

FEASIBILITY OF USING TWO COMMERCIAL BIO-PRODUCTS OF ENTOMOPATHOGENIC NEMATODES, COMPARING TO CADUSAFOS, IN CONTROLLING *MELOIDOGYNE JAVANICA* ON COMMON BEAN IN SAUDI ARABIA

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ABSTRACT

Two commercial bio products of entomopathogenic nematodes (EPNs) namely *Entonem* (*Steinernema feltiae*) and *Larvanem* (*Heterorhabditis bacteriophora*), were tested for their efficacy as alternative biocontrol agents, comparing to the nematicide, *Cadusafos* 10G against the root-knot nematode, *Meloidogyne javanica* on common bean cv. Strike under greenhouse conditions (27±2°C). EPNs at the rates 1000, 2000, 4000 and 8000 J/pot, and *Cadusafos* 10G (*Rugby*®) at 0.1g/pot, were applied immediately after *M. javanica* inoculation. Healthy plants and *M. javanica*-infected ones were served as checks. All treatments were replicated five times and arranged in a complete randomized design in the greenhouse. Disease and plant growth parameters were recorded, 55 days after *M. javanica* inoculation.

Both EPNs significantly decreased number of root galls (29.9-62.1%), egg masses (29.4-62.5%) and reproduction factor (R_f) values (12.5-59.5%) of *M. javanica* on the treated plants. Increasing the application rates of either EPNs, significantly enhanced *M. javanica* suppression. Unfortunately, treatments of either EPNs, almost, did not improve visual growth parameters and pod weights of treated plants as compared to *M. javanica*-infected ones.

Application of *Cadusafos* 10G provided the maximum *M. javanica* reduction in root galls, egg masses and R_f value (97.7%, 98.1% and 97.9% respectively), and significantly improved performance of the treated plants. Therefore, *Entonem* and *Larvanem*, are not accepted as good alternatives to *Cadusafos* 10G in the management of *M. javanica* on common beans cv. Strike in Saudi Arabia in this study.

Key words: Bio-products, biocontrol agents, root-knot nematode, *Meloidogyne javanica*.

INTRODUCTION

Plant-parasitic nematodes, especially root-knot nematodes, *Meloidogyne* spp. are one of the major limiting factors of plant production worldwide. *Meloidogyne javanica* is the most common and widely distributed root-knot nematode in Saudi Arabia and it may cause serious damage potentials to many vegetables and other plant crops (Al-Hazmi *et al.*, 1995).

Entomopathogenic nematodes (EPNs) belonging to Steinernematidae and Heterorhabditidae are being produced commercially and used as biocontrol agents against many soil pests and insects of Coleoptera, Diptera, Lepidoptera, Orthoptera, and Siphonaptera (Hazir *et al.*, 2003). The infective stage of these nematodes is the third stage juveniles (J_3), carries a symbiotic bacterium that is released following infection of the insect host. The bacteria, *Xenorhabdus* spp. are associated with Steinernematids, and *Photorhabdus* spp. are associated with Heterorhabditis spp. Once infection has occurred, the bacteria multiply and produce allelochemicals that kill the insect host within two days (Burnell and Stock, 2000; Hazir *et al.*, 2003).

The use of EPNs has been suggested as one possible alternative in the management of plant-parasitic nematodes on certain crops. Application of EPNs to the soil significantly reduced abundance, diversity and maturity of the nematode community by reducing of genera and abundance of plant-parasitic nematodes (Somasekhar *et al.*, 2002). EPNs had suppressed a number of potentially important plant-

parasitic nematodes, including *Aphelenchoides rhytium* (Hu *et al.*, 1999), *Belonolaimus longicaudatus* and *Criconemella* sp. (Grewal *et al.*, 1997), *Bursaphelenchus xylophilus* (Hu *et al.*, 1995), *Globodera rostochiensis* (Perry *et al.*, 1998), *Hoplolaimus* sp. (Pérez and Lewis, 2006), *Meloidogyne* spp. (Bird and Bird, 1986; Ishibashi and Choi, 1991; Gouge *et al.*, 1994; Hu *et al.*, 1995; Ishibashi and Matsunaga, 1995; Lewis *et al.*, 2001; Fallon *et al.*, 2002; and Pérez and Lewis, 2002 & 2004), *Mesocriconema xenoplax* (Nyczepir *et al.*, 2004), *Pratylenchus coffeae* (Ishibashi and Matsunaga, 1995), and *Tylenchorhynchus* spp. (Smitley *et al.*, 1992). Also, Ishibashi and Kondo (1986) previously observed that the population densities of stubby-root, ring and spiral nematodes were suppressed after addition of *Steinernema glaseri* to the soil.

The aim of this study was to evaluate the efficacy of two commercial bio-products of entomopathogenic nematodes (*Entonem* and *Larvanem*) as biocontrol agents, comparing to the nematicide, *Cadusafos* 10G (*Rugby*®) in controlling *M. javanica* on common bean cv. Strike under greenhouse conditions in Saudi Arabia.

MATERIALS AND METHODS

Meloidogyne javanica Culture and Inoculum:

The root-knot nematode, *Meloidogyne javanica* was originally isolated from naturally infected eggplants growing in Riyadh, and identified according to the morphological characteristics of perineal

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patterns (Hartman and Sasser, 1985). *M. javanica* culture was maintained on tomato (*Lycopersicon esculentum* Mill) cv. Rutgers in the greenhouse ($27\pm 2^\circ\text{C}$). Eggs of *M. javanica* to be used as inoculum were extracted using a sodium hypochlorite method (Hussey and Barker, 1973).

Entomopathogenic Nematodes Inocula:

Two commercial bio-products of entomopathogenic nematodes namely, Entonem (originally *Steinernema feltiae*) and Larvanem (originally *Heterorhabditis bacteriophora*), were purchased from Koppert B. V. Biological Systems, Berkel en Rodenrijs, the Netherlands. About 1g of the contents of either nematode package was suspended in 100ml water into a 250ml glass flask and well-stirred on a magnetic stirrer in order to make a stock suspension of third stage juveniles (J_3) of either nematode for inoculation.

General Methods:

Seeds of common bean (*Phaseolus vulgaris* L.) cv. Strike were planted in 14 cm-d clean plastic pots, filled with an autoclaved mixture of sand and silt (1:1). Fifteen days after germination, seedlings were thinned to a uniformed one/pot, and inoculated with *M. javanica* at 5000 eggs in an aqueous suspension. Nematode eggs were pipetted into 4-5 holes (0.5 cm diameter x 4 cm deep) around plant roots. Inoculations with either entomopathogenic nematodes at 1000, 2000, 4000 and 8000 J_3 /pot were applied immediately after *M. javanica* inoculation in the same holes (Fallon et al., 2002; Pérez and Lewis, 2002). In another treatment, for comparison, granules of the nematicide, Cadusafos 10G (Rugby®) was applied at 0.1g/pot soon after *M. javanica* inoculation. Pots of the non-inoculated (healthy) and *M. javanica*-infected plants were used as checks. All treatments were replicated five times and arranged in a randomized complete design on a clean bench in the greenhouse ($27\pm 2^\circ\text{C}$). Plants were watered and fertilized as needed.

Fifty-five days after treatments, plants were removed from pots and roots were gently washed free of soil particles using tap water. Fresh weights of shoots and roots were determined. Roots of treated plants were stained with an aqueous solution of Phloxin B (0.15 g/l) to emphasize *M. javanica* egg-masses for counting (Holbrook et al., 1983). Counts of root galls and nematode egg-masses were determined and recorded. Final egg population (P_f) of *M. javanica*

was extracted using a sodium hypochlorite method, and counted using eelworm counting slide and recorded (Hussey and Barker, 1973). Then, *M. javanica* reproduction factor (R_f) was calculated according to the formula ($R_f = P_f/P_i$), given by Oostenbrink (1966), where P_i is the initial egg population (5000 eggs).

Statistical Analysis:

Row data including numbers of root galls and nematode egg-masses, R_f values, and fresh weights of shoots and roots of the experimental plants, were subjected to the analysis of variance (ANOVA). Relative efficacy of all treatments comparing to Cadusafos 10G were calculated, and regression models of the effects of different application rates of Entonem and Larvanem on *M. javanica* suppression, were created using SAS software (SAS Institute, 1989).

RESULTS AND DISCUSSION

All tested application rates of Entonem and Larvanem, used in this study, significantly ($p < 0.05$) decreased number of root galls (29.9-62.1%) and egg-masses (29.4-62.5%) and R_f values (12.5-59.5%) of *M. javanica* on common beans cv. Strike (Table 1). It was previously observed that addition of EPNs of the species of *Steinernema* and *Heterorhabditis* to the soil, significantly suppressed the root-knot nematode, *M. javanica* on soybeans (Fallon et al., 2002), and tomatoes (Bird and Bird, 1986; Fallon et al., 2002), *M. hapla* on peanuts (Pérez and Lewis, 2004) and *M. incognita* on cucumbers (Ishibashi and Matsunaga, 1995) and tomatoes (Ishibashi and Choi, 1991; Lewis et al., 2001; Pérez and Lewis 2002 and 2004). On the other hand, application of Cadusafos 10G at 0.1g/pot provided the highest reduction of root galls (97.7%), egg-masses (98.1%) and R_f (97.9%) of *M. javanica* on common beans cv. Strike (Table 1).

It was found that increasing the application rates of Entonem and Larvanem significantly enhanced *M. javanica* suppression on treated common beans (Tables 2-3). This finding is in agreement with results of Pérez and Lewis (2002) showed that increasing the rates of *Heterorhabditis bacteriophora* and *Steinernema feltiae* from 25 to 125 infective third stage juveniles/cm² had improved *M. incognita* suppression on greenhouse tomatoes.

Table 1. Effects of Entonem, Larvanem, and Cadusafos 10G on number of root galls, egg masses, and reproduction factors of *Meloidogyne javanica* (Mj) on common beans cv. Strike, 55 days after inoculation

Treatment	No. /plant		R _f *	% Reduction		
	Root galls	Egg-masses		Root galls	Egg-masses	R _f
Nematode only (Mj)	783.6 a	757.4 a	108 a	—	—	—
Mj + Cadusafos 10G	18.0 h	14.4 h	2.3 f	97.7	98.1	97.9
Mj + Entonem	1000J ₃ /pot	549 b	535 b	94.5 b	29.9	12.5
	2000J ₃ /pot	495 bc	483 bc	84.1 bc	36.8	22.1
	4000J ₃ /pot	457.4 cd	435.2 cd	74.6 c	41.6	30.9
	8000J ₃ /pot	391.4 ef	370.2 ef	55.4 d	50.1	48.7
Mj + Larvanem	1000J ₃ /pot	427 de	406 de	78.7 c	45.5	27.1
	2000J ₃ /pot	352.8 f	330.6 fg	59.9 d	55.0	44.5
	4000J ₃ /pot	345.6 fg	326.6 fg	51.8 de	55.9	52.0
	8000J ₃ /pot	296.8 g	284 g	43.7 e	62.1	59.5

- Values are means of 5 replicates of one plant/pot for each treatment.
 - Means within a column followed by the same alphabetical letter(s) are not significantly different (P<0.05) according to Fisher's protected LSD.
 * R_f= Reproduction factor.

Mechanisms of suppression of plant-parasitic nematodes by EPNs were discussed by some authors (Hu *et al.*, 1996 and 1999; Grewal *et al.*, 1999). The nematicidal effects of allelochemicals produced by various symbiotic bacteria associated with EPNs, such as ammonia (Grewal *et al.*, 1999; Lewis *et al.*, 2001), 3,5 Dihydroxy-4-isopropylstilbene and indole metabolites (Hu *et al.*, 1996 and 1999), could be the causal agents or contributing factors in nematode suppression.

Table 2. Statistical analysis and regression models of the effects of different application rates of Entonem (Em) on *M. javanica* on common beans cv. Strike

Parameter	Regression equations	R ²	F value	T ratios
Root galls (Gs)	logGs=3.216-0.158 logEm	0.75**	55.2	(43.59)**(-7.43)**
Egg-masses (Es)	logEs=3.229-0.168 logEm	0.81**	76.57	(48.46)**(-8.75)**
Reproduction factor (R _f)	logR _f =2.695-0.237 logEm	0.74**	51.79	(23.55)**(-7.20)**

-Values were log transformed prior to statistical analysis.
 R²= Determination coefficient.
 ** significant at P<0.01.

Table 3. Statistical analysis and regression models of the effects of different application rates of Larvanem (Lm) on *Meloidogyne javanica* on common beans cv. Strike

Parameter	Regression equations	R ²	F value	T ratios
Root galls (Gs)	logGs=3.116-0.166 logLm	0.55**	22.31	(25.64)**(-4.72)**
Egg-masses (Es)	logEs=3.079-0.162 logLm	0.52**	19.23	(24.09)**(-4.39)**
Reproduction factor (R _f)	logR _f =2.712-0.277 logLm	0.84**	95.44	(27.57)**(-9.77)**

-Values were log transformed prior to statistical analysis.
 R²= Determination coefficient.
 ** significant at P<0.01.

Regarding to plant performance of the treated common beans, it was clear that application of either Entonem or Larvanem at their tested rates, almost had no effects on improving visual growth parameters and pod weights of treated plants as compared to *M. javanica*-infected ones (Table 4). Findings of Fallon et al., (2002) supported these results. They reported that *Steinernema* spp. did not affect the growth or development of *M. javanica*-infected tomatoes. Also, *Steinernema riobrave* and *H. bacteriophora* did not

improve dry root and shoot weights and shoot lengths of Nemaguard peach trees infected by *Mesocriconema xenoplax* (Nyczepir et al., 2004). Moreover, results of Crow et al., (2006) confirmed that although EPNs may occasionally reduce population densities of plant-parasitic nematodes on turfgrass in Florida, they are so inconsistent in their results, and they could not be considered as an alternative management tactic as compared to the nematicide, fenamiphos treatment.

Table 4. Effects of Entonem, Larvanem, and Cadusafos 10G on visual growth parameters and pod weights of common bean cv. Strike, 55 days after *M. javanica* inoculation

Treatment	Shoot fresh weight (g)	Root fresh weight (g)	Pods weight (g)
Healthy plants (Check)	10.52 a	7.20 ab	4.66 a
Nematode only (<i>Mj</i>)	6.08 b	4.48 d	2.50 b
<i>Mj</i> + Cadusafos 10G	11.34 a	7.80 a	4.90 a
<i>Mj</i> + Entonem	1000J ₃ /pot	6.48 b	5.58 cd
	2000J ₃ /pot	6.64 b	5.60 cd
	4000J ₃ /pot	6.62 b	5.62 cd
	8000J ₃ /pot	6.70 b	5.84 c
<i>Mj</i> + Larvanem	1000J ₃ /pot	7.16 b	5.52 cd
	2000J ₃ /pot	7.02 b	5.48 cd
	4000J ₃ /pot	7.40 b	6.10 bc
	8000J ₃ /pot	7.62 b	6.18 bc

- Values are means of 5 replicates of one plant/pot for each treatment.

- Means within a column followed by the same alphabetical letter(s) are not significantly different (P<0.05) according to Fisher's protected LSD.

Eventually, the present results show that while Entonem and Larvanem relatively suppressed *M. javanica* on common beans cv. Strike, they did not improve visual growth parameters and pod weights of the infected plants, whereas the nematicide Cadusafos 10G provided the maximum *M. javanica* reduction and the best plant performance. Therefore, it can be concluded that Entonem and Larvanem as biocontrol agents are not acceptable alternatives to Cadusafos 10G in controlling *M. javanica* on common bean cv. Strike. However, further trials are needed to study the efficacy of these products on other plant nematodes and other economically important crops, as well to improve their potentials under different conditions of Saudi agriculture.

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المخلص العربي

جدوى استخدام مركبين حيويين تجاريين للنيماتودا المتطفلة على الحشرات ، مقارنة مع المبيد النيماتودي كاديوسافوس ، في مكافحة نيماتودا تعقد الجذور *Meloidogyne javanica* على الفاصوليا في المملكة العربية السعودية

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اختبرت كفاءة مركبين حيويين تجاريين للنيماتودا المتطفلة على الحشرات هما مركب انتونيم (Entonem) نيماتودا (*Steinernema feltiae*) ، ومركب لارفانيم (Larvanem) نيماتودا (*Heterorhabditis bacteriophora*) ، كمركبات مكافحة حيوية مقارنة مع المبيد النيماتودي كاديوسافوس ١٠٪ (الرجبي) ، لمكافحة نيماتودا تعقد الجذور *Meloidogyne javanica* على نباتات الفاصوليا صنف Strike تحت ظروف البيت المحمي (٢٧±٢م°) . وقد استخدمت أربع معدلات من كلا المركبين (١٠٠٠ ، ٢٠٠٠ ، ٤٠٠٠ ، ٨٠٠٠ يرقة طور ثالث للنيماتودا المتطفلة على الحشرات/اصيص) ، بينما استخدم مبيد الراجبي ١٠٪ بمعدل ٠.١ جم/اصيص ، وقد أجريت كل المعاملات مباشرة عقب عدوى نباتات الفاصوليا بالنيماتودا بمعدل *M. javanica* ٥٠٠٠ بيضة/اصيص) . تم تخصيص معاملتين أخيرتين للمقارنة ، إحداهما لنباتات سليمة والأخرى لنباتات مصابة بالنيماتودا فقط ، وتم تكرار المعاملات خمس مرات ، ورتبت الأصص داخل البيت المحمي تبعاً للتصميم العشوائي الكامل ، وأخذت النتائج بعد مرور ٥٥ يوم من العدوى .

أدت المعاملة بكلا المركبين إلى خفض معنوي في أعداد العقد الجذرية بنسبة تراوحت بين ٢٩,٩-٦٢,١٪ ، وأعداد أكياس البيض بنسبة ٢٩,٤-٦٢,٥٪ ، وقيم عامل تكاثر النيماتودا R_r بنسبة ١٢,٥-٥٩,٥٪ ، على نباتات الفاصوليا المعاملة . وقد لوحظ أن زيادة معدل الاستخدام لأي من المركبين قد حسنت معنوياً من تثبيط نيماتودا *M. javanica* . ولسوء الحظ لم تحسن المعاملة بأي من المركبين - غالباً - من مقاييس النمو وأوزان القرون لنباتات الفاصوليا المعاملة مقارنة بالنباتات الأخرى المعاملة بالنيماتودا فقط .

من ناحية أخرى أدت المعاملة بالمبيد النيماتودي كاديوسافوس إلى أعلى تثبيط للنيماتودا *M. javanica* بنسبة ٩٧,٧ ، ٩٨,١ ، ٩٧,٩٪ لكل من أعداد العقد الجذرية ، أعداد أكياس البيض ، وقيم عامل التكاثر R_r ، على التوالي. كما أدت هذه المعاملة إلى تحسن معنوي واضح في مقاييس النمو وأوزان القرون للنباتات المعاملة .

وعلى ذلك ، فإنه لا يمكن قبول هذين المركبين الحيويين كبدايل جيدة للمبيد النيماتودي كاديوسافوس ، في مكافحة نيماتودا تعقد الجذور *M. javanica* على نباتات الفاصوليا في المملكة العربية السعودية.