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Insecticidal and Repellent Activity of *Plectranthus amboinicus* (Lour.) Spreng Leaf Extracts Against *Callosobruchus maculatus* (Fab.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Natural pesticides derived from plant-based substances are effective alternative to conventional pesticides. A study was conducted on the biopesticide chemicals extracted from powdered. *P. amboinicus* leaves using different solvents and their effectiveness against cowpea beetles, *Callosobruchus maculatus.* In this study, leaf powders of *P. amboinicus* were extracted with various solvents and tested qualitatively and quantitatively for phytochemical constituents using GC-MS.

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The highest concentrations of terpenoids, fatty acids, phenolic compounds, and steroids were found in petroleum benzine and ethanolic extracts. This insecticidal effect is attributed in part to the presence of secondary metabolites identified in the extracts. These petroleum benzine extracts of *P. amboinicus* also contained biopesticides such as thymol, beta caryophyllene, farnesol, phytol, Codlelure, (Z)-11-Hexadecenal, Erucic acid, and squalene. A higher repellency (44.8±0.8%) was achieved at a higher concentration (50 mg/ml) of petroleum benzene extract of *P. amboinicus* after 360 minutes of treatment followed by ethanol extract (40.8±1.49%) and, benzene (37.6±0.97%), chloroform (36.8±0.8%) and water extract (34.4±0.97%). We measured the levels of toxicity after 24 hours, 48 hours, and 72 hours of exposure. After 72 hours, Petroleum benzene caused the highest level of toxicity (LD_{50} = 20.89 mg/ml) of *Callosobruchus maculatus*, followed by ethanol (LD_{50}=28.18 mg/ml), water (LD_{50} = 32.35 mg/ml), chloroform (LD_{50} = 40.73mg/ml and Benzene (LD_{50} = 46.77mg/ml). Based on our results, we suggest *P. amboinicus* phytocompounds are helpful in protecting stored grains from *Callosobruchus maculatus*.

Keywords: Bioinsecticide; Callosobruchus maculates; P. amboinicus; phytochemical; repellent activity and toxicity.

1. INTRODUCTION

One of the most important aspects of agriculture is pest management with plant based products. In order to meet the food needs of a growing population and decreasing land availability, crop production must increase significantly. Many pests cause significant damage and losses to plants at every stage of their growth, as well as when they are harvested and stored (Gasic and Tanovic 2013). Around the world, pests, including insects, pathogens, and weeds, cause 27–42% losses in crop production. As a result, a staggering 48–83% of crops are lost without crop protection [1]. Pest control poses a significant challenge around the world because it must not damage the environment. Crop protection continues to be provided by synthetic pesticides that control agricultural pests. The long-term use of these compounds is, however, threatened by their well-documented adverse effects, including carcinogens, teratogenics, high residual toxicity, disruption of mammalian hormonal systems, the long persistence of their effects in the environment, and the presence of residues in food that have become an important issue for consumers [2,3,4]. The development of new synthetic pesticides has also become a significant challenge, leading to very high development costs [5]. Future crop protection products may be biopesticides because they are eco-friendly, safe for humans and non-target organisms, and can be used both individually and as part of integrated pest management (IPM).

As defined by Regnault-Roger and Philogene [6], "biopesticides" can be defined as "specific preparations containing living microorganisms," or more broadly as botanical compounds, semiochemicals (e.g. pheromones) and transgenic products. The US Environmental Protection Agency defines biopesticides as those that are derived from "natural materials such as animals, plants, bacteria, and certain minerals" [7]. Some of these chemicals have been used in the past to manage pests on plants as stationary organisms that must defend themselves against mobile herbivores and pathogens [8]. Phytochemically, plant bioactive compounds can be classified into phenylpropanoids, phenolics, terpenoids, steroids, alkaloids, and nitrogenated compounds. As early as 400 BC, pyrethrum (Tanacetum cinerariaefolium, Asteraceae) was documented for its medicinal properties [9]. As early as the seventeenth century, tobacco leaves were used as the first pure botanical insecticide [9].

The perennial herb *P. amboinicus* (Lour.) Spreng., also known as Indian borage is widely distributed among species in the tropics and warm regions of the world, particularly in Asia, Africa, Australia, and India [10]. Many pharmacological properties have been linked to this herb, including antimicrobial, anti-inflammatory, antitumor and wound-healing effects, anti-epileptic, insecticidal, antioxidant, and analgesic properties [11,12]. Phytochemically, this herb contains two major monoterpenes, namely carvacrol and thymol, that show insecticidal properties [13,14]. Insecticidal properties are found in essential oils from *Plectranthus amboinicus*, including the cowpea weevil (*Callosobruchus maculatus*) [15], mosquito larvae (*Ae. aegypti* and *Anopheles gambiae*) [16,17], red flour beetle (*Tribolium castaneum*) [18], and the termite (*Odontotermes*

obesus), among others [13,14]. Based on above facts, we evaluated the Insecticidal and repellent activity of *P. amboinicus* (Lour.) Spreng leaf extracts against *Callosobruchus maculatus* (Fab.).

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

We collected the leaves of *P. amboinicus* in Kasilingapuram village, in the Thoothukudi district, Tamilnadu, India (8°46'30.4"N 77°53'20.2"E), in October and November (2019). The plant specimens were identified and authenticated by Dr. C. Babu, Head and Associate Professor of Botany at Pioneer Kumaraswamy College, Nagercoil. The leaves were thoroughly rinsed under running water, then shade-dried at room temperature for 7-8 days. After making a fine powder from plant leaves, it was stored in an airtight container for later use.

2.2 Extract Preparation

A Soxhlet extractor was used to dissolve 50 grams of dry leaves in 250 ml petroleum benzene (40-60^{o}C), benzene, chloroform, ethanol, and water. In the Soxhlet loop, solvents are poured into the loop where extraction occurs until the solvent is colorless [19]. As the solvent evaporated, the extracts were concentrated and stored in airtight containers at room temperature. In order to further utilize the solution, it was frozen at 4^{o}C [20].

2.3 Phytochemical Analysis

We tested the phytochemicals in *P. amboinicus* leaves according to a previously described method [21]. Several qualitative chemical tests were conducted on the extracts to determine their chemical composition profiles. Each solvent is tested to determine what phytoconstituents are present in the crude powder extracted using standard procedures. An analysis of a plant is usually performed to determine whether it contains terpenoids, steroids, fatty acids, phenolic compounds, alkaloids, saponins, and flavonoids.

2.4 Gas Chromatography Mass Spectrometry (GC-MS) Analysis

To investigate the phytochemistry of *P. amboinicus* plant extracts from Heber Analytical Instrumentation Facility (HAIF), Bishop Heber College, Trichy-620 017, we conducted GCMS analysis. Analyses were conducted using GC-MS equipment (GC MS QP2020; SHIMADZU), which includes an auto sampler, sample injector, gas chromatograph and mass spectrometer. An SHRxi-5Sil-MS capillary capillary nonpolar column was used (diameter 0.25mm, film thickness 0.25mm, density 100% dimethyl poly siloxane) for GC-MS analysis. In this experiment, electrons were ionized with 70EV ionization energy. The injection was performed with helium gas (99.99%) at a rate of 1.20ml/min and a volume of 5 µl (split ratio: 10). In the oven, the temperature was programmed to rise from 50°C (isothermal for 2 minutes) to 280°C for 10 minutes. We collected mass spectra at 70eV with a scan interval of 0.3 seconds and a scan range of 50 - 500 m/z. There were 21 minutes spent running the GC. Based on the average peak area divided by the total peak area, we calculated each component's percentage. GC-MS real-time software from Shimadzu was used to analyze mass spectra and chromatograms.

2.5 Identification of Components

Gas chromatograms and mass spectra were interpreted using data from NIST [22] and WILEY [23] having more patterns. Based on the NIST and WILEY libraries, we compared the unknown component's spectrum to the known component's spectrum. All components of the test material were identified with their Molecular formulas, names, Molecular weights, and structures.

2.6 Insect Collection and Rearing

The species of *Callosobruchus maculates* (Fab.) was collected from a farmer in the village of Kasilingapuram, Thoothukudi District, Tamil Nadu, India. We conducted all experiments at the PMT College's PG and Research Department of Zoology in Melaneelithanallur, Tenkasi District, Tamil Nadu, India. (8°46'30.4"N 77°53'20.2"E). At the beginning, 50 pairs of adults aged 1–2 days were placed in jars containing cowpea seeds and allowed to mate and lay eggs for a maximum of seven days. Pests were reared using cowpea grains at 28 ± 1^{o}C and 65 ± 5% relative humidity (RH). In order to prevent contamination and insect escape, cowpea seeds containing eggs were removed from their parents and transferred to new cowpea seeds in breeding jars, which were covered with cloth and

fastened with rubber bands. We used progenies of the pest in all of our experiments.

2.7 Repellency Tests

An area partiality method was used to test the repellent effects of the extract (*P. amboinicus*) and some of its individual components against *Callosobruchus maculates* [24]. As a solvent, ethanol was used to prepare the plant extract solutions, and a volume of 1 mL was applied to a half-filter paper disk as uniformly as possible to obtain the required plant extract volume per unit area of 10, 20, 30, 40, and 50 mg/ml. Using ethanol as a vehicle control, the other half of the filter paper was treated with the same amount of ethanol. The testing areas consisted of 9 cm Whatman no. 1 filter paper cut in half (31.8 cm^2). Air-drying was performed for 10 minutes on the treated and control half disks to evaporate the solvent. Moreover, untreated halves were reattached with adhesive tape and placed in Petri dishes 90 mm in diameter. A total of 30 adults of both sexes of Callosobruchus maculates were released at the center of each filter paper disk. Dishes were covered and kept in darkness at 22±2^oC and 75± 10%relative humidity. We counted the number of Callosobruchus maculates on the treated and untreated portions of the experimental paper halves after 60, 120, 180, 240, 300, and 360 seconds. The percentage repellency (PR) for a given exposure time was calculated as follows: PR = [(Nc - Nt)/(Nc+Nt)] X 100, where Nc and Nt were the number of insects on the untreated (control) and treated areas, respectively. A total of five replications were performed for each concentration tested [25].

2.8 Toxicity Assay

The extracts of *P. amboinicus* were used in a toxicity test against adults of *Callosobruchus maculates* at at 28 ± 1^oC and 65 ± 5% RH. In these studies, newly developed adults (1–15 days of age) were used. A leaf extract from *P. amboinicus* was tested for its ability to kill *Callosobruchus maculates* adults in glass jars (replicates) with filter paper (3 x 3 cm) attached to screw caps on the underside. In each jar, 30 insects were applied with plant extracts at concentrations of 10, 20, 30, 40, and 50 mg/ml. Five times were repeated for each treatment and control. The solvent alone was used to treat filter paper pieces as a control. Based on log-concentration mortality regression lines, the lethal concentration causing 50% mortality (LC_{50}) was calculated after 24, 48, and 72 hours for each concentration. As long as the legs or antennae did not move, the insects were considered dead [26] (Muhammad, 2008).

2.9 Statistical Analysis

We conducted a one-way analysis of variance (ANOVA) and a least significant difference (LSD) multiple range test on the data to determine whether there were significant differences between variable concentrations ($P < 0.05$). In the hope of estimating lethal concentrations (LC) and lethal concentrations (LC_{50}), Finney's probit analysis [27] was considered significant when their respective 95% fiducial limits did not overlap.

3. RESULTS AND DISCUSSION

The present study was carried out on the plant of *P. amboinicus* leaves to identify the presence of biopesticide components. Phytochemical tests, being economical and fast, are recommended for the quality control of insecticidal secondary metabolism. In the present study, phytochemicals were confirmed to be present in different solvent extracts of of *P. amboinicus*.

3.1 Qualitative Phytochemical Analysis of *P. amboinicus* Leaves Extract

Plants have a toxicity value due to some chemical substances that possess a strong physiological effect on insects. The most important of these compounds are alkaloids, terpenoids, steroids, fatty acids, and phenols. The qualitative phytochemical analysis of various solvent extracts of of *P. amboinicus* leaves was showed in Table 1. The phytochemical analysis results revealed that the presence of alkaloids, terpenoid, steroid, fatty acid and phenolic compounds. There was high intensity of terpenoids in petroleum benzine extract and low intensity in chloroform and ethanol extracts [28]. Fatty acids were detected in high intensity in petroleum benzene, chloroform, benzene, and ethanol extracts. The presence of steroids was found in high intensity in ethanol and water extracts. The alkaloids were found to be in very low intensity in ethanol extracts. The phenolic compound was present in low intensity in petroleum benzene and benzene extracts. Saponin was found in very small amounts in ethanol and water extracts, while flavonoids were found in very small amounts in benzene, chloroform, and ethanol extracts [29,30].

Table 1. Preliminary phytochemical screening of extract of powdered leaves of *P. amboinicus*

S. No	Phytochemicals	Solvents				
		Petroleum benzine	Benzene	Chloroform	Ethanol	Water
1	Terpenoids	+++	+	+	++	+
2	Steroids	-	-	-	+++	++
3	Fatty acids	+++	+++	+	++	+
4	Phenolic compounds	+	++	-	+++	++
5	Alkaloids	-	-	-	++	-
6	Saponin	-	-	-	++	+
7	Flavonoids	+	-	+	++	-

Note: *+ → present in small concentration; ++ → present in moderately high concentration; +++ → present in very high concentration; - → absent*

3.2 GC-MS Analysis of *P. amboinicus* Leaves Extract

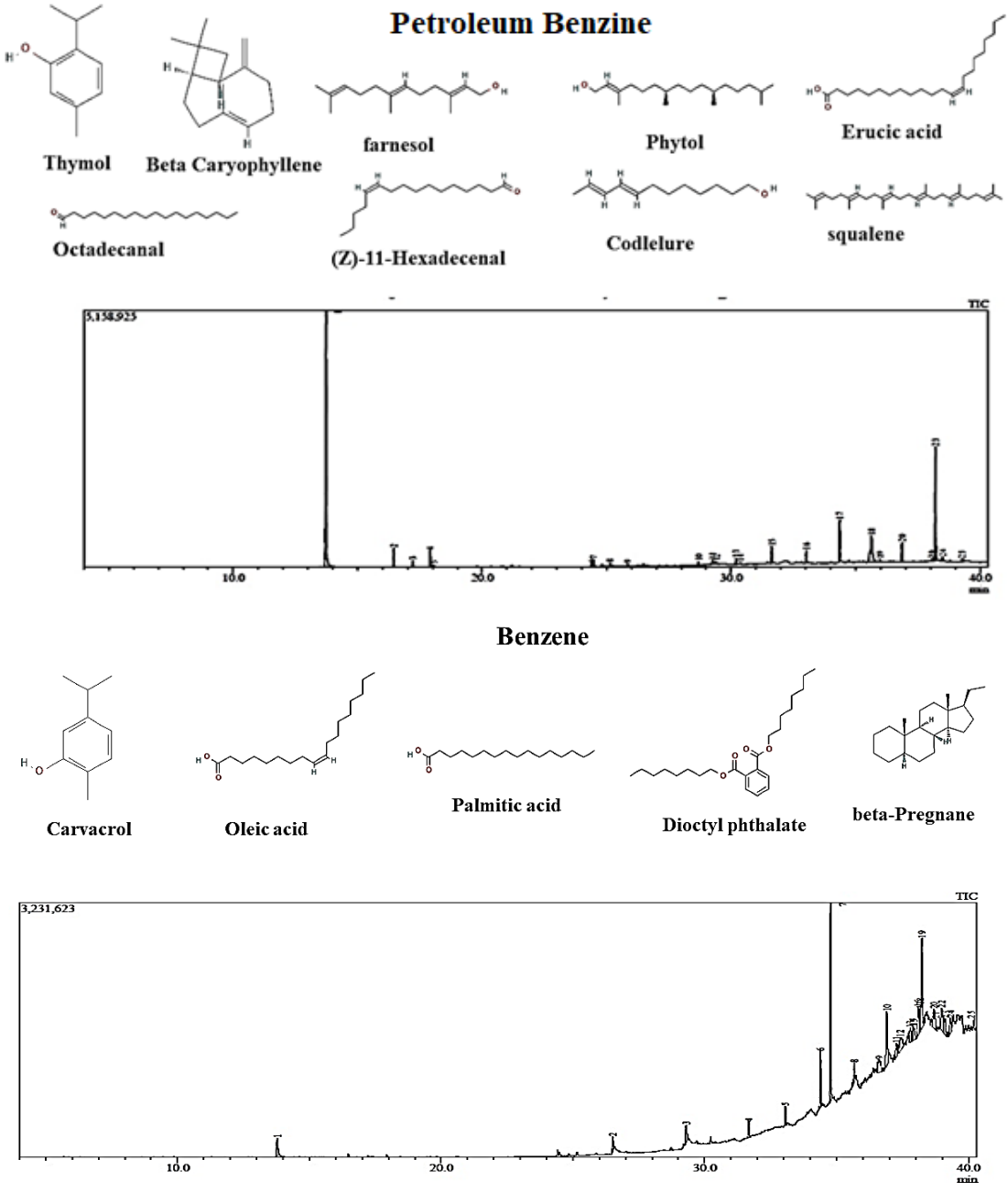
The most effective way to determine the functional groups that make up bioactive constituents of Terpenoids, Steroids, Fatty Acids, Phenolic Compounds, Alkaloids, Saponins, and Flavonoids is through GC-MS. In this study, we analyse the results of Gas Chromatography - Mass Spectroscopy on the various solvent extracts of of *P. amboinicus*, as shown in Table 2 and Fig. 1. Among twenty-five compounds identified in the petroleum benzine extract,nine showed to be toxic in nature. The GC-MS analysis of petroleum benzine extract of *P amboinicus* revealed the presence of toxic compounds like thymol (43.03), beta caryophyllene (3.25), farnesol (1.4), octadecanal (1.2), phytol (0.71), codlelure (0.5), (Z)-11-hexadecenal (1.36), erucic acid (0.71), and squalene (17.45). Benzene extracts, twenty compounds were identified and five of those compounds appeared toxic. The toxic compounds in benzene extracts, such as carvacrol (43.03), Palmitic acid (1.11), Oleic acid (1.36), Dioctyl phthalate (6.65), and beta-Pregnane (2.96). Among the 17 compounds identified in the chloroform extracts, five were toxic. A toxic compound such as phytol (1.92), palmitic acid (11.76), decahydronaphthalene (1.12), (Z)-11-hexadecenal (7.96), and dioctyl phthalate (44.86). The ethanol extracts identified twenty compounds out of which eight were toxic. Toxic compound such as palmitic acid (7.84), 1-dodecanethiol (1.98), 1-dodecene (4.26), 2,5-di-tert-amylhydroquinone (8.15), hexacosanoic acid (6.53), genipin (2.36), and propylparaben (2.33). The water extracts showed that 10 compounds were identified and two of these compounds were toxic, such as 2,4-di-tert-butylthiophenol (7.17) and usnic acid (10.24).

Terpenoids are widely used in the fragrance and food industries, as well as in a wide range of pharmacological applications. Terpenoids are the largest class of natural products derived from plants. They include essential oils, flavours, fragrances, lipid-soluble pigments and toxic compounds [31]. A toxin that causes paralysis and mortality led to the development of the most successful commercial pesticides [32] (Gershenzon and Croteau, 1994). Phenolic compounds possess a hydroxyl (—OH) group, attached to the benzene ring or to another aromatic ring structure, such as catechol, resorcinol, hydroquinone, pyrogallol, etc. In various studies [33], phenolic compounds found in oak have been found to have a negative effect on the growth of gypsy moths. Various studies have shown that plant phenolic compounds act as one of the primary defences against insects [34,35]. Insecticides using fatty acids are natural. Its relatively low toxicity to vertebrates, ease of soil decomposition, and lack of resistance by target insects make it an excellent natural insecticide (Imai *et al.,* 1995). Lauric acid is a saturated fatty acid with a 12-carbon atom chain (medium chain fatty acid) that acts both as a physical and chemical insecticide [36]. Cholecalciferol is an acute (single-feeding) or chronic (multiple-feeding) rodenticide toxicant with unique activity for controlling commensal rodents as well as anticoagulant-resistant rats [37]. An association between the monoterpene, sesquiterpene, and oxygenated monoterpene content of *Hoslundia opposita.* and its insecticidal activity against *Callosobruchus maculates* [38] (Nerio et al. 2010). In recent years, GC-MS has become one of the most recommended tools for monitoring and tracking organic pollutants in the environment. It is the tool used to test for prohibited performance enhancing drugs such as anabolic steroids in athletes' urine samples. It is exclusively used for the analysis of esters, fatty acids, alcohols, aldehydes, terpenes, etc. GC-

MS is an extremely powerful technology that provides a rare opportunity to characterization and identification of new compounds synthesized [39].

3.3 Repellence Activity

Table 3 were showed that repellence activity of methanol, ethyl acetate and hexane extracts of *P. amboinicus*. Highest repellency (48±1.26%) about was achieved at higher concentration (50 mg/ml) of petroleum benzene extract of *P. amboinicus* after 360 minutes of treatment, followed by ethanol extract (47.2±1.49%), water extract (37.6±0.97%), benzene (42.4±0.97%) and chloroform (41.6±0.97%). The highest individual repellency activity was achieved at petroleum benzene extract of *P. amboinicus* against stored grain insect pests *Callosobruchus maculatus*. The individual replicate with mean value was showed that highest repellence activity in petroleum benzene extract at 1 h interval. The repeated measure analysis of *P. amboinicus* against *Callosobruchus maculatus* between various doses of 10, 20, 30, 40 and 50 mg/ml after 1, 2, 3,4, 5and 6 h respectively were significant at $p < 0.05$ level.



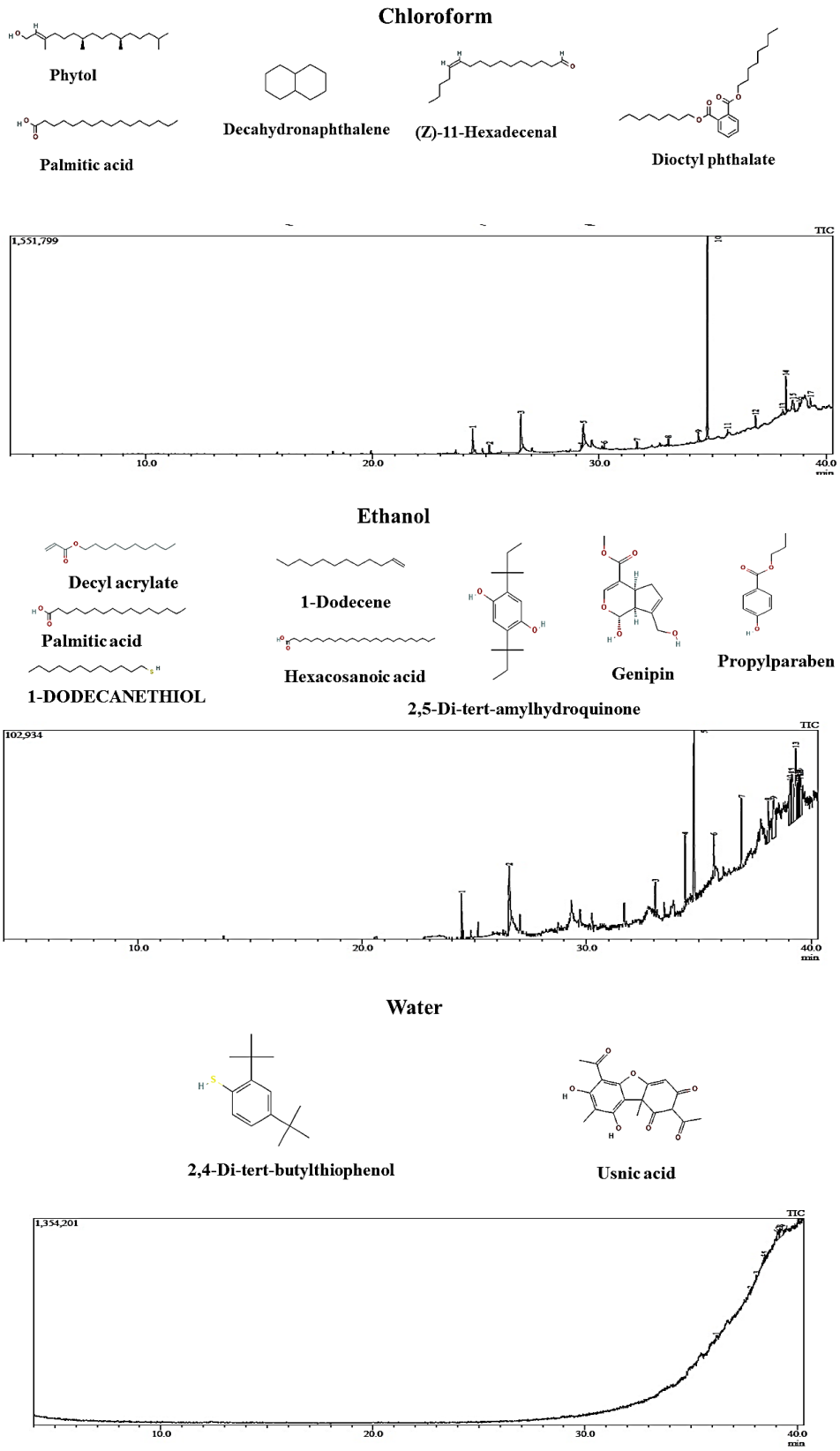


Fig. 1. GC-MS Chromatogram and toxic compounds of *P. amboinicus* leaves different solvent extract

Table 2. Phytochemical analysis of *P. amboinicus* leaves different solvent extract

Solvent	Retention Time (min)	Peak Area	Molecular Weight	Molecular formula	Name of the Compound	Name of the Phytochemical	Toxicity
Petroleum benzine	13.73	43.03	150	$C_{10}H_{14}O$	Thymol	monoterpene	Pesticide- Fungicides, Acute oral toxicity, and Skin corrosion/ irritation
	16.45	3.25	204	$C_{15}H_{24}$	Beta Caryophyllene	Sesquiterpenes	Health Hazard ,
	18.05	1.4	222	$C_{15}H_{26}O$	Farnesol	Fatty Alcohols	Pesticide-Pheromone, Skin and eye corrosion/ irritation
	24.47	1.2	268	$C_{18}H_{36}O$	Octadecanal	aldehyde	Skin and eye corrosion/irritation
	25.12	071	296	$C_{20}H_{40}O$	Phytol	diterpenoid	Skin and eye corrosion/ irritation
	25.83	0.5	296	$C_{12}H_{22}O$	Codlelure	Terpenes	Insecticide, Skin and eye corrosion/ irritation
	29.25	1.36	238	$C_{16}H_{30}O$	(Z)-11-Hexadecenal	Aldehydes	Insecticide, Skin and eye corrosion/ irritation
	29.41	071	338	$C_{22}H_{42}O_2$	Erucic acid	fatty acid	Skin and eye corrosion/ irritation
	38.21	17.45	410	$C_{30}H_{50}$	squalene	Terpenes	Skin and eye corrosion/ irritation
Benzene	13.78	43.03	150	$C_{10}H_{14}O$	Carvacrol	monoterpene	Acute oral toxicity, Skin and eye corrosion/ irritation
	26.51	1.11	256	$C_{16}H_{32}O_2$	Palmitic acid	Fatty acid	Organ toxicity, Skin and eye corrosion/ irritation
	29.29	1.36	282	$C_{18}H_{34}O_2$	Oleic acid	Fatty acid	Acaricides, Herbicides, Insecticides, Organ toxicity, Skin and eye corrosion/ irritation
	34.77	6.65	390	$C_{24}H_{38}O_4$	Dioctyl phthalate	Phthalic Acids derivatives	Health Hazards
	37.89	2.96	288	$C_{21}H_{36}$	5beta-Pregnane	steroid.	Organ toxicity, Skin and eye corrosion/ irritation
Chloroform	25.15	1.92	296	$C_{20}H_{40}O$	Phytol	diterpenoid	Acute oral toxicity, Skin and eye corrosion/irritation
	26.54	11.76	256	$C_{16}H_{32}O_2$	Palmitic acid	Fatty acid	Organ toxicity, Skin and eye corrosion/ irritation
	29.20	1.12	138	$C_{10}H_{18}$	Decahydronaphthalene	bicyclic hydrocarbon	Organ toxicity, Skin and eye corrosion/irritation

Solvent	Retention Time (min)	Peak Area	Molecular Weight	Molecular formula	Name of the Compound	Name of the Phytochemical	Toxicity
	29.30	7.96	238	$C_{16}H_{30}O$	(Z)-11-Hexadecenal	Aldehydes	Insecticide, Skin and eye corrosion/irritation
	34.77	44.86	390	$C_{24}H_{38}O_4$	Dioctyl phthalate	Phthalic Acids derivatives	Health Hazards
Ethanol	24.42	2.84	212	$C_{13}H_{24}O_2$	Decyl acrylate	Ester	Organ toxicity, Skin and eye corrosion/irritation
	26.54	7.84	256	$C_{16}H_{32}O_2$	Palmitic acid	Fatty acid	Organ toxicity, Skin and eye corrosion/irritation
	33.06	1.98	202	$C_{12}H_{26}S$	1-DODECANETHIOL	thiol	Organ toxicity, Skin and eye corrosion/irritation
	38.10	4.26	168	$C_{12}H_{24}$	1-Dodecene	acyclic olefin	Organ toxicity, Skin and eye corrosion/irritation
	38.33	8.15	250	$C_{16}H_{26}O_2$	2,5-Di-tert-amylhydroquinone	phenolic	Acute oral toxicity, Skin and eye corrosion/irritation
	39.17	6.53	396	$C_{26}H_{52}O_2$	Hexacosanoic acid	fattyacid	Skin and eye corrosion/irritation
	39.40	2.36	226	$C_{11}H_{14}O_5$	Genipin	monoterpenoid	Acute oral toxicity,
	39.48	2.33	180	$C_{10}H_{12}O_3$	Propylparaben	phenolic	Acute oral toxicity, Skin and eye corrosion/irritation
Water	38.43	7.17	222	$C_{14}H_{22}S$	2,4-Di-tert-butylthiophenol	thiophenol	Acute oral toxicity, Skin and eye corrosion/irritation
	39.12	10.24	344	$C_{18}H_{16}O_7$	Usnic acid	Phenolic	Acute oral toxicity, Skin and eye corrosion/irritation

Table 3. Repellent Activity of different *Plectranthus amboinicus* extract against cowpea beetle, *Callosobruchus maculates*

Solvent	Concentration (mg/ml)	60	120	180	240	300	360	Average	P Value	Significance
Benzene	10	2.4±0.97	4±1.26	8±1.26	12.8±1.49	17.6±0.97	23.2±0.8	11.33	<0.005	***
	20	4.8±1.49	9.6±0.97	12.8±0.8	16.8±1.49	20±1.26	24.8±0.8	14.80	<0.005	***
	30	9.6±0.97	12.8±0.8	17.6±0.97	20.8±0.8	25.6±0.97	29.6±0.97	19.33	<0.005	***
	40	12.8±0.8	16.8±0.8	22.4±0.97	26.4±0.97	28.8±0.8	33.6±0.97	23.46	<0.005	***
	50	15.2±0.8	20.8±0.8	24.8±0.8	29.6±0.97	34.4±0.97	37.6±0.97	27.06	<0.005	***
Chloroform	10	1.6±0.97	3.2±0.8	7.2±0.8	12±1.26	16±1.26	21.6±1.6	10.26	<0.005	***
	20	4.8±0.8	8±1.26	12±1.26	16.8±0.8	18.4±0.97	24.8±0.8	14.133	<0.005	***
	30	8.8±0.8	12±1.26	16.8±0.8	20±1.26	24±1.26	28.8±0.8	18.4	<0.005	***
	40	12±1.26	16±1.26	20.8±1.49	24.8±1.49	27.2±0.8	32±1.26	22.13	<0.005	***
	50	13.6±1.6	20±1.26	24±1.26	28.8±0.8	33.6±0.97	36.8±0.8	26.13	<0.005	***
Ethanol	10	3.2±0.8	5.6±0.97	8±1.26	14.4±0.97	19.2±1.49	24.8±0.8	12.54	<0.005	***
	20	5.6±0.97	9.6±0.97	15.2±1.49	20±1.26	23.2±1.49	28.8±1.49	17.07	<0.005	***
	30	10.4±0.97	15.2±0.8	20±1.26	24.8±1.49	28±1.26	31.2±1.49	21.60	<0.005	***
	40	14.4±0.97	19.2±0.8	21.6±1.6	27.2±0.8	30.4±0.97	36.8±1.49	24.94	<0.005	***
	50	17.6±0.97	20.8±1.49	25.6±0.97	32±1.26	37.6±0.97	40.8±1.49	29.07	<0.005	***
Petroleum	10	4.8±0.8	7.2±0.8	10.4±0.97	16.8±0.8	21.6±0.97	27.2±1.49	4.8	<0.005	***
Benzine	20	7.2±0.8	10.4±0.97	16±1.26	22.4±1.6	26.4±0.97	32.8±1.49	7.2	<0.005	***
	30	11.2±0.8	16±1.26	20.8±0.8	26.4±0.97	29.6±0.97	35.2±1.49	11.2	<0.005	***
	40	15.2±1.49	20.8±0.8	25.6±0.97	31.2±1.49	30.4±0.97	36.8±1.49	15.2	<0.005	***
	50	19.2±0.8	24.8±1.49	29.6±0.97	35.2±0.8	39.2±1.49	44.8±0.8	19.2	<0.005	***
Water	10	0.8±0.8	2.4±0.97	5.6±0.97	8.8±1.49	14.4±0.97	16.8±0.8	8.133	<0.005	***
	20	4±1.26	8±1.26	9.6±0.97	12±1.26	16.8±1.49	20±1.26	11.73	<0.005	***
	30	6.4±0.97	9.6±0.97	13.6±1.6	15.2±1.49	20.8±0.8	24±1.26	14.93	<0.005	***
	40	9.6±0.97	12±1.26	15.2±1.49	22.4±0.97	24.8±1.49	29.6±0.97	18.93	<0.005	***
	50	9.6±0.97	15.2±1.49	20.8±0.8	26.4±0.97	28.8±1.49	34.4±0.97	22.53	<0.005	***

Each datum represents for five replicates (Mean ± SE, %), adults (n = 30)

Table 4. Toxicity and profit analysis of different *P. amboinicus* extracts againt cowpea beetle, *Callosobruchus maculates*

Plant Material	Concentration (mg/ml)									LC_{50} (mg/ml)
	Hours	10	20	30	40	50	Mean	P value	Significant	
Petroleum	24	15.2±1.49	25.6±1.6	35.2±1.49	45.6±1.6	55.2±1.9	35.36	0.002	***	47.86
Benzine	48	25.6±0.97	35.2±1.49	45.6±1.6	55.2±1.9	65.6±1.6	45.44	0.002	***	31.62
	72	35.2±1.49	45.6±1.6	55.2±1.49	65.6±0.97	75.2±1.49	55.36	0.002	***	20.89
Benzene	24	0±0	6.4±0.97	16±1.26	26.4±0.97	36.8±1.49	17.12	0.005	***	95.49
	48	6.4±0.97	16.8±1.49	26.4±0.97	36.8±1.49	46.4±0.97	26.56	0.001	***	74.13
	72	16±1.26	26.4±0.97	36.8±1.49	46.4±0.97	56.8±0.49	36.48	0.002	***	46.77
Chloroform	24	0±0	8±1.26	18.4±0.97	28.8±1.49	38.4±0.97	18.72	0.002	***	74.13
	48	8.8±0.8	18.4±2.0	28.8±1.49	38.4±0.97	48.8±1.49	28.64	0.004	***	63.09
	72	18.4±0.97	28.8±0.8	38.4±1.6	48.8±1.49	58.4±0.97	38.56	0.002	***	40.73
Ethanol	24	8.8±1.49	18.4±0.97	28.8±1.49	38.4±0.97	48.8±1.49	28.64	0.001	***	56.23
	48	18.4±0.97	28.8±1.49	38.4±0.97	48±1.26	58.4±1.6	38.4	0.001	***	41.68
	72	28.8±1.49	38.4±0.97	48.8±1.49	58.4±0.97	68.8±1.49	48.64	0.002	***	28.18
Water	24	5.6±0.97	15.2±0.8	25.6±0.97	35.2±0.8	45.6±0.97	25.44	0.001	***	60.22
	48	15.2±1.49	25.6±0.97	35.2±0.8	45.6±0.97	55.2±0.8	35.36	0.001	***	46.77
	72	25.6±1.6	35.2±1.49	45.6±0.97	55.2±0.8	65.6±0.97	45.44	0.002	***	32.35

*** highly significant

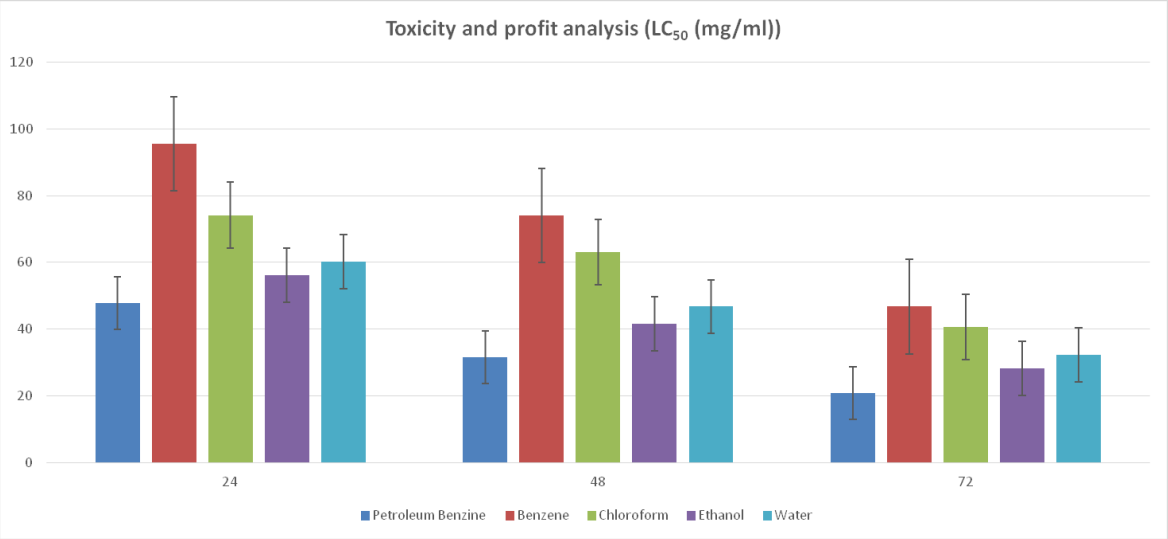


Fig. 2. Toxicity and profit analysis of different *P. amboinicus* extracts againt cowpea beetle, *Callosobruchus maculates*

Some monoterpenes such as α--pinene, cineole, eugenol, limonene, terpinolene, citronellol, citronellal, camphor and thymol are common constituents of a number of EO described in the literature, as presenting mosquito repellent activity [40,41,42] (Yang *et al.,* 2004). Among sesquiterpenes, β-caryophyllene is most cited as a strong repellent against A. aegypti [43]. Although repellent properties of several EO regularly appear to be associated with the presence of monoterpenoids and sesquiterpenes [44,45], other authors [46] have found that phytol, a linear diterpene alcohol, has high repellent activity against Anopheles gambiae. Moreover, the oxygenated compounds phenylethyl alcohol, β-citronellol, cinnamyl alcohol, geraniol, and α-pinene, isolated from the essential oil of Dianthus caryophyllum, showed strong repellent activities against ticks (*I. ricinus*) [47]. In our study, petroleum benzene leaf extract from *Plectranthus amboinicus* was more effective at repelling insects than other extracts such as ethanol, water, chloroform, and benzene. The petroleum benzene extract of *Plectranthus amboinicus* was found to contain eight toxic compounds, including Geranyl acetate, Dihydroactinidiolide, Neophytadiene, Phytol, Ascorbic Acid, Linoleic acid, Octadecanedioic acid and squalene. These compounds act as repellent activity against stored grain pests *Callosobruchus maculatus* [48].

3.4 Toxicity Assay

Petroleum benzine, benzene, chloroform, ethanol, and water extracts of *P.amboinicus* showed excellent bio-pesticide activity against *Callosobruchus maculatus*, and the time needed to cause 50% (LC_{50}) mortality dropped with increased concentration. (Table 4). Plant extracts from the leaves were fumigated against the pest with various concentrations of 10, 20, 30, 40, and 50 mg/ml and exposure times of 24, 48, and 72 hours, respectively. The mean mortality activity of petroleum benzene extract is observed 39.04, 48.16and 60.32in 24, 48 and 72 hours respectively (Fig. 2). The benzene extract mean observed mortality percentage is 10.24 (24hr), 16.32 (48hr) and 24.16 (72hr). Chloroform extract showed the mean value of observed mortality is 12.32, 18.24and 25.12 in 24, 48 and 72 hours respectively. The mean mortality activity of ethanol extracts observed 34.24, 42.72, and 52.48 in 24, 48 and 72 hours respectively. The water extract mean observed mortality percentage is 21.28 (24hr), 24.48 (48hr) and 38.88 (72hr). The highest toxicity (87.2±0.8% and LC_{50} value 16.22 mg/ml) of *Callosobruchus maculatus* was caused by Petroleum benzene, followed by ethanol 76.8±0.8±1.26% & LC_{50} value 23.98 mg/ml), water (52.8±1.49% and 44.66 mg/ml) Benzene (37.6±0.97% and 89.13 mg/ml) and chloroform (38.4±0.97% and 102.32 mg/ml) after 72 hours.

The essential oil of *Vernonia arborea* can be used to manage *Callosobruchus maculatus* and other insect pests in stored products. It acts as an insecticide, reducing the rate of female oviposition, population growth, and development [49]. Novel botanical insecticides, especially their

hexane fraction, have a similar or higher biological activity than the most popular botanical insecticides like *Azadirachta indica* against stored grain pests like *Callosobruchus maculatus* (Kosini *et al.,* 2017). Considering its high levels of toxicity against cowpea weevils in storage, *Hapalosiphon welwitschii* leaves powder extracts should be used as postharvest insecticides of plant origin for stored cowpea management [50].

Analogues of secondary metabolites have the possibility of interfering with various vital components of the cellular signalling system, or interfering with vital enzymes and signals in the nervous system (such as neurotrans- mitter synthesis, storage, release, binding, and re-uptake, receptor activation and function, enzymes involved in signal transduction), or blocking metabolic pathway functions [51]. Toxic effects of essential oils or their constituents in insects and other arthropods point to a neurotoxic mode of action; most prominent symptoms are hyperactivity and hyperexcitation leading to rapid knockdown and immobilization [52].

The results showed that petroleum benzene extract exhibited the highest bio-pesticides activity. This was because petroleum benzene extract contained the highest concentration of terpenoids, fatty acids, and toxic substances such as thymol [53,54]. Furthermore, ethanol extract showed a more toxic effect against *Callosobruchus maculatus*. Ethanol extract exhibits fatty acids, terpenoids, glycosides, acrylate, and cholecalciferol. Water extracts are found in steroids and organometallic compounds and show moderate activity. Cowpea beetle, *Callosobruchus maculatus* responded to benzene and chloroform extracts with the least toxicity. A toxin causes disturbances of the nervous system, which can lead to paralysis and death, giving rise to the most successful commercial pesticides of all time [32,55]. The results of our study concluded that *Plectranthus amboinicus* phytocompounds are highly effective at preventing the growth of *Callosobruchus maculatus* on grains stored in storage [56-60].

4. CONCLUSION

As a result of this study, it has been shown that botanical compounds are effective insecticides that can be used commercially. The powdered leaves of *P. amboinicus* are highly toxic as well as having an inhibitory effect on *Callosobruchus maculatus*. Among various solvent plant extracts, petroleum benzene and ethanol extracts were the most effective at high levels of toxic compounds. Several traditional plant products have proven effective in controlling stored grain pests called *Callosobruchus maculatus*. Using these products reduces the severity of insect pest damage. The *P. amboinicus* extract has shown to be efficient and effective at storing cowpea seeds, and it is affordable and easy to adapt, making it more accessible to farmers. In addition, further investigation is needed to determine the active component of the product, as well as their cost-benefit ratio and ability to control infestations in grain stores.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Oerke EC, Dehne HW. Safeguarding production – losses in major crops and the role of crop protection. Crop Prot. 2004; 23(4):275-85.
DOI: 10.1016/j.cropro.2003.10.001
2. Chandler D, Bailey AS, Tatchell GM, Davidson G, Greaves J, Grant WP. The development, regulation and use of biopesticides for integrated pest management. Philos Trans R Soc Lond B Biol Sci. 2011;366(1573):1987-98.
DOI: 10.1098/rstb.2010.0390, PMID 21624919.
3. Van D, Paveley N, Shaw M, Hobbelen P, Oliver R BF. The dose rate debate: does the risk of fungicide resistance increase or decrease with dose? Plant Pathol. 2011; 60(4):597-606.
DOI: 10.1111/j.1365-3059.2011.02439.x
4. Khater HF. Prospects of botanical biopesticides in insect pest management. Pharmacologia. 2012;3(12):641-56.
DOI:10.5567/pharmacologia.2012.641.656
5. McDougall P. R&D Expenditure in 2007 and expectations for 2012. Final report for Crop Life America and the European Crop Protection Association. Phillips McDougall AgriService. 2010. The cost of new agrochemical product discovery, development and registration in;1995:2000 and 2005-8.
6. Regnault-Roger C, Philogéne BJR. Past and current prospects for the use of botanicals and plant allelochemicals in

integrated pest management. Pharm Biol. 2008;46(1-2):41-52.
DOI: 10.1080/13880200701729794

7. Seiber JN, Coats J, Duke SO, Gross AD. Biopesticides: state of the art and future opportunities. J Agric Food Chem. 2014; 62(48):11613-9.
DOI: 10.1021/jf504252n, PMID 25406111.
8. Miresmailli S, Isman MB. Botanical insecticides inspired by plant-herbivore chemical interactions. Trends Plant Sci. 2014;19(1):29-35.
DOI: 10.1016/j.tplants.2013.10.002, PMID 24216132.
9. Coloma AG, Reina M, Diaz CE, Fraga BM. Natural product-based biopesticides for insect. In comprehensive natural products II. Chem Biol. 2010;3:237-68.
10. Arumugam G, Swamy MK, Sinniah UR. *Plectranthus amboinicus* (Lour.) Spreng: botanical, phytochemical, pharmacological and nutritional significance. Molecules. 2016;21(4):369.
DOI: 10.3390/molecules21040369, PMID 27043511.
11. Wadikar DD, Patki PE. Coleus aromaticus: A therapeutic herb with multiple potentials. J Food Sci Technol. 2016;53(7):2895-901.
DOI: 10.1007/s13197-016-2292-y, PMID 27765960.
12. Jimmy JL. Coleus aromaticus Benth: An update on its bioactive constituents and medicinal properties. All Life. 2021; 14(1):756-73.
DOI: 10.1080/26895293.2021.1968959
13. Singh M, Lal K, Singh SB, Singh M. Effect of calotropis (*Calotropis procera*) extract on infestation of termite (*Odontotermes obesus*) in sugarcane hybrid. Indian J Agric Sci. 2002;72:439-41.
14. Singh G, Singh OP, Prasad YR, de Lampasona MP, Catalan C. Studies on essential oils. Part 33. chemical and insecticidal investigations on leaf oil of Coleus amboinicus (Lour). Flavour Fragr J. 2002;17(6):440-2.
DOI: 10.1002/ffj.1123
15. Satongrod B, Wanna R. Chemical composition and bioactivity of essential oil from Indian borage *(Plectranthus amboincu*s (Lour.) Spreng) against *Callosobruchus maculatus* (f.). Int J Agric Technol. 2020;16:1243-56.
16. Lima MA, Oliveira FFM, Gomes GA, Lavor PL, Santiago GM, Nagao-Dias AT, et al. Evaluation of larvicidal activity of the essential oils of plants species from Brazil against *Aedes aegypti* (Diptera: Culicidae). Afr J Biotechnol. 2011;10:11716-20.
17. Verma RS, Padalia RC, Chauhan A. Essential oil composition of Coleus aromaticus Benth. from Uttarakhand. J Essent Oil Bear Plants. 2012;15(2): 174-9.
DOI: 10.1080/0972060X.2012.10644033
18. Jaya JP, Singh P, Prakash B, Dubey NK. Insecticidal activity of *Ageratum conyzoides* L., Coleus aromaticus Benth. and *Hyptis suaveolens* (L.) Poit essential oils as fumigant against storage grain insect Tribolium castaneum Herbst. J Food Sci Technol. 2014;51(9):2210-5.
DOI: 10.1007/s13197-012-0698-8, PMID 25190885.
19. Radha R, Sermakkani M, Thangapandian V. Evaluation of phytochemical and antimicrobial activity of *Andrographis paniculata* Nees (Acanthaceae) aerial parts. Int J Pharm Life Sci. 2011;2(2):562-7.
20. Kumoro AC, Hasan M, Singh H. Effects of solvent properties on the Soxhlet extraction of diterpenoid lactones from Andrographis paniculate leaves. Sci Asia. 2009;35:306-9.
21. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. J Phytol. 2011;3(12):10-4.
22. Stein S. Mass spectral reference libraries: an ever-expanding resource for chemical identification. Anal Chem. 2012;84(17): 7274-82.
DOI: 10.1021/ac301205z, PMID 22803687.
23. Hübschmann HJ. Handbook of GC-MS: fundamentals and applications. John Wiley & Sons; 2015.
24. Tapondjou AL, Adler C, Fontem DA, Bouda H, Reichmuth C. Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du Val. J Stored Prod Res. 2005;41(1):91-102.
DOI: 10.1016/j.jspr.2004.01.004
25. Nerio LS, Olivero-Verbel J, Stashenko EE. Repellent activity of essential oils from seven aromatic plants grown in Colombia against *Sitophilus zeamais* Motschulsky (Coleoptera). J Stored Prod Res. 2009; 45(3):212-4.
DOI: 10.1016/j.jspr.2009.01.002

26. Abbott WS. A method of computing the effectiveness of an insecticide. J Econ Entomol. 1925;18(2):265-7.
DOI: 10.1093/jee/18.2.265a
27. Finny DJ. Probit analysis. London: Cambridge University Press. 1971;333.
28. Fazil M, Srinivasa Rao A, Shaik Rasheed A, A, Ibrahim M. Phytochemical studies and antioxidant activity of Melia azedarach linn leaves by dpph scavenging assay. Int J Pharm Appl. 2012;3(1):271-6.
29. Leela DJeya, Irudaya Monisha G, Anitha S. Immaculate, A. Rosaline Vimala. Studies on Phytochemical, Nutritional Analysis and Screening of in Vitro Biological activities of Meliadubia Leaf Extract. J. Int J Sci Eng Res. 2016;7(8): 56-68.
30. Nikolaou P, Marciniak P, Adamski Z, Ntalli N. Controlling stored products' pests with plant secondary metabolites: a review. Agriculture. 2021;11(9):879.
DOI: 10.3390/agriculture11090879
31. Wink M, Schimmer O. Modes of action of defensive secondary metabolites. In: Wink M, editor. Functions of plant secondary metabolites and their exploitation in biotechnology. Annual plant Reviews No. 3. Sheffield: Sheffield Academic Press. 1999;17-133.
32. Raffa KF, Priester TM. Synergists as research tools and control agents in agriculture. J Agric Entomol. 1985;2:27-45.
33. Berbehenn RV, Martin MM, Hagerman AE. Reassessment of the roles of the peritrophic envelope and hydrolysis in protecting polyphagous grasshoppers from ingested hydrolyzable tannins. J Chem Ecol. 1996;22(10):1901—4919.
34. Barbehenn RV, Martin MM. Tannin sensitivity in larvae oL Malacosoma Dis stria (Lepidoptera): roles of the pertitrophic envelope and midgut oxidation. J Chem Ecol. 1994;20(8):1985-2001.
DOI: 10.1007/BF02066238, PMID 24242724.
35. Henn MW. Adsorption von Tanninen aus Eichenblattern an Kohlen e hydrathaltiger Verbindungen. Mitt Dtsch Entomol Ges. 1997;11:495-9.
36. Mohamad S. Fathiyah, and S. Aziz, S. Procedia Eng. The susceptibility of aphids, Aphis gossypii glover to lauric acid based natural pesticide. 2013;53:20-8.
37. Marshall Edward F. Cholecalciferol: A unique toxicant for rodent control. In: Proceedings of the eleventh vertebrate pest conference. 1984;22-35.
38. Ayub K. Biopesticides: alternatives for management of *Callosobruchus maculates*. J Biopesticides. 2021;14(1):59-78.37.
39. Al-Rubaye AF, Hameed IH, Kadhim MJ. A Review: Uses of gas chromatography-mass spectrometry (GC-MS) technique for analysis of bioactive natural compounds of some plants. Int J Toxicol Pharmacol Res. 2017;9(1):81-5.
DOI: 10.25258/ijtpr.v9i01.9042
40. Jantan I, Zaki ZM. Development of environment-friendly insect repellents from the leaf oils of selected Malaysian plants. Asean Rev Biodivers Environ Conservafion (ARBEC). 1998;6:1-7.
41. Jaenson TG, Pålsson K, Borg-Karlson AK. Evaluation of extracts and oils of mosquito (Diptera: Culicidae) repellent plants from Sweden and Guinea-Bissau. J Med Entomol. 2006;43(1):113-9.
DOI: 10.1093/jmedent/43.1.113, PMID 16506457.
42. Park BS, Choi WS, Kim JH, Kim KH, Lee SE. Monoterpenes from thyme (*Thymus vulgaris*) as potential mosquito repellents. J Am Mosq Control Assoc. 2005;21(1): 80-3.
DOI:10.2987/8756-971X(2005)21[80:MFTTVA]2.0.CO;2, PMID 15825766.
43. Gillij YG, Gleiser RM, Zygadlo JA. Mosquito repellent activity of essential oils of aromatic plants growing in Argentina. Bioresour Technol. 2008;99(7): 2507-15.
DOI: 10.1016/j.biortech.2007.04.066, PMID 17583499.
44. Kiran SR, Devi PS. Evaluation of mosquitocidal activity of essential oil and sesquiterpenes from leaves of Chloroxylon Swietenia DC. Parasitol Res. 2007; 101(2):413-8.
DOI: 10.1007/s00436-007-0485-z, PMID 17520288.
45. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: A review. J Am Mosq Control Assoc. 1991;7(2):210-37. PMID 1680152.
46. Odalo JO, Omolo MO, Malebo H, Angira J, Njeru PM, Ndiege IO, et al. Repellency of essential oils of some plants from the Kenyan coast against Anopheles gambiae. Acta Trop. 2005;95(3):210-8.

DOI: 10.1016/j.actatropica.2005.06.007, PMID 16018957.

47. Tunón H, Thorsell W, Mikiver A, Malander I. Arthropod repellency,especially tick (*Ixodes ricinus*), exerted by extract from Artemisia abrotanum and essential oil from flowers of Dianthus caryophyllum. Fitoterapia. 2006;77(4):257-61. DOI: 10.1016/j.fitote.2006.02.009, PMID 16624501.
48. Rattan RS. Mechanism of action of insecticidal secondary metabolites of plant origin, rop Protection. 2010;29:943-20.
49. Moura E, Faroni A, Zanuncio LD, Heleno JC, Figueiredo Prates FF, LH. Insecticidal activity of *Vanillosmopsis arborea* essential oil and of its major constituent α- bisabolol against *Callosobruchus maculatus* (Coleoptera: Chrysomelidae). Sci Rep. 2019;9(3723):1-8.
50. Fotso TG, Tofel HK, Abdou JP, Tchao N, Zourmba CMA, Nukenine C, et al. Control of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) using fractionated extracts from Cameroonian *Hemizygia welwitschii* (Lamiaceae) Leaf on stored *Vigna unguiculata* (Fabales: Fabaceae). J Insect Sci. 2019;19(2): 22:1-9. DOI: 10.1093/jisesa/iez029, PMID 31222325.
51. Wink M. Interference of alkaloids with neuroreceptors and ion channels. Stud Nat Prod Chem. 2000;21:3-122. DOI: 10.1016/S1572-5995(00)80004-6
52. Enan EE. Insecticidal activity of essential oils: Octopaminergic sites of action. Comp Biochem Physiol C Toxicol Pharmacol. 2001;130(3):325-37. DOI: 10.1016/s1532-0456(01)00255-1, PMID 11701389.
53. Youssefi MR, Tabari MA, Esfandiari A, Kazemi S, Moghadamnia AA, Sut S, et al. Efficacy of two monoterpenoids, carvacrol and thymol, and their combinations against eggs and larvae of the west Nile vector Culex pipiens. Molecules. 2019;24(10):1867. DOI: 10.3390/molecules24101867, PMID 31096594.
54. Tabari MA, Youssefi MR, Maggi F, Benelli G. Toxic and repellent activity of selected monoterpenoids (thymol, carvacrol and linalool) against the castor bean tick, Ixodes ricinus (Acari: Ixodidae). Vet Parasitol. 2017;245(15):86-91. DOI: 10.1016/j.vetpar.2017.08.012, PMID 28969843.
55. Gershenzon J, Croteau R. Terpenoids. In: Rosenthal GA, Berenbaum MR, editors. Herbivores: Their interactions with secondary plant metabolites. 2nd ed. Orlando, FL: Academic Press. 1991;165-219.
56. Gašić S, Tanović B. Biopesticide formulations, possibility of application and future trends. Pestic Fitomedicina. 2013; 28(2):97-102. DOI: 10.2298/PIF1302097G
57. Imai T, Tsuchiya S, Morita K; 1995. Fatty acid insecticide and insecticidal method using the same. European patent application EP0663147A1. Available:https://www.google.com/patents/EP0663147A1
58. Kosini D, Nukenine EN. Bioactivity of Novel Botanical Insecticide from *Gnidia kaussiana* (Thymeleaceae) against *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) in Stored Vigna subterranea (Fabaceae) Grains. J Insect Sci. 2017;17(1):1-7. DOI: 10.1093/jisesa/iex004, PMID 28423430.
59. Kiewnick S. Practicalities of developing and registering microbial biological control agents. CAB Rev. 2007;2(013):1-11.
60. Yang P, Ma Y. Repellent effect of plant essential oils against *Aedes albopictus*. J Vector Ecol. 2005;30(2):231-4. PMID 16599157.