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Eco-friendly formulation preparation of fungal biopesticide *Metarhizium anisopliae* and its impact on the viability, insecticidal activity and pesticidal compatibility

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Abstract

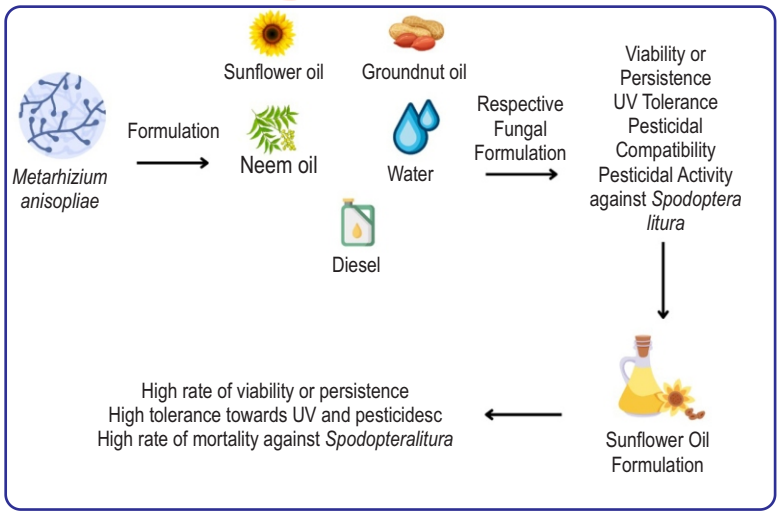
Aim: The present investigation evaluated the effect of various formulations on the viability, pesticide compatibility and biocontrol potential of fungal biopesticidal agent *Metarhizium anisopliae* under laboratory conditions.

Methodology: Soil isolate of *M. anisopliae* biomass was formulated with different oils like sunflower, groundnut, neem oil, water and diesel using green principles.

Results: Sunflower oil recorded maximum viability among the formulations, revealing 91.4% formulation efficacy, followed by groundnut oil, which showed 87.2% efficacy. The viability of the fungal isolate was found to be low in diesel formulation, which showed 69.2% formulation efficacy. A compatibility study revealed that sunflower oil formulation showed high tolerance to all the tested pesticides. Sunflower oil formulation also showed a high rate of mortality against *S. litura.*

Interpretation: The present study suggests using oil formulation as an effective carrier for the fungal biopesticide *Metarhizium anisopliae* with high compatibility and viability.

Key words: Biopesticide, Fungal strains, *Metarhizium anisopliae,* Sunflower oil



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Introduction

Environmental pollution is a worldwide scenario whose impact on human health is of major concern. Before the raise on issue of pollution, it is mandatory to maintain an equal balance between the present scenario of pollution (caused my man-made activities) and its possible consequences. Pesticides are a major class of environmental pollutants devised to eradicate pests. Since their mode of action toward one species is unspecified, they cause adverse effects on living organisms (including humans) other than pests. According to WHO, developing countries have 3 million cases of pesticide poisoning, with up to 220,000 deaths annually (Kaur *et al.,* 2019). Pesticides offer advantages to the productivity and yield of crops, but they also pose toxic effects on food chains, food webs, and ecosystems. Pesticides are associated with insecticides, fungicides, herbicides, rodenticides and other chemicals used in agriculture practices (Relyea, 2012). Though synthetic pesticides are effective against insect pests, indiscriminate use leads to various hazards like destruction of soil, aquatic ecosystems, and beneficial non-target organisms, including host plants.

Most importantly, the insect pests develop resistance to synthetic pesticides. Hence, it is necessary to develop or formulate pesticide agents that should act against target pests without affecting non-target organisms, including humans (Namasivayam and Bharani, 2021). Biological agents and their metabolic products, mainly plants and microorganisms (bacteria, fungi, viruses, protozoa, nematodes), have gained more attention in recent decades. Even though bacterial species (*Bacillus thuringiensis*) and viral species (Nuclear Polyhedrosis Viruses) are broadly utilised in the current study, from the species mentioned above, entomopathogenic fungi have gained considerable attention in the Integrated Pest Management (IPM). These fungal species are lethal to insects, and have the highest potential for pest management (Udayababu and Zacharia 2021). *Entomopathogenic fungi* are bioinsecticides, exhibiting the capacity to kill arthropods. The mechanism of infection involves various phases, dealing with the fungus direct interaction with the targeted insect's substrate. The efficiency of the infection mechanism is affected by various agents (lytic enzymes, secondary metabolites, and adhesins produced by *Entomopathogenic fungi*). Since the efficiency of insecticides in *Entomopathogenic fungi* is maximum, they are regarded as biopesticides in organic farming (Litwin *et al.,* 2020).

Among various entomopathogenic fungi, *Beauveria bassiana*, *B. brongniartii, Metarhizium anisopliae, Lecanicillium lecanii, Hirsutella thompsonii, Cladossporium oxysporium* and *Isaria fumosorosea* are the major fungal species exhibiting pathogenicity to various insects (Induja *et al.,* 2021). For instance, *B. bassiana* with strain PDRL1187 infect Mustard Aphid, *Lipaphis erysimi* and *Aphis craccivora* Koch. While the strain BB-01 infects *Schizaphis graminum*, Rhopalosiphum padi, *Brevicoryne brassicae* and *L. erysimi*. Another fungi, *L. lecanii* with strains V17, PDRL922 infect *M. persicae* and *L. erysimi* along with *A. craccivora* Koch. Mustard aphids, Diamondback moth *L. erysimi* and *Plutella xylostella* are infected by *Paecilomyces fumosoroseus* with the strain n32. *Metarhizium anisopliae* (green muscardine fungi) with the strains L6, M440, PDRL711, PDRL526 infect Cabbage aphid, *Mustard Aphids, Lipaphis erysimi, Aphis gossypii, A. craccivora* Koch respectively. (Maina *et al.,* 2018). Of these, *Metarhizium anisopliae* (Ascomycetes: Hypocreales) or green muscardine fungi is a species found naturally in soils and has been widely employed as a potential biopesticidal agent, as this fungi is infected by diverse insects. *Metarhizium*efficiently removes diverse insects, mainly the *Lepidoptera* (order of winged insects).

They are broadly utilised in regulating various insects (termites, mosquitoes, leafhoppers and beetles) (Namasivayam *et al.,* 2015). However, applying entomopathogenicfungi as a pesticide never yields the desired results due to various abiotic and biotic factors. These species are vital as they mediate as naturally occurring biological control agents of diverse pests (Carruthers and Hural, 1990). Biological control with entomopathogenic fungi is beneficial for, decreasing the density of pest population. Preserving entomopathogens is required, either naturally or by incorporating them to regulate the growth of pests (Moino and Alves 1998). Parallelly, in the Integrated Pest Management scheme, the incorporation of suitable pesticides is a significant step, which has a relation with entomopathogenic fungi, leading to the rise in control efficiency, declining the rate of essential insecticides and lowering the chances of environmental pollution, as well as pest resistance expression (Parjane *et al.,* 2020). Despite its broad usage as a biopesticide, many factors limit its effect. A key constraint to commercialize fungal biopesticides is overcoming difficulties in formulating a viable, lucrative and user-friendly product.

Formulating biocontrol agents, including bioinoculants and biopesticides, is critical in determining crop growth and yield cultivated by organic agriculture practices (Namasivayam *et al.,* 2022). The live nature of the microbial agent underscores the importance of formulation in maintaining the microbial cells in a metabolically and physiologically competent state in order to obtain the desired benefit when applied. Variety of methods to formulate fungal biocontrol agents are commercially available. Diverse natural and synthetic agents can be used in formulating biocontrol agents which protect the cells or spores from various environmental stresses (Perumal *et al*., 2023; Atrchian *et al.,* 2024; Priyanka *et al*., 2023). Oliveira *et al.* (2015) extensively studied and investigated a protocol for estimating the conidial viability of fungal entomopathogens *B. bassiana* and *M. anisopliae* from commercial products using emulsifiable oil formulation. Pesticidal activity of *B. bassiana* and *M. anisopliae* against green stinkbug *Nezaraviridula* (Linn.) was reported by Nada (2015), resulting in *M. Anisopliae* exhibiting highest pesticidal activity. Latifian (2018) studied the physicochemical properties and effects on different oil formulations (canola oil, soybean oil, sesame oil, corn oil and odoriferous oil) of *Metarhizium anisopliae* for controlling date palm horned beetle *Orycetes elegans.*

Increase in the persistence of entomopathogenic fungus *M. anisopliae* for controlling *Aedes aegypti* larvae using neem oil formulation was reported by Paula *et al.* (2019) and concluded that the half-life and virulence of *M. anisopliae* increases on combining with neem oil and testing the mixture against *A. aegypti* larvae under simulated field conditions. Neem oil also shielded the fungus from ultraviolet radiation. Field efficacy of *M. anisopliae* oil formulations (mineral oil and silicon oil) against *Rhipicephalus microplus* ticks using cattle spray was extensively reviewed by Barbieri *et al.* (2023). Based on the previous studies, the present study was undertaken to evaluate the effects of *M. anisopliae,* a fungal biopesticide, formulated with oil, water and diesel and screened for investigating the viability, pesticidal compatibility and activity.

Materials and Methods

Isolation identification of fungal strains: *Metarhizium anisopliae* was isolated from garden soil in SIMATS School of Engineering, Chennai by serial dilution technique (Fernandes *et al.* 2010). The home Garden Soil Sample (1 g) was added and stirred well in 100 m l^{-1} of distilled water. Homogenised content was serially diluted and transferred to the sterile Petri plate. Sterile PDA was poured into the Petri plate, and the content was gently mixed. Seeded plates were incubated at 28°C for 7 days. After incubation, the plates were observed for fungal growth. Fungal colonies were identified through morphological or cultural characteristics. *M. anisopliae* was identified in accordance with the identification criteria proposed by Paul and Clerk (1996). Over a continuous 20-day period, the growth characteristics of isolates on PDA were observed. Parameters such as colony size, mycelial colour, colony reverse, and conidial mass colour were visually assessed. Spore features, including shape and size of *Metarhizium* isolates were scrutinised using wet mount glass slide techniques under a light microscope (Fish Olympus phase contrast microscope).

Preparation of fungal formulation: Fungal formulation using different formulating agents was carried out by the modified method of Namasivayam *et al.* (2022). The fungal inoculum was prepared from a well-sporulated fungal culture 20 days after seedling emergence on the sterile PDA slant for this study. The PDA slant was flooded with sterile distilled water and a Tween-20 mixture under aseptic conditions. The prepared suspension was centrifuged, followed by resuspension of the collected pellet in sterile distilled water, which was used as a source of inoculum. Sunflower oil, groundnut oil, neem oil, diesel and water, screened for formulation, were prepared by dispersing the fungal spore inoculum into the respective oils stirred well with a magnetic stirrer at ambient conditions for 3 hr, followed by transferring respective formulation aseptically to sterile screw cap vial and used for further studies.

Estimation of formulation efficacy

***In vitro* Glass Tube Assay**: A known volume of respective formulation was aded to 100 m l of distilled water and kept in shaker at ambient condition for 30 min. The homogenised suspension was serially diluted and plated. Viable colonies thus formed were recorded, and formulation efficacy was determined from the colony count data.

Phyllosphere Assay: Healthy groundnut plants (20 days after seedling emergence) raised in pots were used for the study. Respective formulation was sprayed on groundnut plants using an ultra-low volume sprayer. Samples were collected from the plants 15 and 30 days after application (top, bottom and middle portion of the plants). Collected phyllosphere samples were brought to the laboratory for further processing. One gram of collected leaves was suspended in 100 ml distilled water. Tween-80 mixture was incubated under shaking conditions (150 rpm for 30 min.). The homogenised suspension was serially diluted. Aliquots were transferred to the plates containing sterile PDA medium. Inoculated plates were incubated at optimum temperature (28°C) for 5 days, and fungal colonies were enumerated after the incubation period. Protein and carbohydrate content in groundnut leaves was estimated by the method of Lowry *et al.,* 1951) and (DuBois *et al.,* (1956).

UV radiation screening: Formulating agents (sunflower oil, groundnut oil, neem oil, water and diesel) were pouned sterile test tubes and exposed to UV in the Laminar Air Flow Chamber for 5 min. After treatment, the formulations were serially diluted and placed on PDA plates. These plates were incubated at 28°C, and fungal colonies were enumerated and recorded after incubation.

Effect of Formulations on Pesticidal Compatibility

Selection of pesticides: Pesticides, Agent Plus (lambda-cyhalothrin 5% EC), Ekalux (Quinolphos 25% EC) and Hostathion (Triazophos 40% EC), were selected for the present study, were procured from a local fertiliser store in Sholinganallur, Chennai. A working solution was initially prepared from the stock solution and reconstituted in distilled water, which was used for further studies.

Compatibility studies: A dual plate assay was adopted to determine the compatibility of sunflower oil, groundnut oil, neem oil, water and diesel with the pesticide. A 2.5 ml each of sunflower oil, groundnut oil, neem oil, water and diesel were mixed, with Agent Plus (lambda-cyhalothrin 5% EC), Ekalux (Quinolphos 25% EC) and Hostathion (Triazophos 40% EC) in a sterile screw cap vial, incubated under static conditions for 3 hr at ambient temperature. Control was maintained without pesticides. After incubation, the content was diluted serially with sterile distilled water under aseptic conditions. Aliquots were incubated on a sterile PDA medium under ambient conditions for 5 days, followed by estimation of fungal colonies. Differences in the colony count of control and treatments revealed the compatibility.

Pesticidal activity: The effect of formulation on the pesticidal activity was investigated against the polyphagous lepidopteron

pest *Spodoptera litura* under laboratory conditions.

Bioassay: Leaf tip method was adopted for determining the pesticidal activity induced by the respective formulation. *S. litura* III instar larvae collected from groundnut fields of Chengalpet were used in this study. Castor leaves were dipped with respective formulations at ambient temperature. Treated leaves were transferred to plastic trays. 10 III instar larvae were introduced into the trays and allowed to feed on the leaves. Control was maintained by dipping the leaves with distilled water. Inoculated trays were incubated at room temperature. Treated groups were observed for mortality. The mortality data was determined Lethal Concentration 50 (LC_{50}) and Lethal Time 50 (Lt_{50}).

Statistical Analysis: All the experimental were carried out in three replicates. The significance of data was analysed by One–way ANOVA.

Results and Discussion

The success of biocontrol agents, including biopesticides, mainly depends on the formulation techniques that enhance the viability, post-treatment persistence and desirable biocontrol potential (Lei *et al.,* 2023). The formulation can be carried out with natural and synthetic agents like oils, detergents, charcoal,talc, milk powder, and gelatin. In this study, the effect of various fungal formulation on the viability or persistence and compatibility with the synthetic pesticide was studied. Among the different formulating agents (sunflower oil, groundnut oil, neem oil, water and diesel), sunflower oil exhibited maximum formulation efficacy (91.4%), followed by groundnut oil (87.2%), neem oil (80%), water (70.1%) and diesel formulation (69.2%) (Fig. 1). These findings are in accordance with Namasivayam *et al.* (2022) who similarly evaluated the formulation (soybean oil, coconut oil, sunflower oil, palm oil, biogel and water formulation) of biocontrol agents (biopesticides and bioinoculants) from *Trichoderma viride, Fusarium oxysporum* and *Alternaria alternata*, alongwith the viability, compatibility with metallic nanoparticles and efficiency of degrading organic wastes and recorded the highest efficacy of formulation in soybean oil + coconut oil, followed by soybean oil + sunflower oil, dry granular biogel and soybean oil + palm oil formulation. Impact of formulation on biocontrol efficacy of *Fusarium oxysporum* and *Alternaria alternata* revealed that the formulating agents (soybean oil, coconut oil, sunflower oil, palm oil, biogel and water) reduced the growth of fungal colonies and biogel formulation.

Ground nut plants, raised in pots, were sprayed with different oil formulations. The perusal of data showed that 15-days-old phyllosphere exhibited the highest number of fungal colonies than 30 days (Fig. 2). Among the treatments, sunflower oil formulation recorded the maximum efficacy. The colony count in sunflower oil was 20×10^8 colonies g^{-1} of leaves and 16×10^8 colonies g^{-1} of leaves in 15 days and 30 days, respectively. Least efficacy was recorded in diesel and water, whose colony count was 12×10^8 colonies g^{-1} of leaves and 10×10^8 colonies g^{-1} of leaves in 15 days respectively. No fungal colonies were found in diesel and water formulation at 30 days treatment. These findings clearly showed that the diesel formulation inactivated or destroyed the fungal spores. The viability of fungal spores was also less in water formulation as the fungal organism was directly exposed to light. Paixão *et al.* (2017) studied the increase in heat tolerance afforded by conidial formulation of *Metarhizium anisopliaes*.I. (IP 46) and *Metarhizium robertsii* (ARSEF 2575) with mineral oil, vegetable oil, canola oil, water and carboxymethyl cellulose gel as the precursor agents. Conidia incorporated into unstable oil-in-water emulsions showed a higher percentage of formulation within oil micellae compared to stable emulsions, which exhibited a higher percentage outside the oil micellae. However, no significant difference in thermotolerance was observed between conidia formulated in stable and unstable emulsions. Similarly, biocompatible formulation of potential fungal biopesticide *Nomuraea rileyi* for the improved post treatment persistence and biocontrol potential was by Namasivayam and Vidyasankar (2014), who reported maximum viability and pesticidal activity in biogel formulation than oil and hydrogel formulation at all the tested temperatures.

The effect of formulation on the biochemical composition of groundnut leaves was studied by measuring the quantity of carbohydrates and proteins. The highest carbohydrates content was found in sunflower oil formulation at 15 and 30 days. Similarly, protein content was elevated significantly in sunflower oil formulation on 15th and 30th days. A significant reduction in both carbohydrate and protein contents was recorded in diesel formulation on 15th and 30th day. (Fig. 3, 4). El-Badawy *et al.* (2018) studied the impact of entomopathogenic fungal infection on biochemical parameters in the haemolymph of cotton leaf worm, *S. littoralis using B. bassiana, M. anisopliae, P. lilacinus* and *L. antillanumin* at different time intervals (1st, 2nd, 3rd and 4th days). The results showed a reduction in the carbohydrate content in the haemolymph of larvae, injected with fungi on 1st, 2nd and 3rd days, in comparison to control insects. Protein content in control larvae and fungal larvae increased on 1st day, while on 2nd and 3rd day, the protein content increased in the larvae injected with *B. bassiana* and *L. antillanum.* On 4th day, carbohydrate and protein content in 5th larval instar haemolymph of *S. littoralis* gradually increase due to the effect of fungal infection. Lipid content also rose gradually in the larvae injected with the fungal species, concluding that the biochemicals in the 5th larval instar haemolymph of *S. littoralis* infected with entomopathogenic fungi differed, leading to modifications in the contents of haemolymph, causing abnormal growth and development of insects.

The test fungal formulations were also screened for UV radiation tolerance under laboratory conditions. The results showed that the sunflower oil and neem oil formulation retained maximum viability, while diesel formulation recorded the least viability when exposed to UV rays for 1-2 hrs. A significant difference in fungal colonies was not observed in groundnut oil and water. It can be seen that various oil formulations protected viable or infective propagules or spores from UV radiation (Fig. 5).

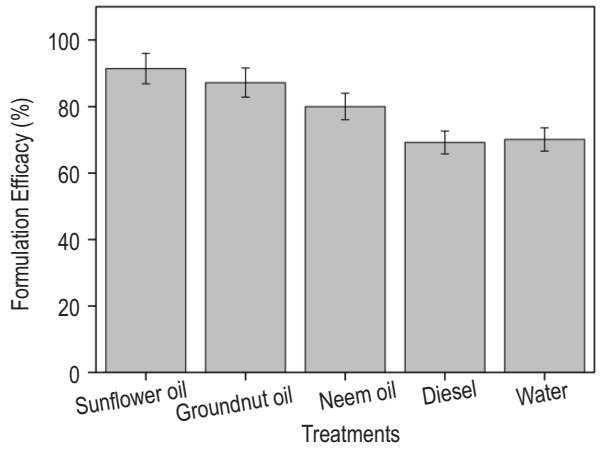

Fig. 1: Effect of Formulating agents on the viability of *M. anisopliae* (*in-vitro* glass tube assay)

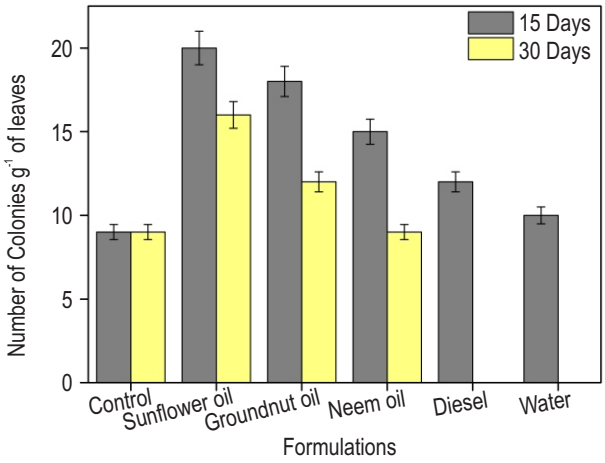

Fig. 2: Effect of Formulating agents on the viability of *M. anisopliae* (Phyllosphere pot assay).

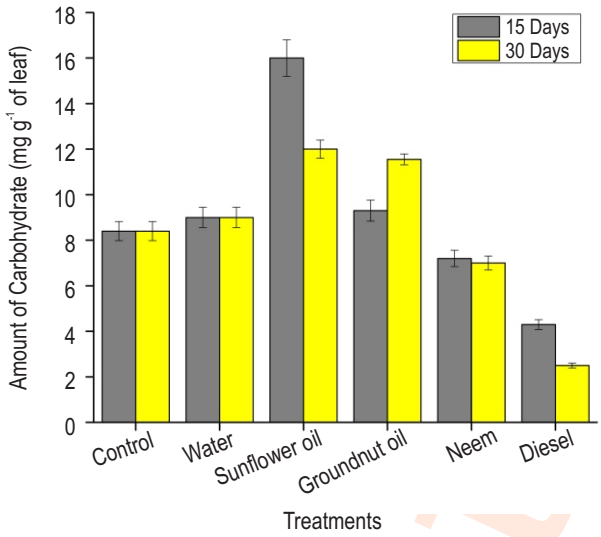

Fig. 3: Effect of formulations on the carbohydrate content of groundnut leaves.

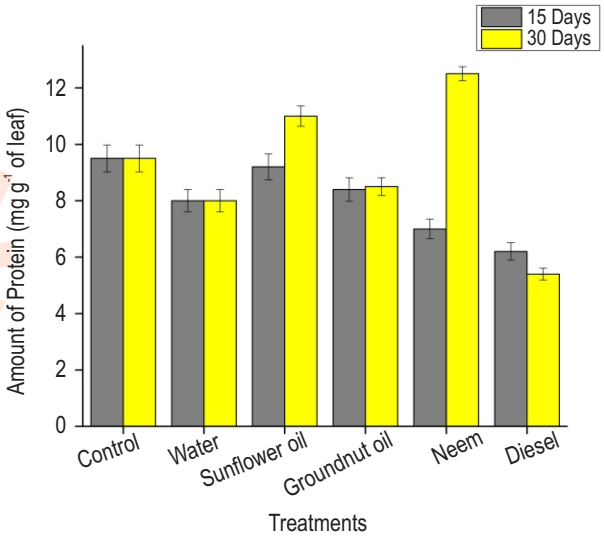

Fig. 4: Effect of formulations on the protein content of groundnut leaves.

Rodrigues *et al.* (2016) studied the bio-insecticidal action of *Beauvaria bassiana* and *Metarhizium anisopliae* on *Diatrea saccharalis.* The detrimental impact, caused by *D. saccharalis* manifested through both direct and indirect mechanisms. The direct mechanism involved the formation of spores within the culm, leading to reduced sap flow and increased vulnerability to wind, rain, drought, and related stresses, potentially causing structural failure, whereas the indirect mechanism involved facilitation of phytopathogenic microorganisms, including *Colletotrichum falcatum* and *Fusarium moniliforme* fungi into the culm (through the holes), inducing red rot and sucrose inversion (Gallo *et al.,* 2002). Their studies revealed that UV radiation poses a toxic impact on pure conidia after exposure for 5 minutes, leading to a drastic reduction in germination from 94% to 52% for *B. bassiana.* At the same time, *M. anisopliae* gradually reduced germination from 96% to 54%. Alginate formulation secured the conidial spores of *B. bassiana* against UV exposure when evaluated at different time intervals (15 min to 48 hr) since the fungi retain viability even after exposure.

The mortality rate was high in dry encapsulated conidial spores of *B. bassiana* (79.6%) and *M. anisopliae* (10%). The effect of temperature and UV radiation on the growth and pathogenicity of *Metarhizium anisopliae* using conidial strains (LBMa-11, LBM-30, LBM-41 and LBM-146) was extensively studied and evaluated by Gutiérrez *et al.* (2022). Various

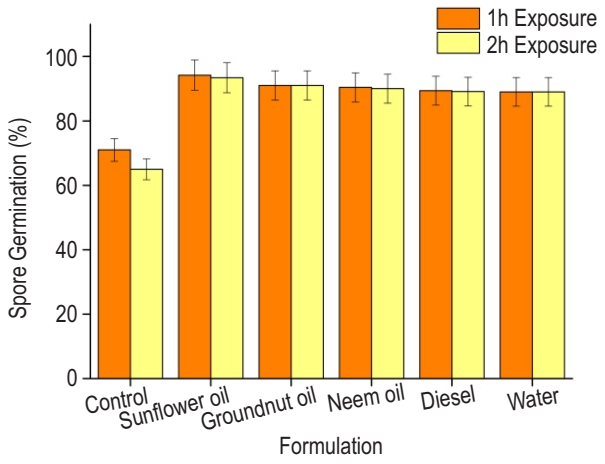

Fig. 5: Effect of UV radiation on the spore germination of *M. anisopliae.*

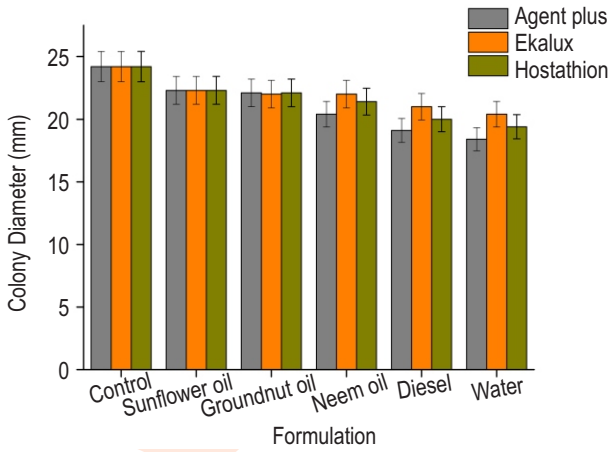

Fig. 6: Effect of compatibility of formulation with pesticides.

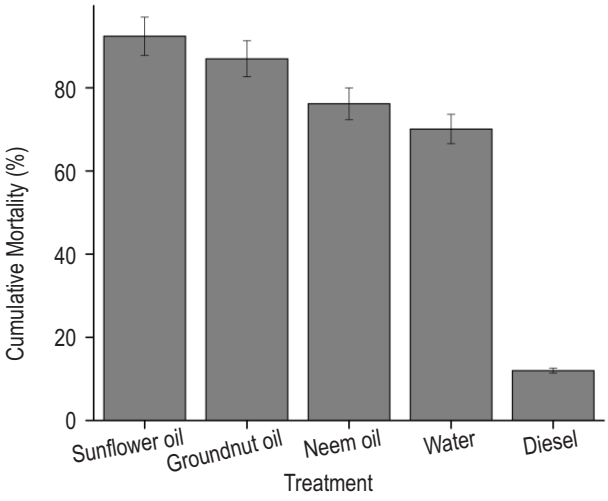

Fig. 7: Effect of formulation on cumulative mortality of *S. litura.*

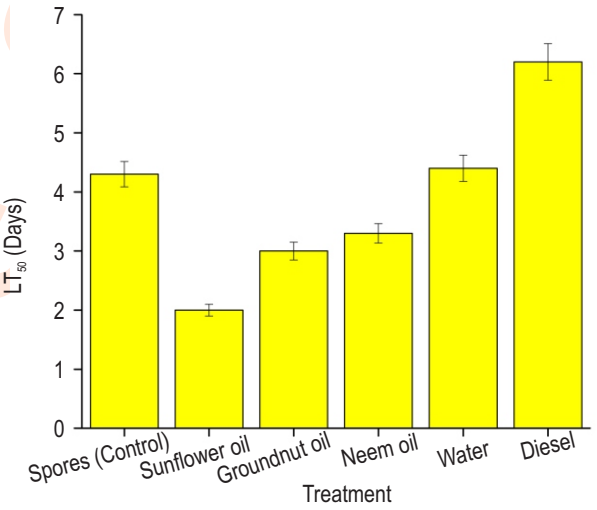

Fig. 8: Effect of formulation on LT_{50} of *S. litura.*

environmental factors, such as temperature, solar radiation, pH, and humidity, can impede the growth and development of *M. anisopliae,* presenting a notable drawback for its field application. Temperature plays a pivotal role in conidial germination, germ tube development, penetration, colonization, and reproductive capabilities (Pereira *et al.,* 2018). Solar radiation significantly influences the survival and efficacy of this fungus as a biocontrol agent, while also contributing to its persistence on treated crops. Ultraviolet light modulates the growth of *Metarhizium* spp. based on the intensity of energy received and duration of exposure, potentially inhibiting or delaying conidial germination through direct (mutations) or indirect genetic damage.

The effect of fungal compatibility was evaluated by measuring the colony diameter of fungi with different pesticides (Agent Plus, Ekalux and Hostathion). In this study, sunflower oil formulation showed the highest compatibility with all the pesticides, followed by groundnut oil. Diesel exhibited the least compatibility among all the tested pesticides (Fig. 6). Sunflower oil formulation exhibited superior pesticidal compatibility due to its hydrophilic-lipophilic balance, facilitating optimal spore dispersion and adhesion. The presence of unsaturated fatty acids enhances membrane fluidity and permeability, enhancing spore germination and infection. Furthermore, its stability and biocompatibility provide an ideal environment for sustaining viability and virulence of *M. anisopliae,* resulting in enhanced pest control efficacy compared to other formulations. The hydrophobic nature of Diesel impedes uniform spore dispersion, while its chemical composition, primarily aliphatic hydrocarbons, may disrupt spore viability. Additionally, its volatility compromises

formulation stability, contrasting with oil-based carriers like sunflower or neem oil, which offer superior longevity and environmental safety for *M. anisopliae* formulations. Moreover, its potential to induce oxidative stress in fungal spores due to its aromatic hydrocarbon content further diminishes its efficacy. The persistence of diesel residues in soil may also pose ecological risks, necessitating careful consideration of environmental impacts. Overall, the combination of these factors underscores the suboptimal suitability of diesel as a formulating agent for *M. anisopliae* formulations.

Sahayaraj *et al.* (2011) evaluated the compatibility of entomopathogenic fungi (*Beauveria bassiana*, *Isaria fumosorosea*, and *Lecanicillium lecanii*) with plant extracts (*Syndrella nodiflora*, *Premna tomentosa, Vitex negundo, Ipomea carnea, Pteridium aquilinum* and *Annona squomosa*) and commercial botanicals (Biospark, Phytophrate, Exodos, Biodos and Neemgold). Their studies revealed that mycelial growth in *B. bassiana* was reduced by botanical agent Biospark, while Phytophrate and Exodos inhibited the mycelial growth in *I. fumosorosea* and *L. lecanii.* However, maximum and minimum growth inhibiting activity against *B. bassiana, I. fumosorosea,* and *L. lecanii* was exhibited by *A. squomosa* and *I. carnea*, respectively. Sohrabi *et al.* (2019) evaluated the toxicity of *B. bassiana, L. lecanii* and *M. anisopliae* along with the botanical insecticides (tondexir and palizin) for controlling *Galleria mellonella.*

Compatibility of Entomopathogenic fungi (*B. bassiana, M. anisopliae* and *L. lecanii*) with Neonicotinoids [Thiamethoxam 25 SG and Dinotefuran 20 SG] against *Nilaparvata lugens* at recommended concentrations was studied by Nagendra Reddy *et al.* (2021). Their studies resulted in the inhibition of fungal growth partially at all test concentrations (0.5RC, 1RC and 1.5RC) by the neonicotinoids. Dinotefuran 20 SG @ 0.4 g/l exhibited medium toxicity to *B. bassiana, M. anisopliae* and *L. lecanii* (20% – 35% reduction in fungal growth) while Thiamethoxam 25 SG exhibited medium toxicity to *B. bassiana* and *M. anisopliae* but, the toxicity was average in *L. lecanii* (35% – 50% reduction in fungal growth). Thiamethoxam 25 SG alone exhibited 65% mortality in brown planthopper, while, in combination with *B. bassiana* in exhibited 86.25% mortality and with *L. lecanii* exhibited 81.25% mortality under glasshouse conditions. Dinotefuran 20 SG alone 98.75% mortality exhibited.

Under laboratory conditions, the effect of formulation on the pesticidal activity against polyphagous lepidopteran pest, *Spodoptera litura* III instar larvae was investigated using bioassay method and this method was screened for evaluation of cumulative mortality rate and LT_{50}. Among the test formulations, sunflower oil (92.4%) showed the maximum pesticidal activity against *S. litura*, confirmed by high mortality rate and lower LT_{50}, followed by groundnut oil (84.1%) and neem oil (79.0%). Reduced pesticide activity was recorded in the diesel formulation (Fig. 7). Significant mortality reduction and increased LT_{50} were inferred from the diesel formulation (P = 0.01). The results show that fungal spores lose virulence by the formulating agent (diesel), reducing mortality. In the case of LT_{50}, the respective formulation exhibited notable variation. Among them, sunflower oil formulation showed lower LT_{50} days than other formulations (Fig. 8). This formulation brought about a marked reduction in LT_{50} as the infectivity of spores dispersed in sunflower oil was enhanced. The pesticidal activity of fungal spores primarily depends on the viability. When the fungal spores are formulated with suitable carriers or adjuvants (mainly oil-based agents), the spores are dispersed uniformly, enhancing the availability of fungal spores. Similarly, potential of *B. bassiana, M. rileyi, I. fumosorosea, I. sinclairii* and *L. muscarium* as biocontrol agents of *Plutella xylostella* II instar larvae and their compatibility with chemical insecticides was reported by Duarte *et al.* (2016). *P. xylostella* is a prominent pest affecting Brassicaceae plants worldwide, exacerbated by its remarkable dispersal capacity, adaptability to adverse weather conditions, and abbreviated life cycle with prolific reproductive rates (Honda *et al.,* 1992). Cabbage (*Brassica oleracea*) stands as a primary target among cultivated species, as its foliage serves as a preferred host, causing yield reduction and plant mortality. Employing various biological control agents, particularly entomopathogenic microorganisms like *B. bassiana, M. anisopliae, M. rileyi, I. sinclairii* and *L. muscarium* presents a viable strategy for managing *P. xylostella* infestations and curtailing associated crop losses.

Development of suitable formulation techniques is crucial to enhance the efficacy of biopesticides. Our present study dealt with the impact of *Metarhizium anisopliae* formulation on various parameters (formulation efficacy, persistence, UV tolerance, pesticidal compatibility and activity against Spodoptera litura). Of the formulatiing agents, sunflower oil exhibited the abovementioned parameters. Further research is needed to explore large-scale production, evaluation, and fresh trials.

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