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Persistence of *Steinernema carpocapsae* (Nematoda: Steinernematidae) and *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) in Soil around Date Palm Trunks and their Effect on Adults of *Rhynchophorus ferrugineus*.

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ABSTRACT

This work has been conducted through a project for biological control of the red palm weevil in Arab Gulf Countries sponsored by Arab Organization for Agricultural Development. *Steinernema carpocapsae* and/or *Beauveria bassiana* were applied in soil around trunks of date palm trees. Successive soil samples were tested for persistence of nematodes and fungi and their role in controlling adults of the red palm weevil, *Rhynchophorus ferrugineus* (RPW) *S. carpocapsae* caused 100% mortality in RPW adults at the first day however, mortality gradually decreased to 6.7% after 16 days. The nematodes survived in the field for longer than that but in lower population density as detected by larvae of *Galleria mellonella. B.bassiana* caused 66.7% mortality at the first day then mortality decreased to 11.1% after 4days. The fungus was detected in the field after that with larvae of *G.mellonella*. The mixture of the nematode and the fungus caused 100% mortality in the first day then mortality decreased gradually to 22.2% after 8days. Results support the possibility of using the nematode and the fungus in management programs of RPW.

Key Words: Entomopathogen, *Steinernema carpocapsae, Beauveria bassiana*, biological control, *Rhynchophorus ferrugineus.*

INTRODUCTION

Entomopathogenic nematodes in genera *Heterorhabditis* and *Steinernema* showed high virulence against the red palm weevil *R. ferrugineus* in the laboratory and the field (Shamseldean and Abdel Gawad 1994, Abbas and Hanounik 1999, Hanounik et al 2000, Abbas et al 2000, Shamseldean 2002 and Saleh and Alheji 2003). The entomopathogenic fungus *Beauveria bassiana* was sprayed on caged date palm trees artificially infested with adults of *R. ferrugineus*. The fungus induced 92.8% mortality in these weevils (Hanounik *et. al.*2000). *B. bassiana* has been successfully used as a bio-control agent for the management of a number of coleopteran insects including Colorado potato beetle, *Leptinotarsa decemlineata* (Miranpuri *et al.*, 1992a), blister beetle, *Lytta nuttalii* (Miranpuri *et al.*, 1994) and flea beetle, *Phyllotreta cruciferae* (Miranpuri *et. al.*, 1992b). Interactions between the nematode *Steinernema carpocapsae* and the fungus *B. bassiana* resulted in greater control in soil born larvae of *Spodoptera exigua* than application of nematode alone (Barbercheck and Kaya 1991).

Females of the red palm weevil lay eggs in leaf bases or directly into the woods of host palms. Immature stages develop in the crown and stem region where conditions are moist. Adult activities occur outside the palm but weevils are crypto and seek harborage (Kalshoven 1950, Sadakathulla 1991). In Arabic Gulf region adults of *R. ferrugineus* were frequently observed aggregating on the basal buried part of the trunk in the soil and among leaf axils for feeding, mating and egg lying. These adults used to spend time in wet soil around date palm trunks escaping high air temperature (Hanounik *et al.* 2000a). Abbas *et al.*, (2000) reported that adults of *R. ferrugineus* occasionally inhabit soil so that 40% of its population in United Arab Emirates was associated by free living nematodes. A considerable portion of (35%) of *R. ferrugineus* infestation in date palm trees in eastern region in Saudi Arabia were found at the soil surface or below (unpublished data). Application of *S. carpocapsae* and *H. bacteriophora* in soil under date palm trees artificially infested with adults of *R. ferrugineus* caused 77.5 and 17.5% mortality in the pest adults within 10 days (Saleh and Alheji 2003). Abbas *et al.* (2000) obtained 100% mortality in *R. ferrugineus* adults in soil using *S. riobravae*.

This work aimed to assess persistence of a nematode (*S. carpocapsae*) and a fungus (*B. bassiana*) and their role in controlling adults of *R. ferrugineus* in soil around date palm trees.

MATERIALS AND METHODS

The nematode

S. carpocapsae S2 (Shamseldean et al 1996) was maintained on wax moth *Galleria mellonella* full grown larvae as descriped by Woodring and Kaya 1988.

The fungus

An oil formulation of local strain (BSA3)of *B. bassiana* from Saudi Arabia was prepared at a concentration of 5 $X10^9$ conidia/ml as descriped by Goettel and Inglis (1994).

The insects

Adults of *R. ferrugineus* were collected from pheromone-kairomone traps (Hanounik *et al.*2000b) distributed in date palm farms in Al Qatif region, Saudi Arabia and maintained in the laboratory in the same region on logs of date palm wood until use.

The experiments

Date palm *Phoenix dactylifera* L. variety "Ekhlas" 7 years old trees at the experimental station of The Agricultural Development Project at Al Qatif, Eastern Province, Saudi Arabia were Choasen for these studies. The trees were treated with the nematode *S.carpocapsae*, the fungus *B. bassiana* or a mixture of both in order to assess the persistance of the nematode, the fungus or the mixture of both and their role in controlling adults of *R. ferrugineus* in soil. The dose of *S.carpocapsae* was $2X10^6$ infective juviniles (IJs) /5L/tree. The dose of *B. bassiana* was 100 ml of oil formulation/5L/tree (1ml of oil formulation contained $5X10^9$ conidia). The dose of the mixture was half of the dose of both the nematode and the fungus. Treatments were appllied to the crown of the tree and the soil around the trunk using a watering cane. Each treatment replicated three times. A date palm tree represented a replicate. Samples of treated soil (1kg/sample) were taken at 0,1,2,4,8 and 16 days after treatment as descriped by Akhurst and Bedding (1975). Each sample was divided into two subsamples each of which was kept in a plastic container (10cm diameter X 6cm high). One subsample received 3 weevils of *R. ferrugineus* and the other received 5 larvae of *G. mellonella*. All experimental containers were kept in 25 °C for a week and mortality of insects was calculated for different treatments. Insect cadavers were transferred to White traps (White 1927) and nematode or fungus infections were recorded.

The combined effect of the nematode and the fungus in their mixture was assessed as coeffiency factor (CF) according to the equation of Mansour *et al.* (1966).

$$CF = \frac{\% \text{ observed} - \% \text{ expected}}{\% \text{ expected}} \times 100$$

Positive factors of 20 or more indicate synergism, negative factors of 20 or less indicate antagonism while intermediate values indicate additive effect.

RESULTS AND DISCUSSION

At the beginning of application of the nematode, *S. carpocapsae*, the fungus *B. bassiana* and the mixture of both to soil under date palm trees, mortality percentages in adults of the red palm weevil *R. ferrugineus* exposed to treated soil were 100, 66.7 and 100% ,respectively (Table 1). After two days in the field, mortality due to the nematode, the fungus and the mixture were 75, 44.4 and 66.7, respectively. By time, mortality gradually decreased but in different rates according to persistence ability of the applied biocontrol agent. The effect of the nematode, *S. carpocapsae* lasted on red palm weevil in the field for 16 days inducing minimum mortality of 6.7%. However, the fungus *B. bassiana* caused its minimum mortality in the red palm weevils (11%) after four days in the field. The effect of the mixture of the nematode and the fungus lasted for 8 days in the field when caused a minimum mortality of 22.2% in the exposed weevils.

The nematode alone (full dose) achieved better results than the fungus alone (full dose) or the nematode-fungus mixture (half dose of the nematode + half dose of the fungus). However, additive values of co efficiency factor (CF) of nematode-fungus mixtures between 10.46 and -8.5 showed no antagonism between the nematode and the fungus in controlling the red palm weevil in the field. Barbercheck and Kaya (1991) reported that they obtained greater control of *Spodoptera exigua* in the soil from *S. carpocapsae- B. bassiana* mixture than that obtained from the nematode alone. These results indicate that the nematode *S. carpocapsae* and the fungus *B. bassiana* could be used in the field with no contradiction.

Although the effect of all treatments against the red palm weevils came to an end after 16 days, there was still a high rate of mortality (68.8-100%) in larvae of *G. mellonella* (Table 2). It is expected therefore that the nematode or the fungus can persist in lower populations in the soil after termination of their effect on adults of the red palm weevils. That indicated that augmentative applications of nematodes or fungi in the

Table (1): Mortality in adults of *Rhynchophorus ferrugineus* in soil under date palm trees after treatments with the nematode *Steinernema carpocapsae*, the fungus *Beauveria bassiana* or combination of both and the values of co-efficiency factor.

	% Mortality after (days)					
	0	1	2	4	8	16
F	66.7	44.4	22.2	11	0	0
N	100	75	62.5	56.3	31.3	6.25
F+N	100	66.7	55.5	22.2	22.2	0
CF	4.99	2.93	7.76	-8.5	10.46	-

Table (2): Mortality in larvae of *Galleria mellonella* in soil under date palm trees after treatments with the nematode *Steinernema carpocapsae*, the fungus *Beauveria bassiana* or combination of both and the values of co-efficiency factor.

	% Mortality after (days)					
	0	1	2	4	8	16
F	100	100	93.3	100	93.3	75
N	100	87.5	81.3	93.3	81.3	68.8
F+N	100	100	100	100	93.3	93.3
CF	0	1.67	3.64	0.8	1.72	7.44

Table (3): Development of the nematode *Steienernema carpocapsae* (N) and the fungus *Beauveria bassina* (F) in cadavers of adults of *Rhynchophorus ferrugineus* in soil under date palm trees after treatment of nematode-fungus mixtures.

Days after treatment	% Mortality	%Development	
		N	F
0	100	66.5	55.5
1	66.7	44.4	22.2
2	55.6	44.4	11.1
4	22.2	22.2	11.1
8	22.2	22.2	22.2

Table (4): Development of the nematode *Steinernema carpocapsae* (N) and the fungus *Beauveria bassiana* (F) in cadavers of larvae of *Galleria mellonella* in soil under date palm trees after treatment of nematode-fungus mixtures.

Days after treatment	% Mortality	% Development	
		N	F
0	100	100	100
1	100	73.3	100
2	100	100	100
4	100	26.7	66.7
8	93.3	6.67	86.7

soil may add to management of the red palm weevils in date palm orchards. The additive values of CF add evidence of the possibility of using the nematode *S. carpocapsae* and the fungus *B. bassiana* in IPM programs.

Studying the development of the nematode *S. carpocapsae* and the fungus *B. bassiana* in cadavers of the red palm weevil *R. ferrugineus* after treatment of nematode-fungus mixtures showed that the nematode and the fungus could develop simultaneously in the same cadaver (Table 3). In most treatments nematode development was relatively higher than fungus development. At the beginning of application, when the mortality in red palm weevils was 100%, 66.5% of the weevils produced nematodes and 55.5% of them showed fungal development. After 8 days when the mortality was 22.2%, Nematode and fungus developed together in all cadavers.

Development of the nematode and the fungus in cadavers of *G. mellonella* treated with nematode-fungus mixture showed greater ability of sharing the same host (Table 4). Almost all infected cadavers produced nematode juveniles and fungal conidia at the beginning of application of their mixture in the field. After 4 days the fungus showed higher rates of development than the nematode did in cadavers of the wax moth. Results of development experiments showed that after treatment with the nematode-fungus mixtures, the nematode could develop better in cadavers of the red palm weevils while the fungus could develop better in cadavers of the wax moth larvae.

The two pathogens developed together in the same cadaver because they had different target tissues. The main target tissue of the nematodes is the haemolymph (Kaya and Gaugler 1993) while the main target tissue of the fungus is the fat body (Lacey and Brooks 1994).

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مدة بقاء النيماتودا شتينرنيما كاربوكابسا والفطر بوفيرياباسيانا في التربة حول جذوع النخيل ودور ذلك في مكافحة الحشرات الكاملة لسوسة النخيل الحمراء

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أجري هذا البحث ضمن إطار مشروع المكافحة الحيوية لسوسة النخيل الحمراء بدول مجلس التعاون الخليجي أحد مشروعات المنظمة العربية للتنمية الزراعية. تمت معاملة التربة حول جذوع النخيل بمنطقة القطيف بالمملكة العربية السعودية بالنيماتودا شتينرنيما كاربوكابسا أو الفطر بوفيريا باسيانا أو بهما معا وأخذت عينات متعاقبة من التربة لاختبار مدة بقاء النيماتودا والفطر في التربة ودور ذلك في مكافحة الحشرات الكاملة لسوسة النخيل الحمراء ووجد أن النيماتودا تقتل ١٠٠% من الحشرات الكاملة لسوسة النخيل الحمراء في اليوم الأول للمعاملة وأن نسبة القتل تنخفض مع استمرار مدة بقاء النيماتودا بالحقل حتى تصل إلى 6.7 % بعد ١٦ يوما في الحقل وأن الفطر يقتل الحشرات الكاملة لسوسة النخيل الحمراء بنسبة 66.7 % ثم تنخفض مع بقاء الفطر بالحقل إلى ١١% في اليوم الرابع. أما مخلوط النيماتودا والفطر فقد أحدث نسبة موت ١٠٠% في الحشرات الكاملة لسوسة النخيل في بدء المعاملة ثم انخفض التأثير حتى وصل ٢٢،٢% في اليوم الثامن. تم رصد النيماتودا والفطر بالتربة بعد تضاؤل تأثيرهما على سوسة النخيل الحمراء. وقد أثبتت التجارب أنه لا تعارض بين استخدام النيماتودا والفطر الممرض للحشرات لمكافحة الحشرات الكاملة لسوسة النخيل في التربة.