Opportunities for biological control of root-knot nematodes in organic farming system: A review

Tewodros Ayalew Dejene

Department of Plant Sciences, College of Agriculture and Natural Resources, Dilla University, Ethiopia

ABSTRACT

Parasitic nematodes are among the main plant parasites causing tremendous yield reduction of food crops. Root-knot nematodes are among the most damaging one in agriculture, causing a significant loss on a wide range of food crops in the world. Moreover, root-knot nematodes attack more than 200 plant species, causing about 100 billion $USD annually worldwide. Furthermore, wide host range colonization ability, short generation times, high reproduction capacity and endoparasitic nature of root-knot nematodes make their control difficult as compared to the plant parasites, having narrow host range. Over the years, several approaches and methods have been researched and evolved in controlling root-knot nematodes. Biological control is a low risk, economically and ecologically viable means to control diseases and pests. The objective of this review study was to evaluate the efficiency of bio-control agents against root-knot nematodes, possible related problems that attended biological control methods as well as the economic and environmental advantages of biological control systems over conventional system. Finally, this desk study concluded that, remarkable success in controlling nematodes and increasing crop yield was found through application of biological control measures. Application of biological control means resulted in no hazardous effect on the environment, human health and water that was caused by the applications of biological agents in a single or in compatible combination use, reduced production cost is also observed. Further investigation is recommended since biological control methods are still in a developmental stage so as to identify the possible related problems that come with biological control methods.
Keywords: biological control, organic farming, root-knot nematodes

Corresponding author: teddy1360@yahoo.com

INTRODUCTION

Plant parasites contribute a large amount to the reduction of food crop production and productivity in the world. Of these parasites, nematodes are the main one causing tremendous yield reduction of food crops. Although the amount varies between crops, environment and severity of infestations, nematodes cause considerable yield loss to a wide range of crops throughout the world (Khan et al., 2011; Sehebani and Hadavi, 2008). According to Khan et al, (2011), parasitic nematodes are categorized as the most important pathogens that damage agricultural crops worldwide. Besides, nematodes contribute to crop yield loss indirectly by affecting the resistance of the host plant and aggravating the diseases caused by other pathogens (Khan, et al. 2011). Nematodes attack crops through different mechanisms. Some of the mechanisms by which nematodes attack the plant are: by injuring and feeding on root hairs, epidermal cells, cortical and stellar cells (Khan et al., 2011).

Root-knot nematodes are among the most damaging pests in agriculture, causing a significant loss on a wide range of food crops in the world (Sehebani and Hadavi, 2008;Kiewnick, 2010; Wang et al., 2010; Hashem et al., 2011; Qasim et al., 2010; Singh and Mathur, 2010). According to Singh and Mathur (2010), root-knot nematode is among the top ten damaging genera of plant parasitic nematodes causing considerable yield loss in food crops. The yield loss caused by root-knot nematodes of course, varies depending on the environment and susceptibility of the particular crop as well as the population level of root-knot nematodes. For instance a yield loss of 27.2% on tomato crop has been reported by Singh and Mathur (2010) in India, whereas Brand et al (2010) reported a yield loss of about 10 to 20% in another part of the world. Particularly, root-knot nematodes are severe in the tropics and sub-tropics. In the temperate climate, it is also considered a more dangerous pest in the green house (Biro and Toth, 2009). Moreover, root-knot nematode attacks more than 200 plant species, which include almost all the cultivated crops. According to Brand et al (2010), the yield loss caused by root-knot nematodes in the world was estimated about 100 billion $USD annually. Furthermore, wide host range colonization ability, short generation times, high reproduction capacity and endoparasitic nature of root-knot nematodes make their control difficult as compared to the plant parasites, having narrow host range (Affokpon, et al. 2011).

Over the years, several approaches and methods have been researched and evolved in controlling root-knot nematodes. Most basic control measures are derived through chemical nematicides and/or biological control agents. Soil fumigants, organophosphate and carbonate nematicides are used widely throughout the world to control parasitic nematodes. The chemical control of root-knot nematodes has its own environmental side effects. The environmental hazard caused by nematicides, coupled with the root-knot nematodes resistance against methyl bromide and other frequently used nematicides as well as the increasing environmental concern in this era increased the inspirationtowards developing and using biological control methods against root-knot nematodes (Kiewnick, 2010; Moosavi et al., 2010; Brand et al., 2010). According to (Sehebani and Hadavi, 2008) biological control could be sustainable control methods against root-knot nematodes. For instance the use of biological agent *Trichoderma harzianum*to control *Meloidogne javanica* has shown to decrease nematode infection and egg hatching level of the nematodes, without or with minimal negative side effects as compared with chemical control methods (Sehebani and Hadavi, 2008).

Biological control is a low risk, economically and ecologically viable means to control diseases and pests (Sheppard et al., 2005 and Moosavi, et al., 2010). Biological control is defined by Brand et al (2010) and Delfosse (2005) as an eco-friendly pest management strategy that utilizes deliberate introduction of living natural enemies, to lower the population level of a target pest. Moreover, the current ever increasing environmental concern of the world, makes biological control methods more visible and a good alternative (Hashem, et al., 2011). Affokpon *et al* (2011), in his study of vegetable production in West-Africa indicated the biological control measures as a best and environmentally viable option for managing root-knot nematodes at low cost level. Furthermore, it is also considered as a long term solution for the ever increasing diseases and pest invasion since biological control agents can be used for a long period, from the time of introduction in the area due to its ecological acceptability (Sheppard *et al*., 2005). Biological control may also reduce the management cost in the long run, since the enemies spread by themselves through the invading populations of the parasites introduced and adapted to the environment (Sheppard *et al.*, 2005). Two kinds of microorganisms are used to control nematodes biologically. They are plant growth promoters and parasites or predators (Kiewnick, 2010). The combined as well as single use of biological control agents was found to reduce the yield loss caused by root-knot nematodes (Hashem *et al*. 2011), but the mixed applications of different biological agents were found to be more efficient at controlling root-knot nematodes than single application of the control agents (Hashem and Abo-Elyousr, 2011).

The efficiency of bio-control agents against nematodes depends on a number of issues; such as compatibility of bio-control agents with the operating production system, production inputs, and the resistance and susceptibility level of the host plant (Kiewnick, 2010). Therefore, to overcome the limitations of biological control system, a combination use of different protection agents could be desirable. This is because the combined use of nematode antagonists may have different modes of action and attack the nematodes at their different developmental stages (Kiewnick, 2010).

Different biological agents have been used to control root-knot nematodes. In this review paper, the use of fungi, bacteria, plant and combination use of these agents against root-knot nematodes control dealt based on the data from previous works or desk study. Moreover, the review focuses on the application and limitations of biological control, over chemical control methods.

METHODOLOGY

To prepare this review paper, different stages of work were followed. At first, relevant literatures that have relations with the topic of the review were searched through different websites. The literatures were categorized according to the topic they dealt with. For instant, the literatures were classified based on the type of biological control agents, biology of nematodes, biological control methods. The second stage was designed to catch the concept of the topic. Finally the different parts of the review were done and summarised in the conclusion part.

Classification of Root-knot Nematodes

Nematodes are round worms, which belong to phylum Nematoda. Because of their ability to occupy different ecological niches and live as parasites in animals, humans and plants nematodes are most abundant in the earth surface (Brand *et al*., 2010).

The root-knot nematodes, *Meloidogyne species* are obligate endoparasites (Wang *et al*., 2010; Hashem *et al*., 2011), which enter to the host plant root and affect the activity of the root and/or the plant through living and feeding on it (Khan *et al*., 2011). The genus *Meloidogyne* got the alternative name root-knot nematode (RKN), since they develop knots in the root of plants (Brand *et al*., 2010). Since root-knot nematodes are soil borne pathogens, they spend part of their life cycle in the soil. The complete life cycle of nematodes consists of three different development stages of egg, juvenile and adult. The eggs of the root-knot nematodes bring forth the infective juvenile stage in the soil. For the survival of juvenile root-knot nematodes ther is the need to find host, which provides food (Wang *et al*., 2010). To fulfil this requirement, the juvenile root-knot nematodes first enters the root part of the plant and immigrates to the vascular cylinder of the plant so as to establish feeding site and also complete the life cycle in the plant (Wang *et al*., 2010 and Brand et al., 2010). Root-knot nematodes, *Meloidogyne spp*., synthesise and release secretory proteins during migration within plant systems, which is used by root-knot nematodes as a parasitism mechanism against the plant (Huang *et al*., 2003). These proteins are ingested by the plant through stylet, the feeding structure of the nematode. Afterwards, they form galls (root knot). These galls serve as a shelter and egg laying place for the female nematode (Brand *et al*., 2010). Nematodes start infecting the host plant with root penetration. This first root penetration starts by secondary-stage juvenile (J2) nematodes, hatched in the soil from the eggs encapsulated in egg laid by female nematodes on the infected plant root (Hashem *et al*., 2011).

Mechanisms of Infestation by Root-knot Nematodes

Root-knot nematodes attack crops of several families from *Solanaceae, Cucumrbitaceae* through fruit crops, causing economic loss (Brand *et al*., 2010). According to (Brand *et al*., 2010) root-knot nematode infected plants shows leaf chlorosis, development of gall on roots, which thereby result in reduction of the root system and finally stunted growth of the whole plant. If no proper protection measures applied on time, witing occurs. The different sized galls formed on the root of the plant inhibit the uptake of water and nutrients (Biro and Toth, 2009), which results in malnutrition, water stressed condition of the infected crop and finally, reduction of yields.

Classification of Biological Control Agents

Several natural enemies exist in nature, which are used as biological control agents against root-knot nematodes. These natural enemies range from bacteria, fungi, plant, and rickettsia to viruses. However, fungus and bacteria won the attention of the researchers as a biological control agent against root-knot nematodes, since they offer a great importance to control nematodes because of their wide field applicability, reproduction ability and abundance (Brand *et al*., 2010). The applicability and formulation of this biological control agent at commercial level however deserves further investigation (Brand et al., 2010). Biological control agents are further classified as natural and induced. This implies the inhibition of a particular population of micro-organisms over nematodes naturally and artificial application of control agents specifically as a biological control agent against nematodes, respectively (Brand *et al*. 2010). According to Hashem *et al*., (2011), root-knot nematodes management could be possible biologically through direct attack of applied micro-organisms over the population of nematode as well as through inhibiting or killing effects of compounds produced by the applied micro-organisms.

Bacteria as a Biological Control Agent

Different microorganisms are used as biological control agents against soil-borne pathogens including root-knot nematodes (Dabire *et al*., 2005). Bacteria are among the main biological control agents used against root-knot nematodes, *Meloidogyne spp*., (Dabire *et al*., 2005; Hashem *et al*., 2011; Qasim *et al*., 2010). According to Hashem and Abo-Elyousr (2011), antagonistic bacteria are among the most promising microorganisms as biological control agents as far as effective control of nematodes are concerned. For instance effective control of root-knot nematodes, *Meloidogyne spp*. through *Pseudomonas aeruginosa bacterium* and other *Pseudomonas species* were reported by Hashem *et al*., (2011) on his study about management of root-knot nematodes *Meloidogyne incognita* through combined application of different bio-control microorganisms. Moreover, *Pseudomonas* produces iron-chelating siderophores, anti-biotics or hydrogen cyanide and these compounds reduce deleterious and pathogenic rhizosphere microorganisms, which forms favourable environment for root growth, thereby promote the growth, vigour and productivity of crop plants. Researchers categorized nematicidal bacteria into two types such as rhizzobacteria and nematode parasites (Brand *et al*., 2010). According to Brand *et al* (2010), *Pasteuriapenetrans* are the most effective obligate endoparasite of *Meloidogyne. Pseudomonas* also provides considerable importance in the control of root-knot nematodes. According to Qasim *et al* (2010), bacteria act in two ways to attack nematodes. Bacteria may enhance the growth of the plant, which thereby increases the vigour and resistance of the plant against root knot nematodes (Choudhary and Johri, 2009). The other group of bacteria directly suppress the nematodes infecting the rhizosphere of the plant, thereby making the plant free of infection.

Fungi as a Biological Control Agent

Fungi are among the main biological control agents used against root-knot nematodes (Kiewnick, 2010). It is one of the promising agents to be relied on to achieve the efforts going on to replace chemical control of nematodes by biological control agents (Regaieg *et al*., 2010). Affokpon *et al* (2011) also identified fungi as attractive and a potential bio-nematicide against root-knot nematodes among the other micro-organisms used as a biological control agent against root-knot nematodes. This same study has indicated the free-living soil fungi, *Trichoderma spp*. as a best example of potential fungi to function as a biological control agent to manage nematodes. This is further proved by Biro and Toth (2009) in their study on the effect of trifender on the number of root-knot nematodes, indicating the potential of *Trichoderma spp*. to control root-knot nematodes. Besides, the wide range of crops rhizosphere colonizing ability of fungi makes it a preferable biological control agent (Atkins *et al*., 2003). Thus, this behaviour or ability of fungi creates an opportunity to use fungi as a control agent for nematodes in a wide range of food crops. Fungi control the development of nematodes by attacking the egg or larvae of the nematodes and using the nematodes as a food source for further growth and reproduction. This has also been seen as of paramount importance since the fungi population increases rapidly by using the nematodes as a nutrition source. As the population of the fungi increases, it develops the capacity to attack over large populations of nematodes within a short period of time.

Fungi develop different mechanisms to attack nematodes. Nematode trapping fungi attack the nematodes, using special structure developed in response to presence of nematodes known as mycelial. The other group of nematodes act as a parasite by growing inside the nematode-endoparasitic fungus (Brand *et al*., 2010). According to Brand *et al*., (2010), there are also groups of fungi that parasitize root-knot nematodes, including eggs and females. Singh and Mathur (2010) concluded that fungi attack nematodes by producing toxic compounds, which affect root-knot nematodes survival, reproduction, development and ability to infest and attack crops or by parasitizing them directly. According to Hashem *et al*., (2011), the application of different fungal strains resulted in inhibition of nematode egg hatching or killing of nematodes in the soil and plant, due to production of growth inhibiting substances. For instance, the lytic enzymes, serine protease and chitinase produced by *Paecilomyces lilacinus* fungus penetrate and attack eggs and cuticles of nematodes. According to Sehebani and Hadavi (2008) fungi also increase the systemic resistance of the plant by inducing plant defence mechanisms. Sehebani and Hadavi (2008) in their study about biological control of root-knot nematodes by *Trichoderma harianum* indicated that the population of root-knot nematodes and disease severity reduced in *Trichoderma harzianum* inoculated potato field.this was due to the suppression effect of *Trichoderma harzianum* onnematodes activities (nematode infection, egg mass, production and number of eggs). On the works of Biro and Toth (2009), a significant increase of pepper yield has been reported due to the application of fungus (*Trifender spp*.).

Fungi, as a biological control agent against root-knot nematodes, are utilized through application in the soil. The fungi spores are applied to the soil in the rhizosphere of the plant to fasten the effect of the fungi on the nematodes, since nematodes are largely available in the rhizosphere of the plants. To achieve the maximum nematicidal efficacies of the fungus against root-knot nematodes, applications need to synchronize the active phase of fungus with the sedentary phase of female nematodes and eggs (Brand *et al*., 2010). The mode of action is brought about by the fungal extracellular enzyme secretion through appressoria (hyphal structure), which inhibits the growth, multiplication and development of nematodes. According to Brand *et al* (2010), the protection level of nematodes by fungus is largely dependent on the rate and time of the application of fungus. A three to four times enhancement in potato yield was recorded due to the application of fungus as compared to the nematode infected plants. Application of fungus 10 days before planting and during planting, to achieve the desired nematode control level had been recommended (Brand et al., 2010).

Plants as a Biological Control Agent

Plant resistance is of the methods used as a control means against nematodes. According to Choudhary and Johri (2009), plants naturally owns a range of defence mechanisms. These active defence mechanisms are expressed during the occurrence of biotic stress. Moreover, the effectiveness of the active defence mechanism of the plant depends on the timing of the response against the pathogen effect. Several crops develop resistant genes against nematodes and provide an important role for plants to cope with nematodes infestation (Brand *et al*., 2010). Additionally, the identity of the species and the diversity contributes to the suppression of nematodes (Sohlenius *et al.*, 2011). The quantity of root material deposited in the soil as well as the chemical composition, varies from one plant species to the other plant species (Sohlenius *et al*., 2011). The behaviour of plants to exude substances that are harmful to nematodes vary. The plant resistance to nematode may be specific or broad over several species. For instance *Mi* gene of tomato confers resistance particularly against root-knot nematodes, *Meloidogyne javanica*, whereas this gene does not provide resistance against *Meloidogyne hapla* (Brand *et al*., 2010).

Plant oils also act as an effective biological control agents against nematodes. According to Kim *et al* (2011), different commercial plant oils have important nematicidal action. The different compounds produced by these oil plants inhibit the activities of the nematodes, which thereby reduce the effect caused by nematodes on the crop (Kim *et al*., 2011). However, the effectiveness of these commercial plant oils as a biological agent depends on the concentration of applied compounds.

This shows the importance of knowing the proper rate of application needed to manage the nematodes at economic threshold level. Furthermore, the nematicidal activities of the essential plant oils depend on the type of plants. For instance, oils from *Gaultheria fragrantissima* and *Zanthoxylu malatum* plant type caused about 100% nematode mortality, whereas *Citrus reticulata* and *Tasmannia lanceolata* resulted with about 25% nematode mortality (Kim *et al*., 2011). This explains the differential mode of action and detrimental effect (the chemical compositions) of different plant oils against parasitic nematodes.

Action of Biological Control Agents in Combination

In general, infection by nematodes results in reduction of fresh as well as dry weight of plants, which thereby contribute to the low yield of crops or low productivity of the system. Application of biological control agents in a mix or in a single form have been observed to minimize yield reduction caused by nematodes (Hashem et al., 2011). The application of biological control agents reduces the yield loss through two mechanisms; firstly by causing lethal effect on nematodes and secondly by enhancing the plant growth. This is because application of biological control agents (for example *Pseudomonas fluorences*, *Paecilomyces lilacinus* and *Pichiagu illiermondii*) enhances the growth of the plant through supplying nutrient elements, thereby inducing the systematic resistance of the plant (Hashem et al., 2011), whereas others attack the nematodes directly. According to Brand *et al* (2010), combined application of *Pseudomonas lilacinus* and chitin resulted in less infection of root-knot nematodes along with suitable growth and development of the tomato plant, than a separate application of *Pseudomonas lilacinusin* in the tomato field.

A study by Singh and Mathur (2010) on biological control of root-knot nematodes indicted that combined application of fungus and chick pea powder have antagonistic effect on the development of root-knot nematodes and also increased plant vigour. This can be explained by the fact that the chick pea powder provides additional nutrients for both plant and biological control agents thereby increasing the activity of the fungus in the soil, which enhances the ability of the fungus to attack the nematodes. The advantage of using combined biological control agents is also explained on the works of Hashem *et al* (2011), which states the chances of having a variety of traits enabling the suppression of root-knot nematodes over a wide range of environmental conditions. However to ensure the efficacy of the combined biological control agents interference and competition between these antagonists need to be considered (Masadeh *et al*., 2004).

Economic and environmental advantages of biological control systems over conventional system

Several studies revealed that different Nematicides were in use for the last many years, to overcome crop damage caused by root-knot nematodes (Delfosse, 2005) and still, chemical control is in use as a control measure (Qasim *et al*., 2010), regardless of the different side effects that resulted from the application of synthetic control measures. According to Brand *et al* (2010), growers relied on chemical pesticides since 1950 to control nematodes, but from the last decade, the utilization of chemical pesticides is being discouraged due to severe environmental and public health related problems caused by the application of these chemicals (Brand *et al*., 2010). The environmental problems caused due to the application of these chemicals are ground water contamination, avian and mammalian toxicity and accumulation of pesticides in the food stuffs, which is called residual effect. Moreover, the widely used nematicide (Methyl bromide) was withdrawn from market due to its adverse effect on the ozone layer (Brand *et al*., 2010 and Atkins *et al*., 2003). Furthermore, the efficacies of the nematicides are declining due to the development of resistance by nematodes against most widely used nematicides, which might result due to the long-time usage of these chemicals against nematodes.

Applicability of chemical control also depends on the time of application. For effective control of nematodes, the chemicals would have to be applied before the nematode penetrates the root. This is because once the nematode penetrates the root, chemical control becomes ineffective. Besides, to manage nematode damage, repeated application of nematicides is a must, which ultimately increases the production cost per plot as well as the environmental damage caused by the applied chemicals (Brand *et al*., 2010). According to Qasim *et al*., (2010), besides the toxicity caused by chemicals, the cost of the chemicals is an important factor, which mandates the need for alternative control measures. The chemical cost, coupled with the cost spent for rehabilitation of the environment and treating human and animal health conditions make chemical control measures ineffective and biological control methods, more reliable and better alternative in the current era. Biological control methods have also economic advantages in addition to the environmental effects it brings. Regarding to cost, the money spent for purchasing chemicals are not the only cost related to chemical control methods. A number of issues come, which force us to spend more money with practicing chemical control methods, such as: cost spent for health treatment, to rehabilitate the environment, to remove the residue of chemicals, to band the chemicals which is no more in use and more, whereas this costs are minimized or absent in case of biological control methods. Moreover, the yield keeps on increasing with the use of biological control methods. For instance, a study by Affokpon *et al* (2011) on the effect of native African arbuscular mycorhiza fungi in protecting vegetable crops against root-knot nematodes, demonstrated a yield increase of 26% and more than 300% of tomato and carrot respectively, due to field application of arbuscular mhicorhizal fungus. This is as compared to the treatments infected with root-knot nematodes or control treatments. This same research stressed the potential of developing fungus based biological pest management system for commercial vegetable production, since it reduces the long term production cost, health and environmental hazard. According to Affokpon *et al* (2011) due to increasing population, the demand for fresh vegetable is rising and production is undermined due to high pest and disease pressure, especially of root-knot nematodes (*Meloidogyne*), therefore biological control is important to increase the yield and address the ever increasing demand for vegetables.

The aforementioned weakness and negative side effects of chemical nematicides have forced researchers throughout the world to look towards new alternative approaches and methods for nematode control (Brand *et al*., 2010). Therefore the use of biological agents has been found to fill this gap, which also contributes to reduced environmental degradation. Besides, the development of effective and commercially viable biological control agents needs to address a number of important issues. Selection of proper strain, knowledge of physiological, ecological and taxonomic characteristics of the biological control agents, pre-test on the applicability and economic feasibility of the selected strain, knowledge on formulation of the agents and risk assessment trials, rate of application (concentration), host range are among the issues, which need immediate solutions (Brand *et al*., 2010).

According to Dabire *et al*., (2005), the frequency and intensity of irrigation during crop production also needs consideration as far as the effectiveness of artificially applied or naturally available bacterial nematicide are concerned. This is because intensive irrigation may result in loss of spore population. Furthermore, availability of a cost-effective production and stabilization technology for manufacturing an effective formulation is an important aspect, which needs consideration during selection of biological control agents for commercial application (Brand *et al*., 2010).

CONCLUSION

Different researches throughout the world have shown that different possible problems attend the application of synthetic chemicals to control nematodes. Whilst Researches in the last couple of years have shown remarkable success in the application of biological control measures on different crops (either in combination or single application) in controlling nematodes and increasing crop yield. Moreover, studies so far have not suggested any hazardous effects on environment, human health and water that was caused by the applications of biological agents in a single or in compatible combination use. Furthermore, the high chemical cost, which is a hinderance to the farmers, especially the farmers in developing countries are minimized in biological control methods. However, since biological control methods are still in developmental stages, further investigations are suggested to identify the possible related problems that would attend biological control methods so as to take safety measures ahead of the occurrence of the problem. This is because different possible problems may emerge during the course of continued usage.

REFERENCES

Affokpon, A., Coyne, D.L., Htay, C.C., Agbede, R.D., Lawouin, L. and Coosemans, J. (2011) ‘ Biocontrol Potencial of Native Trichoderma Isolates against root knot Nematodes in West Africa Vegetable Production Sysstems,’ *Soil Biology and Biochemistry*. 43:600-608.

Affokpon, A., Coyne, D.L, Lawouin, L., Tossou, C., Agbede, R.F. And Coosemans, J. (2011) ‘Effectiveness of Native West African Arbusccularmycorrhizal fungi in rotecting Vegetable Crops Against Root-knot Nematodes,’ *Bio Fertil Soils*. 47:207-217.

Atkins, S.D., Hidalgo-Diaz, L., Kalisz. H., Mauchline, T.H., Hirsch, P.R. and Kerry, B.R. (2003) ‘Development of a New Management Strategy for the

Control of Root-Knot Nematodes (*Meloidogyne sp.p*) in Organic Vegetable Produciton,' *Pest Management Science*. 59:183-189.

Biro, T. and Toth, F. (2009) 'Effect of Trifender (Trichoderma asperellum) on the Number of Root-KnotNematode (Meloidogyne hapla Chtwood),' *Acta phytopathological Entomologica Hungarica*. 44 (2): 363-371.

Brand, D., Soccol, C.R, Sabu, A. and Roussos, S. (2010) 'Production of Fungaal Biological Control Agents through Solid State Fermentation: A Case Study on Paecilomyces lilacinus Agent Root-Knot Nematodes,' *Microbiology Aplicada International*. 22(1):31-48.

Choudhary, D.K. and Johri, B.N. (2009) 'Interactions of Bacillus Spp. and Plans with Special Reference to Induced Systemic Resistance (ISR),' Microbiological Research. 164:493-513).

Dabire, K.R., Ndiaye, S., Chotte, J.L, Fould, S., Thiam, M.D. and Mateille, T. (2005). 'Influences of Irrigation o the Distribution and Control of the Nematode Meloidogyne javanica by the biocontrol bacterium Pasteuria penetrans in the field,' *Biol Fertil Soil*. 41:205-211.

Delfosse, E.S. (2005). 'Risk and Ethics in Biological Control,' *Biological Control*. 35:319-329.

Hashem, M. and Abo-Elyousr (2011), 'Management of root-knot nematode *Meloidogyne incognita* combinations of deferent bio control agents,' *Crop protection*. 30:285-292.

Huang, G., Gao, B., Maier, T., Allen, R. Davis, E.L., Baum, T.J. and Hussey, R.S. (2003), 'A Profile of Putative Parasitism Genes Expressed in the Esophageal Gland Cells of the Root-knot Nematode *Meloidogyne* *incognita*,' the *American Phytopathological Society*. 16:376-381.

Khan, M.R. and Anwer, K.A. (2011). 'Fungal Bionoculants for Plant Disease Management,' *Agricultural and Environmental Application*. DOI 10.1007/978-1-4419-7931-5_17 Springer Science + Business Media, LLC 2011.

Kiewnick, S. (2010) 'Importance of Multitrophic Interactions for Successful biocontrol of Plant Parasitic Nematodes with Paeciomyces lilacinus Strain 251,' Plant Pathology in the 21st Century 1, DOI 10.1007/978-1-4020-8804-9_7, Springer Science + Business Media B.V. 2010.

Kim, J., Seo, S. and Park, K. (2011) 'Nematicidal Activity of Plant Essential Oils and Components from *Gaultheria fragrantissima* and *Zanthoxylumalatum* against the Pine Wood Nematode, *Bursaphelenchusxylophilus,'Nematology*. 13 (1): 87-93.

Masadeh, B., Alten, H.V., Stoecker, G.G. and Sikora, R.A..(2004). 'Bio-control of Root-Knot Nematodes using the arbuscular mycorhizal fungi Glomusintradices and the Antagonist Trichoderma viride in Two Tomato Cultivars in their Susceptibility as Hosts for the Nematodes,' Journal of Plant Diseases and Protection. 111(40): 322-333.

Moosavi, M.R., Zare, R., Zamanizadeh, H.R. and Fatemy, S. (2010). Pathologenicity of Pochonoa Species on Egg of Meloidogyne javanica,' *Journal of Invertebrate Pathology*. 104:125-133.

Regaieg, H., Ciancio, A., Raouani, N.H. And Rosso, L. (2010) 'Detection of Biological Potencial of Verticillium leptobactrum parasitizing Meloidogyne spp.,' *World Journal of Microbiological Biotechnology.*

DOI 10.1007/s11274-010-0615-0.

Qasim, K.M., Waseen, A.M., Javed, M.Z. and Ahmed, S.K. (2010). 'Evaluation of Bacillus thuringiensis Isolates against Root-Knot Nematodes Following Seed Application in Okra and Mungbean,' *Pakistan Journal of Potato*. 42(4): 2903-2910.

Sohlenius, B., Bostrom, S. and Viketoft, M. (2011). 'Effects of Plant Species and Plant Diversity on Soil Nematodes a field Experiment on Grassland Run for Seven Years,' *Nematology*. 13 (1): 115-131.

Sehebani, N. and Hadavi, N. (2008). 'Biological Control of the Root-Knot Nematode Meloidogyne javanica by trichoderma harzianum,' *Soil Biology and Biochemistry*. 40: 2016-2020.

Sheppard, A.W., Shaw, R.H and Sforza, R. (2005). 'Top 20 Environmental Weeds for Classical Biological Control in Europe: a review of opportunities, regulations and other barriers to adoption,' *Weed Research*. 46:93-117.

Singh, S., and Mathur, N. (2010) 'Biological Control of root-Knot Nematodes, Meloidogyne incognita infesting Tomato,' *Biological Science and Technology*, 20(8): 865-874.

Wang, C., Lower, S., Thomas, V.P. and Williamson, V.M. (2010) 'Root-knot nematodes Exhibit Strain-Specific Clumping Behaviour That is inherited as a Simple Genetic trait,' *PLoS ONE.* 5(12):e15148