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Effect of certain commercial compounds in controlling root-knot nematodes infected potato plants

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Abstract

To examine the effect of certain commercial compounds against root-knot nematode (*Meloidogyne incognita*) which infect potato plants (*Solanum tuberosum* L. var. sponta). Two experiments were carried out under laboratory and field conditions during 2012 and 2013 seasons. Eight nematicides were used as treatments *i.e.*, Mocap, Super control, Dento, Nematex, Vertimyl, Oxamyle, Bionematone, and Bioxy$^+$. The successful treatments were chosen due to their effectiveness on percentage reduction in nematode populations, maximize plant production. The chosen treatments applied in soil naturally infected with nematode in the experimental field in Nubaria, Behira Governorate, North Egypt. Results exhibited significantly reduction in all nematode developmental stages in plant and soil, ex. number of egg masses, galls and nematodes in 250 cm^3 soil with all chemical compounds. The consequence of vegetative growth parameters and yield were increased significantly with tested treatments compared with control. Moreover, results of laboratory experiments recorded that 87 to 98% inhibition for egg-masses hatching and 85 to 98% mortality for juveniles, respectively, with 90% for the lethal concentration of Bioxy$^+$, Oxamyl and Vertimyl.

Key words: Chemical control, *Meloidogyne incognita*, fungi, potatoes.

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Introduction

Potato (*Solanum tuberosum* L.**)** is one of the most important crops grown in Egypt for local consumption, export and processing. The area cultivated with potatoes about 212,000 acres producing about 2.2 million tons, with an average of 10.5 tons per acre (Salah, 2009). *Meloidogyne incognita* root-knot nematode is a serious pathogen of potato causing quality defects that result in reduced crop value or crop rejection when soil temperatures are warm, juvenile (J2) invade roots or tubers establish feeding sites, and develop into the adult stage. It is an obligate parasite that must complete its life cycle in a plant host, but eggs are laid in gelatinoids matrix on or just below the root surface and can remain inactive in the absence of a host and/or fallow for months or years.

This nematode is widespread in Egypt, but is usually found in sandy or sandy loam soils. As *M. incognita* larvae enter the plant root, feed and mature, the surrounding cells of the plant root increase in size and divide causing swellings, often referred to as galls, on the roots. The flow of nutrient and water is restricted, and plants wilt quickly when water becomes limiting. If plants are infected when young, they are often severely stunted and chlorotic (Ingham et al., 1999). Potatoes yield losses are often significantly increased as a result of the interaction between nematode and other pests. In this regard, nematodes can elevate disease pathogens to major pest status even though their population levels or pathogenic potential are low. The most well documented example is the root-knot nematode and wilt disease (Noling, 1987). Management has the objective of minimizing economic losses and includes the whole system of care and treatment of crop pests. All registered nonfumigant nematicides are carbamates or organophosphates and 1,3-dichloropropene is a second carcinogenic grade. Potato production problems often involve complexes of nematodes and plant diseases such as fungal wilts and damping off (Ingham et al., 1999). In India for hundred years, the farmers were used the Bioxy^{+}™ deliver unsurpassed broad-spectrum total kill against the most difficult diseases to eradicate pathogens without the corrosive, toxic and dangerous factors associated with competing disinfectants and sporicidal products on the market today. Bioxy products are a powdered formulation that contains sodium carbonate peroxyhydrate /sodium percarbonate, TAED (Tetra-Acetyl-Ethylene-Diamine, and benzalkonium chloride (Quaternary ammonium compound). They are a white powder with no odor. When powder forms of Bioxy are dissolved in water, sodium percarbonate yields mixture of hydrogen peroxide (H_2O_2) and sodium carbonate (Na_2CO_3). Sodium carbonate raises the pH towards alkaline range and as a result of which hydrogen peroxide dissociates rapidly (under alkaline condition) to form perhydroxyl anion/hydroxyl radical (HO_2). These hydroxyls radical do nucleophilic attack on TAED which releases two molecules of peracetic acid (CH_3CO_3H) which act as a disinfectant. This perhydrolysis takes place much faster than the hydrolysis reaction. Benzalkonium chloride is also one of the important biocidal ingredients of Bioxy products. Solution of benzalkonium chloride is fast acting, generally not affected by pH and also has a long duration of action. Also, Hydrogen peroxide remains in the final solution and boost the microbial killing along with peracetic acid (PAA) and benzalkonium chloride. This study aims to evaluate certain commercial compounds against *Meloidogyne incognita* infected potato and soil born fungi associated the nematode infection.

Materials and methods

Evaluation of certain commercial compound against *M. incognita in vitro*: Each of treatment concentrations (Table 1) was added to egg-masses of *M. incognita* (hand-picked) and to nematode. The same egg-masses and juvenile numbers received distilled water to serve as control. Each treatment was applied in three replicates. The inhibition percentage of egg hatching was recorded after three days. The nematode mortality percentage of juvenile was recorded after 48 hours under a stereoscopic microscope.

Isolation of pathogenic fungi: Isolation of pathogenic fungi from potato roots was carried out according to the method described by Saremi (2005). Potato roots were washed by tap water and surface sterilized for two minutes by dipping in 2% sodium hypochlorite solution. Then, the roots were washed several times in sterile distilled water then it's dried between two filter papers. The dried roots were cut into small pieces (0.2 cm) and placed on the surface of the PDA medium in sterile Petri dishes. The plates were incubated for 7 days at 28°C. The growing fungi were identified based on the morphological and cultural characteristics in Mycology and Plant Disease Survey Department, Plant Pathology institute, Agriculture Research Center, Giza, Egypt (Gilman, 1957; Domseh, 1980; Sneh, et al., 1991; Ellis, 1993).The frequency percentages of isolated fungi were counted and calculated according to the following equation:

$$\text{Fungal frequency percentage} = \frac{\text{No. of fungus}}{\text{Total No. of fungi}} \times 100$$

Field experiments: The field experiment was carried out in naturally infested soil with root-knot nematode *Meloidogyne incognita* at Nubariea research station, during two growing 2012 and 2013 seasons to study the influence of treatments on the root-knot nematode and on isolated fungi. The area of the experimental plot equal 1/100 of acre. Sponta potato tubers were coated with the treatments and cultivated. The experiment was carried out in completely randomized design (CRD) and randomized complete block design (RCBD) as outlined by Steel and Torrie (1980). Physical and chemical properties for experimental soil are shown in Table (2).

The plant growth parameters (plant height, number of leaves, number of shoots, fresh and dry weights of shoots and length of root per plant) were determined after 75 days from cultivation. While, number of tubers, average of tuber weight/plant and total yield/acre were measured at end of season (110 days from cultivation). Chemical components of potatoes were analyzed. Also, the roots were washed to get rid of the adhering sand particles to determine the number of nematode larvae in 250 cm^3 of soil (free nematodes in soil), numbers of galls, egg-masses, Reproductive Factor (RF) and Developmental Stages (DS) were calculated according to Norton (1978) as follow:

$$RF = \frac{\text{No. Eggs} + \text{Developmental stages} + \text{Free Nematode in soil}}{\text{Initial Nematode Population}}$$

DS = number of developed juveniles (second, third and fourth stages) embedded in the roots

Statistical Analysis: All data were subjected to statistical analysis according to the procedures "ANOVA" reported by Snedecor and Cochran, (1980). Treatments means were compared by the Least Significant Difference test "LSD" at 5 % level of probability in two seasons of experimentation.

Results and Discussion

Effect of treatments against egg-masses and juveniles nematode *in-vitro*: The tested compounds were applied to evaluate their efficacy on the root-knot nematode *(M. incognita)*. The compounds were utilized according to the recommended dose such as Oxamyl and the antagonistic microorganism. Data in Table 3 showed significant inhibition and mortality as affected by treatments of chemical compounds, ranged from 87 to 98% inhibition for egg-masses hatching and 85 to 98% mortality for juveniles, respectively at 90% for the lethal concentration of Bioxy$^+$, Oxamyl and Vertimyl. The biocontrol compound Bionematon (*P. lilasinus*) recorded 95% inhibition of egg hatching which considered better than many chemical compounds used in this study such as Mocap or Super control. These results were agree with Azam et al., 2012 who stated that the use of *P. lilasinus* one week before nematode inoculation caused an increase in growth and yield characteristics of tomato, and also reduced the reproduction of nematode as compared to other treatments. The results of histological studies indicated that *P. lilasinus* parasitized on the *M. incognita* eggs through the formation of fungal hyphae and conidiophores and caused the disintegration of the eggshells, egg masses and juveniles of *M. incognita*.

Table 1: List of commercial compounds tested in this investigation.

Trade name	Common name	a.i.	Rate of application
Mocap	Ethoprophos	10% G	30 Kg / acre
Super control	Ethoprophos	20% EC	15 L/ acre
Dento	Fenamiphos	40% EC	6 L/ acre
Nematex	Oxamyl	24% SL	2+2 L/ acre
Vertimyl	Oxamyl	24% SL	2+2 L/ acre
Oxamyl	Oxamyl	24% SL	2+2 L/ acre
Bionematone	Pacilomyses lilasinus	1.75% WP	4 Kg/ acre
Bioxy +	Peracetic Acid	0.2 %	2 g/liter

Table 2: Physical and chemical properties for experimental soil.

Physical properties						Chemical properties						
Practical size distribution			Texture	pH	EC(dS/m)	Cation (meq/l)				Anion (meq/l)		
Sand	Silt	Clay				Ca^{++}	Mg^{++}	Na^{++}	K^{+}	HCO_3^{-}	CL^{-}	So_4^{--}
71.2	13.0	15.8	Sand	7.6	2.29	6.01	2.99	10.3	0.15	1.0	2.0	16.25

Isolation of fungi associated with nematode infection: Isolation from root portion of potato plants was done on large scale. The fungi isolated were identified on their typical colony characteristics and are given in Table (4). *Fusarium solani* was isolated with the highest frequency (40.0%) followed by *Rhizoctonia solani* (25%) and *Fusarium oxysporum* (20%). The fungi with lowest frequency were *Alternaria alternata*, *Verticillium albo-atrum* and *Macrophomina phaseolina***,** respectively. Similar fungi have been isolated from tomato seedlings by Gunasekaran et al., (1994)**.** Mitidieri (1994) recorded that the damping off as major disease on tomato and capsicum caused by *Sclerotinia sclerotiorum, Rhizoctonia solani, Sclerotium rolfsii* and *Pythium* spp. MacNish et al., (1995) detected that AG-10 isolate of *Rhizoctonia solani* on potato dextrose agar medium. Kuprashvili (1996) and Lucas et al., (1997) isolated *Alternaria tenuis*. Jiskani et al., (2007) isolated *Rhizoctonia solni*, *Fusarium oxysporum* and *Fusarium solani* from infected tomato plants. Saremi et al. (2011) found that the most frequently soil-borne fungal pathogens on plants are *Fusarium* species that make high economic damages in various agricultural and showed the dominate species isolated from potato were *F. solani*, *F. oxysporum*, *F. pseudograminearum*, *F. moniliforme* and *F. sambucinum*. These results were agreement with those reported by Hooker, (1990), Saremi, (2000) and Saremi and Amiri, (2010). Several *Fusarium* species can cause diseases and make sever damage on potato in several countries, which caused yield losses (Wharton et al., 2006; Saremi, 2005; Saremi & Amiri, 2010). However, many researchers supposed that *F. solani* is the main fungal pathogen isolated from potato tubers and roots all over the world (Hooker, 1990).

Field experiment: Nematicides remain reliable and fast working and can give good economic returns on high-value crops. They may be essential for producing nematode-free export crops. However, in general, nematicides reduce but do not eliminate populations of plant-parasitic nematodes. Therefore, final nematode densities may be too high for a profitable crop to be grown the following season without taking further phytosanitary measures (Hague & Gowen, 1987). Treatments with chemical compounds showed different efficacy compared with untreated treatment. Data in Table (5) showed that the highest effect referred to the treatment with $Bioxy^{+}$ which reduced number of galls to17, number of egg-masses to 14 and number of developmental stages to 20. Moreover, reduced number of soil nematodes to 420 compared with untreated treatment. While, Super control showed the lowest effect. It reduced number of galls to 103, number of egg-masses to 90 and number of developmental stages to 13. Also, reduced number of soil nematode to 620.

Table 3: The inhibition and mortality of *Meloidogyne incognita* after using treatments (combined analysis of two seasons).

Treatments	LC	Inhibition of egg hatching%	Mortality of juvenile %
Mocap	90	92	97
	50	61	74
Super control	90	89	89
	50	48.9	64
Dento	90	98	97
	50	69	70
Nematex	90	94	95
	50	55	75
Vertimyl	90	96	98
	50	68	57
Oxamyle	90	98	95
	50	57	61
Bionematone	90	95	87
	50	75	62
Bioxy+	90	87	85
	50	50	69
Control		0	0
L.S.D. at 5%		9.493	6.604

Table 4: Frequency of fungi isolated from infected potato plants (combined analysis of two seasons).

Isolated fungi	Frequency (%)
Fusarium solani	40
Rhizoctonia solani	25
Fusarium oxysporum	20
Alternaria alternata	7
Verticillium albo-atrum	6
Macrophomina phaseolina	2

Paecilomyces lilasinus, is one of the most widely tested biocontrol agents for the control of plant parasitic nematodes. It is evident from the results of *in vitro* experiments that this fungus had the ability to infect the eggs and female of *Meloidogyne spp*. and destroy their embryos within a week (Esfahani & Ansari Pour, 2006). The production of secondary metabolites like leucinotoxins, chitinase, protease and acetic acid by *P. lilacinus* has been associated with the infection process which reduced the nematode population. This fungus had the unique adaptability to grow on a wide range of soil pH, which makes it a competitive biocontrol agent in the most of the agricultural soil. It establishes himself in the soil very short span of time and become the dominant species in the introduced area.

Table 5: Effect of certain commercial compounds on *M. incognita* infected potatoes under field conditions (combined analysis of two seasons).

Treatments	No./250 cm^3 soil	No. Galls/ plant	No. egg-mass/ root system	DS*	Rf*
Mocap	320	64	31	63	3.2
Super control	620	103	90	13	5.5
Dento	130	75	53	19	4.5
Nematex	540	64	14	23	4.3
Vertimyl	270	86	19	49	2.8
Oxamyle	330	56	17	23	2.8
Bionematone	450	62	31	25	3.8
Bioxy+	250	17	14	20	2.0
Control	6400	684	459	517	50.36
L.S.D at 5%	69.918	48.87	18.22	26.94	2.57

*DS = Developmental Stages, ** Rf = Reproductive Factor.

Obtained data in Table (6) showed that, all chemical compounds significantly reduced the percentage of disease incidence and increased the survival plants of potatoes compared with untreated control. Nematex treatment occupied the first rank in increasing survival plants (99%) followed by Vertimyl and $Bioxy^+$ in both growing seasons (98 %). In contrast, the percentage of root rot disease was decrease by using $Bioxy^+$ treatment which recorded (2.2 and 2.0 %) in both seasons, respectively. Super control showed the lowest effect in two seasons. The effect of chemical compound can be explain by the used nematicides reduced reproduction of the *Meloidogyne* proving their potentiality in controlling this serious pest on potatoes by inhibiting cholinesterase in nematodes e.g. Carbofuran, Oxamyl and Fenamiphos in *M. incognita* and *M. javanica* (Nordmeyer & Dickson, 1990). Carbamates taken up by plants after nematode penetration have an influence on juvenile development and growth in the root. This curative effect may be the result of direct toxic action on nematode physiology or disturbance in nematode nutrition through indirect effects on the activity of the syncytium and the indirect effect was the reduction of disease incidence on potatoes.

Data in Table (7) showed the effect of various examined chemical compound on infected potatoes by *M. incognita*. All chemical compounds significantly raised the tested essential parameters in infected plants over the control. Generally, results indicated that the nematicides increased plant growth parameters compared with untreated treatment. Applied treatments by Super control, Bioxy^{+}, Mocap and Oxamyl arranged descending according to number of shoot plants. While, Bioxy^{+} and Dento obtained highest values of plant height, number of leaves and fresh and dry weights of shoots. Moreover, Dento, Vertimyl and Bioxy^{+} gave the highest yield. While, untreated treatment recorded the highest tuber weights followed by Super control treatment. It was accepted that these compounds acted by the inhibition of acetyl cholinesterase (ACHE) at cholinergic synapses in the nematode nervous system. Inhibition of ACHE was most likely explanation for the observed effect of organosphosphate and carbamate nematicides on the orientation behavior of nematodes (Wright, 1981; Opperman & Chang, 1990). Thus, these chemicals act by impairing nematode neuro-muscular activity, thereby, reducing their movement, invasion, feeding and consequentially the rate of development and reproduction. Also, Azam et al., (2012) studied the interactions of *Paecilomyces lilasinus* with root-knot nematode *Meloidogyne incognita* and their effects on the growth of tomato. The results indicated that the use of *P. lilasinus* one week before nematode inoculation caused an increase in growth and yield characteristics of tomato. Regarding to sugar content, Data in Table (8) showed that the lowest amount recorded in treatment with Mocap (4.9 and 2.5). Whereas, the highest amount recorded in treatment with Bioxy^{+} (12.3 and 3.8) compared with the control treatment which recorded (1. 5 and 3.1) mg/g potato tubers. Amounts of phenolic compounds differed relatively due to different treatments; the highest amount was noticed in treatment with Bioxy^{+} (13.4 and 8.6). While, the lowest was Super control with 9.8 and 3.2. Whereas, the control recorded 6.6 and 2.2 mg/g. Also, the highest amount of total protein recorded with Bioxy^{+} (0.43 mg/g) followed by Bionematon (0.3 mg/g) compared with control which recorded 0.03 mg/g.

Table 6: Effect of certain commercial compounds on the percentage of root rot disease incidence on potatoes under field conditions during 2012 and 2013 seasons.

Treatments	Season 2012	Season 2013
	Root rot (%)	Root rot (%)
ocap	12.1	13.5
Super control	16.7	18.0
Dento	13.3	10.3
Nematex	3.4	2.5
Vertimyl	4.9	4.9
Oxamyle	5.3	3.2
Bionematone	6.9	5.2
Bioxy+	2.2	2.0
Control	53.6	53.6
LSD at 5%	5.88	6.44

This suggests that Sponta cultivar has active post–penetration biochemical defense mechanism which blocked the development and reproduction of the tested root-knot species. The post penetration response of roots was reported by Grundler et al., 1997 and Valette et al., 1998**;** whom worked on different plants and stated that penetration may actively contribute to plant defense against nematode and other pathogens and phenolics are often cited.

Table 7: Effect of certain commercial compounds on plant growth parameters under field conditions (combined analysis of two seasons).

Treatments	No. of shoots plant	Plant height (cm)	No. of leaves	Shoot Fresh weight (g)	Shoot dry weight (g)	No. of tubers /plant	Average tuber weight (g)	Yield ton/ acre
Mocap	7.9	44	39	513	95	12.00	219	13.0
Super control	8.5	46	52	489	92	11.98	230	12.4
Dento	7.8	48	53	543	101	11.98	223	12.8
Nematex	6.7	46	53	526	98	12.02	191	14.9
Vertimyl	6.1	45	52	499	93	12.02	193	14.8
Oxamyle	7.6	44	49	475	90	12.04	199	14.3
Bionematone	7.3	45	51	511	95	11.97	210	13.6
Bioxy+	8.1	48	54	547	102	12.00	194	14.7
Control	4.1	36	31	351	52	11.96	250	11.4
L.S.D at 5%	0.251	1.953	1.327	2.980	21.89	0.673	54.527	0.826

*DS = Developmental Stages, **Rf = Reproductive Factor.

Table 8: Effect of certain commercial compounds on *M. incognita* infected potatoes under field conditions (combined analysis of two seasons).

Treatments	Concentration (mg/g)				
	Total sugars	Reducing sugars	Total phenols	Free phenols	Total protein
Mocap	4.9	2.5	8.1	5.3	0.12
Super control	5.1	2.3	9.8	3.2	0.20
Dento	5.8	2.7	9.7	3.9	0.17
Nematex	4.7	3.8	10.3	8.2	0.14
Vertimyl	7.2	3.4	13.4	8.0	0.16
Oxamyle	8.0	4.8	10.6	8.1	0.28
Bionematone	11.5	4.3	11.3	9.0	0.30
Bioxy+	12.3	3.8	13.4	8.6	0.43
Control	1.5	3.1	6.6	4.2	0.03

*DS= Developmental Stages, ** Rf = Reproductive Factor.

Generally, nematicides treatments had a positive effect on parameters tested through two growing seasons compared with untreated treatment. Our results obtained that Bioxy$^+$ consider the best treatment effect on inhibition egg-masses hatching, mortality for juveniles, number of galls, number of egg-masses, number of developmental stages, number of soil nematodes, percentage of disease incidence, survival plants, vegetative growth parameters, yield, sugar content, phenolic compounds and total protein compared with other treatments. So, Bioxy$^+$ is more suitable to use as nematicide.

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