and Eissa, 2014).

Free living N_2 fixers

Free living and associative nitrogen fixers *Azotobacter* is a free living nitrogen fixing bacterium. It is highly aerobic, heterotrophic bacterium growing in neutral to alkaline p^H. *Azotobacter* is placed along with the genus *Azomonas* in the family *Azotobacteriaceae.* The other species which are reported include *Azotobacter vinelandii, Azotobacter beijerinckii, Azotobacter insignis, and Azotobacter macrocytogenes* (Mishra *et al.*, 2013). The predominant species in local soil is *A. chroococcum*. The occurrence of *Azotobacter* has been reported from the rhizosphere of a many crop plants such as rice, maize, sugarcane, bajra, vegetables, and plantation crops (Wani *et al.*, 2013). *Azotobacter* poses several unique features like formation of cyst containing novel liquid, more than one type of nitrogenase, extreme tolerance to oxygen. *Azotobacter* requires neutral to slightly alkaline pH for growth. *Azotobacter* is the most commonly isolated and researched free living nitrogen fixer contributing to the natural vegetation and soil fertility. *Azotobacter* is said to contribute in a substantially way to the nitrogen content of soil, a number of beneficial characters are present in this organism such as higher nitrogen fixation, ammonia excretion, production of vitamins and growth promoters, production of siderophores, production of antifungal antibiotics. It was reported that Azotobacter secretes substances that inhibit the growth of certain root pathogens and improve root growth and uptake of plant nutrients (Dobereiner *et al.*, 1972; Babalola, 2010).

Phosphate solubilizing microorganisms

Phosphorous is very much essential nutrient for plant growth and development. This nutrient is estimated to be insufficient amounts in most of the Indian soil as available P. The concentration of available P in soil ranges from 0.05 to 10 ppm and average solubility of P in soil solution is 0-1 ppm of which only small part is available to plant at one time. Generally, the insoluble phosphorus is present either as inorganic material such as apatite or as several organic forms such as inositol phosphate (soil phytate), phosphomonoesters, and phosphotriesters (Mahdi *et al.*, 2012). Soluble inorganic phosphorus that is used as chemical fertilizers becomes immobilized soon after it is applied to the field. Hence, it becomes unavailable to plants and therefore cannot be utilized efficiently (Feng *et al.*, 2004; Angus 2012).

Considering all these factors and situation, there was a need to develop cost effective ecofriendly sustainable system where the supply of P nutrients to plants should be ensured. Several research findings have established microbes that are able to solubilize inorganic phosphorus play a major role in phosphorous availability to the plants. Inorganic phosphorus solubilization occurs as a result of the action of low molecular weight organic acids such as gluconic and citric acids which are synthesized by various bacteria present in the soil (Glick, 2012). P solubilizing biofertilizers carrier based preparation containing living cells of microorganisms like bacteria, fungi actinomycetes which may help in increasing crop productivity by way of solubilizing insoluble P. Some strains of bacteria that possess the ability to solubilize insoluble inorganic phosphorus are *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Micrococcus, Acetobacter, Flavobacterium, and Erwinia* has been found that higher concentration of phosphate-solubilizing bacteria (PSB) is found in the rhizosphere as compared to non-rhizosphere soil (Youssef and Eissa,2014). Besides providing phosphorus in soluble form to the plants, PSB also enhances plant growth by stimulating the efficiency of biological nitrogen fixation (BNF) by nitrogen-fixing organisms (Mohammadi and Sohrabi,2012).

Blue green algae (Cyanobacteria)

Cyanobacteria are a group of photosynthetic prokaryotes of which many are capable of fixing atmospheric nitrogen and common feature of this organism is their ability to grow in any environment where there is moisture and light. They increase plant growth by producing auxin, indole acetic acid, gibberllic acid and fix around 20-30 kg N/ha in submerged rice fields (Mishra *et al.*, 2013). They grow abundantly in tropical climate, especially in paddy soils and have been demonstrated to be ideal for biological nitrogen input in rice ecosystem. They have the ability to carry out photosynthesis and nitrogen fixation, when the former process meets the energy needs of the later. Besides, contribution of the nitrogen to the soil, *Cyanobacteria* have other ecological advantages such as their exceptionally good water holding capacity, ability to concentrate nutrients such as phosphorus, fixed carbon and trace elements. *Cyanobacteria* are either single celled or consists of branched or unbranched filaments. They possess a peculiar structure known as heterocyst which is site of N_2 fixation. Some *Cyanobacteria* without heterocyst have also been found to fix nitrogen under low oxygen conditions. The species of *Cyanobacteria* that are generally used for field application are *Aulosira, Nostoc, Tolypothrix, Anabaena*.

Azolla as a biofertiliser

Azolla is a free floating around that floats in water and fix atmospheric nitrogen in association with nitrogen fixing blue green algae, *Anabaena azollae*. Azolla contains 4-5% nitrogen on dry basis and 0.2-0.4% on wet basis and can be a very useful source of organic manure and nitrogen in rice production (Mishra *et al.*, 2013). Azolla can be used as a biofertilizer in the fields prior to rice cultivation. The generally used species of Azolla in India is *Azolla pinnata* and it can be propagated on commercial scale by vegetative means (Mazid and Khan, 2015). Some other species found in India Azolla are *Azolla caroliniana, Azolla microphylla, Azolla filiculoides*, and *Azolla mexicana* have been introduced for their large biomass production (Mishra *et al.,* 2013). Azolla decomposes in soil easily and its nitrogen is efficiently available to the rice plants. It also contributes to provide a significant amount of phosphorus, potassium, zinc, iron, molybdenum, and other micronutrients (Al Abboud *et al.,* 2013).

Effect of biofertilizer in photosynthesis

Higher photosynthesis provides better growth of the plant because around 90 % of plant biomass is derived from CO_2 assimilation through photosynthesis (Long *et al.*, 2006). It was reported that certain test strains of *Rhizobia* significantly increased the surface areas of plant leaves, net photosynthetic rate of plants, stomatal conductance as well as water use efficiency, indicating that rhizobial inoculation of rice can increase photosynthetic capacity of the plant also (Mia and Shamsuddin, 2010). Heidari and Golpayegani (2012) reported that the combination of three bacterial biofertilizer namely *Pseudomonades, Bacillus lentus, and A. brasilense* increased the expression of antioxidant enzymes as well as increased chlorophyll content in leaves under stress. Thus, biofertilizer can foster the photosynthetic activity of the plant which helps the plant to grow well even in stressed condition.

Effect of biofertilizer in amino acid synthesis

A group of rhizosphere bacteria that have the ability to colonize the root environment are called as rhizosphere (Shahaby *et al.*, 2016). Roots secrete some chemicals in the soils which are referred as “root exudates”. These compounds work as chemical attractants for various microorganisms. The exudation of diverse chemical compounds modifies the physicochemical properties of soil and regulates the structure of soil microbial community in the immediate surrounding area of root surface considerably (Bulgarelli *et al.*, 2013; Huang *et al.*, 2014). Thus, the type of amino acids including the composition of root exudates secreted by the plant is dependent upon the species of plants and associated microorganisms to the plant (Bardgett and van der Putten, 2014).

Effect of biofertilizer on bioremediation of metals

Extensive agricultural activities, rapid increase in industrialization and urbanization has led many environmental problems by liberation of pollutants such as heavy metals, toxic wastes, organic contaminants, etc. (Shinwari *et al.*, 2015). The harmful heavy metals in various valence states include zinc(Zn), arsenic (As), chromium (Cr), cadmium (Cd), mercury (Hg), copper (Cu), nickel (Ni), and lead (Pb). Although metals are required by plants as micronutrients, accumulation of heavy metals is harmful for majority of plants. However, high concentrations of heavy metals in soil decreases soil fertility and affect microbial community (Lenart and Wolny-Koładka, 2013). The role of PGPR in bioremediation of metal toxicity as been examined many times and it has been found that a diverse range of microorganisms play a important role in remediation of toxicity of heavy metals (Dixit *et al.*, 2015). *Achromobacter xylosoxidans, A. chroococcum, B. subtilis, B. megaterium, Bradyrhizobium, Pseudomonas sp., Brevibacillus sp., Kluyvera ascorbata, Mesorhizobium, Pseudomonas putida, Pseudomonas aeruginosa, Ralstonia metallidurans, Rhizobium, Sinorhizobium sp., Variovoxparadoxus, Ochrobactrum sp., Psycrobacter sp., and Xanthomonas sp.* are a few PGPR among the wide range of PGPR that play an important role in bioremediation of heavy metal toxicity (Shinwari *et al.*, 2015). Among the various defensive mechanisms exerted by PGPR, the foremost is the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase which reduces the level of stress-inducer hormone ethylene accumulation in plants (Singh *et al.*, 2015). Another effective mechanism shown by PGPR to decrease metal toxicity is the production of microbial siderophores (Radzki *et al.*, 2013).

Effect of biofertilizer in remediation of pesticides

Excessive and persistent use of pesticides becomes detrimental to the environment and possesses a potential threat to the plant kingdom as well as mankind since it can easily pass into tissues of living organisms and rise to bioaccumulation (Kumar and Puri, 2012). It has been reported that the microorganisms like *Azospirillum, Azotobacter, Bacillus, Enterobacter, Gordonia, Klebsiella, Paenibacillus, Pseudomonas, Serratia*, etc. hold the ability to decrease pesticide toxicity (Shaheen and Sundari, 2013). Besides these strains, *Actinomycetes* alsohave the considerable potential for biotransformation and biodegradation of pesticides. For pesticide degradation, the foremost mechanism exerted by microorganisms is enzymatic degradation. Moreover, several enzymes catalyze a wide range of reactions including hydrolysis, oxidation, addition of amino group to a nitro group, dehalogenation, reduction of nitro group to amino group, replacement of sulfur with an oxygen, ring cleavage, and metabolisms of side chains has been reported to reduce the toxicity exerted by pesticides (Ramakrishnan *et al.*, 2011).

Effect of biofertilizers on plant parasitic nematodes

El-Haddad *et al.*, (2011) reported that some bacterial biofertilizers including the nitrogen-fixing bacteria, *Paenibacillus polymyxa* (four strains), the phosphate solubilizing bacteria, *B. megaterium* (three strains) and the potassium-solubilizing bacteria, *Bacillus circulans* (threestrains) were inoculated individually on tomato plants infested with the root-knot nematode *M. incognita* in potted sandy soil and it was found that all the applied microbial biofertilizers showed significant nematicidal activity. Khan *et al.*, (2012) showed that the growth, yield, and quality of nematode-infested chili (*Capsicum annum*) increased when they were inoculated with biological nitrogen fixer (*Azospirillum* and *Azotobacter*).

Effect of biofertilizer on ecosystem

Effects of biofertilizers on non-target members of soil rhizosphere and food web have been studied to a large extent and most studies suggested measurable changes due to the introduction of bio inoculants to the rhizosphere, however, the magnitude of alterations and its significance on the ecological functions remain yet to be reported (Martínez-Viveros *et al.*, 2010). It is reported that the extent of effect of biofertilizers into the soil organisms depends on various factors including soil characteristics, method of application of biofertilizers, different environmental conditions etc. (Dey *et al.,* 2012). Methods which have higher resolution need to be applied along with traditional techniques for multi-dimensional analysis of the efficacy, diversity, and risk assesment studies of biofertilizers before releasing them into the ecosystem (Sharma *et al.,* 2012).

Effect of biofertilizer in reclamation of degraded land

Proper reclamation of mine spoil dump depends upon the development of an active indigenous microbial community which is responsible for the development of soil structure, plant growth and production of plant nutrients through different bio-geochemical cycles (Juwarkar and Singh, 2007; Kumar *et al.,* 2013). Due to mining, the soil of the mining sites becomes highly acidic which is harmful for the plant growth. The pH of these soils can be increased by application of organic amendments which not only increases soil pH but also improve soil quality and water holding capacity and provide a slow release of fertilizer (Diacono and Montemurro, 2010). Thus, biofertilizers can able to reclaim soil of degraded land to some extent.

Effect of biofertilizers in crop production

The incorporation of biofetilizers in soil play major role in improving soil fertility, yield attributing characters and thereby final yield. Biofertilizers increase the nutrient availability to various crops and give better health to plants and soil, hence enhancing crop yields in a sustainable way. *Azolla* biofertilizeris used for rice cultivation because of its quick decomposition in soil and efficient availability of its nitrogen to rice plants (Yadav *et al.,* 2019). Application of *Rhizobium* biofertilizers significantly enhanced the agronomic yield attributes in pulse crops under temperate climatic conditions and *Azospirillum* application in agricultural crops improves the leaf area index, harvest index, and yield attributes (Mahendra Singh *et al.,* 2016).

Mode of application of formulated biofertilizer

There are many ways for applying formulated biofertilizerinto soil and these are (a) seed inoculation with powder formulations, (b) dry biofertilizers mixed with the seeds in the seed hopper, (c) biofertilizer and adhesive are applied as slurry to seeds and coated with ground material like lime, (d) slurry method (the biofertilizer is suspended in water then added to the seeds and mixed), (e) seed pelleting, (f) sprinkle method (a small amount of water mixed with seeds before peat powder is added and mixed), (g) peat suspension in water sprayed into the furrow during sowing, (h) seed treatment or seed inoculation, (i) soil application, and (j) seedling root dip (Bashan,1998).

Liquid biofertilizers-An innovative step towards biofertilizer technology

Liquid biofertilizers are suspension containing desired microorganisms and special cell protectants or chemicals that enhance formation of latent spores or cysts for longer shelf life and tolerance to unfavourable environments (Hegde, 2008). Liquid biofertilizers have gained popularity because of easy handling and application either on seeds or in soil (Herrmann and Lesueur, 2013). The advantages of liquid biofertilizers over powder based are that microorganisms have longer shelf life upto 2 years, generally they circumvent the effect of high temperature, maintain high *cfu* more than 10^9 ml^{-1} upto 12 months and better survive on seeds and soil, in addition, liquid biofertilizers are easy to use, handling and storage by farmers, the dosage is ten times less than that of powder form, it can be packed in different volumes and save carrier materials (Verma *et al.*, 2011; Borkar, 2015). Additionally, liquid formulations are compatible with machinery on large farms, such as air seeders or seed augers (Bashan *et al.*, 2014). However, some limitations have precluded their use in most developing countries nowadays. Biofertilizers based on broth cultures do not have carrier protection and quickly lose viability on seed. However, addition of some other components like sucrose, glycerol etc. may improve survival of microorganisms in liquid condition (John *et al.*, 2011).

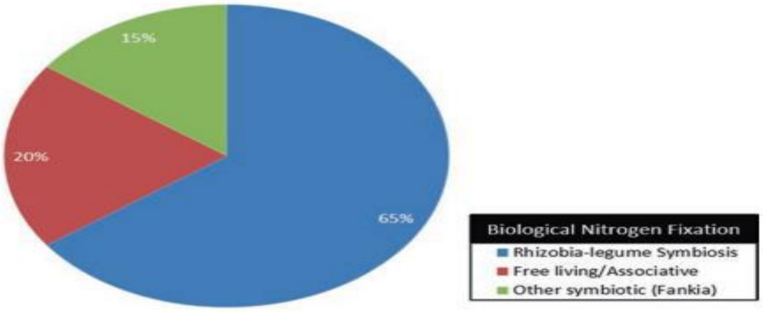
Table.1 Major inoculation groups with inoculant and host plants (Ponmurugan and Gopi, 2006)

Cross inoculation Group	*Rhizobium* species	Host Legume
Pea group	*R. leguminosarum*	Pea, sweet pea
Alfalfa group	*R. meliloti*	Sweet clover
Clover group	*R. trifoli*	Clover / berseem
Bean group	*R. phaseoli*	All beans
Soybean group	*Bradyrhizobium japonicum*	Lupins
Cowpea group	*Rhizobium* sp.	Cowpea, arhar, urd, moong and groundnut

Table.2 Effect of *azotobacter* on crop yield (Dudeja *et al.*, 1981)

Crop	Increase in yield over yields obtained with chemical fertilizers (%)	Crop	Increase in yield over yields obtained with bio fertilizers (%)
Wheat	8-10	Potato	16
Rice	5	Carrot	40
Maize	15-20	Cauliflower	2-24
Sorghum	15-20	Tomato	7-27
Other	13	Cotton	9-24

Fig.1 Biological nitrogen fixation (Modified from Bouizgarne *et al.*, 2015)



Conclusion and future perspectives of biofertilizers

For more widespread utilization of biofertilizers will require addressing few issues with more attention and necessary actions to resolve the issues.

The study of particular strain effectiveness regarding a particular crop and soil and climatic factors. It is needed to strengthen the research and technologies in combination along with extension wing.

Standardization of biofertilizer dose in a particular crop and soil.

Elaborating the use of biofertilizers from laboratory and greenhouse experiments to large-scale commercial use will require a number of advanced new approaches for the growth, storage, shipping, formulation, and application of these bacteria.

A quality control system should exist for the production of inoculants and their application in the field to ensure and explore the benefits of plant-microorganisms' symbiosis. "Bio-fertilizer Act" and strict regulation for quality control in markets and application should be established.

Identification of better carrier material for enhancing the shelf life of strains.

Therefore, it can be said that there is an urgent need to improve the awareness and use of biofertilizers. So, integrated application of biofertilizers along with chemical fertilizers in a sustainable manner can not only able to meet the nutrient need of plant but also maintain the soil health, environmental safety and food security.

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