BIOLOGICAL CONTROL OF THE DISEASE COMPLEX ON POTATO CAUSED BY ROOT-KNOT NEMATODE AND *FUSARIUM* WILT FUNGUS

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Summary. The effect of *Trichoderma koningii* and *Bacillus megaterium* on the control of a disease complex caused by a mixed population (*Meloidogyne javanica* and *Meloidogyne incognita*) of root-knot nematodes and the wilt fungus *Fusarium oxysporum*, and on the growth of potato cv. Nicola, was studied under greenhouse conditions. Application of *T. koningii* and *B. megaterium*, alone or in combination, seven days earlier than soil infestation with *F. oxysporum* and/or the mixed population of *Meloidogyne* spp., significantly reduced *Fusarium* wilt disease incidence and nematode infection on potato and improved plant growth components. Generally, the combination of the two bio-control agents was more effective in controlling the plant disease and improving plant growth components than either of the two organisms used singly.

Key words: *Bacillus megaterium*, *Fusarium oxysporum*, *Meloidogyne incognita*, *M. javanica*, *Trichoderma koningii*.

Potato (*Solanum tuberosum* L.) is one of the most important vegetable crops in Egypt as well as in many other countries; it occupies fourth place in importance amongst major food crops (Scurrah *et al.*, 2005)

During all stages of growth, the potato crop suffers from diseases that cause considerable loss in tuber yield. *Fusarium oxysporum* Schlecht. is a fungus that causes serious disease, with symptoms that include vascular wilt, stunting, chlorosis and eventual plant death in many important crops. The loss of potato yield from this fungus is between 10 and 53% (Thanassoulopouls and Kistos, 1985; Norgues *et al.,* 2002).

Root-knot nematodes (*Meloidogyne* spp.) are one of the major obstacles facing the production of potatoes and cause considerable damage. The loss of production reaches 100 million U.S $/year worldwide (Abd-Allah, 1999; Oka *et al.,* 2000).

The concomitant infection of root-knot nematodes (*Meloidogyne* spp.) and some root pathogenic fungi results in greater damage to the host plant and greater disease severity in a number of crop species than from either pathogen acting alone (Hillock and Marley, 1995; Mahjoub, 1996; Karlsson, 2006). The synergistic interaction between *Verticillium dahlia*e Klep, *Fusarium oxysporum* f. sp. *tuberose* Snyder *et* Hansen and *M. javanica* (Treub) Chitw. resulted in reduction of plant growth and increase in vascular wilt severity (Daami-Remadi *et al.*, 2009).

Because of hazards with the use of pesticides, biological control of plant disease has received increasing attention as a promising supplement. Of the various bioagents, fungi of the genus *Trichoderma* are known to suppress many soil-borne fungi and nematode diseases under greenhouse and field conditions (Bokhari, 2000; Mushtaq, 2011). Application of *Bacillus* spp. to the soil significantly reduced nematode reproduction, disease incidence of pathogenic fungi and improved plant growth (Sunaina and Ajay, 2005; El-Haded, 2011).

Mixtures of biocontrol agents, with different plant colonization patterns, may be useful for control of plant pathogens *via* different mechanisms of disease suppression (Roupach and Klopepper, 1998).

The present work aimed at studying the efficacy of *Trichoderma koningii* Oudem and *Bacillus megaterium* de Bary to control *Fusarium* wilt and root-knot nematode disease complex in potato.

MATERIALS AND METHODS

The work was conducted under greenhouse conditions at the Experimental Farm of the Faculty of Agriculture, Minoufiya University, Shibin El-Kom, Egypt.

Isolation and cultures of Fusarium oxysporum. The isolate of *Fusarium oxysporum* was obtained from potato plants showing *Fusarium* wilt symptoms, according to Otadoh *et al.* (2011). The pure isolate was cultured on Potato Dextrose Agar (PDA) and stored at 5 °C until used.

Preparation of nematode inoculum. Two-months-old nightshade (*Solanum nigrum* L.) roots infected with mixed populations of *Meloidogyne javanica* and *Meloidogyne incognita* were washed with tap water to remove adhering soil particles, cut into small pieces and the eggs collected according to Hussey and Barker (1973). The extracted eggs were transferred to Baermann trays with soft tissue paper at room temperature to allow egg hatching. After 72 hours, the emerging sec-

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ond stage juveniles were counted under a light microscope and the average number per ml calculated.

Source of the bio-control agents. Strains of *Trichoderma koningii* and *Bacillus megaterium*, a plant growth promoting rhizobacteria (PGPR), were obtained from the Agricultural Botany Department, Faculty of Agriculture, Minoufiya University and grown on sand/wheat bran medium (1/1). These strains were selected because they had shown efficacy against several plant pathogenic fungi and root-knot nematode in tests by the Department's researchers (unpublished data).

Effect of Trichoderma koningii and Bacillus megaterium on disease complex on potato. The effects of the bio-agents were tested on the disease complex on potato cv. Nicola. One tuber of potato was planted in each of the 25-cm-diameter plastic pots filled with 5 kg of sterilized clay-sand mixed soil (1 : 1, v/v). After three weeks, the soil was inoculated with *T. koningii* growing on sand/wheat bran medium at the rate of 3% of soil weight. *Bacillus megaterium* was added to the pots by pipetting 10 ml of bacterial suspension (2.0×10^7 CFU). One week later, the soil was infested with *F. oxysporum* growing on PDA at the rate of 3% of soil weight. At the same time, 3,000 J_2 of the mixed *Meloidogyne* spp. population were added by pipette into three or four holes around the growing plant. Treatments were arranged in a completely randomized design with five replicates. Pots were irrigated as needed and fertilized every two weeks with Greinzet NPK solution (50 ml/10 litres water) either added to the soil or sprayed on the leaves (50 ml per pot). The experiment was terminated 90 days after planting. The incidence of *F. oxysporum* was estimated 45, 60, 75 and 90 days after planting as an index of leaf damage (ILD) calculated per potato plant, following the formula of Beye and Lafay (1985)

$$ILD = \sum \text{ratings}/\text{max rating}$$

where ILD = Index of Leaf Damage, $\sum$ ratings = total ratings, max rating = 4.

Ratings were assigned according to a 0-4 scale, where 0 = asymptomatic leaf, 1 = leaf wilted, 2 = leaf with hemiplegic yellowing, 3 = leaf with necrosis, 4 = dead leaf.

At the end of the experiment, root, shoot and tuber weights and plant height were recorded. The soil of each pot was thoroughly mixed and a 250 g sub-sample used to extract nematodes. Second stage juveniles (J_2) were extracted from the soil in each pot by the sieving and modified Baermann technique (Goodey, 1957). The juveniles were counted and referred to as number per pot. Roots were washed under running tap water to remove soil particles, and then weighed. Females and egg masses, as well as the numbers of developmental stages, in the entire plant root were counted after staining the roots in a sodium hypochlorite-acid fuchsin solution according to El-Hazmy (1992).

Statistical analysis. Data were subjected to analysis of variance (ANOVA) using Costat software. The mean differences were compared according to Duncan's Multiple Range Test (DMRT).

RESULTS

The two bio-control agents, *T. koningii* and *B. megaterium*, alone or in combination, reduced significantly the index of leaf damage (ILD) (Table I). The greatest reduction was recorded by the combination of the two bio-agents, which resulted in an ILD of 0.117 after 45 days, 0.268 after 60 days, 0.647 after 75 days and 0.723 after 90 days from planting. The ILD was 0.457 after 45 days, 0.792 after 60 days, 1.701 after 75 days and 2.419 after 90 days in the wilt fungus and nematode treatment only.

The bio-control agents, alone or in combination, also inhibited significantly nematode reproduction and other variables (Table II). Adding *T. koningii* with *B. megaterium* gave the greatest effect on nematode inhibition. The average numbers of J_2 per pot, and numbers of de-

Table I. Effects of the two bio-control agents on index of leaf damage (ILD) of potato plant cv. Nicola.

Treatment	Days after planting			
	45 days	60 days	75 days	90 days
F. oxysporum + *Meloidogyne* spp.+ *T. koningii*	0.24 b	0.54 b	1.08 b	1.30 b
F. oxysporum + *Meloidogyne* spp.+ *B. megaterium*	0.19 c	0.43 c	0.80 c	1.09 c
F. oxysporum + *Meloidogyne* spp.+ *T. koningii* + *B. megaterium*	0.11 d	0.26 d	0.64 d	0.72 d
F. oxysporum + *Meloidogyne* spp.	0.45 a	0.79 a	1.70 a	2.41 a
Control*	0	0	0	0
LSD 5%	0.47	0.04	0.13	0.04

Each figure is the mean of five replicates. Means in a column sharing a letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.
*Control = Healthy plant.
ILD = Index of Leaf Damage.

Table II. Effects of the two bio-control agents on the development of *Meloidogyne* spp. on potato cv. Nicola.

Treatment	J_2s in the soil per pot	Developmental stages (J_2, J_3, J_4) per root	Females per root	Egg masses per root
F. oxysporum + *Meloidogyne* spp.+ *T. koningii*	10,368 b	300 b	100 b	102 b
F. oxysporum + *Meloidogyne* spp.+ *B. megaterium*	5,664 c	220 c	70 c	73 c
F. oxysporum + *Meloidogyne* spp.+ *T. koningii* + *B. megaterium*	3,552 d	86 d	20 d	24 d
F. oxysporum + *Meloidogyne* spp.	16,992 a	360 a	231 a	139 a
Control*	0 e	0 e	0 e	0 e
LSD 5%	488.46	11.50	6.25	3.46

Each figure is t he mean of five replicates. Means in a column followed by the same letters are not significantly different (P ≤ 0.05) according to Duncan's multiple range test.
* Control = Healthy plant.

Table III. Effects of the two bio-control agents on some growth components of infected potato plants of cv. Nicola.

Treatment	Root weight (g)	Shoot weight (g)	Tuber weight (g)	Plant height (cm)
F. oxysporum + *Meloidogyne* spp. + *T. koningii*	14.4 d	70.9 d	108.7 d	44.3 d
F. oxysporum + *Meloidogyne* spp. + *B. Megaterium*	16.8 c	74.9c	120.8 c	47.6 c
F. oxysporum + *Meloidogyne* spp. + *T. koningii* + *B. megaterium*	19.7 b	81.1 b	130.6 b	51.3 b
F. oxysporum + *Meloidogyne* spp.	12.3 e	65.8 e	94.9 e	40.1 e
Control*	21.4 a	85.5 a	138.2 a	54.0 a
LSD 5%	0.60	0.74	0.80	0.77

Each figure is the mean of five replicates. Means in a column followed by the same letters are not significantly different (P ≤ 0.05) according to Duncan's multiple range test.
* Control = Healthy plant.

velopmental stages, females and egg masses per plant were 3552, 86, 20 and 24, respectively, when the bio-control agents were introduced into the soil infested with *F. oxysporum* and the mixture of *Meloidogyne* spp., while the same variables were 16,992, 360, 231 and 139, respectively, when the bio-control organisms were not added.

The bio-control agents increased significantly plant growth components (Table III). Combining *T. koningii* with *B. megaterium* resulted in the best plant growth. Root weight was 19.8 g, shoot weight 81.1 g, tuber weight 130.6 g and plant height 51.3 cm in pots inoculated with bio-control agents, while the same variables were 12.3 g, 65.9 g, 98.6 g and 40.2 cm, respectively, in the soil infested with fungus and nematodes.

DISCUSSION

The application of *T. koningii* and *B. megaterium* to the soil controlled *Fusarium* wilt and root-knot nematode disease under greenhouse conditions and in turn increased plant growth and yield. Diseases incidence was greatly reduced when the antagonists were introduced into the soil one week before inoculating the pathogenic organisms.

Several species of *Trichoderma* have been reported to suppress soil-borne diseases, including *Fusarium* spp. and parasitic nematodes (Siddiqui *et al.*, 1999), because of their ability to produce hydrolytic enzymes, which degrade chitin fungal cell walls (Radwan, 2007), or antibiotics (Sabet, 2000).

Trichoderma harzianum penetrates the nematode egg mass matrix and significantly decreases nematode hatch (Sahebani and Hadavi, 2008). *Trichoderma* spp. also control root-knot nematodes by direct toxic metabolites, which inhibit nematode penetration and development (Bokhari, 2009).

Plant growth promoting rhizobacteria (PGPR) play an important role in controlling soil-borne fungi and plant parasitic nematodes by several mechanisms, such as competition for an ecological niche, production of inhibitory substances or induction of systemic resistance in host plants (Khan *et al.*, 2008). *Bacillus* spp.

produce antibiotics toxic to nematodes, such as fengycin, zwittermycin A (Liefert *et al*., 1995), and antifungal antibiotics, which could inhibit the growth of the wilt pathogen *F. oxysporum* on tomato (Kapoor and Kar, 1989).

In conclusion, the present study has shown that the two bio-control agents gave good control of the disease complex in potato. Therefore, future studies should be focused on the development of economical and efficient mass production of these bio-agents to be used for the management of this disease complex under Egyptian conditions.

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