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Insecticidal activity of the leaf extracts of C*annabis indica* against two major stored grain pest *Callosobruchus chinensis and Sitophilus oryzae*

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ABSTRACT: *Sitophilus oryzae (L.) and Callosobruchus chinensis (L.) are among the most common insect pests found in stored grains around the world. Resisting the insect pests as early as possible is greatly useful to stop the economic loss of the stored grains in terms of both quantity as well as the quality. Crude extracts as well as oil extracts of many plants were earlier tested for their insecticidal and repellent activity for the stored grain pests, but only a few of them have shown to have the activities against these two pest. Here, we tested the repellent and insecticidal activities of crude extract of Cannabis indica against Sitophilus oryzae and Callosobruchus chinensis in two different solvents (Hexane & Chloroform). The crude extract of the plant was applied with two concentrations (3.5 and 4.5 mg/cm^2) to check the insect repellent activity and three concentrations (2.5, 3.5 and 4.5 mg/cm^2) to check mortality.*

Keywords: *Plant Crude extract, Stored grain insect pest, Mortality, Repellent activity*

INTRODUCTION

Insect pest of stored grain were found to be the most important cause for economic loss worldwide. According to the estimates of Food Corporation of India, the insect pests were responsible for economic loss of thousands of crores per year alone in India [20]. *Sitophilus* and *Callosobruchus* Sp. were among the most important insect pest for the stored grains. Traditionally a large number of plant extracts were used to control these insect pests. A range of plants extracts such as *Macaranga* [15], *Psidium, Citrus, Azadirachta* [10], Solanum, Tillandsia [1], Cinnamomum [6] were used to control stored grain pest. The entomopathogenic fungi such as *Metarhizium anisopliae* [17], *Beauveria bassiana, Metarhizium anisopliae, Isaria fumosorosea* [7], were also used as a bio-control agent for *Sitophilus sp*. but were not recommended for long-term use as they are harmful for human as well as for the environment [9], Several synthetic insecticides such as methoprene [10], fenitrothion [4], were also used to control these insect pests worldwide. The extensive use of synthetic insecticides also have disadvantages like, pest recovery, pest resistance, lethal effect on non-target organism and toxic to consumer and user [11]. However, botanical pesticides (plant-based) is less toxic and target specific [14]. Furthermore, they do not have any harmful effect to the higher animals and environment [16]. The plant *Cannabis* has been found to have repellent activity against vertebrates as well as the invertebrates. It has also better results as a companion crop to deter insects, nematodes, fungi and weedy plants. Pure cannabinoids extracted from these plants have been reported to kill or repel mites, insects, nematodes, fungi, protozoan etc. [8]. As there is no proper evidence of using *Cannabis indica* as a bio-control agent against stored grain pest, we tested the repellency activity of *Cannabis indica* against the stored grain pest *Sitophilus oryzae* and *Callosobruchus chinensis.*

MATERIALS AND METHODS

Plant material

The leaves of *Cannabis indica* were collected from the nearby areas of Jorhat, Assam and were air dried in dark. The identification of the plant was confirmed by the Medicinal, Aromatic and Economic Plant Division, CSIR- NEIST, Jorhat, Assam.

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Crude extraction of *Cannabis indica* leaves

The leaves of plant were clean with water properly and dried under shed for a week. After fully drying it was finely powdered. 100g of the powdered sample was extracted twice with 600 mL of the solvent at room temperature for 72 hrs and filtered (Whatman No. 2). The filtrate was concentrated to dryness by rotary evaporation at 40 °C. The yield of chloroform & hexane extraction is calculated by (Dry weight of the solvent extract / dry weight of plant) x100 it was found as 17 and 15% respectively [18].

Insects

For the bioassays, the rice weevil *Sitophilus oryzae* (Coleoptera: Curculionidae) and pulse beetle *Callosobruchus chinensis* (Coleoptera: Bruchidae) were used. The weevils were grown in an insect culture room at 27±2 °C temperature with relative humidity of 75±5%. Jars containing the weevils were covered with thin cloth so that air can pass through it and fed them with rice and pulse grains respectively.

Bioassay

Mortality test: The insecticidal activity of plant extracts was determined by direct contact application [19]. Extracts of *C. indica* in two different solvents (hexane and chloroform) were applied at three concentrations (2.5, 3.5 and 4.5 mg/cm2) to test the mortality. In case of *S. oryzae* the mortality test was done in a petri dish with 8 cm in diameter and for *C. chinensis,* polypropylene cups with 8 cm diameter x 12 cm were used. The Whatman no.1 filter papers were taken and cut accordingly for test against *C. chinensis* and *S. oryzae.*

Test materials were weighed and dissolved in corresponding solvent to get the required concentration. 1 ml of the test material were then applied on the filter papers and kept for air dry to 2-3 hours. Once the solvent is fully dried it is placed in the cup or petri dish and pasted with the help of glue. One ml of corresponding solvents was taken as control. A group of 10 adult insects were taken for each set up of experiments. Mortalities were determined at a regular interval of 24 hours after treatment, up to 5 days. Test insects were considered dead if appendages did not move when prodded with a fine brush.

Repellency test: The repellency test was performed according to Kim *et al.* with some modification [18]. In our experiment, we took two concentrations (3.5 and 4.5 mg/cm^2) of the extract. The repellency test for *C. chinensis,* was performed by connecting two polypropylene cups with same area with a hollow pipe of 0.5 cm in diameter, where one container contain the test material and other one contains the respective solvents. After application of the extract the filter papers were kept for drying and ones fully dried it is paste to the cup with glue. A group of 10 adult insects were placed in the cup containing the extract and the readings were taken in an interval of one hour to check the number of insects moving from the treated container to the control. For the repellency test against *S. oryzae,* petri dishes with 9 cm diameter were taken [16]. The test material was applied in a half cut portion of filter paper and other half with the corresponding solvent to make sure that repellent activity on the insects is not affected due to activity of the solvent. Both the cuts were placed in the petri dish and ten adult weevils were released on the middle of the two half. The weevils present on each half was counted after one hour of interval.

RESULTS

It has been found that the chloroform extracts of *C. indica* showed 100% (Table 1) mortality against *Callosobruchus chinensis* in just two days at 4.5 mg/cm^2, where as it shows highest mortality of 86.66% against *Sitophilus oryzae* on 5th day at same concentration. The extract at concentration 3.5 & 2.5 mg/cm^2 shows 100% mortality on 3rd & 4th respectively against *Callosobruchus chinensis,* while in case of *Sitophilus oryzae* it shows only 56.66% & 50% mortality on 5th day respectively (Fig. 1). The hexane extract of *C. indica* shows 100% mortality at 4.5 mg/cm^2 after 3rd day of treatment and at concentration 3.5 & 2.5 mg/cm^2 it shows 100% mortality on 4th & 5th day respectively against *Callosobruchus chinensis.* On the other hand its shows highest mortality of 76.66, 50 & 36.66 % at 4.5, 3.5 & 2.5 mg/cm^2 concentration on 5th day after treatment against *Sitophilus oryzae.*

For the repellency test on *C. chinensis*, it has been found that chloroform extract show 100% repellency after three hour of treatment at 4.5 mg/cm^2 and 80% repellency at 3.5 mg/cm^2 after 4 hours of treatment (Table 2). Moreover in case of *S. oryzae* it shows 76.66 & 56.66% repellency at 4.5 & 3.5 mg/cm^2 respectively after four hours of treatment. The hexane extract shows 90 & 70% repellency at 4.5 & 3.5 mg/cm^2 concentration respectively after four hours against *C. chinensis,* while in case of *S. oryzae* it shows 63.33 & 50% repellency at 4.5 & 3.5 mg/cm^2 concentration respectively (Fig. 1).

Table 1
Insecticidal activity of chloroform & hexane extract of *C. indica* against *Callosobruchus chinensis* & *Sitophilus oryzae* at different concentrations. (n = 3)

Insect pest	*Solvent*	*Conc. of Extract (mg/cm²)*	*Mortality (%) mean ±SE Days after treatment*				
			1D	*2D*	*3D*	*4D*	*5D*
C. chinensis	Hexane	4.5	43.33 ± 0.33	60 ± 0.577	100 ± 0.33	-	-
		3.5	33.33 ± 0.33	50 ± 0.577	70 ± 0.577	100 ± 0.33	-
		2.5	13.33 ± 0.33	33.33 ± 0.33	46.66 ± 0.33	73.33 ± 0.33	100 ± 0.33
	Chloroform	4.5	63.33 ± 0.33	100 ± 0.00	-	-	-
		3.5	36.66 ± 0.33	80 ± 0.577	100 ± 0.00	-	-
		2.5	16.66 ± 0.33	53.33 ± 0.88	66.6 ± 0.66	100 ± 0.38	-
S. oryzae	Hexane	4.5	23.33 ± 0.33	33.33 ± 0.33	46.66 ± 0.33	63.33 ± 0.33	76.66 ± 0.33
		3.5	10 ± 0.577	23.33 ± 0.66	30 ± 0.577	40 ± 0.577	50 ± 0.577
		2.5	0 ± 0.00	13.33 ± 0.33	23.33 ± 0.33	26.66 ± 0.33	36.66 ± 0.33
	Chloroform	4.5	26.66 ± 0.33	43.33 ± 0.33	56.66 ± 0.33	70 ± 0.577	86.66 ± 0.66
		3.5	20 ± 0.577	30 ± 0.577	36.66 ± 0.33	50 ± 0.577	56.66 ± 0.33
		2.5	6.66 ± 0.33	16.66 ± 0.33	30 ± 0.00	40 ± 0.00	50 ± 0.577

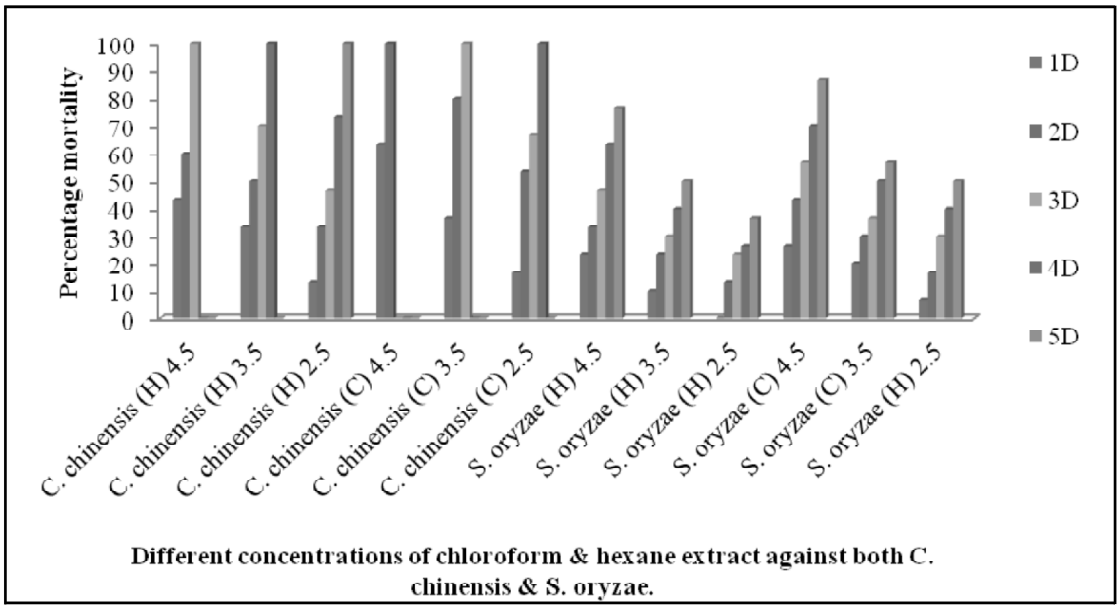

Figure 1: Pesticidal activity of chloroforom & hexane extract of *C. indica* against *C. chinensis* & *S. oryzae* at different concentrations (cm/mg²)

Table 2
Repellency record of *C. indica* extract in two solvents (chloroform & hexane) against *C. chinensis* and *Sitophilus oryzae* (n = 3)

Plant extract	*Solvent*	*Conc. of extract in mg/cm²*	*Readings in hourly interval percentage mean ± SE*			
			1st	*2nd*	*3rd*	*4th*
		4.5	50 ± 0.577	70 ± 0.577	80 ± 0.577	90 ± 0.577
C. chinensis	Hexane	3.5	20 ± 0.66	36.66 ± 0.33	46.66 ± 0.33	70 ± 0.577
		4.5	60 ± 0.577	80 ± 0.577	100 ± 0.00	100 ± 0.00
	Chloroform	3.5	30 ± 0.577	40 ± 0.577	56.66 ± 0.33	80 ± 0.577
		4.5	43.33 ± 0.33	50 ± 0.00	56.66 ± 0.33	63.33 ± 0.33
S. oryzae	Hexane	3.5	23.33 ± 0.33	36.66 ± 0.33	43.33 ± 0.33	50 ± 0.577
		4.5	50 ± 0.577	60 ± 0.577	70 ± 0.577	76.66 ± 0.33
	Chloroform	3.5	30 ± 0.577	36.66 ± 0.33	44.66 ± 0.33	56.66 ± 0.33

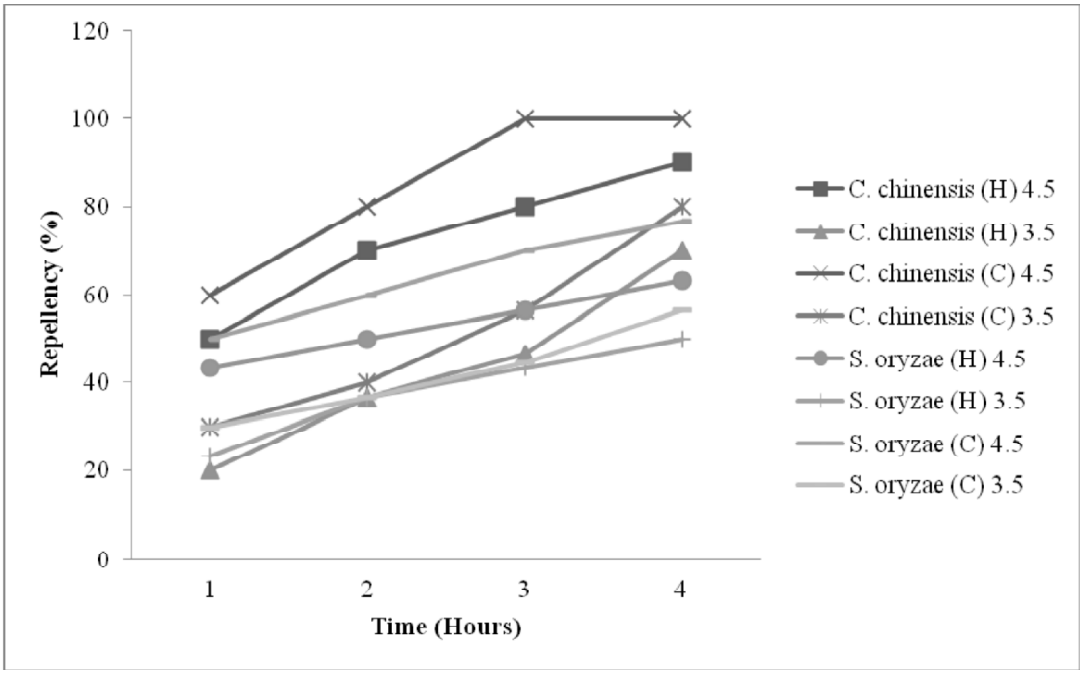

Figure 2: Repellency activity of chloroform (C) & hexane (H) against both *C. chinensis* & *S. oryzae* at different concentrations (mg/cm^2) upto 4 hours

DISCUSSION

There is a growing demand for bio-pesticides now-a-days due to various effects cause by synthetic insecticides such as pest resurgence, pesticide resistance and negative impacts on non-target species including human beings and the environment.

The repellent property was seen in both of the crude extract of different plants with different concentration. The investigations done by Saljogi *et al.* [2] on six different plants, they found *Melia azedirach* as the most effective having the repellent activity of 61.2% (considering % mortality as the main index). Jaiani & Su [5] reported the repellent effect of three extract of common plants in Pakistan, where they found the powdered extract of *Curcuma longa* had the strongest repellency on three stored grain. Kumar et al. [12] reported that the protein-enriched bean flour had a highest repellent activity of 76.3% and 91.2% in 0.1 % and 1% concentration respectively on *Sitophilus oryzae*. As there were no such investigations done on the repellent activity of *Cannabis indica* against the stored grain pest *Sitophilus oryzae* and *Callosobruchus chinensis*, we studied the repellent activity of *Cannabis indica.* Here we found the crude extract of *Cannabis indica* shows excellent insecticidal and repellent activity against both of the test insect-pest.

The selected *Cannabis indica* must have some important chemical compounds which can be isolated to use as pest control. Further studies and research work is required to analyze the effect of insect infestation on the nutritional quality of the stored grains.

CONCLUSION

Cannabis indica is identified as a potential bioactive plant for controlling the stored grain insect pest of pulses and cereals. However, further research works is required to identify the bioactive compound available in *Cannabis indica* for commercial exploitation and developing a herbal formulation against *Sitophilus oryzae* and *Callosobruchus chinensis*.

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