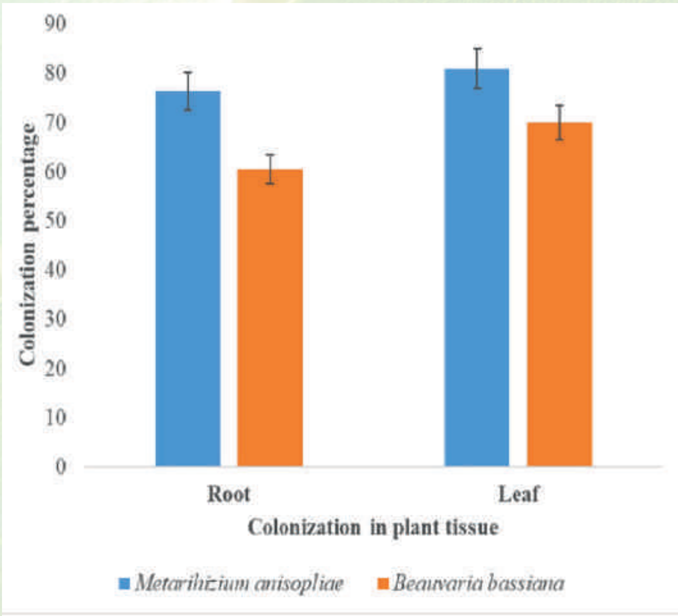
The study demonstrates that *M. anisopliae* is a superior biological control agent for *O. obesus* compared to *B. bassiana M. anisopliae* causes faster termite mortality, with an LT_{50} of 32.84 hours, significantly lower than the 42.48 hours for *B. bassiana*. This faster mortality, combined with a steeper dose-response curve, highlights its greater efficacy. Additionally, *M. anisopliae* shows better endophytic colonization in *D. sissoo* seedlings, achieving 84.31% in foliage, 76.36% in roots, and 80.94% in leaves, surpassing *B. bassiana* rates of 74.32%, 60.52%, and 69.98%, respectively. These results indicate *M. anisopliae* dual benefits: effective termite control and robust plant colonization, making it a promising candidate for integrated pest management in forestry. Further research should explore optimizing *M. anisopliae* use for sustainable pest management strategies.



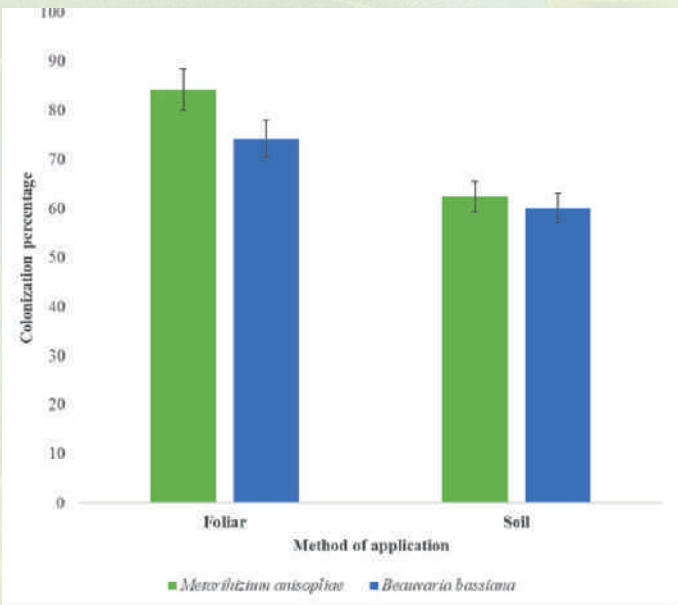


Figure 3. Colonization percentage of entomopathogenic fungi (EPFs) based on (a) different application methods and (b) plant tissue types, specifically root and leaf tissues.

Recommendations and Detailed Guidelines for Farmers, Stakeholders (State Forest Department), and Tree Growers

Preparation of Spray Solution

Prepare a foliar and root zone spray solution with M. anisopliae at a concentration of 1×10^8 conidia/mL.

Ensure the solution is thoroughly mixed to maintain an even concentration of conidia before application.

Application Dosage

Apply 25–30 mL of the prepared solution per seedling.

Ensure uniform coverage on the following plant parts

Ensure uniform coverage on the leaves to promote endophytic colonization and protection, on stems to enhance systemic action and improve plant resilience, and on the soil around roots to target termites and pests while promoting root colonization for improved root health and overall plant growth.

Frequency of Application

Repeat foliar and root zone spraying every 15–20 days during peak termite activity or adjust the timing based on field observations. Regular monitoring plant health and termite presence to fine-tune the application frequency as needed.

Optimal Environmental Conditions

Apply the spray early in the morning or late in the afternoon to minimize conidia degradation from sunlight and avoid heavy rainfall to prevent runoff and ensure better adherence to plant surfaces.

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Endophytic Behavior of Entomopathogenic Fungi in *Dalbergia sissoo* Roxb. ex DC. for Biological Control of Subterranean Termite *Odontotermes obesus* (Rambur, 1842)

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Background

Dalbergia sissoo Roxb. ex DC., commonly known as Indian rosewood, is an economically and ecologically significant tree species in agroforestry and forest ecosystems, particularly in semi-arid and arid regions where afforestation and reforestation efforts are crucial for ecological stability. However, its cultivation and sustainability are increasingly threatened by subterranean termites, particularly *Odontotermes obesus* (Rambur, 1842). These termites cause extensive damage to seedlings and mature trees, leading to significant losses in timber production and reforestation programs. The challenge is more pronounced in semi-arid and arid environments, where soil moisture scarcity and harsh climatic conditions often weaken plant health, making trees more vulnerable to termite infestations. Conventional chemical treatments for termite control pose environmental risks, disrupt soil microbiota, and may lead to the development of resistance in pest populations. Therefore, eco-friendly and sustainable management strategies are urgently needed.

Entomopathogenic fungi (EPF) such as *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschn.) Sorokin, have emerged as potential biocontrol agents against termites due to their ability to infect and kill insect hosts. These fungi have been widely studied for their pathogenicity; however, their ability to establish as endophytes within host plants is a relatively recent and promising area of research. In semi-arid and arid regions, where chemical pesticide use can further degrade soil health, the application of endophytic EPF presents an innovative solution. When EPF colonize plant tissues endophytically, they can enhance plant resistance against insect herbivores by producing bioactive metabolites that deter or kill pests. Additionally, endophytic EPF may contribute to plant growth promotion, water-use efficiency, and systemic resistance—critical factors for tree survival in water-deficient regions.

This study aims to assess the endophytic behavior of entomopathogenic fungi in *D. sissoo* and evaluate their potential in controlling *O. obesus* infestations in semi-arid and arid landscapes. By investigating the ability of EPF to colonize different tissues of *D. sissoo*, their persistence, and their impact on termite mortality, this research seeks to establish an innovative, sustainable, and environmentally friendly strategy for managing subterranean termites. Understanding the interactions between endophytic EPF and *D. sissoo* in semi-arid and arid conditions could pave the way for the development of integrated pest management (IPM) strategies that reduce reliance on chemical pesticides, enhance tree resilience, and support afforestation efforts in water-stressed environments.

Preparation of Fungal Culture, insect bioassay and Raising of Seedlings

O. obesus were collected from infested wood, acclimatized at 26 ± 2°C and 75 ± 5% RH for 1 hour, and only active, uninjured worker termites were used for bioassays. *M. anisopliae* and *B. bassiana* cultures were grown on Sabouraud Dextrose Agar(SDA) and Potato Dextrose Agar(PDA), respectively, at 25 ± 1°C for 2–3 weeks, with conidia harvested, suspended in 0.05% Triton X-100, filtered, and adjusted to 1×10^8 conidia/ml. Worker termites (n=1250 for each EPF) were treated with fungal suspensions using a Potter spray tower, while controls received sterile water with Triton X-100, and mortality was recorded at 24h and every 12h up to 72h, with LT_{50} values estimated (Figure 1). Fungal infection was confirmed by incubating cadavers on sterile filter paper and examining mycosis. *D. sissoo* seeds from ICFRE-AFRI were surface-sterilized and grown in a soil-sand-manure (1:1:1) mix, with seedlings inoculated via foliar spray or soil drench, while control plants received sterile water with Triton X-100. Two months post-inoculation, three seedlings per isolate were analyzed for fungal colonization by plating surface-sterilized tissues on SDA/PDA, with colonization (%) calculated using Petrini & Fisher's formula. Mortality data were corrected using Abbott's formula and analyzed via GLM for LT_{50} estimation, while mycosis and colonization success were assessed using ANOVA with SNK post hoc tests, with probit analysis performed in PoloPlus 2.0 and other statistical analyses in SPSS v22.0.

Findings

The bioassay results demonstrated significant differences in the virulence of *Beauveria bassiana* and *Metarhizium anisopliae* against subterranean termites. For *B. bassiana*, using 1250 termites, the chi-square (X^2) value was 7.55 (df = 3), with a dose-response slope of 3.67 ± 0.23 and a heterogeneity factor of 2.52. The LT_{50} was 42.48 hours (95% FL: 37.09–48.01 h), and LT_{90} was 94.83 hours (95% FL: 76.67–140.46 h). In contrast, M. anisopliae showed greater efficacy, with a lower X^2 value (0.82, df = 3), a steeper dose-response slope (4.88 ± 0.26), and minimal heterogeneity (h = 0.27). The LT_{50} was 32.84 hours (95% FL: 31.32–34.28 h), and LT_{90} was 60.08 hours (95% FL: 56.92–64.02 h) (Figure 2). Control termites exhibited only 4% mortality at 72h. These findings indicate that *M. anisopliae* is more effective in termite mortality than *B. bassiana*, making it a better candidate for termite management in forest plantations.

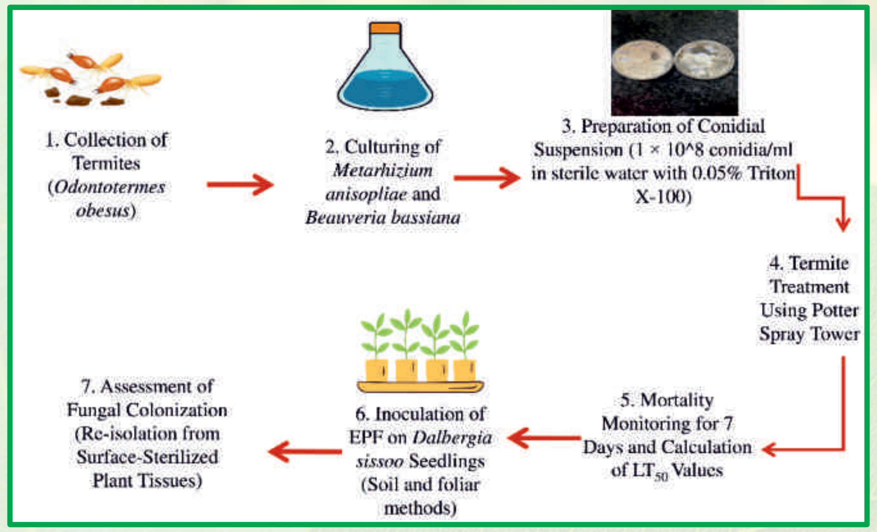

Figure 1. Schematic representation of the use of entomopathogenic fungi as endophytes for biological control of subterranean termites, illustrating the isolation, inoculation, colonization, and termite control process in *Dalbergia sissoo* seedlings.

The colonization of *D. sissoo* seedlings also varied significantly between the fungi. *M. anisopliae* exhibited higher colonization rates, with 84.31% in foliage, 62.45% in soil, 76.36% in roots, and 80.94% in leaves, whereas *B. bassiana* colonized at 74.32%, 60.15%, 60.52%, and 69.98%, respectively. Statistical analysis showed significant differences in root (F = 22.02, P = 0.009) and leaf (F = 21.34, P = 0.0098) colonization (Figure 3). The higher efficacy of *M. anisopliae* may be attributed to its superior enzyme production and adaptability to plant tissues. These results suggest *M. anisopliae* as a more promising biological control agent for subterranean termites in *D. sissoo* plantations.

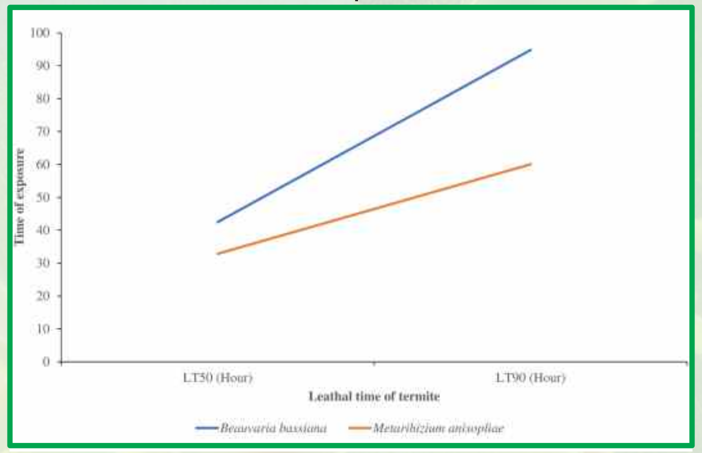

Figure 2. Lethal time (LT_{50} and LT_{90}) of termites exposed to *Beauveria bassiana* and *Metarhizium anisopliae*, showing a higher time of exposure required for *B. bassiana* compared to *M. anisopliae*.