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Research Article

Evaluation of *Beauveria bassiana* (Balsamo) Vuillemin against coriander aphid, *Hyadaphis coriandri* (Das) (Aphididae: Homoptera)

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ABSTRACT: Laboratory bioassays were carried with seven different concentrations of *Beauveria bassiana* against *Hyadaphis coriandri* by using detached leaf bioassay technique with slight modifications. The mortality in treated aphids increased with increase in conidial spore concentration. The highest concentration ($1x10^{10}$ conidia ml^{-1}) caused maximum cumulative mortality (96.85%) at 7 days after treatment compared to the lowest concentration (1×10^4 conidia ml^{-1}) with 67.61 per cent mortality. The median lethal concentration (LC_{50}) value recorded with *B. bassiana* was $1.5x\ 10^4$ conidia ml^{-1} in mixed *H. coriandri* population. Median lethal time (LT_{50}) values were found to be inversely proportional to the spore concentration of *B. bassiana* and were observed as 45.70, 67.60, 71.30, 87.04, 97.72, 120.26 and 141.57 h for 10^{10}, 10^9, 10^8, 10^7, 10^6, 10^5, 10^4 conidia ml^{-1}, respectively.

KEY WORDS: *Hyadaphis coriandri*, *Beauveria bassiana*,bioassay, LC_{50}, pathogenicity, LT_{50}

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INTRODUCTION

Coriander is grown both for green vegetable as well as seed purposes. The seeds and leaves contain essential oil, which accounts for aromatic character of the plant (Sankracharya and Sankaranarayana, 1989). The powdered seeds of coriander are used as condiments and are invariably a major constituent of curry powder employed for flavouring curries, soups, and also used in pickles and spices. Coriander is being attacked by many sucking insect pests *viz.,* aphids, thrips and whitefly, however, coriander aphid, *Hyadaphis coriandri* (Das) is a major pest and responsible for reduction to crop yield (Butani *et al*., 2009). Both nymphs and adults suck plant sap and cause serious damage right from vegetative stage to ripening stage and during flowering yield reduction at great extent observed. Due to heavy infestation, young seedling succumb to death whereas, the older plants show symptoms such as stunting, crinkling and curling of leaves, delayed flowering, shrinking of pods and seeds and finally resulting in heavy yield loss. The damage due to infestation of *H. coriandri* was estimated to be around 18.9% (Meera *et al.,* 2011).

Since, coriander is consumed as leafy vegetable, spraying chemical insecticides is generally not advisable, keeping the adverse effect on human and environmental health in mind. Pest management through biointensive integrated pest management using predators, parasites and different entomopathogens is being encouraged for effective control of *H. coriandri*. Among the different microbial agents, entomopathogenic fungi are gaining importance in recent past for the control of both chewing and sucking pests. More than 750 species of fungi are pathogenic to insects and many of them offer a great potential for the management of sucking insect pests in different crop plants (Rabindra and Ramanujam, 2007). The present study was conducted to evaluate the pathogenicity of *B. bassiana* against *H. coriandri* on coriander plant.

MATERIALS AND METHODS

The experiments were conducted during October, 2007 to March, 2008 in the biocontrol laboratory of Department of Entomology, CCS Haryana Agricultural University, Hisar.

Insect cultures

Nymphs of *H. coriandri* were collected from unsprayed field of coriander and reared in the laboratory conditions. The culture was maintained in an incubator at 25±2°C, >90% relative humidity and 16:8h light: dark.

Beauveria bassiana culture

The culture of HaBa (Hyderabad) strain was raised on Potato Dextrose Agar slants in 250 ml conical flasks following the standard method (Vimala Devi, 2005). Regular passing through the insect hosts was done for

further multiplication and maintenance at 25±2°C, >90% relative humidity and 16:8h light: dark. The virulence of the fungal isolate was maintaines by frequent subculturing often every 5–6 subculturing.

Preparation of *B. bassiana* suspension

Aqueous conidial suspension was made from conidia harvested from the slants preferred in conical flasks after 14 days of inoculation. Tween 80 (0.02%) was used to disperse the conidia uniformly in the solution. The conidial suspension was filtered through double layer muslin cloth to remove the mycelial mat. A suspension of $1x10^{10}$ ml^{-1} conidia concentration was made using haemocytometer counts. The lower conidial concentrations were obtained from the serial dilutions technique for bioassay studies.

Bioassay studies

Bioassay of *B. bassiana* against *H. coriandri* was done under *in vitro* conditions following detached leaf bioassay technique (Yokomi and Gottwald, 1988) with slight modifications. A heavily infested host leaf (plate 1) with all the stages of *H. coriandri* was dipped gently in different spore suspensions (10^{10}, 10^9, 10^8, 10^7, 10^6, 10^5 and 10^4 conidia ml^{-1}) for 2-3 seconds, drained and shade dried completely. The petioles of leaves/ umbels were inserted immediately into water to maintain turgidity of plants and placed inside a bigger container covered with muslin cloth. This whole set up was kept inside a growth chamber for 24h at 25°C and 90 per cent relative humidity.

For further maintenance, inoculated aphids were transferred to clean and fresh leaves/umbels of coriander rinsed initially with distilled water and then dipped in sodium hypochlorite (0.25%) solution for two minutes followed by two rinses in distilled water. After complete drying under aseptic conditions, the clean and fresh leaves of coriander were put individually in Petri plates containing sterilized agar medium (1%). To avoid bacterial contamination, streptomycin sulphate @ 100 ppm was added to the medium before pouring into sterile plates. After 1h of fungal inoculation, 25 live aphids per replication were transferred on leaves/umbels placed over agar medium. The Petri plates were then sealed with parafilm to avoid escape as well as to enhance the settlement of aphids on leaves and maintained in an incubator at 25°C. The newly hatched nymphs were removed from the Petri plates aseptically in laminar flow chamber to avoid confusion in counting. The cadavers showing mycosis were considered to be dead as a result of infection by the fungus. Each treatment was replicated four times. Mortality of aphids was recorded separately at 24 h intervals for seven days. To determine LT_{50} values, the observations on aphid mortality were recorded at 12h interval for 7 days. Mortality data was corrected with that in control by using the Abbott's formula (Abbott, 1925).

Statistical analysis

One-way analysis of variance (ANOVA) was conducted on the mortality data to test the level of significance between the treatments. To assess virulence of the strain, full logarithmic plots of insect mortality against concentration was plotted assuming the probit mode (Finney, 1971). Log concentration, probability regression (including a control mortality correction as an offset for natural mortality) was estimated using probit analysis in each case. These equations allowed us to determine the LC_{50} of the test stage of the insect and LT_{50} of the test insect.

RESULTS AND DISCUSSIONS

Among microbial insecticides, *B. bassiana* was recognized as an important tool for suppression of the several pests (Ferron *et al.,* 1991). The results revealed dose dependent responses in the bioassays with *B. bassiana* were not weaker as evidenced in lower slope values. Shallow dose mortality responses seem to be typical for fungus insect interactions (Prasad *et al.,* 1989). The cumulative corrected mortality was 49.52 per cent at higher concentration ($1x10^{10}$ conidia ml^{-1}) which was 30.91 per cent at low concentration ($1x10^4$ conidia ml^{-1}). The cumulative corrected mortality 96.85 per cent at highest concentration ($1x10^{10}$ conidia ml^{-1}) on seven days after treatment (DAT) whereas at lowest concentration ($1x10^{10}$ conidia ml^{-1}), it was 67.61 percent. It is evident from the data mortality decreased with decreased conidial concentration. As the concentration of conidia of *B. bassiana* increased from 1×10^4 to 1×10^{10} ml^{-1}, the mortality also increased from 30.91 to 49.52 percent. At 7 DAT, the cumulative corrected mortality reached the highest and was 67.61 and 96.85 per cent at $1x10^4$ conidia ml^{-1} and $1x10^{10}$ conidia ml^{-1} respectively. These findings are in agreement with the finding of Vandenberg *et al.,* (2001) who reported that *B. bassiana* causing about 60 per cent mortality in Russian wheat aphid, *Diuraphis noxia* at concentration $1x10^8$ conidia/ml. Similar studies were conducted by Nirmala *et al.,* (2006) which revealed that the isolates of *B. bassiana, M. anisopliae* and *V. lecanii* were pathogenic to *Aphis craccivora, Aphis gossypii* and *Rhophalosiphum maidis* at a concentration of 1×10^7 spores/ml.

The results of the probit analysis including chi-square values, regression coefficients and LC_{50} values are shown in Table 2. The chi-square values indicated good fit of probit regression and absence of heterogeneity in the tested population of the insect pest. Dose dependent responses in the bioassays with *B. bassiana* were not pronounced as evidenced in lower slope values. From the results an inverse relationship was observed between aphid population and its susceptibility to the *B. bassiana*, which was evident from the LC_{50} values.

Table 1. Cumulative corrected percent mortality of *Hyadaphis coriandri* by *Beauveria bassiana* under laboratory conditions

Concentration (spores/ml)	Cumulative corrected percent mortality						
	1DAT*	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT
$1x10^{10}$	49.52 (44.69)	66.28 (54.46)	75.92 (60.30)	79.24 (62.10)	82.27 (65.27)	83.78 (66.22)	96.85 (79.21)
$1x10^{9}$	46.58 (42.83)	61.76 (51.62)	70.66 (57.23)	75.25 (60.30)	80.28 (63.77)	81.78 (64.68)	92.85 (73.82)
$1x10^{8}$	40.60 (39.36)	59.27 (50.45)	64.15 (53.25)	72.48 (58.34)	76.50 (60.97)	78.75 (62.35)	88.85 (70.15)
$1x10^{7}$	38.11 (38.18)	57.87 (49.43)	62.00 (52.07)	67.54 (55.06)	68.25 (55.83)	71.42 (57.71)	79.22 (63.23)
$1x10^{6}$	35.86 (36.40)	53.00 (46.84)	56.32 (48.57)	63.91 (52.81)	64.95 (53.55)	66.74 (54.61)	71.57 (57.71)
$1x10^{5}$	32.65 (34.88)	49.75 (44.83)	52.57 (46.41)	60.64 (51.04)	61.92 (51.77)	63.42 (52.81)	69.76 (56.61)
$1x10^{4}$	30.91 (33.66)	38.16 (38.33)	40.50 (39.79)	54.50 (47.56)	60.05 (51.04)	60.82 (51.04)	67.61 (55.22)
S.Em.±	(0.382)	(0.256)	(0.237)	(0.240)	(0.324)	(0.365)	(0.338)
CD (*P*=0.05)	(1.131)	(0.757)	(0.703)	(0.712)	(0.958)	(1.082)	(1.001)

Table 2. Probit analysis of concentration – mortality response of coriander aphid, *Hyadaphis coriandri* to *Beauveria bassiana* under laboratory conditions

Aphid	Chi-square	Regression equation	LC_{50} (spores/ml)
Hyadaphis coriandri	0.925 NS	Y= 2.2956+0.54X	1.5×10^{4}

NS = Non-significant

Table 3. Probit analysis of concentration – mortality response of exposure time, *Hyadaphis coriandri* to *Beauveria bassiana* under laboratory conditions

Concentration (Spores/ml)	Time mortality response		
	Chi-square	Regression equation	LT_{50} (hrs.)
$1x10^{10}$	10.19NS	Y= 4.057+1.42X	45.70
$1x10^{9}$	4.22 NS	Y= 2.345+1.75X	67.60
$1x10^{8}$	7.70 NS	Y= 3.518+1.73X	71.30
$1x10^{7}$	6.74 NS	Y= 3.259+1.83X	87.04
$1x10^{6}$	9.71 NS	Y= 5.000+1.33X	97.72
$1x10^{5}$	7.45 NS	Y=3.765+1.11X	120.26
$1x10^{4}$	7.42 NS	Y= 4.095+0.78X	141.57

NS = Non-significant

No information is available on the LC_{50} and LT_{50} value of *B. bassiana* to *H. coriandri*. However, some of the related studies recorded LC_{50} values of different strains of *B. bassiana* (CPD 11 and Bb5a) as $1x10^{10}$ conidia/ml (Ekasi *et al.,* 2000) and 6.57 x 10^5 conidia/ml (Nirmala *et al.*, 2006), respectively, for *A. gossypii* nymphs. Butt *et al.* (1999) found that the LC_{50} value of *B. bassiana* was $1x10^{10}$ conidia/ml on green peach aphid, *Myzus persicae*, while during present investigations, the LC_{50} value of *B. bassiana* was 1.5 x 10^4 spores/ml to *H. coriandri* and these finding are very close agreement with that of Liu *et al.* (1999) who reported the LC_{50} values for six aphid derived *B. bassiana* isolates against *M. persicae* to range from 1.2 x 10^4 – 1.55 x 10^6 conidia/ml.

Earlier Miranpuri and Khachatourians (1995) revealed that LT_{50} values of *B. bassiana* ranged from 3-5.4 days on English grain aphid, *Sitobion avenae*, However, during present studies the LT_{50} values of *B. bassiana* were 45.70, 67.60, 71.30, 87.04, 97.72, 120.26 and 141.57 hours at 10^{10}, 10^9, 10^8, 10^7, 10^6, 10^5, 10^4 spores/ml, respectively. These findings are in agreement with that of Nirmala *et al.,* (2006) who reported that the LT_{50} value was highest 9.67 days at lowest dose (10^6 spores/ml) and lowest (1.76 days) at the highest dose ($1x10^9$ spores/ml) while, Vandenberg (1996) reported that the LT_{50} for *B. bassiana* ranged from 4.6 to 12.2 days on *D. noxia* at concentration of $1x10^8$ spores/ml.

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