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Efficacy of *Beauveria bassiana* (Blas.) Vuill. against cabbage aphid *Brevicoryne brassicae* L. (Hem.: Aphididae) in laboratory condition

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In order to replace the conventional chemical pesticides, extensive researches have been done on entomopathogenic fungi. Entomopathogenic fungus *Beauveria bassiana* is an important biocontrol agent against major economic pests and is being employed in Integrated pest management (IPM) along with synthetic pesticides. Cabbage aphid *Brevicoryne brassicae* L. is one of the important pests of Brassicaceae family. Therefore, in this research, the virulence isolate of *B. brassicae* (IRAN 429C) was investigated on adults of cabbage aphid under laboratory conditions. The experiments were conducted at 25 ± 2 °C, 60 ± 10 R. H. and a photoperiod of 16:8 (L: D). After preliminary experiments, the adult aphids were treated with fungal concentrations of 1×10^3 to 1×10^7 spores/ml. Probit analysis was conducted to calculate LC_{50} and LC_{95} values for the isolate. Positive correlation was observed between concentrations and pest mortality. LC_{50} and LC_{95} values calculated for IRAN 429C isolate are 2.04×10^5 and 1.82×10^8, respectively. The mortality was counted one day after the treatment and then continued for 14 days. Cumulative mortality for 14 days after treatment varied from 54% for IRAN 429C at low concentration (10^3 conidia/ml) to 83% at high concentration (10^7 conidia/ml). The lowest LT_{50} was obtained at 7.67 days for IRAN 429C isolate at concentration 1×10^7 spore/ml. According to the insecticidal activity of mentioned fungi on cabbage aphid, it can be used in biocontrol programmes of *B. brassicae*.

Keywords: *Beauveria bassiana*; *Brevicoryne brassicae*; aphids; biological control; entomopathogenic fungi; LC_{50}; LT_{50}

Introduction

Crop protection plays a paramount and integral role in modern day agricultural production. Indiscriminate use of synthetic pesticides results in drastic problems such as environmental pollution, and health hazards, etc. in humans. One of the proven technically and commercially sustainable options for crop protection is the use of biocontrol agents. *Beauveria bassiana* (Bals.) Vuill. is one of the well understood and widely used entomopathogenic fungi (Eleanor & Lockwood, 1991).

At present, aphids are mostly controlled by insecticidal applications. Indiscriminate use of insecticides has resulted in problems such as the development of resistance in this pest against pesticides, pest resurgence and adverse effect on biocontrol agents, environmental pollution and accumulation of toxic residues in the natural ecosystem. Biological control is an alternative control method for insect pests.

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Entomopathogenic fungi are of great importance in microbial control. Entomopathogenic fungi can provide long-lasting insect control without damage to the environment or non-target organisms (Khetan, 2001).

Entomopathogenic fungi are lethal to insects, and at present these fungi are used as biocontrol agents for insects. The known entomopathogenic fungi, such as *Beauveria*, can kill insect pests of the order Lepidoptera (Soetopo, 2004), Coleoptera (Lord, 2001; Wraight & Ramos, 2002) and Homoptera (Wraight et al. 1998). Entomopathogenic fungi are pathogenic to insects and are widely used as biocontrol agents for insect pests. The aim of this research was to study the virulence of *B. bassiana.*

Despite of many studies in other countries, there are few studies on the control of aphids with entomopathogenic fungi in Iran. The present study was conducted to select highly virulent fungal isolates against cabbage aphid.

Materials and methods

Insect

The colonies of *B. brassicae* were maintained on cabbage plants The plants were maintained at temperature 25 ± 2 °C, relative humidity 60 ± 10 and photoperiod 8:16 (dark: light).

Ten aphids were transferred to cabbage plants for reproduction, after 24 h, adults aphids were removed from cabbage and newborn nymphs that were even-aged were kept in a breeding room to develop. The nymphs which matured were used for testing.

Fungi

The fungi were cultured at 25 ± 1 °C on SDA (Sabouraud Dextrose Agar) for two weeks. Then, it was left to generate spores. The concentration of fungi that cultured, spores with sterile scalpel were scratched and transferred to testtube with sterile distilled water containing 0.05% Tween 80, the tubes were tightly closed and the tubes were shaken well and then, were passed through several layers tiffany to filtered conidial suspension to remove hyphal debris. For conidial concentration, Neubaurer haemoytometer was used, and the spore concentration was determined by the addition of sterile distilled water, which was provided to the original suspension.

Bioassay

Serial dilutions (1×10^3–1×10^7 conidia/ml) of each fungi isolate was prepared. Each concentration was considered for one treatment. The detached leaf method was used for the treatment of aphids with conidial concentrations. The cabbage leaves were sterilised, and then the leaves were placed in plastic Petri dishes. Ten adult aphids were dipped in each concentration for 10 s and kept on each leaf. The aphids of the control were treated with 0.05% Tween 80. The experiment was conducted with 10 replications for each treatment and mortality of aphids was recorded daily up to 10 days.

Results

The results show that the concentration of conidia affected the mortality of aphids differently ($p < 0.001$). The LC_{50} of this fungal isolate against the adults of aphids 14 days after conidial treatment was determined to be 2.04×10^5 conidia/ml (Table 1).

Table 1. LC_{50}, LC_{95} values of IRAN441C isolate against *B. brassicae* adults.

LC_{95}	LC_{50}	Slope ± SE	Intercept (a)+5	*p*	d*f*	Insect species
1.82×10^8 (5.76×10^7,8.84×10^8)	2.04×10^5 (1.23×10^5, 3.48×10^5)	0.558 ± 0.048	2.036	0.932	3	*B. brassicae*

Table 2. Effect of conidial concentration of *B. bassiana* (IRAN441C) against *B. brassicae* adults.

Classify to groups with Tukey test in statistic level 1%					
Group E	Group D	Group C	Group B	Group A	Concentrations (Spore/Ml)
15.07					10^3
	29.01				10^4
		42.6			10^5
			52.59		10^6
				65.87	10^7

Table 3. LT_{50} values of IRAN441C isolate against *B. brassicae* adults.

LT_{50}	Slope ± SE	Intercept (a)	χ^2	*p*	d*f*	Concentrations (Spore/Ml)
7.67 (7.31–8.05)	3.70 ± 0.20	1.72	3.67	0.98	12	10^7
9.37 (8.86–9.96)	3.14 ± 0.20	1.94	8.13	0.77	12	10^6
11.16 (10.50–11.97)	3.26 ± 0.22	1.59	3.41	0.992	12	10^5
12.35 (11.63–13.27)	3.78 ± 0/27	0.86	5.09	0.95	12	10^4
13.24 (12.43–14.33)	3.98 ± 0.30	0.53	10.28	0.59	12	10^3

The mortality increased from day 12 to 14. The cumulative mortality caused by *B. bassiana* (IRAN429C) at the end of the observation (day 14) was 65.87–15.07 (Table 2).

The mortality increased with increase in spore concentration and exposure time.LC_{50} and LT_{50} studies revealed dose-dependent mortality of *B. brassicae*. The respective estimated LT_{50} values of IRAN 429C for *B. brassicae* adults at 10^7, 10^6, 10^5, 10^4 and 10^3 conidia/ml were 7.67, 9.37, 11.16, 12.35 and 13.24 days, respectively (Table 3).

Discussion

Entomopathogenic fungi have been observed to cause mortality in pest populations and thus, investigated for their potential as biological control agents (Hesketh et al. 2008) or successfully developed as biocontrol agent for a number of different pests, including aphids (Shah & Pell, 2003; De Faria & Wraight, 2007).

Different concentrations of fungal isolate were tested against the adults of aphids. The concentration of conidia affected the mortality of aphids differently ($p < 0.001$). The LC50 of the fungal isolate *B. bassiana* (IRAN429C) was observed to be 2.04×10^5 conidia ml^{-1},14 days after inoculation against the aphid. Previous studies demonstrated that conidia of *V. lecanii* were highly pathogenic against aphids (Fournier

& Brodeur, 2000; Kim et al. 2001).The LC50 of *V. lecanii* (VL10) against *Myzus persicae* was observed to be 1.65×10^6 conidia ml^{-1}.

After six days of treatment (Yokomi & Gottwald, 1988), Vu et al. (2007) also reported that *V. lecanii* (41,185) with LC50 value of 6.55×10^5 conidia ml^{-1} and its other strains were more lethal to aphid than fungal isolates of *P. farinosus* and *M. anisopliae*.

Time-dose dependent mortality response experiments were designed as a measure of mortality of different fungal isolates against aphids. The mortality observed was low on day 1 and 2, after the treatment the mortality then dramatically increased from day 7 to 9. The mortality in infected aphids with fungal isolate increased with increase in spore concentration of conidial suspensions and exposure time. The susceptibility of target insect to fungal infection is dose dependent (Liu et al. 2002; Wright et al. 2005).

Ansari et al. (2004) also found that the mortality depended on the concentration of conidial suspension, exposure time and temperature. The susceptibility of same aphid species may vary to different fungal strains. Even biotypes or different colons of the same aphid species may have varying susceptibility to fungal infection (Blanford et al. 2003).

The results (Table 2) show that mortality caused by concentration of 1×10^7 conidia ml^{-1} of fungi isolates was significantly higher ($p < 0.001$) than those of 1×10^5, 1×10^4 and 1×10^3 conidia ml^{-1}. Therefore, concentration of 1×10^7 conidia ml^{-1} is the recommended concentration to control aphids (Vu et al. 2007).

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