International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(11): 283-287
www.biochemjournal.com
Received: 14-08-2025
Accepted: 18-09-2025

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Compatibility of *Metarhizium anisopliae* and *Heterorhabditis indica* in different formulations

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i11Sd.6229

Abstract
Study on "Compatibility and efficacy of *Metarhizium anisopliae* and *Heterorhabditis indica* in different formulations against white grubs in sugarcane." is carried out in 2024-2025, at the RCSM College of Agriculture in Kolhapur. The main objective was to assess the compatibility of *M. anisopliae* and *H. indica* in different formulations as well as their effectiveness against white grub in pot culture. In the present study, sodium alginate gel observed as the most suitable and effective carrier, supporting a mean CFU of 2.78×10^7 g^{-1} for *M. anisopliae* and achieving 95.44% survival of *H. indica*. Comparatively lower viability and survival rates were recorded in other carriers, such as talc and cocopeat. Pot culture studies showed a dose- and time-dependent increase in grub mortality, with the highest mortality (80.00%) recorded at 21 days after treatment (DAT) with T_5 (10 ml L^{-1}). T_4 (8 ml L^{-1}) also showed comparable results (77.78%). Mean mortality followed a similar trend: T_5 (65.26%), T_4 (61.48%), and T_3 (51.85%), while the untreated control recorded no mortality. The findings suggest a complementary interaction between the EPN (early action) and the fungus (sustained infection), with alginate gel encapsulation enhancing persistence and controlled release. Overall, the alginate gel formulation proved to be most effective for maintaining microbial viability and achieving significant white grub management, indicating strong potential for field application.

Keywords: *Metarhizium*, *Heterorhabditis*, Alginate, Biological Control Agents, Pest Control, Biological/methods, Formulation Technology, Sugarcane/parasitiology

Introduction
Sugarcane (*Saccharum officinarum L.*) is one of the most important commercial crops in India, India is the world's second-largest producer of sugarcane. During 2023-24, the country produced about 453.16 million tonnes of cane with an average productivity of 78.95 t/ha (DAFW, 2025). Among the major producing regions, Uttar Pradesh, Maharashtra, and Karnataka contribute the largest shares. Apart from crystalline sugar, sugarcane sustains traditional sweetener industries such as jaggery and khandsari, with Kolhapur alone accounting for nearly 22.5% of Maharashtra's total jaggery production (Patil *et al*., 2017) [13]. In addition, by-products such as molasses, bagasse, and press mud serve as valuable raw materials for alcohol, biofuel, and organic fertilizer industries. Despite its economic importance, sugarcane suffers heavy losses from pest infestations. More than 228 insect pests are known to attack the crop, with white grubs (Coleoptera: Scarabaeidae) being among the most destructive. Yield losses are commonly estimated at around 20%, but in some regions grubs can cause complete crop failure (Visalakshi *et al*., 2015) [15]. The most prevalent species in India include *Leucopholis lepidophora*, *Holotrichia serrata*, and *Anomala bengalensis*. These pests primarily damage roots, with the third instar stage causing the greatest injury (Chandel *et al*., 2015) [3].
Conventional insecticide-based strategies have proven inadequate and pose environmental risks, increasing the emphasis on safer, eco-friendly solutions. Among these, the entomopathogenic fungus *Metarhizium anisopliae* and the entomopathogenic nematode *Heterorhabditis indica* have shown considerable potential. *M. anisopliae* provides long-term control by infecting insects through cuticle penetration and toxin production, while *H. indica* causes rapid mortality through its symbiotic bacterium *Photorhabdus luminescens* (Kaya & Gaugler, 1993; Zimmermann, 2007) [9, 16]. When used together, they may act synergistically, although their success depends on environmental conditions and formulation methods

(Ansari *et al*., 2008) [1]. Recent advances in carrier technologies, such as alginate beads and encapsulated systems, further enhance their stability and field delivery (Lacey & Shapiro-Ilan, 2008) [11]. Given the economic importance of sugarcane and the severe threat posed by white grubs, evaluating the compatibility of *M. anisopliae* and *H. indica* under Indian agro-climatic conditions is essential for developing sustainable and eco-friendly pest management strategies.

Materials and methods

For formulation preparation, 3 mL of *Metarhizium anisopliae* (2×10^8 conidia mL^{-1}) and 5 mL *Heterorhabditis indica* (4000 IJs mL^{-1}) were mixed gently to avoid mechanical injury the nematodes. This biocontrol mixture was incorporated into 20 g of sterilized substrates like sawdust-vermicompost-sand-talc (1:1:1:2), diatomaceous earth, talc, talc-DE mix (2:3), cocopeat, sodium alginate gel, and distilled water. Each substrate was aseptically loaded in Petri plates, mixed uniformly, packed, and stored under controlled conditions. *M. anisopliae* viability was determined via CFU counts on PDA plates after serial dilution, incubated at 25 ± 2°C for 5-7 days. *H. indica* survival was measured by counting live IJs under a stereomicroscope at 7-day intervals up to 21 days. Statistical analysis for both CFU and survival data was conducted using Microsoft Excel and validated through OPSTAT at a 5% significance level.

In the pot culture experiment, sodium alginate gel was used at different concentrations of 2, 4, 6, 8, and 10 g/L to prepare treatment admixtures of *M. anisopliae* and *H. indica*. Third-instar white grubs were introduced into pots containing a soil and FYM in 2:1 ratio and sugarcane seedlings, with three replicates per treatment under controlled conditions (25 ± 2 °C, 65 ± 5% RH). Grub mortality was recorded at 7, 14, and 21 days after treatment. Percent mortality was calculated and angular-transformed for analysis. Data were statistically analyzed using one-way ANOVA and validated with the OPSTAT package at a 1% significance level.

Results and Discussion

Compatibility of *M. anisopliae* and *H. indica* in Different Formulations

The viability of *M. anisopliae* conidia and survival of *H. indica* were determined in several carriers. Among these, sodium alginate gel (T_6) consistently showed the highest conidial viability (mean 2.78×10^7 CFU g^{-1}), followed closely by distilled water (2.77×10^7 CFU g^{-1}) and cocopeat (2.74×10^7 CFU g^{-1}). Moderate viability was recorded in talc-based carriers ($2.67\text{-}2.73 \times 10^7$ CFU g^{-1}), while diatomaceous earth (2.56×10^7 CFU g^{-1}) and sawdust-vermicompost-sand-talc mixture (2.63×10^7 CFU g^{-1}) were showed the least viability. The better performance of alginate gel was due to its semi-moist environment, which helps shield the conidia from drying out and from UV damage, thereby extending their shelf life. For nematode survival, the alginate gel formulation (T_6) proved most effective, maintaining an average survival rate of 95.44%, followed closely by the sawdust-based mixture at 94.00%. Distilled water also supported relatively good survival (88.78%). In comparison, talc (78.00%), talc + DE mixture (84.56%), and DE alone (74.83%) showed only moderate performance, while cocopeat resulted in the lowest survival (63.33%). The success of alginate gel can be linked to its ability to provide a balanced microenvironment with controlled gas exchange, while the sawdust-vermicompost combination may have helped by reducing the risk of desiccation. In contrast, cocopeat appeared unsuitable, likely due to uneven moisture distribution that created localized dry zones, leading to reduced nematode survival. Overall, sodium alginate gel emerged as the most reliable carrier, supporting the viability of both fungi and nematodes. Its semi-permeable structure maintained stable moisture and aeration, contributing to longer shelf life and sustained infectivity compared with talc, DE, or organic substrates.

The findings of this study reaffirm that alginate gel remains the most reliable carrier for maintaining the viability of *M. anisopliae*, while talc-based formulations provided moderately good results. In contrast, organic mixtures and diatomaceous earth were less suitable due to their inconsistent ability to sustain CFU levels over time. These observations align with earlier reports, which emphasize that the choice of formulation plays a critical role in determining the shelf life, infectivity, and field effectiveness of entomopathogenic fungi (Jaronski, 2010; Mascarin & Jaronski, 2016) [8, 12].

Treatment No.	Treatment details	Plate count (1×10^7 CFU /gm)			
		Day 7	Day 14	Day 21	Mean
T_1	Sawdust, vermicompost, sand and talc powder mixture	2.65 (1.77)	2.63 (1.77)	2.62 (1.77)	2.63 (1.77)
T_2	Diatomaceous earth	2.60 (1.76)	2.57 (1.75)	2.50 (1.73)	2.56 (1.75)
T_3	Talc powder	2.73 (1.80)	2.68 (1.78)	2.60 (1.76)	2.67 (1.78)
T_4	Talc and Diatomaceous earth mixture	2.77 (1.81)	2.72 (1.79)	2.70 (1.79)	2.73 (1.80)
T_5	Cocopeat	2.78 (1.81)	2.73 (1.80)	2.72 (1.79)	2.74 (1.80)
T_6	Sodium alginate gel	2.83 (1.83)	2.78 (1.81)	2.73 (1.80)	2.78 (1.81)
T_7	Distilled water	2.80 (1.82)	2.75 (1.80)	2.77 (1.81)	2.77 (1.81)
	S. E. (±)	0.01	0.01	0.01	-
	C. D. (5%)	0.03	0.03	0.03	-
	C. V.	0.95	0.89	1.1	-

The present study highlights the importance of formulation in maintaining the viability of entomopathogenic nematodes (EPNs) during storage. The high survival rate recorded in alginate gel is in line with earlier findings, where alginate-based encapsulation was shown to create a favourable microenvironment that conserves nematode moisture and reduces metabolic stress (Grewal, 2002; Kaya & Gaugler, 1993) [6, 9]. Functioning as a semi-permeable matrix, alginate beads allow a regulated exchange of gases and moisture, thereby protecting nematodes from rapid desiccation and extending their shelf life.

An additional advantage of alginate gel lies in its dual compatibility with both fungal and nematode entomopathogens, making it a versatile carrier system. The ability to maintain infectivity and extend shelf life for both microbial agents (Ehlers, 2001; Kaya & Gaugler, 1993) [5, 9]

is largely attributed to the semi-permeable properties of alginate beads, which provide balanced hydration, controlled aeration, and adequate nutrient availability.

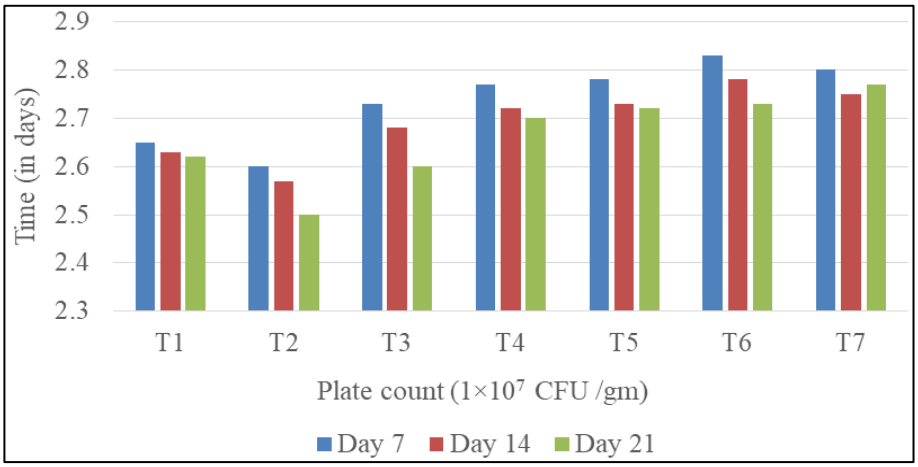

Fig 1: Plate Count (1×107 CFU /gm) of *Metarhizium anisopliae* in Different Formulations

Treatment No.	Treatment details	Survival (%)			
		Day 7	Day 14	Day 21	Mean
T_1	Sawdust, vermicompost, sand and talc powder mixture	99.17 (85.74)	95.83 (78.31)	87.00 (68.88)	94.00 (77.29)
T_2	Diatomaceous earth	79.67 (63.21)	76.33 (60.90)	68.50 (55.89)	74.83 (59.98)
T_3	Talc powder	83.17 (65.82)	78.50 (62.39)	72.33 (58.30)	78.00 (62.14)
T_4	Talc and Diatomaceous earth mixture	89.67 (71.32)	85.17 (67.39)	78.83 (62.63)	84.56 (67.07)
T_5	Cocopeat	68.17 (55.67)	63.50 (52.84)	58.33 (49.80)	63.33 (52.76)
T_6	Alginate gel	99.83 (88.65)	96.83 (79.95)	89.67 (71.30)	95.44 (79.54)
T_7	Distilled water	93.33 (75.07)	90.33 (71.97)	82.67 (65.42)	88.78 (70.77)
	S. E. (±)	1.3	1.1	1.13	-
	C. D. (5%)	3.87	3.32	3.42	-
	C. V.	3.06	2.80	3.16	-

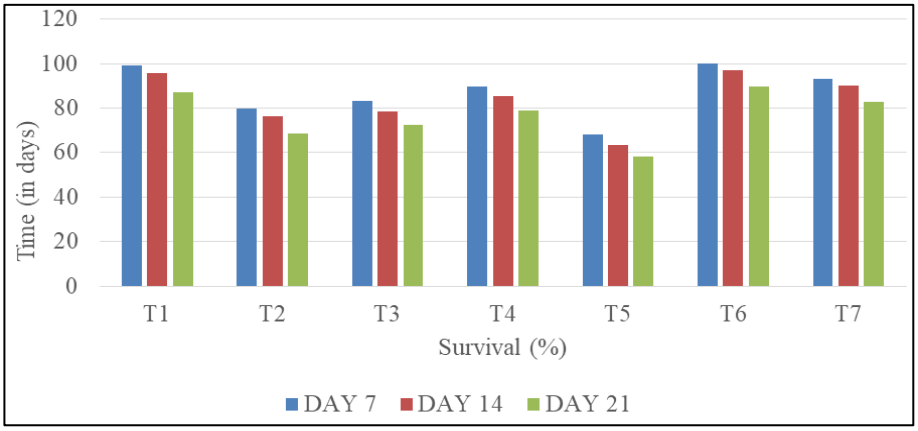

Fig 2: Survival (%) 0f EPN in different formulations

Efficacy of Sodium Alginate Gel Formulation against White Grubs in Pot Culture

Pot culture studies revealed a clear dose-dependent increase in grub mortality with alginate gel formulations containing both *M. anisopliae* and *H. indica*. At 7 days after treatment (DAT), mortality ranged from 17.78% at the lowest dose (2 ml L^{-1}) to 51.11% at the highest (10 ml L^{-1}). Mortality further increased at 14 DAT (24.44-64.66%) and peaked by 21 DAT (48.88-80.00%). The 8-10 ml L^{-1} treatments consistently recorded the highest mortality (77-80%) and were statistically at par, confirming superior efficacy. No mortality was observed in control pots. The results indicate a synergistic action between nematodes and fungus. *H. indica* caused early mortality within 7 days by penetrating host openings and releasing symbiotic bacteria (*Photorhabdus* spp.), while *M. anisopliae* contributed to delayed but sustained mortality through cuticle penetration and systemic colonization. The alginate gel matrix enhanced this dual action by protecting propagules from desiccation and ensuring gradual release, thus extending the infection window. These findings highlight sodium alginate gel as the most reliable formulation for combined delivery of *M. anisopliae* and *H. indica*, ensuring high shelf life, compatibility, and effective suppression of white grubs in pot culture conditions.

Treatment No.	Concentration (ml/lit)	Per cent Mortality			Mean
		Day 7	Day 14	Day 21	
T_1	2	17.78 (24.85)	24.44 (29.58)	48.88 (44.36)	30.37 (32.98)
T_2	4	24.44 (29.58)	35.55 (36.59)	62.22(52.09)	40.74 (39.43)
T_3	6	35.55 (36.59)	48.88 (44.36)	71.11 (57.63)	51.85 (46.15)
T_4	8	44.44 (41.80)	62.22 (52.09)	77.78 (61.93)	61.48 (51.92)
T_5	10	51.11 (45.63)	64.66 (53.54)	80.00 (63.64)	65.26 (54.20)
T_6	Control	0	0	0	0
	S. E. (±)	1.31	1.21	1.91	-
	C. D. (1%)	5.06	5.24	8.25	-
	C. V.	7.61	5.82	7.1	-

By penetrating through natural orifices and releasing symbiotic bacteria (Photorhabdus spp.), which proliferate fast in the hemocoel and produce septicemia (Kaya & Gaugler, 1993; Shapiro-Ilan *et al*., 2012) [9, 14], entomopathogenic nematodes such as *H. indica* provide early infection and quick death. This possibly influenced the first levels of grub death recorded within 7 days. *M. anisopliae*, on the other hand, has a slower infection process necessitating conidia adhesion, germination, cuticle penetration, and systemic colonization (Inglis *et al*., 2001) [7]. This clarifies the greater cumulative mortality observed at 14 and 21 days as fungal infection strengthened nematode-induced death. The simultaneous action of fungus and nematode within the same alginate matrix guaranteed both fast early mortality (driven by nematodes) and persistent long-term mortality (driven by fungal colonisation). Several studies have shown synergistic or additive effects when EPNs and entomopathogenic fungi are used together, therefore providing better pest suppression than individually (Ansari *et al*., 2004; Koppenhöfer & Kaya, 1996) [2, 10]. These findings are in line with the aforementioned ones, demonstrating that alginate gel allows for the effective co-formulation and administration of both drugs.

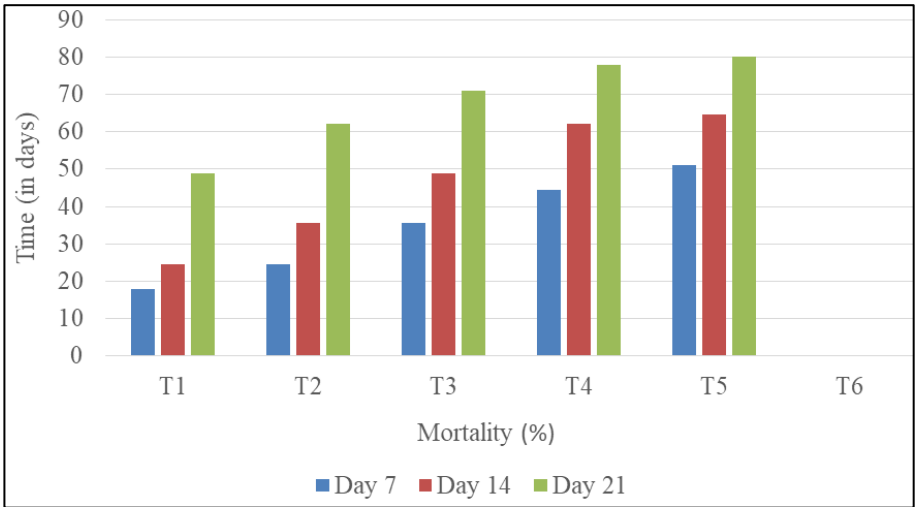

Fig 3: Per cent larval mortality post treatment

Conclusion

This study clearly demonstrates that formulation methods strongly influence the survival and effectiveness of microbial biocontrol agents. Among all carriers tested, sodium alginate gel consistently maintained high viability of both *Metarhizium anisopliae* and *Heterorhabditis indica*, supporting fungal colony stability and nematode juvenile survival under storage. Compared to traditional carriers such as cocopeat, talc, and diatomaceous earth, gel-based and encapsulated formulations performed significantly better, showing higher microbial persistence and efficacy. Pot culture trials further validated its superiority, with a clear dose-response relationship culminating in 80% white grub mortality at 10 ml L^{-1} after 21 days. These results highlight sodium alginate gel as a robust, eco-friendly, and farmer-compatible carrier that ensures compatibility between fungi and nematodes while offering sustained release and long-term control. Overall, it presents a promising alternative to chemical pesticides for sustainable white grub management in cropping systems.

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