

Original Research Article

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Isolation and Identification of Entomopathogenic Fungi from Rhizosphere Soil and *invivo* study on Insecticidal activity against the Mealybugs

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ABSTRACT

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Mealy bugs are pests that mainly infect plants in the Solanaceae family. These bugs generally act as parasites and also as vectors that transmit pathogens. These bugs are controlled only through the roguing process, so the need to develop a pest control agent could prevent crop loss. Meanwhile, the extensive use of chemical pesticides was already causing environmental concerns and posing adverse effects on soil organisms. Hence, developing a biological agent may prevent these damages and also enhance plant growth. *Trichoderma hamatum* is an entomopathogenic fungus that targets only insects and not plants. In this study, we isolated this fungus from the rhizosphere soil and cultivated it in Oatmeal- cTAB media. The morphological and molecular characterization was done to confirm the fungal species. After which, a fungal spore assay was done on the mealybugs reared under laboratory conditions; reduction of bugs was prominently recorded by day 14. Hence, the findings provide evidences on potential insecticidal activity of *Trichoderma hamatum* towards mealybugs.

Introduction

Insects play a major role in damaging plants and causing a decrease in yield rate, which is faced as an important problem in agricultural fields (Kauppi *et al.*, 2021). They act as a vector for the transfer of diseases. In order to control the insect pest, farmers are currently using chemical pesticides that contain many harmful chemicals that cause damage to the environment. The pesticides washed out from the fields are let into water bodies that can cause harmful effects for the living organisms in the water bodies. These chemical pesticides contain heavy

metals that could deposit in soil and make the soil sterile over a period of time. To avoid such consequences caused by chemical pesticides, biological pest control and insect control agents called as biopesticides or bioinsecticides are used. These biological agents can be bacteria, fungi, insects and viruses that could kill the insect pest. The biopesticides are environmentally friendly, more specific and can degrade without leaving residues. Hence, biopesticides could be a better alternative to chemical pesticides. (Borges *et al.*, 2021). Mealy bugs are insects that belong to the family *Pseudococcidae* and have a cottony appearance small,

soft-bodied sucking insects. The adult mealy bugs are found on leaves, stems, and roots and are covered with a white, waxy substance. They feed on the sap of different plants. These bugs have a waxy outer coat that helps in adherence to the plant easily and protects the insect from external environmental factors. These insects also act as a vector to transfer the plant pathogen from an infected plant to a healthy plant. The mealy bugs generally infect many fruits, vegetables and ornamental plants that could lead to a reduction in yield following economical loss. Since this feed on the plant sap that contains basic sugars, the insect excretes a substance known as honeydew. The excess plant sap attracts the ants, which transfer the mealybugs from soil to the plant or from one plant to another. The honeydew substance also causes fungal growth that covers the outer covering of plants, including leaves the in turn inhibits photosynthesis and ultimately leads to the death of plants due to lack of nutrition. Mealy bug infestation changes with climatic conditions. Therefore, control of mealy bugs could help prevent infestation of pests and diseases (Tanwar *et al.*, 2007). The honeydew of mealy bugs and *Phlebotomus portentosus* forms fungal gall disease in the roots of infested plants. (Fang *et al.*, 2020) Mealy bugs do not have a complex diet, and they simply rely on plant sap, whereas the metabolism of the mealy bug is supported by a nested symbiotic relationship of the bacteria present inside it. These bacteria help in producing the essential amino acids which are required for the growth of mealy bugs (Sza bo *et al.*, 2017).

Fungi are a group of micro-organisms that are beneficial in many areas and are also used in biopesticide production. The fungal biopesticides are used against many disease-causing microbes and insect pests. They are effective against pests such as aphids. Some fungal biopesticides are *Beauveria* species and *Metarhizium* species. *Trichoderma* is a free-living fungal species of the family *Hypocreaceae* that can directly or indirectly perform mycoparasitism towards phytopathogens and other insects that infest plants, which makes it a potential biocontrol agent. (Poveda 2021). Unlike chemical fungicides, *Trichoderma spp.* provide long-term protection without adverse side effects. *Trichoderma hamatum* is one of the species in this genus that has numerous biological activities and is also considered an alternative to chemical pesticides due to its wide range of biological activities. *T.hamatum* has been known to exhibit nematocidal activity (Yao *et al.*, 2023). Hence, this study aimed to reveal the insecticidal activity of the entomopathogenic fungi isolated from the soil towards

the mealy bugs that were causing yield reduction and was carried out with an in-vitro analysis.

Materials and Methods

Isolation of entomopathogenic fungi from rhizosphere soil

Formulation of media

The media were formulated by minor modification of Posadas *et al.*, 2012. In this, 4 g of rolled oats was added to 200 ml of water and boiled to 100ml; the filtrate with a solidifying agent was sterilised, and 1 ml cetyl trimethyl ammonium bromide (CTAB) (0.6g/ L concentration) and 1ml of Chloramphenicol antibiotic (0.5/L concentration) were added to the media for isolating the entomopathogenic fungi from serially diluted soil samples.

Isolation and identification of Fungi

The primary screening of the fungi was done using the macroscopic and microscopic appearance (Liu *et al.*, 2020), which were then characterised using MALDI-TOF MS for confirmation of the fungal species based on the 16s ribosomal proteins at the molecular level (Motteu *et al.*, 2022) using MALDI Bio typer, and MSP-matched patterns were generated based on the score value obtained and matched by comparison to the library of filamentous fungi.

Rearing of mealy bugs (Dhobi and Mehta 2018)

The mealy bugs were collected from the *Murraya koenigii* plant infested with these bugs. The collected bugs were transferred to the sprouted potato, which was washed and surface sterilised. The sprouted potato acts as a medium for the mealy bugs and is used for their maintenance in a healthy state for spore infection assay.

Cultivation of *Trichoderma hamatum* for collecting spores

Firstly, the inoculum was prepared using the Oatmeal-CTAB broth. 2ml of prepared inoculum was then inoculated into 200ml of Sabouraud Dextrose Broth with chloramphenicol and incubated at 24°C on a rotary shaker for 7 days (Bhadauria *et al.*, 2012).

Spore infection assay (Lana *et al.*, 2023)

Spore infection assay under lab conditions

The broth was filtered and kept at 90°C in a water bath for 5 minutes to obtain fungal spores, and then applied topically to the reared mealy bugs under laboratory conditions. A separate sprouted potato was used to maintain the mealy bugs, which were used as a control. The fungal spores were sprayed on consecutive days and observed for the results.

Results and Discussion

Isolation and identification of *Trichoderma hamatum*

Different fungal colonies were isolated from the serially diluted soil sample.

A primary screening was done through macroscopic and microscopic examination, from which a single colony with similar physical characteristics to *Trichoderma spp.* was isolated as shown in Figure 1.

Cultural characteristics of *Trichoderma hamatum*

Macroscopic appearance:

The colonies are white to cream-coloured and develop a green or yellow tint with maturation. They appear to be fluffy or cottony with a velvety texture and become powdery in appearance due to sporulation, with a smooth or slightly wrinkled surface with well-defined margins. (Figure 2).

Similarly, Liu *et al.*, (2020) isolated *Trichoderma hamatum* from the rhizosphere soil of *Syringa oblata* and identified it through macroscopic and microscopic observations.

Microscopic appearance

The conidiophores are erect, septate and branched, and bear conidia at the tips. Conidia are typically ellipsoid or cylindrical in shape, with a round or tapered end, are hyaline and smooth-walled, and are produced in chains. The size of the conidia varies but ranges between 3-5 µm in width and 5- 10 µm in length. (Figure 3 A, B, C)

Characteristics in the broth

Trichoderma hamatum, when inoculated in Sabouraud Dextrose Broth, is observed to be fluffy, cottony, ball-like in appearance. (Figure 4 A, B)

Molecular Identification

Identification of Fungi based on MALDI-TOF MS Analysis

MALDI-TOF MS was performed for the identification of fungi based on the 16s ribosomal protein. The genus and species of the fungal culture. *Trichoderma hamatum* CBS 123063 was confirmed by matching with a score value of 1.10, which refers to secure genome identification and probable species identification when compared with library filamentous fungi that is been depicted in Table1 and Figure 5. The fungi were confirmed as *Trichoderma hamatum* by MALDI-TOF MS; likewise, Motteu *et al.*, 2022, have also reported that MALDI-TOF MS could be implemented as an added value to classical morphology identification.

Spore infection assay

By topical application of the fungal spores under lab conditions, a significant reduction in mealybug adults was observed from day 8, and complete reduction of mealybugs was observed after 14 days. Similarly, Lana *et al.*, (2023) performed a spore assay by topical application on *Spodoptera littoralis*. But no infection of the sprouts was noted, as the mealybugs are first reared on sprouted potato. In like manner, Dhobi and Mehta 2018 studied that sprouted potato medium would be a suitable medium for rearing the mealybugs.

The spores started to cover the surface of the mealybugs, and the penetration of mycelium was microscopically observed, which might be a reason behind the death of mealybugs and their reduction. The insecticidal activity of *Trichoderma hamatum* was studied from day 0 to day 14. (Figure 6) *Trichoderma spp.* was reported to have pesticidal activity towards wide range of plant pathogens and some insects which was described by Li *et al.*, 2022 and Baazeem *et al.*, 2021. In the present study the insecticidal activity of *Trichoderma hamatum* against mealybugs was tested by performing a spore infection assay as described by Lana *et al.*, 2023

Table.1 Identification of fungi by MALDI-TOF MS using 16s ribosomal protein

Rank (Quality)	Matched Pattern	Score value	NCBI Identifier
1 (-)	<i>Trichoderma hamatum</i> CBS 123063 CBS	<u>1.10</u>	<u>49224</u>
2 (-)	<i>Scedosporium apiospermum</i> D 16 256 6 6LLH	<u>1.07</u>	<u>41687</u>
3 (-)	<i>Fusarium oxysporum</i> D 16 256 6 5 LLH	<u>1.01</u>	<u>5507</u>
4 (-)	<i>Monilinia laxa</i> CBS 488_5CBS	<u>1.0</u>	<u>61186</u>
5 (-)	<i>Trichoderma longibrachiatum</i> DSM 63060 DSM mod	<u>0.99</u>	<u>5548</u>
6 (-)	<i>Scedosporium apiospermum</i> 11137023 MVD	<u>0.96</u>	<u>41687</u>
7 (-)	<i>Aspergillus pulvinus</i> DSM 63373 DSM mod	<u>0.95</u>	<u>38239</u>
8 (-)	<i>Dichotomopilus funicola</i> 1267 MPA	<u>0.92</u>	<u>79816</u>
9 (-)	<i>Penicillium roqueforti</i> DSM 1812 DSM	<u>0.92</u>	<u>5082</u>
10 (-)	<i>Fusarium equiseti</i> DSM 62204 DSM	<u>0.90</u>	<u>61235</u>

Figure.1 *Trichoderma hamatum*

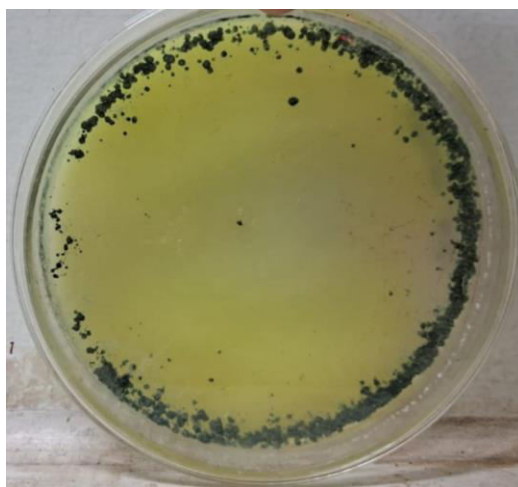
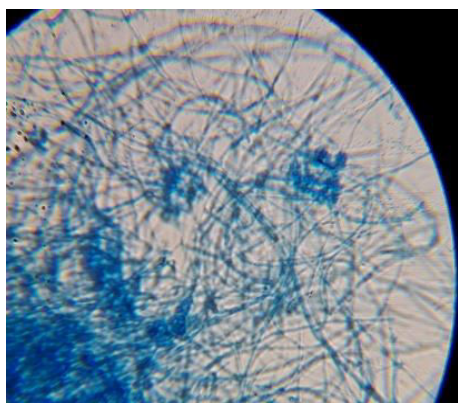


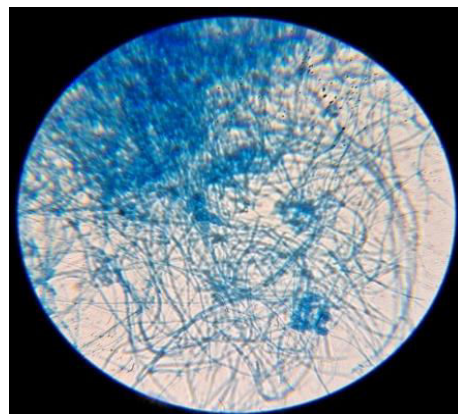
Figure.2 White fluffy colonies of *Trichoderma hamatum* with a green and yellow tint



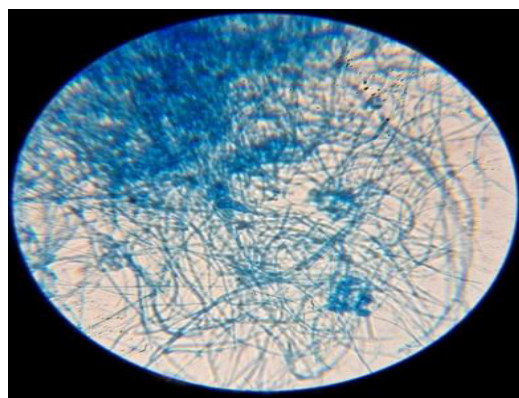
Figure.3 A, B and C: microscopic appearance of the fungal culture showing erect, branched conidiophores, often septate, bearing conidia at the tips, which are ellipsoid or cylindrical in shape with a round end and smooth-walled



A



B



C

Figure.4 A and B Fluffy cottony growth appearance of *Trichoderma hamatum*



A



B

Figure.5 MALDI TOF MS analysis of the fungi was inferred by score value of 1.10

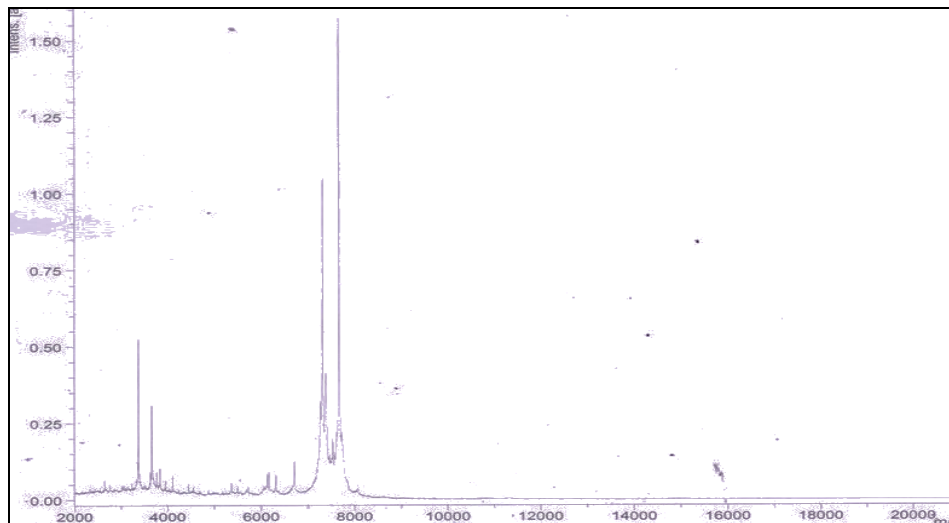
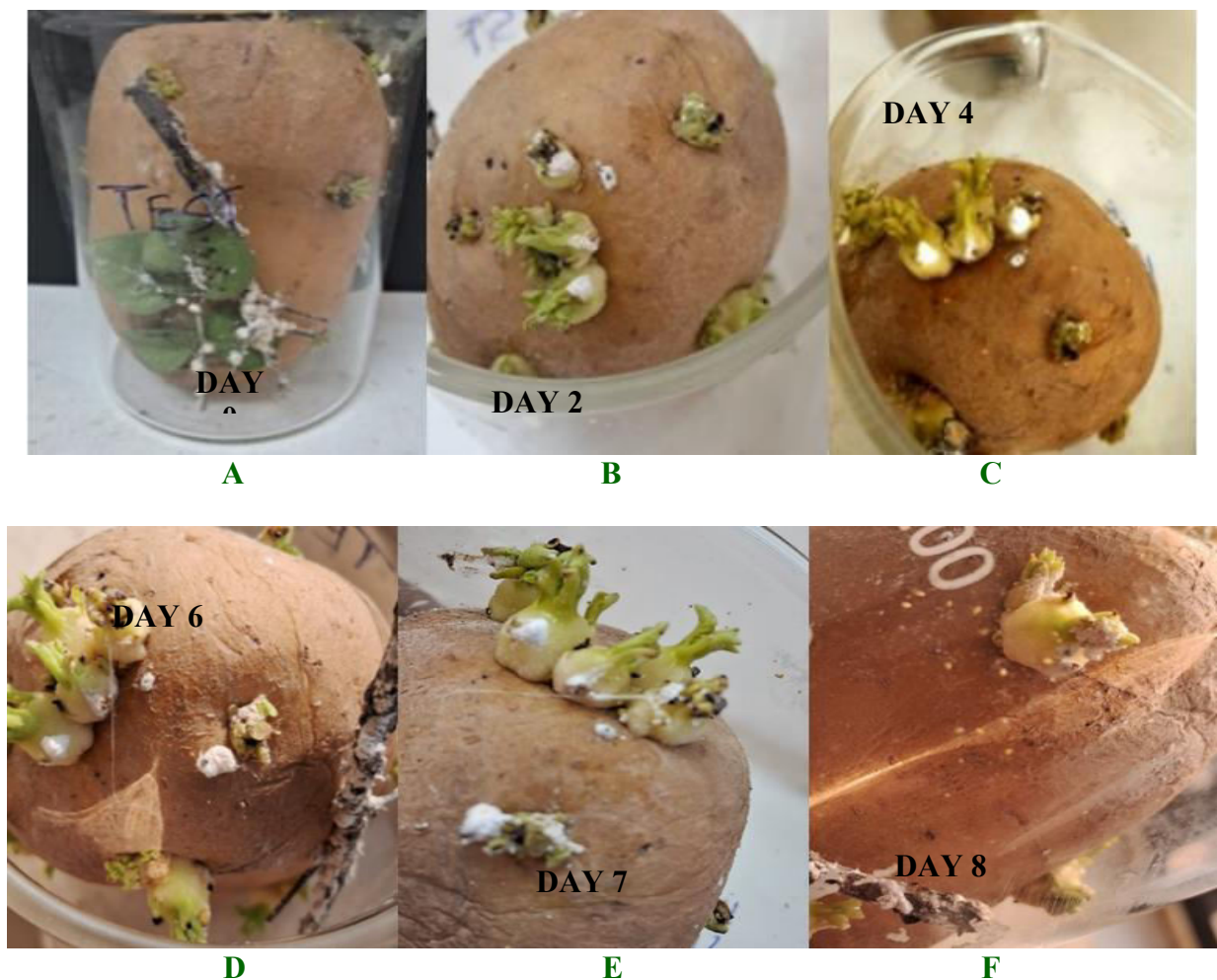


Figure.6 Fungal spores on mealy bugs on sprouted potato media under lab conditions on Day 0(A), Day 2 (B), Day 4(C), Day 6(D), Day 7(E), Day 8(F), Day 9 (G), Day 14 (H and I)



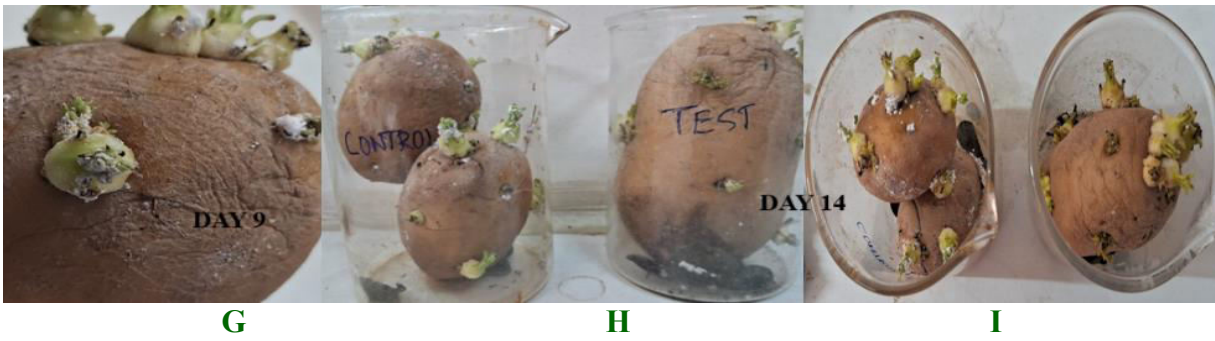
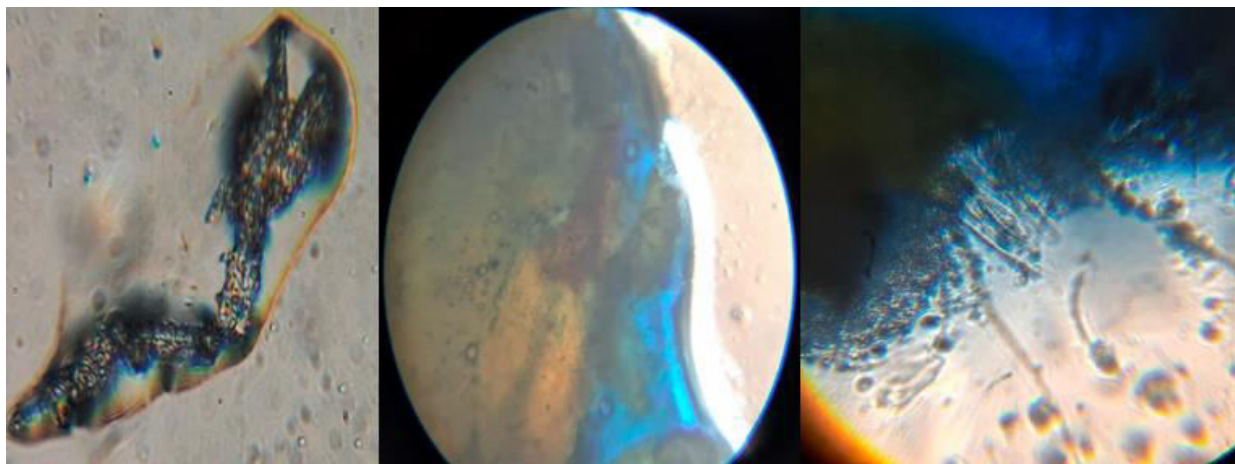
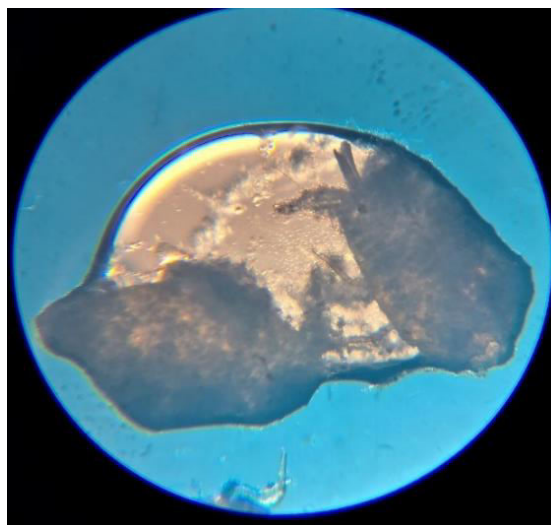


Figure.7 A. Microscopic Appearance of the infected mealybug from test sample and B. Uninfected mealybug as control



A



B

In this study, the entomopathogenic fungi isolated from the soil were identified as *Trichoderma hamatum*, which was then cultivated in Sabouraud Dextrose Broth, and

the spores collected showed effective insecticidal activity against mealybugs. Hence, *Trichoderma hamatum* spores could be used as an effective bioinsecticide in the field of

agriculture. Further, *Trichoderma hamatum* can be formulated into an effective bioinsecticide by combining fungal spores with the enzymes of the fungi as a biological formulation. Hence, the results could be achieved faster. Other than mealybugs, *Trichoderma hamatum* can also be targeted towards other insects and pests, and this fungal species can be developed into a broad-spectrum bioinsecticide in the future.

Author Contributions

Arunanandhini Manivannan: Conceived the original idea and designed the model and wrote the manuscript. K. Nithya: Designed the model and the computational framework and analysed the data.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

- Abera Daba, Gezahegn Berecha, Mekuria Tadesse, Addisie Belay. Evaluation of the herbicidal potential of some fungal species against *Bidens pilosa*, the coffee farming weeds. Saudi J Biol Sci 2021; 28: 6408–6416
<https://doi.org/10.1016/j.sjbs.2021.07.011>
- Ahmad Nawaz, Muhammad Dildar Gogi, Muhammad Naveed, Muhammad Arshad, Muhammad Sufyan, Muhammad Binyameen, Shams Ul Islam, Muhammad Waseem, Muhammad Bilal Ayyub, Muhammad Jalal Arif and Habib Ali. *In vivo* and *in vitro* assessment of *Trichoderma species* and *Bacillus thuringiensis* integration to mitigate insect pests of brinjal (*Solanum melongena* L.). Egyptian J Biol Pest Control 2020; 30 (60): 1-7
<https://doi.org/10.1186/s41938-020-00258-5>
- Ahmed Abdelkhalek, Abdulaziz A Al-Askar, Amr A Arishi, Said I Behiry. *Trichoderma hamatum* Strain Th23 Promotes Tomato Growth and Induces Systemic Resistance against Tobacco Mosaic Virus. J Fungi 2022; 8 (228): 1-20
<https://doi.org/10.3390/jof8030228>
- Alaa Baazeem, Abdulaziz Almanea, Palanisamy Manikandan, Mohammed Alorabi, Ponnuswamy Vijayaraghavan Ahmed Abdel-Hadi. *In Vitro* Antibacterial, Antifungal, Nematocidal and Growth Promoting Activities of *Trichoderma hamatum* FB10 and Its Secondary Metabolites. J Fungi 2021; 7 (331): 1-15
<https://doi.org/10.3390/jof7050331>
- Bhanu Pratap Bhaduria, Smita Puri Singh PK. Mass Production of Entomopathogenic Fungi Using Agricultural Products. The Bioscan 2012; 2: 229-232
<https://thebioscan.com/index.php/pub/article/view/1005>
- Bin Liu, Shida Jib, Huifang Zhang, Yucheng Wang, Zhihua Liu. Isolation of *Trichoderma* in the rhizosphere soil of *Syringa oblata* from Harbin and their biocontrol and growth promotion function. Microbiol Res 2020; 235: 1-10
<https://doi.org/10.1016/j.micres.2020.126445>
- Chase AR, Osborne LS, Ferguson VM. Selective Isolation of the Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae* from an artificial potting medium. The Fla Entomol. 1986; 69 (2): 285-292
<https://doi.org/10.2307/3494930>
- Dhobi CB and Mehta DM Comparative Biology of Mealy bug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) on Different Host under Laboratory Condition. Int J Agric Sci 2018; 10(16): 7021-7024
<https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217>
- Fabian Felipe Fernandez Daza, Ginna Rodriguez Roman, Marino Valencia Rodriguez, Ivan Andres Gonzalez Vargas, Heiber Cardenas Heano, Marney Pascoli Cereda, Raul Alberto Cuervo Mulet. Spores of *Beauveria bassiana* and *Trichoderma lignorum* as a bioinsecticide for the control of *Atta cephalotes*. Biological Res. 2019; 52 (51):1-8
<https://doi.org/10.1186/s40659-019-0259-y>
- Gitta Szabó, Frederik Schulz, Elena Toenshoff, Jean-Marie Volland, Omri Finkel, Shimshon Belkin, Matthias Horn. Convergent patterns in the evolution of mealybug symbioses involving different intra

- bacterial symbionts. The ISME J 2017; 11: 715–726.
<https://doi.org/10.1038/ismej.2016.148>
- Hongpeng Xiong, Jiacheng Cai, Xuan Chen, Shiping Liang, Xiujun Wen, Cai Wang. The Effects of *Trichoderma* Fungi on the Tunneling, Aggregation, and Colony-Initiation Preferences of Black-Winged Subterranean Termites, *Odontotermes formosanus* (Blattodea: Termitidae). Forests 2019; 10: 1-14
<https://doi.org/10.3390/f10111020>
- Jorge Poveda. *Trichoderma* as biocontrol agent against pests: New uses for a mycoparasite Biol Control 2021; 159: 1-8
<https://doi.org/10.1016/j.biocontrol.2021.104634>
- Julieta Posadas, Ricardo Comerio, Jorge Mini, Ana Nussenbaum Roberto Lecuona A novel dodine-free selective medium based on the use of cetyl trimethyl ammonium bromide (CTAB) to isolate *Beauveria bassiana*, *Metarhizium anisopliae* sensu lato and *Paecilomyces lilacinus* from soil. Mycol 2012; 104(4): 974–980. <https://doi.org/10.3852/11-234>
- Katja Kauppi, Ari Rajala, Erja Huusela, Janne Kaseva, Pentti Ruuttunen, Heikki Jalli, Laura Alakukku, Marja Jalli. Impact of Pests on Cereal Grain and Nutrient Yield in Boreal Growing Conditions. Agron. 2021; 11(592): 1-16
<https://doi.org/10.3390/agronomy11030592>
- Maite Lana, Oihane Simón, Pablo Velasco, Víctor M. Rodríguez, Primitivo Caballero, Jorge Poveda. First study on the root endophytic fungus *Trichoderma hamatum* as an entomopathogen: Development of a fungal bioinsecticide against cotton leafworm (*Spodoptera littoralis*). Microbiol Res 2023; 270: 1-8
<https://doi.org/10.1016/j.micres.2023.127334>
- Mohammad Manjur Shah and Hamisu Afiya. Introductory Chapter: Identification and Isolation of *Trichoderma* spp. – Their Significance in Agriculture, Human Health, Industrial and Environmental Application. *Trichoderma* - The Most Widely Used Fungicide. Intechopen 2019; 1-12
<https://doi.org/10.5772/intechopen.83528>
- Natacha Motteu, Berdieke Goemaere, Sandrine Bladt Ann Packeu. Implementation of MALDI-TOF Mass Spectrometry to Identify Fungi from the Indoor Environment as an Added Value to the Classical Morphology-Based Identification Tool. Front Allergy 2022; 3: 1-8
<https://doi.org/10.3389/falgy.2022.826148>
- Pablo Velasco, Víctor Manuel Rodríguez, Pilar Soengas Jorge Poveda. *Trichoderma hamatum* Increases Productivity, Glucosinolate Content and Antioxidant Potential of Different Leafy Brassica Vegetables. Plants 2021; 10: 1-13
<https://doi.org/10.3390/plants10112449>
- Rathna Silviya Lodi, Chune Peng, Xiaodan Dong, Peng Deng Lizeng Peng. *Trichoderma hamatum* and Its Benefits. J Fungi 2023; 9 (994): 1-17
<https://doi.org/10.3390/jof9100994>
- Rudsamee Wasuwan, Natnapha Phosrithong, Boonhiang Promdonkoy, Duangjai Sangsrakru, Chutima Sonthirod, Sithichoke Tangphatsornruang, Somsak Likhitrattanasapal, Supawadee Ingsriswang, Chettida Srisuksam, Kewarin Klamchao, Malinee Suksangpanomrung, Thipmanee Hlepongpanich, Sareeya Reungpatthanaphong, Morakot Tanticharoen, Alongkorn Amnuay kanjanasin. The Fungus *Metarhizium* spp. BCC 4849 Is an Effective and Safe Mycoinsecticide for the Management of Spider Mites and Other Insect Pests. Insects 2021; 13 (42): 1-18
<https://doi.org/10.3390/insects13010042>
- Shannon Borges, Abdulrahim Alkassab, Elizabeth Collison, Silvia Hinarejos, Ben Jones, Emily McVey, Ivo Roessink, Thomas Steeger, Maryam Sultan, Jacoba Wassenberg. Overview of the testing and assessment of effects of microbial pesticides on bees: strengths, challenges and perspectives. Apidologie 2021; 52: 1256–1277.
<https://doi.org/10.1007/s13592-021-00900-7>
- Tanwar, R. K., Jeyakumar, P., & Monga, D. (2007). *Mealybugs and their management*. National Centre for Integrated Pest Management. https://nriipm.res.in/NCIPMPDFs/Publication/BulletinMealybugsEnglish_.pdf
- Xiaojiao Li, Jinsong Leng, Longfeng Yu, Haidong Bai, Xiaojun Li, Michael Wisniewski, Jia Liu, Yuan Sui. Efficacy of the biocontrol agent *Trichoderma hamatum* against *Lasiodiplodia theobromae* on macadamia. Front in Microbiol 2022; 13:1-9
<https://doi.org/10.3389/fmicb.2022.994422>
- Xin Yao, Hailin Guo, Kaixuan Zhang, Mengyu Zhao, Jingjun Ruan, Jie Chen. *Trichoderma* and its role in biological control of plant fungal and nematode disease. Front in Microbiol 2023; 14:1-15
<https://doi.org/10.3389/fmicb.2023.1160551>
- Yi-Wei Fang, Wen-Bing Wang, Ming-Xia He, Xin-Jing Xu, Feng Gao, Jing Liu, Tian-Wei Yang, Yang Cao, Tao Yang, Yun Wang, Chun-Xia Zhang. Relationship between the honeydew of mealy bugs and the growth of *Phlebotomus portentosus*. PLoS ONE 2020; 15(6): 1-10
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