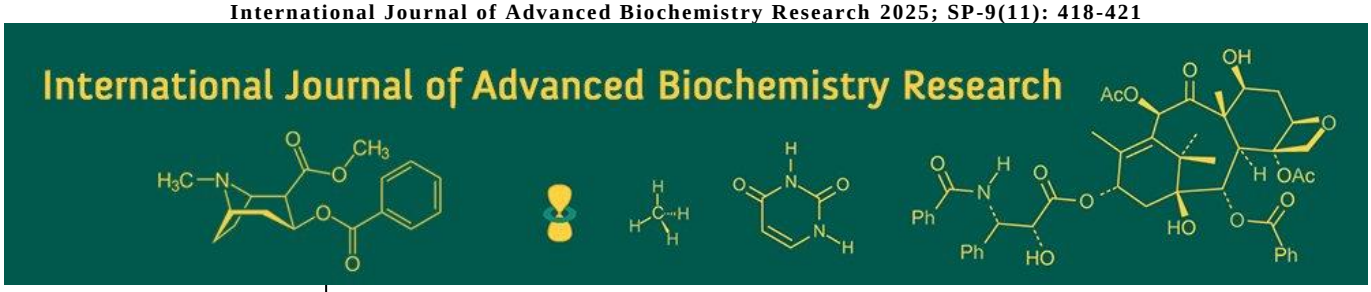

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Effect of different strains of *Metarhizium anisopliae* (Metschn.) Sorokin against white grubs in laboratory and field conditions

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Abstract
The present study entitled *"*Effect of different strains of *Metarhizium anisopliae* (Metschn.) Sorokin against white grub in laboratory and pot culture conditions*"* was conducted during 2024-25 at the Bio-control Laboratory and Entomology Section, RCSM College of Agriculture, Kolhapur. The experiment comprised five treatments including four strains of *M. anisopliae* [Grub Guard, Kalichakra, Protectam, NBAIR-Ma4] and an untreated control, each replicated four times under a Completely Randomized Design. In the laboratory experiment, larval mortality ranged from 0.00 to 82.50% at 7, 15, and 30 days after treatment (DAT), with *M. anisopliae* (NBAIR-Ma4) recording the highest efficacy (82.50%), followed by Grub Guard (80.00%), Kalichakra (60.00%), and Protectam (50.00%). In the pot culture experiment, mortality ranged from 0.00 to 75.00%, with NBAIR-Ma4 again superior (75.00%), followed by Grub Guard (72.50%), Kalichakra (52.50%), and Protectam (42.50%). In the pot culture experiment 0.00 to 75 percent mortality of the white grub was observed at 7 DAT, 15DAT and 30 DAT. The efficacy of biopesticides against white grub infesting sugarcane under pot culture experiment revealed that *M. anisopliae* (NBAIR-Ma4) was the most effective in controlling white grub with 75.00 percent of total mortality followed by treatment with *M. anisopliae* (Grub Guard) 72.50 percent total larval mortality followed by treatment *M.anisopliae* (Kalichakra) with 52.50 percent total larval mortality. The treatments *M. anisopliae* (Protectam) performed consistently less with 42.50 percent total mortality while the control treatment recorded no mortality. All strains were significantly superior over untreated control. Overall, *M. anisopliae* (NBAIR-Ma4) proved most effective and may serve as a promising biocontrol agent against white grubs under integrated pest management strategies.

Keywords: White grubs, entomopathogenic fungi, *Metarhizium anisopliae*, *Holotrichia serrate* strains

Introduction
Sugarcane (*Saccharum officinarum* L.) is a major cash crop and raw material for agro-based industries, contributing significantly to India's economy. However, its production is affected by several insect pests, with white grubs (Coleoptera: Scarabaeidae) being the most destructive, especially in Maharashtra. These root-feeding larvae cause wilting, reduced tillering, and even crop failure, with losses ranging from 10-40% and sometimes reaching 100% (Visalakshi *et al.*, 2015) [8]. Chemical control of white grubs is often ineffective due to their subterranean habitat and long larval stage (Khagta *et al.*, 2006) [3]. It also poses threat to the environment and non-target organisms. Hence, eco-friendly alternatives are essential. Entomopathogenic fungi, particularly *Metarhizium anisopliae* (Metschn.) Sorokin is promising as biocontrol agent because of their high host specificity and non-toxicity to the environment, unique mode of action and appreciable shelf. Also, *Metarhizium* grows easily in artificial media (Milner *et al.*, 1992) [5]. Thus, suitable pathogenic strains that can thrive under local soil and climatic conditions can be mass produced and used in fields. *M. anisopliae* shows promising potential as a biological control agent against various soil-dwelling insect pests; however, its effectiveness varies with the strain, exposure duration, and insect developmental stage (Saharwat *et al.*, 2021) [7]
Despite the availability of different *M. anisopliae* strains, their comparative efficacy against white grubs under local agro-climatic conditions is not well established. Therefore, the present study was undertaken to evaluate the performance of different strains of *M.*

anisopliae against white grubs under laboratory and pot culture conditions.

Materials and Methods

Experimental site and insect collection

The study was conducted during 2024-25 at the Bio-control and Biopesticides Production Laboratories, Division of Entomology, RCSM College of Agriculture, Kolhapur (Maharashtra, India). Second instar larvae of *Holotrichia serrata* were collected from infested sugarcane fields and maintained individually in vials with moist field soil and potato slices at 25±2 °C and 65±5% RH until use.

Isolation of fungal strain

Four strains of *Metarhizium anisopliae* (Grub Guard, Kalichakra, Protectam, and NBAIR-Ma4) were isolated from the respective products using serial dilution method and were initially grown on PDA till the pure culture of each strain is obtained. For mass multiplication, fungal isolates were transferred to jaggery-based medium and incubated at 25±1 °C for 14 days to obtain conidia.

Conidial suspension preparation

The conidial suspension of each *Metarhizium anisopliae* strain was prepared following the methodology of Grewal *et al.* (2015) with slight modifications. Conidia were harvested from fungal mats produced on jaggery medium and transferred into 100 mL of distilled water containing 0.1% Tween-80. The conidial concentration was adjusted to 1 × 108 spores/ml using Neubauer's improved haemocytometer.

Laboratory bioassay

The experiment was laid out in a Completely Randomized Design (CRD) with five treatments and four replications, each replication comprising ten second instar grubs. Larvae were immersed for 5s in the conidial suspensions of respective strains, air-dried, and transferred individually to vials with potato food. Mortality was recorded at 7, 15, and 30 days after treatment (DAT). The percent mortality of the grubs in each strain were calculated using the following formula.

$$\% \text{ larval mortality} = \frac{\text{No. of dead larvae}}{\text{Total number of treated larvae}} \times 100$$

The data were subjected to one-way analysis of variance (ANOVA) and validated with the OPSTAT package at a 1% significance level.

Pot culture experiment

Pot culture studies were carried out under CRD with five treatments and four replications to assess the efficacy of *M. anisopliae* strains under semi-natural conditions. Soil and FYM were mixed (2:1) and filled into pots planted with sugarcane setts. Talc-based formulations of each strain were prepared by blending fungal biomass (mycelium + conidia) grown on jaggery medium with sterile talc. The formulations were cured, stored, and later suspended in sterile water at the rate of 5 g L^{-1} for soil application. The suspension was drenched uniformly around the base of each plant to ensure thorough distribution of the fungus in the rhizosphere. Ten healthy second instar grubs were released per pot by placing them near the root zone and covering them with soil. Control pots received only distilled water. Grub mortality was recorded at 7, 15, and 30 DAT. Percent mortality was calculated and analyzed statistically to determine treatment efficacy.

Results and Discussion

Effect of different strains of *Metarhizium anisopliae* against second instar grub of *Holotrichia serrata* under laboratory conditions

The pathogenicity of four *Metarhizium anisopliae* strains was evaluated against second instar grub of *H. serrata* under laboratory conditions and mortality was recorded at 7, 15 and 30 days after treatment (DAT).

All the treatments were superior over untreated control (Table 1). At 7 DAT, M. anisopliae (NBAIR-Ma4) recorded highest mortality (30%), followed by M. anisopliae (Grub Guard) 22.50% and M. anisopliae (Kalichakra) 20%. At 15 DAT, mortality increased significantly, reaching 55.00% in M. anisopliae (Grub Guard) and 60.00% in M. anisopliae (NBAIR-Ma4). By 30 DAT,

M. anisopliae (NBAIR-Ma4) once more showed the highest mortality rate (82.50%), followed by Grub Guard (80.00%). The study reveals that *M. anisopliae* (NBAIR-Ma4) was the most effective strain, maintaining high pathogenicity throughout the experimental period. The slightly reduced mortality beyond 15 DAT in NBAIR-Ma4 might be due to the early death of susceptible larvae. These findings corroborate with those of Bohara *et al.* (2018) [1] and Fofana (2023) [2], who reported 65-100% grub mortality using various *M. anisopliae* isolates under similar laboratory conditions.

Table 1: Cumulative and total larval mortality post treatment in laboratory experiment

Tr. No	Treatments	Cumulative mortality			Total Mortality (%)
		7 DAT	15 DAT	30 DAT	
T_1	*M. anisopliae* (Grub Guard)	22.50 (28.23)*	55.00 (47.88)	80.00 (63.43)	80.00 (63.43)
T_2	*M. anisopliae* (Kalichakra)	20.00 (26.57)	35.00 (36.22)	60.00 (50.77)	60.00 (50.77)
T_3	*M. anisopliae* (Protectam)	10.00 (18.43)	30.00 (33.21)	50.00 (45.00)	50.00 (45.00)
T_4	*M. anisopliae* (NBAIR-Ma4)	30.00 (33.21)	60.00 (50.77)	82.50 (65.47)	82.50 (65.47)
T_5	Untreated control	0.00	0.00	0.00	0.00
		S.E. (±)			0.90
	C.D (1%)				3.79
		C.V			4.04

DAT = Days after treatment, *Figures in parenthesis are arcsine transformed value

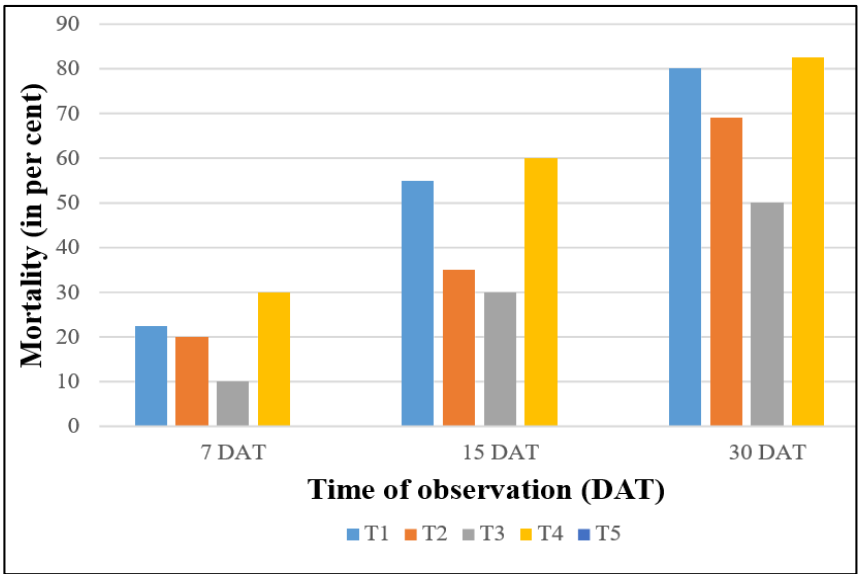

Fig 1: Cumulative larval mortality (in percent) post treatment in laboratory conditions

Effect of different strains of *Metarhizium anisopliae* against second instar grub of *Holotrichia serrata* under pot culture conditions

Under pot culture conditions, all four *M. anisopliae* strains were tested to assess their virulence under a more natural soil environment. The results revealed similar trends to laboratory trials but with slightly lower overall mortality (Table 2).

At 7 DAT, *M. anisopliae* (NBAIR-Ma4) showed the highest mortality (27.50%), followed by Grub Guard (20.00%) and Kalichakra (17.50%). Mortality increased with time, reaching 55.00% and 52.50% at 15 DAT for NBAIR-Ma4 and Grub Guard, respectively. At 30 DAT, NBAIR-Ma4 again recorded maximum grub mortality (75.00%), followed by Grub Guard (72.50%) and Kalichakra (52.50%). The untreated control recorded 0.00% mortality.

The results clearly demonstrate that *M. anisopliae* (NBAIR-Ma4) performed consistently well in both environments. The reduced mortality percentages in pot culture compared to laboratory trials may be attributed to soil factors affecting fungal proliferation and conidial contact with the host.

These findings align with Ramanujam *et al.* (2020), who reported 100% mortality with *M. anisopliae* (NBAIR-Ma4) under semi-field conditions, and Mane and Mohite (2015), who observed 34-62% mortality depending on fungal concentration and soil conditions.

Table 2: Cumulative and total larval mortality post treatment in pot culture

Tr. No.	Treatments	Cumulative mortality			Total Mortality (%)
		7 DAT	15 DAT	30 DAT	
T_1	*M. anisopliae* (Grub Guard)	20.00 (26.57)*	52.50 (46.44)	72.50 (58.45)	72.50 (58.45)
T_2	*M. anisopliae* (Kalichakra)	17.50 (24.53)	40.00 (39.23)	52.50 (46.44)	52.50 (46.44)
T_3	*M. anisopliae* (Protectam)	10.00 (18.43)	32.50 (34.72)	42.50 (40.67)	42.50 (40.67)
T_4	*M. anisopliae* (NBAIR-Ma4)	27.50 (31.55)	55.00 (47.88)	75.00 (60.11)	75.00 (60.11)
T_5	Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E. (±)				1.45
	C.D. (1%)				6.07
	C.V				7.07

DAT = Days after treatment, *Figures in parenthesis are arcsine transformed value

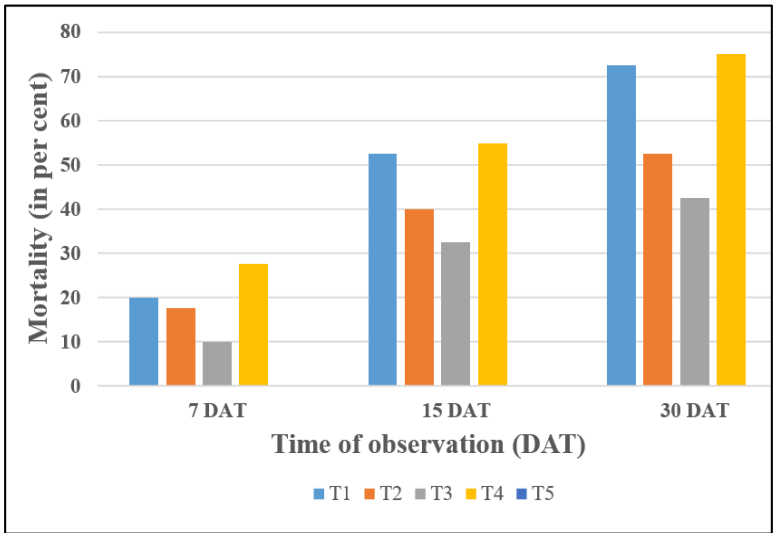

Fig 2: Cumulative larval mortality (in percent) post treatment in pot culture experiment

Conclusion

The present study demonstrated that all tested strains of *Metarhizium anisopliae* were effective against the second instar grubs of *Holotrichia serrata* under both laboratory and pot culture conditions. Among the strains, *M. anisopliae* (NBAIR-Ma4) consistently recorded the highest grub mortality, followed by *M. anisopliae* (Grub Guard). The superior performance of these strains indicates their strong virulence and potential for effective biocontrol of white grubs. Thus, *M. anisopliae* (NBAIR-Ma4) can be recommended as a promising entomopathogenic fungus for the eco-friendly management of *H. serrata*.

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