Environmental Sustainability (2018) 1:295–301
https://doi.org/10.1007/s42398-018-00029-x

ORIGINAL ARTICLE

Botanical insecticides effectively control chickpea weevil, *Callosobruchus maculatus*

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Received: 9 April 2018 / Revised: 21 September 2018 / Accepted: 19 October 2018 / Published online: 5 November 2018

Abstract

Health hazards associated with the excess use of chemical insecticides used for the post-harvest preservation of food grains has prompted the emphasis on the use of natural insecticides in agriculture. In this regards, botanical insecticides have emerged as one of the best, safer, green, eco-friendly, promising and sustainable alternatives for controlling insect pests. Therefore, present study was aimed to evaluate the insecticidal and repellent activities of extracts of various plants such as *Azadirachta indica* (kernel), *Allium sativum* (bulb), *Curcuma longa* (rhizome), *Citrus sinensis* (peel), *Citrus limon* (peel), *Murraya koenigii* (leaves) and *Eucalyptus* sp. (leaves) against the chickpea weevil, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae). These extracts exhibited potent insecticidal and repellent effects on *C. maculatus*. After 5 days treatment, all these extracts showed varying degree of insecticidal and repellent activity against *C. maculatus*; *A. indica* produced highest mortality (58%) followed by *A. sativum* (52.66% mortality) and *C. longa* (49.33% mortality) whereas *C. sinensis* exerted the lowest mortality (12.45%) against *C. maculatus*. All these extracts also showed good repellent activity, *A. indica* showed the highest index of repellency (IR) (0.53) followed by *A. sativum* (IR = 0.66), *C. longa* (IR = 0.73) and *M. koenigii* (IR = 0.86). These plant extracts also protected the chickpea grains from damage due to the beetle, *A. indica* provided 95.63% protection to chickpea grains against damage. Among the various extracts, extracts of *A. indica* (kernel), *A. sativum* (bulb) and *C. longa* (rhizome) proved to be promising insecticides and repellent against chickpea insect pest and can be used as effective, safer and green insecticides and repellents to preserve and protect chickpea grains against coleopteran pest.

Keywords Botanical insecticides · *Callosobrucus maculatus* · Mortality · Repellency · *Azadirachta indica*

Introduction

Chickpea, *Cicer arietinum* is an important food legume with a good source of protein for human consumption. Worldwide, chickpea ranks third among the pulse crops and accounting for 10.1 million tons annually (Muehlbauer and Sarker 2017). Chickpea is produced in over 50 countries with India having the largest production and accounting for over 70% of total world production (FAO 2015). It is a highly nutritious and an inexpensive source of proteins; it provides 24% proteins, 60–65% carbohydrates and 6% fat and is a good source of minerals and essential B vitamins (http://www.fao.org/statistics/en/). Although the exact loss estimate in farmers' storage system is not available, it is reported that approximately 50–80% of cowpea grains are lost during the storage stage due to insect pest infestations (Akami et al. 2016, 2017). Chickpea is mainly infected by bruchid beetle *Callosobruchus maculatus* and *Callosobruchus chinensis*. These beetles cause physical damage to the grains and also affect the nutritional quality of grains (Hamdia et al. 2017; Verkaart et al. 2017). *C. maculatus* and *C. chinensis* are major storage pests of chickpea crops and cause considerable economic losses worldwide (Sharma and Thakur 2014). During early onset of infection, immature stages of insect are not normally seen, by the time, the visible symptoms occur, i.e., appearance of round holes in the grains due to the emergence of the insect through windows, the grain is completely damaged and lost (Moreira et al. 2012). In order to protect the post-harvest storage losses of

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chickpea chemicals such as cellphones are commonly and traditionally used as insecticides and fumigants (Kosini et al. 2015). Though the chemical insecticides are effective, they are carcinogenic, hazardous to non-target organisms, the health of plants, human and environment and lead to the development of insecticide resistance in insect pest (Kosini and Nukenine 2017). Many beetle populations have been reported to exhibit a higher degree (200-fold) of resistance to more than 52 different types of insecticides (Mahmood 2007; Alyokhin et al. 2008). This warrants the urgent need to search, develop and use eco-friendly, cost-effective, safe insecticides for protecting stored chickpea against bruchid weevil. Extracts of various plants such as *Azadirachta indica* (neem), *Allium sativum, Curcuma longa, Citrus limon* etc. have been reported to show insecticidal, anti-ovipositional and insect repellent effects against *C. maculatus* and other bruchid pests (Radha and Susheela 2014). The volatile extract (oil) of *A. indica* and other plants possesses great persistent and fumigant effect and hence can be directly applied to the seeds (Tamgno and Ngamo 2014). Though these botanicals are effective and best insecticides and repellents, there is limited information on the use of the plant products as bioinsecticides for controlling weevils in storage. Therefore, the present work was aimed at determining the toxic, anti-ovipositional and repellency effect of *A. indica*, *A. sativum*, *C. longa*, *Citrus sinensis*, *C. limon*, *Murraya koenigii* and *Eucalyptus* sp. against the storage chickpea weevil, *C. maculatus* (Coleoptera: Chrysomelidae) with a view of the fact that the use of the indigenous plants will be a promising approach to reduce the bruchid population effectively.

Materials and methods

Laboratory rearing of *C. maculatus*

Infested seeds of chickpea (variety, Dollar) procured from the local market were brought to the laboratory. *C. maculatus* were collected from infected seeds and raised in the laboratory on chickpea seeds in a plastic container covered with a muslin cloth. The parental stock of *C. maculatus* was reared and bred under laboratory conditions on the seeds of chickpea in a growth chamber at 30 ± 2 °C at 70% relative humidity. Initially, 50 pairs of 1–2 day old male and female adults of *C. maculatus* were placed in a jar containing chickpea seeds. Jars were sealed and incubated at 30 ± 2 °C at 70% relative humidity for 7 days to allow mating and oviposition (George and Verma 1999). Parent stocks were removed and chickpea seeds containing eggs were transferred to fresh seeds in the breeding jars covered with pieces of cloth and fastened with a rubber band to prevent contamination and escape of beetles. The subsequent progenies of beetles were used for further studies (Fatima et al. 2016).

Test plant materials

The kernel of *A. indica* and leaves of *M. koenigii* and *Eucalyptus* were collected from plants grown in the botanical garden of Bareilly College, Bareilly, (UP), India, (28°21′26.51°N, 79.25′2.91E/28.3573639°N 79.42). Thoroughly washed and air-dried leaves; were manually grind into powder followed by passing the resulting powder through a mesh sieve to obtain a fine dust. The bulb of *A. sativum* (variety Pant-Lohit) and rhizome of *C. longa* (variety Pant-Pitabh) were procured from local market. Peels of *C. limon* and *C. sinensis* were separately dried and grind into powder. The plant parts were extracted in petroleum ether followed by evaporation of the solvent in a rotary evaporator at 40 °C (Kosini et al. 2015). After complete evaporation of the solvent, the crude extract was weighed and preserved in sealed bottles in the refrigerator until used for insect bioassays. During use, each extract was diluted with petroleum ether at the concentration of 20 mg mL^{-1}.

Insect bioassays

Mortality test

Mortality test of various plant extract against *C. maculatus* adult was checked by contact/residual method (Bacci et al. 2009; Fatope and Mann 1995). In this method, grains of chickpea were separately treated with each extract at a dosage concentration of 10 mL kg^{-1}. Chickpea grains treated with 10 mL kg^{-1} of petroleum ether alone were used as a control. A 100 g of chickpea grains along with extract of each plant were separately taken in 250 mL glass jars and continuously stirred for 1 min to ensure uniform coating of the extract on each seed grain. The jar was kept at 30 ± 2 °C for 3 h to allow the complete evaporation of the solvent. Ten adult beetles were placed on the treated grains and each jar was covered with a nylon mesh and kept at 30 ± 2 °C for 2 and 5 days (Pandey and Singh 1995) and observed for mortality effects.

Repellency test

Insect pest repellency of each extract was assessed by using the area preference method (Obeng-Ofori et al. 1998; Ngamo et al. 2007). Whatman filter paper (10 cm of diameter) was cut into a half disc, 1 mL of the extract was applied to a half filter paper disc and the other half of disc was treated with petroleum ether (control). Both i.e., extract treated and control parts of the paper were air dried to evaporate the solvent. The treated half disc of paper was attached to an untreated half disc with the help of Teflon and placed in Petri dish followed by adding 10 adult weevils on each half. The percentage of insects present on treated (G) and control (P) area were recorded after 30 min and index of repellency (IR) was calculated by using following formula.

$$IR = 2G/G + P,$$

where G is the percentage of insects present on filter paper treated with the extract, P is the percentage of insects present on untreated filter paper (control).

The repellency index was classified in terms of value, the value of < 1 repellency indicated repellent effect, the value of 1 indicated neutral effect while the value of > 1 indicated the attractive effect of the extract.

Damage assessment and F1 progeny bioassay

In order to assess the damage in chickpea grains caused due to *C. maculatus* infestation, infested grains separately treated with each extract were incubated at 30 ± 2 °C for 30 days along with untreated infested grains and control (uninfected–untreated). Following the incubation, the extent of damage to the grains in each treatment was assessed using the exit-hole method (Ajayi and Adedire 1996), the holes were counted and expressed as percentage damage (PD) by using following formula (Chowdhury et al. 2008).

$$PD = \frac{\text{Total number of treated grains perforated}}{\text{Total number of grains}} \times 100$$

Only those grains that were riddle with exit-holes were counted for measurement of weevil perforation index (WPI) by using the following formulae (Ajayi and Adedire 1996; Fatope and Mann 1995).

$$WPI = \% \text{ of treated grains perforated } \times 100$$

$$\% \text{ of control grains perforated } + \% \text{ of treated grains perforated}$$

Statistical analysis

Data obtained from each experiment was statistically analyzed and the mean of three replicates was taken into consideration. Each mean value was subjected to the analysis of variance (ANOVA) followed by Tukey's multiple comparison tests for comparison of all treated groups with the control group (Parker 1979). Values of $P \leq 0.05$ were taken as statistically significant values.

Results

Laboratory rearing of *C. maculatus*

Oviposition and an increase in the number of insect population were observed in a growth chamber, indicative of the good growth of *C. maculatus*.

Insect bioassay

Mortality test

The grains treated with extracts of *A. indica* kernel, bulb of *A. sativum* and rhizome of *C. longa* produced significant insecticidal effect against *C. maculatus*. A mortality rate of 49.33–58% was evident after 5 days of exposure ($P < 0.001$) of infested grains to these extracts. Among the various extracts, *A. indica* kernel extract appeared to be the most effective and potent insecticidal preparation, as it caused 32.33% and 58% mortality in *C. maculatus*, after 2 days and 5 days of storage respectively. Extracts of other plants also exhibited a varying degree of mortality and repellency. Peels of *C. limon* and *C. sinensis* and *Eucalyptus* (leaves) did not show insecticidal effects (Table 1).

Repellent effect

Treatment of infected chickpea bean with extracts of all plants except *C. limon* and *C. sinensis* exhibited a varying degree of repellent effect on *C. maculatus*. Highest repellency index (0.53) was obtained with *A. indica* extract followed by *A. sativum* (IR = 0.66) and *C. longa* (IR = 0.73). While the extracts of *Eucalyptus* leaves, *C. sinensis,* and *C. limon*, did not show any repellent effect, on the contrary, they exhibited attractive behavior towards *C. maculatus* (Table 1).

Damage assessment and F1 progeny bioassay

During 30 days storage at 30 °C, chickpea grains treated with various extracts showed a marked reduction in the grain damage. The extract of *A. indica* kernel resulted in

Table 1 Effect of the petroleum ether extracts on mortality and repellency of *C. maculatus*

Plant extracts	Percent mortality of adult weevil		Repellency index (RI)
	After 2 days	After 5 days	
A. indica (kernel)	32.33 ± 1.17***	58.00 ± 0.95***	0.53
A. sativum (bulb)	27.33 ± 1.69***	52.66 ± 1.90***	0.66
C. longa (rhizome)	22.33 ± 1.17***	49.33 ± 3.24***	0.73
M. koenigii (leaves)	17.33 ± 1.69**	32.66 ± 1.90**	0.86
C. limon (peel)	0.00 ± 0.00^{ns}	0.00 ± 0.00^{ns}	1.40
C. sinensis (peel)	0.00 ± 0.00^{ns}	12.45 ± 0.54^{ns}	1.10
Eucalyptus (leaves)	0.00 ± 0.00^{ns}	0.00 ± 0.00^{ns}	1.40
Control	0.00 ± 0.00	0.00 ± 0.00	1.00

Values are expressed as mean ± SEM (standard error mean)

Data were analysed by one way ANOVA followed by Tukey's *t* test

Comparison of all treated groups made with control group ***p < 0.001, **p < 0.05, ns: non-significant

significant decrease (95.63%) in grain damage; only 4.73% grain were found damaged, followed by *A. sativum* bulb, with 7.42% damage (92.58% reduction) *M. koenigii* (11.20% damage) (88.80% reduction) while lowest protection (15.52%) was obtained in case of *C. longa* rhizome treated grains. The percent damage values with other plant extracts were insignificant and did not show heterogeneity of the mortality data in comparison to control. *A. indica* kernel extract was highly effective in preventing the formation of holes in stored grains as it resulted in minimum (10.22%) WPI as compared to all other preparations including the control (50% WPI) (Table 2).

Discussion

Organically or naturally treated food items have attracted consumers, farmers and researchers, hence are gaining market potential. Insecticidal, pesticidal, fumigant and repellent effects of plant extracts are in practice since ancient times for pre and post-harvest control of insect pests (Dubey et al. 2008; Benzi et al. 2009). A wide variety of extracts, powders and essential oil from different plants have been reported either as insecticides or insect repellents against a wide variety of stored grain insect pests (Tunaz et al. 2009; Moreira et al. 2012). In the present study extract of *A. indica*, *A. sativum* and *C. longa* exhibited multiple properties including insecticidal effects, repellent activity, anti-ovipositional feature and persistent protectant property against *C. maculatus*. *A. indica* extract showed better insecticidal features and proved useful in preventing the grain damage during storage.

The insect repellent test revealed the potential of *A. indica* kernel and *A. sativum* bulb. Kosini and Nukenine (2017) reported that hexane extract of *Gnidia kaussiana* and neem seed oil were potent for *Callosobruchus* and averted weevil damage. Radha and Susheela (2014) have also reported that an extract of *A. indica* leaves caused a considerable reduction in the number of weevils. Dubey et al. (2008) have claimed azadirachtin of *A. indica* to affect the feeding, growth, molting, and reproduction of insects. Pandey and Singh (1995) reported that a petroleum ether extract of *A. indica* leaves and twigs mixed with green gram seeds inhibited the oviposition of *C. chinensis*. Studies of Boeke et al. (2004), Nukenine et al. (2009) and Kosini et al. (2015) have also reported protective effects of *A. sativum* against *C. maculatus*. Padin et al. (2013) showed the repellent effect of many medicinal plants against *Tribolium castaneum*. Upadhyay and Singh (2012) found that lectins present in *A. sativum* damage the mid-gut enzymes leading to the death of insects. Meriga et al. (2012) reported insecticidal activity of *A. sativum* bulbs against the larvae of *Spodoptera litura*.

Ali et al. (2014a, b) and Mobki et al. (2014) have studied the insecticidal activity of *C. longa* and *A. sativum* extracts against *Tribolium castaneum* and obtained a significant reduction in the insect population and crop infestations. Tavares et al. (2016) claimed ar-turmerone of *C. longa* rhizome as an effective insecticide against agricultural insect-pests. Lee et al. (2001) reported 64% mortality at 500 ppm concentrations of sesquiterpene ketone *ar*-turmerone of the *Curcuma* rhizome against *C. chinensis* and other insects. Plata-Rueda et al. (2017) found intoxication and necrosis effect of essential oils of garlic on the larva, pupa, and adult of mealworm beetle. Gangwar and Tiwari (2017) evaluated the insecticidal activity of *C. longa* against insects of Coleoptera order and claimed that the insecticidal/fumigation activity of *C. longa* was due to its essential oil that exhibited fumigant action. Ali et al. (2014a, b) found the insecticidal activity of turmeric and garlic extracts against red flour beetle and claimed these botanicals as a safe alternative to the insecticides in stored commodities. Oboh et al. (2017) observed inhibition of acetylcholin esterase and Na^{+}/

Table 2 Effect of plant extracts on grain damage

Plant extracts	Total grains	No. of perforated grains	Percentage grain damage (PD)	Reduction in grain damage (%)	WPI
A.indica (kernel)	226.33 ± 0.71	10.66 ± 0.73***	4.73 ± 0.32***	95.27	10.22 ± 0.58***
A. sativum (bulb)	242.33 ± 1.17	18.00 ± 1.25***	7.42 ± 0.44***	92.58	15.14 ± 0.83***
C. longa (rhizome)	247.00 ± 0.94	38.33 ± 0.32**	15.52 ± 0.12**	84.48	27.23 ± 0.23**
M. koenigii(leaves)	247.00 ± 0.94	27.66 ± 0.45***	11.20 ± 0.15***	88.80	21.22 ± 0.52***
C. limon (peel)	245.33 ± 1.19	50.33 ± 1.32^{ns}	20.52 ± 0.15^{ns}	79.48	33.05 ± 0.32^{ns}
C. sinensis (peel)	236.33 ± 1.17	93.33 ± 0.26^{ns}	39.49 ± 0.23^{ns}	60.51	48.78 ± 0.24^{ns}
Eucalyptus (leaves)	236.33 ± 1.17	95.00 ± 1.69^{ns}	40.20 ± 0.86^{ns}	59.80	49.21 ± 0.56^{ns}
Control	232.33 ± 0.98	96.33 ± 0.72	41.56 ± 0.16	58.44	50.00 ± 0.00

Values are expressed as mean ± SEM (standard error mean)

Data were analyzed by one way ANOVA followed by Tukey's t test

Comparison of all treated groups made with control group ***$p < 0.001$, **$p < 0.05$, ns: non-significant

K^+-ATPase activities in *C. maculatus* and other insects due to an essential oil present in orange peel.

The insecticidal potential of *A. indica, A. sativum,* and *C. longa* is attributed to their principal and active ingredients such as azadirachtin, allicin, and curcumin, respectively. All these ingredients are known to possess antimicrobial properties, insecticidal activities, and repellent potential. Extracts from botanicals are responsible for the insecticidal activities that include toxicity, growth inhibition, anti-ovipositional and grain protection against stored insect pests (Isman 2008). Various plant extracts tested in the present study with the aim to control *C. maculatus* proved to be effective and eco-friendly insecticides. However, extract of *A. indica* kernel was found to be most effective botanical insecticide as it resulted in highest mortality of *C. maculatus*, reduced the grain damage (due to *C. maculatus*) during storage as well as protected the chickpea grains from formation of holes. The insecticidal effect of these extracts may be mostly attributed to alkaloid contents in the these extracts. Alkaloids are toxic secondary metabolites which block ion channels, inhibit enzymes or interfere with neurotransmission leading to the death of insect pests (Aniszewski 2007). This suggests that *C. maculatus* is more sensitive to alkaloids (azadirachtin) content of *A. indica*. To be an effective insecticide, persistence of activity is prerequisite for its long term application. Activity and efficiency of insecticidal products decrease with the time due to the evaporation of volatile active compounds or because of the autooxidation of non-volatile compounds. However, *A. indica* extract had great persistent because of the low volatility of its main insecticidal compound, azadirachtin and it could protect chickpea against *C. maculatus* infestation for at least 30 days (Tamgno and Ngamo 2014).

The plant extracts of *A. indica, A. sativum,* and *C. longa* showed insect repellent activity against *C. maculatus*, the highest repellency index was obtained with *A. indica* kernel extract. In earlier studies (Nukenine et al. 2009; Kosini et al. 2015) plant extracts were reported as insect repellents against storage grain insect pests. The insect repellent property is one of the essential features of insecticidal preparations for the effective management of insect pests during storage, since it directly prevents the egg-laying and hence the emergence of adults, thus preventing the establishment of pest populations in stored grains.

In addition to the toxic effects, the extract of *A. indica* kernel, *A. sativum* bulb, and *M. koenigii* resulted in significant decrease in grain damage. This may be attributed to the grain protectant effects of these preparations that inhibited oviposition or killed the *C. maculatus* larvae at developmental stages. Crude extracts of *A. indica* kernel and *A. sativum* bulb are known to retard the development and cause mortality of larvae, causing damage to the endocrine system which regulates the growth of larvae (Acheuk and Doumandji-Mitiche 2013). Azadirachtin is also known to block the synthesis and release of molting hormones, ecdysteroids from the prothoracic gland of insect, thus preventing ecdysis in immature insects (El-Wakeil 2013).

Conclusion

The use of botanical insecticides should be encouraged in small farm storage, as they are a rich source of novel natural phytochemical substances that can be used to develop environmentally safe methods for insect control. Present investigation also suggests the use of botanical insecticides to minimize the severe damage caused by insect pests during storage. The botanical insecticides seem to be eco-friendly, safe, cost-effective and potent insecticides, repellents and preservatives to prevent insect pest infestations during post-harvest storage.

Observations recorded during present study confirm that bruchid beetle, *C. maculatus*, can be effectively controlled by *A. indica* seed kernel extract and *A. sativum* bulb extract. These extracts could be a potential toxicant, grain protectant and total suppresser of *C. maculatus* progeny emergence in treated grains. The persistence of the toxicity of *A. indica* extract indicated the stability and potential of this extract to protect chickpea grains against insect pests infestation for at least 30 days. However, further studies are needed towards the isolation and identification of the active principle component and finally, it is important to ensure the good quality and non-toxicity of the insecticidal preparation to humans before commercialization.

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