

Microbial Products and Biofertilizers in Improving Growth and Productivity of Apple – a Review

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Abstract

The excessive use of mineral fertilizers causes many negative consequences for the environment as well as potentially dangerous effects of chemical residues in plant tissues on the health of human and animal consumers. Bio-fertilizers are formulations of beneficial microorganisms, which upon application can increase the availability of nutrients by their biological activity and help to improve soil health. Microbes involved in the formulation of bio-fertilizers not only mobilize N and P but mediate the process of producing crops and foods naturally. This method avoids the use of synthetic chemical fertilizers and genetically modified organisms to influence the growth of crops. In addition to their role in enhancing the growth of the plants, biofertilizers can act as biocontrol agents in the rhizosphere at the same time. Biofertilizers are very safe for human, animal and environment. The use of *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Burkholderia*, *Bacillus*, *Paenibacillus* and some members of the *Enterobacteriaceae* is gaining worldwide importance and acceptance and appears to be the trend for the future.

Key words: apple productivity, biocontrol, biofertilization, bioproducts

Introduction

Apple (*Malus domestica* Borkh.) is the 3rd most important fruit crop worldwide, after citrus and banana (FAOSTAT, 2013). In 2013, the total apple production and harvest in the world was estimated at 80.8 million tons and 5.2 million hectares, respectively. Its cropping has expanded into subtropical and tropical zones (Karakurt and Aslantas, 2010) and is one of the most important cropped and consumed fruits in the world (Brown, 2012). Intensive farming practices, that warrant high yield and quality, require the extensive use of chemical fertilizers and pesticides, which are costly and create environmental problems. Hence, there has been a resurgence of interest in environmental friendly, sustainable and eco-friendly agricultural practices (Esitken *et al.*, 2002). One potential way to decrease negative environmental impacts resulting from continued use of chemical fertilizers is inoculation with plant growth promoting rhizobacteria (PGPR). These bacteria improve nutrient (N, P, K, Fe, and Zn) bio-availability (Table I) and exert beneficial effects on plant growth and development, and therefore may be used

as biofertilizers for agriculture. The natural role of the PGPR in maintaining soil fertility is more important than in conventional agriculture where higher use of agrochemicals minimizes their significance (Canbolat *et al.*, 2006). Moreover, the applications of biofertilizers containing beneficial microorganisms instead of synthetic chemicals are known to improve fixation of nutrients in the rhizosphere, produce growth stimulants for plants, improve soil stability, provide biological control, biodegrade substances, recycle nutrients, promote mycorrhiza symbiosis, and develop bioremediation processes in soils contaminated with toxic, xenobiotic and recalcitrant substances (Rivera-Cruz *et al.*, 2008). So the use of more sustainable technologies, such as biofertilization, is inevitable for the mitigation of environmental damage (Karakurt and Aslantas, 2010).

The influence of biofertilizers in improving apple growth and productivity

Applications of bio-fertilizers containing beneficial microorganisms instead of synthetic chemicals are known to improve plant growth through the supply

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Table I
Plant Growth Promoting Rhizobacterial (PGPR) strains improving nutrient (N, P, K, Fe and Zn) bioavailability

	Bacteria	References
N – Nitrogen, P – Phosphorus, K – Potassium, Fe – Iron	<i>Rhizobium leguminosarum</i>	Biswas <i>et al.</i> , 2000
	<i>Bradyrhizobium japonicum</i> UCM B-6018	Tytova <i>et al.</i> , 2013
N – Nitrogen, P – Phosphorus, Fe – Iron	<i>Pseudomonas aeruginosa</i> BS8	Goswami <i>et al.</i> , 2015
N – Nitrogen, P – Phosphorus	<i>Bacillus megaterium</i> , <i>Bacillus mucilaginosus</i>	Han and Lee, 2005
N – Nitrogen, Fe – Iron	<i>Pseudomonas</i> strain GRP3	Sharma <i>et al.</i> , 2003
	<i>Pseudomonas fluorescens</i> C7	Vansuyt <i>et al.</i> , 2007
N – Nitrogen	<i>Azospirillum</i> spp.	Bashan and De-Bashan, 2010
	<i>Pseudomonas alcaligenes</i> PsA15, <i>Mycobacterium phlei</i> MbP18	Egamberdiyeva and Höflich, 2004
	<i>Azospirillum lipoferum</i> , <i>Azospirillum brasilense</i>	Malik <i>et al.</i> , 2002
	<i>Klebsiella pneumonia</i> , <i>Pantoea agglomerans</i>	Riggs <i>et al.</i> , 2001
	<i>Azotobacter</i> spp.	Mrkovacki and Milic, 2001
	<i>Azotobacter chroococcum</i>	Wu <i>et al.</i> , 2005
P – Phosphorus	<i>Streptomyces</i> spp.	Chang and Yang, 2009
	<i>Micrococcus</i> spp.	Dastager <i>et al.</i> , 2010
	<i>Achromobacter</i> spp.	Ma <i>et al.</i> , 2009
	<i>Bacillus</i> spp., <i>Burkholderia</i> spp.	Tao <i>et al.</i> , 2008
	<i>Bacillus megaterium</i>	Wu <i>et al.</i> , 2005
	<i>Pseudomonas alcaligenes</i>	Zhang <i>et al.</i> , 2014
	<i>Pseudomonas aeruginosa</i>	Yadav <i>et al.</i> , 2014
K – Potassium	<i>Bacillus edaphicus</i>	Sheng and He, 2006
Zn – Zinc	<i>Serratia</i> spp.	Abaid-Ullah <i>et al.</i> , 2011
	<i>Pseudomonas fluorescens</i>	Di Simine <i>et al.</i> , 1998
	<i>Pseudomonas aeruginosa</i>	Fasim <i>et al.</i> , 2002
	<i>Flavobacterium</i> spp.	He <i>et al.</i> , 2010
	<i>Pseudomonas</i> spp. PsM6, <i>P. jessenii</i> PjM15	Rajkumar and Freitas, 2008
	<i>Acetobacter diazotrophicus</i>	Saravanan <i>et al.</i> , 2007
	<i>Rhizobia</i> spp.	Wani <i>et al.</i> , 2008
	<i>Pseudomonas</i> sp. Z5	Yasmin, 2011

of plant nutrients and may help to sustain environmental health and soil productivity (O'Connell, 1992). A biofertilizer is a substance which contains living microorganisms which, when applied to seeds, 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 (Vessey, 2003). Biofertilization is now a very important method for providing the plants with their nutritional requirements without having an undesirable impact on the environment (Abou El-Yazied and Sellim, 2007). Additionally, the use of biofertilizers can improve productivity per unit area in a relatively short time, consume smaller amounts of energy, mitigate contamination of soil and water, increase soil fertility, and promote antagonism and biological control of phytopathogenic organisms (Corpoica *et al.*, 2007). Moreover, biofertilizers are known to improve fixation of nutrients in the rhizosphere, produce growth stimulants

for plants, improve soil stability and provide biological control. They also biodegrade substances, recycle nutrients, promote mycorrhiza symbiosis and develop bioremediation processes in soils contaminated with toxic, xenobiotic and recalcitrant substances (Rivera-Cruz *et al.*, 2008). Raghuwanshi (2012) stated that biofertilizers have a great potential as supplementary, renewable and environmental friendly sources of plant nutrients. Furthermore, they are an important component of integrated nutrient management and plant nutrition system. Application of biological potassium fertilizers (BPF), as preparation of silicate bacteria (liquid solution, containing two million bacteria per 1 ml, or packages of 500 g of peat-moss substrate, contains 2 million bacteria) and Azobacterin increased trunk cross-sectional area, mean shoot length, mean leaf area, total leaf area, number of fruits per tree, mean fruit weight and yield of "Charavnitsa" apple variety (Ryabtseva *et al.*, 2005). Von-Bennwitz and Hlusek (2006) reported that

biofertilization is beneficial in stimulating the growth and fruiting of pome and stone fruits. Treatment of 'Topaz' and 'Ariva' apple trees with the biopreparations: Micosat F, Humus UP, Humus Active + Aktywit PM, BioFeed Amin, Vinassa, Florovit Eko and Florovit Pro Natura produced positive effects on the growth of apple roots and their mycorrhizal frequency, and the size of the populations of microorganisms in the rhizosphere soil (Derkowska *et al.*, 2014). Besides, Rozpara *et al.* (2014) found also that Biofeed Amin preparation had a positive influence on the growth and development of 'Ariwa' apple trees growing. Tree trunk sectional area and yield of "Topaz" apple trees was improved with Florovit Natura and Yeast combined with *Pantoea* spp., *Pseudomonas fluorescens*, *Klebsiella oxytoca* and *Rhizobium* spp. bacteria species respectively as compared to NPK chemical fertilization (Mosa *et al.*, 2016).

The effect of mycorrhiza on apple growth and yield

Arbuscular mycorrhizal (AM) fungi are associated with the roots of over 90% of terrestrial plant species (Gadkar *et al.*, 2001). They are a very important component within the rich biodiversity of microorganisms occurring in the rhizosphere (Turnau and Haselwandter, 2002). Xavier and Boyetchko (2002) have found that mycorrhizal fungi, in particular endomycorrhizal have a beneficial effect on plant growth and development, and that effect can be likened to the effects of biostimulators and biofertilizers on plants. Root inoculation with two biopreparations, Vambac® (VA-mycorrhiza genus *Glomus*, *Gigaspora* and the rhizospheric bacteria *Agrobacterium radiobacter*) and Amalgerol® (composed of vegetative and sea-algae oils and extracts) enhanced the uptake of phosphorus and vegetative growth of two-year-old apple trees cv. "Jonagold" grown on M.9 root stock (Von Bennowitz and Hlusek, 2006). Cavallazzi *et al.* (2007) stated that apple (*Malus prunifolia*) Colonization by *Glomus etunicatum* SCT110, *Scutellospora pellucida* SCT111, *Acaulospora scrobiculata* SCT112 and *Scutellospora heterogama* SCT113 fungal isolates significantly affected plant height, shoot and root dry weights, and root: shoot ratio. Moreover, mycorrhizal inoculation also significantly altered tissue concentrations of P, Zn, Cu, Ca, S, Na, N, K, Fe and Al.

Overall, *G. etunicatum* and *S. pellucida* were the most effective isolates to promote plant growth and nutrient uptake. Many investigations shows that AM symbiosis contributes to plant growth, nutrient uptake and improve fruit quality (Miransari, 2010). The positive and beneficial effects of AM fungi such as growth promotion, increased root length, leaf area and stem diameter (Sharma *et al.*, 2011), transplant performance

and tolerance to abiotic (water, nutrition) stresses (Göhre and Paszkowski, 2006), could be due to a positive interaction between AM fungi and other associated microorganisms such as *Azotobacter chroococcum* in a particular edaphic and agro-climatic conditions. Sharma *et al.* (2012) reported significant improvement in the vegetative growth parameters of 'Royal Delicious' apple saplings by using single and/or dual application of soil inoculation of *Glomus fasciculatum*, *Glomus mosseae*, and *A. chroococcum* strains namely, *A. chroococcum* strain-I (AZ₁) and *A. chroococcum* strain-II (AZ₂) at nursery stage under reduced inorganic fertilization. Grzyb *et al.* (2015) found that Florovit Eko + mycorrhizal fungi improved the tree trunk diameter of maiden trees of apple cv. "Topaz". Inoculation of three Arbuscular Mycorrhizal Fungi (AMF) species; *Glomus versiforme*, *Claroideoglomus etunicatum* and *Rhizophagus intraradices* could increase apple root-stocks (M.9, M.7 and MM.106) shoot height, stem diameter, leaf area, shoot fresh and dry weight and root fresh and dry weight and the concentration of N, P, Ca, Mg, Zn, and Fe compared to those of non – mycorrhizal control plants (Hosseini and Gharaghani, 2015). Mosa *et al.* (2016) noticed that the combination of mycorrhizal fungi (*G. mosseae* and *Glomus intraradices*) and plant growth promoting bacteria (*Pantoea* sp., *P. fluorescens*, *K. oxytoca* and *Rhizobium* sp) improved the tree trunk, number and weight of fruits per tree of "Topaz" apple cultivar.

The influence of mycorrhiza in alleviating biotic and abiotic stresses in apple orchard

Runjin (1989) mentioned that sterilized soil inoculated with *G. versiforme* and *Glomus macrocarpum* improved water status and drought tolerance of the plants. Furthermore, arbuscular mycorrhiza colonization in sterilized soils reduced the stomatal resistance and the permanent wilting as well enhanced the rate of recovery of the plant from the water stress. This was probably due to enhancing absorption and translocation of water by the external hyphae. Kaldorf and Ludwig-Müller (2000) observed that mycorrhiza-covered roots were better developed; especially the number of lateral and fine roots was significantly greater. The presence of mycorrhiza in the roots intensifies uptake of water and minerals from the soil by the root system. Al-Karaki (2004) showed that mycorrhizal fungi colonized more readily the roots of plants growing in an area with high water deficiency, and that the use of mycorrhizal inocula in dry areas had a favourable effect on the size and quality of the crop. Hamel (2004) reported that the network of extraradical mycorrhizal hyphae facilitate nutrient acquisition and transport

many ions to roots, particularly less mobile ions such as P, N, K, S, Ca and Zn. Arbuscular mycorrhizal fungi (*Glomus deserticola*) decreased soil EC and organic carbon and increased soil availability of N, P and K as well as leaf nutrient status of “Kinnow” mandarin (Usha *et al.*, 2004). Inoculation of cherry rootstocks, ‘Edabriz’ and ‘Gisela 5’, plantlets with *Glomus clarum*, *Glomus caledonium*, *G. etunicatum*, *G. intraradices*, *G. mosseae* and mixture of these species increased Zn and P nutrient uptake than non-mycorrhizal plantlets (Aka-kaçar *et al.*, 2010). It has been found that AM fungi can alleviate the unfavourable effects on plant growth of stresses such as heavy metals, soil compaction, salinity and drought (Miransari, 2010). Yang *et al.* (2014) studied the influence of *G. versiforme* on increasing one-year-old “Red Fuji” apple seedlings (*Malus hupehensis* Rehd. root stock) salt tolerance. They noticed that arbuscular mycorrhizal fungi significantly increased the root length colonization of mycorrhizal apple plants under 2‰, and 4‰ salinity stress levels as compared to non-mycorrhizal plants. However, percent root colonization reduced as saline stress increased. Salinity levels were found to negatively correlate with leaf relative turgidity, osmotic potential irrespective of non-mycorrhizal and mycorrhizal apple plants, but the decreased mycorrhizal leaf turgidity maintained relative normal values under 2‰ and 4‰ salt concentrations. Under salt stress condition, Cl⁻ and Na⁺ concentrations clearly increased and K⁺ contents obviously decreased in non-mycorrhizal roots in comparison to mycorrhizal plants, this caused mycorrhizal plants to have a relatively higher K⁺/Na⁺ ratio in the root. Ascorbate peroxidase and catalase activities increased in mycorrhizal more than in non-mycorrhizal plants.

The role of some beneficial bacterial strains in improving nutrient uptake, soil fertility, apple growth and productivity

Use of biofertilizers containing beneficial microorganisms instead of synthetic chemical is known to improve plant growth through supply of plant nutrients and may help to sustain environmental health and soil productivity (O’Connell, 1992). In field trials, preplant inoculation with both *G. intraradices* and *G. mosseae* increased rootstock growth and leaf concentrations of P, Mg, Zn and Cu in fumigated plots but not in non-fumigated plots, indicating that colonization by native AM fungi in non-fumigated plots may have been sufficient for adequate nutrient acquisition (Forge *et al.*, 2001). The plant promoting effect of the PGPB is mostly explained by the release of metabolites directly stimulating growth. The mechanisms by which PGPB promote plant growth are not fully understood, but are thought to include: (a) the ability to produce plant hor-

mones, such as gibberellins (Gutierrez-Manero *et al.*, 2001), cytokinins (De Salamone *et al.*, 2001) and auxins (Egamberdiyeva, 2005) and inhibit ethylene production (Glick *et al.*, 1995); (b) asymbiotic N₂ fixation (Sahin *et al.*, 2004); (c) solubilization of inorganic phosphate and mineralization of organic phosphate and/or other nutrients (Jeon *et al.*, 2003). Esitken *et al.* (2003) found that *Bacillus* strains; OSU-142 and M-3 stimulated macro and micro-nutrient uptake such as N, P, K, Ca, Mg, Fe, Mn, Zn and Cu in apricot (*Prunus armeniaca* L. cv. Hacihaliloglu). Tenuta (2003) found that *Rhizobium*, *Bacillus* and *Pseudomonas* improve the uptake of nutrients like nitrogen, phosphorus, potassium, sulphur and iron. Recent studies confirmed that, a number of bacterial species mostly associated with the plant rhizosphere, are found to be beneficial for plant growth, yield and crop quality. They have been called ‘Plant Growth Promoting Bacteria (PGPB)’ including the strains in the genera *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium* and *Serratia* (Bashan and de-Bashan, 2005). Orhan *et al.* (2006) reported that *Bacillus* M3 (N₂-fixing and phosphate solubilizing) alone or in combination with *Bacillus* OSU-142 (N₂-fixing) increased the total N, available P, K, Ca, Mg, Fe, Mn, Zn contents in the soil and Fe and Mn contents in the leaves of raspberry cv. “Heritage”. Aslantas *et al.* (2007) mentioned that floral and foliar applications of *Pseudomonas* BA-8 and *Bacillus* OSU-142 alone or in combination have the potential to increase yield, growth and nutrition of apple cultivars “Granny Smith and Stark Spur Golden”. Karlidag *et al.* (2007) noticed that *Bacillus* M3, *Bacillus* OSU-142 and *Microbacterium* FS01 combinations stimulated plant growth and resulted in significant yield increases in apple cv. “Granny smith” by promoting abilities for auxin and cytokinin production, N₂-fixation, phosphate solubilization and antimicrobial substance production. Karakurt and Aslantas (2010) evaluate the effects of four strains of plant growth promoting rhizobacteria (*Agrobacterium rubi* A-18, *Bacillus subtilis* OSU-142, *Burkholderia gladioli* OSU-7 and *Pseudomonas putida* BA-8) on growth and leaf nutrient content of ‘Starking Delicious’, ‘Granny Smith’, ‘Starkrimson Delicious’, ‘Starkspur Golden Delicious’ and ‘Golden Delicious’ apple cultivars grafted on semi-dwarf rootstock MM-106. They found that bacteria applications showed the desirable effects on plant growth and plant nutrient element contents. Mosa *et al.* (2016) showed the improvement in the growth, yield and fruit quality of “Topaz” apple trees following the addition of *Pantoea* sp., *P. fluorescens*, *K. oxytoca* and *Rhizobium* sp. bacteria species to Fertigo, Micosat, Humus UP, BioFeed Quality, BioFeed Amin, Yeast, Vinassa and Florovit Eko as compared to chemical NPK fertilization.

Some beneficial roles of bacterial strains in apple trees pest management

Biological control is considered a promising strategy for the management of fire blight and several biological control agents are now commercially available, including *P. fluorescens* A506 (Wilson and Lindow, 1993), *Pantoea agglomerans* E325 (Pusey, 1999), *B. subtilis* QST713 (Aldwinckle *et al.*, 2002), *P. agglomerans* P10c (Vanneste *et al.*, 2002) and *B. subtilis* BD170 (Broggini-Schärer *et al.*, 2005) and *Pantoea vagans* C9-1 (Smits *et al.*, 2010). *In vitro* – bacterized plantlets not only grew faster than nonbacterized controls but also were sturdier, with a better-developed root system and significantly greater capacities for withstanding biotic (Barka *et al.*, 2000) and abiotic (Bensalim *et al.*, 1998) stresses. Ramamoorthy *et al.* (2001) showed that some plant growth-promoting rhizobacteria (PGPR) induce systemic resistance by strengthening the physical and mechanical strength of the cell wall, as well as altering the biochemical and physiological reaction of the host plant that leads to the synthesis of chemical defense against the pathogen. Plant growth-promoting rhizobacteria can disrupt phytopathogen organization (Barka *et al.*, 2002), stimulate developmental changes in host plants, induce systemic resistance to pathogens, affect phytohormone production, and improve nutrient and water management (Compant *et al.*, 2005). *Pseudomonas* strains MRS23 and CRP55b inhibited the growth of pathogenic fungi, *i.e.* *Aspergillus* sp., *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani* under culture condition (Goel *et al.*, 2002). Commercial formulations combining bacteria antagonistic to plant pathogenic microbes with ice nucleation-active bacteria have been utilized as an environmentally safe method to manage biotic and abiotic stress in plants (Lindow and Leveau, 2002). In addition, some of these bacteria, such as epiphytic or endophytic plant growth-promoting rhizobacteria, enhance plant growth while improving their resistance to stress (Dobbelaere *et al.*, 2003). *Pseudomonas*, *Bacillus*, *Burkholderia*, *Agrobacterium* and *Streptomyces* suppress plant disease by production of antibiotics, siderophores, or by induction of systemic resistance or any other mechanism (Tenuta, 2003). The plant promoting effect of the PGPR are thought to do antagonism against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes and/or fungicidal compounds and competition with detrimental microorganisms (Lucy *et al.*, 2004). Lactic acid – (LAB) active against *Erwinia amylovora* could be a novel approach for fire blight control, because they have been reported in the field of food technology as biopreservatives (Vermeire *et al.*, 2004), including fermented vegetables or fruit juices (Gomez *et al.*, 2002). The capacities of certain

species of LAB isolated from fresh plant products to control food-borne human pathogenic bacteria and postharvest fungi have been studied (Trias *et al.*, 2008a; 2008b; 2008c). Also, strains of LAB have been reported as antagonistic to the plant pathogenic bacteria *Pectobacterium carotovorum*, *Xanthomonas campestris* and *Pseudomonas syringae* (Trias *et al.*, 2008c). This antagonistic and bioprotective capacity is mainly due to a wide diversity of mechanisms of action including not only antibiosis (Cleveland *et al.*, 2001), but also pre-emptive colonization of wounds and cuts (Trias *et al.*, 2008a). In addition, LAB are not perceived as environmental and health hazards, because they have been considered with the status of “generally recognized as safe” (GRAS) by the Food and Drug Administration (FDA, USA) and with the “qualified presumption of safety” (QPS) status by the European Food Safety Agency (EFSA). Ongena *et al.* (2005) showed the ability of *B. subtilis* strain M4, an important producer of a wide variety of fengycin-type lipopeptides, to protect wounded apple fruits against mold disease caused by *Botrytis cinerea*. The resistance of plants to root diseases as well as efficient nutrient assimilation is profoundly influenced by the presence and activity of beneficial microorganisms in the soil (Picardi *et al.*, 2005). Orchard application of biological potassium fertilizers (BPF) increased the resistance of “Charavnitsa” apple trees to viral and bacterial diseases and to the sucking pests (Ryabtseva *et al.*, 2005). Rhizobacteria are soil bacteria that colonize plant roots; they are able to multiply and occupy all the ecological niches found on the roots at all stages of plant growth (Antoun and Prévost, 2006). Such bacteria may negatively interact with plants, directly by competing for nutrients. Alternatively, the relationship between rhizobacteria and the host plant can be positive. For example, the bacteria may compete with pathogens for survival in the rhizosphere or they may promote mutualistic relationships with plants they were associated, allowing nutrient exchange and stimulating antibiotic production against phytopathogenic agents (Siddiki, 2006). Floral and foliar applications of *Bacillus* OSU-142 and BA-8 and OSU-142 decreased shot-hole disease in “Granny Smith” and “Star Spur Golden” young apple trees (Aslantas *et al.*, 2007). Over 400 species of fungi and more than 90 species of bacteria which infect insects have been described including *Bacillus thuringiensis*, varieties of which are manufactured and sold throughout the world primarily for the control of caterpillar pests and more recently mosquitoes and black flies. So far, more than 40000 species of *B. thuringiensis* have been isolated and identified belonging to 39 serotypes. These organisms are active against either *Lepidoptera*, *Diptera* or *Coleoptera* pests (Moazami, 2007). *Burkholderia* species are able to synthesize a remarkable array of metabolites, including

siderophores, antibiotics, and phytohormones (Vial *et al.*, 2007), and many strains belonging to this genus exhibit activities involved in bioremediation or biological control *in vitro* (Caballero-Mellado *et al.*, 2007). Beneduzi *et al.* (2012) mentioned that bacteria that colonize plant roots and promote plant growth are referred to as plant growth-promoting rhizobacteria (PGPR). Their effects can occur *via* local antagonism to soil-borne pathogens or by induction of systemic resistance against pathogens throughout the entire plant.

The effect of biocontrol agents in nematode control

Different fungal strains isolated from nematodes, soil and plants were shown to produce substances that inhibit nematode egg hatch or kill nematodes (Nitao *et al.*, 1999). Khan *et al.* (2003) showed that the fungus *Paecilomyces lilacinus* penetrates nematode eggs and cuticles through the production of the lytic enzymes serine protease and chitinase. *Pseudomonas aeruginosa* (Siddiqui *et al.*, 2000) and *Pseudomonas* spp. (Ali *et al.*, 2002) have shown good results for the control of *Meloidogyne* spp. Besides, antagonistic bacteria have been repeatedly shown to be promising microorganisms for the biological control of plant-parasitic nematodes (Giannakou *et al.*, 2004). Furthermore, many attempts have been made to use antagonistic bacteria and fungi to control root-knot nematodes (Khan *et al.*, 2008). The damage caused by root-knot nematodes could be managed by application of microorganisms antagonistic to *Meloidogyne* spp., or compounds produced by these microbes (Ashraf and Khan, 2010). Mazzola *et al.* (2009) mentioned that the root-lesion nematode *Pratylenchus penetrans* is the most important nematode affecting apple production. This lesion may exhibit poor growth of "Gala" young apple trees grown on M26 stock apple, stunting and a gradual decline in yields. Severely infected root systems may lack feeder roots. Moreover, the author stated that this lesion can be controlled by MeloCon WG (*P. lilacinus* strain 251) at 2 to 4 lb/A plus a soil wetting agent to established plants, although it might be better used when applied to plants just before planting.

Conclusions

- Biofertilizers are important components of integrated nutrients management and renewable source of plant nutrients to supplement chemical fertilizers in sustainable agricultural system.
- Biological fertilizers would play key role in productivity and sustainability of soil and also protect the

environment as ecofriendly and cost effective inputs for the farmers.

- Beneficial microorganisms can be used as a tool in the apple orchard to improve greatly growth, yield and fruit quality.
- Biological pest management can be aim to reduce the usage of insecticides and maintain a clean environment and food safety, and then human health.

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