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APPLICATION OF DELIVERY METHODS FOR FUNGAL PATHOGENS AND INSECTICIDES AGAINST CHIRONJI (*BUCHANANIA LANZAN*) STEM BORER, *PLOCAEDERUS OBESUS* GAHN

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Abstract: Field trials were conducted to assess the efficacy of *Beauvaria bassiana* and *Metarhizium anisopliae* against chironji (*Buchanania lanzan*) stem borer, *Plocaederus obesus* Gahn (Coleoptera: Cerambycidae). These fungi treatments were compare with conventional prophylactic insecticide treatments. *Buchanania lanzan* trees with early phase of borer infestation were marked and the treatments were applied sequentially. Different delivery methods were adopted viz. swabbing of saturated conidial- mud slurry over the tree trunk, pouring saturated aqueous suspension of conidia through grub entry holes and soil incorporation of fungal spawn, after removing the grubs and cleaning out the frass material. The effectiveness of treatments was assessed, based on the extent of recovery of infested trees. All the treatments were superior to the untreated control in reducing borer infestation. Variations among the treatments could be observed with a higher coefficient of variation of 47.15 per cent. The efficacy of *B. bassiana* and *M. anisopliae* were on a par, but the delivery methods varied significantly. Pouring conidial suspension affected a 16.0–25.0% recovery of the infested trees followed by swabbing conidial slurry with a 17.0–20.0% recovery and soil application with 13.0–14.0% recovery of trees. Conventional insecticidal treatments remained superior with 38.0–43.0% recovery of infested trees. However, implementation of fungal application in integrated control of *P. obesus* should be considered. Fungi would not only be safer to non target organisms, but it would also be more effective in the long term pest control programme.

Key words: *Buchanania lanzan*, *Plocaederus obesus*, fungal entomopathogens, delivery methods

INTRODUCTION

Buchanania lanzan (Spreng) is commonly called Chironji. It is an economically important forest tree species in central India. Seeds are used as an expectorant tonic for the body and brain. The flesh of ripe fruits and kernels are edible and fetch quite a high rate as a substitute for almonds in flavoring sweetmeat, confectionary and betel nut powder. The root is used as a cure for blood diseases. The juice of the leaves is digestive, expectorant and purgative (Anonymous 1952). Due to indiscriminate harvesting, cut branches and lopped trees attract the infestation of insect pests which adversely affect the growth and productivity. The trees have suffered due to some biotic factors *i.e.* grazing, repeated fire, indiscriminate harvesting (lopping and cutting), disease and insect pests (Beeson 1941; Bhasin *et al.* 1958; Joshi 1992; Meshram 2005). The productivity of this economically important forest tree species is threatened by insect pests. Of them, stem borer *Plocaederus obesus* is the key insect pest and the most harmful one in central India. The adults are dark brown longicorn beetles. The adult female beetle lays eggs in the crevices of loose bark on the trunk. The emerging grubs bore into the living bark and causes significant damage to the vascular system by feeding and tunneling in the inner layer of bark. These actions by the stem borer cause the tree to have reduced nutrient uptake, premature leaf senescence, gradual shedding of leaves and eventually the trees die. The intensity of infestation and extent of damage varies widely across the regions. On the average, this borer kills about 2–5% of productive trees every year. Severely attacked trees die within a period of two years. Such a fast attack causes capital loss to the forest departments and farmers who must uproot and replace the infested trees. Existing pest management strategies utilizing cultural, mechanical and chemical methods have met with limited success mainly due to the cryptic life cycle of the borer inside the trunk and roots. After cleaning the ejection holes, either 5 g of crystal or 5 ml of a saturated solution of paradichlorobenzene in kerosene should be poured inside holes and sealed with moist soil (Anonymous 1952). Cultural and mechanical control tend to be labour intensive. The use of pesticides is often met with control failure which make repeated application necessary. The variable response of the existing control

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measures has prompted an investigation for alternative targeting strategies. Use of fungal entomopathogens as an alternative strategy seemed to be a potential measure particularly for concealed pests like the stem borer. The environment within the grub tunnels is a quite congenial for the development of mycoses. The biostages of *P. obesus* in dead trees were often found naturally infected by *Metarhizium anisopliae* (Metsch.) Sorok., and *Beauvaria bassiana* (Bals) Vuill., at enzootic levels (Bhat and Prasad 1996). Diverse isolates of *M. anisopliae* and *B. bassiana* were reported to be highly pathogenic to cashew stem borer, *P. ferrugineus* under laboratory bioassay studies (Ambethgar *et al.* 1999). However, their potential, following artificial introduction in fields, was seldom explored due to inadequate application technology.

In the present study, the efficacy of *M. anisopliae* and *B. bassiana* against *P. obesus* was assessed under field conditions *i.e.* natural stand of *B. lanzan* using different delivery methods, and were compared with the recommended chemical insecticides.

MATERIALS AND METHODS

Source of fungal pathogens

The fungal pathogens, *M. anisopliae* and *B. basiana* were initially isolated from naturally infected cadavers of *P. obesus* grubs. The pathogens were selected based on laboratory bioassay adopting two ways screening viz., using an initial single dose assay with a standard concentration of 1×10^8 conidia per ml in 0.02% of sporulated spawn.

Production and preparation of inoculum

The fungal isolates were initially passed through the grubs of *P. obsess* and the type isolates were re-isolated on Sabouraud Dextrose Agar medium with 0.25% w/v yeast extract (SDAY). After re-isolation, the fungi were inoculated separately in autoclavable polythene bags each containing 250 g of 24 h presoaked sorghum grains and autoclaved at 15 lbs.cm^2 pressure for 30 minutes. The fungus inoculated grains were incubated at 28°C±2°C until fluffy sporulation. The conidia were harvested from 2–3 week old sporulated spawn by crushing and mixing in sterile distilled water containing 0.02 per cent sporulated spawn. Viability of conidia was determined by direct examination at 200 x with a phase contrast microscope, prior to use.

Field trial

Field trials were conducted during the years 2007–2008 on the efficacy of *M. anisopliae* and *B. bassiana*. The three delivery methods were used in controlling *P. obesus* infestation and the methods were compared with the standard check monocrotophos (0.2%) and neem oil (5%) swab as an environment friendly component in the natural stand of *Buchanania lanzan* at Batkakhapa, East Forest Division, Chhindwara, Madhya Pradesh, India. The trial was conducted in Completely Randomized Block Design (CRBD) using nine treatments viz. T_1. Swabbing the trunk with saturated conidia of *M. anisopliae* in mud slurry; T_2. Swabbing the trunk with saturated conidia of *B. bassiana* in mud slurry; T_3. Pouring a saturated conidial suspension of *M. anisopliae* through entry holes of borers; T_4. Pouring a saturated conidial suspension of *B. bassiana* through entry holes of borers; T_5. Soil application of *M. anisopliae* spawn 250 g + Farm Yard Manure (FYM) 50 kg/tree under the canopy; T_6. Soil application of *B. bassiana* spawn 250 g + FYM 50 kg/tree under the canopy; T_7. Spraying the trunk with Monocrotophos 0.2%; T_8. Spraying the trunk with crude neem oil 5% and T_9. The control (untreated). Early phase borer infested trees with symptoms of gummosis and extrusion of frass were marked. Before imposition of the treatments, the grubs residing in the trees were removed mechanically. The expelled remains of the frass and resin in the damaged portion of the bark were cleaned. The treatments were applied to the deformed trees adopting the following delivery methods described:

1) Swabbing: Two litres of mud slurry containing saturated conidia of respective fungus were swabbed thoroughly on the exposed roots and trunk from the collar to one meter high after brushing the scaly bark to dislodge the eggs.
2) Pouring conidial suspension: Two litres of saturated aqueous spore suspension of respective fungus containing 0.02% of sporulated spawn was poured through borer entry holes all around the collar region of trunk until the point of saturation.
3) Soil application: Crushed spawn (250 g) containing conidia and mycelia was mixed with 50 kg of well decomposed farm yard manure and incorporated into soil up to 20 cm deep and to a 1.0 m radius from the tree base under the canopy area. The treatments were imposed one by one sequentially, when infested trees were available. The sequential treatments refer to T-1 on the first infested tree; T-2 on the second infested tree. T-3 on the third infested tree and like wise up to T-9 on the ninth infested tree. Then again, T-1 on the tenth infested tree, T-2 on the eleventh infested tree and so on. In this way, 11–13 trees were used per treatment and with a total of 110 infested trees received different treatments throughout the period of the study as shown in table 1.

Observations recorded

Observations on active holes of the stem borer were recorded one day prior to treatment. Post observations were made three months after imposing the treatments. Borer entry holes in which the of frass extrusion/gum exudation was stopped after treatment, were treated as dead holes. Holes with fresh frass and continuous gum exudation were treated as active holes. The effectiveness of treatments was assessed based on the extent of recovery of infested trees out of the total number of trees treated per treatment over the entire study period. The recovery per cent for each treatment was worked out using the following formula:

$$\text{Recovery per cent} = \frac{\text{Number of trees recovered}}{\text{Total number of trees treated}} \times 100$$

Mean and coefficient of variations were worked out and the data were analyzed statistically (Gomez and Gomez 1984).

RESULTS

The data on the efficacy of *M. anisopliae* and *B. bassiana* against *P. obesus* are presented on table 1 and figure 1. All the treatments were superior to the untreated control in reducing the borer infestation at varying levels. Variations among the treatments were evident, as indicated by the higher coefficient of variation (47.15%), exhibiting superiority over the untreated control. Even though the efficacy of *M. anisopliae* and *B. basiana* was on a par with each other, the performance of delivery methods varied. Among three delivery methods, direct pouring of saturated suspension of conidia through borer' entry holes registered a maximum of 25.0 and 16.0% recovery of infested trees for *M. anisopliae* and *B. bassiana* respectively, which were on a par with each other. Pouring suspension through the entry holes was significantly superior to rest of the treatments, viz., swabbing the tree trunk with mud slurry containing a saturated concentration of conidia (with 20.0 and 17.0 per cent recovery) and soil application of crushed fungal spawn under tree canopy, with 14.0 and 13.0 per cent recovery of trees of *M. anisopliae* and *B. bassiana* respectively. None of the untreated trees recovered (Fig. 1).

Table 1. Effect of fungal pathogens and insecticides on *P. obesus* under field conditions

Treatments	No. of trees	2007–2008	2008–2009	2009–2010	Total No. of trees treated/recovered	Recovery [%]
T1. Swabbing with *M. anisopliae* in mud slurry	treated recovered	2 1	2 0	1 0	5 1	20.00
T2. Swabbing with *B. bassiana* in mud slurry	treated recovered	2 1	2 0	2 0	6 1	17.00
T3. Pouring *M. anisopliae* saturated conidial suspension through borer entry holes	treated recovered	2 1	1 0	1 0	4 1	25.00
T4. Pouring *B. bassiana* saturated conidial suspension through borer entry holes	treated recovered	2 1	2 0	2 0	6 1	16.00
T5. Soil application of *M. anisopliae* spawn 250 g + FYM 50 kg/tree	treated recovered	2 1	2 0	3 0	7 1	14.00
T6. Soil application of *B. bassiana* spawn 250 g + FYM 50 kg /tree	treated recovered	4 1	2 0	2 0	8 1	13.00
T7. Spraying with monocrotophos 0.2%	treated recovered	2 1	2 1	3 1	7 3	43.00
T8. Spraying with 5% crude Neem oil	treated recovered	2 1	2 1	4 1	8 3	38.00
T9. Control (only removal of grubs)	–	2 0	2 0	3 0	7 0	0.00
Total	–	–	–	–	58 12	–
Mean	–	–	–	–	–	20.68
±SE	–	–	–	–	–	04.17
CD at 5%	–	–	–	–	–	47.15

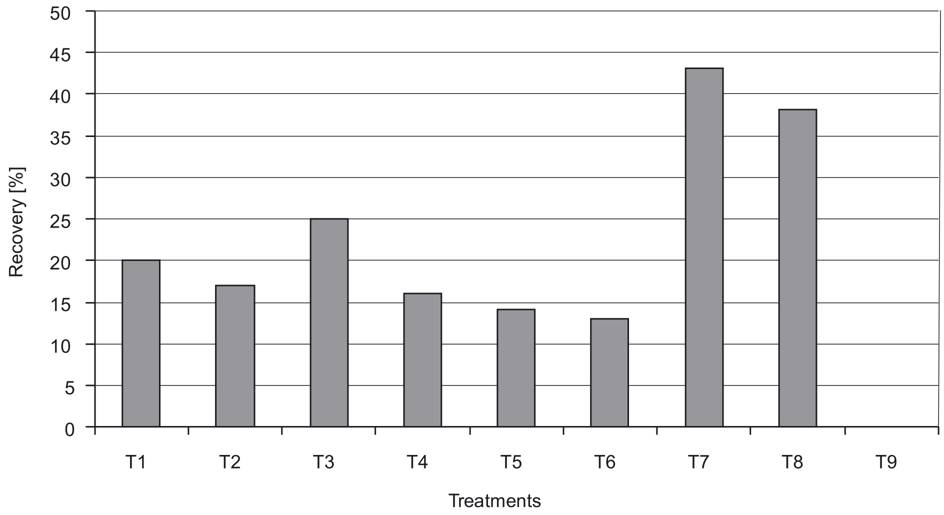

Fig. 1. Effect of fungal pathogens and insecticides on *P. obesus* under field condition

DISCUSSION

The result of the present study agrees with the findings of the recent field studies. Pouring spore suspension of *M. anisopliae* and *B. bassiana* through borer holes was effective compared to swabbing and soil application of spores (Saminathan *et al.* 2004). Soil application of 250 g of *M. anisopliae* and *B. bassiana* spawn in combination with 500 g of neem cake in October and November, minimized the borer infestation to 7.40% and 11.10% respectively as against 20.35% infestation in the untreated control (Sahu and Sharma 2008). However, swabbing conidial suspension of *M. anisopliae* as prophylactic measure was not satisfactory and had less of an effect on the borer (Mohapatra and Jena 2008). In the present study, phytosanitation by mechanical removal of grubs followed by pouring fungal inoculum on the infested portion have improved the efficiency of *M. anisopliae* and *B. bassiana* by realizing 16.0–25.0% recovery of the infested trees. The overall recovery per cent in each of the treatment confirmed the performance of the delivery methods in the following descending order: pouring through infestation holes > swabbing the trunk and exposed roots > soil application under the tree canopy. The conventional chemical treatments with different delivery methods remained superior to the fungal pathogens (Table 1). Post-prophylactic treatment with monocrotophos 0.2% registered maximum of 43.0% recovery of infested trees, followed by crude neem oil 5% with 38.0% recovery. The control trees which used only mechanical removal of grubs had a 0% recovery. The complete failure met with mechanical removal of grubs from the infested trees was due to reinfestation resulting from volatile chemicals, particularly of kairomones released from the wounded portion of trees. The volatile chemicals might have served as a promoter factor to attract gravid beetles for egg laying and subsequent re-infestation (Chakraborti 2006). It is seemed that the insecticides (monocrotophos and neem oil) performed as maskers for the tree volatiles and thereby prevented re-infestation by 43 per cent. The mortality of the remaining 43% of treated trees might be due to the progression of larval tunneling which prevented the toxicants from reaching the target sites where the residual grub population resided inside the trees. Mortality might also have taken place because the trees may have been in the state beyond recovery phase, when the treatment was imposed. Prior reports indicated that infested trees in the early phase of borer attack respond to insecticidal treatments, but succumb in the advanced phase of attack (Mohapatra and Jena 2008). Despite more convincing results of chemical insecticides, arrived at in the present study, absolute control of the pest could not be achieved with conventional insecticides or using fungal entomopathogens. Compared to insecticides, entomofungi were least effective. Implementation of fungal application in the integrated control programme of *P. obesus*, however, should be considered, as there could be great logistic advantages. Repeated introduction of fungi could kill as many grubs as possible over the years and would reduce the pest incidence in that locality in the long run. Mixing the fungal spawn with organic carriers like farm yard manure, vermicompost and *B. lanzan* chunk increase the spore load and improve the persistence for at least three months under field conditions. If these entomofungi are made to be naturalized in *B. lanzan* planting, they might represent a practical and safe method of suppressing the pest complex. This could be a safe method because fungi would not unnecessarily go after non-target organisms, and fungi would be more effective in the long term by displaying self-replicative mechanisms in the natural ecosystems.

CONCLUSION

In the present study, phytosanitation by mechanical removal of grubs followed by pouring fungal inoculum on to the infested portion have improved the efficiency of *M. anisopliae* and *B. bassiana* by realizing 16.0–25.0% recovery of infested trees. The overall recovery per cent in each of the treatments confirmed the performance of the delivery methods in the following descending order: pouring through infestation holes > swabbing the trunk and exposed roots > soil application under the tree canopy. The conventional chemical treatments remained superior to different delivery methods using fungal pathogens.

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