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Efficacy of Entomopathogenic Nematode, *Steinernema carpocapsae* and its Interaction with *Beauveria bassiana* against *Pieris rapae* L. (Lepidoptera: Pieridae)

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ABSTRACT

Pieris rapae L. is one of the most economically important pests of Brassicaceae causing extensive defoliation of plants. Entomopathogenic fungi and nematodes are promising biological control agents. The virulence of each of them tested alone against 4th instar larvae and pupa of *P. rapae*. The entomopathogenic fungi, Beauveria *bassiana* showed efficiency against 4th instar larvae and pupae with LC_{50}: $28.857x10^3$ and $175.406x10^3$ spore/ml, respectively. Also, the entomopathogenic nematode, *Steinernema carpocapsae* was revealed efficacy against 4th instar larvae and pupae with LC_{50}: 18.148 and 38.96 IJs/larva and pupa, respectively. In addition, the interaction of *S. carpocapsae* with *Beauveria bassiana* against *P. rapae* larvae was studied. An antagonistic effect was clearly seen when *S. carpocapsae* nematode was applied immediately after the *B. bassiana*. However, the synergistic or the additive effect was seen when the application of the EPNs 12h after their treatment with *B. bassiana*.

Keywords: *Pieris rapae*, *Beauveria bassiana*, *Steinernema carpocapsae*, interaction

INTRODUCTION

The cabbage butterfly, *Pieris rapae* L. (Lepidoptera: Pieridae) is the most common pest of cruciferous crops including cabbage, cauliflower, broccoli, brussel sprouts and others. The larvae are herbivorous causing extensive defoliation and even kill the plant. Also, it contaminates plants with large quantities of faces (Hill, 1987 and Alford, 1990).

The extensive use of synthetic insecticides resulted in many problems to the environment, human health and non-target organisms (Sifakis *et al.* 2011 and Fantke *et al.* 2012). Biological control offers many alternative agents to the traditional insecticides.

Entomopathogenic fungi offered themselves as promising biocontrol agents for insect pest control (Gottel *et al*., 1990 and Ferron *et al*., 1991) due to their good epizootic and over dependence on suitable environmental factors. In addition, they don't have to be ingested by the insect host but can invade by contact with the insect cuticle (Boucias *et al*., 1988).

Also, entomopathogenic nematodes especially in families Steinernematidae and heterorhabditidae can be used as biological control agents (Gaugler, 1981; Kaya, 1985 a; Poinar, 1986; Gaugler and Kaya, 1990; and Kaya and Gaugler, 1993). In spite of steinernematid and heterorhabitid nematodes can parasitize broad host range of economic insect pests, they show no mammalian pathogenicity (Gaugler and Boush, 1979; and Boemare *et al*., 1996). The present study was carried out to evaluate the pathogenicity of entomopathogenic fungi, *Beauveria bassiana* and *Steinernema carpocapsae* nematode as biological control agents against *P. rapae* and also studying the interaction between them in case of combined application of them.

MATERIALS AND METHODS

Entomopathogenic Fungi

Wettable powder formulation of *Beauveria bassiana* was provided from Insect Pathogen Production Unit (IPPU), at Plant Protection Institute, ARC, Ministry of Agriculture, Egypt. It was grown on Sabouraud dextrose yeast extract agar (SDYA) [10g/l peptone, 40g/l dextrose, 10g/l yeast extract and 20g/l agar] and incubated at 25± 2C° and 80 ±5% RH until further growth. The spores were harvested and counted, then the tested fungal concentrations were prepared.

Entomopathogenic Nematodes

Infective juveniles (IJs) of *Steinernema carpocapsae* were obtained friendly from Plant Protection Institute, ARC, Egypt.

Rearing of *Galleria mellonella* and nematode propagation

The greater wax moth larvae of *Galleria mellonella* L. were used as a host to produce progeny of the tested nematode. The insect culture was reared in glass beakers closed with filter paper and a metal screen at 25±2 °C on an artificial diet (Ehlers, 2001). The mature females laid their eggs on the filter paper. Eggs hatched within 3-4 days and larvae were fed on the diet till reach the last instars within 5-6 weeks, then they were collected and inoculated with *S. carpocapsae* nematode and let nematodes to propagate inside them. Infective juveniles (IJs) were collected in White traps (White, 1927), then washed three times and stored in sterilized distilled water at 5°C for two weeks at the most.

Rearing of cabbage butterfly

A pure culture of *P. rapae* larvae was obtained from the farm of Faculty of Agriculture, Mansoura Univ. which is known that was free from any insecticides. The larvae reared on cabbage, *Brassica oleracea* var. capitata under plastic greenhouse conditions of 25±2 C°, 70±5 RH and 14h Light: 10h dark. They were let to develop to adults and gave new generations. The 4th instar larvae of *P. rapae* were used for the laboratorial bioassays

Laboratory bioassay

1. Sterilization of *P. rapae* tested stages and cabbage leaves

Cabbage leaves were sterilized by fast immersing in 70% alcohol then in sterilized water then 5% sodium hypochlorite for 90 seconds, then in three changes of sterile water (Clair *et al*., 1997). Plant leaves were dried and then used as a nutrition source.

Also, *P. rapae* 4th instar larvae and pupae were sterilized by 1% sodium hypochlorite and then washed by sterile water.

2. Susceptibility of *P. rapae* larvae and pupae to the entomopathogenic fungi, *Beauveria bassiana*

Every ten sterilized pupae or 4th instar larvae were transferred to glass jar (10x10x5 cm) containing sterilized cabbage leaf to provide larvae with nutrition, then sprayed with the fungal concentration then sealed and incubated at 22 ±2C°, 70± 5 % RH., and photoperiod 16 L: 8D. Each concentration had three replicates and another three replicates sprayed only with water and 0.05% aqueous Tween 80 to be considered as control. In case of larval treatment, cabbage leaves were replaced by another fresh ones after the first three days of the treatment to provide nutrition source. Mortality percentage were recorded daily and the experiment continued for seven days.

3. Susceptibility of *P. rapae* larvae and pupae to the entomopathogenic nematode, *S. carpocapsae*

Each Petri-dish lined with filter paper (Whatman No. 1) was inoculated with 2ml of nematode species at the tested concentrations. Ten larvae with a piece of cabbage leaf and/or ten pupae were transferred into the petri-dish, then sealed and incubated at 22±2 C°, 70± 5 RH and 14h Light: 10h dark. Each concentration represented by three replicates and another three as control. Mortality percentage was recorded daily along the experimental period (7 days).

Combination of *S. carpocapsae* with the entomopathogenic fungi, *B. bassiana* against *P. rapae* larvae

To determine the compatibility of *B. bassiana* with *S. carpocapsae*, ten *P. rapae* 4th instar larvae with a piece of cabbage leaf were sprayed with LC_{50} of *B. bassiana* then immediately or after 12 hours, they placed onto glass jar lined with filter paper already had been inoculated with LC_{50} of *S. carpocapsae* and maintained under experimental conditions at 22 ± 2 °C, 70 ± 5% RH and a (14: 10) L: D hour photoperiod. They daily observed and the mortality percentages were recorded. The remaining living larvae were let to pupate and continue their development.

Statistical analysis

The average of mortality percentages of both larval and pupal stages were estimated and corrected using Abbott's formula (1925), then, calculated according to Finney (1971). The corresponding concentration probit lines (LC-p lines) were estimated in addition to determination of LC_{50}, LC_{90}, slope values and the efficiency of tested pathogens using Sun's equation (1950).

RESULTS AND DISCUSSION

Efficiency of the *B. bassiana* fungi against 4th instar larvae and pupae of *P. rapae*

Data in Table 1 and Fig. 1&2 showed that the cumulative mortality percent increased with increasing concentrations of the tested fungi. *B. bassiana* didn't reveal activity rather against *P. rapae* larvae nor pupae at the first day of application but the mortality percent increased with increasing the time elapsed after treatments of different concentrations of the tested fungi. This slow action due to the nature mechanism of fungi which depends on the time intervals for tissues invasion, consumption of the body of the host insect and accumulation of toxins (Ibrahim, 2012). Also, it showed more slow action against pupae this may be due to the chitinized sheath of pupa which require more time for invading it by the entomopathogenic fungi. Both of LC_{50} and LC_{90} values were obtained from probit analysis for mortality values. It was clear that larvae were more susceptible than pupae. It showed LC_{50}: $28.857x10^3$ spore/ml, whereas, pupae showed LC_{50} of $175.406x10^3$ spore/ml.

Table 1. Efficiency of the *B. bassiana* fungi against 4th instar larvae and pupae of *P. rapae* under laboratory conditions of 22 ± 2 C0, 70 ± 5% RH.

Treatment	Conc. (spore /ml)	Mortality %at indicated day after treatment.				LC_{50} (spore/ml) and confidence limits at 95%		LC_{90} (spore/ml) and confidence limits at 95%		Slope
		1 day	3 day	5day	7day					
						4th instar larvae				
B. bassiana	$16x10^3$	0	16.67	40.00	46.67	$28.857x10^3$		$31.66383x10^5$		0.6281± 0.1352
	$16x10^4$	0	23.33	50.00	63.33					
	$16x10^5$	0	36.67	93.33	96.67	$4.398x10^3$	$81.805x10^3$	$9.747x10^5$	$33.9773x10^6$	
	$16x10^6$	0	56.67	93.33	96.67					
						Pupae				
	$16x10^3$	0	0	10.00	30.00	$175.406x10^3$		$380.759x10^5$		0.5485± 0.1161
	$16x10^4$	0	0	16.67	46.67					
	$16x10^5$	0	0	30.00	70.00					
	$16x10^6$	0	0	36.67	86.67	$45.579x10^3$	$486.406x10^3$	$76.650x10^5$	$1277.083x10^6$	

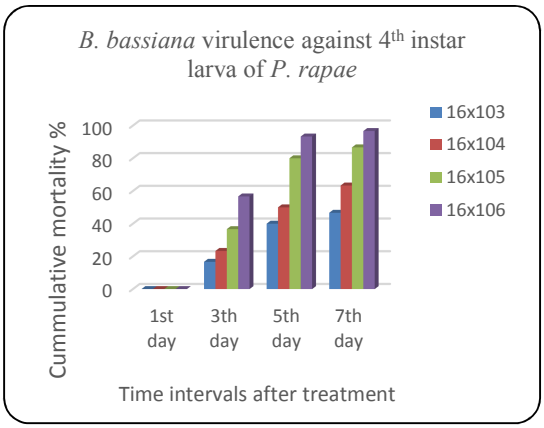

Fig. 1. Virulence of *B. bassiana* against *P. rapae* 4th instar larvae under laboratory conditions of 22 ± 2^0 C, 70 ± 5% RH.

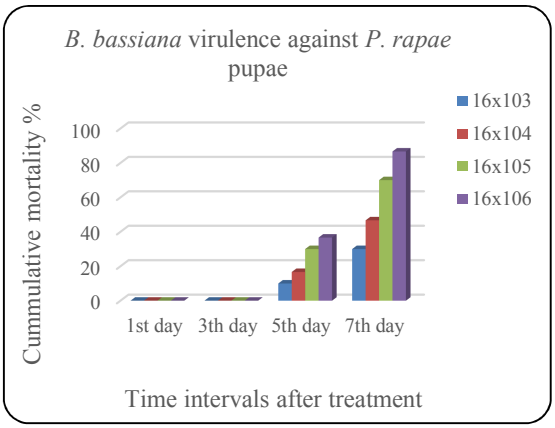

Fig. 2. Virulence of *B. bassiana* against *P. rapae* pupae under laboratory conditions of 22 ± 2^0 C, 70 ± 5% RH.

Efficiency of the *S. carpocapsae* nematode against 4th instar larvae and pupae of *P. rapae*

Data in Table 2 and Fig. 3&4 showed that the cumulative mortality percent increased with increasing concentrations of the tested nematodes. *S. carpocapsae* revealed activity within few hours after treatment of larvae and pupa. The 4th instar larvae showed more susceptibility than pupae with LC_{50}: 18.148 and 38.96 IJs/larva, respectively. The infective juveniles (IJs), actively seek out hosts and penetrate the insect body

usually through natural openings, then invade the host haemocoel releasing their symbiont bacteria, causing septicaemia and, ultimately, killing the host (Forst and Clarke, 2002).

Table 2. Efficiency of the *S. carpocapsae* nematode against 4th instar larvae and pupae of *P. rapae* under laboratory conditions of 22 ± 2 C0, 70 ± 5% RH.

Treatment	Conc. (IJs/ larva)	Mortality %at indicated day after treatment.			LC_{50} (IJs/ larva) and confidence limits at 95%		LC_{90} (IJs/ larva) and confidence limits at 95%		Slope
		1 day	3 day	5day					
	4th instar larvae								
	10	6.67	33.33	36.67					
	20	13.33	33.33	43.33	18.148		71.163		2.1597± 0.4135
	40	23.33	60	73.33					
	80	40	90	96.67	12.623	23.729	48.878	147.494	
S. carpocapsae	Pupae								
	25	20.00	30.00	33.33					
	50	23.33	40.00	53.33	38.96		101.92		3.0688± 0.5856
	75	40.00	70.00	76.67					
	100	36.67	83.33	96.67	29.40	47.22	79.339	163.72	

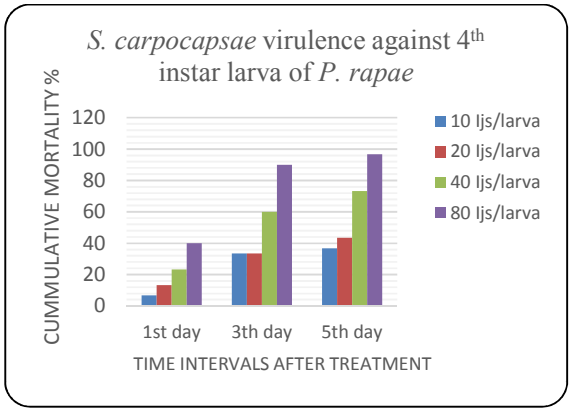

Fig. 3. Virulence of *S. carpocapsae* against *P. rapae* 4th instar larvae under laboratory conditions of 22 ± 2⁰ C, 70 ± 5% RH.

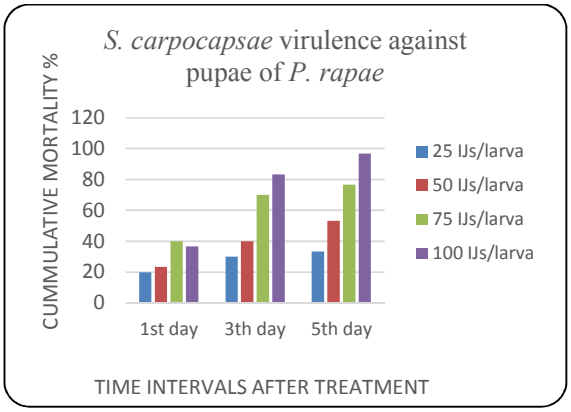

Fig. 4. Virulence of *S. carpocapsae* against *P. rapae* pupae under laboratory conditions of 22 ± 2⁰ C, 70 ± 5% RH

Interaction between *B. bassiana* and *S. carpocapsae*

The present study evaluated the combined effects of the tested entomopathogenic nematode with the entomopathogenic fungi *B. bassiana* on the 4th instar larvae of *P. rapae*. Data in Fig. 5&6 showed that the results were entirely depended on the time interval. A simultaneous combination of both *S. carpocapsae* and *B. bassiana* caused marked antagonistic effect. This antagonistic effect may be due to the nature of each agent mechanism. In contrast, the combination of *B. bassiana* with *S. carpocapsae* after 12hrs caused additive and synergistic effects on the 4th instar larvae due to the stress effect caused by *B. bassiana*. There was higher efficacy of the combined application than when either the nematode or the fungus was used alone. This agreed with Abdolmaleki *et al*., (2017) they illustrated the additive or synergistic effects of entomopathogenic nematode 24 h after treatment, but the antagonistic effect was seen when the EPNs were applied immediately after the *B. bassiana*.

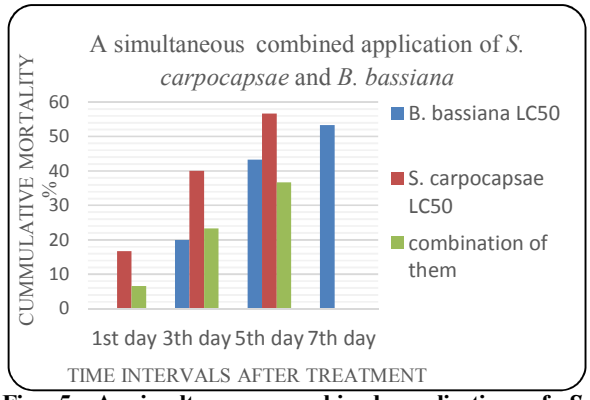

Fig. 5. A simultaneous combined application of *S. carpocapsae* and *B. bassiana* on P, rapae 4th instar larvae under laboratory conditions of 22 ± 2⁰ C, 70 ± 5% RH

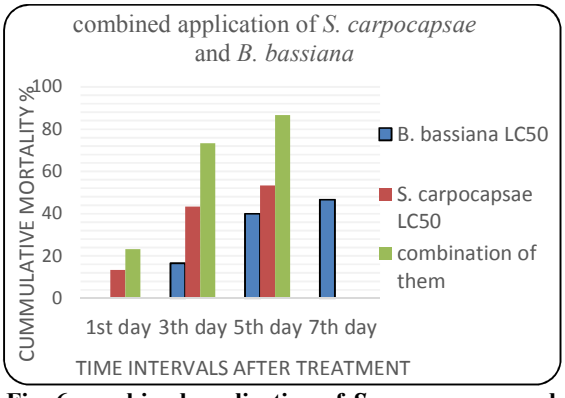

Fig. 6. combined application of *S. carpocapsae* and *B. bassiana* on *P, rapae* 4th instar larvae after 12 hrs. under laboratory conditions of 22 ± 2⁰ C, 70 ± 5% RH

Also, it was noticed that the treated larvae which didn't seem to be infected, they developed to the pupal stage but couldn't develop to the adults. This may be due to the action of one of the agents. Entomopathogenic nematodes

have several deleterious effects on their hosts including sterility, reduced fecundity, longevity and flight activity, delayed development, or other behavioral, physiological and morphological aberrations and in some cases, rapid mortality. (Vashisth *et al*., 2013).

In conclusion, the combination of the entomopathogenic nematode, *S. carpocapsae* with *B. bassiana* on *P. rapae* increase their efficiency with taking into consideration the inoculation time required for fungi to establish themselves into the insect body.

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كفاءة النيماتودا الممرضة للحشرات *Steinernema carpocapsae* و فطر *Beauveria bassiana* ,وتفاعلهما لمكافحة أبو دقيق الكرنب *Pieris rapae* L.

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تعتبر حشرة أبو دقيق الكرنب واحدة من أهم الحشرات الاقتصادية التي تصيب عائلة الكرنبيات مسببة الفقد الحاد في أوراق النبات. وتعتبر الفطريات والنيماتودا الممرضة للحشرات من أهم العوامل الواعدة في المكافحة الحيوية للحشرات لذلك تم اختبار القدرة الامراضية لكل من فطر *Beauveria bassiana* و نيماتودا *Steinernema carpocapsae* كل على حدى ضد الطور الرابع من يرقات وعذاري ابو دقيق الكرنب ووجد ان فطر البيوفاريا يعطي كفاءة جيدة على كليهما ب LC_{50}: LC_{50} : $28.857x10^3$ and $175.406x10^3$ spore/ml للبرقات والعذاري على التوالي. أيضا النيماتودا أعطت كفاءة عالية ب 18.148 and 38.96 IJs/larva and pupa للبرقات والعذاري على التوالي. أيضا تم اختبار التفاعل بين الفطر والنيماتودا وذلك برشهما سويا في نفس التوقيت على اليرقات أو بفاصل زمني 12 ساعة. ولقد وجد أن التطبيق المتزامن يسبب تضاد في عملهما مؤثرا على كفاءتهما بينما الفاصل الزمني بين استخدامهما يعطي اضافة وزيادة في الكفاءة الابادية.