Journal homepage
www.ajas.uoanbar.edu.iq
Anbar Journal of Agricultural Sciences
(University of Anbar – College of Agriculture)

EFFECT OF SECONDARY METABOLISMS OF THE TRICHODERMA HARZIANUM FUNGUS ON THE SECOND LARVAL STAGE OF THE MUSCA DOMESTICA L.

S. M. Hardan* **Th. A. Muhsen**

College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad

***Correspondence to:** Saffanah M. Hardan, Dept of Biology, College of Education for Pure Science (Ibn Al-Haitham, University of Baghdad, Iraq.
Email: safana.mushrif1102a@ihcoedu.uobaghdad.edu.iq

Article info

Received: 2024-05-18
Accepted: 2024-08-01
Published: 2024-12-31

DOI-Crossref:
10.32649/ajas.2024.184477

Cite as:
Hardan, S. M., and Muhsen, Th. A. (2024). Effect of secondary metabolisms of the trichoderma harzianum fungus on the second larval stage of the musca domestica l.. Anbar Journal of Agricultural Sciences, 22(2): 1202-1213.

Abstract

The experiment in this study aimed to detect secondary metabolic chemical compounds in the alcoholic extract of the *Trichoderma harzianum* fungus and to demonstrate the effect of this extract on *Musca domestica* L. in the second larval stage. Gas chromatography-mass spectrometry (GC-MS) was used to analyze the raw extract of *T. harzianum*. The analysis revealed that the extract contained a wide range of chemical compounds. Among these, 15 secondary metabolites were identified, with ethanol, 1-(2-butoxyethoxy), showing being the highest at 13.43%. Decanol and carbonochloridic acid, and decyl ester were also significant at 11.80% each. The molecule benzenepropanamine, alpha-methyl had the lowest presence at 1.48%. The study also tested the efficacy of the crude extract on the second stage of housefly larvae using both direct spray and food methods at three concentration levels (25%, 50%, and 75%). Significant variations in mortality rates were observed at a probability level of less than 0.05. The 75% concentration produced the best results achieving a 100% mortality rate by the fifth day compared to 13.3% on the first day when applied as a food treatment, and reaching 100% by the fourth day with direct spraying.

Keywords: Musca domestica, Entomopathogenic Fungi, T. harzianum, Secondary Metabolism, GC-MS technology.

تأثير الأيض الثانوي لفطر Trichoderma harzianum في مرحلة اليرقة الثانية لحشرة Musca domestica L.

سفانة مشرف حردان* ثامر عبدالشهيد محسن

كلية التربية ابن الهيثم، قسم علوم الحياة، جامعة بغداد

*المراسلة الى: سفانة مشرف حردان، قسم علوم الحياة، كلية التربية ابن الهيثم، جامعة بغداد، العراق.

البريد الالكتروني: safana.mushrif1102a@ihcoedu.uobaghdad.edu.iq

الخلاصة

هدفت التجربة في هذه الدراسة إلى اكتشاف المركبات الكيميائية الأيضية الثانوية في المستخلص الكحولي لفطر *Trichoderma harzianum* وإظهار تأثير هذا المستخلص على يرقات الطور الثاني لحشرة *Musca domestica* L.. تم استخدام كروماتوجرافيا الغاز–مطياف الكتلة (GC–MS) لتحليل المستخلص الخام من *T. harzianum.* كشف التحليل أن المستخلص يحتوي على مجموعة واسعة من المركبات الكيميائية. من بين هذه المركبات، تم تحديد 15مستقلبًا ثانويًا، أظهر الإيثانول، 1–(2–butoxyethoxy) أعلى معدل تواجد بنسبة 13.43%، كما كان Decanol وحمض Carbonochloridic، decyl ester، مهمين، حيث بلغ معدل وجود كل منهما 11.80%. كانت جزيئة Benzenepropanamine, alpha–methyl، الأقل تواجدًا بنسبة 1.48%، كما اختبرت الدراسة فعالية المستخلص الخام على يرقات الطور الثاني للذبابة المنزلية باستخدام طريقتي الرش المباشر ومعاملة الغذاء وبثلاثة تراكيز (25%، 50%، و75%)، لوحظ اختلافات كبيرة في معدلات الوفيات عند التركيزات المختلفة، بمستوى احتمالية أقل من 0.05. حقق تركيز 75% أفضل النتائج، إذ حقق معدل وفاة بنسبة 100% بحلول اليوم الخامس، بينما كان معدل الوفاة لنفس التركيز في اليوم الأول 13.3% عند معاملة البيئة الغذائية ووصل إلى 100% بحلول اليوم الرابع مع الرش المباشر.

كلمات مفتاحية: الذبابة المنزلية، Musca domestica، الفطريات الممرضة للحشرات، T. harzianum، أيض ثانوي وتقنية GC–MS.

Introduction

The housefly (*Musca domestica* L.) is a common insect of the *Muscidae* Order *Diptera* family. The relationship between humans and flies is historically ancient, with 90% found in human dwellings being houseflies. They pose a significant nuisance and danger to public health, especially where decomposing organic waste and garbage accumulate. Houseflies are most commonly found in animal production sites, but are also abundant in urban areas where larvae develop amidst a wide range of decomposing plant materials, feces, and household garbage. The common factor among the suitable growth conditions for larvae is the presence of abundant live microorganisms (1, 10 and 11). *M. domestica* is the most prevalent and well-known

type of fly found in human dwellings, and among the most dangerous insect categories due to its ability to carry and transmit dangerous pathogens. Estimates indicate that it is a disease vector for a hundred pathogens. In response to it being a global pest affecting humans and animals, transmitting dozens of pathogens, and causing significant economic losses, scientific research has focused on identifying the microorganisms carried by this fly, their distribution, their impact on other living organisms, and their methods of interaction. This is to understand the challenges posed by flies to various life systems (10).

Insect-pathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana* have received considerable attention in the field of biological pest control due to their parasitic capabilities and the ability to select strains that can cause disease in the targeted pest. These pathogenic fungi have shown encouraging results as methods for controlling arthropods in general (1), and promising results in controlling populations of adult houseflies (20 and 28). Hywel-Jones (14) and Blackwell (6) estimate that there is a huge number of species of fungi pathogenic to insects, indicating their crucial role in the ecosystem. Despite this, they have not received the necessary attention compared to their counterparts in plant pathogens (6). Therefore, many aspects can be explored regarding infection and transmission methods, natural products, and the response secretions during the interaction between pathogenic fungi and their hosts. Among the few fungi species studied mechanically are *B. bassiana* and various species of *Metarhizium* (4 and 24). These distinct species represent fungi that kill and consume their hosts within days of infection without causing noticeable adaptive behavioral changes to the host. Thus, these fungi have an infection pattern that contrasts with other types that manipulate host behavior, have a limited host range, and may spend more time in a symbiotic relationship with the host insect before killing it (27). Some entomopathogens' secondary metabolites have been shown to be beneficial in IPM programs against a variety of insect pests (13).

The fungus *Trichoderma harzianum*, which belongs to the Ascomycota division, is one of the most important fungal species used in biological control. It is used directly or indirectly as a bio stimulant, allowing for the reduction of chemical use (17). T. *harzianum* is known to control aphids and other pests due to its production of cuticle-degrading enzymes like protease, lipase, and chitinase, which break down the insect cuticle and release various mycotoxins. Over the past decade, it has been observed that plants colonized by T. *harzianum* have enhanced indirect defense mechanisms against aphids (8). These findings align with research by (5), which demonstrated that the culture filtrate of T. *harzianum* exhibited significant toxicity against *Helicoverpa armigera*, acting as a strong antifeedant. This reduced the larvae's feeding rate and body weight, decreased successful pupation, and increased larval and pupal mortality.

In light of the above, this study investigated and characterized the secondary metabolite compounds in the crude extract of T. *harzianum*, as well as assessed the impact of this extract on housefly larvae.

Materials and Methods

This experiment was conducted at the central laboratory of the Department of Biology at the College of Education, Ibn Al-Haytham, University of Baghdad.

Collection and rearing of insects: Housefly adults were collected from various farms and fields in Baghdad Governorate using an aerial net and placed in wooden cages covered with light fabric (tulle). They were provided with a petri dish containing about 50 g of powdered sugar and powdered milk (1:1 w/w) and another petri dish containing cotton soaked with water for the purpose of feeding the adult insects to encourage their multiplication. To facilitate egg-laying and larval feeding, each cage contained a 200-ml plastic container filled with ground dried sheep manure sterilized with an autoclave and sterilized dried bread powder in a 2:1 ratio. This mixture was moistened with distilled and sterilized water. The insects were reared for four generations before conducting experiments on them (9 and 18). The houseflies were then sent to the Natural History Museum of Iraq for accurate identification and classification.

Preparation of potato dextrose agar (PDA): The PDA medium was prepared according to the manufacturer's instructions, autoclaved for 15 minutes, and left to cool. The antibiotic chloramphenicol was added, and the medium poured into sterile plates. The T. *harzianum* fungal isolates were accurately identified and obtained from the soil of the agricultural area in the city of Ramadi. The plates were incubated at 25 ± 5°C for seven days (18).

Preparation of fungal extract in potato dextrose broth (PDB): The PDB medium was prepared according to the manufacturer's instructions by dissolving 30 grams of dry powder in 1 liter of distilled water in a glass flask. It was thoroughly mixed, autoclaved for 15 minutes, allowed to cool, and then supplemented with antibiotics.

Determining secondary metabolites and toxins in crude extract of T. *harzianum* by GC-MS device: After gathering the biomass of the T. *harzianum* fungus growing on PDB culture media, it was dried completely in an oven at 65°C. Next, 10 milliliters of pure methanol (99.99%) was added to it and shaken with a Vortex device. After that, it was placed in an ultrasonic device for 30 minutes and left for 24 hours. Three milliliters were then taken out and put in a sterile, clean vial for the GC Mas device, and one microliter was injected (18).

Production of fungal toxic proteins and secondary metabolites in the PDB medium: To extract secondary metabolites from the T. *harzianum* extract, a piece of fungal colony grown on PDA medium for three days was taken, and using a sterile scalpel, a 1 cm piece was transferred into 500 ml of previously prepared PDB medium. The flask was then incubated at 25±2°C with 80% humidity in a shaking incubator for 10-12 days.

The method involved maceration. After incubation, the fungal biomass was separated from the culture medium using soft gauze to retain the broth, which contained toxins, secondary metabolites, and spores. Crude secondary metabolites were extracted by filtering the light-yellow organic phase of the fungal mat using Whatman filter paper No.1. For cold extraction, 500 ml of broth was mixed with 750 ml of a solvent mixture (1:1 ethyl acetate and methanol) and shaken for 2-3 days at 28°C. The solvent was separated from the aqueous phase using a separating funnel and then filtered through a 0.2 µm micropore filter. A vacuum evaporator at 80°C

was employed to remove the solvent from the concentrated extracts. The crude extract was then dissolved in deionized water and Tween 80 (12).

Evaluation of the fungal extract's efficacy against the second larval stage of *M. domestica*: This experiment was conducted following the method described by (20) with some modifications, employing two methods: direct spray application of the fungal extract on insects and incorporation into food. Three concentrations of the crude fungal extract (25%, 50%, and 75%) were used by dissolving the methanolic crude extract in deionized water and Tween 80 to separate and dissolve the compound.

Direct spray technique: Ninety instar housefly larvae were used with 3 replicates of 30 larvae per concentration, and 10 larvae per replicate were sprayed with the fungal extract at concentrations of 25%, 50%, and 75%. The treated larvae were placed in medium-sized cages in an incubator at 25±2°C and 70% relative humidity. Mortality was recorded and averaged after 1, 2, 3, 4, and 5 days post-treatment.

Indirect technique (treated rearing medium): Five grams of housefly rearing medium were used and placed in a 50 ml container. The rearing medium was sprayed with the fungal extract and transferred to untreated containers at the three concentrations of 25%, 50%, and 75%, with three replicates per concentration as described in the direct method. 30 insects were placed in each concentration. The larvae were then incubated at 25±2°C and 70% humidity. Mortality was recorded after 1, 2, 3, 4, and 5 days (18).

Statistical analysis: All experiments were conducted using the completely randomized design (CRD). Mortality percentages were transformed using angular transformation before statistical analysis. Mean comparisons were performed using the least significant difference (LSD) test at a significance level of 0.05 (3).

Results and Discussion

Detecting chemical compounds using GC-MS technique in the crude extract of T. *harzianum:* Figure 1 shows the peaks detected in the crude methanolic extract (99.99%) of T. *harzianum* fungus analyzed by GC-MS.

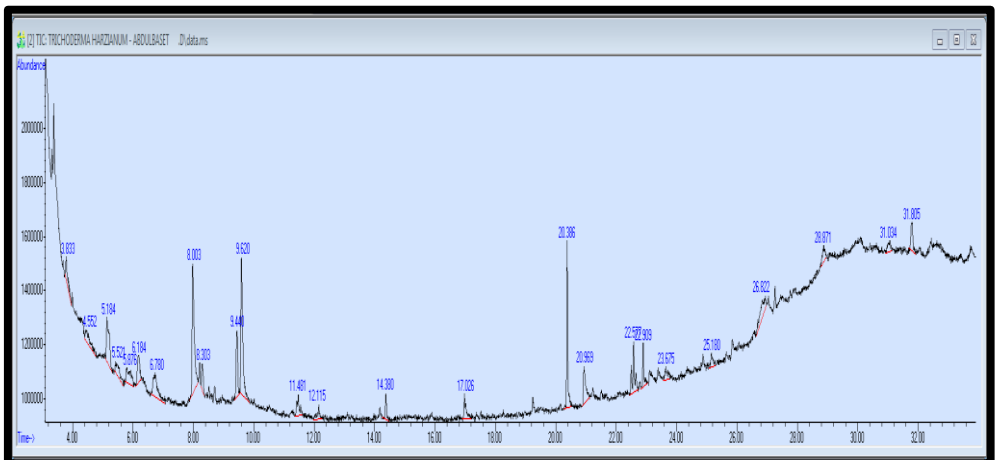
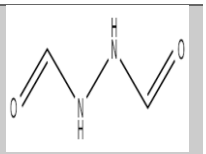
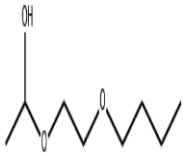
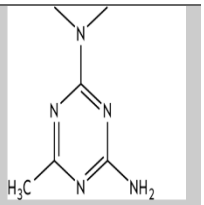
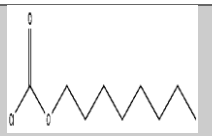
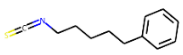
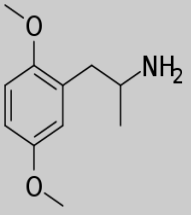
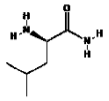
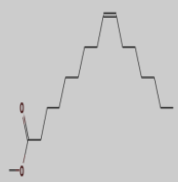
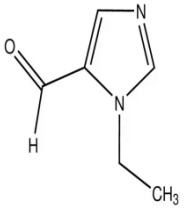
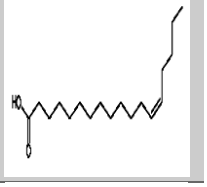
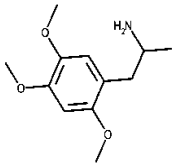
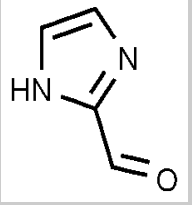
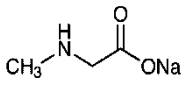
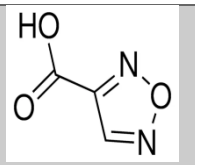


Figure 1: Peak curves in the crude fungal extract of T. *harzianum* using GC-MS technology.

Table 1 illustrates the secondary metabolites in the fungal extract identified by GC-MS, totaling 15 secondary metabolites. The compound ethanol, 1-(2-utoxyethoxy)- exhibited the highest presence at 13.43%. Decanol and carbonochloridic acid, and decyl ester ranked second at 11.80% each, with benzenepropanamine, alpha-methyl having the lowest presence in the fungal extract at 1.48%

Table 1: Secondary metabolite compounds in the crude methanolic fungal extract of T. *harzianum* using GC-MS technology.

NO	Name	Peak No.	R.T.	Area %	Classification	Mol. Weight	Mol. Formula	Structure
1	1,2-Hydrazinedicarboxaldehyde	1	3.831	2.29	Aldehyde	88.07	C2H4N2O2	
2	Ethanol, 1-(2-utoxyethoxy)-	2	8.003	13.43	Alcohol	190.31	C8H18O3	
3	Amino-4-dimethylaminomethylenepe	3	8.306	2.11	aromatic organic	371.43	C23H21N3O2	
4	Decanol	4	9.622	11.80	fatty alcohol	158.2811	C10H22O	
5	Carbonochloridic acid, decyl ester	5	9.622	11.80	carbonic acid	220.736	C11H21ClO2	
6	Benzenepropanamine, .alpha.-methyl	6	12.114	1.48	ammonium ion derivative	149.2328	C10H15N	
7	2,3-Dimethoxyamphetamine	7	17.031	2.25	Benzenoids	195.2582	C11H17NO	
8	2-Amino-4-dimethylaminomethylenepe	8	8.306	2.11	Amines	122.17	C7H10N2	

9	Hexadecanoic acid, methyl ester	9	20.389	8.94	fatty acid methyl esters	270.4507	C17H34O	
10	1-Imidazolidinecarboxaldehyde, 5-h	10	20.969	4.29	Benzyl	96.09	C4H4N2O	
11	trans-13-Octadecenoic acid, methyl	11	22.579	4.94	fatty acid	296.4879	C19H36O	
12	Benzenethanamine, 4-methoxy-.alpha	12	22.908	2.49	Amines	165.2322	C10H15NO	
13	1-Imidazolidinecarboxaldehyde	13	23.678	1.70	Benzyl	110.116	C4H4N2O	
14	Sarcosine, N-valeryl-, pentyl este	14	25.184	1.51	amino acid	243.3425	C13H25NO3	
15	1,2,5-Oxadiazol-3-carboxamide	15	25.184	1.51	Azoles	159.10	C4H5N3O4	

This research demonstrated that the crude extract of T. *harzianum* functions effectively as a pesticide against *Musca domestica* L. larvae, owing to its specific compounds and enzymes identified via gas chromatography-mass spectrometry analysis. The filtrate's impact was found to be dependent on both dosage and exposure time, with increased effectiveness observed at higher concentrations and extended application periods. Fungi are known to produce numerous secondary metabolites due to the diversity of fungal species and the variability of genes responsible for secondary metabolite production. This capability enables fungi to synthesize a wide range of chemical compounds, contributing to their widespread presence in various environments. Filamentous fungi, in particular, are renowned for

producing secondary metabolites that can exhibit biological effects on various organisms.

Our results are consistent with (13) who found crude extracts of secondary metabolites from *T. harzianum* affected *Myzus persicae*. These findings also align with those of (16), who demonstrated T. *harzianum's* ability to produce effective bioactive secondary metabolites, which serve as alternatives to hazardous industrial chemicals.

Moreover, studies analyzing extracts from T. *harzianum* using various solvents such as ethyl acetate and butanol have revealed several chemical compounds, including flavonoids, alkaloids, tannins, peptides, saturated and unsaturated fatty acids, phenols, and terpenoids. These secondary metabolites present in raw extracts from various organisms such as plants, fungi, and algae can exhibit numerous direct or indirect biological effects on other organisms. These compounds are utilized in antibiotic resistance, induced resistance, and can mitigate various environmental issues, including plant diseases and pest control (7, 15 and 19).

Evaluation of the fungal extract on housefly larvae: The results of evaluating the crude fungal extract of T. *harzianum* on the second instar larvae of houseflies using direct spraying and indirect (rearing medium) method revealed higher mortality rates using the food method compared to direct spraying (Table 2). The evaluation showed significant differences at a 0.05% probability level in the concentrations used in the experiment over time compared to the control group. Results indicate that the 75% concentration produced the highest mortality rates over time with a high significant difference, reaching 100% mortality on the fifth day. The cumulative effect of treatments for this concentration over five days was 61% using the food method, whereas the 25% concentration showed the highest mortality rate of 7.1% on the fourth day, with a cumulative effect of 2.76% over five days.

Table 2: Effect of T. *harzianum* fungal extract on second instar larvae of houseflies using the food method.

No.	Concentration (%)	Destruction rate/Day (%)					Transaction impact rate (%)
		Day 1	Day 2	Day 3	Day 4	Day 5	
1	25	0.0	3.3	3.4	7.1	0.0	2.76
2	50	6.6	14.2	29.1	52.0	62.5	32.88
3	75	16.6	44.0	71.4	75.0	100.0	61
4	Control	0.0	0.0	0.0	0.0	0.0	-
LSD <0.05	1.69						

Table 3 also shows the impact of the fungal extract on second instar larvae of houseflies via direct spraying on insects. There were significant differences at the 0.05 probability level among treatments with the highest mortality rate at the 75% concentration level, reaching 13.3% on the first day and 100% on the fourth. At 25% concentration, mortality was 3.3% on the first day, with no fatalities on the fourth and fifth days.

Table 3: Effect of the *T. harzianum* fungal extract on the second instar larvae of the housefly using the direct spray method.

No.	Concentration (%)	Destruction rate/Day (%)					Transaction impact rate (%)
		Day 1	Day 2	Day 3	Day 4	Day 5	
1	25	3.3	6.8	7.4	0.0	0.0	3.5
2	50	10.0	11.1	20.8	26.6	18.8	17.4
3	75	13.3	34.6	47.0	100.0		48.7
4	Control	0.0	0.0	0.0	0.0	0.0	-
LSD <0.05	1.07						

Crude extracts of T. *harzianum* have been utilized in biological control to combat diseases and environmental problems, particularly insect pests, due to their bioactive compounds, i.e., secondary metabolites. These extracts have been employed as alternative biopesticides to synthetic chemicals (2, 23 and 25). Studies evaluating the effect of T. *harzianum* extract on insects show an increased mortality rate in treated insects, whether applied directly or mixed with food (18 and 26).

The *Trichoderma* species have long been acknowledged as biocontrol agents (BCAs) that prevent and manage plant diseases while promoting plant growth and development. This is achieved through the release of a wide array of secondary metabolites, both volatile and non-volatile, which have adverse effects on numerous species (22). Formulations based on *Trichoderma* represent highly effective biofungicides in integrated pest management, comprising over 60% of all registered biofungicides (15).

As for the mechanism for the effect of toxic metabolic compounds on insects, the crude extract of the fungus contains compounds that play a role in the synthesis of antimicrobial peptides and resistance to phagocytosis. Additionally, these compounds induce oxidative stress, which causes the destruction of many antioxidant enzyme systems in the host (insect) (21).

Conclusions

This study demonstrated the effectiveness of the methanolic crude extract containing secondary metabolites and toxins from the local isolate of the T. *harzianum* fungus in controlling the housefly, an insect of medical importance. Therefore, it is considered an important biological control agent for limiting the spread of insects and disease vectors, as well as reducing the use of environmentally harmful chemical pesticides.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Author Saffanah M. Hardan: methodology, writing and original draft preparation; Thamer A. A. Muhsen writing, review and editing. All authors have read and agreed to the published version of the manuscript.

Funding:

This research received no external funding.

Institutional Review Board Statement:

The study was conducted following the protocol authorized by the Head of the Ethics Committee, University of Baghdad, Iraq Republic.

Informed Consent Statement:

No Informed Consent Statement.

Data Availability Statement:

No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflict of interest.

Acknowledgments:

The authors thank the Department of Biology at the College of Education, Ibn Al-Haytham, University of Baghdad.

Disclaimer/Journal's Note:

The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of AJAS and/or the editor(s). AJAS and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

References

1. Aljoboory, R. K. I., and Saber, A. J. (2022). A Survey of the Types of Insects Present on the Medicago Sativa Crop in Baghdad. Journal of Pharmaceutical Negative Results, 13(7): 198-200.
2. Alkafaji, A. H., and Alzubaidi, F. S. (2014). The Effect of Alkaloids Extract of Amaranthus gracilis (L.) on Some Biological Aspects of House Fly Musca domestica (L.). Iraqi Journal of Science, 55(4A): 1472-1476.
3. Al-Rawi, K. M., and Khalaf Allah, A. M. (1980). Design and analysis of agricultural experiments. El Mousel Univ., Iraq, 19: 487.
4. Bilgo, E., Lovett, B., St. Leger, R. J., Sanon, A., Dabiré, R. K., and Diabaté, A. (2018). Native entomopathogenic Metarhizium spp. from Burkina Faso and their virulence against the malaria vector Anopheles coluzzii and non-target insects. Parasites and vectors, 11: 1-6. https://doi.org/10.1186/s13071-018-2796-6.
5. Binod, P., Sukumaran, R. K., Shirke, S. V., Rajput, J. C., and Pandey, A. (2007). Evaluation of fungal culture filtrate containing chitinase as a biocontrol agent against Helicoverpa armigera. Journal of applied microbiology, 103(5): 1845-1852. https://doi.org/10.1111/j.1365-2672.2007.03428.x.
6. Blackwell, M. (2011). The Fungi: 1, 2, 3… 5.1 million species?. American journal of botany, 98(3): 426-438. https://doi.org/10.3732/ajb.1000298.
7. Contreras-Cornejo, H. A., Orozco-Granados, O., Ramírez-Ordorica, A., García-Juárez, P., López-Bucio, J., and Macías-Rodríguez, L. (2022). Light and mycelial injury influences the volatile and non-volatile metabolites and the biocontrol properties of Trichoderma atroviride. Rhizosphere, 22: 100511. https://doi.org/10.1016/j.rhisph.2022.100511.
8. Coppola, M., Cascone, P., Chiusano, M. L., Colantuono, C., Lorito, M., Pennacchio, F., ... and Digilio, M. C. (2017). Trichoderma harzianum enhances

tomato indirect defense against aphids. Insect science, 24(6): 1025-1033. https://doi.org/10.1111/1744-7917.12475.

9. Farooq, M., and Freed, S. (2018). Insecticidal activity of toxic crude proteins secreted by entomopathogenic fungi against Musca domestica L. (Diptera: Muscidae). Kuwait Journal of Science, 45(2).
10. Farhan, K., O., W. Ibade, Kh., & A. Kream, T. (2024). Efficiency Alcoholic Extract Of Nightshade Wild And Potassium Silicate In Normal And Nano To Control Of Downy Mildew Disease In Cucumber. Anbar Journal Of Agricultural Sciences, 22(1), 706–718. https://doi.org/10.32649/ajas.2024.183806.
11. Gogarten, J. F., Düx, A., Mubemba, B., Pléh, K., Hoffmann, C., Mielke, A., ... and Leendertz, F. H. (2019). Tropical rainforest flies carrying pathogens form stable associations with social nonhuman primates. Molecular ecology, 28(18): 4242-4258. https://doi.org/10.1111/mec.15145.
12. Hammed, A. A., AlShammari, H. I., and S. A. Kathiar. (2022). Effect of secondary metabolite crude of Metarhizum anisopliea fungus on the second larval stage of the housefly Musca domestica. Baghdad Science Journal, 19(6) (Suppl.): 1493-1501. http://dx.doi.org/10.21123/bsj.2022.19.4.ID0000.
13. Hatem, R. B., Al-Jedawi Y. D. R., and Mohmed, A. S. (2020). Lethal effects of crude secondary metabolites of Trichoderma harzianum against Myzus Persicae (Homoptera: Aphididae). Plant Archives. 20(2): 4997-5000.
14. Hywel-Jones, N. L. (1993). A systematic survey of insect fungi from natural, tropical forest in Thailand, p 300–301. In Isaac S, Frankland, J. C., Watling, R., Whalley, A.J. S. (ed), Aspects of tropical mycology. Cambridge University Press, Cambridge, United Kingdom.
15. Ibrahim, T. M., Nayyef, R. A. H., and Al-Magdamy, B. A. A. H. (2020). Effect of Algal Extracts on the Growth of Tow Bacterial Types Isloated from Pollutants Discharge. Indian Journal of Forensic Medicine and Toxicology, 14(1): 741-744. DOI: 10.37506/v14/i1/2020/ijfmt/192991.
16. Lakhdari, W., Benyahia, I., Bouhenna, M. M., Bendif, H., Khelafi, H., Bachir, H., ... and Dehliz, A. (2023). Exploration and evaluation of secondary metabolites from Trichoderma harzianum: GC-MS analysis, phytochemical profiling, antifungal and antioxidant activity assessment. Molecules, 28(13): 5025. https://doi.org/10.3390/molecules28135025.
17. Loreto, R. G., Elliot, S. L., Freitas, M. L., Pereira, T. M., and Hughes, D. P. (2014). Long-term disease dynamics for a specialized parasite of ant societies: a field study. PloS one, 9(8): e103516. https://doi.org/10.1371/journal.pone.0103516.
18. Manickkam, J., and Moses, J. P. (2023). Immunotoxicity of methanol extract of Entomopathogenic fungi, Aspergillus niger (OM514698) against House fly (Musca domestica) larva, 1-16. https://doi.org/10.21203/rs.3.rs-2432721/v1.
19. Mishra, S., and Malik, A. (2013). Nutritional optimization of a native Beauveria bassiana isolate (HQ917687) pathogenic to housefly, Musca domestica L. Journal of parasitic diseases, 37: 199-207. https://doi.org/10.1007/s12639-012-0165-5.

20. Mnyone, L. L., Ng'habi, K. R., Mazigo, H. D., Katakweba, A. A., and Lyimo, I. N. (2012). Entomopathogenic fungi, Metarhizium anisopliae and Beauveria bassiana reduce the survival of Xenopsylla brasiliensis larvae (Siphonaptera: Pulicidae). Parasites and vectors, 5, 204. https://doi.org/10.1186/1756-3305-5-204.
21. Molnár, I., Gibson, D. M., and Krasnoff, S. B. (2010). Secondary metabolites from entomopathogenic Hypocrealean fungi. Natural product reports, 27(9): 1241-1275. https://doi.org/10.1039/C001459C.
22. Muhsen, T. A., Hawar, S. N., Mahdi, T. S., and Khaleel, R. (2020). Effect of Eucalyptus and Myrtus extracts identification by gas chromatography-mass spectrometry on some species of Candida as amodel of medical plants. Ann. Trop. Med. and Public Health, 23(S10): 1-11. http://doi.org/10.36295/ASRO.2020.231032.
23. Poveda, J. (2021). Trichoderma as biocontrol agent against pests: New uses for a mycoparasite. Biological Control, 159: 104634. https://doi.org/10.1016/j.biocontrol.2021.104634.
24. Vega, F. E., and Blackwell, M. (Eds.). (2005). Insect-fungal associations: ecology and evolution. Oxford University Press.
25. Verma, M., Brar, S. K., Tyagi, R. D., Surampalli, R. N., and Valero, J. R. (2007). Antagonistic fungi, Trichoderma spp.: panoply of biological control. Biochemical Engineering Journal, 37(1): 1-20. https://doi.org/10.1016/j.bej.2007.05.012.
26. Vivekanandhan, P., Kavitha, T., Karthi, S., Senthil-Nathan, S., and Shivakumar, M. S. (2018). Toxicity of Beauveria bassiana-28 mycelial extracts on larvae of Culex quinquefasciatus mosquito (Diptera: Culicidae). International journal of environmental research and public health, 15(3): 440. https://doi.org/10.3390/ijerph15030440.
27. Wang, J., Lovett, B., and Leger, R. J. S. (2019). The secretome and chemistry of Metarhizium; a genus of entomopathogenic fungi. Fungal Ecology, 38: 7-11. https://doi.org/10.1016/j.funeco.2018.04.001.
28. White, R. L., Geden, C. J., and Kaufman, P. E. (2021). Exposure timing and method affect Beauveria bassiana (Hypocreales: Cordycipitaceae) efficacy against house fly (Diptera: Muscidae) larvae. Journal of Medical Entomology, 58(1): 372-378. https://doi.org/10.1093/jme/tjaa156.