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Extraction and Characterization of Bio Pesticide from Custard Apple (Annona Squamosa) Seed Kernels

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Abstract— *Bio-pesticides, derived from natural sources that target pest's nervous systems directly, represent a sustainable alternative to chemical pesticides. The widespread use of chemical pesticides has led to profound ecological and health concerns, including biodiversity decline, genetic variability in plant populations, water pollution, and human health risks like cancer. This study focuses on extracting an environmentally friendly bio-pesticide from custard apple seed kernels, emphasizing high yield and efficacy. The extraction process employed advanced batch processing using Soxhlet extractor and rota-evaporator, ensuring efficient separation and purity of the bio-active compounds. Gas Chromatography (GC) was employed to meticulously characterize the chemical composition of the extracted samples. The effect of solvent, particle size and time of contact were determined. Furthermore, the bio-pesticide's effectiveness against pests infesting soil and leaf surfaces was rigorously evaluated. The results of GC analysis revealed that Oleic acid and Palmitic acid are prominent bio-active components responsible for insect mortality. Furthermore, it is noteworthy that hexane extraction yielded the highest percentage (70%) of bio-pesticide. This research advances sustainable pest management strategies by* *developing natural bio-pesticides from agricultural by-products. Emphasizing eco-friendly solutions, the study aims to mitigate the environmental impact of conventional pesticides while promoting agricultural sustainability and ecosystem health.*

Index Terms—*Acetone, biopesticide, custard apple seeds, gas chromatography, hexane, soxhlet extraction*

I. INTRODUCTION

The history of agricultural pest control has seen a notable evolution from traditional methods to more sophisticated biological approaches. Early records cite the use of plant extracts such as nicotine for managing plum beetles as far back as the 17th century. By the 19th century, experiments with mineral oils and the discovery of *Beauveria bassiana's* potential to infect silkworms marked significant milestones in biological control research. The rapid expansion of agricultural research institutions in the early 20th century further propelled advancements in bio-control strategies, leading to the development of bio-pesticides [1].

Bio-pesticides, also known as biological pesticides, encompass a diverse array of living organisms ranging from bacteria, plants, and minerals to animals. These substances are naturally occurring or derived from living organisms, each possessing unique modes of action that target specific pests and diseases. Their use is gaining prominence as they offer effective and environmentally friendly alternatives to conventional chemical pesticides. Unlike conventional chemical pesticides, bio-pesticides generally pose lower environmental risks, break down more rapidly, and require smaller quantities to achieve effective pest control. However, their optimal application often requires a comprehensive understanding and integration into Integrated Pest Management (IPM) strategies [2], [3]. Bio-pesticides are recognized for their potential as reduced-risk alternatives to conventional chemical pesticides, contributing significantly to sustainable agricultural practices [1], [4].

The focus of this study is on the extraction and evaluation of a bio-pesticide derived from custard apple (*Annona squamosa*) seed kernels. Custard apple seeds have been historically utilized in folk medicine for their rich content of bioactive compounds such as alkaloids, flavonoids, phenolic compounds, acetogenins, and cyclopeptides [2]. Recent research highlights their diverse biological activities including antioxidant, antibacterial, antiviral, hepato-protective, anticancer, and anthelmintic properties [3]. These properties underscore custard apple seeds as promising candidates for bio-pesticide development. The rich bioactive profile of custard apple seeds positions them as promising natural alternatives for sustainable pest management strategies in agriculture.

This study contributes to advancing sustainable pest management strategies by exploring the efficacy of custard apple seed-derived bio-pesticides. By emphasizing eco-friendly alternatives to chemical pesticides and promoting agricultural sustainability, this research underscores the importance of integrating bio-pesticides into modern farming practices to enhance ecosystem health and food security. Integrating bio-pesticides into modern farming practices not only supports sustainable agriculture but also contributes to ensuring long-term food security by reducing reliance on synthetic chemicals and fostering resilient agricultural systems capable of adapting to environmental changes.

II. MATERIALS AND METHODOLGY

A. Materials and Equipment

Dried custard apple seeds served as the primary raw material for the study. Hexane and acetone were employed as solvents, while sodium hydroxide was utilized for determining the acid value. Experimental procedures involved the use of a Soxhlet apparatus for extraction, a rotary evaporator for solvent removal, a mechanical grinder for particle size reduction, a sieve shaker for particle separation, and a Gas Chromatograph for chemical analysis.

B. Experimental Procedure

The raw material, custard apple seeds (250g) were collected and dried in the sun for twoweeks. The seeds were ground in the grinder and then separated into three different particle sizes of 75, 150, 600µm by passing through a sieve shaker. Experiments were also conducted by mixing all particles sizes. The bio-pesticide extraction process was carried out in a Soxhlet apparatus. For each batch 10g of dried custard apple seed powder was weighed and transferred to the thimble,inside the holder. Extraction was done using two different solvents, with hexane at 70°C and acetone and 60°C. The extraction temperature was set above the boiling point of the two solvents. 250 ml of the solvent was transferred to the flask and the apparatus setup was connected. As the time progressed, the vapors wereproduced, condensed in the form of drops, then fell to the round bottom flask andmixed with the solvent. After sufficient time, which ranges between 3 to 6 hours, the extractedsamplewastakentorotary evaporatorforseparationof solvent from the extracted oil.Theyieldofbio-pesticide extracted was expressed as percentage of the weight of extracts obtained from extractionprocessrelative tothe weightofcustard appleseed powder. The separated oil was characterized using gas chromatography technique.

III. RESULT AND DISCUSSION

A. Chemical and physical properties of Biopesticide

The bio-pesticide sample extracted underwent a detailed assessment to determine its physical and chemical characteristics. When extracted using acetone as the solvent, the bio-pesticide exhibited a dark yellow-brownish color, indicating the presence of various pigments and compounds extracted by this solvent. In contrast, extraction with hexane resulted in a lighter yellowish-brown color, suggesting a different composition or concentration of extractable substances compared to acetone.

The density of the bio-pesticide extract was measured at 0.440 g/cm³. This parameter is crucial as it reflects the concentration and purity of the extracted bioactive compounds. The viscosity of the bio-pesticide was found to be 0.193 stokes, which describes its resistance to flow. This property is significant for understanding how easily the bio-pesticide can be applied and distributed in agricultural settings.

Furthermore, the acid value of the bio-pesticide was determined to be 4.769 ml/g. This value provides insights into the amount of free fatty acids present in the bio-pesticide extract, which can influence its stability and effectiveness as a pesticide.

B. Characterization using Gas Chromatography

The identification of the bio-pesticide components was carried out using Gas chromatograph Clarus 500. GC analysis was done for two samples of bio- pesticide, one extracted with hexane solvent and the other with acetone. The components with their peaks are shown in Fig 1 and Fig 2. The list of identified components is shown in table 1 and Table 2. Notably, Oleic acid emerged as the predominant peak in both analyses, underscoring its significant presence within the bio-pesticide extract.These analytical results provide a comprehensive profile of the bioactive compounds present in the custard apple seed-derived bio-pesticide, highlighting Oleic acid's potential role and contributing to further understanding its bio-pesticidal properties.

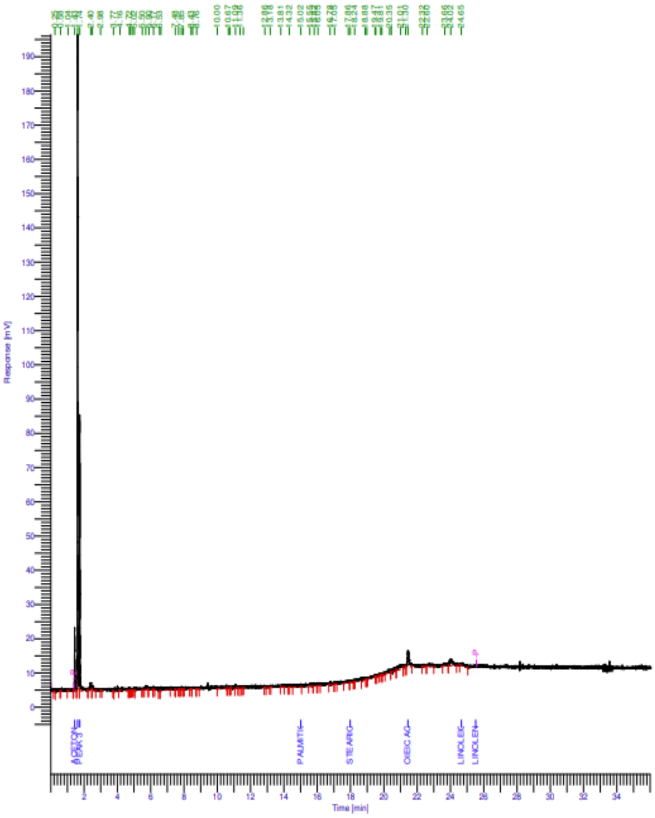

Fig 1 Gas chromatographic analysis of biopesticide using Acetone solvent

Table 1 Components of the biopesticide using Acetone solvent

Peak #	Component Name	Time [min]	Area [µV*sec]	Height [µV]	Area [%]
4	Acetone	1.44	39860.3	18562.8	6.57
5	Hexane	1.61	363588.6	191891.1	59.89
6	Peak 3	1.74	135381.2	80815.7	22.30
39	Palmitic Acid	15.02	13315.8	878.4	2.19
46	Stearic Acid	17.99	5288.3	713.8	0.87
58	Oleic Acid	21.45	36592.3	4769.6	6.03
63	Linoleic Acid	24.65	13064.9	683.4	2.15
			607091.4	298314.9	100.00

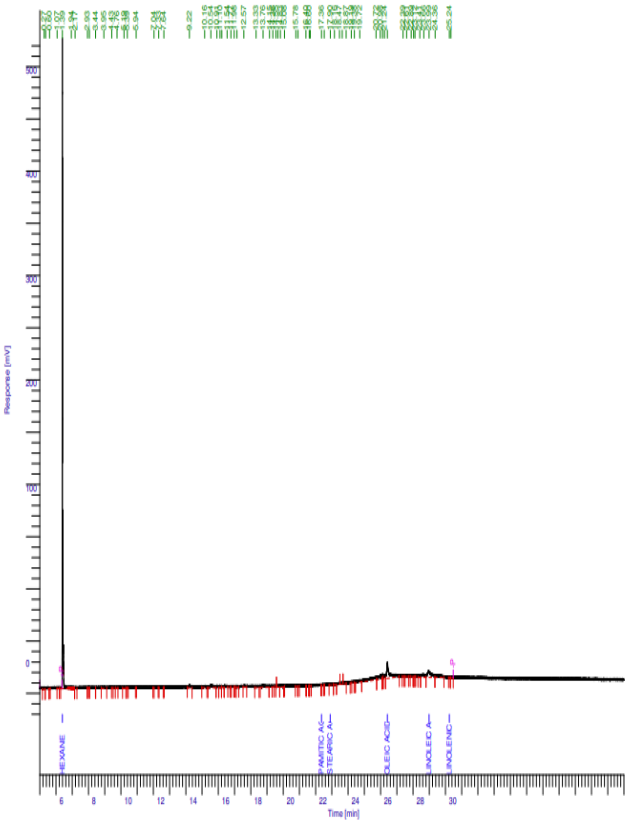

Fig 2 Gas chromatographic analysis of biopesticide using hexane solvent

Table 2 Components of biopesticide using hexane solvent

Peak #	Component Name	Time [min]	Area [µV*sec]	Height [µV]	Area [%]
5	Hexane	1.61	879613.3	622522.5	77.76
44	Pamitic Acid	17.36	1872.8	708.5	0.17
46	Stearic Acid	17.90	6740.3	701.3	0.60
58	Oleic Acid	21.42	154831.6	14674.5	13.69
66	Linoleic Acid	23.99	86970.6	5363.6	7.69
68	Linoleic Acid	25.24	1160.5	615.9	0.10
			1131189.1	644586.4	100.0

C. Effect of process time

The effect of time on the extraction of oils from seeds in a Soxhlet extractor is crucial as it directly impacts the efficiency and yield of the extraction process. It can be observed in Fig 3 and Fig 4 that the yield of biopesticide increases as the time of contact of solvents increases with the custard apple seed particles. Maximum yield was obtained after contact of six hours.Generally, longer extraction times in a Soxhlet extractor led to higher yields of extracted oils [5]. This is because extending the extraction time allows more opportunities for the solvent to come in contact with the seed material, dissolve the oil components, and transfer them into the solvent. Moreover, the process of extraction in a Soxhlet extractor continues in a cyclic manner: as more solvent is vaporized and condensed, it repeatedly extracts oils from the seed material. This cycling ensures that fresh solvent continually remains in contact with the seed material, maintaining a high concentration gradient and facilitating ongoing extraction. The extraction process in a Soxhlet extractor works towards establishing equilibrium between the oil components in the seeds and the solvent. Initially, the extraction rate is high as the solvent rapidly dissolves accessible oil. Over time, as more oil is extracted, the rate of extraction slows down and eventually reaches a point of diminishing returns. Further longer extraction times facilitate deeper penetration of the solvent into the seed matrix and allow for more thorough diffusion of the dissolved oil components from within the seeds to the bulk solvent. This is particularly important for seeds with a complex structure where oil might be entrapped deep within the solid matrix. The yield of biopesticide using hexane solvent for a duration of 7 hours run was more than acetone.

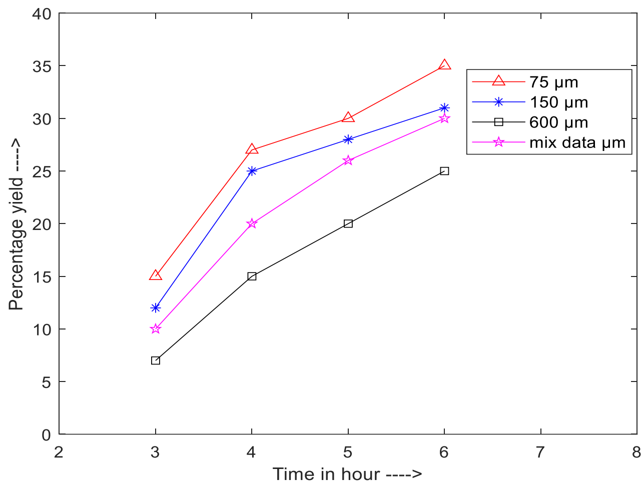

Fig 3. Effect of process time on percentage yield of biopesticide using acetone as solvent

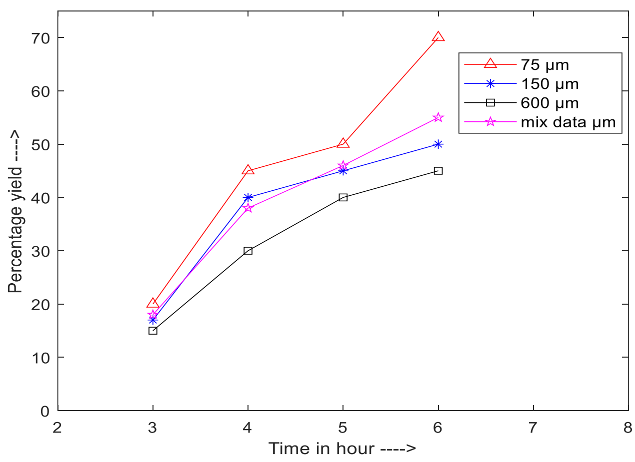

Fig 4. Effect of extraction time on percentage yield of biopesticide using hexane as solvent

D. Effect of Solvent

The process of mass transfer of oil components from the ground custard apple seeds into the solvent in the Soxhlet extractor began with the solvent penetrating the solid matrix of the ground seeds. The type of solvent used for extraction greatly affects the yield of the soluble components [6]. In this study two common solvents, hexane having boiling point 69°C and acetone having boiling point 56°C were chosen for the extraction process. These have been reported to selectively dissolve oils while leaving behind non-oil components. This dissolution process occurs because the oil components have higher solubility in this solvent compared to other seed components like cellulose, proteins, and carbohydrates. From the experimental findings of this study, it was revealed that hexane proved to be a better solvent as compared to acetone as the oil components of the custard apple seeds showed greater solubility in hexane than acetone.

From Fig 5 it is observed that 70% yield of biopesticide was achieved with hexane as compared to 35% yield with acetone. Hexane is commonly used due to its efficiency in extracting oils and its relatively low boiling point (69°C). Hexane has good penetration ability and can swell the seeds, which helps in exposing the oil-rich parts of the seeds to the solvent [7], [8]. As the solvent penetrates inside the seed material it not only selectively dissolves the oil components but also interacts with them through various intermolecular forces (like van der Waals forces or hydrogen bonding depending on the solvent). These interactions stabilize the dissolved oil-solvent mixture, aiding in the extraction process. Diffusion within the solid matrix of the seeds allows the dissolved oils to migrate towards the surface of the seed particles. Diffusion is driven by concentration gradients, moving the oil-solvent mixture from areas of higher concentration (inside the seeds) to areas of lower concentration (in the bulk solvent) [9].

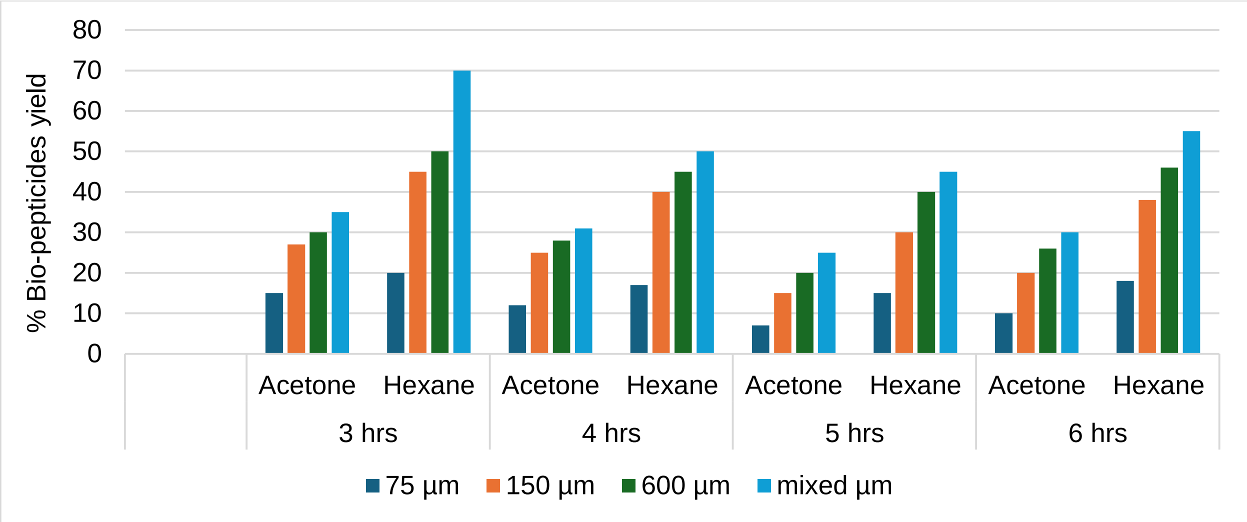

Fig 5. Effect of solvent on the percentage yield of biopesticide

E. Effect of particle size

The effect of particle size on the bio-pesticide yield can again be observed from Fig 5. Itcan beseen with both types of solvents maximum yield was obtained with smallest particle size which was 75 µm in this study. This can be attributed to the fact that the smaller the particle size larger the surface area thus facilitating better contact of the solvent with the seed components and hence increased efficiency of extraction[9-11].

F. Testifying the efficacy of the biopesticide

Thebio-pesticidesamplewasappliedoninsects in the soil and on the surface of leaf throughaspraytestto check for its effectiveness as depicted in Fig (6).

(a) (b)

Fig 6. Dead insect in soil (a) and on surface of leaves (b) after the application of bio-pesticide

It was noticed that once the bio-pesticide fell on theinsect body, ittooktwo minutesforthe insectto die. The components which make the oils acts as a bio-pesticide are Oleic andPalmitic acid. With the scientific researches, it has been approved that these two acids aretoxictowards insect bodies andmaketheirbody stopfunction[3], [12], [13].

IV. CONCLUSIONS

Bio pesticides are eco-friendly as they are biodegradable and not harmful for the crops.Optimum extraction conditions for biopesticide using hexane and acetone were investigated and it was found that hexane serves as a more effective solvent than acetone yielding 70% biopesticide as compared to 35% yield with acetone. It was found that as the particle size and the process time increases, the percentage yield of bio-pesticide increases. The extracted bio-pesticide has been analyzed by GS technique. The activecomponents identified are Oleic acid and Palmitic acid which are responsible for the insecticidalproperty. The Spray Test proved to be effective as it caused the insect to die within 2minutes. Overall, it can be concluded that the efficiency of extraction depends on factors such as solvent choice, particle size of the seeds, and extraction time. In essence, the mass transfer of oil components from seeds into the solvent in a Soxhlet extractor is driven by solvent penetration, solubility of oils in the solvent, diffusion within the seed matrix, and continuous solvent circulation. These factors together enable efficient extraction of oils from seeds, making Soxhlet extraction a widely used method in laboratories and industries for this purpose.

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REFERENCES

[1] Chaudhary, S., Kanwar, R.K., Sehgal, A., Cahill, D.M., Barrow, C.J., Sehgal, R. and Kanwar, J.R., 2017. Progress on Azadirachta indica Based Biopesticides in Replacing Synthetic Toxic Pesticides. *Frontiers in Plant Science*, [online] 8, p.610. Available at: https://www.frontiersin.org/articles/10.3389/fpls.2017.00610/full

[2] Gupta, S. and Dikshit, A.K., 2010. Biopesticides: An ecofriendly approach for pest control. *Journal of Biopesticides*, [online] 3(1), pp.186-188.

[3] Gawali, S., Deotale, P. and Shaikh, S., 2017. *Annona squamosa*: A Source of Natural Pesticide. *International Advanced Research Journal in Science, Engineering and Technology*, [online] 4(3), pp.10-12.

[4] Lengai, G.M.W. and Muthomi, J.W., 2018. Biopesticides and Their Role in Sustainable Agricultural Production. *Journal of Biosciences and Medicines*, [online] 6(6), pp.1-10. Available at: https://www.scirp.org/journal/PaperInformation.aspx?PaperID=85395

[5] Pareek, C.M., Jadhav, M.V., Rokade, H.N., Jatale, A.U., and Pavitrakar, N.R., 2018. Laboratory Evaluation of Custard apple Seed Extract against Pigeon pea Pod borer *Helicoverpaarmigera* (Hubner). *Journal of Entomology and Zoology Studies*, [e-journal] 6(1), pp. 541-543.

[6] da Silva, G., de Oliveira Henriques, J.D., and Martinez, P.F., 2022. Sugarcane wax extraction using hexane and limonene mixtures. *Engenharia na agricultura*, [e-journal] Vol. 30, pp. 13-18.

[7] Hu, Y., Chen, X., Mu, S., and Li, Q., 2022. Extraction and separation of petroleum pollutants from oil-based drilling cuttings using methanol/n-hexane solvent. *Chemical Engineering Research & Design*, [e-journal] Vol. 168, pp. 760-767.

[8] Kumar, S.P.J., Prasad, S.R., Banerjee, R., Agarwal, D.K., Kulkarni, K.S., and Ramesh, K.V., 2017. Green solvents and technologies for oil extraction from oilseeds. *Chemistry Central Journal*, 11, p. 9. doi: 10.1186/s13065-017-0238-8.

[9] Al-Sumri, A., Al-Siyabi, N., Al-Saadi, R., Al-Rasbi, S., Al-Dallal, A., 2016. Study on the Extraction of Date Palm Seed Oil using Soxhlet Apparatus. *International Journal of Scientific & Engineering Research*, [e-journal] Volume 7, Issue 12, December, pp. 1266-1270.

[10] Gayathri, P.K., Arun, D., Sripriya, R., Soniya, E., Vennila, R. and Felcitta Aarthi, R., 2016. Effects of four biopesticides on three different species – A Comparative Study. *Journal of Chemical and Pharmaceutical Sciences*, July - September 2016, [e-journal] 9(3), pp.1531-1534.

[11] Manyele, S.V. and Kahemela, I.F., 2008. Investigation of the Effect of Particle size on Groundnut – Oil Solvent Extraction. *Tanzania Journal of Engineering and Technology (TJET),* [e-journal] Vol. 31(2), December, pp. 1-13.

[12] Aguilar-Marcelino, L., Pineda-Alegría, J.A., Salinas-Sánchez, D.O., Hernández-Velázquez, V.M., Silva-Aguayo, G.I., Navarro-Tito, N., Sotelo-Leyva, C., 2022. In Vitro Insecticidal Effect of Commercial Fatty Acids, β-Sitosterol, and Rutin against the Sugarcane Aphid, MelanaphissacchariZehntner (Hemiptera: Aphididae). Journal of Food Protection, Volume 85, Issue 4, April, pp. 671-675.

[13] Opondo, F.A., K'Owino, I.O., Chepkwony, S.C. and Kosgei, V.J., 2022. In Vitro Antibacterial Activity of Essential Oils from *Tithonia diversifolia* Leaves and Flowers Against *Ralstonia solanacearum*. *European Journal of Applied Sciences*, 10(2), pp.523-539.

AUTHORS PROFILE

Manahil Msellem Al Khusaibi is a proficient chemical engineer with a robust educational foundation and extensive research accomplishments. She holds a bachelor's degree in chemical engineering and a master's degree in chemical process engineering from the National University of Science and Technology, Muscat, Oman. As a research assistant in the university's Waste to Energy Lab, she has significantly contributed to NURG and GRG MOHERI projects and has published several research papers.

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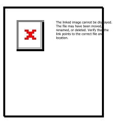

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