TERPENES PROPERTIES AS BIOPESTICIDES

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Abstract: Biopesticide consists of many different types like plants, fungi, bacteria, microalgae and nowadays it is not yet widely introduced and rarely available in the market; common available pesticides are chemical-based pesticides that harm not only the environment but also humans. Plant essential oils are created from different plant resources, most of them are members of the mint family (Lamiaceae) and a multiple combination of a class of terpenes that consist of two-isoprene units or called monoterpenes composed of oils. It linked an aromatic compound with a molecular formula of C6H5OH or called phenols and a sesquiterpenes. Further research is needed in the emerging or happening of organic pesticides with showing the possible control agents, formulation, delivery and commercialization. Since the availability of biopesticides are minimal, the researchers come up with the idea of synthesizing a prototype of biopesticides from lemon peel, neem leaves, cinnamon bark and garlic using steam distillation as a mode of extraction of the essential property which is terpenes that holds a promising role in killing pest particularly aphids. The findings of this study aim to test the efficacy of the prototype made by the researchers which is the biopesticides that has extracts from lemon peel, neem leaves, garlic and cinnamon bark. The researchers are recommending the application of the prototype to the other pests and insects in order to know the effectiveness of it besides aphids.

Key Words: control agent; terpenes; aphids; biopesticides; steam distillation

1. INTRODUCTION

1.1. Background of the Study

Throughout the world, pesticides are widely used to secure a variety of crops (Desai et. al., 2017). There is a harmful impact on using chemical pesticides and fertilizers that causes impotence of the soil, water hardness, genetic differentiation in plants, development of insect resistance, increase in toxic remains through food chain and animal feed that makes an escalation in health issues and many more (Srijita, 2015).

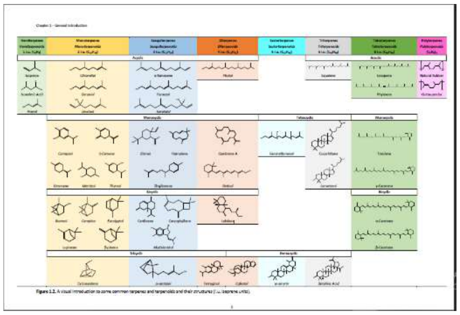
Due to the presence of pests that leads to damage of plant crops, the use of synthetic pesticides raises the call for secured foods as well as the ecological costs that it brings, which only shows the status of emerging studies in the field of biopesticides (Costa et al., 2019).

Residues that came from pesticide may cause a remarkable source of contamination of ecological factors such as air, soil, and water (Jayaraj et al., 2016). They have been reported to contaminate our environment as their residues accumulate in air, soil, water, animal tissue samples, and humans around the world (Desai et al., 2017). More usage by pesticides for the increased agricultural manufacture that brought to rise pollution of environmental sections (Jayaraj et al., 2016). Regardless of their repressive effects on pests hazardous to plants and animals, pesticides can also be dangerous to human health and contaminate the environment (Mostafalou & Abdollahi, 2017; Albuquerque et al., 2018; Gomiero, 2018 cited by Costa et al., 2019).

A key role acts during this contact is that the Plant Secondary Metabolites (PSM) that may also act as nurturing deterrents through controlling the food intake of herbivores (Dearing et al., 2005, cited by Costa et al., 2019), changing hunting actions (Roy & Bergeron, 1989, cited by Costa et al., 2019) or breeding (Tran & Hinds, 2012, cited by Costa et al., 2019). Essential oils are believed to be one in all the very pleasing botanical pesticides because they are nontoxic to mammals, similarly not harmless within the environment (Isman, 2000 cited by Costa et al., 2019).

In 1995, the study by Pimentel presented that just a small percentage (0.3%) of valuable pesticides were set to the target pest; however the 99.7% moved anywhere else in the environment (Jayaraj et al., 2016). According to Aneja et al. (2016), further research is needed in the emerging or happening of organic pesticides with showing the possible control agents, formulation, delivery and commercialization.

1.2. Central Problem



Due to pest infestation in crops and plants non-organic pesticides are invented and are widely used in the society and as an effect its residues leave traces in soils, air, and bodies of water that is adding to the pollution and more importantly causes harm to us.

On the other hand, plants are everywhere and most of all it has a lesser amount of danger to use. Based on the previous published researches terpenes properties are abundant in plants and have the possibility to be a component of biopesticide. In line with this, the researchers aim to synthesize a prototype of biopesticide with the property mentioned above which is terpenes.

1.3. Theoretical Framework

Multiple combination of a class of terpenes that consist of two isoprene units or called monoterpenes composing the oils. It linked an aromatic organic compound with a molecular formula of C6H5OH or called phenols and a sesquiterpenes (Nnamonu & Onekutu, 2015).

Essential oils (EO) biological activity and their components on pest insects comprise behavior and changes in feeding behavior, soap toxicity, and lethal toxicity via contact was reported by Castro et al. Their favorable mammalian toxicity and nonpersistance in the environment is the most attractive aspect of using Eos, that makes it exempted from registration in the United States of America (Vickers et al., 2009, cited by Boncan et al., 2020).

Volatile oils can be used for plants matrices using any kind of method categorized as conventional like using distillation with the use of water by heat as a way to bring out the total important material, and advanced which focus on the development in extraction competence by reducing extraction time, usage of energy, solvent, and CO2 emission (de Matos et al., 2019).

Modes of EO extraction are precise to their hydrophobic and volatile nature. Hydro distillation and steam distillation that is accommodated in usual ways are for the majority of herb parts, and cold expression for citrus rind (Pejin et al., 2011, cited by Maes et al., 2019).

Figure 2: Pesticidal Properties

Agriculture	
Pesticides	Pyrethrins, limonene
Plant protectors	Farnesene
Animal feed	Zeaxanthin
Phytohormones	Fusicoccanes, abscisic acid

1.4. Existing Model

Figure 1: A visual introduction to some common terpenes and terpenoid and their structures (i.e., isoprene units).

These theories are also applied in the study of terpenes properties as biopesticides as well as the possibility that is related to these theories.

Hence, this study proposes that these theories can be true to explore the said topic through experimentation, and development of a prototype that will lead to answer the following questions.

Figure 3: Raw materials with pesticidal properties

Brazil nut family (Lecythidaceae)	S-methylmethionine,	Wood-boring longicorn beetles (Cerambycidae)	deterrent to specialist beetle seeking oviposition sites	[81]
Lavender (*Lavandula angustifolia*)	β-trans-ocimene, (+)-R-limonene	Aphids	deterrent to pest	[82]
Cucumber (*Cucumis sativus*)	Tetracyclic terpenes: Cucurbitacins	Spider mite (*Tetranychus urticae*)	antibiotic effect on spider mites but attractive to the pest cucumber beetle	[83,84]
Cinnamon and clove	Eugenol, caryophyllene oxide, α-pinene, α-humulene and α-phellandrene	*Sitophilus granarius*	toxic and repellent effects to adult pest	[58]
Water primrose (*Ludwigia octovalvis*)	α-pinene, linalool oxide, geraniol, and phytol	Weber (*Altica cyanea*)	attractive to pest females	[85]
Rice (*Oryza sativa*).	(S)-linalool, 4,8-dimethyl-1,3,7-nonatriene, (E)-caryophyllene, and (R/S)-(E)-nerolidol	African rice gall midge (*Orseolia oryzivora*)	attractive to mated female pest in intact rice, but repellent with different concentrations of the same volatiles in infested plant	[60]
Eucalyptus grandis	α-pinene, γ-terpinene	*Leptocybe invasa*	potentially attractive to pest	[86]
Various plant species	Geraniol	*Bemisia tabaci*	encapsulated geraniol shows attraction to *B. tabaci*	[87]

1.5. Research Questions

1.5.1. What is the effect of terpenes on the plant's aphids after applying it for 7 days?

1.5.2. Is there a significant difference between the result of treated and untreated?

1.5.3. Is there a significant difference between the results in three cases (mild, moderate, and extreme)?

1.5.4. What is the effect of terpenes on the leaves?

1.6. Significance of the study

This study will help:

Farmers

- this study can help them in minimizing the population of the pest.

-give them knowledge about terpenes properties.

Businessman

-this will give them an opportunity to develop biopesticide and improve what's in the market.

Experts

-this will serve as a reference for them to innovate ideas and the possibility of terpenes as biopesticide.

Future Researchers

-will give them additional ideas about the topic of biopesticide as well as the terpenes properties.

1.7. Scope and Delimitation Scope

The study through meta-synthesis and meta-analysis wherein the researchers gathered review of related literatures and synthesizes it to explore their chosen topic which is terpenes properties as biopesticides and produce a prototype out of it focuses on raw materials, formulation and the properties of the developed biopesticides.

Delimitation

The study limits only on terpenes properties and the formulation of biopesticides which means that the researchers will only collect information about raw materials connected to the topic. It also limits on the mentioned focuses above.

2. METHODOLOGY

This study aims to test the efficacy of the prototype made by the researchers which is the biopesticide that has extracts from lemon peel, neem leaves, garlic and cinnamon bark in controlling tomato plant's aphids.

2.1. Prototype of Terpenes Properties as Biopesticide

Figure 4. Sample of the Prototype of Terpenes Properties as Biopesticide

2.2 Research Design

The researcher used posttest only control design wherein the treated tomato leaves will be observed as well as the untreated to have a comparison between the two data after experimentation.

2.3. Experimental Design

2.3.1. Sampling procedure for the selection

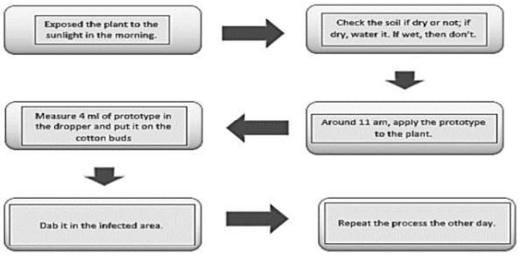
The cases are classified as mild, moderate, and extreme wherein specific measurements are assigned:

Fig. 5.1. Mild - (1-15 mm of aphids)

Fig. 5.2. Moderate - (16-30 mm of aphids)

Fig. 5.3. Extreme - (31-45 mm of aphids)

2.3.2 Application of Terpenes Properties as Biopesticides



A prototype of pesticide is being tested on the leaves of tomatoes. The control group of leaves to be treated will be classified as a) mild case, b) moderate case, c) extreme case.

2.3.3. Data Gathering Procedure

Using the validated observation sheets, the researchers proceeded to the experimentation, in a span of seven days the tomato leaves' color and aphids' infection were observed as well as the untreated with continuous application of the prototype. After the data gathering, the collected data was analyzed through statistical tools.

Cases	Day of Application						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
NT							
T							

Cases							
Changes	Day(s)						
	1	2	3	4	5	6	7
Green							
Light Green							
Yellow							
Brown							
Dried Up							

2.4. Data Analysis

The data gathered were tallied in a tabular form using Microsoft Excel and it is analyzed using non-parametric (frequency, percentage and mean) and parametric (two sample t-test and one-way analysis of variance) statistics.

2.4.1. Formula

2.4.1.1. Two-sample T-test

$$t = \frac{x_1 - x_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

2.4.1.2. One-way Analysis of Variance

$$SS_{total} = \sum_{j=1}^{p} \sum_{i=1}^{n_j} (x_{ij} - \overline{x})^2$$

$$SS_{between} = \sum_{j=1}^{p} n_j (\overline{x}_j - \overline{x})^2$$

$$SS_{within} = \sum_{j=1}^{p} \sum_{i=1}^{n_j} (x_{ij} - \overline{x}_j)^2$$

3. RESULTS AND DISCUSSION

Based on the research questions the following data are presented:
3.1. What is the effect of terpenes on the plant's aphids after applying it for 7 days?
3.2. Is there a significant difference between the result of treated and untreated?
Presentation of Data

Table 1. Observation Sheets (Aphid's Growth Infection – Mild Case)

Cases	Day of Application						
Mild	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
NT	14	20	35	51	56	71	100
T	14	7	5	2	0	0	0
Moderate	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
NT	28	38	48	62	100	100	100
T	25	11	8	5	5	0	0
Extreme	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
NT	38	38	47	55	66	100	100
T	35	24	14	9	3	1	0

T – Treated NT – Not Treated

Fig. 6.1. Graph that shows the growth of aphid's infection in mild case.

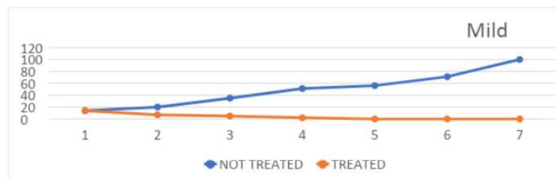


Fig. 6.2. Graph that shows the growth of aphid's infection in moderate case.

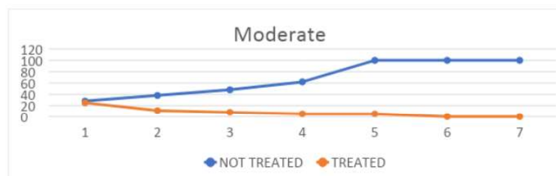


Fig. 6.3. Graph that shows the growth of aphid's infection in severe case.

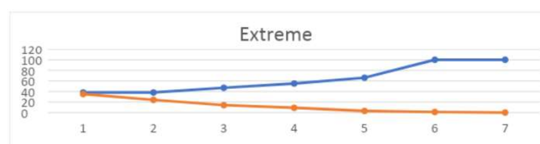


Table 2. Normal Distribution Data Table

Mild	n	x̄	s	df	Computed T-value	Critical Value
NT	7	49.6	30.37			
T	7	4	5.19	6	3.92	1.94
Moderate	n	x̄	s	df	Computed T-value	Critical Value
NT	7	1001.33	31.64			
T	7	73.9	8.60	6	4.87	1.94
Extreme	n	x̄	s	df	Computed T-value	Critical Value
NT	7	459.3	21.43			
T	7	171.9	13.11	6	4.74	1.94

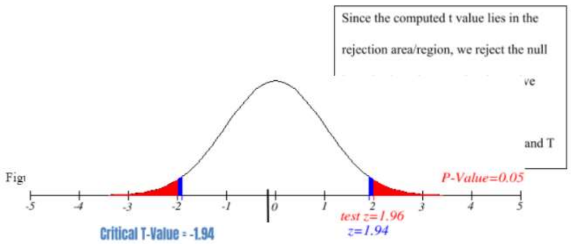


Table 1 shows the progression and comparison of the aphid's growth infection between the treated and untreated leaves of every case. While figures 6.1, 6.2, and 6.3 illustrate the data of the aphids between the said two groups of mild, moderate, and extreme. Lastly is the normal distribution table in table 2 that contains the data in order to use the student's t-test formula.

Test T = 1.96
Critical T-Value = 1.94

3.2. Is there a significant difference between the result in three cases (mild, moderate, and extreme)?

Table 3. Anova Single Factor Data Table

Anova Single Factor						
Summary						
Groups	Count	Sum	Average	Variance		
T1	7	28	4	27		
T2	7	54	7.714286	73.90476		
T3	7	68	12.28571	171.9048		
Anova						
Source of Variation	SS	df	MS	F	P-Value	F-Crit
Between Groups	241.1429	2	120.5714	1.325886	0.290293	3.554557
Within Groups	1636.857	18	90.93651			
Total	1878	20				

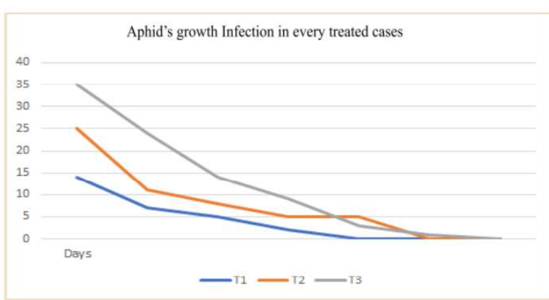


Fig. 7. Graph that shows the growth of aphid's infection in every treated case.

Table 3 shows the data computed using Microsoft Excel one way anova single factor and figure 7 shows the downfall of the growth of pest.

3.3 What is the effect of terpenes on the leaves?

Table 4.1. Effects of Terpenes on Leaves – Mild Case

Mild							
Changes	Day(s)						
	1	2	3	4	5	6	7
Green	+	+	+	+	+		
Light Green						+	+
Yellow							
Brown							
Dried Up							

Table 4.2. Effects of Terpenes on Leaves – Moderate Case

Moderate							
Changes	Day(s)						
	1	2	3	4	5	6	7
Green	+	+	+	+	+		
Light Green						+	+
Yellow							
Brown							
Dried Up							

Table 4.3. Effects of Terpenes on Leaves – Severe Case

Extreme							
Changes	Day(s)						
	1	2	3	4	5	6	7
Green							
Light Green	+						
Yellow		+	+	+	+	+	+
Brown							
Dried Up							

Tables 4.1, 4.2 and 4.3 indicate the changes on leaves using a heat map wherein color of leaves are being observed.

Observation Sheet (Aphid's Growth Infection)

The result of data shows that there is a significant difference between the treated and untreated cases which are mild, moderate and severe. As the experimentation goes by the aphid's growth infection in the treated cases are already gone while the untreated cases continue to increase the number of pests on its leaves.

Effects of Terpenes on leaves

The researchers observed discoloration on leaves as it lightens all throughout the experimentation process, factors such as the condition of plants, changes in temperature, breaking down of chlorophyll and such should be considered.

4. CONCLUSIONS

The study found out that the biopesticide prototype showed an enormous significant difference between the treated and untreated tomato leaves in every case. The outcome of the experiment revealed a huge decrease of the population of aphids as the day passed until it wiped out all the aphids in the treated leaves. In the same effect, data showed that between the three cases number of aphids it showed that there is no significant difference. This is only indicated that the biopesticide prototype is effective in whatever cases (mild, moderate, and extreme).
However, mild discoloration in leaves observed when the biopesticide prototype was applied. Out of all the results gathered the researchers concluded that the prototype terpenes properties as biopesticides has

potential to be a controlling agent for aphids in plants.

5. ACKNOWLEDGMENTS

The researchers recommend the following based on the result of the study:
A further research is needed wherein factors like aphid's growth infection, discoloration on leaves should be modified in order to increase the accuracy of the prototype. More efficient way of applying the prototype on the infected leaves by aphids.

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6. REFERENCES

Aneja, K. R., S. A. Khan, and A. Aneja. "Biopesticides an eco-friendly pest management approach in agriculture: status and prospects." Kavaka 47 (2016): 145-154

Boncan, D. A. T., Tsang, S. S., Li, C., Lee, I. H., Lam, H. M., Chan, T. F., & Hui, J. H. (2020). Terpenes and Terpenoids in Plants: Interactions with Environment and Insects. International Journal of Molecular Sciences, 21(19), 7382.

Costa, Jorge Alberto Vieira, et al. "Potential of microalgae as biopesticides to contribute to sustainable agriculture and environmental development." Journal of Environmental Science and Health, Part B 54.5 (2019): 366-375.

de Matos, Sheila P., et al. "Essential oils and isolated terpenes in nanosystems designed for topical administration: A review." Biomolecules 9.4 (2019): 138.

Desai, Ketaki R., et al. "MITIGATION OF EARLY DELTAMETHRIN INDUCED HEPATOTOXICITY IN MALE MICE OF SWISS STRAIN BY ALLIUM SATIVUM." (2017).

Jayaraj, Ravindran, Pankajshan Megha, and Puthur Sreedev. "Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment." Interdisciplinary toxicology 9.3-4 (2016): 90-100.

Maes, Chloë, Sandrine Bouquillon, and Marie-Laure Fauconnier. "Encapsulation of essential oils for the development of biosourced pesticides with controlled release: A review." Molecules 24.14 (2019): 2539.

Malini, Desak Made, M. Madihah, and Euis Julaeha. "Histological Structure of Mice (Mus Musculus L.) Liver after Administration of Ethanol Extract and Spinasterol from Senggugu (Clerodendron Serratum L) Leaves." (2015).

Martins, M. A. R. (2017). Studies for the development of new separation processes with terpenes and their environmental distribution (Doctoral dissertation, Universidade de Aveiro (Portugal).

Nnamonu, Lami A., and Amana Onekutu. "Green pesticides in Nigeria: an overview."Journal of Biology, Agriculture and healthcare 5.9 (2015): 48-62.

Srijita, Dutta. "Biopesticides: An ecofriendly approach for pest control." World Journal of Pharmacy and Pharmaceutical Sciences (WJPPS) 4.6 (2015): 250-265.