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Management of Potato Production cv. Diamond Infected with Root Knot Nematode, *Meloidogyne arenaria* Using Biological Process under Field Conditions.

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ABSTRACT

A field experiment designed in a complete randomized block was conducted to evaluate the potentiality of certain commercial bioproducts (biofertilizers and bionematicides) in combination for their effectiveness against root knot nematode, *Meloidogyne arenaria* infecting potato cv. Diamond in naturally infested field in the Research Station of NRC in Nubaryia, Beheira governorate, Egypt. The commercial biofertilizers under investigation were Nitrobein (the N_2-fixing containing *Azospirillium sp.* and *Azotobacter sp.*), Potassiumag (containing the potassium solubilizing bacteria *Bacillus circulanes*) and the Stanes Symbion VAM plus (containing the vascular arbiscular mycorrhiza, *Glomus fasciculatum*) promote the uptake of phosphorus and the bionematicides were Stanes Bionematon (containing the egg pathogenic fungus *Paecilomyces lilacinus*) and Stanes Sting (containing the rhizobacteria, *Bacillus subtilis*). Obtained results showed that all the tested combinations reduced *Meloidogyne arenaria* second stage juveniles (J_2) both in soil and roots as well as root galling, egg masses and female's counts and increased potato yield production as compared to untreated control treatment. The highest increase in potato yield production 68.7% over control resulted from the application of the bionematicides, Stanes Bionematon in combination with the biofertilizers.

Key words: Bionematicides, biofertilizers, potato, *Meloidogyne arenaria*.

Introduction

Potato is a carbohydrate-rich food highly popular worldwide and prepared and served in a variety of ways. Root-knot nematode species are reported to be destructive to potato production. Infected potato tubers become rough and bumpy on the surface and inside the potato, the adult nematode females produce brown spots that discolor further during frying. These deformations and blemishes make tubers unmarketable and, more importantly facilitate dissemination of the pathogen in infected seed tubers to new areas (Vovlas *et al*., 2005). Producers have relied mainly on nematicides and chemical fertilizers to control plant parasitic nematodes and increase crop yield, but their applications are associated with myriads of problems on human health, phytotoxicity, soil and water pollution, extermination of beneficial organisms and development of resistance to nematicides (Kohli *et al.,* 1999). It is well known that, rhizosphere organisms provide an initial barrier against pathogens attacking the root (Weller 1988), such microorganisms that can grow in the rhizosphere are ideal for use as biocontrol and biofertilizer agents. Bacteria are the most abundant microorganisms of which about 2 to 5% are rhizobacteria exert beneficial effects on plant growth and therefore, are termed as plant growth promoting rhizobacteria (PGPR) (Kloepper and Schroth, 1978). Many studies on PGPR have focused on their effect as biological agents of plant diseases than on growth promotion (Kloepper *et al*., 1989). *Bacillus subtilis* is among the PGPR that have been assessed and have multiple modes of action against nematodes including: production of toxic substances, chitinolytic enzymes, reduction of the activity of egg hatching factors, alteration of root exudates and inhibition of nematode penetration into the roots as well as improving the plant growth (Tian *et al*., 2007, Kavitha *et al*., 2013 and Ameen *et al*., 2013). The egg-parasitic fungus, *Paecilomyces lilacinus* is one of the most widely tested soil hyphomycetes for the biological control of plant-parasitic nematodes, is capable of parasitizing nematode eggs, juveniles and females, reducing soil population of plant parasitic nematodes. It was first discovered in soil and observed to control root knot nematodes on potato (Jatala *et al*., 1979) and on tomato (Lara *et al*., 1996). Arbuscular mycorrhizal fungi (AMF) are endophytic fungi that grow within plant tissues without causing disease and can play a protective role against parasitic nematodes by forming distinct symbiotic structures (Ozgonen *et al.,* 1999) and are of great value in promoting the uptake of phosphorus, minor elements and water (Allen, 1996) . They also influence the severity of several plant pathogens by altering plant–pathogen interactions (Siddiqui and Mahmood, 1995).

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This study was conducted to examine the integration between some microorganisms which known to have potential as biocontrol agents against nematodes, bionematicides (containing egg-parasitic fungi, *Paecilomyces lilacinus* and rhizobacteria, *Bacillus subtilis*) and biofertilizers (containing phosphate solubilizing fungus VAM, *Glomus fasciculatum*; N_2-fixing bacteria, *Azospirillium sp.* and *Azotobacter sp*. and the potassium solubilizing bacteria, *Bacillus circulanes*) to manage potato production grown in field naturally infested with *Meloidogyne arenaria*.

Materials and Methods

This experiment was conducted to manage potato production crop cv. Diamond grown in the Research Station of National Research Centre in Nubaryia, Beheira governorate in field naturally infested with *Meloidogyne arenaria* using an integration between some biocontrol agents and biofertilizers as mentioned in Table (1). The experimental field was divided into plots each comprises rows of 15 m long and 75cm apart and the distance between plants was 50cm. The experiment was set up in a completely randomized block design with four treatments and 90 replicates (plants) for each one. The cattle manure was incorporated to soil before planting potato eyes with leaving one row without addition in each plot to serve as control, all plots were irrigated immediately. Two weeks later potato eyes were treated by each of the biocontrol and biofertilizer products as seed dressing as labeled for potato cultivation and planted in hills at 50cm apart. *Meloidogyne arenaria* second stage juveniles (J_2) initial soil population was determined prior to incorporation the cattle manure and at harvest time from each treatment. Nematodes were extracted from 200g soil according to Barker (1985). 1 g roots from each treatment was incubated in water for one week according to Young (1954).Four months later at harvest time five plants chosen at random from every row were carefully uprooted and potato tubers were hand-harvested and yield was determined for each treatment. The following data were recorded, number of root galls, egg masses, females as well as the hatched *M. arenaria* J_2/1g roots. The final nematode populations were expressed as nematodes/kg soil. Data were subjected to analyses of variance and means were tested statistically. Percentage nematode reduction in soil was determined according to Handerson and Tilton formula (Puntener, 1981) as follows:

$$\text{Nematode reduction (\%)} = [1-(\frac{PTA}{PTB} X \frac{PCB}{PCA})] X 100$$

Where:
PTA= Population in the treated plot after application,
PTB= Population in the treated plot before application,
PCB= Population in the check plot before application and
PCA= Population in the check plot after application.

Table 1: List of commercial bioproducts used in controlling *Meloidogyne arenaria* infesting potato cv. Diamond under field conditions.

No.	Commercial Name	Bioagents	Source	Rates/Concentrations
1	Stanes Symbion VAM plus	*Glomus fasciculatum*	Gaara Company	15g/plant $2x10^2$ infective propagates
2	Nitrobein	*Azospirillium sp.* + *Azoobacter sp*	Agric. Res. Centre, Giza, Egypt	300g/ treatment
3	Potassiumag	*Bacillus circulanes*	Agric. Res. Centre, Giza, Egypt	5L/600L water /feddan
4	Stanes Sting	*Bacillus subtilis*	Gaara Company	$1x10^9$ cells/1ml
5	Stanes Bionematone	*Paecilomyces lilacinus*	Gaara Company	$1x10^9$ cfu/1ml 1L/500L Water/feddan

Results and Discussion

In general, our results revealed a promising effect of the tested treatments in reducing final population of *M. arenaria* J_2 in soil and improving potato yield production by different rates, as compared with the untreated control treatment (Tables 2&3).

Data showed that application of the three biofertlizers (Stanes Symbion VAM plus, Nitrobien and Potassiumag induced a significant reduction in final nematode population up to 48.8% and increased potato yield by 20.5% as compared to untreated control (Table 2). These are in accordance with results obtained by Bagyaraj *et al*. (1979) who stated that *Glomus intraradices* has the potential to improve plant growth of nematode-infected plants by reducing nematode multiplication and altering physiology of the roots, including the exudates responsible for chemotactic attraction of nematodes and offset yield loss normally caused by them. Increasing the uptake of phosphorous and other nutrients leading to improvement of plant vigor growth as reported by Hussey and Roncadori (1982). Kirkpatrick *et al.* (1964) reported that the greater availability of phosphorus made the host plant strong enough to tolerate pathogen attack and also phosphorus itself played a vital role in building self defense of plants against nematodes. Cohen (1980) found that both dry weight and nitrogen contents in corn increased by 50-100% when corn seeds were inoculated with *Azospirillium* sp. before planting. Data in Table (2) also, showed that the highest percentage decrease in nematode soil population 83.4% was associated with the application of biofertilizers plus *B. subtilis* and improved potato yield by 60.1%, as compared to untreated control treatment. Such findings are in agreement with that of Vasudeva *et al*. (1958) who found that *B. subtilis* was able to produce the antibiotic bulbiformin which directly affected nematodes and that of Brodbent *et al.* (1977) who reported that, the use of *B. subtilis* improve plant growth by suppressing parasitic root pathogens or by the production of biologically active substances and transform the unavailable mineral and organic compounds in forms available to plants. Also, (Lin *et al.*, 2005, Dawar *et al*., 2008 and Aranjo and Marchest, 2009) stated that application of *Bacillus spp.* significantly reduced larval hatching and reproduction of *Meloidogyne javanica* and promoted the growth of tomato plants. Good biocontrol was also achieved in tomato plants by *B. subtilis* with over 60% reduction in numbers of galls and over 70% reduction in numbers of eggmasses (Adam *et al.,* 2014).Other protocols including *B. subtilis* with soil fertilizers such as cow manure showed significant protection of *Phaseolus vulgaris* against root knot nematode. The gall index was found to be 1.6 in the combination treatment compared with 4.5 in the control group (Wepuhkhulu *et al*., 2011).

Table 2: Effects of some biocontrol and biofertilizer products against *Meloidogyne arenaria* infecting potato cv. Diamond and yield production under field conditions.

Treatments	Initial J_2/1kg soil	Final J_2/1kg soil	% Red.	Potato yield Ton /Feddan	% inc.
Control	3740	3682a*	-	3.7 c*	-
Cattle manure + biofertilizers(Stanes Symbion VAM plus +Nitrobein+Potassiumag)	2180	1098 b	48.8	4.5 bc	20.5
Cattle manure+ biofertilizers(Stanes Symbion VAM plus +Nitrobein +Potassiumag) +Stanes Sting	1950	318 b	83.4	6.0 ab	60.1
Cattle manure + biofertilizers(Stanes Symbion VAM plus +Nitrobein +Potassiumag)+Stanes Bionematone	2052	843 b	58.3	6.3 a	68.7

**The mean difference is significant at 0.05 level according to Duncan's Multiple Range Test.*
% decrease according to Handerson and Tilton formula (Puntener, 1981).

Table 3: Effects of some biocontrol and biofertilizer products on *Meloidogyne arenaria* J_2, root galls, egg masses and females in 1g root of potato cv. Diamond under field conditions.

Treatments	No. of *M. arenaria* J_2/ 1g root	% Red.	No. of galls / 1g root	% Red.	No. of egg masses / 1g root	% Red.	No. of females / 1g root	% Red.
Control	4858	--	193	--	344	--	249	--
Cattle manure + biofertilizers (Stanes Symbion VAM plus +Nitrobein+ Potassiumag)	2304	52.6	92	52.3	94	72.7	96	61.5
Cattle manure+ biofertilizers(Stanes Symbion VAM plus +Nitrobein + Potassiumag) + Stanes Sting	1769	63.6	94	51.3	114	66.9	117	53.0
Cattle manure + biofertilizers(Stanes Symbion VAM plus +Nitrobein + Potassiumag) +Stanes Bionematone	2747	43.5	37	80.8	43	87.5	54	78.3

Regarding the application of the egg parasitic fungus *P. lilacinus* plus the biofertilizers, it resulted in 58.3% reduction in nematode soil population and 68.7% increase in potato production as compared to untreated control (Table 2). Also, this combination (fungus and biofertilizers) recorded the highest percentages reduction, 80.8, 87.5 and 78.3% in the number of root galls, egg masses and females, respectively. While the highest percentage reduction in hatched J_2 was 63.6% achieved by the application of *B. subtilis* and biofertilizers (Table 3). Jatala *et al.* (1980) found that potato plants grown in plots inoculated with fungus *P. lilacinus* had a significant lower root galling index than those grown in plots to which organic matter and nematicides had been applied. Also, Jatala, (1986) suggested that the use of *P. lilacinus* as a promising and practicable biocontrol agents against different stages (eggs, juveniles and adult females).

From our results and others previous studies we can conclude that, application of biocontrol agents that proved potent nematicidal activity in combination with biofertilizers characterized by nitrogen fixation as well as potassium and phosphorus properties may be a good management of root knot nematode infecting potato crops. However further researches are recommended to isolate new bioactive races of bacteria and fungus for optimum integration protocols.

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