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**RESEARCH ARTICLE**

# ENTOMOPATHOGENIC FUNGI OF *ALBIZIA LEBBECK* SEED BORER, *BRUCHUS BILINEATOPYGUS* (COLEOPTERA: BRUCHIDAE)

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**ABSTRACT**

Pulse beetle*, Bruchus bilineatopygous* is one the key insect pest of *Albizia lebbeck* and causes about 80% damage to seeds. Thirteen entomopathogenic fungi viz., *Absidia corymbifera*, *Aspergillus amtelodami, A. niger, Fusarium oxysporum* (3 strains), *F. solani* (4 strains), *F. udum*, *Myrothecium roridum*, and *Trichoderma harizanum* were isolated from the *A. lebbeck* seed borer, *B. bilineatopygus* and identified. Out of 13 entomopathogenic fungi, *F. oxysporum* followed by *Absidia corymbifera* was found to be the most effective against *B. bilineatopygus* after 5 days of application in laboratory condition. Application of entomopathogenic fungi as a potential microbial tool in the insect management programme for sustainable management systems.



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## INTRODUCTION

*Albizia lebbeck* (kala sirus) is one of the most promising fodder trees for semi-arid regions. It has leaves during a large part of the rainy season and digestibility of the twigs is considerably higher than that of most fodder trees. It is an excellent fuelwood and charcoal species and the wood is suitable for construction, furniture and veneer. The shallow root system makes it a good soil binder and recommendable for soil conservation and erosion control (Joker, 2000). Large quantities of seeds are collected for the purpose of raising the seedlings for afforestation programme. The requirement of good quality seeds in adequate quantities for the purpose of raising the nursery stock is well recognized. Seeds are attacked by a number of insect pests. Their incidence varies from 5 to 80 per cent. Pulse beetles, weevils, bugs and other lepidopterous larvae are serious pests of harvested and stored seeds of forest trees are in storage which are attacked by a variety of insects and other pests (Beeson, 1941; Meshram *et al.,* 1986). Bruchids are the major problem not only in storage of seed kept for propagation and consumption but also in development seeds inside the pods. The damage caused by Bruchids reduce the weight, quality, nutritive value and viability of seeds. Bruchids are very serious pests of seeds, especially *Albizia* spp. (Mathur *et al*., 1958) in storage as well as to pods on trees. In India eight species of Bruchids have been reported to damage *Albizia* seeds (Joshi *et al*., 1990). Out of these *B. bilineatopygous* Pic. causes about 80% damage to the seeds of *A. lebbeck* and *A. procera* (Singh *et al*., 1983). Harsh and Joshi (1993) have reported 70% damage to *Albizia* seeds due to insect and diseases, out of which 40% was due to solely to the insect, *B. bilineatopygous* and *B. sparsemacculatus*.

Reduced viability and germination failure of these damaged seeds have been reported (Ponnuswamy *et al.*, 1990). Control of this borer in developing pods and seed in storage is an essential element in pest management, It involves primarily minimizing of the infestation in seed while they are inside pods as well as in storage. Beeson, (1941) who suggested sun drying of seed after harvest to prevent the attack of seed border in stored seed and Joshi (1992) who suggested mixing of folidol dusts with seed of *A. catechu* in the ratio of 1:100, none studied a technique of controlling this seed borer by using entomopathogenic fungal suspension. The chemical

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insecticides used against insect pests in agriculture and forestry have contributed undesirable side effects to animals, plants, and the environment. The increasing concern about these side effects has necessitated a change in strategies to manage insect pests in an ecologically acceptable manner. Now a days biological control as a practical science is very appreciated and as a solvent for long term usage of chemical pesticides problem is completely notified. There has been an increasing interest in employing fungal pathogens to combat insect pests. The fungus is a facultative parasite which as an entomopathogen can affect a group of insects. The exact number of entomopathogenic genera and species is indefinite but in some reviews about 90 genera and 700 belonging species were reported (Onofre *et al*., 2001).

Many strains of entomopathogenic fungi have been isolated and tested on different insect pests in a variety of cropping systems (Liu and Bauer, 2008). Investigation are going on throughout the world to discover safer and effective methods to control the insect pest, entomopathogenic bacteria, viruses, fungi and nematodes have been reported as plant protection agents against several insects (Rosell *et al*., 2008) biological control particularly by entomopathogenic fungi is important for reducing the population (Oliveira *et al*., 2003). Entomopathogenic fungi have been experimented with success against several stored products insect species in both laboratory and field tests (Sabbour and Abdul-El-Aziz, 2007). Recently some chemical insecticides are banned and their toxic effect on the seed germination. In the present study 13 entomopathogenic fungi of *Albizia* seed borer, *B. bilineatopygus* were isolated and their potential for pathogenicity to this seed borer was tested.

## MATERIALS AND METHODS

### Study site and sample collection

Survey was conducted in the campus of Tropical Forest Research Institute (TFRI), Jabalpur, Madhya Pradesh. TFRI, is situated between 23°5'37" to 23°6'10"N latitude and 79°59'49" to 79°59'42"E longitude. Pods of Kala siris, were collected for laboratory study. Infected pods were observed in laboratory during January to February 2015 in clean bags for isolation of fungi.

### Isolation of fungi

Isolation of fungi was done by moist blotter paper technique. In these techniques 10 cm Petri plates were covered with blotter paper, infected grubs were placed in plate, maintain moisture condition and incubated at 27ºC. After 7 days of incubation period the surface of the specimen shows the fungal growth. These growths were transfer into the potato dextrose agar (PDA) media it's supplemented with 30mg/l streptomycin to inhibit the growth of Bacteria. After 5 days of incubation at 27±2°C, whitish wooly growth of fungal colonies appeared in Petri dishes, which were observed directly under stereo-binocular microscope.

### Identification of fungi

The cultures were identified on the basis of morphological study of colony including colour, texture, shape and diameter, and microscopic characteristic like spore bearing fruiting body, spore size, growth rate, and presence of specific reproductive structures (Barnet and Hunter, 1998; Booth, 1971, 1977; Nagmani *et al*., 2006; Subramanian, 1971; Verma *et al*., 2008). After the identification pure culture was deposited in Forest Pathology Division, Culture collection in TFRI, Jabalpur for accession number and used in this study.

### Presentation of data

The general distribution of fungi, isolated from each rhizospheric soil sample collected from different tree. The frequency and abundance of fungi was calculated following the method of Saksena (1955).

The observations were recorded from following expressed classes for frequency:

$$\% \text{ of frequency} = \frac{\text{Number of observation in which species appeared}}{\text{Total number of observation}} \times 100$$

Frequency class- the term frequency is used to refer to the frequency of a fungus during the entire sampling period. The fungus is grouped as:

| | |
|---|---|
| Class 1 – Species occurring in 1 – 20% | R – Rare |
| Class 2 – Species occurring in 21 – 40% | O – Occasional |
| Class 3 – Species occurring in 41 – 60% | F – Frequent |
| Class 4 – Species occurring in 61 – 80% | C – Common |
| Class 5 – Species occurring in 81 – 100% | D – Dominant |

$$\% \text{ of Abundance} = \frac{\text{Number of colonies of species in all observation}}{\text{Total number of colonies in all observation}} \times 100$$

### Bioassay

In the laboratory, 3rd and 4th instars grubs (larvae) of seed borer, *B. bilineatopygus* were carefully transferred from the rearing chambers and placed into sterile Petri dishes (10 larvae per Petri dish). The spore concentration was determined with a Neubauer hemocytometer and adjusted to the desired concentrations. The grubs were sprayed with one ml of the $1\times10^8$ germinating conidia of appropriate fungus suspension and with the same amount of distilled $H_2O$ (control). Mortality of grubs was recorded after 3 and 5 days respectively. Each treatment was conducted in triplicate. The fungi grown on cadavers were observed under a microscope, then isolated in pure culture on PDA medium to confirm fungus assignment to the species. Re-identification was made basing on the micro-morphology and using the keys as indicated above. The slides were prepared in the same way as for conidial germination analysis. All the data on the mortality of grubs were compiled and analyzed statistically by using MS Excel.

## RESULTS

The results revealed that from the infected grubs (larvae) of *A. lebbeck* seed borer, *B. bilineatopygus*, entomopathogenic fungi isolated includes 7 strains of *Fusarium,* 2 species of *Aspergillus* followed by *Absidia corymbifera, T. harzianum* and *Myrothecium roridum,* one species each (Table 1). Amongst 13 entomopathogenic fungi isolated from infected larvae of *B. bilineatopygus,* the highest frequency and abundances were shown by *A. niger*, *F. solani* strain (ii) followed by *F. oxysporum* strain (i), *F. solani* strain (iv) and lowest was recorded in five fungi namely, *A. amstelodami*, *F. oxysporum* strain (iii), *Absidia corymbifera, Myrothecium roridum* and *F. udum* (Table 1).

**Table 1. Name of isolates, accession number, frequency and abundance of fungi isolated**

| S. N | Name of fungi isolates | Accession number | Frequency | Abundance |
|---|---|---|---|---|
| 1. | *Absidia corymbifera* (Cohn) Sacc. & Trotter. | TFC -87 | I | 1.47 |
| 2. | *Aspergillus amstelodami* Thom & Church | TFC - 79 | I | 1.47 |
| 3. | *A. niger* van Tieghem | TFC -86 | V | 20.58 |
| 4. | *F. oxysporum* (strain ii) | TFC - 83 | II | 2.94 |
| 5. | *F. oxysporum* (strain iii) | TFC -84 | I | 1.47 |
| 6. | *F. solani* (strain ii) | TFC - 80 | V | 19.11 |
| 7. | *F. solani* (Mart.) Sacc. (strain i) | TFC - 78 | II | 4.41 |
| 8. | *F. solani* (strain iii) | TFC - 82 | II | 2.94 |
| 9. | *F. solani* (strain iv) | TFC -85 | V | 10.29 |
| 10. | *F. udum* Butler | TFC -90 | I | 1.47 |
| 11. | *Fusarium oxysporum* Schltdl. (strain i) | TFC - 81 | V | 13.23 |
| 12. | *Myrothecium roridum* Tode | TFC -89 | I | 1.47 |
| 13. | *Trichoderma harzianum* Rifai | TFC -88 | II | 4.41 |

**Table 2. Pathogenecity test against larvae of seed borer, *Bruchus bilineatopygus***

| S.No. | Fungi Isolated | Inoculated insects | % mortality of grubs after treatments | | |
|---|---|---|---|---|---|
| | | | 3 days | 5 days | Pooled Mean |
| 1. | *Absidia corymbifera* | 10 | 9.5 | 9.5 | 9.5 |
| 2. | *Aspergillus amstelodami* | 10 | 7.5 | 8.5 | 8.0 |
| 3. | *A. niger* | 10 | 6.5 | 8.5 | 7.5 |
| 4. | *Fusarium oxysporum* (Strain i) | 10 | 7.5 | 9.0 | 8.25 |
| 5. | *F. oxysporum* (Strain ii) | 10 | 8.5 | 9.5 | 9.0 |
| 6. | *F. oxysporum* (Strain iii) | 10 | 8.0 | 10.0 | 9.0 |
| 7. | *F. solani* (Strain i) | 10 | 7.5 | 8.0 | 7.75 |
| 8. | *F. solani* (Strain ii) | 10 | 8.0 | 9.5 | 8.75 |
| 9. | *F. solani* (Strain iii) | 10 | 8.0 | 9.0 | 8.5 |
| 10. | *F. solani* (Strain iv) | 10 | 7.5 | 9.0 | 8.25 |
| 11. | *F. udum* | 10 | 8.0 | 9.5 | 8.75 |
| 12. | *Myrothecium roridum* | 10 | 6.5 | 9.5 | 8.0 |
| 13. | *Trichoderma harzianum* | 10 | 8.0 | 9.0 | 8.5 |

The results on the pathogenecity test against the seed borer, showed that out of 13 isolated fungi, *Absidia corymbifera, F. oxysporum* strain (ii), (iii) followed by *F. solani* strain (ii), *F. udum*, *Trichoderma harzianum* and *F. solani* (iv) were found to be most effective fungi against *B. bilineatopygus*. The pooled means (average of 3 days and 5 days of treatment) of mortality per cent of grubs were ranges from 7.5 - 9.5. The fungus *Absidia corymbifera* was shown the highest mortality while the lowest mortality was recorded in *A. niger* (Table 2).

## DISSCUSSION

Entomopathogenic fungi are host specific with a very low risk of attaching non-target organism or beneficial insect (Shahid *et al*., 2012). Many enthomopathogenic fungi have been recorded in all over world viz., *Beauveria*, *Conidiobolus*, *Lecanicillium*, *Metarhizium*, *Neozygites*, *Paecilomyces*, *Tolypocladium* (Bridge *et al*., 2005); *Aspergillus*, *Collectrichum*, *Fusarium* and *Penicillum* (Gouli *et al*., 2013; Marcelino *et al*., 2009). *Fusarium* species isolated from larvae and adult insects and were reported as insect pathogens (Claydon and Groove 1984) and as soil opportunistic pathogens to insects in several studies (Ali- Shtayeh *et al.,* 2002; Sun *et al.*, 2008; Abdullah and Mohamed Amin, 2009). According to Evlakhova (1974) species of genus *Aspergillus* were not typical entomopathogens and even according to Humber (1997) they were contaminants growing on cadavers after insect death.

Member of Hyphomycetes are generally considered to be opportunistic pathogens infecting many species in a range of insect order and host death is commonly associated with toxic production which over whelms the host defense response (Samson *et al*., 1988). Hypomycetes can be hemibiotrophic with well defined parasitic phase within insect host and saprophytic phase on the death of their roots (Augustyniuk-Kram, 2012). Pathogenecity test against seed borer observed in Laboratory. Laboratory test do not always coincide later with their practical use, but provide valuable information on the activity of entomopathogenic fungi and their potential role in biological control of many dangerous pests (Augustyniuk-Kram, 2012). All isolates give very good % mortality of grubs after treatments and all most 70% grubs die in 3 days. Entomopathogenic fungi penetrate the host cuticle shortly after germination or after limited hypal growth (Butt *et al*., 1988; St. Leger, 1993).

This can occur between 24 to 48 h under ideal condition (Wraight *et al*., 1990). Proteins are major components of insect cuticle and a recyclable resource for the insect. Therefore both insect and entomopathogenic fungi produce a variety of cuticle degrading protease (Samuels and Paterson, 1995). A number of cuticle degrading enzyme are produced during penetration of the host (Smith *et al*., 1981). The life cycle of entomopathogenic fungus consists of a parasitic phase (from host infection to its death) and a saprophytic phase (after host death). In these phase fungi emit some chemical which show adverse effect in insect. *A. niger* produced nigragillin which

caused immediate knockdown (Isogai *et al*., 1975), *Myrothecium* sp. secrete rugulosin which inhibited growth of insect (Calhoun *et al*., 1992). *Myrothecium roridum* secrete one compound which reduced feeding and caused mortality when introduced to the pre oral cavity of *E. varivestis* (Kishaba *et al*., 1962).

Different species of *Fusarium* produced a sterol which has ecdysteroidal effect and general toxic effect against caterpillar and beetles (Dowd *et al*., 1992). Trichothecenes (Kishaba *et al*., 1962), Diacetoxyscirpenol and neosolaniol (anti-feedant), increasing T2 concentration and caused a sigmoidal response in the inhibition of glucose 6-phosphate dehydrogenase (Joffe, 1986). Eugenio *et al*. (1970) fed zearalenone persisted in the insect throughout metamorphosis. Rao *et al*. (1971) studied various fungus metabolites to determine their effect on survival and reproduction and observed toxic effect on mortality. *F. solani* produced secondary metabolites with insecticidal properties (Wright *et al*., 1982). A detailed study on *Fusarium* toxicity against insect has been conducted (Joffe, 1986). Chemical insecticides are commonly used in plant protection.

The consequences of this are to increase the resistance of insect of various chemical substance contained in plant protection product. Over 500 arthropod species now show resistance to one or more type of chemical (Mota- Sanchez *et al*., 2002). Biological control of pests with entomopathogenic fungi is an attractive alternative to the use of conventional pesticides, mainly because these fungi are safer for plants, animals, and the environment (Khetan, 2001). Entomopathogenic fungi are constantly present in populations of insect hosts but when density of the host population is normal infections occur sporadically (enzootic phase of insect diseases). However, during insects outbreaks fungi that infect insects can increase their numbers enough to spread in the environment and contribute to the reduction of insect's population (epizootic phase) (Fuxa and Tanada, 1987).

**Conclusion**

On the basis of present study, it can be concluded out of 13 entomopathogenic fungi studied, *Fusarium oxysporum*, *Absidia corymbifera* were found to be effective against larvae of seed borer, *Bruchus bilineatopygus*. These entomopathogenic fungi may be used in management of this pest.

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