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Evaluation of bio-agents against Ambrosia fungal symbiont associated with coffee shot-hole borer, *Xylosandrus compactus* Eichhoff

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Shot hole borer (SHB), X*ylosandrus compactus* Eichhoff (Coleoptera: Scolytidae) is a pest of concern especially in robusta coffee attacking young and succulent twigs.The infestation causes damages to coffee plant through disruption of flow of nutrients in the young branches leading to crop loss. The Ambrosia fungus and SHB have a strong symbiotic relationship and are the only food source for developing grubs. The successful development of SHB colony mainly depends on the establishment of the fungal symbiont Ambrosia fungus. Arresting the establishment of fungal symbiont in the tunnels of twigs is the key steps which will directly affect the life cycle/developmental stages of the SHB. In this view efforts have been made to evaluate various biocontrol agents against the symbiotic fungus. Six native strains of biocontrol agents*viz.*, *Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanii, Trichoderma harzianum, Bacillus subtilis,*and *B. cereus* were screened against the Ambrosia fungus *in-vitro* by followingdual culture technique. The *in vitro* study revealed that, *T. harzianum* and *B. subtilis* were found to be the most efficient antagonist compared to other tested bioagents.

Key words: Antagonist, Ambrosia fungus, bio-agents, coffee, shot hole borer

INTRODUCTION

Coffee is one of the major plantation crop grown predominantly in the southern states (Karnataka, Kerala and Tamil Nadu) and to a limited extent in the North-Eastern states of India. There are two commercially cultivated species of coffee *viz*., arabica coffee (*Coffea arabica*) and robusta coffee (*Coffea canephora*).Coffee eco system provides a congenial habitat for various valuable species of soil micro-flora fauna, insects, birds and mammals. More than a hundred insect pests have been reported on coffee, but only a few are economically significant.

Shot Hole Borer (SHB), *Xylosandrus compactus* Eichhoff (Coleoptera: Scolytidae) is a highly polyphagous and an emerging pest of concern for robusta coffee that destroys young and succulent twigs.

Due to the disruption of sap flow and severity of infestation, the leaves turn yellow andthe affected succulent twigs gradually dry out, leading to loss of bearing wood (Anonymous, 2014). SHB infests more than 225 plant species from about 62 families (CABI, 2020). SHB is known to have a symbiotic relationship with Ambrosia fungus and the development of SHB colony within the twigs depends mainly on the growth of fungus, as it is the only source of food for the grubs of beetles. The female beetle disperses in search of a suitable place to infest the twigs, carrying fungal spores in a specializedstructure called mycangium. The presence of *Ambrosiella xylebori* in association with *X. compactus* on robusta coffee in India has been confirmed. The ambrosia fungi are in the order Endomycetales (especially Endomycopsis and Ascoidae) and Deuteromycota (especially *Monilla* and *Ambrosiella*). The hyphae of the fungi enter through the twigs, while surface of the tunnels and galleries are filled with a coating of conidiophoresand yeast-like sprout cells that bud from conidia. The yeast like cells constitutes the ambrosial stage on which the beetles feed. This symbiotic relationship between the beetle and fungus is obligate, as neither the Ambrosia fungi nor the beetles are found without the other in nature.

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After creating the galleries, fungal spores are inoculated by beetle and germinate into mycelia (Fig.1).

The grubs eat the fungus growing in these galleries and thus life cycle continues. Additionally, among Ambrosia beetles, *X. compactus* is one of the few that attacks both healthy and plants under stress conditions caused by drought, recent transplanting, or pruning. As they are cryptic in nature, the control strategies include preventive cultural approaches and fungicide spray for young plants. Due to the unavailability of sufficient skilled laborers in coffee plantations, it has become difficult to carry out estate operations in time resulting in flare-up of this pest. Although the pest has enormous economic impacts and has been intensively studied in many regions around the world, effective and sustainable control methods have yet to be devised. As a result, one of the critical components for managing SHB infestation is arresting the fungal growth within the twigs by the use of beneficial mycoparasites. Besides this, biological control is generally considered as a potential weapon nowadays since it is selective in action and does not have any negative impact on environment.

The objective of this study was to generate baseline laboratory data on the potential use of mycoparasitic fungi and bacteria and to study the antagonistic behaviour of these bioagents against Ambrosia fungus of shot hole borer.

MATERIALS AND METHODS

The bio-control agents *viz.*, *Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanii, Trichoderma harzianum, Bacillus subtilis* and *Bacillus cereus* isolated from coffee rhizosphere soil of Central Coffee Research Institute (CCRI) farm maintained in the Division of Entomology were used for the *in vitro* experiment.

Isolation of Ambrosia fungus from shot hole borer infested twigs

Shot Hole Borer infested twigs were collected from the field and brought to the laboratory for isolation of Ambrosia.The infested twigs were surface sterilized with sodium hypochlorite (0.5%) and subsequently washed with distilled water. The gallery was then cut open under the aseptic condition to isolate the fungal symbiont Ambrosia with the help of sterile needle, fungal symbionts grown on walls of the tunnel, both from the entryhole and inner side of the tunnel were transferred on to petri-dishes containing potato dextrose agar (PDA) Medium. The Petri dishes were incubated at 24(±1)^{0}C. Further, the cultures were purified by following serial-dilution technique.Pure colonies obtained were observed under stereo microscope for morphological character such as colony colour, growth pattern and development of conidial rings, conidial colour.

In-vitro screening

Efficacy of the bio-agents in inhibiting the mycelial growth of Ambrosia fungus was evaluated by following the dual culturemethod (Aneja 2012).The fungal and bacterial bio-agents were cultured on the PDA medium and Nutrient Agar medium respectively. For testing the efficacy of fungal bio-agents 5mm diameter mycelia disc of actively growing cultures of the Ambrosia fungus as well as antagonist were placed on Petri dishes(90 mm dia.) containing PDA medium in a straight line at equal distance of 5mm from the periphery. To evaluate the bacterial antagonist, the bacteriium was streaked in triangle manner at 3cm apart from centre of Petri plate and the test fungus was placed at centre. The plate with test fungus alone was maintained as control. Three replicates were maintained for each treatment and were incubated at 24±1^{0}C in BOD incubator. The experiment was arranged in a Completely Randomised Block Design. After obtaining the maximum mycelial growth in the control i.e., 30 days after incubation, the observation on radial growth was recorded and per cent inhibition was computed by using Vincent's formula.

Percentage of inhibition (PI) = (C-T) /C*100
Where,
PI=Percentage of growth inhibition, C=Colony diameter/radial growth of pathogen in control
and T=Colony diameter/radial growth of pathogen in treatment.

RESULTS AND DISCUSSION

The morphological characters of the pure culture of the Ambrosia fungus isolated from the galleries were similar as described earlier, hence confirmed the identity of the *Ambrosiella* sp.

The ecological and biological features, such as fungus-farming, haplodiploidy, and a wide host range, make the Xyleborini one of the most successful colonizers. The symbiotic relationship between Ambrosia beetles and their associated fungi is one of the most important ecological mutualisms of insects and the most remarkable feature in this relationship is that laying eggs by Ambrosia beetles is obligated by the development of symbiotic fungi in their galleries (Ranger *et al.* 2016). The disruption of thismutualistic relationship creates difficulties in completing the life cycle of the insect which provides a new perspective for the development of environment friendly management strategies. The natural infection of the entomopathogenic fungus *B. bassiana*, was also reported in *X. compactus* and sprays of a conidial suspension of the fungus at 10^7efu/ml caused 21% of the colonies to be infected after 2 weeks. However, there are limited studies on the efficacy of bio-agents against fungus associated with shot hole borer. Since the beetles depend on the Ambrosia fungus for survival (Greco and Wright, 2015), present study was taken up to target the symbiotic fungus using biological control agents that are successful against several fungal symbionts.Microorganisms associated with the rhizosphere are of particular importance in the search for successful biological agents since they secrete a wide range of substances that could act in the suppression of pathogens (Compant *et al.,* 2010).

Table 1: *In vitro* bio-efficacy of bioagents against Ambrosia fungus

Bioagents	Percent mycelial inhibition
Beauveria bassiana	64.79 (53.6)
Metarhizium anisopliae	52.15 (46.3)
Lecanicillium lecanii	32.08 (34.5)
Trichoderma harzianum	100 (90.0)
Bacillus subtilis	100 (90.0)
Bacillus cereus	34.20 (35.8)
S.Em±	0.93
CD (P=0.01)	(3.77)

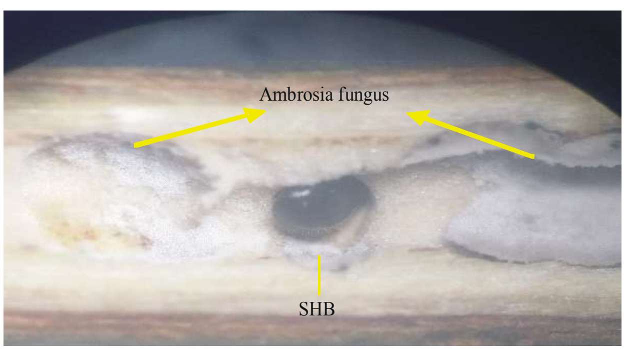

Fig. 1 : Cross section of Shot hole borer infested twig showing a gallery with Ambrosia fungus and adult beetle. (40X)

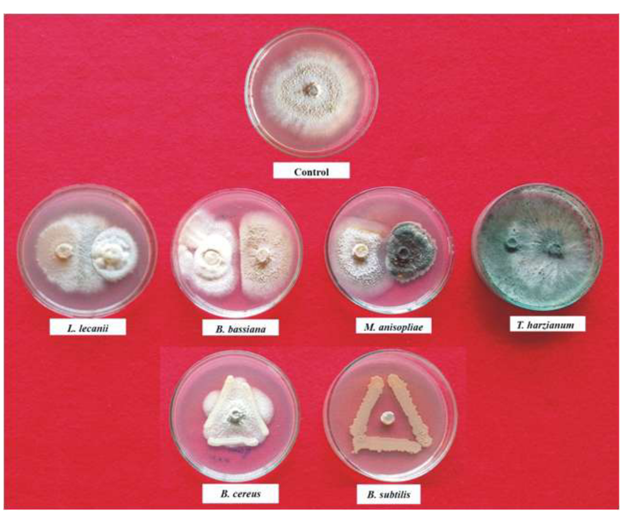

Fig. 2 : Dual culture assay of Ambrosia fungus with bioagents

The results of the antagonistic assay presented in the table revealed that evaluated biological control agents inhibited mycelial growth of Ambrosia fungus, with varying degrees of efficacy. The inhibition of mycelial development in different treatments ranged from 32 to 100 percent compared to control. Among the bio-control agents screened, *T. harzianum* and *B. subtilis* inhibited 100 percent mycelial growth of targeted fungus, Ambrosia followed by *B. bassiana* (65 %), *M. anisopliae* (52%) and *B. cereus* (36%), while the least inhibition was recorded in *L. lecanii* (32%).Hence,*T. harzianum* and *B. subtilis* were found to be mostefficient among the tested bio-agents Ambrosia fungus *in vitro* (Table 1 & Fig.2)

T.harzianum, the predominantly used bio fungicide proved its efficacy in the present study also in inhibiting mycelial growth of Ambrosia fungus and exceeded the growth target fungus by covering 100 per cent area on the petri-dishes at the end of 3rd day of incubation. These results were in accordance with the results of Castrillo *et al.* (2016), where the *in vitro* assays showed *T. harzianum* outcompeted different strains of *Ambrosiella roeperi* and *A. grosmanniae* associated with Asian Ambrosia beetle, *Xyllosandrus crassius culus* and Alnus Ambrosia beetle, *X. germanus*, respectively and in contrast, entomopathogenic fungi *B. bassiana* and *Metarhizium brunneum* blocked the spread of symbionts only in primary competition assays. The bioagents *B. bassiana* and *M. anisopliae* which showed the inhibition rate up to 65% and 52% re spectively. Even though a clear zone has formed in the centre of the tested isolates of *B. bassiana* and *M. anisopliae* by 10th day as well, but it is not successful in out competing the test fungus.

In the present study, the bacterial isolates *B. subtilis and B. cereus* registered contrast results in terms of efficacy against the test fungus. *B. subtilis* significantly inhibited the mycelial growth in 4 days after treatment and expressed the mycoparasitic nature with complete suppression of Ambrosia fungus.The results support the *in vitro* findings of Guevara-Avendano *et al*., (2018) in which *B. subtilis* was found to cause mycelial inhibition against polyphagous shot hole borer fungal symbionts. Similarly, Francis (2016) reported that, strains of *B. subtilis*, isolated from California sycamore (*Platanus racemosa* Nutt.) and avocado wood samples, significantly inhibited the growth of *Fusarium euwallaceae*.

Pest management using bioagents such as entomopathogenic fungi and plant-associated bacteria is one alternative that may contribute to decrease in pesticide usage. Native isolates of beneficial microorganisms that are particularly effective against pathogens can be multiplied in artificial media for their possible use in crop pest management. Ambrosia beetles are causing serious problems in many crop plants, fruits and timber trees around the world. The use of biological control agents, especially mycoparasitic fungi and bacteria, strengthens the management options for SHB beetles by targeting not only the insects but also the symbiotic fungi and developing brood inside the tunnel.

In conclusion, the present study has demonstrated the effectiveness of antagonists such as *T. harzianum* and *B. subtilis* followed by *B. bassiana* in suppression of Ambrosia fungus associated with coffee shot hole borer under *in vitro* conditions. However, further studies are necessary to confirm the potentiality of these bio-agents under field conditions.

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REFERENCES

Aneja, K. R. 2012. Experiments in Microbiology, Plant Pathology and biotechnology. New Age International (P) Limited, New Delhi, p.607.

Anonymous. 2014. *Coffee guide*. Central Coffee Research Institute, Chikkamagalur, India;. p. 85-87.

CABI. 2020. Invasive species compendium: *Xylosandrus compactus* (shot-hole borer). Available at: http://www.cabi.org/isc/datasheet/57234 [Accessed 1 December,2020].

Castrillo, D., Dunlap, C. A., Avery, P. B., Navarrete, J., Duncan, R. E., Jackson, M. A., Behle R.W., Cave, R. D., Crane, J. Rooney, A. P., Pena J, E. 2015. Entomopathogenic fungi as biological control agents for the vector of the laurel wilt disease, the red bay ambrosia beetle, *Xyleborus glabratus*(Coleoptera: Curculionidae). *Bio. Control*.**81:**44–50.

Castrillo, L. A., Michael, H., Griggs, B., John, D., Vandenberg. 2016. Competition between biological control fungi and fungal symbionts of ambrosia beetles *Xylosandrus crassiusculus* and *X. germanus* (Coleoptera: Curculionidae): Mycelial interactions and impact on beetle brood Production. *Bio. Control*.**103:**138–146.

Compant, S., Clement, C., Sessitsch, A. 2010. Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol. Biochem*. **42:**669–678. https://doi.org/ 10.1016/j.soilbio.2009.11.024

Francis, Na. 2016. Identification of two novel fungal species associated with Kuroshio shot hole borer (*Euwallacea* sp.) and evaluation of novel biological control method to inhibit the fungal associates of the invasive ambrosia beetle species in California. Available from Pro Quest Dissertations and Theses Global. (1836080563).

Greco, E., Wright, M. G. 2015. Ecology, biology, and management of *Xylosandrus compactus* (Coleoptera: Curculionidae: Scolytinae) with emphasis on coffee in Hawaii. *J. of Integr. Pest Manag*. **6:** 7. doi: 10.1093/jipm/pmv007

Guevara-Avendano, E., Carrillo, J.D., Ndinga-Muniania, C., Moreno, K., Méndez-Bravo, A., Guerrero-Analco, J.A., Eskalen, A., Reverchon, F. 2018. Antifungal activity of avocado rhizobacteria against *Fusarium euwallaceae* and *Graphium* spp., associated with *Euwallacea* spp. nr. *fornicatus*, and *Phytophthora cinnamomi*. *Antonie van Leeuwenhoek*.**111:** 563-572.

Ranger, C. M., Reding, M. E., Schultz, P. B., Oliver J. B., Frank, S. D., Addesso, K. M. 2016. Biology, ecology, and management of nonnative ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) in ornamental plant nurseries. *J. Integr. Pest Manag.* **7:**1–23.