An eco-friendly biopesticide from Cassia auriculata leaf extract as an alternative pesticide against the stored grain pest Callosobruchus maculatus

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Abstract- A biopesticide is a critical component to integrated pest management, as they are a more eco-friendly and safer alternative to chemical pesticides. We investigated the biopesticide properties of leaf powders of Cassia auriculata using different solvents as well as identified the phytochemicals using qualitative analysis and gas chromatography-mass spectrometry (GC-MS). From the extracts of Cassia auriculata based petroleum benzene and ethanol the concentration of terpenoids, fatty acids, phenolic compounds, and steroids were noted in the highest quantity. Cassia auriculata leaves were extracted using various solvents to determine the ability of their toxic compounds to control the cowpea beetle, Callosobruchus maculatus. Toxicity was recorded after the exposure times of 24, 48 and 72 hours. The highest toxicity (60.32& LC50 19.05 mg/ml) of callosobruchus maculatus was caused by petroleum benzene, followed by ethanol (52.48% & LC50 23.98 mg/ml), water (39.04% & LC50 45.70 mg/ml), chloroform (25.79% & LC50 89.13 mg/ml) and benzene (23.84% & LC50 91.2 mg/ml) after 72 hours. As a result of our study, we concluded that cassia auriculata phytocompounds protect stored cowpea against callosobruchus maculatus. It is possible to recommend cassia auriculata phytocompounds as a viable alternative to synthetic pesticides, since they are economical, available at farmer level, and eco-friendly

Key words: Bio pesticide, Callosobruchus maculatus, Cassia Auriculata, phytocompound and toxicity

INTRODUCTION

Since insect infestations can result in a 20-30% loss in food production and stored products, or even a total loss in severe cases, they directly affect food production [1]. The use of pesticides on agricultural crops and stored grain accumulates toxic residue on food grains that can cause health problems. Pesticide poisoning is also responsible for a high death rate around the world [2]. Pesticide exposure poses a serious health risk to the large number of people exposed to pesticides, including production workers, sprayers, mixers, and loaders on farms [3]. In many countries around the world, insect pesticides have long been used to control insect pests. However, these pesticides are not biodegradable and cause harm to non-target insects, amphibians, fish, birds, and even humans [4]. As a result of continuous chemical pesticide use, resistant insects have developed along with new insects emerging. These chemicals also result in resurgences and new species of insects. Pesticide pollution poses a threat to ecosystems because harmful effects are caused on beneficial insects, microbes, plants, fishes, birds, and animals [5]. Using natural compounds that have bioactivity against insects is seen as an economical, biodegradable, and easy-to-use alternative to industrial pesticides when controlling pests on food crops and stored products [6].

The larva of Calosobruchus maculates Fab. damage the grains of leguminous cowpea that are economically important for the crop. The grains are unfit for consumption, replanting, or sprouting. The genetic resistance of insect species, residual toxicities, vertebrate toxicity, widespread environmental hazards, and the high cost of synthetic pesticides make it necessary for biodegradable pesticides to be developed [7, 8]. A growing awareness of this issue has spurred interest in finding alternative strategies to control pests and diseases, such as reexamining the use of plant derivatives [9]. Degradation of plant-derived materials will be faster, the material will be less toxic to mammals, will be more selective and will retard the emergence of resistance. However, they can be derived from both crude and partially purified extracts, which can be readily made and produced by farmers and small-scale producers. They have the main advantage of being easily and inexpensively produced by farmers and small-scale manufacturers as crude, or as partially purified extracts. A considerable amount of energy and time has been spent over the past three decades in the search for new botanical insecticides to replace existing insecticides [10]. In order to reduce oviposition and seed damage, the stored grains were mixed with plant extracts powder [11].

Plant extracts have shown great promise for pest management due to their secondary plant chemistry [12]. Numerous plant compounds can be used to control multiple insect pests, including limonoids from the meliaceae, which are environmentally friendly and can effectively kill a variety of insects [13]. Several researchers have studied the insecticidal effects of Cassia species extracts, mainly on mosquito larvae and pests attacking vegetables and storage products [14]. In view of above maintaining facts, we were seeking an eco-friendly biopesticide from Cassia auriculata leaf extract as an alternative pesticide against the stored grain pest Callosobruchus maculatus.

MATERIALS AND METHODS

Collection and authentication of plant material

Cassia auriculata leaves were collected during the months of October and November (2019) from Kasilingapuram village, Thoothukudi district, Tamilnadu, India. Plant specimens have been identified and authenticated by Dr. C. Babu, Head and Associate Professor of Botany, Pioneer Kumaraswamy College, Nagercoil. To remove dust from leaves, the leaves were thoroughly rinsed under running water, then shade-dried at room temperature for 7-8 days. Plant leaves were then ground into a fine powder and stored in an air-tight container for future use.

Extract Preparation

Extracts were prepared by dissolving 50 grams of dry leaves in 250 ml of petroleum benzene (40-60 oC), benzene, chloroform, ethanol, and water in a Soxhlet extractor. As the solvent is poured into the Soxhlet loop, extraction occurs until it is colourless [15]. In order to allow the solvent to evaporate, the extracts were made into a concentrated state at room temperature and stored in an airtight container. For further use, the solution was frozen at 4 °C [16].

Phytochemical analysis

The leaves of Cassia auriculata were used to test for phytochemicals using a previously described method [17]. In order to establish the chemical composition profile of the extracts, numerous qualitative chemical tests were conducted on the individual extracts. Following standard procedures, the crude powder is extracted with different solvents and tested to determine what phytoconstituents are present in each one. Generally, tests are performed to determine if terpenoids, steroids, fatty acids, phenolic compounds, alkaloids, saponins, and flavonoids are present.

Gas Chromatography Mass Spectrum (GC-MS) Analysis

We used GCMS analysis of Cassia auriculata plant extracts from Heber Analytical Instrumentation Facility (HAIF), Bishop Heber College, Trichy-620 017 to investigate its phytochemistry. The analyses were performed with GC-MS equipment (GC MS QP2020; SHIMADZU), which consists of an autosampler, a sample injector, a gas chromatograph (GC-2010) and a mass spectrometer. GC-MS system was composed of SHRxi-5Sil-MS capillary standard non-polar column (Dimension: 30.0 m, Diameter: 0.25mm, Film thickness: 0.25µm which is 100% Dimethyl poly siloxane The electron ionization energy system used had an ionization energy of 70eV. It was carried out with helium gas (99.99%) at a rate of 1.20ml/min and an injection volume of 5µl (split ratio: 10). The oven temperature was programmed from 50°C (isothermal for 2 min.), increasing to 280°C for 10 min. Mass spectra were taken at 70eV at a scan interval of 0.3 seconds with scan range of 50 - 500 m/z. A total of 21 minutes were spent running the GC. We calculated the percentage of each component based on its average peak area divided by the total peak area. To analyze mass spectra and chromatograms, we used Shimadzu's GC-MS real-time software package.

Identification of Components

The interpretation of GC-MS mass spectra was done using data from the National Institute Standard and Technique (NIST14) [18] and WILEY8 [19] having more patterns. We compared the spectrum of the unknown component to the spectrum of the known components from the NIST14 and WILEY8 libraries. Molecular formula, Name, Molecular weight, and Structure were identified for each component of the test material.

Insect Collection and Rearing

Callosobruchus maculates Fab. was collected from a farmer in Kasilingapuram village. All experiments were conducted at the PG and Research Department of Zoology PMT College, Melaneelithanallur. In the beginning, 50 pairs of adults aged 1–2 days were placed in jars containing cowpea seeds, sealed, and allowed a maximum of 7 days for mating and oviposition, the pest reared on cowpea grains in the laboratory at 28 ± 1oC and 65 ± 5% relative humidity (RH). The parents were removed and cowpea seeds containing eggs were transferred into new cowpea seeds in breeding jars, which were covered with cloth fastened with rubber bands to prevent contamination and insect escape. All experiments were carried out using progenies of the pest.

Toxicity Assay

In toxicity assay, the Cassia auriculata extracts were used against adults of Callosobruchus maculates at 28 ± 1oC and 65 ± 5% RH. Newly developed adults (1–15 days old) were used in these studies. Cassia auriculata leaf extracts were tested against Callosobruchus maculates adults by using glass jars (1L) as chambers (replicates) and filter paper pieces (3 x 3 cm) connected to the underside of the screw caps. Each jar contained 30 insects and the plant extracts were applied by 10, 20, 30, 40, and 50 mg/ml to filter paper pieces. The treatment and control were repeated five times. Filter paper pieces were treated with solvent alone as a control. An insect mortality percentage was observed and recorded after 24, 48, and 72 hours for each concentration, and the lethal concentration causing 50% mortality (LC50) were calculated from log-concentration mortality regression lines. The insects were considered dead if they did not move their legs or antennae [20, 21].

Statistical Analysis

One-way analysis of variance (ANOVA) and the least significant difference (LSD) multiple range test were performed on the data to determine significant ($P < 0.05$) differences among variable concentrations. It was hope that Finney's probit analysis [22] would help estimate lethal concentration (LC) and LC50 values were considered significantly different when their respective 95% fiducial limits did not overlap.

RESULTS AND DISCUSSION

The present study was carried out on the plant Cassia auriculata 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 Cassia auriculata.

Qualitative phytochemical analysis of *Cassia auriculata leaves e*xtracts

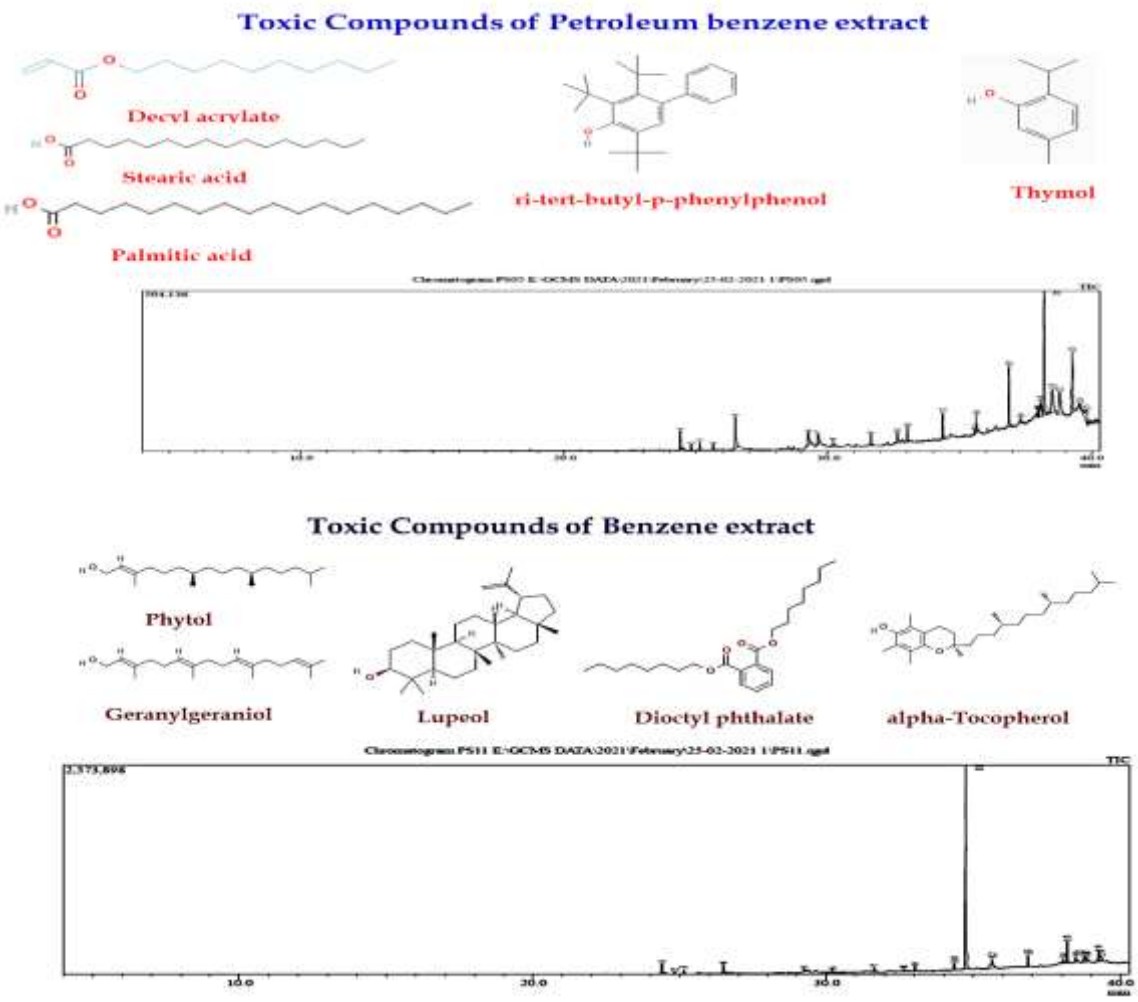
Plants have a toxicity value due to some chemical substances that possess a strong physiological effect on insects. The most important of these compounds are alkaloids, terpenoids, steroids, fatty acids, and phenols. The qualitative phytochemical analysis of various solvent extracts of Cassia auriculata 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 benzene extract and low intensity in chloroform and ethanol extracts [23].

Table 1: Preliminary phytochemical screening of extract of powdered leaves of *Cassia auriculata*

S. No	Phytochemicals	Solvents				
		Petroleum benzene	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



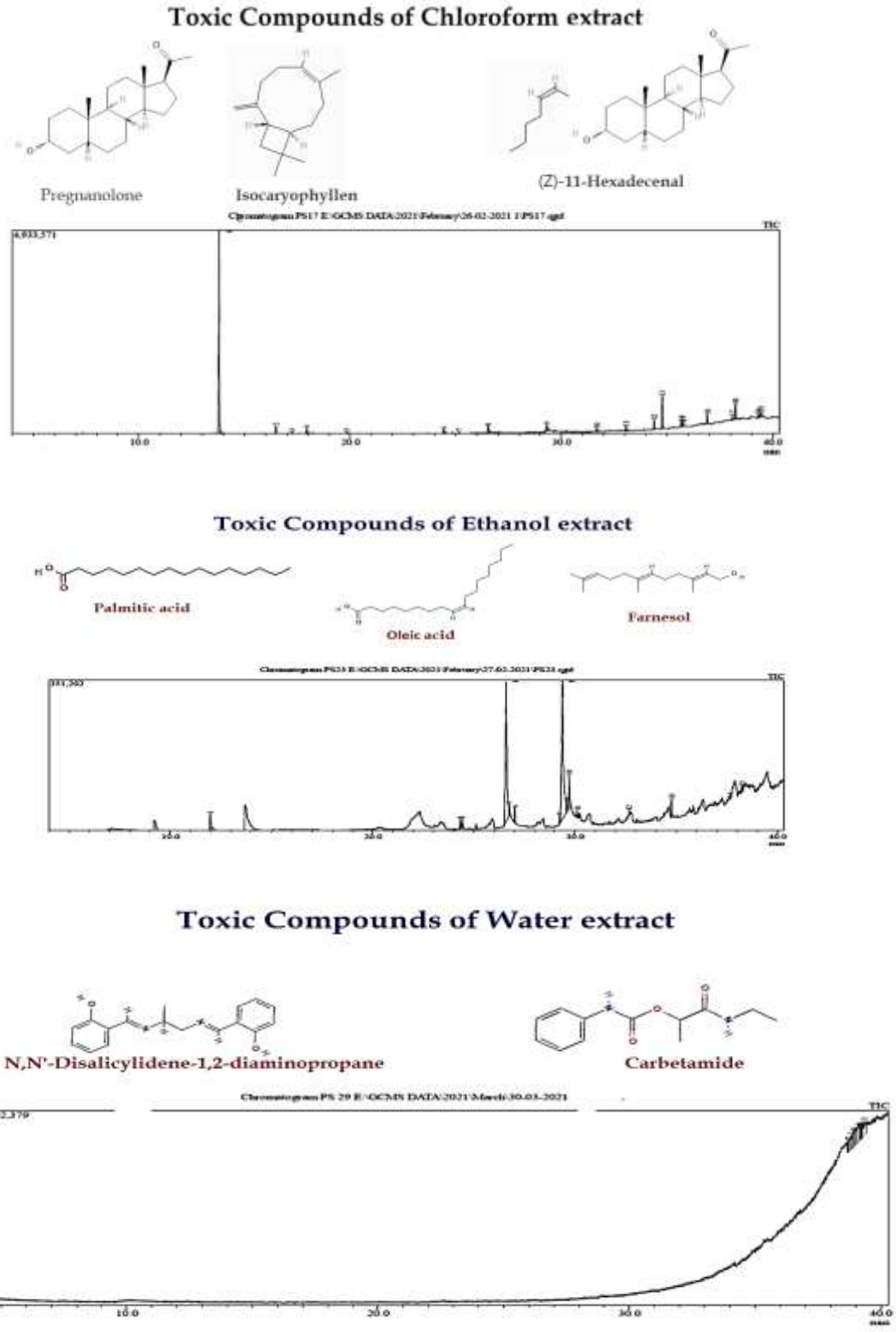


Fig. 1 GC-MS Chromatogram and toxic compounds of Cassia *auriculata* leaves different solvent extract

Table 2. Phytochemical analysis of *Cassia auriculata* leaves different solvent extract

Solvent	Retention Time (min)	Peak Area	Molecular Weight	Molecular formula	Name of the Compound	Name of the Phytochemical	Toxicity
Petroleum benzene	24.815	2.73	212	$C_{13}H_{24}O_2$	Decyl acrylate	acrylate	Skin and eye irritation
	25.645	1.31	262	$C_{18}H_{13}O$	Tri-tert-butyl-p-phenylphenol	Phenolic	Acute oral toxicity
	26.485	6.84	256	$C_{16}H_{32}O_2$	Palmitic acid	long-chain saturated fatty acid	Skin and eye irritation
	29.615	2.61	284	$C_{18}H_{36}O_2$	Stearic acid	saturated long-chain fatty acid	Skin and eye irritation
	32.635	17.37	290	$C_{20}H_{34}O$	Thymol	monoterpene	Acute oral toxicity
Benzene	25.125	1.24	296	$C_{20}H_{40}O$	Phytol	diterpene	Skin and eye irritation
	31.635	0.59	290	$C_{20}H_{34}O$	Geranylgeraniol	diterpenoid	Skin and eye irritation
	32.630	0.71	426	$C_{30}H_{50}O$	Lupeol	Steroids	Acute oral toxicity
	35.630	6.13	430	$C_{29}H_{50}O_2$	alpha-Tocopherol	Hydroxy compounds	Skin and eye irritation
Chloroform	13.800	1.17	150	$C_{10}H_{14}O$	Pregnanolon	Steroids	Acute oral toxicity
	16.480	1.99	204	$C_{15}H_{24}$	Isocaryophyllene	Sesquiterpenes	Acute oral toxicity
	29.310	2.58	238	$C_{16}H_{30}O$	(Z)-11-Hexadecenal	Fatty aldehyde	Acute oral toxicity
Ethanol	26.595	1.4	256	$C_{16}H_{32}O_2$	Palmitic acid	long-chain saturated fatty acid	Skin and eye irritation
	29.370	43.32	282	$C_{18}H_{34}O_2$	Oleic acid	Fatty acid	Skin and eye irritation
	32.665	0.42	222	$C_{15}H_{26}O$	Farnesol	Fatty alcohol	Pesticide
Water	38.710	4.391	282	$C_{17}H_{18}N_2O_2$	N,N'-Disalicylidene-1,2-diaminopropane	Phenolic	Acute oral toxicity
	39.260	10.5	236	$C_{12}H_{16}N_2O_3$	Carbetamide	Phenylcarbamates	Acute oral toxicity

Table 3. Toxicity and probit analysis of different extract to cowpea beetle, *Callosobruchus maculatus*

Plant Material	Concentration (mg/ml)									LC_{50} (mg/ml)
	Hours	10	20	30	40	50	Mean	P value	Significant	
Petroleum Benzene	24	14.4±0.98	27.2±0.8	42.4±0.1.26	55.2±0.8	63.2±1.49	40.64	<0.005	***	35.48
	48	23.2±0.8	38.4±0.98	49.6±01.26	60.8±1.49	72.8±0.8	48.96	<0.005	***	27.54
	72	31.2±1.49	48±1.26	61.6±0.98	73.3±0.98	87.2±0.8	60.32	<0.005	***	19.05
Benzene	24	3.2±0.8	8.8±1.26	10.4±0.98	16.8±0.8	23.2±1.49	12.48	<0.005	***	165.95
	48	7.2±1.26	12.8±0.8	20.8±1.49	25.6±0.98	35.2±0.8	20.32	<0.005	***	97.72
	72	15.2±0.8	21.6±0.98	25.6±1.26	30.4±0.98	36.0±0.98	23.84	<0.005	***	91.20
Chloroform	24	4.8±0.8	10.4±0.98	12.8±1.49	19.2±1.49	24.8±0.8	14.4	<0.005	***	181.97
	48	6.4±1.26	11.2±0.8	19.2±0.8	24±1.26	33.6±0.98	18.88	<0.005	***	107.15
	72	12.0±0.8	18.4±0.98	27.2±1.49	32.8±0.8	38.4±0.98	25.76	<0.005	***	89.13
Ethanol	24	12.8±0.8	21.6±0.98	36.8±1.26	47.2±0.8	57.6±0.98	32.2	<0.005	***	43.65
	48	19.2±0.8	33.6±0.98	46.4±0.98	60.8±1.26	66.4±0.98	45.32	<0.005	***	30.90
	72	25.6±0.98	40.8±0.8	54.4±1.6	65.6±0.98	76.8±1.26	52.64	<0.005	***	23.98
Water	24	6.4±0.98	14.4±0.98	23.2±1.26	30.4±1.49	35.2±0.8	21.92	<0.005	***	85.11
	48	10.4±1.49	16.8±1.26	23.4±0.8	33.6±0.98	41.6±1.26	25.12	<0.005	***	77.62
	72	21.6±1.26	32±0.98	39.2±1.49	48.8±1.26	54.4±0.98	39.04	<0.005	***	45.70

*** highly significant

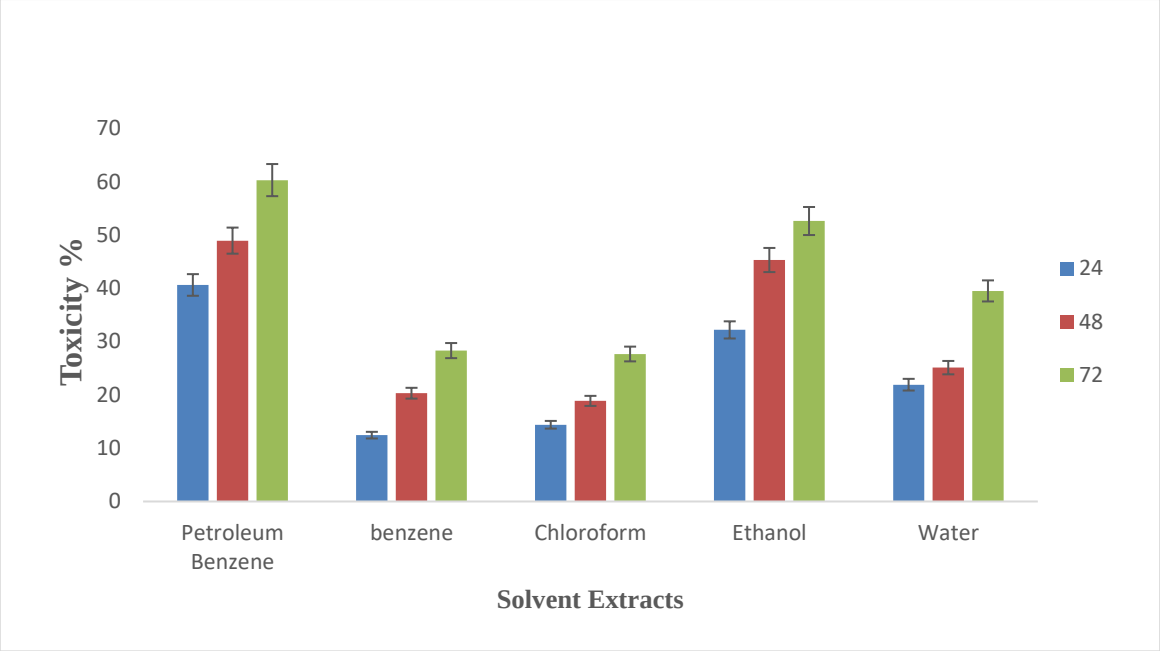


Figure 2. Toxicity of *Callosobruchus maculatus* when treated with *Cassia auriculata* plant extracts

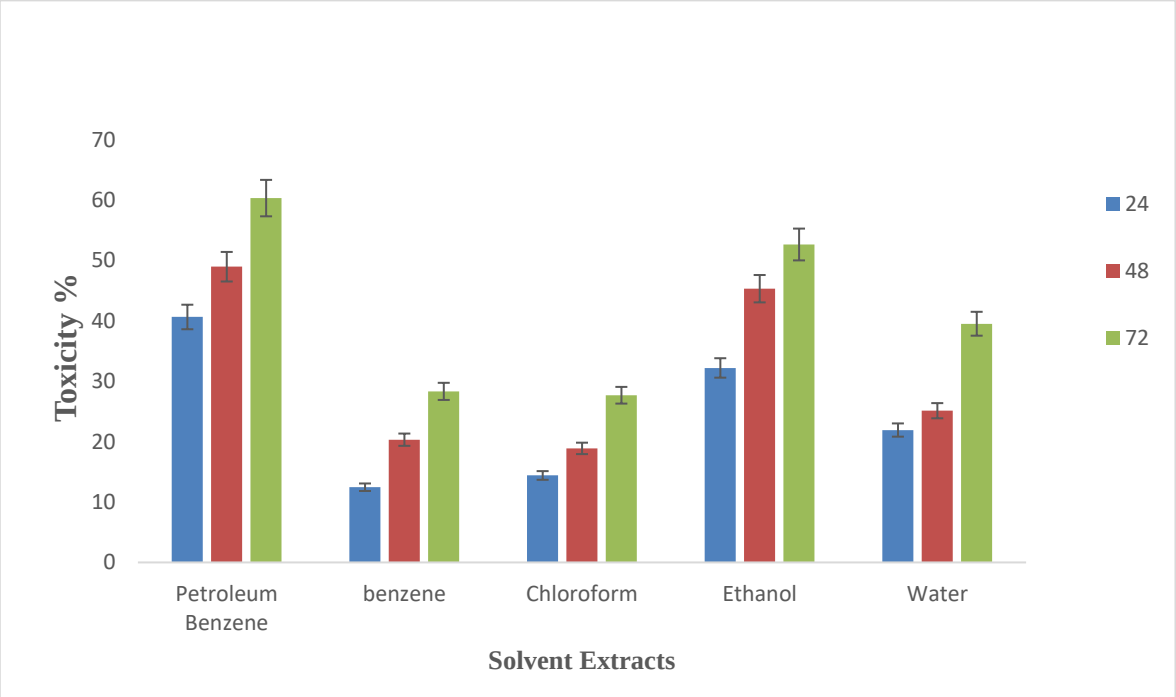


Figure 3. LC_{50} Value of *Cassia auriculata* leaf extracts against cowpea pest *Callosobruchus maculatus*

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 [24, 25].

GC-MS analysis of Cassia auriculata 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 Cassia auriculata, as shown in Table.2 and Fig. 1. Among twenty-five compounds identified in the petroleum benzene extract, five showed to be toxic in nature. The GC-MS analysis of petroleum benzene extract of Cassia auriculata revealed the presence of toxic compounds like Decyl acrylate (2.73), Tri-tert-butyl-p-phenylphenol (1.31), Palmitic acid (6.84), Stearic acid (2.61) and Thymol (17.37). Benzene extracts, twenty compounds were identified and four of those compounds appeared toxic. The toxic compounds in benzene extracts, such as Phytol (1.24), Geranylgeraniol (0.59), Lupeol (0.71), and alpha-Tocopherol (6.13). Among the 20 compounds identified in the chloroform extracts, three were toxic. A toxic compound such as Pregnanolone (3.17), Isocaryophyllene (1.99), and (Z)-11-Hexadecenal (2.88). The ethanol extracts identified fifteen compounds out of which three were toxic. Toxic compound such as Palmitic acid (1.4), Oleic acid (43.32), and Farnesol (0.42). The water extracts showed that 10 compounds were identified and two of these compounds were toxic, such as N,N'-Disalicylidene-1,2-diaminopropane (4.3) and Carbetamide (10.5).

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 [26]. A toxin that causes paralysis and mortality led to the development of the most successful commercial pesticides [27, 28]. 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 [29], 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 [30, 31]. 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 [32]. 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 [33]. 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 [34]. An association between the monoterpene, sesquiterpene, and oxygenated monoterpene content of H. opposita and its insecticidal activity against Callosobruchus maculatus [35, 36]. 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 [37].

Toxicity assay

Petroleum benzene, benzene, chloroform, ethanol, and water extracts of Cassia auriculata showed excellent fumigant activity against Callosobruchus maculatus, and the time needed to cause 50% (LC50) mortality dropped with increased concentration. (Tables 3 and Fig. 3 & 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 40.64, 48.96 and 60.32 in 24, 48 and 72 hours respectively (Fig. 2). The benzene extract mean observed mortality percentage is 12.48 (24hr), 20.32 (48hr) and 23.84 (72hr). Chloroform extract showed the mean value of observed mortality is 14.4, 18.88 and 25.76 in 24, 48 and 72 hours respectively. The mean mortality activity of ethanol extracts observed 32.2, 45.32 and 52.64 in 24, 48 and 72 hours respectively. The water extract mean observed mortality percentage is 21.92 (24hr), 25.12 (48hr) and 39.52 (72hr). The highest toxicity (87.2±0.8% and LC50 value 19.05 mg/ml) of Callosobruchus maculatus was caused by Petroleum benzene, followed by ethanol (76.8±0.8% & LC50 value 23.98 mg/ml), water (54.4±0.98% and 45.70 mg/ml) Benzene (36.6±0.98% and 61.20 mg/ml) and chloroform (38.4±0.98% and 89.13 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 [38]. 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 [39]. 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 [40].

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 [41]. Several monoterpenoids, which have been identified as important components of essential oils, have been evaluated for neurotoxicity against the house fly and the German cockroach [42]. 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 [43]. A variety of essential oils, monoterpenes, and naturally derived products have been shown to act as AChE inhibitors against a variety of insect species [44]. The interference with GABA-gated chloride channels of insects is another potential target for essential oils [45]. As a naturally occurring biogenic amine, octopamine serves a critical role in invertebrates as a neurotransmitter, neuromodulator, and neuro-hormone, with the same physiological role as nornephrine within vertebrates [46]. Essential oil compounds have acute and sub-lethal behavioural effects on insects and other vertebrates, suggesting that they target an octopaminergic target site, by blocking octopamine receptors in insects [47]. The data from various studies clearly indicates that some compounds, such as thymol or eugenol, may be able to block octopamine receptors and/or function using tyramine receptor cascades [48,49].

The results showed that petroleum benzene extract exhibited the highest fumigant toxicity. This was because petroleum benzene extract contained the highest concentration of terpenoids, fatty acids, and toxic substances such as thymol [50,51]. 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 [52, 53]. The results of our study concluded that Cassia auriculata phytocompounds are highly effective at preventing the growth of Callosobruchus maculatus on grains stored in storage.

CONCLUSION

The present investigation indicates that there is a potential power for botanical compounds to be used commercially as insects control agents. Cassia auriculata leaves powdered extract showed a higher level of toxic and inhibitory activity against Callosobruchus maculatus. Among the various solvent plant extracts, the petroleum benzene and ethanol were the most effective with high toxic compounds. In order to reduce the severe damage caused by insect pests, traditional plant products have proved to be highly effective in controlling the stored grain pest Callosobruchus maculatus. The application of Cassia auriculata extracts to cowpea seeds for storage is an inexpensive and effective method and its ease of adaptability will give it additional advantages leading to its acceptance by farmers. In spite of the promising results, further study is needed, especially to isolate and identify the active principal component of the product, and to assess both its cost-benefit ratio and its ability to control insect infestations in grain stores.

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