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Review

Biofertilizers and their role in management of plant parasitic nematodes. A review

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A biofertilizer is a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the anterior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant or bio-fertilizer contains biological means, living organisms that synthesis the atmospheric plant nutrient in the soil or in the plant body, or create such an atmosphere in the soil or in the medium in which the organisms are kept. Biofertilizers are applied as seedling root dip, seed or soil treatments. Many researches showed that the growth, yield and quality parameters of certain plants significantly increased with biofertilizers containing bacterial nitrogen fixer, phosphate and potassium solubilizing bacteria and microbial strains of some bacteria. These bacteria reduced the population of *Meloidogyne incognita* infecting chilli and tomato and *Tylenchulus semipenetrans* on Washington navel orange. Also, Six new commercial Egyptian bio-fertilizers viz.,nitrobien, rizobacterin, cerealin, phosphorine, microbien, blue green algae, and five new commercial Egyptian plant nutrients viz., nuftarein, potassein F, citrein, kotangein and kapronite as for the control of root-knot nematode, *Meloidogyne incognita* on sunflower. All the tested products significantly reduced the numbers of juveniles in soil, and galls, females, eggmasses on roots. Also, nitrobien and phosphorine were effective in reducing *M. incognita* population infecting cowpea and enhancing plant growth criteria.

Key words: Control; Biofertilizers; Plant Parasitic Nematodes.

INTRODUCTION

Increasing use of chemical fertilizers in agriculture make country self- dependent in food production but it deteriorate environment and cause harmful impacts on living beings. Due to insufficient uptake of these chemical fertilizers by plants, they reach into water bodies through rain water, cause eutrophication in water bodies and affect living beings including growth inhabiting microorganis*m.* The excess uses of chemical fertilizers in agriculture are costly and also have various adverse effects on soils as depletion of water holding capacity, soil fertility and disparity in soil nutrients. It was felt from a long time to develop some low cost effective and eco-friendly fertilizers which work without disturbing nature. Now, certain species of microorganism are widely used which have unique properties to provide natural products, and serve as a good substitute of chemical fertilizers (Deepali and Gangwar, 2010). Hence, the present review throws the light on the necessity of biofertilizers and their role in management of plant parasitic nematodes.

Field and vegetable and vegetable crops

Biofertilizers

Plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. A biofertilizer (also, bio-fertilizer) is a substance which contains living microorganisms which, when applied to seed, plant surface, or soil, colonizes the rhizosphere or the anterior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. (Gaur, 2010).

What is an artificial fertilizer?

Nitrogen, phosphorus and potassium are the most

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important among 17 essential plant nutrients. The plant absorbs nitrogen only in the form of solid. Conservation of gaseous form of nitrogen into solid form is called nitrogen fixation. Fixation of nitrogen in chemical fertilizers in factories called artificial nitrogen fixation (artificial fertilizers) (Gauer, 2010).

What are the differences between biofertilizer and organic fertilizer?

Bio-fertilizer itself explains fertilizer that contains biological means, living organisms that synthesize the atmospheric plant nutrient in the soil or in the plant body, or create such an atmosphere in the soil or in the medium (in which the organisms are kept) which are helpful for the plants. The biofertilizers may be in solid or liquid medium and micro organisms are in huge numbers, (e.g. 10-7 cells /g). All these mean that the nutrients made available to the plants by the help of microorganisms are bio-fertilizers. Bio-fertilizers are element specific. Organic manure is the manure prepared from the animal and plant wastes after properly decomposing the raw material. It may contain all necessary plant nutrients in small quantities which are required in large quantities and these may be the medium for bio-fertilizers (Anonymous, 2012a).

Why biofertilizers?

Biofertilizers are supposed to be safe alternative to chemical fertilizers to minimize the ecological disturbance. Biofertilizers are cost effective, eco-friendly and when they are required in bulk, can be generated at the farm itself. They increase crop yield by 10-40% and fix nitrogen up to 40-50%. The other plus points that, after using 34 years continuously, there is no need of application of biofertilizers because parental inoculums are sufficient for growth and multiplication. They improve soil texture, pH, and other properties of soil (Anonymous, 2012b).

Benefit of Biofertilizers (Gaur, 2010)

1-Cheap source of nutrients; 2-Suppliers of microelements, 3- Suppliers of micro nutrients, 4-Suppliers of organic matter,5-Counteracting negative impact of chemical fertilizers,6-Secretion of growth hormones.

Disadvantages of biofertilizers

Applying biofertilizers is unlikely to harm plant life or the environment in any way, but there is little to guarantee that they will help either. This is a distinct disadvantage compared to nutrient-based fertilizers that reliably provide quantifiable results. The reason for this lies in the myriad factors that have to be aligned for the microbes in biofertilizers to be effective for the purpose they are prescribed. Their effectiveness is a product of complex chemical and biological interactions that are themselves affected by moisture, temperature, pH and other environmental variables. If the conditions aren't right for the microbes to multiply and do their work, their populations are likely to peter out, and the user will have wasted time and money on a product that was not suitable for the soil conditions (Anonymous, 2013):

Carrier-based biofertilizers

Carrier-based biofertilizers are prepared with the help of activated charcoal, which act as a carrier for microbial inoculants. Biofertilizer consumption is not very satisfactory due to certain disadvantages associated with carrier-based biofertilizers like low shelf life (3-4 months), storage conditions (stored in cool temperature) as it is temperature sensitive, bulky to transport, therefore, high transport cost, less scope for export, more chances of contamination, problem of proper packing, poor cell protection, poor moisture retention capacity and restriction on use of charcoal as a measure of conservation (Verma *et.al.,* 2011).

Applications of biofertilizers to crops: (Anonymous, 2010)

Seedling root dip

This method is applied to rice crop. A bed of water is spread on the land where the crop has to grow. The seedlings of rice are planted in the water and are kept there for eight to ten hours.

Seed treatment

The nitrogen and phosphorus fertilizers are mixed together in the water. Then, seeds are dipped in the mixture. After that, seeds are dried and they have to be sown as soon as possible before they get damaged by harmful microorganisms.

Soil treatment

All the biofertilizers along with the compost fertilizers are mixed together. They are kept for one night. Then, the next day; this mixture is spread on the soil where seeds have to be sown.

Microbes used as Biofertilizer(s) (Deepali and Gangwar, 2010)

Microbes are effective in inducing plant growth as they secrets plant growth promoters (auxins, abscisic acid, gibberellic acid, cytokinins, and ethylene) and enhance seed germination and root growth. They also play a considerable role in decomposition of organic materials and enrichment of compost and include:

Nitrogen fixing Bacteria

Rhizobia

Legume plants have root nodules, where atmospheric nitrogen fixation is done by bacteria belonging to genera, *Rhizobium*, *Bradyshzodium*, *Sinorhizobium*, *Azorhizobium* and *Mesorhizobium* collectively called as rhizobia. When rhizobial culture is inoculated in field, pulse crops yield can be increased due to rhizobial symbiosis (Dubey, 2006). Rhizobium can fix 15-20 kg N/ha and increase crop yield up to 20%.

Azorhizobium

It is a stem nodule forming bacteria and fixes nitrogen symbionts of the stem nodule. Also, it produce large amount of indole acetic acid (IAA) that promotes plant growth.

Bradyrhizobium

Bradyrhizobium is reported as a good nitrogen fixer. Bradyrhizobium strain inoculation with mucuna seeds enhances total organic carbon, N2, phosphorus and potassium in the soil, increases plant growth and consequently plant biomass, reduction in the weed population and increased soil microbial population.

Diazotrophs

These are aerobic chemolithotrophs and anaerobic photoautotrophs. These are non-nodule forming bacteria and include numbers of the families:

Azotobacteracae: e.g. Azotobacter

They are the free living aerobic, photoautotrophic, non-symbiotic bacteria. They secrete vitamin-B complex, gibberellins, napthalene, acetic acid and other substances that inhibit certain root pathogens and improve root growth and uptake of plant nutrients. It occurred in the roots of *Paspalum notatum* (tropical grasses) and other species and added 15-93 Kg N/ha/annum on *P. notatum* roots (Dobereiner *et al.,* 1973). *Azotobacter* indicum occurs in acidic soil in sugarcane plant roots. It can be applied in cereals, millets, vegetables and flowers through seed, seedling and soil treatments.

Spirillaceae: e.g. Azospirillum and Herbaspirillum

These are gram- negative, free living, associative symbiotic and non-nodule forming, aerobic bacteria, occurs in the roots of dicots and monocot plants i.e. corn, sorghum, wheat etc. It is easy to culture and identify. *Azospirillum* is found to be very effective in increasing 10-15% yield of cereal crops and fixing N2 up to 20-40 Kg N/ha. Different A. brasiliense strains inoculation in the wheat seed causes increase in seed germination, plant growth, plumule and radicle length. *Herbaspirillum* species occurs in roots, stems and leaves of sugarcane and rice. They produce growth promoters (IAA, gibberillins, cytokinins) and enhance root development and uptake of plant nutrients (N, P & K).

Acetobacter diazotrophicus

Another diazotroph is *Acetobacter diazotrophicus* occurs in roots, stem and leaves of sugarcane and sugar beet crops as nitrogen fixer and applied through soil treatment. It also produces growth promoters e.g. IAA and helps in nutrients uptake, seed germination, and root growth. This bacterium fixed nitrogen up to 15kgN/ha/year and enhanced up to 0.5 – 1% crop yield (Gahukar, 2005-06).

Cyanobacteria (Blue green algae)

Nostoc, *Anabaena*, Oscillatoria, Aulosira, Lyngbya etc. are the prokaryotic organisms and phototropic in nature. They play an important role in enriching paddy field soil by fixing atmospheric nitrogen and supply vitamin B complex and growth promoting substances which make the plant to grow vigorously. Cyanobacteria fix 20-30 Kg/N/ha and increase 10-15% crop yield when applied at 10 Kg/ha. Youssef and Ali (1998) reported that three blue green algae, *Anabena oryzae*, Nostoc calcicola and *Spirulina* sp. Reduced number of galls and eggmasses caused by the root knot nematode ,*Meloidogyne incognita* infecting cowpea and improved plant growth criteria.

Azolla – Anabaena symbiosis

It is a free floating, aquatic fern found on water surface having a cyanobacterial symbiont, *Anabaena azollae* in its leaves. It fixes atmospheric nitrogen in paddy field and excrete organic nitrogen in water during its growth and also immediately upon trampling. Azolla contributes nitrogen, phosphorus (15-20 Kg/ha/month), potassium (20-25 kg/ha/month) and organic carbon etc. and increases 10-20% yield of paddy crops and also suppresses weed growth. Azolla also absorbs traces of potassium from irrigation water and can be used as green manure before rice planting. *Azolla* spp. are metal tolerant, hence can be applied near heavy metal polluted areas.

Phosphate Solubilizing Bacteria

Pseudomonas fluorescens, *Bacillus megatherium* var. *phosphaticum*, *Acrobacter acrogens*, *nitrobacter spp*., *Escherichia freundii*, *Serratia* spp., *Pseudomonas* striata, Bacillus *polymyxa* are the bacteria which have phosphate solubilizing ability. Phosphobacterin are the bacterial fertilizers containing cells of *Bacillus megatherium* var. phosphaticum, prepared firstly by USSR scientists. They increased about 10 to 20 % crop yield (Cooper 1959) and also produced plant growth promoting hormones which helped in phosphate solubilizing activity of soil. Al-Rehiayani et.al. (1999) found that B. megaterium reduced penetration of *M.* chitwoodi and Pratylenchus penetrans into potato roots by 50%. Padgham and Sikora (2007) stated that treatment with Bacillus megatherium resulted in 40% reduction in nematode penetration and gall formation compared with non treated rice plants. Khan *et al*., (2007) stated that biofertilizers, based on plant growth microorganisms, particularly phosphate -solubilizing microorganisms in place of inorganic fertilizers, could also be used in nematode disease management.

Phosphate solubilizing fungi

Some fungi also have phosphate dissolving ability e.g. *Aspergillus niger*, *Aspergillus awamori*, *Penicillium digitatum* etc.

Plant Growth Promoting Rhizobacteria (Pgpr)

They are also called as microbial pesticides e.g. Bacillus spp. and Pseudomonas fluorescens. Serratia spp. and Ochrobactrum spp. are able to promote growth of plants. P. fluorescens application to the black pepper enhance uptake of nutrients which increase plant biomass. Fluorescent rhizobacteria improve the growth of Hevea brasiliensis plants. Bevivino *et.al.,(*1998) found that rhizobacteria could stimulate plant growth directly by producing growth hormones and improving nutrient uptake and indirectly by changing microbial balance in the rhizoshere in favour of beneficial microorganisms.

Mycorrhizae

Mycorrhizae are developed due to the symbiosis between some specific root inhabiting fungi and plant roots and used as biofertilizers. They absorb nutrients such as manganese, phosphorus, iron, sulphur, zinc etc. from the soil and pass them to the plant. Mycorrhizal fungus increases the yield of crops by 30-40% and also produces plant growth promoting substances

VAM fungi or endo- mycorrhizae

They occur commonly in the roots of crop plants. VAM fungal hyphae enhance the uptake of phosphorus and other nutrients that are responsible for plant growth stimulation including roots and shoot length. VAM also enhances the growth of black pepper and protects from Phytophthora capsici, Radopholus similis and *Meloidogyne incognita* (Anandraj *et al.,* 2001). VAM fungi enhance water uptake in plants and also provide heavy metals tolerance to plants. Bagyaraj et.al. (1979) reported that inoculation of tomato roots with root knot nematodes enhanced infection and spore production by vesicular arbuscular Mycorrhizal fungus, Glomus fasciculatus. Inoculation of tomato plants with this fungus significantly reduced the number and size of the root knot galls produced by root knot nematode, *Meloidogyne incognita* and improved plant growth criteria. Suresh *et.al.,* (1885) showed that the number of giant cells caused by *M. incognita* and formed in Mycorrhizal plants were significantly low, Root extract from the Mycorrhizal plants brought about 50% mortality of the nematode larvae in four days. Hajra *et.al.,(*2013) reported that leaf area and plant height were increased in Mycorrhizal plants than non- mycorrhizal, while they showed a shrp decrease in nematode infected plants.The same plants showed less water content due to xylem vessel damage. In mycorrhizal plants, roots had large amount of carbohydrates indicating transfer of photosynthates to fungal partner. Nematode- infected roots have least amount of carbohydrates showing a great sink of carbon to rhizoshere.

Effect of biofertilizers on plant parasitic nematodes

Volatile compounds, fatty acids, hydrogen sulfide, enzymes, hormones, alcohol and phenolic compounds are among the bacterial products implicated in the control of plant parasitic nematodes (Mishra *et.al.,*1987). Such products may be toxic to nematodes directly or it may be indirectly suppress nematode population by modifying the rhizoshere environment.

Effect of some bacterial biofertilizers on the root knot nematode, *Meloidogyne incognita* infecting some vegetable crops

Khan et al., (2012) showed that the growth, yield and quality parameters of chilli (Capsicum annum L.), infested with plant parasitic nematodes, significantly increased with the inoculation of biological nitrogen fixer using Azosperillum and *Azotobacter*. Performance of Azosperillum was found better as compared to *Azotobacter*. Simultaneous inoculation with biofertilizers (100% recommended dose of N-fertilizer at 100kg N / ha and farmyard manure at 15 tons/ha) resulted in the maximum growth, yield and quality parameters. This helps to save 25% nitrogenous fertilizers in chilli crop. Also, there were increased contents in plant nitrogen, phosphate and potash, leaf chlorophyll and residual available soil nitrogen, phosphate and potash with dual inoculation with biological nitrogen fixers along with recommended full dose of nitrogen fertilizer. El-Haddad et.al.(2011)reported that the nematicidal effect of some bacterial biofertilizers including the nitrogen fixing bacteria, Paenibacillus polymyxa (four strains), the phosphate solubilizing bacteria, *Bacillus megatherium* (three strains) and the potassium solubilizing bacteria, B. circulans (three strains) were evaluated individually on tomato plants infested with the root-knot nematode *Meloidogyne incognita* in potted sandy soil. Comparing with the uninoculated nematode-infested control, the inoculation with P. polymyxa NFB7, B. megatherium PSB2 and B. circulans KSB2 increased the counts of total bacteria and total bacterial spores in plants potted soil from 1.2 to 2.6 folds estimated 60 days post-inoculation. Consequently, the inoculation with P. polymyxa NFB7 significantly increased the shoot length (cm), number of leaves / plant, shoot dry weight (g) / plant and root dry weight (g) / plant by 32.6 %, 30.8 %, 70.3 % and 14.2%, respectively. Generally, the majority treatments significantly reduced the nematode multiplication which was more obvious after 60 days of inoculation. Among the applied strains, P. polymyxa NFB7, B. megatherium PSB2 and B. circulans KSB2 inoculations resulted in the highest reduction in nematode population comparing with the uninoculated nematode-infested control. They recorded the highest reduction in numbers of hatched juveniles/root by 95.8 %, females/root by 63.75 % and juveniles/1kg soil by 57.8 %. These results indicated that these bacterial biofertilizers are promising double purpose microorganisms for mobilizing of soil nutrients (nitrogen, phosphate and potassium) and for the biological control of *M. incognita*.

Effect of some bacterial biofertilizers on the citrus nematode, *Tylenchulus semipenetrans* infesting citrus trees

Shamseldin *et. al.,* (2010) tested the ability of microbial strains of Pseudomonas fluorescens strain 843 and Azospirillum brasilense strain W24 to improve Washington navel orange fruit quality and to control the persistence of nematode in the soil. Strains were applied one time monthly during the period of experiment to trees at two levels 300 ml and 500 ml per tree with 10-8 cells ml^{-1}. Bio-fertilizer inoculation with Pseudomonas fluorescens strain 843 as growth promoting rhizobacterium significantly improved fruit quality as well as increased fruit yield, fruit weight, fruit length, TSS (Total soluble solids) and juice volumes, while inoculation with Azospirillum brasilense strain W24 increased, but not significantly fruit quantity and quality of Washington navel orange. Commonly, three types of nematodes were detected in the roots including *Tylenchulus* spp., saprophytic nematodes and *Pratylenchus* spp., while the dominant species was *Tylenchulus semipenetrans*. Generally there is a reduction in the number of nematodes with the two examined strains, while the addition of Pseudomonas f. strain 843 was successfully greater to inhibit the growth of nematodes than *Azospirillum* b. strain W24 suggesting that this strain can be used as a bio-fertilizer for promoting citrus growth and bio-control for reducing the distribution and propagation of nematodes associated with citrus. Enhancement and maintenance of soil fertility and conservation of the soil's health through bio-fertilizer applications will be a vital role and occupy significant concern for many of researcher in the future as an unique key for sustainable agriculture in developing countries.

Effect of some commercial biofertilizers and nutrients on the root knot nematode, M. *incognita* infesting some field and vegetable and vegetable crops

Ismail and Hasabo (2000) tested six new commercial Egyptian bio-fertilizers (BF) viz., nitrobien, rizobacterin, serealin, Phosphorine, microbien, bluegreen algae; and five new commercial Egyptian plant nutrients viz., nuftarein, potassein F, citrein, kotangein and kapronite as well as nemaless a new biocide at three different rates (on base lower rate, recommended rate and higher rate) for the control of *Meloidogyne incognita* and improvement of sunflower cv. Giza 101 under greenhouse conditions at 35 ± 5oC. All the tested product significantly reduced ($P \leq 0.05$ and/or 0.01) the numbers of juveniles in soil, females, eggmasses, the rate of nematode build-up, gall formation on roots and consequently gall and eggmass indices. The highest suppression in the nematode populations, galls and its build-up was achieved with seed coating by rizobacterin followed by phosphorine and nitrobien as biofertilizers, while the least reduction was obtained by using nemaless as a biocide followed by blue-green algae as biofertilizer. Different plant nutrients, kapronite as soil amendment and Kotangein as seed coating showed better effect in reducing the previous nematode stages followed by potassein F and citrein, whereas nuftarein as foliar spray nutrient was the least effective one. El-Gindi et al., *(*2005).reported that when cowpea plants infected with *Meloidogyne incognita* were treated with nirobien and phosphorine as biofertilizers, no significant differences were found between nitrobien and phosphorine in reducing nematode population as well as plant growth responses. However, nitrobien seems to be more effective than phosphorine the above mentioned parameters.

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