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GC-MS Analysis of bioactive compounds in bush tea leaf, *Hyptis suaveolens Poit.*

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ABSTRACT

The use of synthetic pesticides in crop pest control programs around the world had caused tremendous damage to the environment, human, livestock, aquatic and many other non-target organisms, including beneficiary living things. The scientists have spurred the application of medicinal plants. The current study presents the GC-FID profiling of the bioactive compounds in N-Hexane extracts of bush tea, *Hyptis suaveolen*. Results of bioactive constituents in bush tea, *Hyptis suaveolens* belong to the group of organic compound terpenes, esters, sterols and triterpenoids known to be active for plant protection and human health enhancement properties. The phytochemical constituents showed the potential of the extracts to act as antibacterial and antifungal, anti-inflammatory, anti-cancer, could be further exploited as hepato-protective, amelioration of hyperglycemia and dyslipidemia, and in reduction of atherogenic risk factor in humans. The biological bioactive compounds in bush tea have insecticidal, nematicidal, antibacterial and antifungal properties.

Keywords: Bush tea, organic compounds. Biopesticide, pest management

INTRODUCTION

Over years, chemical pesticides had made a great contribution to fight against pests and diseases. However, their widespread and long-term use have resulted to insecticide resistance and biomagnifications of insecticides, which in turn resulted in restrictions on their export. Problems, like soil, water contamination and dramatic increase of the harmful residues in many primary and derived agricultural products arose, which endangered both the general environment and human health. It is estimated that the financial cost of the damage to the environment and social economy is about $ 8.1 billion per year (Shen and Zhang, 2000). The use of synthetic organic insecticides in crop pest control programs around the world had caused tremendous damage to the environment, pest resurgence, pest resistance to insecticides, and lethal effects on non-target organisms (Abudulai *et al*., 2001).

Bio pesticides are natural occurring source or agent gotten from living organisms such as animals, plants, and microorganism. The naturally occurring formulations made from the substances that control pests by non-toxic mechanisms and in ecofriendly manner are not newer technologies, they have been used in various forms since human civilization. Bio pesticides being a living organism (natural enemies) or products corresponding less threat to the environment and to human health, hence it can be used for the management of pests. (Kumar S, Singh A (2015). Biopesticides are very effective in the agricultural pest control without causing serious harm to ecological chain or worsening environmental pollution. The research and development of practical applications in the field of biopesticides greatly mitigate environmental pollution caused by chemical pesticide residues and promotes sustainable development of agriculture. Since the advent of biopesticides, a large number of products have been released, several of them have already played dominant roles in the market. The development of biopesticides stimulates modernization of agriculture which gradually replace chemical pesticides. Many biopesticides are ideal substitutes for their traditional chemical counterparts in pollution-free in agricultural production, while some of them display certain toxicity; which should be taken into consideration by the researchers in the field.

Hyptis suaveolens belongs to family Lamiaceae with medicinally important phytochemicals like essential oils, tannins, saponins, phenols, flavonoids, terpenoids, alkaloids, and sterols. Hyptis is one of the largest genus with more than 300 species of herbs, shrubs, and small trees. It belongs to the sub-family Nepetoideae under family Lamiaceae]. *H. suaveolens* (L.) Poit. Itis an annual or herb exhibiting characteristic of genotypic polymorphism, plasticity in morphological and physiological in attributes (Barbosa *et al.*, 2013). It can grow luxuriantly in diverse environmental conditions due to the presence of variety of chemical compounds that provide it adaptive and survival mechanism. *Hyptis suaveolens* has recently been shown to possess insecticidal properties [Singh *et a*l., 1993] as well as grain protectant from Cowpea weevil during storage [Fatope *et al.,* 1995]. The terpenes content of distilled volatile oils have been known to differ enormously due to different geographical locations and between different species of the same plant (Luz *et al.,* 1984). This genus possesses a diverse range of biological activities that led us to investigate the chemical composition of essential oil obtained by hydro-distillation from the leaves of *Hyptis suaveolens*.. The current research aimed to assess the biological pesticide in bush tea, *Hyptis suaveolens.*

MATERIALS AND METHODS

Selection of Plants for Biopesticide

The leaves of bush tea, *Hyptis suaveolens* were collected within the premises of Lagos State University of Science and Technology, Ikorodu.

Methods of Preparation

The leaves were collected and aired dried for four weeks on the laboratory bench, grinded into powder, soaked N-Hexane (10g leaf into 20 ml N-Hexane), sieved with filter paper, kept in 100 ml sample bottles and taken to Nigeria Stored Product Research Institute (NSPRI) Ilorin for chemical composition assessment.

Chromatographic Analyses of the plant extracts

The chemical composition of the essential oils extracted was performed by gas chromatography coupled with mass spectrometry (GC-FID). The GC analysis was performed using a chromatography equipped with a flame ionization detector (FID) and two capillary columns of different polarities OV type: 101 (25 m x 0.22 mm x 0.25 mm) and Carbowax 20 M (25 m x 0.22 mm x 0.25 µm). The carrier gas was Helium with a flow rate of 0.8 ml/minand the oven programming temperature was between 50 and 200°C with a gradient of 5°C/min. CPG/MS coupling was performed on a DB1-type fused silica capillary column (25 m x 0.23 mm x 0.25 µm) with Helium as a carrier gas and temperature programming identical to that of the GC (AOAC, 2019).

Identification of chemical constituents

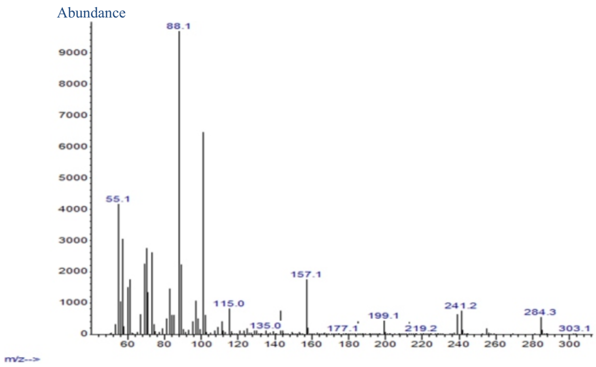
Bioactive compounds extracted from the plant extracts were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC-FID systems).

RESULT AND DISCUSSION

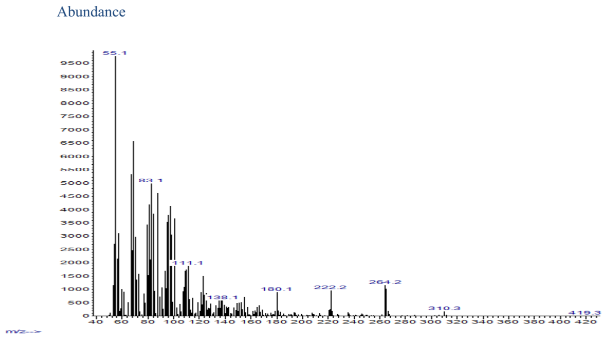
Result

The result of active ingredient in *Hyptis suaveolens* were presented in graph 1 to 4, showing the relative abundance of the technical grade materials (chemical composition) in the plant.

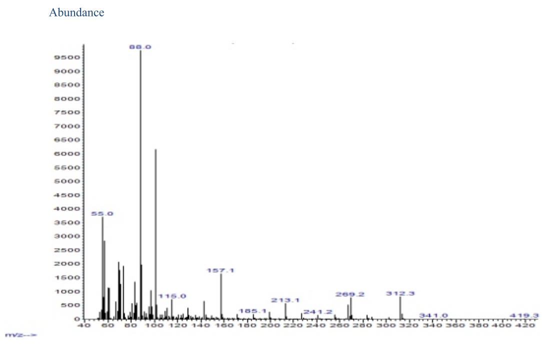
SPECTRUM-1



Graph 1: Resonance of different active ingredient in *Hyptis suaveolens*

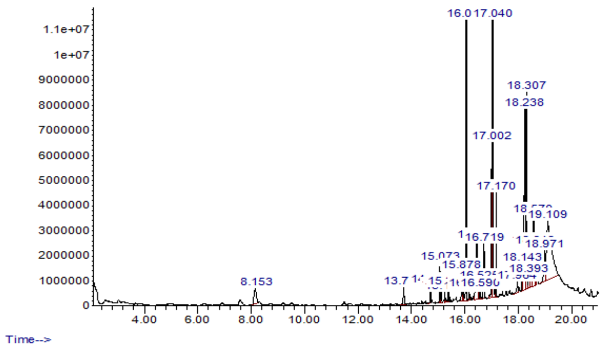


Graph 2:Resonance of different active ingredient in *Hyptis suaveolens*



Graph 3:Resonance of different active ingredient in *Hyptis suaveolens*

Abundance



Graph 4:Resonance of different active ingredient in *Hyptis suaveolens*

Graph 4:Resonance of different active ingredient in *Hyptis suaveolens*

Table 1 elicits the real bioactive compositions (serial number 1-28) in *Hyptis suaveolens* as revealed by GC-FID.

Table 1: The Chemical Composition of *Hyptis suaveolens*

S/N	Retention Time	Area %	Library ID	Ref	CAS	Qual
1	8.154	1.99	Caryophyllene	68513	00087-44-5	97
			Caryophyllene	68509	00087-44-5	95
			Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl8-methylene-		013877	
2	13.716	0.93	Bergamotol, Z-.alpha.-trans-	83571	088034-74-6	91
			trans-.alpha.-Bergamotene	68640	013474-59-4	74
			1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-,		026560-14-5	72
3			Tetradecanoic acid, ethyl ester	117465	000124-06-1	96
			Undecanoic acid, ethyl ester	78055	000627-90-7	91
			Ethyl tridecanoate	104283	028267-29-0	91
4	15.071	1.13	Neophytadiene	138502	00504-96-1	94
			Bicyclo [3.1.1] heptane, 2, 6, 6-trimethyl-, (1.alpha, 2.beta, 5.alpha.)	17424	006876-13-7	90
			Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	17379	000473-55-2	52
5	15.123	0.40	Pentadecanone, 6,10,14-trimethyl	128824	000502-69-2	52
			2-Pentadecanone, 6,10,14-trimethyl	128827	000502-69-2	49
			2-Heptanone, 5-methyl-	12801	018217-12-4	46
6	15.257	0.28	Hexadecyne	85921	000629-74-3	62
			Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)	17424	006876-13-7	60
			Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	17379	000473-55-2	55
7	15.375	0.49	Neophytadiene	138502	000504-96-1	60
			1-Methoxy-3-(2-hydroxyethyl)nonane	66682	070928-44-8	52
			3,7,11,15-Tetramethyl-2-hexadecen-1-o	155865	102608	43
8	15.879	1.49	[1,2,4]Triazolo[4,3-b][1,2,4]triazine, 7-(4fluorophenyl)-	78726	1000338-28-8	12
				81741	000562-23-2	12
			1(2H)-Naphthalenone, 3,4,4a,5,6,7-hexahydro-4a,5-dimethyl-3-(1-methylethenyl)-, [3S-(3.alpha.,4a.alpha .,5.alpha.)]- Longipinocarvone	81635	10001512 87-1	12
9	15.938	0.28	Ethyl 9-hexadecenoate	142078	054546-22-4	99
			9-Octadecenoic acid	142074	002027-47-6	90
			4-Tetradecene, 2,3,4-trimethyl-	1000624	05510-81-6	80
10	16.057	12.10	Hexadecanoic acid, ethyl ester	144309	000628-97-7	98
			Hexadecanoic acid, ethyl ester	144304	000628-97-7	94
			Ethyl tridecanoate		028267-29-0	87
11	16.160	0.44	3.alpha.,17.beta.-dihydroxyestr-4-ene	136616	035950-87-9	12
			Bicyclo[10.8.0]eicosa-1(12),14,18-triene	133023	10000155-83-0	11
			Cyclopropanemethanol, 2-methyl-2-(4-methyl-3-pentenyl)-	38232	098678-70-7	11
12	16.449	5.45	Squalene	243219	000111-02-4	91
			Supraene	243217	007683-64-9	91
			Squalene		000111-02-4	90
13	16.523	0.73	Phenanthrene, 1,2,3,4,4a,9,10,10a-octahydro1,1,4a-trimethyl-7-(1-methylethyl)-, (4aStrans)-	131010	109407-28-4	99
			7-Isopropyl-1,1,4a-trimethyl1,2,3,4,4a,9,10,10a-octahydrophenanthrene	131007	109680-01-5	50
			7-Isopropyl-1,1,4a-trimethyl1,2,3,4,4a,9,10,10a-octahydrophenanthrene	131008	109680-01-5	38
14	16.590	0.29	Heptadecanoic acid, ethyl ester	157927	014010-23-2	97
			Heptadecanoic acid, ethyl ester	157926	014010-23-2	94
			Tetradecanoic acid, ethyl ester	117465	000124-06-1	93
15	16.716	1.94	Phytol	155853	000150-86-7	91
			Phytol	155851	000150-86-7	91
			Phytol	155849	000150-86-7	86
16	17.005	5.07	9,12-Octadecadienoic acid, ethyl ester	167389	007619-08-1	99
			Linoleic acid ethyl ester	167367	000544-35-4	99
			Linoleic acid ethyl ester	167366	00544-35-4	99
17	17.042	12.70	Ethyl Oleate	169277	000111-62-6	99
			(E)-9-Octadecenoic acid ethyl ester	169327	006114-18-7	99
			(E)-9-Octadecenoic acid ethyl ester		006114-18-7	97
18	17.168	3.10	Octadecanoic acid, ethyl ester	171409	000111-61-5	98
			Octadecanoic acid, ethyl ester	171414	000111-61-5	98
			Octadecanoic acid, ethyl ester	171412	000111-61-5	98
19	17.967	0.66	2.alpha., 4a.alpha., 8a.alpha.-Decahydro-2naphthalenol-	27820	001424-36-8	50
			o-Methoxy-.alpha.-phenethylamine	25724	1000334-51-1	49
			Tricyclo[5.2.1.0(4,8)]decane		042836-61-3	49
20	18.145	1.35	Phenanthrene, 1,2,3,4,4a,9,10,10a-octahydro-1,1,4a-trimethyl-	91630	055090-42-1	38
			1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-, [1S-(1.alpha.,4a.alpha.,10a.beta.)]-	106200	000727-20-8	37
			9-Methyltricyclo[4.2.1.1(2,5)]deca -3,7-diene-9,10-diol	46013	078323-73-6	25
21	18.241	9.42	Benzene, 1-(1-buten-3-yl)-4-pentyl	66859	1000161-70-6	22
			Propane, 1,2,2,3-tetrachloro-	48103	013116-53-5	12
			9-Methyltricyclo[4.2.1.1(2,5)]deca -3,7-diene-9,10-diol	46013	078323-73-6	12
22	18.308	11.28	1-Phenanthrenemethanol, 1,2,3,4,4a,5,6,9,10,10a-decahydro-1,4adimethyl-7-(1-methylethyl)-, [1R-(1.alpha.,4a.beta.,10a.alpha.)]-	148284	021414-53-9	99
			Silane, dimethyl(3,7-dimethyloct-3yloxy)butoxy,.	147953	1000346-78-1	35

			Silane, dimethyl(2-methylbutoxy)nonyloxy-	147943	1000347-02-1	22
23	18.390	0.66	Phenanthrene, 1,2,3,4,4a,9,10,10a-octahydro1,1,4a-trimethyl-	91630	055090-42-35 1	
			Androstane-2,11-dione, (5.alpha.)-17-	148165	001449-57-25 6	
			Octadecene-9,11-diynoic acid, 8-oxo-, methyl ester	161752	075112-83-3	22
24	18.501	2.94	9-Octadecenamide, (Z)-	141029	000301-02-98 0	
			9-Octadecenamide, (Z)-	141032	000301-02-98 0	
			9-Octadecenamide, (Z)-	141028	000301-02-97 0	
25	18.567	3.80	Benzene, 1,2-bis(1-buten-3-yl)-	53083	1000162-73-9	38
			Spiro[2.3]hexan-4-one, 5,5-diethyl-6-phenyl-	91600	1000152-23-2	30
			4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2methylenebicyclo[4.1.0]heptane	66881	079718-83-30 5	
26	18.649	1.93	1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1R(1.alpha.,4a.beta.,10a.alpha.)]-	146494	003772-55-99 2	
			1-Phenanthrenemethanol, 1,2,3,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl),[1R(1.alpha.,4a.beta.,10a.alpha.)]-	146196	003772-55-99 2	
			1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1S-(1.alpha.,4a.alpha.,10a.beta.)]-	146495	024035-43-70 6	
27	18.975	2.212	((1R,4aR,4bS,10aR)-7-Isopropyl-1,4adimethyl-1,2,3,4,4a,4b,5,9,10,10 a-decahydrophenanthren-1-yl)methan ol	148279	097640-46-38 5	
			6,9-Octadecadiynoic acid, methyl ester	150118	056847-03-22 1	
			Carbonic acid, propargyl 2,2,2-trichloroethyl ester		1000325-65-3	16
28	19.108	16.56	1-Hydroxy-3,7,8-trimethoxyxanthen- 9-one	161320	020882-69-38 3	
			Xanthen-9-one, 1-hydroxy-3,5,8-trimethoxy-	161321	049599-09-35 9	
			Phenol, 2,4-bis(1-phenylethyl)-	161935	002769-94-20 0	

Discussion

GC-FID chromatogram of extracts revealed that twenty-six (28) bioactive compounds are present in A (*Hyptis suaveolens)* The GC showed the concentrations of the various compounds as a function of retention time while the peak height showed the relative concentrations of the components present in the extract, while the compounds were identified through mass spectrometry attached with the gas chromatography. The identified compounds with their name, % peak area, and retention time were categorized and summarized into five chemical groups Terpenes, fatty acid, hydrocarbons, esters.

Neophytadiene, a sesquiterpene lactone present in the essential oil of various plants such as the neem tree (*Azadirachta indica*), holds promise for numerous applications in medicine, agriculture, and cosmetics (Bhardwaj *et al*., 2020). This naturally occurring compound has undergone thorough investigation due to its potential as an insecticide, fungicide, anti-inflammatory, and antibacterial agent (Younis *et al*., 2022). Extending its effectiveness to a broad spectrum, neophyte diene has demonstrated efficacy against diverse insects, fungi, and bacteria, including the malaria-causing *Plasmodium falciparum* (Bhardwaj *et al*., 2020). The phytochemical constituents present in extracts as revealed by the GC-MS analysis showed the potential of the extracts to act as antibacterial and antifungal, anti-inflammatory, anti-cancer, and could further be exploited as hepato-protective, amelioration of hyperglycemia and dyslipidemia, and in reduction of atherogenic risk factor in humans (Ali, Al-Rahwi, & Lindequist, 2004; Sofowora, 2005). Terpenes like Bergamotol, alpha-trans-> (α-trans-Bergamotenol) was detected in the hyptis (0.93%). The presence of this molecule has not been discussed widely in the management of stored products pest management. The Bergamotenes, are important bioactive compounds derived from the Essential Oils of various plant species. Bergamotenes attract predators; as a result, plants are able to defend themselves against attack by herbivorous insects. The presence of Bergamotol, one of the pheromones signals another vital active ingredient in the extract which posit the possibility of extracts efficacy as plant protection. It is one of the eco-friendly pest management substance of the age.

Phytol is an acyclic diterpene alcohol and a constituent of chlorophyll which is commonly used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1. Furthermore, phytol also was shown to modulate transcription in cells via transcription factors PPAR-alpha and retinoid X receptor (RXR) (Saikia *et al*., 2010). According to the report of Moraes et al. Phytol is gaining more usefulness as a food additive and in medicinal fields, as its manifest promising antischistosomal properties in vitro and in a mouse model of schistosomiasis mansoni (Moraes *et al.,* 2014). Linoleic acid ethyl ester is an esterified form of linoleic acid.It is active against the bacteria S. mutans, A. actinomycetemcomitans, P. gingivalis, S. gordonii, and S. sanguis and the fungus C. albicans when used at a concentration of 25 µg/ml. Linoleic acid ethyl ester (3 mg/kg) prevents arteriosclerosis in rabbits fed a high-cholesterol diet (Huang *et al*., 2010). Silane was another active compound revealed. Silanes are a group of silicon-based organic–inorganic materials, which are biocompatible.

Another active ingredient of interest revealed is stigmasterol, an important plant sterol otherwise regarded as unsaturated phytosterol occurring in plant with potential to inhibit the development of various cancerous cells by inhibiting the promotion and growth of cancer cell lines including but not limited to stomach, lung, ovary, and estrogen-dependent human breast cancers (Bradford and Awad, 2007). It is of great interest finding this extract as one of the sources of stigmasterols that is regarded as the anti-cancer therapy of the future due to its ability to scavenge cancer-causing substances (Guarneri *et al*., 2006). Copaene (COP) is a tricyclic sesquiterpenes derived from different plants; leaves (Afoulous *et al*., 2013). Strongly attractive to and agricultural pest. (Adolhamid *et al.,*2009). Myristic Acid (MA) (Tetradecanoic acid) is a common saturated fatty acid with the molecular formula CH3(CH2)12COOH. Its salts and esters are referred to as myristates or tetradecanoates. MA is one of the most abundant fatty acids in milk fat (10%), alternatively obtained from plant sources such as palm oil, coconut oil. MA occurs as hard, faintly yellow or white, glossy crystalline solid or as yellow-white or white powder. Studies depict that diet rich in MA significantly increase concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the liver and blood plasma (Balvers *et al*., 2010).

Caryophyllene oxide is an oxygenated sesquiterpenoid, well-recognized as a protective compound in nutrition, medications and cosmetics. Caryophyllene oxide has some pharmacological potentials like anticholinesterase, analgesic, anti-inflammatory, antifungal activities (Chavan *et al*., 2010).caryophyllene oxide (CPO) is a sesquiterpene isolated from essential oils of medicinal plants such as clove (Eugenia caryophyllata), black pepper (Piper nigrum L.) , oregano (Origanumvulgare L.), guava (Psidiumguajava) and cinnamo Cinnamomum spp.) , and act as a strong anticancer agent also can stop the proliferation of a wide variety of tumor cells, consisting of breast, lung, liver, and prostate adenocarcinoma as reported in several studies (Park K-R *et al*., 2011). Vanillic acid (VA) (4-hydroxy-3-methoxybenzoic acid) is a dietetic phenolic composite present in plants and fruits. VA is a metabolic formation of caffeic acid that leads to the development of urine in an inhabitant. Consequent to intake of coffee, chocolate or green tea, and it can demonstrate antioxidant, antimicrobial, antigenotoxic, (M.G Erdem *et al*., 2012) and antitumor activities (Gong *et al*., 2019). VA can suppress hepatic fibrosis in chronic liver injury, and it also exhibits chemopreventive effects in experimentally induced carcinogenesis in rats (M. C. Kim *et al*., 2011; A. Vinoth *et al*., 2018). 1,2,3-Trimethoxybenzene (TMB) is an organic compound containing three methoxy groups in benzene ring having a molecular formula C9H12O3 and molecular weight of 168.19. These compounds can be used in pharmaceuticals (analgesics, antiseptics), biocides, formulating insect attractants, flavoring agents, etc. They are also useful in the preparation of antioxidants, stabilizers, UV absorbers for plastics and rubbers (Trivedi *et al*, 2015; Yoshida J-I, 2008).

CONCLUSION

In conclusion, the chemical composition of biopesticides is diverse, comprising various natural ingredients. They offer environmentally friendly pest control options with reduced risks to non-target organisms. While biopesticides have specific target pest control and are biodegradable, they may require frequent application and face limitations in persistence and efficacy. Overall, biopesticides play a valuable role in integrated pest management strategies, promoting sustainable agricultural practices and reducing reliance on synthetic pesticides.

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