International Journal Of

Recent Scientific Research

ISSN: 0976-3031

Volume: 7(4) April -2016

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THE OFFICIAL PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR)
http://www.recentscientific.com/ recentscientific@gmail.com

ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 4, pp. 10713-10717, April, 2016

International Journal of Recent Scientific Research

Research Article

COLLECTION, CHARACTERIZATION AND SCREENING OF ENTOMOPATHOGENIC FUNGI AGAINST FIELDBEAN POD BORER (*HELICOVERPA ARMIGERA*, HUBNER)

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ARTICLE INFO

Article History:

Received 15th January, 2016
Received in revised form 21st February, 2016
Accepted 06th March, 2016
Published online 28th April, 2016

Keywords:

Entomopathogenic Fungi, Bioassay, *Helicoverpa armigera,* Lethal Time,

ABSTRACT

Private An attempt was made to identify, evaluate and standardize entomopathogenic fungi for control of *Helicoverpa armigera*. During survey, for fieldbean pod borer incidence, 7 fungal infected larvae were collected and as many as seven fungal isolates were isolated form dead and infected larvae and they were designated HAF-1, HAF-2, HAF-3, HAF-4, HAF-5, HAF-6 and HAF-7. Based on colony and conidial morphology all fungal isolates were characterized up to genus level. The isolates HAF - 4, HAF - 6 and HAF - 7 were characterized as *Penicillium sp,* HAF - 1 as *Asperigillus sp.,* HAF - 2 as *Metarhizium sp.,* HAF - 3 as *Nomuraea* sp., and HAF – 5 as *Beauveria sp.,*. Further, all fungal isolates were screened against III instar larvae of *H. armigera* to assess their pathogenicity. The HAF-5 (*Beauveria sp.)* showed significantly superior infection in resulting highest per cent mortality to the tune of 60.50 percent followed by HAF-2 with 55.00 per cent mortality. Further, in the experiments to establish lethal time for 50 per cent and 99 per cent larval mortality response between *H. armigera* and HAF – 5 at six spore concentrations against III instars larvae of *H. armigera* using aqueous formulation. Among the six spore concentrations of HAF-5 evaluated, lower lethal time of 7.47 days was observed at 10^8 spore concentration/ml followed by 10^7 spore concentration/ml (8.26 days) and the maximum medium lethal time (LT_{50}) of 22.15 days was observed at 10^3 spore concentration/ml. Similarly, low LT_{99} values of 10.57 days were observed at 10^8 spore concentration/ml followed by 10^7 spore concentration/ml indicating the mean lethal time of HAF-5 against III instar larvae of *H. armigera* decrease with increase in concentration of spores per ml. However, there was no significant decrease in lethal time where the low spore concentration per ml was used.

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INTRODUCTION

The fieldbean or Dolichos bean, (*Lablab purpureus)* belongs to the family Fabaceae is one of an ancient pulse crop among cultivated plants. Presently, fieldbean is grown throughout the tropical region in Asia, Africa and America. It is cultivated either as a pure crop or mixed with ragi, groundnut, castor, maize and sorghum. The crop is mainly used for its green pods and seeds, while the dry seeds are used in various vegetable food preparations. Among many factors responsible for low yields of fieldbean in India, insect pests are the major. Fieldbean is infested with a number of insect pests. Govindan (1974) recorded 55 species of pests feeding on the crop from seedling stage till harvest of crop. Of these, pod borers are the major pests. The damage due to borers was noted up to 80 per cent (Katagihallimath and Siddappaji, 1962). Since, it is a low value crop, control of pod borers using chemicals is very expensive and wide spread use of insecticides is known to produce undesirable consequences such as development of resistance by the pests, environmental pollution and destruction of natural enemies in pest resurgences.

Increased reliance on chemical pesticides for crop protection has led to several serious concerns of pollution, development of pesticide resistance in large number of insect and mite pests, induction of resurgence in target and non target pests and unmanageable rise in the cost of development of new pesticide molecules and ultimately cost of crop protection. Increased public awareness of pesticide hazards and legislative restrictions against introduction of new pesticides would eventually limit the use of chemical pesticides to a large extent

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in the near future. In this perspective one of the biological approaches to suppress the serious pests like *Helicoverpa armigera* would be the use of microbials, which are very promising and non polluting. The biological control employing fungi, bacteria and viruses is expected to help keep the population of pod borer below economic injury level with minimum expenditure.

The entomopathogenic fungi, with chitinous walls (hyphal bodies) spread throughout the insect obtaining nutrients, leading to the death of the host by physiological starvation about 3 to 7 days after infection (Glare and Milner, 1991). With hyphomycete fungi, circulation within the insect haemolymph and toxin production is carried out by yeast like cells which resemble hyphal bodies but are termed blastospores with this fungal group (Samson *et al.* 1988).

Metarhizium anisopliae var. minor and *Nomuraea rileyi* are reported infecting mature larvae of the *Helicoverpa armigera* for the first time in India. In the first case, *H. armigera* was collected from tomato and in the second from fieldbeans [*Phaseolus vulgaris*], both in Karnataka. In pathogenicity tests in the laboratory, *M. anisopliae var. minor* caused 100 per cent mortality of 1^{st} - 4^{th} instar larvae of *H. armigera* and 80 per cent mortality of 5^{th} instar larvae. Exposure of 3^{rd} instar larvae to *Nomuraea rileyi* resulted in 100 per cent mortality (Gopalakrishnan and Narayanan, 1988).

Gopalakrishnan and Narayanan (1990) tested *Beauveria bassiana* at concentrations of $1x10^{10}$, $1x10^{9}$, $1x10^{8}$ and $1x10^{7}$ conidia/ml against 2^{nd}, 3^{rd} and 4^{th} instar larvae of *Helicoverpa armigera*. The fungus was found pathogenic to all the instars inflicting 60-100 per cent mortality and the susceptibility decreased with an increase in the age of the larvae.

Wadyackar *et al*. (2003) studied pathogenicity test against 1^{st}, 2^{nd}, 3^{rd} and 4^{th} instar larvae of *H. armigera*. He reported that spore concentration of *Metarhizium anisopliae* @ $1x10^{8}$ conidia/ml induced the larval mortality of 100, 90, 76.67 and 70 per cent respectively on 1^{st}, 2^{nd}, 3^{rd} and 4^{th} instar larvae. Bioassay analysed by log dose probit mortality revealed LC_{50} values of 6×10^{4}, 1.2×10^{6}, 4.8×10^{6} and 5.0×10^{7} spores /ml for 1^{st}, 2^{nd}, 3^{rd} and 4^{th} instar larvae of *H. armigera*, respectively.

Based on the past work done by different researchers and in view of greater need for developing microbial insecticides to control pod borer of fieldbean, the present investigation was undertaken to isolate, screen and select the entomopathogenic fungi against the larvae of *Helicoverpa armigera* from the dead and infected larvae of fieldbean pod borer.

MATERIALS AND METHODS

Survey for the pest incidence of Helicoverpa armigera of fieldbean

A survey was carried out on the incidence of *Helicoverpa armigera,* and its natural enemies in the major fieldbean growing areas like Kunigal, Magadi, Nelamangala, Chinthamani, Ramanagaram, Kanakapura, Mandya and Mysore. Observations were recorded on fieldbean with regard to intensity of attack of pod borer, availability of natural enemies or the death caused due to them.

In order to study the incidence of the entomopathogenic microorganisms, about 50 larvae were collected from each locality and reared in the laboratory until emergence of adults. The resultant per cent pupation and adult emergence were recorded in order to determine the survivability of the pest.

Fungal infected dead larvae were identified by cottony white mycelial growth found on the mummified dead larvae. The live and dead larvae were collected in separate plastic containers provided with aerated lids and brought to the laboratory for further investigations. The dead and infected larvae were collected in glass vials and kept in freezer for further studies. The dead larvae were crushed and their extract was forcibly fed to healthy larvae to determine the causal organisms of death to prove the Koch's postulates.

Isolation of entomopathogenic fungi

The collected dead and infected larvae showing the symptoms of white mycelial growth on the surface of the body were surface sterilized with 0.5 per cent sodium hypochlorite solution for two minutes and washed with sterile distilled water to remove the traces of sodium hypochlorite to prevent toxicity to fungi. A bit of diseased larvae was taken from surface sterilized specimen and transferred to Sabouraud's dextrose agar (SDA) plates. The plates were prepared by pouring 20 ml of SDA media per plates. The inoculated plates were incubated at room temperature ($25^{0} \pm 2^{0}$C) for a week time for the development of the fungi. The fungi were developed as white fluffy mass. A small quantity of the culture was taken with sterile inoculated needle and streaked on to separate plates and incubated to get pure culture of the fungal isolates for subsequent studies (Raghupati, 1995).

Identification of entomopathogenic fungal isolates

The identification of the efficient fungus is often made by recognition of characterization structures seen in culture. The colony appearance (plate culture), morphology, hyphae colour and spore colour. Based on all the above mention characters the isolated fungal isolates were characterized up to genus level tentatively (Gilman, 1961; Aneja, 1994).

Mass multiplication of fungal isolates

The entomopathogenic fungal isolates were mass multiplied in Sabouraud's dextrose agar supplemented with 1 % yeast extract and were incubated for 3 weeks at 25^{0}C under natural daylight conditions. Spores were harvested by scraping them from the agar surface using a loop and stored at 4^{0}C until they were used. Further clamps of spores were then dispersed in 0.5 % Tween 80. Prior to each bioassay, a sample of spores was taken to determine viability by germinating conidia on thin plates of Sabouraud's dextrose agar with 0.1 % chloramphenicol. After the confirmation of the germination of conidia under microscopic field the spore suspension was further used for subsequent studies (Nahar *et al.* 2004).

In vitro evaluation of entomopathogenic fungal isolates against Helicoverpa armigera

Bioassay tests were conducted to test the efficacy of the entomopathogenic fungal isolates against the *Helicoverpa armigera* under laboratory conditions. For conducting bioassay

tests, fresh culture of host insect was maintained in the laboratory.

Laboratory maintenance of host insect and bioassay studies

To initiate, large numbers of *Helicoverpa armigera* larvae was collected from field and were reared on fresh fieldbean seeds and pods in individual vials to overcome the canabolism till pupation. After pupation, the pupae were taken in petriplates containing fine saw dust and kept for moth emergence. Immediately after emergence, adults were released into earthen pots placed in plastic basins surrounded by moist sand to half the height of basin. Ten per cent of honey or sugar solution was provided as adult food. The top portion of the pots were covered with black muslin cloth for oviposition started, the cloths was changed after egg laying and kept separately, after hatching, the larvae of same age were used for bioassay studies or pathogenicity test.

In vitro evaluation of entomopathogenic fungal isolates against Helicoverpa armigera

In all bioassay studies, third instar larvae of specific hosts reared individually were used. The leaf bioassay has been the most common procedure for assisting *Helicoverpa armigera* susceptibility to the entomopathogenic fungal isolates (Tabarshink, *et al.* 1990: Shelton *et al.* 1993).

Efficacy of entomopathogenic fungal isolates against Helicoverpa armigera

The third instar larvae of *Helicoverpa armigera* were evaluated for their sensitivity to the action of the isolated entomopathogenic fungi. The fungal isolates were tried at spore load of 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 conidia/ml. later, the third instar larvae were treated individually with known quantity of fungal spore suspension containing differential spore load. The infected larvae were reared individually on the spore treated fresh seeds of fieldbean in the vials. Untreated larvae served as control. The bioassay was carried out in replications with ten larvae each. *Helicoverpa armigera* larval mortality was examined at 24 hour interval up to 120 hrs the per cent mortality was calculated by the formula given by Finney, 1974.

$$\text{Per cent mortality} = \frac{\text{Number of larvae dead due to fungal isolates}}{\text{Total number treated}} \times 100$$

Determination of LT_{50}

Mortality of the larvae due to fungal infection was recorded as per the symptoms and the results were subjected to Probit Analysis (Finney, 1974) for each concentration of the efficient fungal isolate with four replications. The larval mortality was recorded at 24 hours intervals for 10 days to determine LT_{50} and LT_{99} values. Further, the fungal isolates which showed better results in bioassay studies were further characterized and used for its efficacy under greenhouse studies.

RESULTS AND DISCUSSION

The survey carried out over different fieldbean growing locations in Karnataka revealed the incidence of the *H. armigera* in fieldbean fields was maximum during Kharif (March-May) and minimum during winter (November-January) in Ramanagram and its adjoining regions. The pod borer incidence is more Ramanagram (75 %) indicating the pest activity is seasonal bounded. Similar observations were observed by Krishnamurthy and Appanna, 1948 where the infestation of pod borer of field bean was very high (30.5 larvae/50blooms) in the optimum parameter such as minimum and maximum temperature.

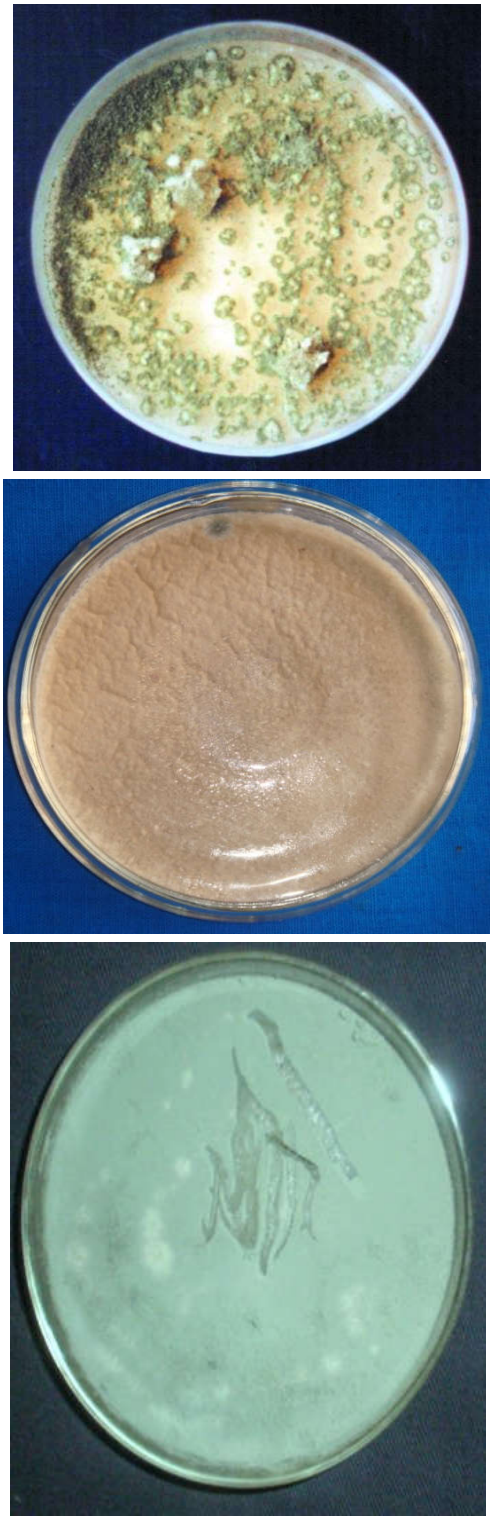

Plate 1 Entomopathogenic fungal isolates

During the course of survey for the fieldbean pod borer incidence in the fieldbean growing areas of South Karnataka (India), as many as 7 fungal infected larvae was collected based on the exhibited symptoms. Of all infected larvae collected, seven fungal isolates were isolated and to mark the isolates for

further studies, they were designated as HAF-1, HAF-2, HAF-3 HAF-4, HAF-5, HAF-6, and HAF-7. Further, all the fungal isolates obtained from dead and infected larvae were characterized tentatively up to genus level based on the colony appearance (plate culture) morphology, hyphae colour and spore colour and were identified. The Isolate number HAF - 4, HAF - 6 and HAF – 7 were identified as *Penicillium sp,* similarly, HAF – 1, HAF – 2, HAF – 3 and HAF – 5 were identified as *Asperigillus sp., Metarhizium sp., Nomuraea* sp., and *Beauveria sp.* respectively (Table 1).

Table 1 Morphological characters of entomopathogenic fungal isolates

Sl. No.	Isolates	Conidial morphology	Microscopic appearance	Identified fungal isolates
1	HAF-1	White colonies become greenish blue, black or brown as culture matures	Single celled spores (conidia) in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophore, the vesicle long conidiophore arise from a septate mycelium.	*Aspergillus flavus*
2	HAF-2	White colonies become greenish as culture matures	Produces green conidia (phialospores) from closely packed and parallely oriented conidiogenous cells born upon a sporodochium–like mass of hyphae.	*Metarhizium anisopliae*
3	HAF-3	White colonies later turn to pale green to malachite green colour	Produces green conidia (phialospores) from closely packed and parallely oriented conidiogenous cells born upon a sporodochium–like mass of hyphae.	*Nomuraea rileyi*
4	HAF-4	Matured cultures usually greenish or blue green.	Single celled spores (conidia) in chains develop at the end of the sterigma arising from the metula of the conidiophore: branching conidiophores arise from a septate mycelium.	*Penicillium spp.*
5	HAF-5	White mycelial colonies as matures	Produces white colour conidia from conidiophores, mycelium is septate and well branched.	*Beauveria bassiana*
6	HAF-6	Matured cultures usually greenish or blue green.	Single celled spores (conidia) in chains develop at the end of the sterigma arising from the metula of the conidiophore: branching conidiophores arise from a septate mycelium.	*Penicillium spp.*
7	HAF-7	Matured cultures usually greenish or blue green.	Single celled spores (conidia) in chains develop at the end of the sterigma arising from the metula of the conidiophore: branching conidiophores arise from a septate mycelium.	*Penicillium spp.*

The different fungal isolates were also obtained from dead and infected larvae. Out of 7 fungal isolates, mainly three of them were potential entomopathogens which are widely used in recent days. It was observed from Table 1 that, the characters of HAF-2 resembled *Metarhizium anisopliae* and HAF-3 was *Nomuraea rileyi* and HAF-5 was *Beauveria bassiana* (Plate 1) where as the others were *Aspergillus flavus* and *Penicillum spp.* which may be the secondary containments of the dead and infected larvae. The results are in agreement with the findings of Maddox (1994) who isolated and characterized many of the fungal isolates *viz., Entomopathora, Beauveria, Metarhizium and Aspergillus* from different source including dead larval samples of lepidopterous pests.

Table 2 Pathogenicity of different entomopathogenic fungal isolates on third instar larvae of pod borer

Sl. No	Fungal isolates	Mortality (%)
1	HAF-1	40.00
2	HAF-2	55.00
3	HAF-3	50.00
4	HAF-4	48.00
5	HAF-5	60.50
6	HAF-6	44.00
7	HAF-7	48.50

Screening and selection of the fungal isolates against H. armigera

This test was conducted using all the 7 fungal isolates against III instar larvae of *H. armigera* to assess their pathogenicity. The details of per cent larval mortality due to different fungal isolates are presented in table 2. The pathogenicity test revealed that *Beauveria bassiana* HAF-5 showed significantly superior infection in producing highest per cent mortality to the tune of 60.50 per cent followed by HAF-2 with 55.00 per cent mortality and HAF-3 with 50.00 per cent mortality. Least mortality of 40.00 per cent was found in HAF-1. The isolate HAF-5 performed well in the pathogenicity test. Hence, it was selected for further bioassay studies. The findings of the present investigation are in close agreement with the findings of Ramanujam *et al.* (2002) who also reported that, among tested three entomopathogenic fungi namely *B. bassiana*, *M. anisopliae* and *N. rileyi* against larvae of *H. armigera* and *S. litura.*

Among them *N. riley* showed the maximum mean mortality of *H. armigera* (54.44 per cent) and *S. litura* (76.66 per cent).

LT_{50} and LT_{99} values

The results of the time mortality response of III instar larvae of *H. armigera* and *Beauveria bassiana* HAF-5 at six aqueous concentrations are given in Table 3. The mean lethal time against III instar larvae decreased with increase in concentration of spores per ml. However, there was no significant decrease in lethal time where the low spore concentration per ml was used. Among the six spore concentrations of *Beauveria bassiana* HAF-5 against III instar larvae lower lethal time of 7.47 days was observed at 10^8 spore concentration/ml followed by 10^7 spore concentration/ml (8.26 days). The maximum medium lethal time of 22.15 days was observed at 10^3 spore concentration/ml. The same pattern of results was obtained for 99 per cent lethal time. Here also, low LT_{99} values of 10.57 days were observed at 10^8 spore concentration/ml followed by 10^7 spore concentration/ml.

The analysis of time mortality response of 3rd instar larvae of *H. armigera* to *Beauveria bassiana* HAF-5 is inversely proportional to the concentration of the spores in the bioassay experiments. LT_{50} values for *Beauveria bassiana* HAF-5 were 7.47 days at the maximum fungal spore concentrations of 10^8 followed by 10^7 concentrations (8.26 days). In contrast, the LT_{99} values also followed the same pattern. LT_{99} values were 10.75 days at 10^8 fungal spores/ml followed by 11.55 days at 10^7 spores/ml. Similar findings were also reported by Kulat *et al.*(2003) who reported that, the LC_{50} and LT_{50} values for *M. anisopliae* against 2nd instar larvae of *H. armigera.* The LC_{50} value was 1.47 x 10^5 conidial concentration of the inoculum.

Table 3 Probit analysis of time - mortality response of 3rd instars larvae of pod borer to *B. bassiana HAF – 5*

Sl. No.	Fungal spores /ml	χ^{2*} (n-2)	Regression equation	LT_{50} (days)	Fiducial limits (days at 95% CI)	LT_{99} (days)
1	10^8	3.89	Y= - 6.70+ 7.08 X	7.47	7.04- 8.25	10.75
2	10^7	2.87	Y= - 7.02 + 7.21 X	8.26	7.48 -8.45	11.55
3	10^6	2.10	Y= - 5.40 + 5.14 X	12.97	11.11 -14.78	15.65
4	10^5	1.17	Y= - 4.13 + 3.70 X	14.38	12.89 – 16.12	19.00
5	10^4	1.80	Y= - 5.19 + 4.00X	18.34	17.87 – 20.01	23.57
6	10^3	3.82	Y= - 6.83+ 6.20X	22.15	19.45 – 24.12	28.05

*non-significant, table χ^2 (P<0.05) @ 8df =15.50

In conclusion, the findings revealed that susceptibility to infection by fungal pathogens decreased as the concentration decreased and LT_{50} values were inversely proportional to the conidial concentration of the inoculum. This may be also due to inherent variations in the susceptibility of the host insect for a particular fungal pathogen. Thus, the study has come out with efficient entomopathogenic isolates capable of controlling field bean pod borer. Scale up studies is required to evaluate *Beauveria bassiana* HAF-5 formulations against *H. armigera* in both greenhouse and field conditions.

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How to cite this article:

Nandish M.S., Suchitha Y and Shivaprakash M.K., Collection, Characterization and Screening of Entomopathogenic Fungi Against Fieldbean pod Borer (Helicoverpa Armigera, HUBNER). *Int J Recent Sci Res.* 7(4), pp. 10713-10717.

TSSN 0976-3031