



SCREENING FOR INSECTICIDAL POTENTIAL OF THE CHICKPEA PLANT *CICER ARIETINUM* (L.) AGAINST THREE STORED GRAIN PESTS *CALLOSBRUCHUS CHINENSIS* (L.), *SITOPHILUS ORYZAE* (L.) AND *TRIBOLIUM CASTANEUM* (HERBST) ADULTS UNDER LABORATORY CONDITIONS

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Abstract

Petroleum ether (PE), chloroform (CHCl₃), and methanol (CH₃OH) extracts of the whole chickpea plant *Cicer arietinum* (L.) have been tested against adult beetles of *Callosobruchus chinensis* (L.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) through dose-mortality and repellent activity tests. In dose mortality tests, the PE extracts of *C. arietinum* gave LD₅₀ values of 3.466, 3.292, 2.721, 2.398, 1.887, 1.663 and 1.428 mg/cm² against *C. chinensis* and 4.297, 3.415, 2.246, 1.725, 1.384, 0.984 and 0.722 mg/cm² against *S. oryzae* both after 12, 18, 24, 30, 36, 42 and 48 h of exposures, respectively. The CHCl₃ extracts gave LD₅₀ values of 5.242, 4.844, 3.472, 2.945, 2.540, 1.875, 1.295 and 1.053 mg/cm² against *C. chinensis* after 6, 12, 18, 24, 30, 36, 42 and 48 h of exposures, respectively. The CH₃OH extracts, on the other hand, yielded LD₅₀ values of 0.923, 0.757, 0.649, 0.515, 0.400, 0.303, 0.247 and 0.197 mg/cm² against *C. chinensis*, 4.227, 3.528, 3.454, 2.933, 2.493, 2.187, 1.938 and 1.724 mg/cm² against *S. oryzae* and 3.485, 3.176, 2.851, 1.531, 1.113, 0.756, 0.534 and 0.478 mg/cm² against *T. castaneum* adults after 6, 12, 18, 24, 30, 36, 42 and 48 h of exposures, respectively. However, the PE extracts of *C. arietinum* did not show any mortality against *T. castaneum* and the CHCl₃ extracts were inactive against *S. oryzae* and *T. castaneum* adults. In repellency tests, all three organic extracts did not show any repellency against the test beetles. According to their intensity of activities, the extracts could be arranged in the following descending order: CH₃OH extracts against *C. chinensis* > CH₃OH extracts against *T. castaneum* > PE extracts against *S. oryzae* > CHCl₃ extracts against *C. chinensis* > PE extracts against *C. chinensis* > CH₃OH extracts against *S. oryzae* adults. The present results indicate that the test plant *C. arietinum* contains bioactive compounds and could be used as a source of control agents for the stored product pests.

Key words: *Cicer arietinum*, *Callosobruchus chinensis*, Dose-mortality, Repellency, *Sitophilus oryzae*, *Tribolium castaneum*

Introduction

Cicer arietinum (L.) also known as chickpea, Bengal gram, or Indian gram, is an edible legume belonging to the Fabaceae family. They are high in protein and are one of the earliest cultivated vegetables (Sandeep et al. 2012). This plant was one of the first coordinator yields of modern horticulture, with origins in southeast Turkey and Syria (Van der Maesen 1987). It is mostly grown as a winter crop in Asia, Europe, Australia, and

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North America; Southeast Asia accounts for over 80% of global output, with India being the leading producer (Shukla et al. 2012; Rachwa-Rosiak et al. 2015). In the world, nearly 11.12 million hectares of land are used to grow chickpeas, contributing 8.62 million tonnes of grain to the world's food supply (Ahmed et al. 2014). This plant is grown in semi-dry conditions and on poor agricultural grade soils, which, gotten together with its vulnerability to drought and devastating parasitic contaminations, results in yields of less than 1 ton per hectare, which is amazingly under the speculative potential. It has several branches from the base and stems that are erect, angular, or winged, the leaves are also densely glandular-pubescent (Holm 1920). There are many medicinal properties in the roots and seeds of this plant (Singh and Gahlot 2018). This plant's leaves are sour and astringent, and they increase taste and appetite while also curing bronchitis and causing flatulence (Vadnere et al. 2012). Although there is evidence of this species to have antifungal (Anjum et al. 2006), antioxidant (Vadnere et al. 2012), and anticancer (Qureshi et al. 2006) activities. However, this plant's insecticidal properties have not been broadly explored yet. The test beetle *C. chinensis* is a member of the family Bruchidae (Coleoptera), and is also known as the pulse beetle, Chinese bruchid, and cowpea bruchid (Islam et al. 2019). This species is one of the harmful beetles to stored grain products for its general food habits and ecological range (Yanagi et al. 2013). Bangladesh is one of the many countries that report significant annual losses of pulses due to bruchid infestations (Bangladesh Agricultural Research Institute, 1984), causing entomologists to work on preventing their spread. The pest *C. chinensis* has been recorded as a major problem in Bangladesh for pulse seeds (Islam and Khan 2000). The formative period from egg to grown-up requires 20-25 days (Raina 2013; Beck and Blumer 2014). The rice weevil, *S. oryzae* (Coleoptera: Curculionidae) is another major stored product pest that is widely distributed and can infect a variety of crops around the world (Bhuiyah et al. 1990). In warmer regions, it is the most common insect pest of stored rice (Akhtar et al. 2015). The adult weevil is a dull rosy earthy coloured to shading. The larval weevil finishes its advancement inside the seed grain. The hatchling creates inside the seed, burrowing it out while taking care of it. The out life cycle from egg to grown-up took 34 to 49 days with a normal of 42 days at 15 to 34°C temperature and 58 to 89 percent relative humidity (Bhuiyah et al. 1990). In distribution around the world, red flour beetles *T. castaneum* (Coleoptera: Tenebrionidae) are the most devastating pests of stored goods (Devi and Devi 2015). It is estimated that the larvae devour 12.5-14.60 percent of the individual seeds in their maturation, which means that the larvae invade almost 88 grains per larva. It causes significant grain loss and lowers viability (Mustary et al. 2020). The ideal temperature for the growth of *Tribolium* spp. is typically considered to be 30°C (Khatun et al. 2010). Larvae in this species are slender, yellowish-white in color, and measure approximately 1 mm long. The pupae are lighter in colour, white to yellowish, and the adult is a small reddish-brown beetle. The size of the adult beetle is about 3.5 mm in length and about 1.2 mm in width (Bousquet 1990). Also, an allergic reaction might be revealed by the red flour beetle (Alanko et al. 2000). It has been discovered that several types of plant proteins have toxic or detrimental effects on insects besides crude extracts and volatile oils. A less or more pronounced entomotoxic or insecticidal effect of some plant lectins has been reported by previous studies, although not all (Peumans and Van Damme 1995; Carlini and Grossi-De-Sa 2002). The fertility and egg viability of pest insects that attack stored goods can be successfully reduced by plant extracts from kamini and jamalgota (Jilani et al. 2011). Since the use of chemical pesticides against storage pests by farmers in developing countries like Bangladesh is often discouraged, the development of alternative pest management strategies involving plant extracts is likely to be pursued nowadays, which led to design and explore the present investigation. The current examination therefore was intended to discover the impacts of the whole plant concentrates of *C. arietinum* on dose-mortality response and repellent actions against three stored grain pests viz., *Callosobruchus chinensis* (L.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) under laboratory conditions.

Materials and Methods

Collection and preparation of test materials

In January 2020, whole plants of *C. arietinum* were collected from crop fields of the Rajshahi University Campus and the voucher specimens were identified and preserved in the herbarium at the Department of Botany, University of Rajshahi. The soil from around the roots was removed without the use of water. The leaves and other parts were slashed into tiny fragments, then dried under shade and powdered using an electric grinder, gauged, and put into conical flasks to be used as and when needed. Three organic solvents viz., petroleum ether (PE), chloroform (CHCl₃), and methanol (CH₃OH) were used at 100 g × 300 ml × two times progressively, each one of which was kept for 48 h on a shaker. For each concentrate, filtration was done by filter paper at 24 h of stretch in a similar jar followed by the vanishing of the solvent until the concentrate was left as dirt. After extraction, the concentrates were transferred to glass vials and preserved with appropriate labeling until used.

Collection and culture of the test insects

Adults of *C. chinensis*, *S. oryzae*, and *T. castaneum* were collected from the cultures maintained at the Crop Protection and Toxicology Laboratory, Department of Zoology, University of Rajshahi, Bangladesh. Because of their short life-cycles and hassle-free culture techniques, the test insects of similar age groups were available and employed for this investigation.

Dose-mortality tests on *C. chinensis* and *S. oryzae*

An *ad hoc* experiment was set to determine the final concentrations for dose-mortality selection. The concentrations of PE extracts were 2.546, 2.292, 2.037, 1.783, 1.528 and 1.273 mg/cm² against *C. chinensis* and 3.056, 2.546, 2.037, 1.528, 1.019 and 0.509 mg/cm² against *S. oryzae*. For CHCl₃ extracts the concentrations were 3.056, 2.546, 2.037, 1.528, 1.019 and 0.509 mg/cm² against *C. chinensis*, and the concentrations for the CH₃OH extracts were 1.146, 0.891, 0.637, 0.382 and 0.127 mg/cm² against *C. chinensis* and those were 2.801, 2.546, 2.292, 2.037, 1.783, and 1.528 mg/cm² against *S. oryzae*. However, the CHCl₃ extracts did not show any activity against *S. oryzae*. In experimentation, 1 ml of the ready solution was blended with the respective food grains and allowed to dry out. Being volatile the solvent disappeared shortly after application. 10 insects of similar age were released on the treated food in triplicates, and a control batch was also maintained with the similar number of insects released in the grains treated with only solvent and dried. The preparations were kept in an incubator at a similar temperature as the insects were raised in stock cultures, and the mortality of the insects was recorded after 6 h of exposure and continued up to 48 h with 6 h intervals.

Dose-mortality tests on *T. castaneum*

The trial for insecticidal test on *T. castaneum* was different from that on *C. chinensis* and *S. oryzae* owing to the feeding uniqueness of the former species. The *ad hoc* trials were set to find out the final doses. The last fixations applied in this trial were 1.528, 1.273, 1.019, 0.764, 0.509, and 0.255 mg/cm² for the CH₃OH extracts. For each of the experiments, 1 ml of each of the doses was dropped on 50 mm Petri dishes to make a uniform film over the surface of the containers and allowed to dry before the release of the test

insects. The factual extract present in 1 ml solution was calculated by dividing the value by the area of the petri dish, and thus the dose per square cm was determined. 10 adult beetles (3-4 days old) were released in each of the petri dishes, and the whole experiment was set in triplicates. A control group was also set up with the similar number of beetles. The treated petri dishes were placed in an incubator at a temperature similar to that of the stock cultures and the mortality was recorded after 6 h of exposure and continued up to 48 h with 6 h intervals.

Statistical analysis

The percent mortality (%) was calculated using Abbott's equation (Abbott 1925) $P_r = \frac{P_o - P_c}{100 - P_c} \times 100$; where, P_r = corrected mortality, P_o = observed mortality, and P_c = mortality within the control. The experimental data were subjected to probit analysis (Finney 1947; Busvine 1971) for achieving the dose-mortality response of the extracts under study.

Repellent activity

The procedure for the repellency test was maintained using the formula of McDonald et al. (1970) with considerable changes. An overall concentration for each one of the extracts was chosen as stock dosage for the repellency test applied against the adults of *C. chinensis* and *S. oryzae* to make other successive dosages by serial dilution to give 0.629, 0.314, 0.157, 0.079 and 0.039 mg/cm² and for *T. castaneum* the dosages were set up as the same as of the previous one. The experiments on *C. chinensis* and *S. oryzae* on 9-cm in diameter Petri dishes were partitioned into three sections separated by two narrow sticks fixed with sticky tapes. Both sides were loaded up with food where one side was treated with food and the other side was untreated followed by the release of 10 adult insects on the center of the Petri dish. For *T. castaneum* half channel paper circles (Whatman filter paper, No. 40) were arranged and chosen doses were independently applied onto every one of the half-disc and set up to dry out as kept uncovered for about 20 min. Each prepared half-disc was then attached the long way, edge-to-edge, to a control half-disc with sticky tape and set in 9 cm diameter Petri dish. For each one of the test samples, three replicates were set. Since the solvent was volatile, it was evaporated out within a few minutes. After that, ten insects were released in each of the paper circles. Repellency was noticed for one-hour intervals and up to five successive hours. In the occurrence of *C. chinensis* and *S. oryzae*, just by checking the number of insects from the non-treated part and the centerpiece of the 90 mm Petri dish floor. While for *T. castaneum*, by checking the number of insects from the non-treated piece of the paper circle on the base of the 90 mm petri dish. The values in the recorded information were calculated for percent repulsion, which was again evolved by arcsine transformation for the calculation of analysis of variance (ANOVA). The common of the counts were changed over to percent repellency (PR) applied according to the formula of Talukder and Howse: $PR = (N_c - 5) \times 20$; where, N_c was that the regular hourly observation of insects on the untreated portion of the disc (Talukder and House 1993; Talukder and Howse 1995).

Results

Dose mortality effects of *C. arietinum* extracts on *C. chinensis*, *S. oryzae*, and *T. castaneum*

The results of dose-mortality assay of the PE, CHCl₃, and CH₃OH extracts of *C. arietinum* against three test beetles are represented in Table 1.

Table 1: LD₅₀ values of the extract of *C. arietinum* against *C. chinensis*, *S. oryzae*, and *T. castaneum* adults.

Plant	Test insects	Solvents of extraction	LD ₅₀ (mg/cm ²) after hours of exposure							
			6 h	12 h	18 h	24 h	30 h	36 h	42 h	48 h
<i>C. arietinum</i>	<i>C. chinensis</i>	PE	-	3.466	3.292	2.721	2.398	1.887	1.663	1.428
		CHCl ₃	5.242	4.844	3.472	2.945	2.540	1.875	1.295	1.053
		CH ₃ OH	0.923	0.757	0.649	0.515	0.400	0.303	0.247	0.197
	<i>S. oryzae</i>	PE	-	4.297	3.415	2.246	1.725	1.384	0.984	0.722
		CHCl ₃	N/A							
		CH ₃ OH	4.227	3.528	3.454	2.933	2.493	2.187	1.938	1.724
	<i>T. castaneum</i>	PE	N/A							
		CHCl ₃	N/A							
		CH ₃ OH	3.485	3.176	2.851	1.531	1.113	0.756	0.534	0.478

N/A=Not active.

Against *C. chinensis* [Fig. 1 (a-f): Regression lines] the PE extract gave LD₅₀ values 3.466, 3.292, 2.721, 2.398, 1.887, 1.663 and 1.428 mg/cm² after 12, 18, 24, 30, 36, 42 and 48 h of exposures; the CHCl₃ extract gave 5.242, 4.844, 3.472, 2.945, 2.540, 1.875, 1.295 and 1.053 mg/cm² and the CH₃OH extract gave 0.923, 0.757, 0.649, 0.515, 0.400, 0.303, 0.247 and 0.197 mg/cm² after 6, 12, 18, 24, 30, 36, 42 and 48 h of exposures respectively. Against *S. oryzae* [Fig. 2 (a-d): Regression lines] the PE extract gave LD₅₀ values 4.297, 3.415, 2.246, 1.725, 1.384, 0.984 and 0.722 mg/cm² after 12, 18, 24, 30, 36, 42, 48 h of exposures; the CH₃OH extract gave 4.227, 3.528, 3.454, 2.933, 2.493, 2.187, 1.938 and 1.724 mg/cm² after 6, 12, 18, 24, 30, 36, 42, and 48 h of exposures. Against *T. castaneum* [Fig. 3 (a-b): Regression lines] the CH₃OH extract gave LD₅₀ values 3.485, 3.176, 2.851, 1.531, 1.113, 0.756, 0.534 and 0.478 mg/cm² after 6, 12, 18, 24, 30, 36, 42 and 48 h of exposures. However, against *S. oryzae* the CHCl₃ extract and against *T. castaneum* the PE and CHCl₃ extract did not show any mortality. According to the intensity of activity the extracts could be arranged in the descending order: CH₃OH extract against *C. chinensis* > CH₃OH extract against *T. castaneum* > PE extract against *S. oryzae* > CHCl₃ extract against *C. chinensis* > PE extract against *C. chinensis* > CH₃OH extract against *S. oryzae*.

Respective regression lines of the LD₅₀ values at the minimum and maximum hours of exposure in Table 1 are given in Fig. 1 (a-f), Fig. 2 (a-d), and Fig. 3 (a-b) below.

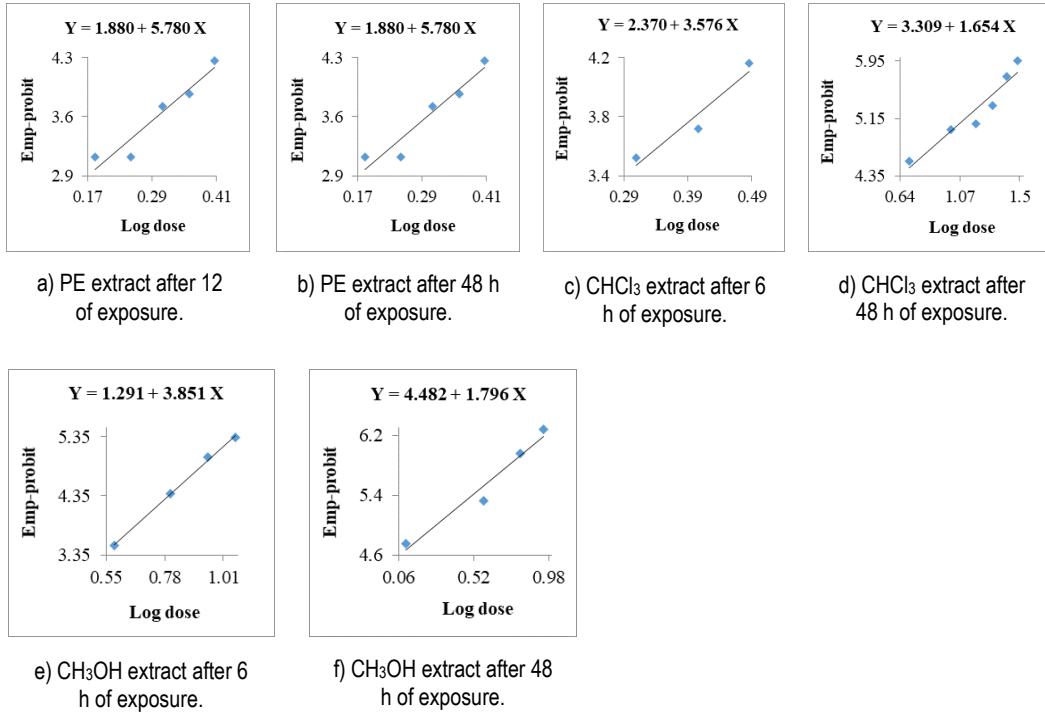


Fig. 1 (a-f): Regression lines of *C. arietinum* extracts against *C. chinensis* at the minimum and maximum hours of exposure.

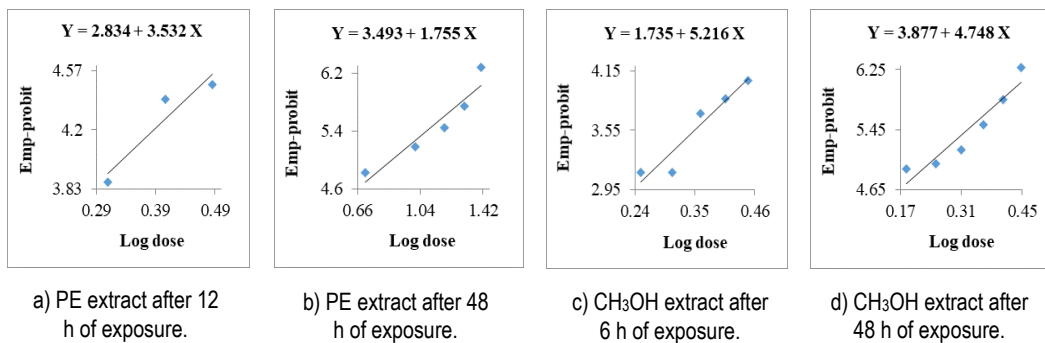


Fig. 2 (a-d): Regression lines of *C. arietinum* extracts against *S. oryzae* at the minimum and maximum hours of exposure.

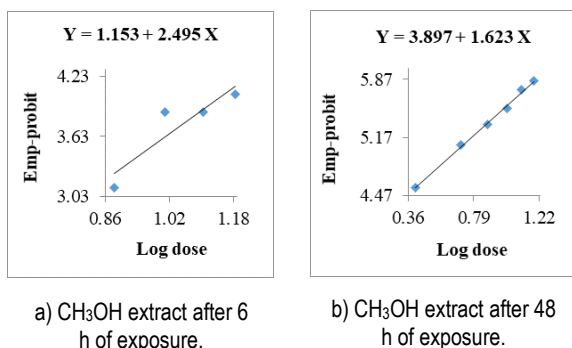


Fig. 3 (a-b): Regression lines of *C. arietinum* extracts against *T. castaneum* at the minimum and maximum hours of exposure.

Repellent effects of *C. arietinum* on *C. chinensis*, *S. oryzae*, and *T. castaneum*

The results of repellent activity of the PE, CHCl₃, and CH₃OH extracts of *C. arietinum* against three test beetles are represented in Table 2.

Table 2: ANOVA results of the repellent activities of the PE, CHCl₃ and CH₃OH extracts of *C. arietinum*.

Plant	Test insects	Solvents of extraction	Sources of variance	SS	df	MS	F-values	P-values
<i>C. arietinum</i>	<i>C. chinensis</i>	PE	Between doses	1544.203	4	386.051	2.128 ^{ns}	0.124
			Between time interval	863.322	4	215.830	1.190 ^{ns}	0.353
		CHCl ₃	Between doses	21.023	4	5.256	0.055 ^{ns}	0.994
			Between time interval	1259.666	4	314.916	3.279 ^{ns}	0.038
		CH ₃ OH	Between doses	2823.125	4	705.781	1.905 ^{ns}	0.159
			Between time interval	3598.080	4	899.520	2.428 ^{ns}	0.090
	<i>S. oryzae</i>	PE	Between doses	2550.044	4	637.511	1.798 ^{ns}	0.179
			Between time interval	1404.050	4	351.012	0.990 ^{ns}	0.441
		CHCl ₃	Between doses	1984.735	4	496.184	3.548 ^{ns}	0.030
			Between time interval	3949.045	4	987.261	7.060 ^{ns}	0.002
CH ₃ OH	Between doses	14395.084	4	3598.771	8.395 ^{ns}	0.001		

Contd. (Table 2)

			Between time interval	1435.499	4	358.875	0.837 ^{ns}	0.521
	T. castaneum	PE	Between doses	7993.522	4	1998.381	3.113 ^{ns}	0.045
			Between time interval	4025.411	4	1006.353	1.568 ^{ns}	0.231
		CHCl ₃	Between doses	12390.930	4	3097.732	4.791 ^{ns}	0.010
			Between time interval	8962.655	4	2240.664	3.465 ^{ns}	0.032
		CH ₃ OH	Between doses	5483.574	4	1370.894	2.516 ^{ns}	0.082
			Between time interval	1660.414	4	415.104	0.762 ^{ns}	0.565

** = Significant at 1% level ($P < 0.01$), * = Significant at 5% level ($P < 0.05$), ns = Non-significant.

The repellency tests of the PE, CHCl₃ and CH₃OH extracts of *C. arietinum* were executed on the experimental beetles (Table 2). However, none of the extracts of *C. arietinum* showed any repellent activity against these test insects at all.

Discussion

The outcomes of the current research receive support from the experiments involving *C. arietinum* and its related species by previous researchers. The species bears the value of good interest; however, the plant is heavily studied, and has some of the most sensitive species to disease and pests. Chickpea is additionally assaulted by different insect species, most remarkably the pod drill, *Helicoverpa armigera* (Lepidoptera: Noctuidae) (Stevenson and Aslam 2006), and some works depicted insecticidal activity of T₀ transgenic chickpea plants showing Bt-Cry protein was evaluated through leaf-feeding bioassay using second instar larvae of *H. armigera* (Mehrotra et al. 2011), which supports this investigation's findings. Additionally, the most trademark actual quality of *Cicer* is the abundant exudation from leaf hairs of certain species, particularly *C. arietinum*. The organization of this exudate is exceptionally acidic (pH < 2) has been viewed as a significant segment in the obstruction of the plant to pests (Simmonds and Stevenson 2001; Rembold 1981). The current investigation's results are likewise supported by the findings of Arisawa et al. (1985) which reported that chickpea oil contains tocotrienols, tocopherols, and steroids that have anti-ulcerative, anti-bacterial, anti-fungal, and anti-tumor properties. Furthermore, this plant and its seeds proteins have a sufficient equilibrium of amino acids, standing apart Glu, Asp, Arg, Leu, Phe, Lys, and Ser in a minor extent are His, Gly, Tre, Ala, Tyr, Val, Ile (Xiao et al. 2015, Cortes-Giraldo et al. 2016). Pangamic acid (6-0-(dimethylaminoacetyl)-D-gluconic acid) has also been isolated from *C. arietinum* and it is accounted to have anti-stress and anti-hyperlipidemic activity (Singh et al. 1983). Also, there are numerous confirmations of cancer prevention agents, antimicrobial, phytotoxic, and properties of *C. arietinum* (Chino et al. 2017, Chalamaiah et al. 2018). Antioxidant and anti-proliferative effects also have been identified in this plant (Marcela et al. 2016). Plants, on the other hand, have been used to prevent or treat infectious diseases for a long time; henceforth, somewhat recently, some antimicrobial vegetal proteins have been recognized that can be used to prevent the advancement of microorganisms (Kan et al. 2010). A few years ago, two peptides were secluded from chickpea seeds, called cicerin (8.2 kDa) and arietin (5.6 kDa); these peptides introduced antifungal action against *Mycosphaerella arachidicola*, *Fusarium oxysporum*, and *Botrytis cinerea*; curiously,

and the arietin showed preferred activity over cicerin (Ye et al. 2002). Finally, the findings of Rao et al. (2014) revealed that the extract of *C. arietinum* was shown to have anti-inflammatory and analgesic effects on rats which also support the results of the present investigation.

Conclusion

The results of the dose-mortality and repellency of the plant *C. arietinum* against *C. chinensis*, *S. oryzae*, and *T. castaneum* eventually lead to the conclusion that the plant contains potent bioactive compounds that might be successful in controlling the stored product pests. Since these plant compounds are natural, they could be biodegradable, and therefore, safe and sustainable for the environment as well as economically viable. Furthermore, additional studies are required to isolate and characterize the active principles that produce the insecticidal effect as well as to understand the mechanisms of its action.

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