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# Greenhouse evaluation of *Beauveria bassiana* (Balsamo) Vuillemin against *Aphis craccivora* (Das) on Fenugreek

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**Abstract:** The bioassay studies were carried out to determine the $LC_{50}$ and $LT_{50}$ of *Beauveria bassiana* against *Aphis craccivora* on fenugreek under greenhouse conditions. The results revealed that, the cumulative corrected mortality (CCM) was 43.50% at higher concentration ($1\times10^{10}$ spores/ml) and it was 20.85% at lowest concentration ($1\times10^{4}$ spores/ml) at one day after treatment (DAT). The CCM decreased with decreasing conidial spore concentration. Likewise, at 2, 3, 4, 5, 6 and 7 DAT, almost same trend was observed. At 7 DAT, the CCM was 85.04% and 55.21% at $1\times10^{10}$ spores/ml and $1\times10^{4}$ spores/ml, respectively. The $LC_{50}$ value of *B. bassiana* against *A. craccivora* was $1.2\times10^{8}$ spores/ml. Mean lethal time ($LT_{50}$) values were worked out 73, 89, 97, 112, 126, 138 and 157 hours for $10^{10}$, $10^{9}$, $10^{8}$, $10^{7}$, $10^{6}$, $10^{5}$ and $10^{4}$ spores/ml, respectively. By testing the field efficacy of *B. bassiana* against *A. craccivora*, this insect pathogenic fungus can be used as potential biocontrol agent for the sustainable management of aphid in fenugreek crop.

**Keywords:** *Aphis craccivora*, *Beauveria bassiana*, $LC_{50,}$ $LT_{50}$ value, Pathogenicity

## INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an important seed spice crop in India and mainly used as spice for flavouring almost all vegetable dishes and pickles. The fresh tender leaves and stems of this crop are consumed as curried vegetable (Yadev *et al.,* 2013). The green as well as mature stalks of fenugreek constitute a good fodder for farm animals, and also widely used as a green manure (Singh *et al.,* 2008). Besides, seeds reported to possess several medicinal properties (Meghwal and Goswani, 2012). The fenugreek leaf extracts have been reported to show a repellent activity against various stored grain insect pests (Farhana *et al.,* 2006), antifeedant property against some insect pests (Thomas *et al.,* 2002) and also have fungitoxic principle (AI-Rahmah *et al.,* 2013). Fenugreek crop is mainly attacked by aphid, alfalfa weevil, leaf miner. Among these insect pests, *Aphis craccivora* (Koch) was the most important in India and quite often proved as the yield limiting factors for the successful seed production. *A. craccivora* is not only as it causes quantitative and qualitative losses in the grain yield but also deteriorate the quality of green leaves by sucking cell sap and secreting honeydew. The honeydew secretion of the aphids provide suitable media for the development of the sooty moulds and fungi which ultimately hamper the process of photosynthesis. Yield losses caused by *A. craccivora* on fenugreek were even up to 68.80% (Sharma and Kalra, 2002; Deshwal, 2007).

At present, fenugreek aphid is mostly controlled by chemical insecticidal applications. Fenugreek leaves are often consumed as leafy vegetables. So the growing demand for reducing chemical inputs in fenugreek crop ecosystem and increased resistance to insecticides have provided great impetus to the development of alternative forms of aphid control in fenugreek crop. Moreover, it is a difficult pest to control with insecticides because of its polyphagous nature with very short life cycle and high reproduction rates. Major emphasis is given on biocontrol approaches in the present context of environmental safety. Biological control of insect pests using entomopathogenic fungi (EPF) are gaining importance due to their target specificity, self-perpetuity and obvious environmental safety. The use of fungal biological control agents is a rapidly developing field and is increasingly being adopted and accepted worldwide for management of agricultural pests (Hajek and Delalibera, 2010).

Fungi have been considered the principal group of aphid pathogens, the most prevalent and widely encountered species belonging to the order Zygomycetes. In particular environments (Greenhouse/tropical regions) Deutermycetous species also significantly reduce aphid numbers (Suresh *et al.,* 2012). Fungi are the only insect pathogens currently used for the control of aphids (Latge and Papierok, 1988) and species like,

*Beauveria bassiana*, *Fusarium pallidoroseum*, *Lacanicilium lecanii* and *Isaria fumosoroseus* were responsible for epizootics that often successfully regulate aphid population (Milner, 1997; Chen and Feng, 1999; Kim *et al.*, 2008). Therefore, *B. bassiana* could be more appropriate for management of insect pests with piercing sucking mouth parts which are unlikely to ingest microbes upon their feeding (Wraight and Carruthers, 2010). However, full potential of EPF is yet to be exploited for management of many aphid species infesting several cultivated crop plants. Keeping these in view, the present study was undertaken to study pathogenicity of *B. bassiana* against *A. craccivora* on fenugreek under greenhouse conditions.

## MATERIALS AND METHODS

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

**Culture of *Beauveria bassiana***: The culture of aphid specific *B. bassiana* HaBa (Hyderabad) strain was raised on potato dextrose agar slants in 250 ml conical flasks following the standard method (Vimaladevi, 2005). Regular passage was done for further multiplication and maintenance which was done at 25±2$^{o}$C, >90% relative humidity and 16:8h light: dark. The fungal isolate was sub-cultured once in a month and after every 5-6 sub-culturing again re-isolated for further studies to maintain the virulence of the strain.

**Preparation of *B. bassiana* spore 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 $1\times10^{10}$ conidia ml$^{-1}$ concentration was made using haemocytometer counts. The lower conidial concentrations were obtained from the serial dilutions technique for bioassay studies.

**Bioassay studies:** For bioassay studies 32 fenugreek plants were grown in pots (one plant/pot). All agronomic practices were practiced in greenhouse under semi natural conditions. The aphid culture was maintained until all experiments were completed. Seven treatments comprising various spore suspensions ($10^{10}$, $10^{9}$, $10^{8}$, $10^{7}$, $10^{6}$, $10^{5}$ and $10^{4}$ spores/ml) were sprayed on the infested twigs of fenugreek plant having counted number of aphids and each treatment were replicated four times. One control treatment (water + Tween 80 (0.02%) spray only) was maintained alongside. The greenhouse temperature and relative humidity was 27±2$^{o}$C, >90% respectively and 16:8h light: dark.

**Determination of $LC_{50}$ and $LT_{50}$:** Daily observations on aphid mortality were continued for 7 days. The cadavers showing mycosis were considered to be dead as a result of infection by the fungus. To determine $LT_{50}$ values, the observations on aphid mortality were recorded at 24 hours interval for 7 days. Mortality data was corrected with that in control by using the Abbott's formula (Abbott, 1925).

**Statistical analysis:** The data was then analyzed by probit analysis (Finney, 1971). One-way analysis of variance (ANOVA) was conducted on the mortality data to test the level of significance of the difference in response between the treatments. To assess virulence of the strain, full logarithmic plots of insect mortality against concentration was analyzed 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 $LC_{50}$ i.e. lethal concentration required for 50 % mortality of the test stage of the insect and $LT_{50}$ lethal time required for 50 % mortality of the test stage of the insect.

## RESULTS AND DISCUSSION

Entomopathogenic fungi that parasitize insects are valuable weapons for biocontrol and play an important role in promoting integrated pest management. To date, various strains of EPF such as *Lecanicillium* sp., *B. bassiana*, *Metarhizium anisopliae*, *Paecilomyces* sp. and *Nomuraea rileyi* have been used to control aphids (Vu *et al.*, 2007; Kim *et al.*, 2008; Selvaraj *et al.*, 2010). It is well known for EPF isolates of the same species to exhibit different biological and ecological differences when challenged against the same insect species. Therefore, one of the first important steps in the development of an effective microbial control agent is careful evaluation and selection of the appropriate isolate based on virulence against the target pest. Moreover, large discrepancies between field and laboratory results have made it difficult to predict the real effects these fungi have on both target and non-target insects due to several factors are differences in exposures and differences in environmental conditions. Present investigation targeted to study the effect of greenhouse conditions on pathogenicity of *A. craccivora*. There are several biopesticides based on EPF available in the market for use against insect pests in greenhouse ecosystems. Mycoinsecticide is the use of fungi in biological processes to lower the insect density with the aim of reducing disease producing activity and consequently crop damage.

**Pathogenicity of *B. bassiana* against *A. craccivora:*** During the period of study, the population buildup of aphids was noticed vegetative stage to flowering stage that it ranged between 50 to 110 numbers per plant. In present studies, the results revealed that one day after treatment the cumulative corrected mortality was 43.50% at higher concentration ($1\times10^{10}$ spores/ml) and it was 20.85% at low concentration ($1\times10^{4}$ spores/ml)

**Table 1.** Cumulative corrected mortality (%) of *A. craccivora* by *B. bassiana* under greenhouse conditions**.**

| Concentration (spores/ml) | Cumulative corrected mortality (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1DAT* | 2 DAT | 3 DAT | 4 DAT | 5 DAT | 6 DAT | 7 DAT |
| $1\times10^{10}$ | 43.50 (41.24)** | 52.52 (44.12) | 57.36 (49.29) | 63.76 (52.81) | 68.53 (55.22) | 73.64 (58.67) | 85.04 (67.59) |
| $1\times10^{9}$ | 40.88 (39.50) | 50.76 (42.40) | 53.07 (46.98) | 59.48 (50.45) | 62.23 (52.21) | 72.84 (58.34) | 79.19 (66.79) |
| $1\times10^{8}$ | 35.28 (36.55) | 44.04 (39.50) | 49.14 (44.69) | 55.89 (48.13) | 58.09 (49.87) | 71.88 (56.45) | 75.39 (64.50) |
| $1\times10^{7}$ | 30.54 (33.50) | 41.02 (37.15) | 46.41 (42.97) | 50.72 (45.26) | 56.91 (48.71) | 63.47 (52.81) | 70.84 (58.99) |
| $1\times10^{6}$ | 28.81 (32.25) | 32.75 (35.35) | 41.78 (40.09) | 47.75 (43.54) | 54.71 (47.56) | 59.07 (50.48) | 64.99 (57.71) |
| $1\times10^{5}$ | 26.81 (30.96) | 32.26 (34.74) | 34.51 (35.95) | 43.95 (41.24) | 49.34 (44.69) | 52.73 (46.41) | 60.88 (54.00) |
| $1\times10^{4}$ | 20.85 (26.90) | 21.51 (27.61) | 32.26 (34.74) | 41.23 (40.09) | 40.22 (39.36) | 50.21 (45.26) | 55.21 (51.04) |
| S.Em.± | 0.18 | 0.71 | 0.46 | 0.16 | 0.16 | 0.18 | 0.20 |
| CD $_{(P=0.05)}$ | 0.53 | 0.51 | 0.56 | 0.49 | 0.49 | 0.55 | 0.59 |

*DAT-Days after treatment; ** Values given in parenthesis are arc sin transformed.

**Table 2.** Probit analysis of concentration-mortality response of coriander aphid, *A. craccivora* to *B. bassiana* under greenhouse conditions.

| Aphid | Chi-square | Regression equation | $LC_{50}$ (spores/ml) |
|---|---|---|---|
| *A. craccivora* | 2.638 NS* | Y= 3.5885+0.245X | $1.2\times10^{8}$ |

(Table 1). It is evident from the data that the insect species was susceptible to fungal infection. The CCM was decreased with decreasing fungal conidial concentration. Similarly, at 2, 3,4,5,6 and 7 DAT almost same trend was observed. Moreover, the CCM was increased with increased exposure time. At 7 DAT, the highest CCM (85.04%) was observed at the highest concentration ($1\times10^{10}$spores/ml) and at lowest concentration ($1\times10^{4}$ spores/ml), it was 55.21%. The results described herin are in accordance with Nirmala *et al.* (2006) who reported the twelve fungal isolates belonging to *B. bassiana, M. anisopliae,* and *Verticillium lecanii* were pathogenic to *A. craccivora, Aphis gossypii* and *Rhapalosiphum maidis.* Similar results were obtained when individual of *Schizaphis graminum* and *Rhopalosiphum padi* were exposed to the *B. bassiana* and the highest concentration i.e. $1\times10^{8}$ spores/ml showed the maximum percent mortality on the seventh day of fungus treatment (Akmal *et al*., 2013). Treatment of green peach aphid adults with isolates of *B. bassiana* in the greenhouse resulted in 30.7 to 48.3% infection rates. The highest infection rate was achieved using the isolate GHA followed by BAU018, BAU019 and BAU004 with no significant differences among them (Al-alawi and Obeidat, 2014).

**Determination of $LC_{50}$ and $LT_{50}$ value for *A. craccivora:*** In the present studies, the $LC_{50}$ value of *B. bassiana* was $1.2\times10^{8}$spores/ml against *A. craciivora* using the data on 3DAT (Table 2). Feng *et al.* (1990) reported that the $LC_{50}$ values for their *B. bassiana* strain were $8.2\times10^{4}$ conidia/ml on *Diuraphis noxia*; $2.1\times10^{5}$ on *Schizaphis graminum*; $3.3\times10^{5}$ on *Metapolophium dirhodum*; $1.1\times10^{5}$ on *Sitobion avenae*; $2.1\times10^{6}$ on *Rhopalosiphum maidis* and $3.3\times10^{6}$ on *R. padi* under greenhouse conditions. Aswini (2006) found that *Fusarium semitectum* were effective against the early and late instars of sugarcane woolly aphid at $2.7\times10^{9}$ spores/ml (62% mortality) under both laboratory and greenhouse trials. Saranya *et al*. (2010) recorded the lowest $LC_{50}$ value of $2.5x10^{4}$ spores /ml for *V. lecanii* and *Hirsutella thompsonii* isolates, which showed higher virulence as compared to other isolates against *A. craccivora*. The $LC_{50}$ values of *B. bassiana*, *M. anisopliae* and *Cladosporium oxysporum* were $4.5x10^{4}$, $8.9x10^{5}$ and $7.4x10^{5}$ spores/ml respectively. $LT_{50}$ value of *B. bassiana* was 73, 89, 96, 112, 126, 138 and 157 hours for $10^{10}$, $10^{9}$, $10^{8}$, $10^{7}$, $10^{6}$, $10^{5}$, $10^{4}$ spores/ml, respectively (Table 3). $LT_{50}$ values decreased with increasing concentration of *B. bassiana* and at highest concentration of $1\times10^{10}$ spores/ml, 50% mortality was achieved in 73.24 hours after treatment and it was 157.39 hours at lowest concentration of $1\times10^{4}$ spores/ml. The mortality in infected aphids with *B. bassiana* increased with increase in spore concentration of conidial suspensions and exposure time. The $LT_{50}$ values were found to be inversely proportional to the spore concentrations. These findings were in close agreement with Liu *et al.*

**Table 3.** Probit analysis of time-mortality response of *A. craccivora* to *B. bassiana* under greenhouse conditions.

| Concentration (Spores/ml) | Time mortality response | | |
|---|---|---|---|
| | Chi-square | Regression equation | $LT_{50}$ (hrs.) |
| $1\times10^{10}$ | 19.37 | Y=0.682+4.9634X | 73 |
| $1\times10^{9}$ | 33.11 | Y= 1.415+3.94X | 89 |
| $1\times10^{8}$ | 21.67 | Y= 2.2334+2.84X | 97 |
| $1\times10^{7}$ | 28.96 | Y= 3.2791+1.21X | 112 |
| $1\times10^{6}$ | 25.87 | Y= 0.6104+3.97X | 126 |
| $1\times10^{5}$ | 30.82 | Y= 3.603+1.25X | 138 |
| $1\times10^{4}$ | 2.04 NS* | Y= 0.011+4.167X | 157 |

NS=Non-significant

(2002) and Wright *et al*. (2005) who found the susceptibility of target insect to fungal infection is dose -time dependent. Median $LT_{50}$ values for *B. bassiana, H. thompsonii, V. lecanii, C. oxysporum* and *M. anisopliae* were 3.63, 3.64, 3.90, 5.24 and 5.54 days, respectively (Saranya *et al.*, 2010). The $LT_{50}$ values at all concentrations varied between the pathogen and aphid host with *B. bassiana* tending to kill aphid more rapidly. Ibrahim *et al*. (2011) estimated $LT_{50}$ values of *B. bassiana* strains LIM1 and $LIB_1$ for *M. persicae* adults at $10^5$ conidia $ml^{-1}$ were 6.8 and 5.6 days, respectively. Increase of conidial concentration by a 1000 fold significantly reduced aphid population by 50% in less than one day. Opined that significant variations between the times required for each isolate to acquire 50% death were observed 7 days after inoculation. However, all isolates appeared to be fast killing fungi requiring one or two days for reducing aphid population by half.

These findings suggest that *B. bassiana* is ideal for further development as a microbial pesticide to control of aphids. *B. bassiana* might be an excellent candidate for the development as a microbial control agent against *A. craccivora* in fenugreek crop. Aphids possess piercing sucking mouth parts by which they suck plant sap from the conductive tissues. This feeding behavior might result in avoidance of the ingestion of many microbial control agents such as bacteria and viruses which need to be ingested to infect their hosts. On the contrary, EPF cause infection by direct penetration through their host cuticle which makes them excellent candidates as microbial control agents against pests with piercing sucking feeding behavior (Wraight and Carruthers, 2010).

## Conclusion

It is evident from the results that the *A. craccivora* was susceptible to infection of *B. bassiana* isolates under greenhouse conditions. The mortality observed was low on day 1-2 after treatment in all fungal concentrations, it increased gradually and maximum mortality was obtained on day 4-7. Further, the mortality in infected aphids with *B. bassiana* increased with increase in spore concentration of conidial suspensions and exposure time. So study clear says that different concentration of *B. bassiana* isolates against *A. craccivora* is dose-time dependent mortality response after application of the pathogenic fungus. By testing their field efficacy, they can be used as a potential entomopathogenic fungus for the sustainable management of *A. craccivora* in fenugreek crop. Furthermore, *B. bassiana* is considered safer and ecofriendly than chemical insecticides, has wide host range attacking a variety of important pests, can be cultured on relatively inexpensive media and have long shelf life.

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