Nematicidal Activity of *Beauveria bassiana* (Bals.-Criv.) Vuill. Against Root-Knot Nematodes on Tomato Grown under Natural Conditions

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ABSTRACT

Plant parasitic nematodes such as root-knot nematodes (RKNs) are a major threat to vegetables, fruits and ornamental plants which cause yield loss. One of the best alternative methods of nematode control is using the entomopathogenic fungi (EPF). The aim of this study was to evaluate the effects of two Turkish isolates of entomopathogenic fungi, *Beauveria bassiana* (Bals.-Criv.) Vuill. (F-56 and F-63), against naturally infected mixed populations of *Meloidogyne incognita* and *Meloidogyne javanica* on tomatoes under natural conditions. The experiment contained 3 conidial suspensions (10^6, 10^7 and 10^8 cfu ml^{-1}) with 4 replicates of each treatment. A total of 32 plants were arranged in a randomized block. EPF were applied 4 times (15 days before planting, planting, 15, and 30 days after planting). Nematicide [*Paecilomyces lilacinus* strain 251 (BioAct®)] was used as a positive control. At the end of the growing season, the efficiency of the applications was determined according to the root gall index (I) and yields of tomatoes (II). The fungi concentration of 10^8 cfu ml^{-1} was significantly greater than in the others. This concentration from both isolates [F-56 (4.59±0.54) and F-63 (2.77±0.59)] controlled *M. incognita* and *M. javanica* and increased the yield of tomatoes. The results demonstrated the suitability of using *B. bassiana* isolate F-56 and F-63 for reducing RKNs on tomatoes under greenhouse conditions.

Key words: Root-knot nematodes, *Meloidogyne incognita*, *M. javanica*, *Beauveria bassiana*, Tomato, Biocontrol.

INTRODUCTION

Plant parasitic nematodes (PPN) usually cause yield loss on many agricultural products around the world. One of the most important groups is the root-knot nematodes (RKNs) (Perry and Moens, 2006 and Kepenekci, 2012). RKNs can be suppressed by chemical control, but its use has been restricted due to its impact on environmental problems, human health and non-target organisms. Therefore, alternative methods are needed to expand effective management strategies for plant parasitic nematodes.

Entomopathogenic fungi (EPF) are among the effective biological control agents (Butt *et al*., 2001). They are environmentally more acceptable than chemicals (Arici *et al*., 2012) and also can be used when pests have developed resistance to chemical pesticides (Butt, 2002). Some fungi have shown promising results as a biocontrol agent against the destructive RKNs (Jatala *et al*., 1979; Stirling, 1991 and Tigrano-Milani *et al*., 1995). More than 30 genera and 80 species such as *Arthrobotrys* spp., *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., *Purpureocillium lilacinum*, and *Verticillium chlamydosporium* have been reported (Viaene and Abawi, 2000; Sun *et al.,* 2006 and Bakr *et al.,* 2014).

Beauveria bassiana (Bals.-Criv.) Vuill. is a well known biopesticide for insects, but few studies have done on PPN. *B. bassiana* grows saprotrophically within the host and produced metabolites such as; beauvericin, bassianin, beauverolides, bassianolide and oosporein. This fungus invades the insect body and its toxins act as an insect platelet aggregation inhibitor (Butt *et al*., 2001).

The aim of this study was to evaluate two Turkish isolates of EPF, *B. bassiana* F-56 and *B. bassiana* F-63 on the mixed population of *Meloidogyne incognita* and *M. javanica* under greenhouse conditions.

MATERIALS AND METHODS

Nematodes

A greenhouse (about one and half decares), planted with tomato naturally contaminated with the root-knot nematodes, *M. incognita* and *M. javanica* in the province of Fethiye, Muğla, Turkey, was selected. New tomato plants, infected with knots, were washed carefully to obtain occupant RKNs on the roots in the greenhouse. After examination of the root galls, abundant mature females were found attached to the roots. On the basis of the perennial pattern of mature females (Southey, 1986), *M. incognita* and *M. javanica* were identified (Jepson, 1987 and Eisenback and Triantaphyllou, 1991).

Fungus isolates

Cultures of the Turkish isolates, EPFs, *B. bassiana* F-56 and *B. bassiana* F-63, were provided by the

Gaziosmanpaşa University, Faculty of Agriculture, Department of Plant Protection, Tokat, Turkey via the Project of GOU-BAP (Project No: 2012/25) from Kelkit Valley in Gümüşhane, Turkey. They were stored at 4ºC on Potato Dextrose Agar (PDA) and at –80ºC. The fungi were subcultured on PDA (Difco TM, Becton Dickinson and Company, USA) with the help of a sterilized bacteriological loop. The dishes were closed by a parafilm at 25±1°C for 7-14 days. The conidia were harvested, using a sterilized rubber loop, attached to 1 ml borosilicate pipette at the angle of 45°. Scraped material was shifted into sterilized Petri dishes and stored at 4°C in refrigerator. The harvested fungal conidia were incorporated to sterile 0.05% Tween-80 solution and the material was stirred for complete homogeneity (Wakil *et al*., 2012). Serial dilutions were prepared and the number of conidia was measured by a haemocytometer to achieve the concentrations of 10^6, 10^7 and 10^8cfu ml^{-1}.

Greenhouse experiments

The experiment was conducted between the 5th of August 2014 and the 8th of January 2015. Ilgin F1-tomatoe (*Solanum lycopersicum*) was used as a susceptible variety to RKN. BioAct® (nematicide based on entomopathogenic fungus, *Paecilomyces lilacinus* strain 251) was used as a positive control. Only water was applied as a negative control via drip irrigation. The experiment contained 3 different conidial concentrations (1×10^6, 1×10^7 and 1×10^8cfu ml^{-1}). One parcel had 2 rows and each row contained 16 plants, totaling 32 plants were arranged (8 m×1. 5 m =12 m^2) in a randomized block design. Four replicates of each treatment were performed. The 2.5 m safety lanes were left between parcels. Entomopathogenic fungi (EPF) were applied 4 times: 15 days before planting, planting, 15 and 30 days after planting, with a watering pot (the extracts were applied 1 ml plant^{-1}). After application, drip irrigation was opened approximately 1 hr for diffusing the bio pesticide in the soil. The parameter of gall index (root knot), yield (kg plant^{-1}) and the effect (%) were evaluated. Temperature and relative humidity were recorded by HOBO [15.57-43.12ºC (24.85ºC ±6.88) and R.H.% of 67.33-96.97 (86.24±8.70%)] during the experiment. To evaluate the effect of treatments on yield, mature (red color) tomatoes were harvested regularly. The first fruits were harvested on the 12th of September 2014 and completed on the 8th of January 2015. Twenty tomato plants were selected randomly from each treatment and harvested tomatoes were weighed and recorded for each plant for the entire season (8 to 10 times). At the end of the season, mature tomatoes on the plants were collected and plants were uprooted the same day. The damage caused by RKN was evaluated according to Zeck galling scale. Twenty plants were selected randomly from each plot and galling damage was rated on a 0-to-10 scale, as 0= no galls, 1= very few small galls, 2= numerous small galls, 3= numerous small galls of which some were grown together, 4= numerous small and some big galls, 5= 25% of roots severely galled, 6= 50% of roots severely galled, 7= 75% of roots severely galled, 8= no healthy roots but plant was still green, 9= roots rotting and plant dying, and 10= plant and roots dead (Zeck, 1971).

Statistical analysis

All data were analyzed using ANOVA by SPSS package program. The mean separations were performed by the Duncan's Multiple Range Test and difference at $p\leq0.05$ was considered as significant (SPSS, 1999).

RESULTS AND DISCUSSION

The results showed that the concentration of 1×10^8 cfu ml^{-1} of *B. bassiana* F-56 and F-63 were found more effective than other concentrations (1×10^7 and 1×10^6). The highest effect on gall index was recorded on the concentration of 1×10^8 cfu ml^{-1}of *B. bassiana* F-63 (2.77±0.59) and found statistically the same group with positive control (1.34±0.35). Another effective application was found in the concentration of 1×10^8 cfu ml^{-1} of *B. bassiana* F-56 (4.58±0.54). Negative control recorded the highest gall index value (8.66±0.45) compared to the others ($F= 19.57$; df: 7.31; $P<0.05$) (Fig. 1). The concentration of 1×10^8cfu ml^{-1} of *B. bassiana* F-63 (66.60±9.25%) showed the highest effect on gall index compared to the other concentrations. The highest yield was demonstrated by *B. bassiana* F-56 and F-63 (5.00±0.09 and 4.95±0.31 Kg plant^{-1}, respectively). The lowest yield was found on negative control parcels (3.17±0.69 Kg plant^{-1}); while the highest yield (62.04±28.88) was obtained at the concentration of 1×10^8 cfu ml^{-1} of *B. bassiana* F-56, followed by the concentration of 1×10^8 cfu ml^{-1} of *B. bassiana* F-63 (61.02±33.82%). The lowest effects were recorded at the lowest concentrations of both *B. bassiana* F-63 and F-56 (Fig. 1). It was found that the most virulence of the EPF depends on increasing the concentration.

Entomopathogenic fungi are one of the effective methods to control the plant parasitic nematodes (Butt *et al*., 2001). Khalil *et al*. (2012) reported that *P. lilacinus* [*Purpureocillium lilacinum* (syn: *Paecilomyces lilacinus*)] was the most effective treatment against *M. incognita,* a pot experiment under greenhouse conditions, recorded 88.23% gall and 76.94% egg mass reduction. Zakaria *et al*. (2013) found that *Verticillium chlamydosporium* and symbiotic bacterium *Photorhabdus luminescens* were alone or combined or with soil amendment

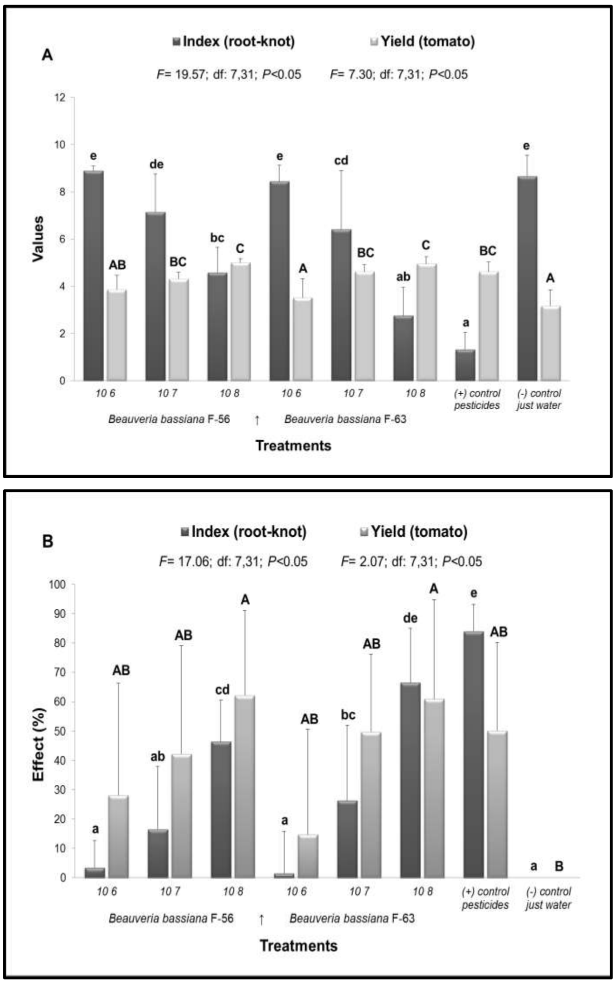

Fig. (1): Effect of different concentrations of *Beauveria bassiana* on *Meloidogyne incognita* and *M. javanica* infection of tomato plants (A) root gall index and yield, (B) percentage of efficacy of *B. bassiana*. Capital (yield) and small (root gall index) letters denote within the column.

significantly reduced nematode infections. A few studies have been done on the effect of *B. bassiana* on the plant parasitic nematodes. Ekanayake and Jayasundara, (1994) found that *P. lilacinus* was more effective than *B. bassiana* as toxin beauvericin produced from *B. bassiana* showed weak nematicidal activity against *M. incognita*.

Chen *et al*. (1996) evaluated 21 isolates of 18 fungal species and found that *B. bassiana* had less parasitism of nematode eggs, but reduced hatching of *Heterodera glycines* in comparison to *P. lilacinus* that found moderately pathogenic to the egg and reduction of hatching. Sun *et al*. (2006) determined microflora on egg and females of *Meloidogyne* spp. and 455 fungal isolates were obtained. The predominant fungal species observed was *P. lilacinus* (49.3% of the isolates) and *B. bassiana* was found only in one location. Most of the isolates were collected from *Meloidogyne* spp. eggs. *In vitro* study showed that *B. bassiana* parasitized (100%) of the eggs, the egg hatching rate was (36%) and juvenile mortality rate was (18.1%) of *M. hapla*. Liu *et al*. (2008) evaluated culture filtrate of isolate *B. bassiana* against eggs and juveniles of *M. hapla in vitro* and glass-house conditions. They found the filtrate of *B. bassiana* successfully inhibited the invasion of *M. hapla* juveniles and 1× dilution of *B. bassiana* isolate showed better inhibition (98.61%) rate compared with the chemical pesticide of Aldicarb (88.89%). Although the 1× dilution of *B. bassiana* isolate had showed the highest effect on *M. hapla,* also inhibited tomato plant growth. 5× dilutions of *B. bassiana* isolate inhibited nematodes like Aldicarb, but also better plant growth were recorded. Bustos *et al*. (2014) evaluated a crude filtrate from the strain of *Purpureocillium* sp. UdeA0106 against *M. incognita* and *M. javanica* in *in vitro* conditions and found the full strength filtering (100%) was the most effective in inhibiting both egg hatching and juvenile mobility.

As a conclusion, *B. bassiana* showed that the concentration (10^8cfu ml^{-1}) of F-56 and F-63 had a promised nematicidal activity of root-knot nematodes as well a positive effect on yield. This result indicated that applying the biological control agent inoculum level was important for biocontrol efficacy. These native isolates of *B. bassiana* could be suggested as biological control agents of *Meloidogyne* spp.

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REFERENCES

Arici, S. E., I., Gülmez, H. Demirekin, H. Zahmekıran and I. Karaca. 2012. Efficiency of entomopathogenic fungus *Fusarium subglutinans* against *Aphis fabae* Scopoli (Hemiptera: Aphididae). Turkish Journal of Biologial Control, 3:89-96.

Bakr, R. A., M. E. Mahdy and E. M. Mousa. 2014. Biological control of root-knot nematode *Meloidogyne incognita* by *Arthrobotrys oligospora*. Egyptian J.Plant Prot., 9(1):1-11.

Bustos, N. L. C., H. Pavas and E. P. Fernández. 2014. Efecto del filtradocrudo de *Purpureocillium* sp. (Cepa UdeA0106), sobre la eclosión de huevos y movilidad de juveniles de *Meloidogyne incognita-javanica.* Rev. Colomb.Biote., XVI 2:37-44.

Butt, T. M., C. Jackson and N. Magan. 2001. Introduction-fungal biological control agents: progress, problem and potential. In: Butt, T. M., C. Jackson and N. Magan (Eds.). Fungi as biocontrol agents, CABI, pp. 1-9.

Butt, T. M. 2002. Use of entomogenous fungi for the control of insect pests. In: F. Kempken (Ed.), Themycota XI agricultural applications. Springer, Berlin Heidelberg, 111-134.

Chen, S. Y., D. W. Dickson and D. J. Mitchell. 1996. Pathogenicity of fungi to eggs of *Heterodera glycines*. J. Nematol., 28:148–158.

Eisenback, J. D. and H. H. Triantaphyllou. 1991. Root-knot nematodes: *Meloidogyne* species and races. In: Nickle, W.R. (Ed.). Manual of agricultural Nematology. Marcell Dekker: New York, pp. 191-274.

Ekanayake, H. M. R. K. and N. J. Jayasundara. 1994. Effect of *Paecilomyces lilacinus* and *Beauveria bassiana* in controlling *Meloidogyne incognita* on tomato in Sri Lanka Nematol. Mediterr., 22:87-88.

Jatala, P., R. Kaltenbach and M. Bocangel. 1979. Biological control of *Meloidogyne incognita acrita* and *Globodera pallida* on potatoes. J. Nematol., 11:303.

Jepson, S. B. 1987. Identification of root-knot nematodes (*Meloidogyne* species). CAB International, Wallingford, Oxon, OX 8DE, United Kingdom, 265 pp.

Kepenekci, İ. 2012. Nematoloji (Bitki Paraziti ve Entomopatojen Nematodlar) [Genel Nematoloji (Cilt-I), Taksonomik Nematoloji (Cilt-II)], Eğitim, Yayım_ve Yayımlar Dairesi Başkanlığı, Tarım Bilim Serisi Yayın, No: 3 (2012/3), LIV+1155 sayfa.

Khalil, M. S. H., A. F. G. Allam and A. S. T. Barakat 2012. Nematicidal activity of some biopesticide agents and microorganisms against root-knot nematodes on tomato plants under greenhouse conditions. J. Plant Prot. Res., 52:47-52.

Liu, T., L. Wang, Y. X. Duan and X. Wang. 2008. Nematicidal activity of culture filtrates of *Beauveria bassiana* against *Meloidogyne hapla*. World J. Microbiol. Biotechnol., 24:113-118.

Perry, R. N. and M. Moens. 2006. Plant Nematology. CABI, Wallingford, UK, 447 pp.

Southey, J. F. 1986. Laboratory methods for work with plant and soil nematodes. Ministry of Agriculture, Fisheries and Food Reference Book, 402. Her Majesty Stationary Office, London, 202 pp.

SPSS. 1999. SPSS for Windows, release 10.0.1. Chicago, IL, USA: SPSS.

Stirling, G. R. 1991. Biological control of plant parasitic nematodes. UK: CABI Publishing. 282 pp.

Sun, M. H., L. Gao, Y. X. Shi, B. J. Li and X. Z. Liu. 2006. Fungi and actinomycetes associated with *Meloidogyne* spp. eggs and females in China and their biocontrol potential. J. Invertebr. Pathol., 93:22-28.

Tigrano-Milani, M. S., R. G. Carneiro, M. R. de Faria, H. S. Frazão and C. W. McCoy. 1995. Isozyme characterization and pathogenicity of *Paecilomyces fumosoroseus* and *P. lilacinus* to *Diabrotica speciosa* (Coleoptera: Chrysomelidae) and *Meloidogyne javanica* (Nematode: Tylenchidae). Biol. Control, 5(3):378-382.

Viaene, N. M. and G. S. Abawi. 2000. *Hirsutella rhossiliensis* and *Verticillium chlamydosporium* as biocontrol agents of the root-knot nematode *Meloidogyne hapla* on Lettuce. J. Nemat., 32:85-100.

Wakil, W., M. U. Ghazanfar, Y. J. Kwon, E. Ullah, S. Islam and K. Ali. 2012. Testing *Paecilomyces lilacinus*, diatomaceous earth and *Azadirachta indica* alone and in combination against cotton aphid (*Aphis gossypii* Glover) (Insecta: Homoptera: Aphididae). Afr. J. Biotechnol., 11(4):821-828.

Zakaria, H. M., A. S. Kassab, M. M. Shamseldean, M. M. Oraby and M. M. F. El-Mourshedy. 2013. Controlling the root-knot nematode, *Meloidogyne incognita* in cucumber plants using some soil bioagents and some amendments under simulated field conditions. Ann. Agric. Sci., 58(1):77-82.

Zeck, W. M., 1971. A rating scheme for field evaluation of root-knot nematode infestation. Pflanzenschutz Nachrichten, Bayer. Published by Farbenfabriken Ag. Leverkusen, 10:141-144.