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Increasing the Efficacy of *Metarhizium anisopliae* var. *acridum* (Metchnikoff) Soroken and *Beauveria bassiana* (Bals.) Vuill. Using Certain Essential Oils against Desert Locust and Grasshoppers

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ABSTRACT

Three plant essential oils namely parsley, cumin and onion seed oil were tasted at three concentrations (0.1, 0.5 and 1 % V/V) in combination with the entomopathoginic fungi *Metarhizium anisopliae* var. *acridum* and *Beauveria bassiana* in laboratory against *Schistocerca gregaria* (Forskal) and *Euprepocnemis plorans* (Charpentier). Parsley oil was used at the concentration (0.1%) in combination with the two fungi at rate of 25 g spores/ha, as well as the two fungi separately at rates of 50 and 25 g spores/ha against common grasshoppers at Baharia Oasis. Laboratory results showed that parsley and cumin oils showed better effects for *M. anisopliae* var. *acridum* and *B. bassiana*, while onion oil caused antagonism. In the field, using Parsley oil at the concentration 0.1% in combination with the two fungi, better control was marked.

Key words: *Schistocerca gregaria, Euprepocnemis plorans,* Parsley, Cumin, Onion, *Metarhizium anisopliae, Beauveria bassiana.*

INTRODUCTION

Interest of using pathogens as biological control agents against locust and grasshoppers has grown since the last major locust plague of the 1980s. Millions litres of chemical pesticides were used to combat this plague, which led to an outcry among environmental groups (Greathead 1992a). The most extensively studied pathogens are the protozoa, *Nosema locustae* Canning and deuteromycete fungi such as *Metarhizium anisopliae*, *M. anisopliae* var. acridum (=*Metarhizium flavoviridae*) and *Beauveria bassiana* (Greathead 1992 b). The use of pathogens may offer an environmentally sound method for the management of grasshoppers and locusts, hyphomycete fungi are the most promising candidates (Perior and Greathead, 1989). Essential oils are volatile, natural, complex compounds characterized by a strong odour and are formed by plants as secondary metabolites. In nature, essential oils play an important role in the protection of the plants as antibacterials, antivirals, antifungals, insecticides and also against herbivorous by reducing their appetite for such plants. They also may attract some insects to favour the dispersion of pollens and seeds, or repel undesirable others (Bakkali *et al.* 2008). Unlike chemical pesticides, entomopathogens require time to induce their effect, so there is a great need to increase their efficacy after application. Onion, parsley and cumin seed oils showed acceptable toxicity level against desert locust *S. gregaria*, Onion oil caused potentate to Carbosulfan, Methomyl and Fenitrothion and antagonism to Fenvalerate when used as mixture against desert locust, while parsley oil caused potency to Methomyl and Fenitrothion and additive effect to Carbosulfan and Fenvalerate, in contrast cumin oil caused antagonistic effect to the four insecticides (Abd El-Hamid and Neama, 2006).

The present work aims to study the effect of onion, parsley and cumin oils at low concentrations (to avoid their antimicrobial effects) on the efficacy of two fungi; *M. anisopliae* var. *acridum* and *B. bassiana* against *Schistocerca gregaria* and *Eupropocnemis plorans* in the laboratory as well as against the common grasshoppers in in the field experimented at Baharia Oasis.

MATERIALS AND METHODS

Tested insects

Insects used in the laboratory bioassay were the 3rd nymphal instar of the locust, *S. gregaria* and the clover grasshopper, *E. plorans* (Orthoptera: Acrididae). The individuals were taken from stock culture maintained for several generations at Locust and Grasshopper Research Department, Plant Protection Research Institute, Agricultural Research Center (A.R.C.), Dokki, Giza, Egypt. The insects were reared and handled as described by Hunter-Jones (1966). The culture is usually fortified with some insects brought from the field every year.

Entomopathogenic fungi

The conidiospores of *M. anisopliae* var. *acridum* used in this study were from isolate IMI330189, kindly provided by (Biological Control Products), South Africa. The spores of *B. bassiana* were originally isolated from adult cadavers of the red palm weevil *Rhynchophorus ferrugineus* Oliv. collected from El-Kassasien area, Ismaalia

Governorate (Heikal, 2001), this isolate was propagated in locust and grasshoppers for several generations as following: an oil formulation of *B. bassiana* spores was used to infect both nymphs of the desert locust, *S. gregaria* and the grasshopper, *E. plorans*. The infected nymphs were kept under 31° C and observed for mortality. Cadavers were removed, sterilized according to (Lacey and Brooks 1997) and kept in Petri dishes to dry for 24 h. Then each cadaver was placed in a Petri dish provided with moistened cotton pieces. Petri dishes were incubated at 27° C and observed for the fungus growth. The conidiospores grown on the cadavers were collected and suspended in 1ml. of sterile sunflower oil for 24 hours, and used to contaminate 10 Petri dishes contained selective media, consists of 0.36 g. KH_2PO_4, 1.42 g. Na_2HPO_4 $12H_2O$, 0.62 g. $MgSO_4$ $7H_2O$, 1 g. KCL, 0.70 g. NH_4NO_3, 10 g. maltose, 5 g. yeast extract, 18 g. agar-agar and 0.5 g. chloramphenicol per 1000 ml distilled water. The Petri dishes were incubated at 27° C for 21 days. Then *B. bassiana* spores were collected by using small brush.

Essential oils

Seed oils of parsley (*Petroselinum sativum*), cumin (*Cuminum cyminum*) and onion (*Allium cepa*) were obtained from the products commercially produced by (CAP PHARM, El-Obour City, Cairo, Egypt)

Laboratory bioassay

M. anisopliae var. *acridum* and *B. bassiana* were used at a dose of $10^{3,}$ spores/nymph against *S. gregaria* and *E. plorans* 3rd instar nymphs. Sixty insects were used for each treatment, divided into 4 groups in glass cylinder ø 15 cm. in diameter covered with a piece of white light cloth. The conidiospores of both fungi at rates of 10^6 spores/ml were suspended in sunflower oil or in mixture of parsley or cumin or onion oils at 3 different concentrations, which were 0.1, 0.5, 1.0 % (v/v in sunflower oil); 1µl of each treatment, were placed by using micro pipette under the pronotum of tested insects (according to Prior *et al.*, 1995); while the oils were used alone against desert locust and clover grasshopper, another sixty insects of both test species were treated with sunflower oil only as control. Morality among treated nymphs were observed daily and recorded. The mortality was corrected according to Abbott (1925). The combined action of the mixtures was calculated in term of co-toxicity factor according to the equation given by Mansour *et al.* (1966):

$$\text{Co-toxicity factor} = \frac{\text{Observed \% mortality} - \text{Expected \% mortality}}{\text{Expected \% mortality}} \times 100$$

where:

Observed % mortality: the mortality percentage among treated insects with fungi suspended in oil mixtures.

Expected % mortality: the sum of mortality percentage among treated insects with fungi suspended in sunflower oil and among treated insects with each oil mixture alone.

This factor was used to differentiate the results into three categories:

1. Potency (a positive factor of + 20 or more)
2. Antagonism (a negative factor of - 20 or more)
3. Additive (an intermediate values *i.e.* between +20 and – 20)

Effect of the essential oils on the spores' viability

To determine the effect of the used essential oils on the viability of the two fungi conidiospores, series of dilutions were prepared by using the pre-mentioned essential oils at the used concentrations under sterile conditions, then 100 micro litter of each dilution were spread in Petri dish containing thin layer of the previous selective media, 3 Petri dishes of each dilution were examined using light microscope for spore germination after 24 hours at 27° C. The viability was calculated as percentage of germination by examination of 100 spores, this was repeated three times in each Petri dish.

Field trials

Field trials were conducted at Baharia Oasis against grasshoppers. The most dominant species of grasshoppers were *Aiolopus strepens* (Latreille) and *Acrotylus insubricus* (Scopoli) (Orthoptera: Acrididae), the percentages of occurrence of each species were 51.4 and 33.6 % respectively, while the grasshopper species *Chrotogonus homalodemus* (Blanchard)(Ortoptera: Pyrgomorphidae), *Heteracris annulosa* Walker and *Truxalis nasuta* (Linnaeus) (Orthoptera: Acrididae) slightly occurred, the grasshoppers were in different stages (3rd, 4th, 5th nymphal instars and adults) and the mean number of grasshoppers individuals/ m^2 was 12.59. The two fungi were applied at the rates of 50, 25 g spores/ha, suspended in sunflower oil, 25 g spores/ha diluted in mixture of sunflower oil + parsley oil at concentration of 0.1 % (v/v) and sunflower oil alone (control treatment). Each treatment was applied into 50 X 50 meter plots, cultivated with Alfalfa (*Medicage sativum*) in sandy soil by using hand-held spinning disc sprayer (Micron Ulva +), the final volume of oil used, was at the rate of 2 liter/ha. Then, 100 grasshoppers collected immediately from the centre of each plot area, divided into 4 groups in a plastic cage (bottomless 2 liter plastic jar covered with white long tube of white textile). The cages were kept under field conditions and the insects were fed daily with Alfalfa from the relevant plot area. Mortality was recorded till the 17th day post treatment.

RESULTS AND DISCUSSION

Laboratory bioassay

Data in table (1) demonstrate the effect of mixing the tested entomopathogens with parsley, cumin and onion oils at the tested concentrations against *S. gregaria* after 5, 10 and 15 days post treatment. It's clear that parsley oil caused the greatest increase in the efficacy of the two fungi, where the increase of the co-toxicity of *M. anisopliae* var. *acridum* was obtained when mixed with parsley oil at the concentration 0.1% (40 % increase in the efficacy of *Metarnizium* after 5 days post infection). At the same line, parsley oil caused increase in the efficacy of *B. bassiana*, where co-toxicity of *Beauveria*, mixed with parsley oil at the concentration of 0.1% was equal to 25%, such increase in the efficacy decreased by the increase of the concentration. Onion oil caused decrease in the efficacy of the two fungi and the amount of decrease increased by concentration increase. The cumin oil caused increase to the efficacy of the two fungi but less than parsley oil in most cases. It's clear that the most effective treatment was parsley at the concentration of 0.1 % + *Metarhizium*. It could be concluded that parsley and cumin oils caused potency effects to *Metarhizium* and *Beauveria* by the 5th day post treatment at all used concentrations except in case of *Metarhizium* with cumin oil at the concentration of 1%. It was only positive additive effect. The onion oil caused negative effect to both fungi ranged between -5.41 and -57.14%.

Table (2) illustrates the effect of mixing the two fungi with the essential oils used in the present study at the concentrations of 0.1, 0.5 and 1 % (v/v), after 5, 10 and 15 days post treatment on the grasshopper *E. plorans*. The same trend resulted as the results presented in table (1). The greatest co-toxicity 28.57% was obtained by mixing *Metarhizium* with 0.1% parsley oil. It could be noticed that parsley caused potency effects to both fungi, only at concentration 0.1% while at the other concentrations as well as cumin all concentrations caused positive additive effects. Also, in case of grasshopper, *E. plorans* the onion oil caused negative effects to both fungi ranged between -5.71 and -56-67 %.

Spores viability

Figure (1) shows the effect of used essential oils on the spore viability (as percent of spores' germination) for the two fungi after mixing with the used essential oils at the tested three concentrations. It's obvious that the parsley oil at the concentration 0.1% showed lowest effect on the spores' germination of *M. anisopliae* var. *acridum* and *B.*

Table (1): Effect of mixing *Metarhizium anisopliae* var. *acridum* and *Beauveria bassiana* with parsley, cumin and onion oils at the concentrations (0.1, 0.5 and 1% v/v) on *Schistocerca gregaria* at 5, 10 and 15 days post treatment.

Fungi + plant essential oil	0.1%			0.5%			1%		
	expected [a]	observed [b]	Co-toxicity	expected [a]	observed [b]	Co-toxicity	expected [a]	observed [b]	Co-toxicity
					5 days				
Metarhizium anisopliae + parsley	8.33	11.67	40.00	10.00	13.33	33.33	11.67	15.00	28.57
Metarhizium anisopliae + cumin	6.67	8.33	25.00	8.33	10.00	20.00	10.00	11.67	16.67
Metarhizium anisopliae + onion	10.00	6.67	-33.33	11.67	6.67	-42.86	15.00	8.33	-44.44
Beauveria bassiana + parsley	6.67	8.33	25.00	8.33	10.00	20.00	10.00	13.33	33.33
Beauveria bassiana + cumin	5.00	6.67	33.33	6.67	8.33	25.00	8.33	10.00	20.00
Beauveria bassiana + onion	8.33	6.67	-20.00	10.00	8.33	-16.67	13.33	10.00	-25.00
					10 days				
Metarhizium anisopliae + parsley	33.33	40.00	20.00	40.00	43.33	8.33	43.33	45.00	3.85
Metarhizium anisopliae + cumin	31.67	31.67	0.00	38.33	38.33	0.00	40.00	41.67	4.17
Metarhizium anisopliae + onion	36.67	26.67	-27.27	46.67	20.00	-57.14	50.00	25.00	-50.00
Beauveria bassiana + parsley	30.00	35.00	16.67	36.67	38.33	4.55	40.00	41.67	4.17
Beauveria bassiana + cumin	28.33	28.33	0.00	35.00	35.00	0.00	36.67	36.67	0.00
Beauveria bassiana + onion	33.33	28.33	-15.00	43.33	35.00	-19.23	46.67	38.33	-17.86
					15 days				
Metarhizium anisopliae + parsley	61.67	73.33	18.92	73.33	75.00	2.27	80.00	81.67	2.08
Metarhizium anisopliae + cumin	58.33	63.33	8.57	66.67	68.33	2.50	76.67	78.33	2.17
Metarhizium anisopliae + onion	68.33	50.00	-26.83	75.00	45.00	-40.00	83.33	50.00	-40.00
Beauveria bassiana + parsley	48.33	60.00	24.14	60.00	63.33	5.56	66.67	68.33	2.50
Beauveria bassiana + cumin	45.00	50.00	11.11	53.33	60.00	12.50	63.33	70.00	10.53
Beauveria bassiana + onion	55.00	51.67	-6.06	61.67	58.33	-5.41	70.00	65.00	-7.14

[a] expected = the sum of mortality percentage among treated insects with fungi suspended in sunflower oil and among treated insects with each oil mixture alone.

[b] observed = the mortality percentage among treated insects with fungi suspended in oil mixtures.

Table (2): Effect of mixing *Metarhizium anisopliae* var. *acridum* and *Beauveria bassiana* with parsley, cumin and onion oils at the concentrations (0.1, 0.5 and 1% v/v) on *Euprepocnemis plorans* at 5, 10 and 15 days post treatment.

Fungi + plant essential oil	0.1%			0.5%			1%		
	expected [a]	observed [b]	Co-toxicity	expected [a]	observed [b]	Co-toxicity	expected [a]	observed [b]	Co-toxicity
					5 days				
Metarhizium anisopliae + parsley	11.67	15.00	28.57	11.67	13.33	14.29	13.33	15.00	12.50
Metarhizium anisopliae + cumin	10.00	11.67	16.67	11.67	13.33	14.29	13.33	15.00	12.50
Metarhizium anisopliae + onion	13.33	8.33	-37.50	15.00	10.00	-33.33	18.33	13.33	-27.27
Beauveria bassiana + parsley	10.00	13.33	33.33	10.00	11.67	16.67	11.67	13.33	14.29
Beauveria bassiana + cumin	8.33	8.33	0.00	10.00	11.67	16.67	11.67	13.33	14.29
Beauveria bassiana + onion	11.67	10.00	-14.29	13.33	10.00	-25.00	16.67	11.67	-30.00
					10 days				
Metarhizium anisopliae + parsley	36.67	45.00	22.73	46.67	50.00	7.14	46.67	51.67	10.71
Metarhizium anisopliae + cumin	35.00	35.00	0.00	43.33	43.33	0.00	45.00	45.00	0.00
Metarhizium anisopliae + onion	40.00	28.33	-29.17	50.00	21.67	-56.67	53.33	28.33	-46.88
Beauveria bassiana + parsley	33.33	40.00	20.00	43.33	46.67	7.69	43.33	48.33	11.54
Beauveria bassiana + cumin	31.67	33.33	5.26	40.00	41.67	4.17	41.67	43.33	4.00
Beauveria bassiana + onion	36.67	31.67	-13.64	46.67	36.67	-21.43	50.00	40.00	-20.00
					15 days				
Metarhizium anisopliae + parsley	65.00	78.33	20.51	76.67	78.33	2.17	83.33	85.00	2.00
Metarhizium anisopliae + cumin	63.33	65.00	2.63	71.67	73.33	2.33	80.00	81.67	2.08
Metarhizium anisopliae + onion	71.67	51.67	-27.91	78.33	36.67	-53.19	86.67	40.00	-53.85
Beauveria bassiana + parsley	51.67	63.33	22.58	63.33	65.00	2.63	70.00	71.67	2.38
Beauveria bassiana + cumin	50.00	55.00	10.00	58.33	63.33	8.57	66.67	70.00	5.00
Beauveria bassiana + onion	58.33	55.00	-5.71	65.00	58.33	-10.26	73.33	66.67	-9.09

[a] expected = the sum of mortality percentage among treated insects with fungi suspended in sunflower oil and among treated insects with each oil mixture alone.

[b] observed = the mortality percentage among treated insects with fungi suspended in oil mixtures.

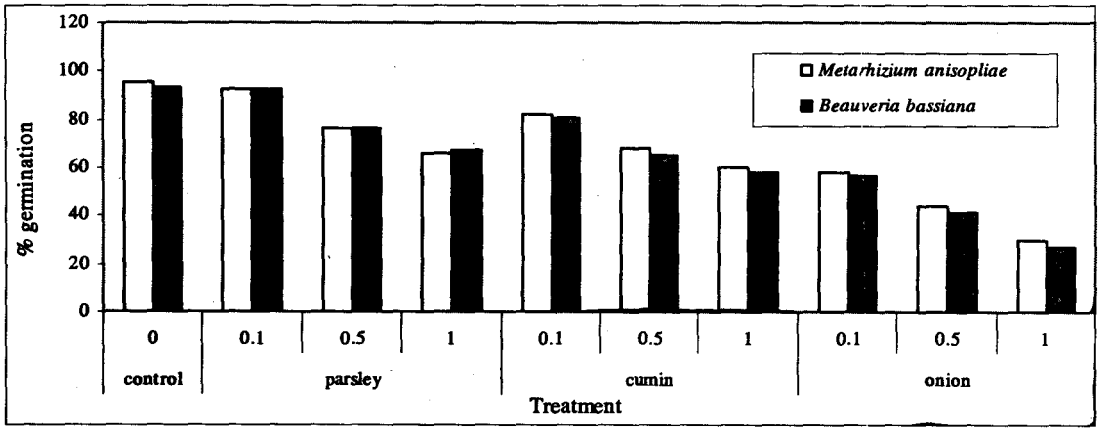

Figure (1): Effect of parsley, cumin and onion oils on the viability of *Metarhizium anisopliae* var. *acridum* and *Beauveria bassiana* spores.

bassiana, while the onion caused the highest effect. Also, it could be noticed that such effects on spores' germination increased as the dose increases.

Laboratory experiments showed that the onion oil had a strong effect to inhibition of spores germination of both fungi, which explain the antagonism effect of mixing the two fungi with onion oil. There are many reports about the antifungal effects of onion oil, for example Phay *et al.*, 1999 against several fungal species, as well as Irkin & Korukluoglu 2007 on *Aspergillus niger*. In the present study cumin oil had intermediate effect on the spores germination, such effect corresponded with its effect on activation of the tested fungi, the antifungal effects of cumin oil was reported by Lee *et al.*, 2007 against *Botrytis cinerea, Fusarium oxysporum, Pythium ultimum* and *Rhizoctonia solani* but not against *Colletotrichum gloeosporioides*. The same authors reported also that parsley oil did not affect these fungal species. In contrast to this finding in the present study, parsley oil showed slight effect on the germination of both tested fungi spores, such effect increased as the dose increases. According to these results, both fungal species were tested against local grasshoppers at Baharia Oasis at the recommended doses, half recommended dose suspended in sunflower oil and half recommended dose suspended in parsley oil (0.1% v/v in sunflower oil).

Field trials

Figures 2 and 3 show field trials results of *M. anisopliae* var. *acridum* and *B. bassiana* against

the common grasshoppers at Baharia Oasis. The two fungi were used at the recommended dose (50 g spores /ha.) and at half of the recommended the dose (25 g spores /ha.) suspended in sunflower oil, as well as at dose 25 g spores /ha. suspended in sunflower oil mixed with parsley oil at the concentration of 0.1 % (v/v). It could be noticed that using sunflower oil mixed with parsley oil increased the efficacy of *M. anisopliae* var. *acridum* and *B. bassiana* against the local grasshoppers in Baharia Oasis. This increase caused increase in the efficacy of *M. anisopliae* var. *acridum* at the half dose to be more than the recommended dose and accelerated the mortality as well. While in case of *B. bassiana,* the mixture caused increase at the efficacy of the half dose to be as the recommended dose.

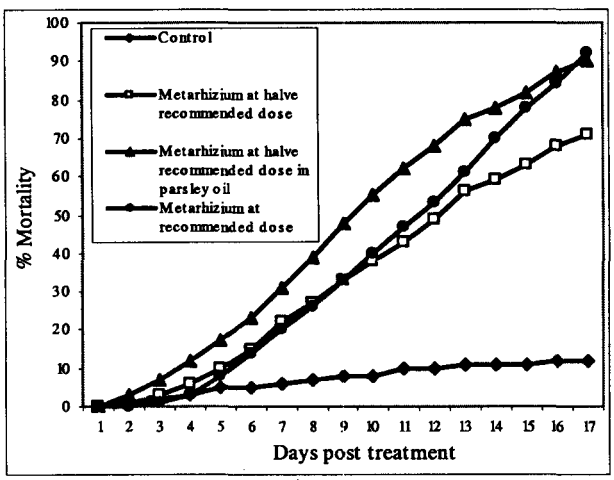

Figure (2): Effect of using *Metarhizium anisopliae* spores suspended in sunflower at the recommended dose and half recommended dose in sun flower oil and at half recommended dose in parsley oil against the grasshoppers in the field at Baharia Oasis.

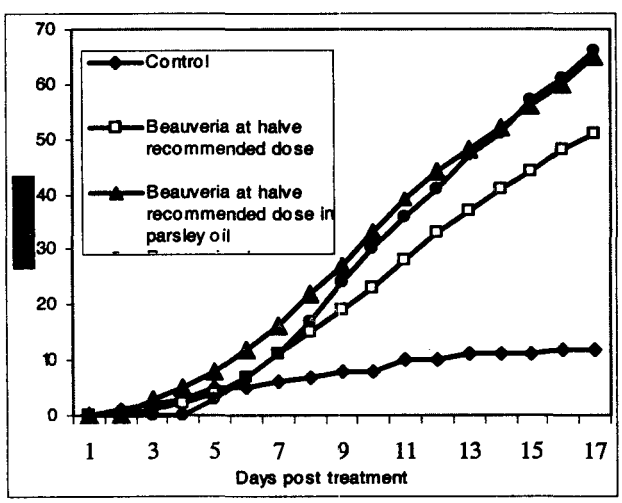

Figure (3): Effect of using *Beauveria bassiana* spores suspended in sunflower at the recommended dose and half recommended dose in sun flower oil and at half recommended dose in parsley oil against the grasshoppers in the field at Baharia Oasis.

In the field study, *M. anisopliae* var. *acridium* showed greater virulence in comparison with *B. bassiana,* where the percentages of mortality among treatments with *Metarhizium* ranged between 70-90% while in *Beauveria* treatments they were 50 to less than 70 %. This finding may be due to that *B. bassiana* required lower temperature to develop than that required by *M. anisopliae* var. *acridium* as reported by Rezk *et al.,* 2008 in their work about the effect of temperature on the efficacy of *M. anisopliae* var. *acridium* and *B. bassiana* against desert locust. Also, Prior and Greathead, (1989) reported that all fungi isolates that have been tested were from genus *Metarhizium* and had originated from Orthoptera were highly virulent to *S. gregaria.*

Many authors reported the toxicity of the essential oils which were used during the present study, onion oil against *Tetranychus urticae* (Barakat *et al,.* 1985), cumin oil against mosquito females (Mansour *et al.,* 1985) and against the desert locust (Abd El-Hamid 2006).

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