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Integrated Action Between Entomopathogenic Fungi and Some Essential Oils on The Grasshopper, *Chrotogonus homalodemus* (Blanchard) (Orthoptera: Pyrgomorphidae)

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Abstract: *Beauveria bassiana*, *Metarhizium anisopliae* in the form of bio-insecticides under experiment and two concentrations of five essential oils; parsley oil, onion oil, mustard oil, clove oil and Eucalyptus oil were applied sequentially on grasshopper, *Chrotogonus homalodemus* under field cage conditions to determine the interaction between these entomopathogenic fungi and the essential oils. The laboratory experiment determined the most suitable concentrations of essential oils for use in the field experiment. Two concentrations of five essential oils (0.5 and 1%) and a recommended concentration of the entomopathogenic fungi (1x 10^8 CFU's /gm) were sprayed on third, fourth and fifth nymphal instars of *C. homalodemus* in the field by knapsack motorized sprayer. Entomopathogenic fungi and essential oils were applied on grasshopper each alone then they were applied together. When grasshoppers were exposed to essential oils mixed with fungi all treatments demonstrated additive interaction after 15 days of application. LT_{50} was reduced in case of use of the entomopathogenic fungi mixed with essential oils. The additive interaction that was noted in our study between entomopathogenic fungi and the essential oils would lead us to optimize the mixture in the future to reach high levels of grasshoppers control, especially in agricultural crops.

Key words: *Chrotogonus homalodemus* · Entomopathogenic fungi · *Beauveria bassiana* · *Metarhizium anisopliae* and Essential oils

INTRODUCTION

Grasshoppers (Orthoptera: Acrididae: Caelifera) currently consist of 12023 valid species [1], where 108 species are recorded in Egypt from which, 93 (86%) belong to the family Acrididae [2]. The locust and grasshoppers cause significant damage to crops [3]. Grasshoppers have become critical pest in Egypt in particular inside the newly reclaimed regions [4]. The Berseem grasshopper, *Euprepocnemis plorans plorans* is the most economic species that brought about a severe harm. This species occasioned 95% harm to planted vegetation of the El-farafra Oases, El-Bahria Oases and Nile Delta [5].

Existing control techniques depend on chemical insecticides proved to be important to prevent damage to crops. The significant control technique embraced against the desert grasshopper depends on the usage of pesticides. The continuous utilization of pesticides towards pests has led dramatically to a resistance to the insecticides and a speedy increase of insect tolerance occurred against any type of neurotoxic insecticide [6]. Similarly, the intensive use of these synthetic compounds caused problems such as residual toxicity (pollutants) and hazardous consequences on helpful insects, people and their domestic animals. Such problems have turn out to be a motive for attempting to find safe pesticides [7].

Lately, the bio-control, in particular, the use of entomopathogenic agents through their diverse species, easy spreading around, specificity of action and persistence in the environment is a very promising alternative to make sure effective pest control. The microorganisms utilized in microbial control belong to several taxa particularly; bacteria, viruses, fungi, nematodes and protozoa [8].

Fungal biological control agents have been shown to offer alternative and rapid prospects for implementation against grasshoppers and locusts [9]. Among these entomopathogenic fungi is *Beauveria bassiana* (Balsamo) Vuillemin, which had demonstrated considerable potential for the management of a variety of insect pests, including Acrididae [10].

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A criticism of the entomopathogenic fungi is that they act too slowly [9]. The combined use of essential oils and entomopathogenic fungi could counter this deficiency.

Aim of the present work is to use more than one tool as safe insecticides for grasshopper control aiming more effective result than using each tool separately. This approach deserve further research and analysis.

MATERIALS AND METHODS

Insect: During the summer and spring seasons (2018, 2019) numerous ecological surveys were done in to assess the major insect pest of family Acrididae predominant in Baharyia Oasis, (29°01'13.1"N 29°32'09.1"E), the Western desert of Egypt. It was found that the grasshopper *Chrotogonus Homalodemus* was the most prevailing insect in this area. Appropriate infested sites with the grasshoppers were chosen. Those sites have been characterized by excessive population density of grasshoppers (in excess of 20 insect/m^2). The nymphs are most vigorous feeders in the third, fourth and fifth instars. Thus, overlapping instars of grasshopper nymphs was taken into consideration for evaluation. Then, treated fresh clover leaves (*Medicago sativa*) were used after application.

Entomopathogenic Fungi: Two isolates of the entomopathogenic fungi; *Beauveria bassiana* (AUMC 9896) (Biossiana) and *Metarhizium anisopliae* (AUMC 9884) (BioMet) were isolated in Bio-insectcide Production Unit, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt and were identified in Mycological Center, Faculty of Science, Assiut University, Assiut, Egypt [11]. these entomopathogenic fungi were cultured on Sabouraud's dextrose agar with 1% yeast extract (SDAY) plates in several Petri dishes (9 cm in diameter) and were grown for 15 days at 25±1°C. The conidia were harvested by scraping the surface of 14-15 days old culture gently with inoculation needle. The conidia were suspended in distilled water containing 0.1% Tween-80. The mixture was stirred with a magnetic shaker for ten minutes.

Essential Oils: Five commercial essential oils; parsley, onion, mustard, clove and eucalyptus oil were tested each with four concentrations (3%, 2%, 1%, 0.5%). Each concentration was sterilized and added to autoclaved 100 ml. Dox medium into several 250 ml. Erlenmeyer flasks, then the media were poured into sterile petri-dishes and inoculated with $1x10^4$ spores of *B. bassiana* and *M. anisopliae* and incubated at 25±1°C For 15 days. Each treatment was replicated five times, the plates of control contained inoculated medium without essential oils. The quantification comparison was carried out between fungi growth of the plates with essential oils and without it by the total count of spores per petri-dish using a hemocytometer (Neubauer improved HBG, Germany).

Trials under Field Cage Conditions: The field triasl were carried out with two concentrations of each essential oil that showed higher spores production of fungi while mixing them under laboratory conditions.

Application Equipment: The motor spray spare was Nozzle, number 3 was used in all treatments, spraying height was 0.5 m above the plants. The walking speed was 40 m/min. Swath width was 3 m according to wind velocity. The weather conditions at applications; Wind was 3-5 m/sec, Humidity was 30-40%. The temperature was 28-30°C maximum and 18-20°C minimum. The spraying was done between 8 and 10.5 am early in the morning.

Experimental Design: Wettable Powder formulation of Entomopathogenic fungi spores (250g/100L water) were sprayed in a separate plot after dilution with water on Egyptian clover plants. Essential oils were sprayed alone at two concentrations 1% and 0.5% with 0.05% Tween 80 as an emulsifier after dilution by water or combined with the entomopathogenic fungi. Every approximate plot was 525 m^2 (35m x 15m). The plots have been disengaged by a belt of150 m^2 (10m x 15m) to avert the drifting spray and block the immigration of treated insects to the other plots and the control. Plots laying upwind of treatments had been utilized as a control. The untreated plot was sprayed with water. Three replicate cages 0.5m x 0.5m were represented to each treatment. Directly after application, insects were collected randomly from the same treatment by utilizing sweep-net and placed inside the cages. The cages have been left in the region of treatment under the trees. Each cage was provided with fifty nymphs to feed on the treated cover. Mortality checks were even 15 days post-treatment. Routine work includes removing the previous uneaten food, dead nymphs, faces and counting the living insects was executed daily before introducing the fresh food.

Statistical Analysis: Data were analyzed using "Ldp Line" software [12].

RESULTS AND DISCUSSION

Effect of Essential Oils on Spores Production of the Entomopathoginic Fungi: Four concentrations of five commercial essential oils; parsley, onion, mustard, clove and eucalyptus oil were added on *B. bassiana* and *M. anisopliae*. Laboratory results showed that the two concentrations (0.5. % and 1%) of all essential oils were given convergent growth from control; the result ranged from 82 to 95%, while the remaining concentrations gave a sharp decrease in the growth. So we used these concentrations (1% and 0.5%) in the field experiment.

Trials under Field Cage Conditions: After 15 days of treatment the results showed the efficacy of the entomopathogenic fungi *B. bassiana* (Biossiana) and *M. anisopliae* (BioMeta), 1x 108 CFU's /gm on grasshopper nymphs that, LT_{50} was 10.88 days and 10.77 days, respectively. *M. anisopliae* fungus gave a higher total mortality rate than *B. bassiana* (Table 1). Also five essential oils in two concentrations 1% and 0.5% were applied on grasshoppers in the field and the mortality percentages were calculated after 15 days (Table 1). Essential oils concentration (1%) resulted mortality percentages of 16.66% for parsely oil, 10% for

Table 1: Effects of entomopathogenic fungi and essential oils individually on *C. Homalodemus* nymphs after 15 days of treatment

Treatment	Total mortality %	Linear probit	Slope	LT_{50}	Lower limit	Upper limit
Biossiana 1× 10^8 CFU's /gm	62.07	5.46	3.30±0.206	10.88	10.307	11.56
BioMeta 1× 108 CFU's /gm	70.00	5.57	4.016±0.241	10.78	10.3	11.32
Parsley oil 1%	16.67	4.07	4.016±0.242	25.5	21.202	35.31
Parsley oil 0.5%	13.33	3.83	4.016±0.243	54.9	35.45	130.38
Onion oil 1%	10.00	3.75	4.016±.244	33.79	25.07	64.88
Onion oil 0.5%	6.67	3.67	4.016±0.245	38.79	27.15	87.5
Mustard oil 1%	10.00	3.64	4.016±0.246	44.56	29.61	115.3
Mustard oil 0.5%	6.67	3.36	4.016±0.247	910.95	169.88	268856
Clove oil 1%	13.33	3.88	4.016±0.250	38.24	27.76	70.72
Clove oil 0.5%	10.00	3.77	4.016±0.251	44.68	30.43	98.73
Eucalyptus oil 1%	13.33	4.04	4.016±0.248	32.16	24.96	50.1
Eucalyptus oil 0.5%	10.00	3.71	4.016±0.249	37.63	26.78	79.69

Table 2: Mixing effects of Biossiana (*Beauveria bassiana*) and two concentrations of essential oils on *C. Homalodemus* nymphs after 15 days of treatment

Treatment	Total mortality %	Linear probit	Slope	LT_{50}	Lower limit	Upper limit
Biossiana + 1% parsley oil	83.33	5.9149	4.848±0.26	9.71	9.35	10.09
Biossiana+0.5% parsley oil	76.66	5.7228	4.2139±0.24	10.10	9.68	10.56
Biossiana +1% onion oil	80.00	5.7701	5.0141±0.29	10.53	10.14	10.95
Biossiana +0.5% onion oil	73.33	5.6284	4.5164±0.28	10.88	10.45	11.37
Biossiana+ 1% mustard oil	80.00	5.8473	4.6941±0.26	9.89	9.52	10.30
Biossiana+ 0.5% mustard oil	73.33	5.6903	4.5984±0.27	10.61	10.19	11.07
Biossiana+ 1% clove oil	80.00	5.8	4.4496±0.25	9.91	9.52	10.33
Biossiana+ 0.5% clove oil	76.66	5.639	3.9748±0.23	10.35	9.90	10.86
Biossiana+ 1% eucalyptus oil	80.00	5.7646	4.1271±0.23	9.79	9.37	10.23
Biossiana+ 0, 5% eucalyptus oil	76.667	5.6945	3.9064±0.22	9.96	9.52	10.44

Table 3: Mixing effects of BioMeta (*Metarhizium anisopliae*) and two concentrations of essential oils on *C. Homalodemus* nymphs after 15 days of treatment

Treatment	Total mortality%	Linear probit	Slope	LT_{50}	Lower limit	Upper limit
BioMeta+ 1% parsley oil	86.66	5.8039	4.0289±0.22	9.47	9.07	9.90
BioMeta+ 0.5% parsley oil	83.33	5.7	3.7205±0.21	9.72	9.28	10.21
BioMeta+ 1% onion oil	83.33	5.7824	4.078±0.23	9.64	9.23	10.08
BioMeta+ 0.5% onion oil	76.66	5.7552	3.916±0.23	9.62	9.17	10.11
BioMeta+ 1% mustard oil	80.00	5.8177	4.1334±.023	9.51	9.11	9.93
BioMeta+ 0.5% mustard oil	80.00	5.7157	4.2154±0.24	10.14	9.72	10.60
BioMeta +1% clove oil	86.66	5.8606	4.5928±0.25	9.74	9.36	10.14
BioMeta +0.5% clove oil	83.33	5.742	3.8483±0.22	9.62	9.19	10.08
BioMeta+ 1% eucalyptus oil	86.66	6.0268	4.5142±0.24	8.88	8.53	9.24
BioMeta+ 0.5% eucalyptus oil	80.00	5.8043	4.4062±0.245	9.85	9.45	10.27

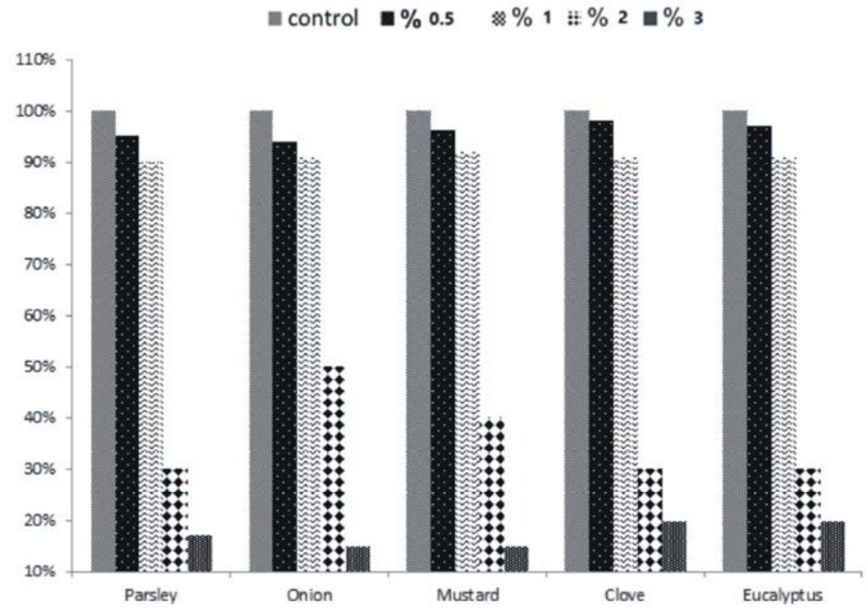

Fig. 1: Percentage of spores production of *Beauveria bassiana* with and without essential oils

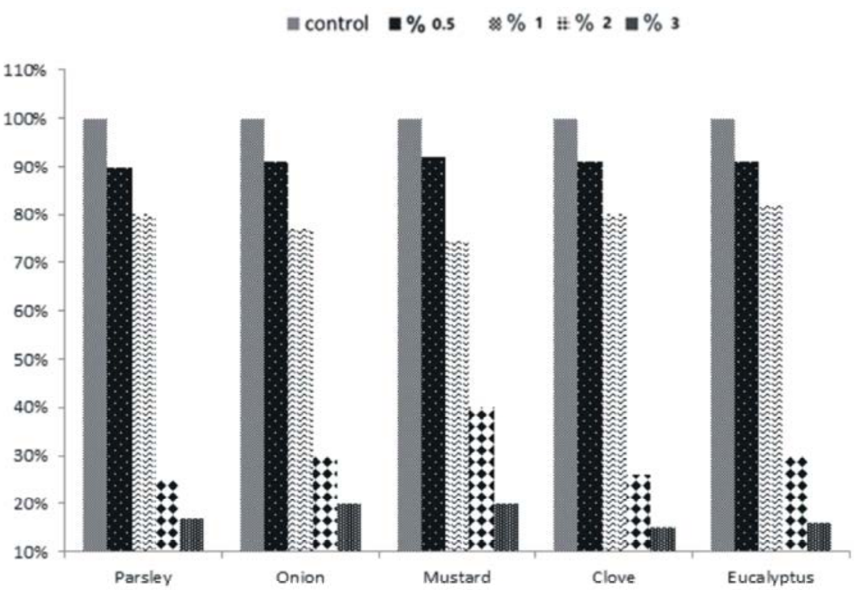

Fig. 2: Percentage of spores production of *Metarhizium anisopliae* with and without essential oils

onion & mustard oils, 13.33% for clove & eucalyptus oils. But the concentration (0.5%) recorded 13.33% for parsley oil, 6.67% for onion and mustard oils and 10% for clove and eucalyptus oils.

The accumulative percentage mortalities of nymphs in Tables (2&3) revealed a significant increase by mixing entomopathogenic fungi with essential oils compared to individual use. Mixture between *M. anisopliae* with 1% parsely oil or eucalyptus oil or clove oil gave the highest mortality percentage (86.66%) after 15 days of application on the other hand, mortality percentages for *B. bassiana* with 0.5% onion oil or mustard oil were 73.33%. LT_{50} value was reduced to 9.47 days with mixing *M. anisopliae* with 1% parsley oil instead of 10.77 days for *M. anisopliae* alone. Also in the *B. bassiana* there was a reduction in LT_{50} from 10.88 days without mixture to 9.71 days after mixed with1% parsley (Tables 1, 2 & 3).

Generally, the two concentrations of essential oils resulted low effects on the targeted insects when used individually. The total mortality rate did not exceed 17%

at the best effect, compared to the use of fungi, which gave relatively high mortality rates when used alone as well. When entomopathogenic fungi were mixed with essential oils they gave mortality rates exceeding 80% in most treatments.

Therefore, it is recommended to mix the entomopathogenic fungi with specific concentrations of some essential oils, which increase the mortality rate of insects and reduce the value of LT_{50}.

There have been many studies that have tested the effects of essential oils on many insects as well as mixing them with other compounds to increase effectiveness. Awad [13] described a prolongation in the life of treated insects of *Agrotis ipsilon* and a decrease in its weight when fed on leaves treated with garlic oil. As well as Shairra and Awad [14] Showed that after the 5th nymphal instar of *Schistocerca gregaria* was fed on clover leaves treated with garlic oil, percentage mortality increased after 48 hrs after application. Also Sharaby *et al*. [15] applied Three essential oils on the first nymphal instar of *Heritiera littoralis* the LC50 values were as follows: garlic 0.067 > eucalyptus 0.075 > mint 0.084 ml./100ml of diet. Pelizza *et al*. [16] demonstrated that the combination of canola oil with wheat bran makes the bait poisoned with conidia of *B. bassiana* more palatable for nymphs of *Dorycera maculipennis*. This was reflected in the higher mortality and lower MST values for this species of grasshoppers, both in laboratory bioassays and those carried out under field cage conditions. Studies carried out by Latchininsky *et al*. [17] reported that there are certain vegetable oils such as olive, canola, corn and flax that have phagostimulant properties on nymphs and adults of the North American grasshopper pest *Melanoplus sanguinipes* (Fabricius).

So, both essential oils and entomopathogenic fungi might be applied on wider together in programs of integrated pest management on insect pests in the field, since those bio-insecticides are safer to the environment than insecticides.

CONCLUSIONS

In this work, it was demonstrated that the combination of *Metarhizium anisopliae* (formulation BioMeta) with 1% from parsley oil or clove oil or eucalyptus oil and *Beauveria bassiana* (formulation Biossiana) with 1% parsley oil, gave a strong effect to control *Chrotogonus homalodemus* nymphs. Results are recommended for use as safe combinations to be applied in the integrated pest management systems of grasshoppers to reduce the use of pesticides.

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