

Potential Antimicrobial and Pathogenic Properties of *Oscheius* spp. and *Beauveria Bassiana*: Implications for Medical Microbiology

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ABSTRACT

Background: The potato (*Solanum tuberosum*) is a staple food crop worldwide, contributing significantly to global food security. However, the potato tuber moth (*Phthorimaea operculella*) is a major agricultural pest that causes severe economic losses, particularly in Ecuador. This study aimed to evaluate the efficacy of entomopathogenic nematodes (*Oscheius myriophilus* and *Oscheius tipulae*) and the fungal biocontrol agent *Beauveria bassiana* in controlling *P. operculella* larvae and pupae under laboratory conditions. **Methods:** The bioefficacy of both commercial and locally isolated *Oscheius* spp. nematodes was assessed at four infective juvenile (IJ) concentrations (25, 50, 75, and 100 IJs/mL). Additionally, *Beauveria bassiana* spores were tested at three concentrations (1×10^5 , 1×10^7 , and 1×10^9 spores/mL), both individually and in combination with nematodes. Compatibility between the fungal and nematode biocontrol agents was examined microscopically to assess any inhibitory effects on growth or efficacy. **Results:** The combined use of *B. bassiana* at 1×10^9 spores/mL with 100 IJs/mL of commercial nematodes resulted in the highest mortality rates of *P. operculella*, with 76.66% mortality in larvae and 73.33% in pupae. Similarly, the combination of *B. bassiana* at 1×10^9 spores/mL with 100 IJs/mL of local nematodes showed mortality rates of 68.33% for *O. myriophilus* (73.33% larvae, 63.33% pupae) and 73.33% for *O. tipulae* (80% larvae, 66.66% pupae). The results demonstrated that *P. operculella* larvae were more susceptible to biological control agents than pupae, indicating a higher effectiveness of biocontrol strategies at the larval stage. **Conclusion:** The findings of this study suggest that *Oscheius* spp. and *B. bassiana* exhibit significant potential as biological control agents against *P. operculella*, particularly when used in combination. The compatibility between these bioagents further supports their integrated use in sustainable pest management strategies. Further research is recommended to explore field applications and optimize biocontrol formulations for large-scale implementation.

Keywords: Entomopathogenic Nematodes, *Oscheius*, Potato Tuber Moth, *Phthorimaea Operculella*, Lepidoptera Gelechiidae, *O. Myriophilus*, *O. Tipulae*

INTRODUCTION

Potato is a common and preferred host for potato tuber moth (Zeller *Phthorimaea operculella*) which is along with it [1]. The potato tuber moth is considered a major potato cosmopolitan pest that belongs to the Lepidoptera order and the Gelechiidae family. It attacks potatoes (*Solanum tuberosum*) and other solanaceous plants cultivated including tobacco (*Nicotiana tabacum*), tomato (*Lycopersicon esculentum*), eggplant (*Solanum melonguene*), and bell pepper (*Capasicum annuum*) [2]. There are three potato tuber moths (Lepidoptera; Gelechiidae) species; the Andean potato tuber moth (*Symmetrischema tangolias Gye*), Guatemalan potato tuber moth (*Tecia solanivora* Povolny), and the common specie potato tuber moth (*Phthorimaea operculella* Zeller) that cause significant losses economically and present in region of Ecuador [3]. For many years, usage the chemicals synthetic pesticides as agricultural insect pest control that causes damage to durable crops of food in fields and storage. However, the application of these synthetic chemical insecticides continuously may cause considerable problems includes develop insects' resistance, the environmental pollution, handling toxic hazards. Thus, need to development alternatives measures that easy in preparent, cheap, safe using, and available locally [4]. The application of insecticide always to effectively control larvae that are concealed in tubers is impractical. Thus, environmentally friendly and more effective tactics control are a persistent need [5]. The use of a parasitoid addressed to host-finding affects the parasitoid's ability to

control pests. By parasitoid finding of the host depends on responses to cues of the host itself and the host habitat [6]. Promising results have appeared by using Entomopathogenic nematodes (EPNs) as alternative control measures for many pests of soil and biological control [7]. Globally, *Beauveria bassiana* which is an arthropod-pathogenic fungus has been used against many pests of crops as a biological control agent and human disease vectors. Moreover, *B. bassiana* (entomopathogenic fungi) current use as biopesticides that have been focused primarily on approach of nundative biocontrol [8]. Interestingly, preliminary studies and experiments revealed that under laboratory conditions, nematodes including *Oscheius* spp. infect and kill two species of insects; *Tenebrio molitor* and *Pieris rapae*. This makes potentially the nematodes and their species vital agents for biological control. Furthermore, in China more specifically Chongming Island, *Oscheius microvilli* showed a high effectiveness level [9]. At present, many studies report some rhabditid nematodes capable of acting as potential agents of biocontrol to various pests of invertebrate control [10]. Some *Oscheius* species' entomopathogenic actions have been linked to bacteria of Serratia that during the infection play an essential role and causing presumably the host insect's death [11]. There are many *Oscheius* species have been revealed to reproduce in the insects and kill them such as *O. tipulae* [12], *O. safricana* [13], *O. myriophilus* [14], *O. microvilli* [15], and *O. basothovii* [16]. So, this study aims to evaluate the compatibility

between *Oscheius* (species *O. myriophilus* and *O. tipulae*) and *Beauveria Bassiana* together in controlling potato tuber moth larvae and pupae individually under laboratory conditions.

MATERIALS AND METHODS

Insect, Bioagent Entomopathogenic Nematodes and Fungal isolates

Potato tubers contaminated with *Phthorimaea operculella* larvae and pupae under laboratory conditions were obtained from infested potatoes available at the Entomology laboratory of the Department of

Agricultural Research - Department of Biological Control laboratories- the Ministry of Science and Technology, in Baghdad governorate.

This study was conducted in September 2023. The laboratory environmental conditions were maintained at $20\pm 25^{\circ}\text{C}$ and 60-70% relative humidity (R.H.) and photoperiod (12:12 light: dark) and providing daily fresh food and temperature and humidity check. *Beauveria bassiana*, Local and commercial nematode were obtained also from Entomology laboratory of Department of Agricultural Research - Department of Biological Control laboratories- the Ministry of Science and Technology, in Baghdad governorate.



FIGURE 1: P. Operculella (pupae)

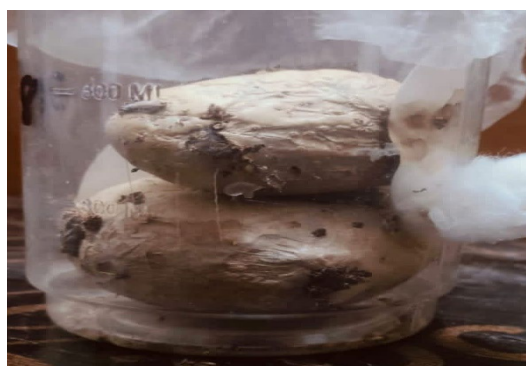


FIGURE 2: P. operculella (larvae)



FIGURE 3: P. operculella (adult)



FIGURE 3: Rearing cages of P. Operculella

In the experiment, Petri dishes (diameter 5 cm) filled with filter paper discs were used to carry the *Galleria mellonella* in continuous contact with the two *Oscheius* nematode species (*O. myriophilus* and *O. tipulae*). The larvae of the *Galleria mellonella* insect were used as a medium for feeding and reproducing the nematodes. The infective

juveniles then were used and converted into suspension via added sterile distilled water to the Petri dish that contained the infective juveniles. After this step, it was withdrawn by using a pipette and transferred to a Tissue Flask with 25 milliliters size and stored in the refrigerator at a temperature ranging between 8 -10°C.

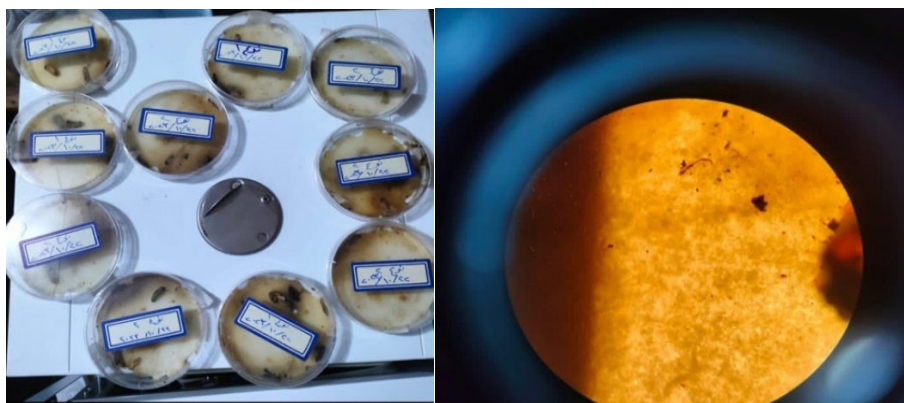


FIGURE 3: Entomopathogenic Nematodes

Preparation Nematode Suspension

The commercial and local types of two *Oscheius* nematode species (*O. myriophilus* and *O. tipulae*) were used to identify the efficiency of these types on *Phthorimaea operculella*. The suspension preparation that contains nematode infective juvenile stage (IJS) was initially

prepared by using a fine pipette to take 100 microliters volume of the suspension and count the stars.

The value of commercial and local types was of 92 IJS and 85 IJS respectively. To calculate the concentration of IJS for a 25 milliliters volume: $c_1/v_1 = c_2/v_2$. Where:

Table 1: Preparation Nematode Suspension

C1	The IJS Concentration in 25 Milliliters of Nematode Suspension
V1	The volume of Tissue Flask (25 milliliters).
C2	The IJS concentration in 100 microliters
V2	The volume of nematode suspension that was withdrawn with the micropipette.

The experiments were conducted in Petri dishes containing a PDA medium (40 grams of PDA, and 1000 milliliters of sterile distilled water).

Cotton swabs were covered on the upper opening of Petri dishes to prevent the sample from escaping and immediately sealed tight and were placed in an autoclave device to perform the process of sterilization under appropriate laboratory conditions (up to 121 °C.,

up to 15 psi of pressure for 20 minutes. Then the medium was transferred to Petri dishes and leaves to solidify. Then, a swab was added to the center of a container (dish) that contained fungal growth of the *Beauveria bassiana* to inoculate. The containers were incubated set at $25 \pm 2^\circ\text{C}$, RH $70 \pm 2\%$ for 7-9 days. After the growth of the fungi was confirmed, the dishes were stored using the refrigerator at an appropriate temperature (4°C) until use.

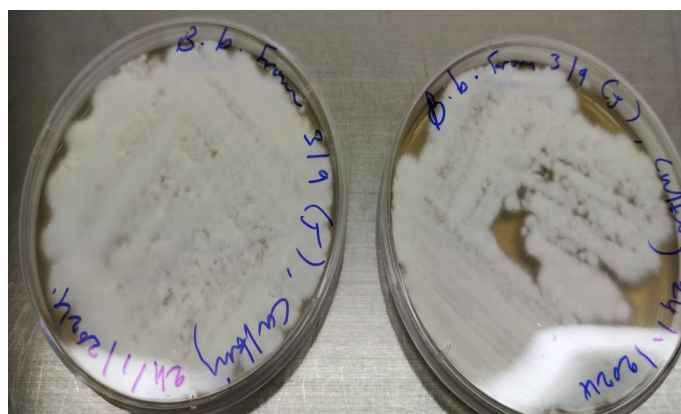


FIGURE 6: Beauveria bassiana

Preparation of the Spore Suspension

The fungal suspension was prepared after activating the biological control agent *B. bassiana* on PDA for 14 days, and then adding sterile distilled water on the surface of culture, the spores on the dish surface scraped off by using an L-shaped lobe, the dish contents poured into a

beaker and complete to 100 ml after filtration, and this called stock solution (S). The concentration of stock solution was calculated by Haemocytometer and by the following equation : Spore count per milliliter = spore count per square $\times 2.5 \times 10^5$. The stock solution was diluted by sterile distilled water to prepare S/2 and S/4 solutions,

these three concentrations of the fungus *B. bassiana* (S, S/2, S/4) were used against potato tuber moth.

Evaluation the Compatibility of Local and Commercial Nematodes with the Fungus

To evaluates the compatibility of local and commercial nematodes with the fungus as agents of biological control. Both agents were mixed without a negative effect of one on the effectiveness and activity of the other agent. The best local and commercial nematode concentrations were mixed with the best concentrations of fungus that achieved the highest percentage of killing for the larvae and pupae of *Phthorimaea operculella*.

As a process of preparation in this experiment, in a Petri dish filed with filter paper that provides the biological control agent with appropriate humidity, were placed 1-milliliter suspension of nematode at 100 infectious stages/ml concentration, and 1 ml of fungal suspension at 1×10^9 spores/ml concentration at the rate of three dishes. These dishes then were placed into an incubator at 25 ± 2 of temperature and 90% RH. Continuous monitoring of the dish to track the effect extent one of the agents of biological control on the other organism and results were recorded.

Evaluation the Compatibility of the Fungus with Nematodes as Control Agents Against the *P. Operculella*

After determining the compatibility of the two agents of biological control and the effect of one on the effectiveness and activity of the other agent, the insect stages of larvae and pupae were exposed to a mix of the two agents of biological control to determine their effectiveness in insect pest elimination. In this experiment, the 25 infectious stages/ ml of nematode suspension which is the highest concentration was mixed with three of the fungal suspension concentrations (1×10^5 spores/ml, 1×10^7 spores/ml, 1×10^9 spores/ml) respectively. The same steps of the process were repeated, by replacing the commercial nematodes with the local ones, as shown in tables (1,2,3). The mix of both biological control agents was placed in a Petri dish filled with filter paper. The filter paper was sprayed with a solution that contained nematode suspension and fungi and then placed 10 larvae or pupae in each dish with three replicates for each treatment. These dishes were covered thoroughly by using parafilm and placed in an incubator at appropriate conditions (temperature of 25 ± 2 and an RH of 90%) and daily monitored the dishes. The results were recorded after passing 14 days.

Table 2: The Nematodes Concentrations (Commercial) and Sizes and Fungus, along with the Number of Larvae and Pupae that Were Used in the Experiments.

Sample Type	Conc. of Ijs, ^{Ijs} /ml	Ijs Susp. Volume, ml	Conc. of Fungi Spores' ^{spore} /ml	Fungi Spores' Volume ml
Control	-	-	-	-
Larvae	100	360	1×10^9	1
	100	360	1×10^7	1
	100	360	1×10^5	1
Pupae	100	360	1×10^9	1
	100	360	1×10^7	1
	100	360	1×10^5	1

Table 3: The nematodes concentrations (local *O. myriophilus*) and sizes and fungus, along with the number of larvae and pupae that were used in the experiments.

Sample Type	Conc. of Ijs, ^{Ijs} /ml	Ijs Susp. Volume, ml	Conc. of Fungi spores' ^{spore} /ml	Fungi Spores' Volume ml
Control	-	-	-	-
Larvae	100	188	1×10^9	1
	100	188	1×10^7	1
	100	188	1×10^5	1
Pupae	100	188	1×10^9	1
	100	188	1×10^7	1
	100	188	1×10^5	1

Table 4: The nematodes concentrations (local *O. tipulae*) and sizes and fungus, along with the number of larvae and pupae that were used in the experiments.

Sample Type	Conc. of Ijs, ^{Ijs} /ml	Ijs Susp. Volume, ml	Conc. of Fungi Spores' ^{spore} /ml	Fungi Spores' Volume ml
Control	-	-	-	-
Larvae	100	280	1×10^9	1
	100	280	1×10^7	1
	100	280	1×10^5	1
Pupae	100	280	1×10^9	1
	100	280	1×10^7	1
	100	280	1×10^5	1

Statistical Analysis

The GenStat program (version 3) was used in the process of statistical analysis to test all laboratory experiment results. The rates of killing were corrected using Abbott's correction (Abbott, 1925). Additionally, one-way ANOVA was used to analyze the rates of killing and compared them by using the least significant difference test (LSD test).

The statistical analysis of compatibility and interaction between the different concentration parameters for nematodes and fungi was done by using a multifactor ANOVA design and the Fisher LSD test was used to compare the total rates with a confidence probability of 95% ($P \leq 0.05$).

Results and Discussion

The effect of the local *Beauveria Bassiana* Fungus on the Larvae and Pupae of the *Phthorimaea Operculella*

In terms of results of testing the effect of the *Beauveria bassiana* fungus on *Phthorimaea operculella* growth and reproduction showed that the *Beauveria bassiana* affected larvae and pupae at all concentrations used and that the effect percentage was proportional to the concentration used. The highest death percentage was recorded for both stages (larvae and pupae), which was 76.66% and 70%, respectively at the concentration 1×10^9 . This means 23 out of 30 larvae and 21 out of 30 pupae and the remaining have completed their

growth and turned into adults. Regarding the treatment with the second concentration, which is 1×10^7 , the killing percentage of both larvae and pupae was 50% and 43.33%, respectively, meaning that the number of infected larvae that were killed by the fungus was 15 out of 30 larvae, and the rest of the larvae completed their growth normally. As for the infected pupae, their number was 13 out of 30 divided into 3 plates. The third concentration, which is (1×10^5) , recorded a killing percentage for both larvae and pupae was 36.66% and 30% respectively, meaning that the number of infected larvae that were killed by the fungus was 11 out of 30, and the rest of the larvae completed their growth normally. As for the infected pupae, their number was 9 out of 30 divided into 3 plates. The study also showed in comparison between the larvae and pupae, the larvae were more sensitive to fungus as the rate of mortality was 54.44% for larvae compared to 47.77% for pupae. The growth of the fungus appeared on the larvae 3 days after treatment, and gradually increased the growth of the fungus until it was dense on 9 days of treatment. The result of this study indicated that the period required to kill and control the larvae and pupae by using spore suspension in biological control of larvae and pupae of the *Phthorimaea operculella* was shorter than it was in the case of using nematodes. In terms of spore suspension concentration, increases in concentration lead to an increased killing rate for the larvae and pupae

as the killing percentage achieved by the spore suspension (base) stock suspension 1×10^9 spores/ml is higher than the killing percentages achieved by less concentrated dilutions of spores, and this result is consistent with the result reached by Yuan *et al.* (2018), who found that treated larvae of *P. operculella* with *B. bassiana* showed a high mortality rate in comparison with control treatment due to their effect on the growth, and development of the offspring of *P. operculella* and survival. Also, they found *Beauveria bassiana* has a potential role in the control of *P. operculella* at a concentration of 1×10^7 [17]. Another study done by Yulin Gao indicated that *Beauveria bassiana* significantly reduces larvae of potato tuberworm survival and it is highly virulent to *P. operculella* [18]. It has also been noted that the larvae are more sensitive and affected by the biological control agent *Beauveria bassiana* than the pupae.

Fatih Dadaşoğlu *et al.* [18] tested the efficacy of *B. bassiana* isolates against PTM larvae and eggs and suggested that it gave the various efficacy levels results (98.33%-100%) against PTM. Similarly, Zhang *et al.* [19] determined that the survival of the larvae of *P. operculella* fed on potato plants inoculated with *B. bassiana* and the number of eggs laid by the females were quite low, and they noted that *B. bassiana* could be a potential biological control agent in the control of this species.

Table 5: The Effect of the Local *Beauveria Bassiana* Fungus on the larvae and Pupae of the Potato Tuber Moth

Conc. of Fungi spores' spore/ml	Mortality percentages (%)		Rate
	The Larvae	Pupae	
1×10^9	76.66	70	73.33
1×10^7	50	43.33	46.66
1×10^5	36.66	30	33.33
Control	0	0	
Rate	54.44	47.77	
LSD (0.05)	33.51	30.26	

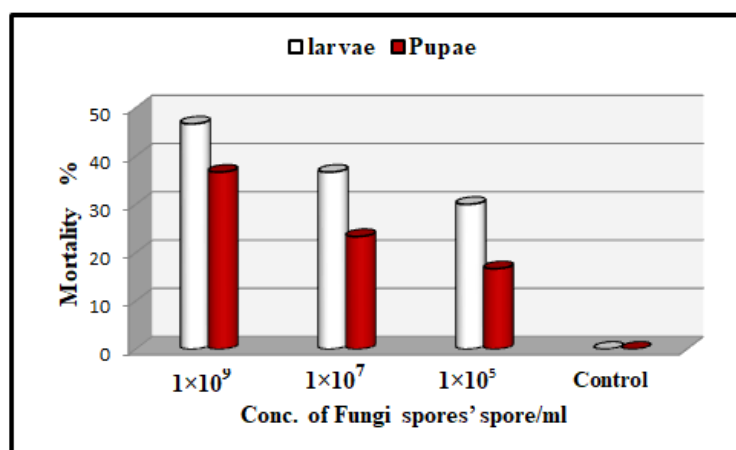


Figure 7: Percentage of Larval and Pupal Mortality of Treatment with *Beauveria Bassiana* Fungus.

Compatibility of the fungus with Nematodes as Abiogenist Against the *P. Operculella*

The result of this study revealed that there is no adverse effect between them. The activity of the nematodes and their movement was at a normal level. Gradually, the growth became dense and could be seen with the naked eye after the 5 days of conducting the test. By microscopic examination, the nematode's movement and activity were very normal and not affected by the growth of fungus. The density of the growth of the fungus gradually increased until it became very dense after 15 days of this experimental. Diverse effects can result when combining two biocontrol agents on the pest mortality target

such as additive effects can occur due to acting the biocontrol organisms independently without interaction [20]. Several studies have explored the compatibility of the combination of EPF *Beauveria* and EPNs towards various field and forest [21, 22]. Another study reported the compatibility of EPNs *Steinernema carpocapsae* with EPF *Beauveria bassiana* [23].

The Fungus (*B. bassiana*) and the Nematode Effect of on *P. Operculella*

The effect of the combination of the *Beauveria bassiana* fungus and commercial nematodes on the *Phthorimaea operculella*: The effect of the combination of two biological control agents, namely the

Beauveria bassiana fungus and commercial nematodes on the larvae and pupae of the *Phthorimaea operculella* had been presented in Table (5): When mixed the two biological agents at 1×10^9 concentration for fungus and a concentration of 100 infective stages/ml for commercial nematode and tested on larvae and pupae of the *Phthorimaea operculella*, the number of larvae that were infected and killed was 10 out of 30 larvae, distributed evenly on 3 Petri dishes. Thus, the larvae death percentage was 76.66%.

For pupae treated with the same concentration, the number of pupae that were infected and killed was 8 out of 30 pupae, distributed evenly on 3 Petri dishes. Thus, the pupae death percentage was 70%. The rate of infection for larvae and pupae treated with the two concentrations mentioned above was 73.33%. In the case of mixing the two biological agents at 1×10^7 concentration for fungus and a concentration of 100 infective stages/ml for commercial nematode as mixed and tested on larvae and pupae of the *Phthorimaea operculella*, the number of larvae that were infected and killed was 14 out of 30

larvae, distributed evenly on 3 Petri dishes. Thus, the larvae death percentage was 50%. For pupae treated with the same concentration, the number of pupae that were infected and killed was 12 out of 30 pupae, distributed evenly on 3 Petri dishes.

Thus, the pupae death percentage was 43.33%. The rate of infection for larvae and pupae treated with the two concentrations mentioned above was 46.66%. In the case of mixing the two biological agents at 1×10^5 concentration for fungus and a concentration of 100 infective stages/ml for commercial nematode and tested on larvae and pupae of the *Phthorimaea operculella*, the number of larvae that were infected and killed was 23 out of 30 larvae, distributed evenly on 3 Petri dishes. Thus, the larvae death percentage was 36.33%. For pupae treated with the same concentration, the number of pupae that were infected and killed was 22 out of 30 pupae, distributed evenly on 3 Petri dishes. Thus, the pupae death percentage was 30%. The rate of infection for larvae and pupae treated with the two concentrations mentioned above was 33.33%.

Table 6: The Combined Effect of the *Beauveria bassiana* Fungus and Commercial Nematodes on the *Phthorimaea operculella*

Code	Fungi + ijs 25 Infective juveniles / μ L	Mortality Percentages (%)		Rate
		The larvae	Pupae	
A	fungi 1×10^5 spore / μ L + Commercial ijs	33.33	26.66	29.99
B	fungi 1×10^7 spore / μ L + Commercial ijs	46.66	40	43.33
C	fungi 1×10^9 spore / μ L + Commercial ijs	76.66	73.33	74.99
D	Control	0	0	0
	Rate	52.21	46.66	
	LSD (0.05)	9.41	15.37	

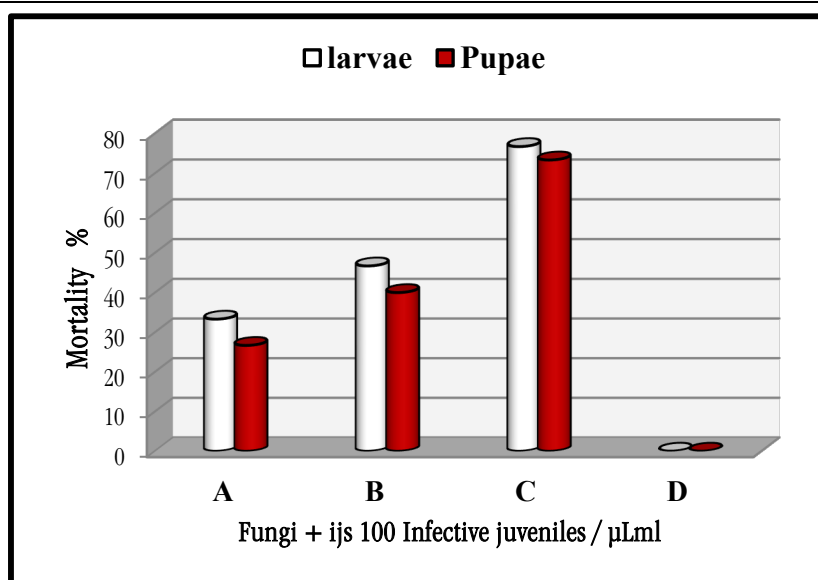


Figure 8: Percentage of Larval and Pupal Mortality of Treatment with Combination of the *Beauveria bassiana* Fungus and Commercial Nematodes

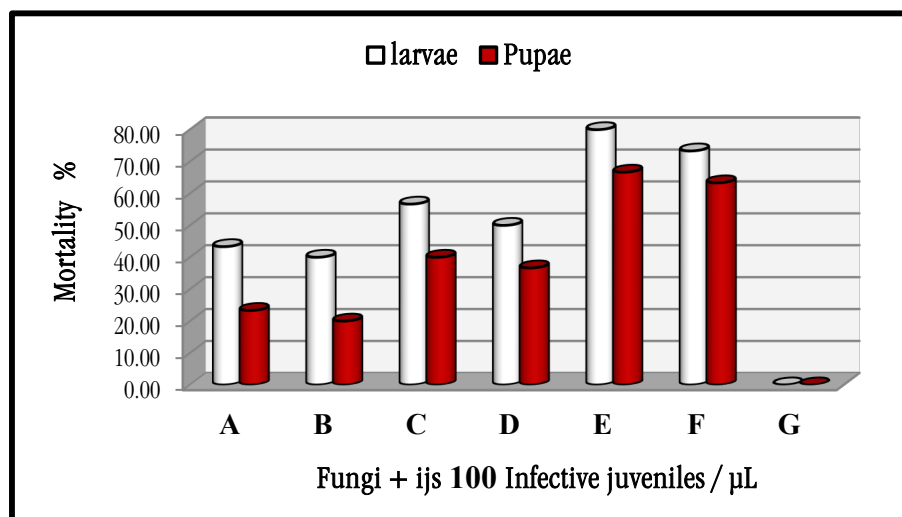
The fungi and nematodes combined use (either delayed the application of *H. bacteriophora* or applied simultaneously) resulted in an enhanced level of virulence in a synergistic fashion or an additive. Synergistic virulence levels were noted greatly in combinations *H. bacteriophora* and *B. bassiana* [24]. The EPN's interaction with EPF is influenced by several factors such as the insect host target, parameters application, environmental conditions, as well as EPF and EPNs strains and species [25]. In 2014, Wu et al. studied the combined *H. megidis* and *H. bacteriophora* with *M. anisopliae* s.s. and *B. bassiana* s.s. in greenhouse experiments and laboratory against *Cyclocephala lurida* Bland (the third instar). In experiments in the laboratory, they found additive interactions between *B. bassiana* and *H. megidis* [26]. Another suggestion by Ansari et al. (2008) who

reported that the synergy mechanism in the infected insects with the nematodes after being infected with the fungus may be due to fungal infection could stress the insect host by affecting body homeostasis and its food intake.

Consequently, this process decreases its mechanisms overcome to infection of the nematode which in healthy grubs is very effective [27]. The combined effect of the *Beauveria bassiana* fungus and local nematodes on the *Phthorimaea operculella*: The effect of the combination of two biological control agents, namely the *Beauveria bassiana* fungus and local nematodes, on the larvae and pupae of the *Phthorimaea operculella*, had been presented in Table (6): The result showed that the combination was more effective than each one alone and the effect increase with the increasing of concentration.

Table 7: The Effect of the Combination of the *Beauveria Bassiana* Fungus and Local Nematodes on the *Phthorimaea Operculella*

Code	Fungi + ijs 100Infective juveniles / μL	Mortality Percentages (%)		Rate
		The Larvae	Pupae	
A	fungi 1×10^5 spore / μL + <i>O. tipulae</i>	43.33	23.33	33.33
B	fungi 1×10^5 spore / μL + <i>O. myriophilus</i>	40.00	20.00	30
C	fungi 1×10^7 spore / μL + <i>O. tipulae</i>	56.67	40.00	48.33
D	fungi 1×10^7 spore / μL + <i>O. myriophilus</i>	50.00	36.67	43.33
E	fungi 1×10^9 spore / μL + <i>O. tipulae</i>	80.00	66.67	73.33
F	fungi 1×10^9 spore / μL + <i>O. myriophilus</i>	73.33	63.33	68.33
G	Control	0	0	0
	Rate	57.22	41.66	
	LSD (0.05)	11.46	10.11	

**Figure 9:** Percentage of Larval and Pupal Mortality of Treatment with Combination of the *Beauveria Bassiana* Fungus and Local Nematodes

The results of fungus treatment at a concentration of 1×10^5 spore / μL with a concentration of 100 Infective juveniles / μL of local nematode (*O. myriophilus* and *O. tipulae*) on *Phthorimaea operculella* larvae and pupae showed that the rate of killing was 30% of *O. myriophilus* (40 % of larvae and 20% of pupae), and 33.33% of *O. tipulae* (43.33% of larvae and 23.33% of pupae), meaning *O. myriophilus* were killed 12 larvae and 6 pupae were killed after the treatment out of 30. While *O. tipulae* were killed 13 larvae and 7 pupae were killed after the treatment out of 30. Regarding the treatment of *Phthorimaea operculella* larvae and pupae with fungus at a concentration of 1×10^7 spore / μL and local nematodes *O. myriophilus* and *O. tipulae* at a concentration of 100 Infective juveniles / μL , the rate of killing was 43.33% of *O. myriophilus* (50% of larvae and 36.66% of pupae), and 48.33% of *O. tipulae* (56.66% of larvae and 40% of pupae), meaning *O. myriophilus* were killed 15 larvae and 11 pupae were killed after the treatment out of 30.

While *O. tipulae* were killed 17 larvae and 12 pupae were killed after the treatment out of 30. Treating the *Phthorimaea operculella* larvae and pupae with fungus at a concentration of 1×10^9 spore / μL and local nematodes *O. myriophilus* and *O. tipulae* at a concentration of 100 Infective juveniles / μL showed that the *O. myriophilus* were killed 22 larvae and 19 pupae were killed after the treatment out of 30. While *O. tipulae* were killed 24 larvae and 20 pupae out of 30 were killed after the treatment, distributed evenly on three plates. The rate of killing was 68.33% of *O. myriophilus* (73.33% of larvae and 63.33% of pupae), and 73.33% of *O. tipulae* (80% of larvae and 66.66% of pupae), as shown in Figure (3). The result of this study showed that the combination is more effective than each bioagent alone and this may be due to that the fungus make the target pest weak and more susceptible to the nematode in addition that the insect affected by

fungus attract the nematode. The combination of EPNs and EPF is considered an effective approach and interesting in microbial pest control. Studies recently mainly have focused on these biological control agent groups' uses to increase their insecticidal efficacy under conditions in the laboratory [28]. In 2004, Shapiro-Ilan et al. observed in their study that *S. carpocapsae* and *B. bassiana* have an additive effect against *Curculio caryae* Horn depending on the doses of the application [29], and this was reported again later in the study done by Ibrahim [29] for *Agrotis ipsilon* [30]. The combination of *S. carpocapsae* and *B. bassiana* additive effect has been reported by many studies for *Hylobius abietis* [31, 32]. Similarly, another study done by Koppenhöfer and Grewal [32] revealed that the application of the nematode fungus tends to have an additive effect produce. Experiments in the laboratory were conducted to successfully assessment of reproductive of *Heterorhabditis bacteriophora* Poinar or *Steinernema carpocapsae* in soilborne *Spodoptera exigua* that was exposed to *Beauveria bassiana* previously. *B. bassiana* plus *H. bacteriophora* treatments could result in higher *S. exigua* total mortality in soil than either fungus treatments alone or nematode alone [33]. Recent study have been developed a method of biological control for the cabbage maggot *Delia radicum* by three biocontrol agents, the bacteria *Pseudomonas chlororaphis*, the nematode *Steinernema feltiae* and the fungus *Metarhizium brunneum* and the result revealed a synergistic effects of *Pseudomonas*-nematode and *Pseudomonas*-fungus combinations against maggot and the triple combination reduced both pest survival in greenhouse experiments and maggot-induced damage on radish bulbs in the field [34]. Another study was evaluate the effect of the combination of the fungus *Beauveria bassiana* and the nematodes *Heterorhabditis indica* and *Steinernema siamkayai* against armyworm, *Spodoptera frugiperda* and the result showed that the combination was more effective than

each one alone and the result also showed that spraying entomopathogenic nematodes followed by fungus was more effective than spraying entomopathogenic fungus followed by nematodes [35].

Conclusions

The finding study indicated that the combined use of nematodes with fungus is more compatible since neither of them affected the efficacy and growth of the other which was observed during microscopic examinations. Increase the mortality rate of *Phthorimaea operculella* larvae and pupae due to the combined use of commercial nematodes with fungi, especially at 1×10^9 concentration. As well as, increase the mortality rate of *Phthorimaea operculella* larvae and pupae due to the combined use of local nematodes with fungi, especially at 1×10^9 concentration and the mortality rate was higher

compared to the combined use of commercial nematodes. Fungi and nematodes as biological control agents have a potential role in the control of *P. operculella* larvae at all concentrations and treated larvae of *P. operculella* with *B. bassiana* showed a high mortality rate in comparison with pupae, especially when combined use the fungus at of 1×10^6 with nematodes at a concentration of 100 Infective juveniles / μL . Use the highest concentrations of combined use of fungi 1×10^9 with nematodes at a concentration of 100 Infective juveniles / μL , since they have great efficiency as give the highest mortality rates for *P. operculella* larvae and pupae. Test and assessment of other types of nematodes and fungi in *P. operculella* control and combined use them to increase the mortality rate of agricultural pests Also, test and assessment of other types of nematodes and fungi in *P. operculella* control and combined use them to increase the mortality rate of agricultural pests as use them in integrated pests' management.

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