Efficacy of bio-pesticide *Beauveria bassiana* against kharsu oak stem and wood borer *Xylotrechus basifuliginosus* Heller, 1926 (Coleoptera: Cerambycidae) in the Garhwal region, Western Himalaya, India

Gaurav Chand Ramola* and Arun Pratap Singh

Entomology Branch, Forest Protection Division, Forest Research Institute, Dehradun 248 006, India

***Xylotrechus basifuliginosus* is a secondary stem borer of stressed, weakened, and dying kharsu oak trees. Recently, it has been found to cause significant infestation in kharsu oak forests in Chakrata Forest Division, Dehradun district, Uttarakhand, India. The susceptibility of eggs and adult stages of *X. basifuliginosus* was tested against the entomopathogenic fungus, 'TAG VERIA' *Beauveria bassiana* (1×10^8 CFU/g minimum) under both laboratory and field conditions. *B. bassiana* was found to be effective against both stages (adults and eggs) of *X. basifuliginosus.* It caused 71.34% mortality in adults and 86% inhibition in the eggs after 12 days of exposure under laboratory conditions, and 38.4% mortality in adults under field conditions in the Deoban Reserve Forest, Chakrata Forest Division, suggesting it to be a promising bio-pesticide against this wood borer.**

Keywords: *Beauveria bassiana*, biocontrol, oak trees, mortality, *Xylotrechus basifuliginosus.*

LARGE-scale mortality of kharsu oak trees by stem borer *Xylotrechus basifuliginosus* (Coleoptera: Cerambycidae) has been recently reported from Chakrata Forest Division, Dehradun district, Uttarakhand, India[1,2]. *X. basifuliginosus* Heller, 1926, is a secondary stem borer that causes considerable infestation on stressed, weakened, dead and dying kharsu oak (*Quercus semecarpifolia*) trees in moist temperate forests in Uttarakhand[1]. Soon after mating, *X. basifuliginosus* females lay eggs in the crevices of kharsu oak bark in June–July, and the fully grown larvae bore into the sapwood, which causes serious damage to oak when compared to other larval instars, by making galleries in the stems and branches and thus degrading the wood quality[2]. In another species of the genus *Xylotrechus*, i.e. *X. arvicola*, adults, eggs and neonate larvae have been identified as the most susceptible stages to insecticide treatment, but eggs are usually protected by crevices[3]. The fecundity and viability of the eggs are extended over a long period of time, and the location of the eggs allows the emerging larvae to easily enter the wood. Once the larvae enter the wood, they become inaccessible to foliar-applied chemicals, which do not penetrate the larval galleries[3]. Insecticides can only reach the larvae of *X. arvicola* within the first 24 h after hatching. Therefore, plant-protection products usually impact only adults and eggs. Another problem encountered in the management of *X. arvicola* adults using insecticide is their staggered pattern of emergence in time[4] as well as the need to minimize side effects on the predators or parasitoids and also the need to improve the environmental cost/benefit ratio of insecticide treatment. However, in *X. basifulginiosus*, the larval galleries are tightly packed with frass, which prevents the entry of chemicals; besides, each larva has a separate gallery system[2]. Thus, it is necessary to develop management strategies to control this pest, especially those involving the use of biological control agents like bio-pesticides.

Choosing bio-pesticides with different modes of action, greater selectivity and fewer chances of developing resistance is a priority for controlling insect pests. As an effective biological control agent, the entomopathogenic fungus *Beauveria bassiana* has played an important role in the control of agricultural and forest insect pests, with significant effect. *B. bassiana* is known to be highly effective in controlling Cerambycid beetles, i.e. *Enaphalodes rufulus*[5], *Monochamus alternus*[6] and *Anoplophora glabripennis*[7]. Previous studies have shown that the genus *Xylotrechus* is most susceptible to *B. bassiana*. It is being used worldwide for the biocontrol of *Xylotrechus quadripes* on *Coffee arabica* in southern India[8] and in China[9]; against *Xylotrechus rusticus* on poplar trees in China[10,11], and against *Xylotrechus arvicola* on *Vitis vinifera* in Spain[12], so as to develop alternative methods of management, keeping in view the health and environmental hazards caused by pesticide usage.

In the present study, the efficacy of *B. bassiana* was tested against *X. basifuliginosus* (eggs and adult stages, as the insecticides are not effective after the larvae have tunnelled and packed with frass) infesting kharsu oak trees and logs under both laboratory and natural conditions.

Experiments were carried out in the laboratory at the Forest Research Institute (FRI), Dehradun (670 m), Uttarakhand, India, in April and during the beetle emergence period (June–July) in the field at Deoban Reserve Forest (2815 m), Chakrata Forest Division (30.74806N; 77.86639E; 2815 m), Dehradun district, Uttarakhand. Under laboratory conditions, newly emerged beetles from kharsu logs, which were collected during June–July 2020 from the Deoban Reserve Forest, were used for the experiment. A small batch of beetles (7♂ and 5♀) was released in a wooden glass cage (40 l × 40 h × 40 w cm) in the laboratory. In order to facilitate beetle movement and oviposition, three kharsu logs (measuring 33, 34 and 37 cm in length) were kept inside the wooden cage. For feeding, cotton wads soaked in 10% honey solution were provided, while cotton wads soaked in water were placed on the logs to maintain humidity inside the cage. Under field conditions, one borer-infested and fallen kharsu oak dead tree (98 cm girth at breast height) was selected, and a total of 13 newly emerged beetles of *X. basifuliginosus* were collected and chosen for the study.

*For correspondence. (e-mail: gauravramola30@gmail.com)

Commercial strain 'TAG VERIA' *B. bassiana* (1×10^8 CFU/ml) as 1.15% wettable powder was made into suspension by mixing 10 g of *B. bassiana* (0.01%) with 1 litre of water, and the suspension was sprayed on the released beetles inside the wooden-glass cage ($40 \times 40 \times 40$ cm) as well as on kharsu logs in the cage with a spray nozzle in the laboratory. Whereas in the field, *B. bassiana* suspension was sprayed on the beetles on borer-infested kharsu oak trees, and the treated trunk section was wrapped with nylon mesh (1×1 m) to make a cylindrical cage and tied from all sides so that the adults cannot escape. *B. bassiana* formulation was sprayed for three days in succession in the morning (9.30–10.0 h) under both conditions.

After treatment, both cages were examined daily for 12 days (23.1–27.4°C temperature and 43–62% relative humidity (RH)) to count the mortality of all beetles and observe the growth of mycelia on their body surfaces under the microscope. The adults who had external mycelium or white spores on their bodies were counted as infected individuals. Under both conditions, a control experiment was also set up with 12 adults each, on whom only distilled water was sprayed.

Eggs of *X. basifuliginosus* were also treated with *B. bassiana*. Eggs used in the experiment were obtained by pairing *X. basifuliginosus* adults, which emerged from the kharsu oak logs and were captured in a plastic jar. The bottom of the jar was covered with filter paper for oviposition. The oviposition substrates were reviewed daily. After egg-laying, 12 eggs were collected, transferred with the help of a brush, and placed into 90 mm diameter Petri dishes. One millimetre spore suspension (1×10^8 CFU/ml) of *B. bassiana* was applied directly to the eggs. Then the Petri dishes were sealed and kept at 50–60% RH for 12 days in June. The same procedure was followed for the control experiment, except that the eggs ($n = 12$) were only treated with tap water. For 12 days following the treatment, daily monitoring was done by counting the inhibition of the eggs as they shrank or the growth of the mycelium. Percentage corrected mortality was calculated using the Abbott formula to distinguish the effects of *B. bassiana* treatment from those caused by natural factors or control conditions[13].

$$\% \text{ Corrected mortality} = \frac{\% \text{ Test mortality} - \% \text{ Control mortality}}{100 - \% \text{ Control mortality}} \times 100.$$

After 12 days, a small mycelium sample of the fungus was collected with the help of a needle from the infected adult bodies and treated eggs and placed in the centre of the slide. Next, a drop of 70% alcohol was added, followed by a drop of lactophenol solution. Then a cover slip was placed over the mount according to the Lacey method[14]. Observations were made under binocular microscope at 100× magnification for identification confirmed the fungal pathogen that caused mycosis in *X. basifuliginosus* adults and eggs as *B. bassiana* by FRI.

The concentration (1×10^8 CFU/ml) of *B. bassiana* was effective in controlling *X. basifuliginosus* adults under laboratory conditions. In the first four days after treatment, no signs of infection were detected on the bodies of the beetles. There were no significant changes in their external appearance or behaviour. However, on the fifth day, white *B. bassiana* hyphae start appearing on the legs (Figure 1 *a*). After seven days, conidia accumulated at the weak points on the body of the beetles (base of surface sensory hairs, mouthparts) that penetrated the integument, which is composed of a thick chitin layer and epidermal cells (Figure 1 *b* and *c*). In the experiment, exposing the beetles to *B. bassiana* resulted in 25% mortality after 7 days, 62.5% mortality after 9 days, and 83.3% mortality in 12 days, whereas in the control experiment, the mortality was 8.3% after 8 days, 25% from days 9 to 11, and 41.66% mortality on the 12th day (Figure 2). The percentage corrected mortality of adults was found to be 71.34 under laboratory conditions.

Under field conditions in the Deoban Reserve Forest, after 12 days of treatment, 8 among 13 beetles were found infected with mycelia growth on the adult bodies (Figure 1 *d* and *e*), which accounted for 61.5% mortality. Mortality started on the fifth day at 7.6% and reached 61.5% on the 12th day. In case of the control experiment, only five beetles died due to natural causes with no sign of infection detected in their bodies, with 37.5% cumulative mortality after 12 days (Figure 2). Percentage corrected mortality was applied, which showed that only 38.4% of adult mortality was due to the effect of *B. bassiana* treatment.

B. bassiana was also effective on ovicidal control, inhibiting 91.6% of eggs from days 2 to 12, but especially after six days of exposure after treatment. The egg of *X. basifuliginosus* normally hatched five days after oviposition. After two days of treatment, no signs of infection were detected on the eggs. However, on the third day, mycelia started appearing, which resulted in 8.3% unhatched eggs, while some eggs were totally covered with mycelia growth on days 4 and 5, which resulted in 25% mortality. More than half (58%) of the unhatched eggs were observed on the sixth day, and the highest percentage (92) of unhatched dead eggs from days 10 to 12 (Figure 2). Most of the infected eggs were wholly covered with mycelia and turned rigid (Figure 1 *f* and *g*). In control experiment, 60% of eggs hatched in 12 days, whereas the remaining 40% dried due to desiccation. Also, 86% corrected mortality of eggs was reported due to the effect of *B. bassiana* treatment alone.

B. bassiana (1×10^8 CFU/ml) was found to be effective against both stages (adults and eggs) of *X. basifuliginosus*. It caused up 83.3% cumulative mortality in adults and 91.6% inhibition in eggs, after 12 days of exposure. In a similar study by De La Rosa[15], *B. bassiana* caused 40.6% mortality in 30 days on coffee berry borer *Hypothenemus hampei* in Soconusco, Chapus, Mexico. Up to 95–97% inhibition and 100% cumulative mortality in adults of

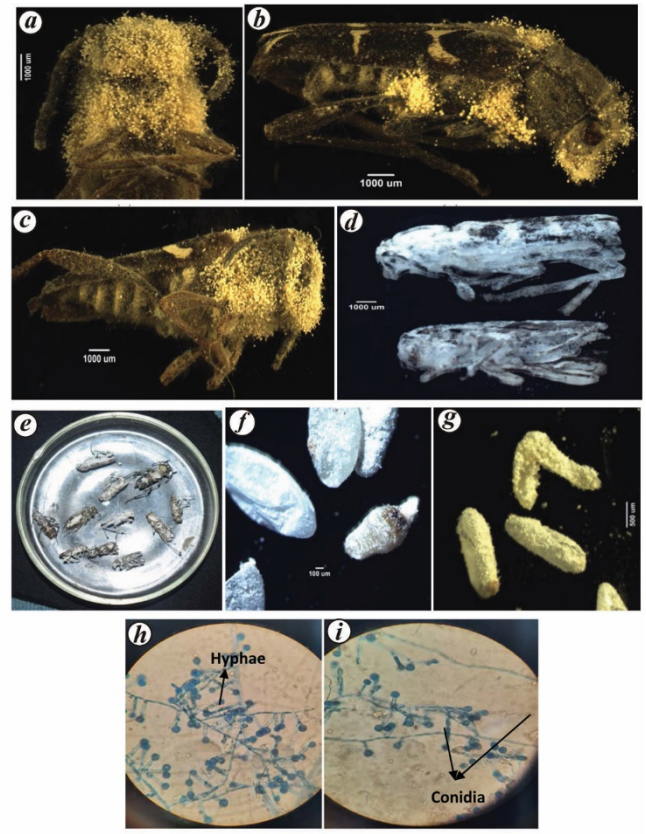

Figure 1. Sporulation of *Beauveria bassiana* on *Xylotrechus basifuliginosus* adults and eggs. ***a***, Dorsal view: Mouth parts covered with conidia. ***b***, ***c***, Lateral view: conidia on pronotum, legs and mouth parts after five days. ***d***, ***e***, *B. bassiana* infected adults after 12 days. ***f***, ***g***, *B. bassiana*-infected eggs with external mycosis under microscope. ***h***, ***i***, Conidia of *B. bassiana* under the microscope.

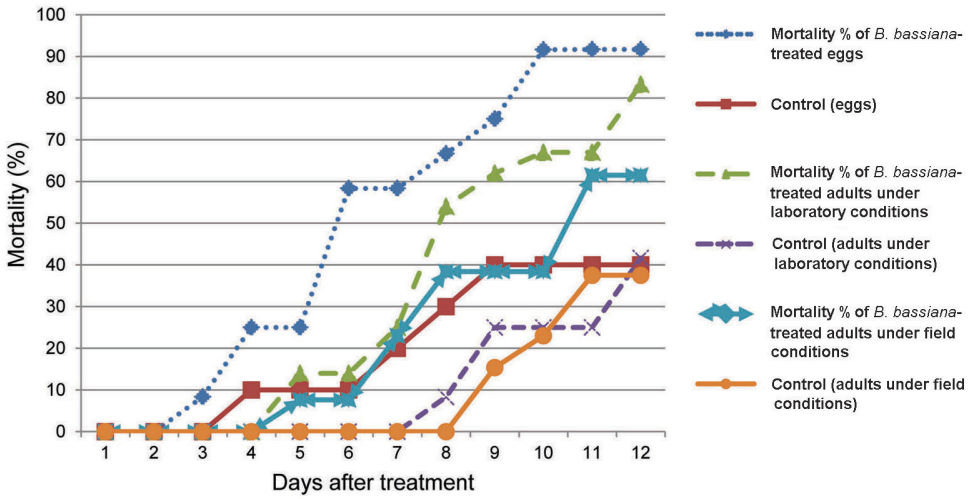

Figure 2. Fungal virulence of *B. bassiana* on *X. basifuliginosus* eggs and adults under both laboratory and field conditions.

X. arvicola on *Vitis vinifera* by *B. bassiana* has been reported in Spain[4,12], while 90–100% cumulative mortality of *X. quadripes* bettles on *Coffee arabica* has been reported from Yunnan, China[9,16].

The eggs of *X. basifuliginosus* are more susceptible to *B. bassiana* than adults because they are thinner than the adult cuticle. The infected adults showed external mycosis, whereas the eggs turned rigid and also showed external mycosis. The adults infected by *B. bassiana* generally lack symptoms in the first few days of infection, but later development inside the insect's body results in their eventual death. Low corrected mortality percentage of adults by

B. bassiana was observed in the field trials compared to the laboratory, mainly because of variation in temperature and RH[15], cage set-up and other abiotic factors[17].

B. bassiana is considered safe on non-target organisms and beneficial insects such as predators, parasitoids and honeybees in the field and forest ecosystem[18], as after application on plants or in the soil, it is able to survive and maintain itself for biocontrol activity. However, it should not interfere with the resident microbiota, which makes it more attractive to conventional pest control products[17]. *B. bassiana* colonizes plants endophytically and protects them from herbivory and disease. A concentration of 10^8 conidia ml^{-1} (in water) of *B. bassiana* applied as a foliar spray or soil drench reduced pests and disease attacks in a common bean (*Phaseolus vulgaris*)[19]. *B. bassiana* insecticidal activity is faster compared to that of other entomopathogenic agents with a longer lifespan. The conidia can persist in the environment through the spread of enzootic or epizootic diseases. Also, insects cannot develop resistance to *B. bassiana* because the fungus uses several modes of action simultaneously and, as a living organism, it can adapt to various host changes[20].

Therefore, the present study suggests that *B. bassiana* should be applied during the emergence period (June–July) of *X. basifuliginosus*, as female beetles lay eggs soon after emergence in the crevices of dead and dying kharsu oak trees. So there will be higher chances of egg mortality due to direct exposure to *B. bassiana*. Whereas in the case of emerged beetles, the first borer on the infested kharsu oak stands should be selected, and then *B. bassiana* sprayed on all the infested trees as well as on the emerged beetles. In order to maintain the level of mortality in the beetles, it would be necessary to carry out a second spraying after 15 days because *B. bassiana* spores are sensitive to UV rays, which will further decrease the efficiency of infecting the beetles. However, due to certain constraints faced in the forest ecosystem, further research is required on the optimization of bio-pesticide formulations, enhancing fungal persistence, developing better attract-infect techniques for field use, and methods best suited for forestry applications.

1. Ramola, G. C. and Singh, A. P., Relationship between Cerambyciid borer (Insecta: Coleoptera) infestation and human-induced biotic interferences causing mortality of kharsu (*Quercus semecarpifolia* Smith in Rees) oak trees in Garhwal, Western Himalaya, India. *Curr. Sci*., 2022, **122**(3), 327–332.
2. Ramola, G. C. and Singh, A. P., Biology of *Xylotrechus basifuliginosus* Heller – a borer of Kharsu oak trees in the Western Himalaya. *Indian J. Entomol.*, 2022; doi:10.55446/IJE.2022.520.
3. Pelaez, H., Hernández, J., Martín, M., Moreno, C. and Santiago, Determinación de lascaracterísticas del huevo de *Xylotrechus arvicola* (Coleoptera: Cerambycidae, Olivier, 1795). Libro de Actasdel X CongresoIbérico de Entomología: Zamora, Spain. In *X CongresoIbérico de Entomología*, 2002, p. 52 (in Russian).
4. Rodríguez-González, Á., Mayo, S., González-López, Ó., Reinoso, B., Gutierrez, S. and Casquero, P. A., Inhibitory activity of *Beauveria bassiana* and *Trichoderma* spp. on the insect pests *Xylotrechus arvicola* (Coleoptera: Cerambycidae) and *Acanthoscelides obtectus* (Coleoptera: Chrisomelidae: Bruchinae). *Environ. Monit. Assess.*, 2017, **189**(1), 1–8.
5. Meyers, J. M., Stephen, F. M., Haavik, L. J. and Steinkraus, D. C., Laboratory and field bioassays on the effects of *Beauveria bassiana* Vuillemin (Hypocreales: Cordycipitaceae) on red oak borer *Enaphalodes rufulus* (Haldeman) (Coleoptera: Cerambycidae). *Biol. Control*, 2013, **65**, 258–264.
6. Maehara, N. and Kanzaki, N., Effect of aging in adult *Monochamus alternatus* (Coleoptera: Cerambycidae) on the susceptibility of the beetle to *Beauveria bassiana* (Ascomycota: Hypocreales). *J. For. Res.*, 2013, **19**(3), 357–360.
7. Hajek, A. E., Huang, B., Dubois, T., Smith, M. T. and Li, Z. Z., Field studies of control of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) using fiber bands containing the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria brongniartii*. *Biocontrol Sci. Technol.*, 2006, **16**, 329–343.
8. Manikandan, K. R., Muthuswami, M., Chitra, N. and Ananthan, M., Management of coffee white stem borer *Xylotrechus quadripes* (Chevrolat, 1863) (Coleoptera: Cerambycidae) in the Lower Pulney Hills, India. *Int. J. Curr. Microbiol. Appl. Sci.*, 2019, **8**(6), 1–11.
9. Jia-ning, W. and Rongping, K., Biological control of coffee stem borers, *Xylotrechus quardripes* and *Acalolepla cervznus*, by *Beauveria bassiana* preparation. *Insect Sci.*, 2002, **9**(2), 43–50.
10. Ding, J. N., Zhang, H. H. and Chi, D. F., Effects of a pathogenic *Beauveria bassiana* (Hypocreales: Cordycipitaceae) strain on detoxifying and protective enzyme activities in *Xylotrechus rusticus* (Coleoptera: Cerambycidae) larvae. *Fla. Entomol.*, 2015, **98**(4), 1148–1156.
11. Ding, J. N. and Chi, D. F., Ultrastructural observations of *Beauveria bassiana* infection in *Xylotrechus rusticus* larvae. *Entomol. Res.*, 2018, **48**(3), 204–213.
12. Rodríguez-González, A., Pelaez, H. J., Mayo, S., González-López, O. and Casquero, P. A., Egg development and toxicity of insecticides to eggs, neonate larvae and adults of *Xylotrechus arvicola*, a pest in Iberian grapevines. *Vitis*, 2016, **55**(2), 83–93.
13. Abbott, W. S., A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, 1925, **18**, 265–267.
14. Lacey, L., *Manual of Techniques in Insect Pathology*, Biological Techniques Series, 1997, p. 395.
15. De La Rosa, W., Alatorre, R., Barrera, J. F. and Toriello, C., Effect of *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycetes) upon the coffee berry borer (Coleoptera: Scolytidae) under field conditions. *J. Econ. Entomol.*, 2000, **93**(5), 1409–1414.
16. Wei, J. N. and Kuang, R. P., Application of capture–recapture models for estimating coffee stem borers (Coleoptera: Cerambycidae) abundance. *Int. J. Trop. Insect Sci.*, 2000, **1**, 39–43.
17. Liu, H. and Bauer, L. S., Susceptibility of *Agrilus planipennis* (Coleoptera: Buprestidae) to *Beauveria bassiana* and *Metarhizium anisopliae*. *J. Econ. Entomol.*, 2006, **99**(4), 1096–1103.
18. Dunkel, F. V. and Jaronski, S. T., Development of a bioassay system for the predator, *Xylocoris flavipes* (Heteroptera: Anthocoridae), and its use in subchronic toxicity/pathogenicity studies of *Beauveria bassiana* strain GHA. *J. Econ. Entomol.*, 2003, **96**, 1045–1053.
19. Parsa, S., Viviana, O. and Fernando, E., Establishing fungal entomopathogens as endophytes: towards endophytic biological control. *J. Vis. Exp.*, 2013, **74**, e50360.
20. Sabbahi, R., Use of the entomopathogenic fungus *Beauveria bassiana* in a strategy of phytosanitary management of the main insect pests in strawberry plantations. University of Quebec, Quebec, Canada, 2008.

Received 20 July 2022; revised accepted 23 March 2023

doi: 10.18520/cs/v124/i11/1339-1342