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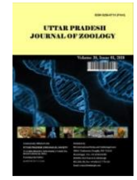

INSECTICIDAL ACTIVITY OF *Syzygium aromaticum* AND *Cinnamomum cassia* PLANT VOLATILE OIL AGAINST THE PULSE BEETLE, *Callosobruchus maculatus* [Fabricius] [Coleoptera : Chrysomelidae]

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author JS conducted and collected the data. Author KE designed and prepared the manuscript. Authors NJN and SMP gave their ideas towards the completion of this work. This work was done by author JS as a part of her Ph.D Thesis in University of Madras. All authors read and approved the final manuscript.

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Original Research Article

ABSTRACT

In the present study, insecticidal activity of two plant volatile oils such as *Syzygium aromaticum* and *Cinnamomum cassia* were tested for their efficacy against the important stored grain coleoptera pest, the pulse beetle, *Callosobruchus maculatus* in a conducive environment at laboraroty. The plant volatile oils were individually tested with 50, 100, 150 and 200 mg/ml concentrations on the adult *C. maculatus*. Initially the grain, *Vigna unguiculata* were uniformly coated with the selected concentration of the plant volatile oil, air dried, then introduced the adult beetles. After 24, 48, 72 hrs of exposure, the number of insects dead were counted and the percent mortality was calculated by using the standard formula. In the present study, the insecticidal activity of *S. aromaticum* indicates that the significant mortalities were observed at 200 mg/mL concentration of the *S. aromaticum* against the insects exposed to 24hrs [82.6%], 48 hrs [88.4%] and 72 Hrs [97.8%]. The trend was followed by 150, 100, 50 mg/mL concentrations with 64.2%, 72.8%, 76.8%; 38.2%, 46.6%, 52.6%; 27.6%, 34.8%, 36.8% at 24, 48 and 72 Hrs respectively. In the same way, the insecticidal activity of *C. cassia* indicates that the significant mortalities were observed at 200 mg/mL concentration of the *C. cassia* against the insects exposed to 24 hrs [80.6%], 48 hrs [86.6%] and 72 Hrs [96.4%]. The trend was followed by 150, 100, 50 mg/mL concentrations with 62.2%, 70.4%, 72.6%; 36.4%, 48.4%, 56.8%; 26.8%, 32.2%, 34.6% at 24, 48 and 72 Hrs respectively. It is inferred that the plant oils induced remarkable insect mortality on the test insect. Thus, it can be possibly utilized to mitigate the infestation of the pest in stored condition and also phytochemicals are eco-friendly, safer to non-target organisms.

Keywords: Plant volatile oil; *Callosobruchus maculatus*; *Syzygium aromaticum*; *Cinnamomum cassia*; insecticidal activity.

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1. INTRODUCTION

Among botanical extracts used as insecticides, plant volatile oils are promising alternatives to chemical insecticides. Plant oils are synthesized by plants, and they play a key role in plant signaling processes including also attractiveness toward pollinators and beneficial insects. Plant species producing essential oils [over 17,000 species] are called aromatic plants and are distributed worldwide. Our review aims to evaluate research studies published in the last 15 years concerning the use of EOs in stored product protection. More than 50% of the retrieved manuscripts have been published by authors from Eastern countries [Iran, China, India, and Pakistan], investigating different aspects related to insect pest management [exposure route, the effect on the target pest, and mode of action]. Coleoptera was the most studied insect order [85.41%] followed by Lepidoptera [11.49%], whereas few studies targeted new emerging pests [e.g., Psocoptera]. Almost all the trials were carried out under laboratory conditions, while no experiments were conducted under real operating conditions. Future research studies concerning the use of EOs as insecticides should focus on the development of insecticide formulations which could be successfully applied to different production realities.

The widespread use of synthetic insecticides to control the pests in agriculture has directed researchers to find worthwhile alternatives that are more environmentally friendly than chemical pesticides. In this context, the use of pesticides based on botanical extracts is attracting substantial interest in recent years. Among botanical extracts used as pesticides, plant volatile oils are a hopeful alternative because of their availability worldwide and cost-effectiveness.

Plant volatile oils are metabolites synthesized by plants secondarily for their defense [1–4]. These oils are mainly constituted by various phylogroups such as monoterpenes and sesquiterpenes Many of research so far conducted was aimed to assess the insecticidal activity of EOs against various crop pests as well as against disease vectors [1,5], but less attention has been paid to stored product pests. In recent years, several research studies focused on the insecticidal activity of plant volatile oils through contact and ingestion routes.

Abdelgaleil et al. [6] evaluated the toxic impact of 20 plant volatile oils against *Sitophilus oryzae* [7]. Several researchers reported the the toxicity of plant volatile oils. To quote few *Acorus calamus* [8], *Coriandrum sativum* [9], *Aster ageratoides* [10], *Dracocephalum moldavica* [11] and *Litsea* [12], *Atalantia guillauminii* [13], *Eucalyptus procera* [14] *Perilla frutescens* oil was found to control the infestation rate of bruchid weevils to a considerable extent [15] and also *A. calamus* and *E. procera* too [8, 14]. Earlier several researchers reported that the plant derived volatile oils have showed significant insecticidal activity on various groups of insects [9,16-20]. These plant volatile oils may act on insects as contact poison or systemic poison [21–26]. For instance, few studies claimed the ingestion toxicity of EOs [27,28]. It is interesting to note that the phytoconstituents of a oil is strongly subjected to variations according to their geographic origin [6, 7,29,30].

2. METHODOLOGY

2.1 Rearing and Maintenance of Stored Grain Pest

Callosobruchus maculatus was collected from infested grains. The beetles were reared on cowpea in our laboratory for about a year [Approximately 10 generations] before the experiments. The insect culture was done in a climate chamber at 30 ±1ºC with 12h photoperiod at R.H [50-80%] For the experiment, newly emerged [1-6 h] insects were used. In the experiments, the day of death of the adult beetles was determined as the day the antennae and legs did not move upon gentle disturbance with forceps.

2.2 Procurement of Beans

Cowpea [*Vigna unguiculata*] was procured from the local market commercially. Then they were stored in a freezer at -20ºC for a week and subsequently dried in an oven at 60ºC for about a week to guarantee the absence of viable insects. The beans were stored in airtight plastic containers at room temperature before use. Only visually uninfected beans were uses for the experiments.

2.3 Effect of Plant Volatile Oils on Weevil Mortality

The toxic effect of *Syzigium aromaticum* and *Cinnamomum cassia* plant oils were tested for their insecticidal activity on adult *C. maculates* was accomplished in Petri-dishes [9 cm diameter] containing 25 g of cowpea with concentrations of 50, 100, 150 and 200 mg/ml. The beans were thoroughly coated by gentle shaking for 5-10 min to ensure uniform coating. The dishes were left open for approximately 30 min to allow traces of oils to dry off; after which 20 newly emerged adult *C. maculatus* were introduced into the dishes and mortality was observed daily for three days. Grains that were solvent treated served as the control experiment. Adults were considered dead where no response was observed after probing them with forceps.

3. RESULTS

3.1 Insecticidal Activity of *Syzygium aromaticum* Tested against *C. maculatus*

In the present study, the insecticidal activity of *S. aromaticum* and *C. cassia* were tested individually against the adults of *C. maculatus* with different concentrations such as 50, 100, 150 and 200 mg/mL. A perusal of the data indicates that the significant mortalities were observed at 200 mg/mL concentration of the *S. aromaticum* against the insects exposed to 24hrs [82.6%], 48 hrs [88.4%] and 72 Hrs [97.8%]. The trend was followed by 150, 100, 50 mg/mL concentrations with 64.2%, 72.8%, 76.8%; 38.2%, 46.6%, 52.6%; 27.6%, 34.8%, 36.8% at 24, 48 and 72 Hrs respectively [Fig. 1].

The lethal concentration of *S. aromaticum* against the *C. maculatus* when exposed to 24 hrs was calculated and the values are shown in Table 1. The LC_{50} value of 115.90 mg/mL with the LCL of 102.65 mg/mL and UCL of 128.42 mg/mL was recorded and at the same time the LC_{90} value was found to be 237.82 mg/mL with the LCL of 213.33 mg/mL and UCL of 275.45 mg/mL were also recorded from the above data [Table 1]. Similarly, the lethal concentration of *S. aromaticum* against the *C. maculatus* when exposed to 48 hrs was calculated and the values are shown in Table 1. The LC_{50} value of 115.90 mg/mL with the LCL of 102.65 mg/mL and UCL of 128.42 mg/mL was recorded and at the same time the LC90 value was found to be 237.82 mg/mL with the LCL of 213.33 mg/mL and UCL of 275.45 mg/mL were also recorded from the above data [Table 1]. In the same way, the lethal concentration of *S. aromaticum* against the *C. maculatus* when exposed to 72 hrs was calculated and the values are shown in Table 1. The LC_{50} value of 115.90 mg/mL with the LCL of 102.65 mg/mL and UCL of 128.42 mg/mL was recorded and at the same time the LC_{90} value was found to be 237.82 mg/mL with the LCL of 213.33 mg/mL and UCL of 275.45 mg/mL were also recorded from the above data [Table 1].

3.2 Insecticidal Activity of *Cinnamonam cassia* Tested against *C. maculatus*

In the present study, the insecticidal activity of *C. cassia* was tested against the adults of *C. maculatus* with different concentrations such as 50, 100, 150 and 200 mg/mL. A perusal of the data indicates that the significant mortalities were observed at 200 mg/mL concentration of the *C. cassia* against the insects exposed to 24hrs [80.6%], 48 hrs [86.6%] and 72 Hrs [96.4%]. The trend was followed by 150, 100, 50 mg/mL concentrations with 62.2%, 70.4%, 72.6%; 36.4%, 48.4%, 56.8%; 26.8%, 32.2%, 34.6% at 24, 48 and 72 Hrs respectively [Fig. 2]. The lethal concentration of *C. cassia* against the *C. maculatus* when exposed to 24 hrs was calculated and the values are shown in Table 2. The LC_{50} value of 120.46 mg/mL with the LCL of 107.14 mg/mL and UCL of 133.42 mg/mL was recorded and at the same time the LC_{90} value was found to be 245.90 mg/mL with the LCL of 219.78 mg/mL and UCL of 286.56 mg/mL were also recorded from the above data [Table 2]. Similarly, lethal concentration of *C. cassia* against the *C. maculatus* when exposed to 48 hrs was calculated and the values are shown in Table 2. The LC_{50} value of 96.24 mg/mL with the LCL of 83.18 mg/mL and UCL of 110.93 mg/mL was recorded and at the same time the LC_{90} value was found to be 220.16 mg/mL with the LCL of 197.65 mg/mL and UCL of 254.66 mg/mL were also recorded from the above data [Table 2]. In the same way, the lethal concentration of *C. cassia* against the *C. maculatus* when exposed to 72 hrs was calculated and the values are shown in Table 2. The LC_{50} value of 86.27 mg/mL with the LCL of 72.63 mg/mL and UCL of 97.52 mg/mL was recorded and at the same time the LC_{90} value was found to be 186.81 mg/mL with the LCL of 170.18 mg/mL and UCL of 187.10 mg/mL were also recorded from the above data [Table 2]. The probit responses for the above said two plant volatile oils have been derived statistically from the data and the results are depicted in Fig. 3 A-F. Their respective r2 values were also shown in the figures.

Table 1. Determined lethal concentration values of *Syzygium aromaticum* tested against *C. maculatus*

Exposure periods [days]	LC_{50}	95 % fiducial limit		LC_{90}	95% fiducial limit		χ^2
		LCL	UCL		LCL	UCL	
24	115.90	102.65	128.42	237.82	213.33	275.33	1.768
48	95.01	79.96	107.54	213.92	192.48	246.43	1.935
72	85.04	41.05	127.39	179.56	134.81	471.33	6.106

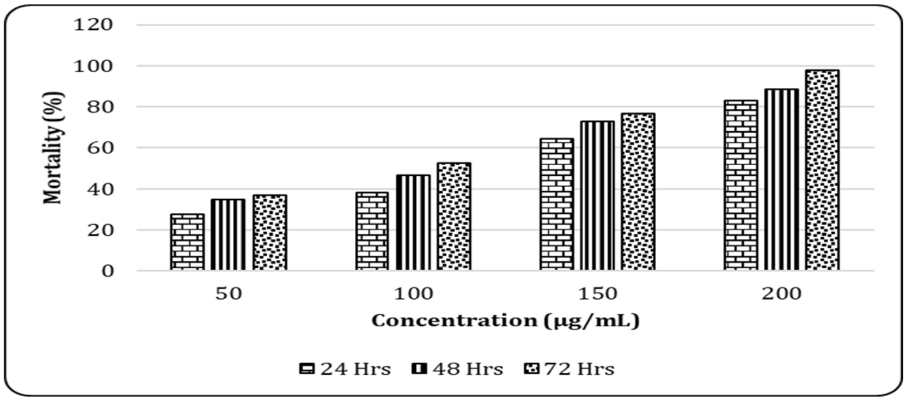


Fig. 1. Insecticidal activity of *Syzygium aromaticum* oil tested against the pulse beetle, *Callosobruchus maculatus*

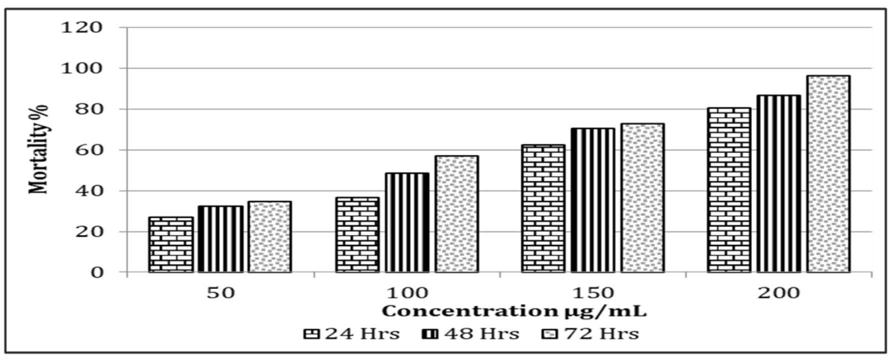


Fig. 2. Insecticidal activity of *Cinnamomum cassia* oil tested against the pulse beetle, *Callosobruchus maculatus*

Table 2. Determined lethal concentration values of *Cinnamomum cassia* oil tested against the pulse beetle, *Callosobruchus maculatus*

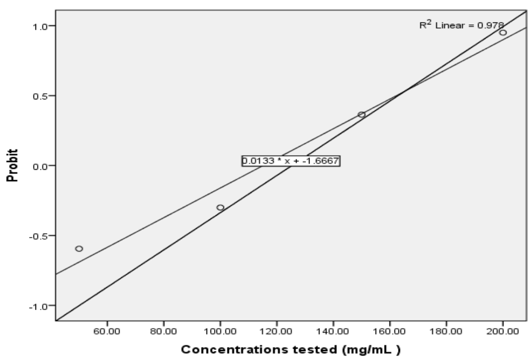
Exposure periods [days]	LC_{50}	95 % fiducial limit		LC_{90}	95% fiducial limit		χ^2
		LCL	UCL		LCL	UCL	
24	120.46	107.14	133.42	245.90	219.78	286.56	1.867
48	96.24	83.18	110.93	220.16	197.65	254.66	4.955
72	86.27	72.63	97.52	186.81	170.18	187.10	0.400

4. DISCUSSION

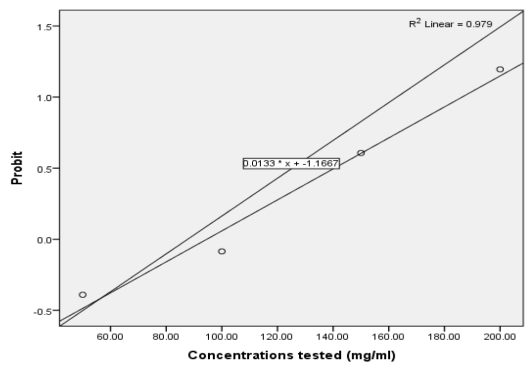
The contact toxicity of plant volatile oils toward stored product pests as insecticide is due to their volatility and thus meager persistency. These characteristics forced the researchers with repeated applications. In the present investigation also the selected volatile oils showed remarkable insecticidal activities against the selected stored grain pest, *C. maculatus*. Our findings are in agreeing with the results of several earlier researhers who have been reported that the insecticidal activity of plant oils against spectrum of stored product insects either individually or in combination [synergistics] with other oils. To quote a few, *Ocimum gratissimum* protected the grains from *Sitophilus zeamais* [31-62].

Many life-history traits of stored product pests may be slightly affected or deeply altered by plant oil treatments. Indeed, phyto-compounds may even not directly kill insects but could cause pertinent reduction of the reproductive performances, as well as several developmental impairments, because, during the experiments, we observed many gravid females didn't lay their eggs on the grains whey they were exposed to 72 hrs treatment. The possible reason we can conclude here is the volatile nature of the oil would interfere with the eclosion behavior of the test organisms. However, when the young ones emerged from the eggs even laid by the test insects while in contact with oil-treated grains, the presence of insecticidal molecules could also cause neonatal insect mortalities [un published data] [63,64]. Several researchers previously opined that the longevity of pests after exposure to the oils may be significantly reduced in stored product pests [65,66]. The intrinsic properties of plant volatile oils affect the basic metabolic, biochemical, and physiological functions of insect pests [66,67]. Several previous research reports state that the neurotoxic actions of plant volatile oils, causing insect paralysis followed by death by inhibiting the acetylcholinesterase [68,20, 69-76].

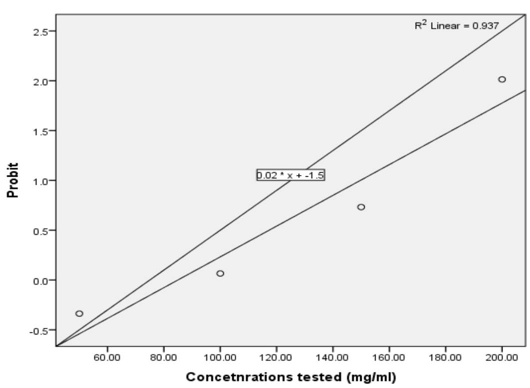
A. *Syzygium aromaticum* at 24 hrs



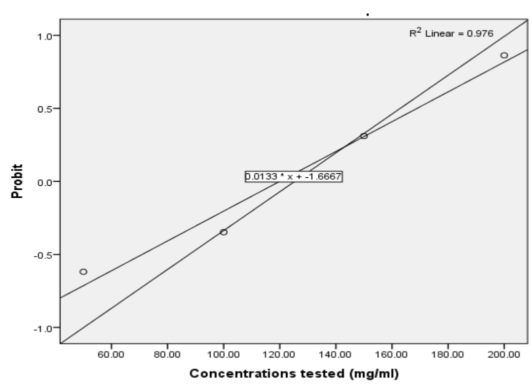
B. *Syzygium aromaticum* at 48 hrs



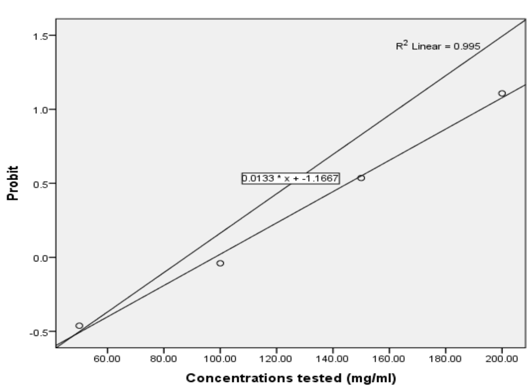
C. *Syzygium aromaticum* at 72 hrs



A. *Cinnamomum cassia* at 24 hrs



B. *Cinnamomum cassia* at 48 hrs



C. *Cinnamomum cassia* at 72 hrs

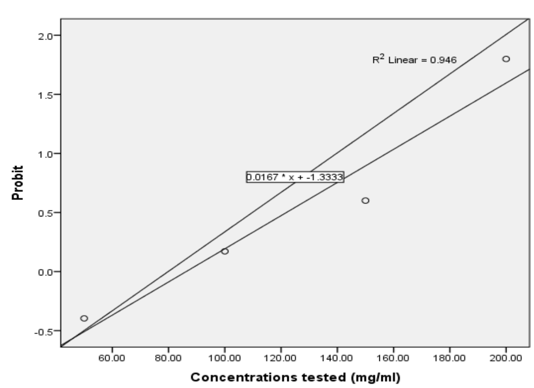


Fig. 3. Probit transformed responses calculated for the oils tested against the pulse beetle, *Callosobruchus maculatus*

5. CONCLUSION

In the last few decades, synthetic pesticides played a major role to control the insect pests in field and post harvesting process. Indiscriminate use of such chemical pesticides once used to control the insect pests caused several deleterious effects in the environment and as well as in the health of man and other non target organisms. Thus, scientist from all over the world tried for an alternative approach to control the pest. Though there are several empherical methods are available, the application of plant based technology has gained a real niche. As a part of it, in the present investigation the insecticidal action of selected two plant volatile oils are well documented. Further, it is also informed that the application of plant oils to control the pest infestation is safer to the environment and the non target organisms also.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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