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Potential of four indigenous plant extracts against the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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ABSTRACT

Potential of fourindigenous plant extracts against the 5th instar larvae of red flour beetle, *Tribolium castaneum* (Herbst), one of the very destructive and serious pests of many stored grain and flours, were carried out in the laboratory conditions (30 ±1.0ºC and 70 ±5% RH). Five concentrations (7.07, 3.54, 1.77, 0.88 and 0.44 mg/cm^2) along with control were made with methanol solvent to evaluate the toxicity effect of neem, thorn apple,custard apple and castor plant extracts at different time intervals. The plant extracts applied were effective in checking insect infestation and had different toxicity against the 5th instar larvae.Larval mortality indicated that castor plant extract showed the highest toxic effect (average mortality 45.77%) but the lowest toxicity was found in the thorn apple leaf extracts (average mortality 25.14%).The larval mortality significantly differed among the concentrations and dose dependent. No larval mortality was observed at untreated control up to 72 HATs. Larval mortality was also observed directly proportional to the level of concentrations and to the exposure period. Probit analysis also revealed the highest toxicity of castor plant extract.The order of effectiveness of plant extract was found as: castor > custard apple > neem> thorn apple. From the results, we concluded that extract of castor gave comparatively the highest mortality of 5th instar larvae after exposure of 72 hours and the lowest were recorded in the extract of thorn apple**.**

Keywords: Insect mortality, larvae, LD_{50},*Tribolium castaneum*(Herbst)

INTRODUCTION

Red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is one of the most comprehensive and destructive pests of stored products, feeding on different stored grain and grain products (Mishra et al. 2012a, 2012b). This species is found in association with different types of commodities such as grains, peas, beans, cocoa, nuts, dried fruits and spices, but milled grain products such as flour is the most preferred food (Hill 2002). Red flour beetles are believed to have Indo-Australian roots and prefer temperate areas, but can survive colder temperatures as well. Flour beetles get their name because they most commonly infest flour and other grains. This pest is not able to eat whole grains, because their mouthparts are not adapted to feed on hard whole grain. They do feed on dockage, fines and grain dust. They are attracted to grain with high moisture content where they encourage mold growth and produce a displeasing, musty odor (Hussain et al. 1994). *Tribolium castaneum* causes damage directly to kernels (germ and endosperm). Direct feeding damage by insects reduces grain weight, nutritional value and germination of stored grain. Infestation also causes contamination, odor, mold and heat-damage problems that reduce the quality of the grain and may make it unfit for processing into feed for humans and animals (Mondal 1994). The fourth and fifth instars larvae are highly active in the rainy season and cause a very high infestation. Commercial grain buyers may refuse to accept delivery of insect contaminated grain or may pay a reduced price. Therefore, safe storage of grains and food products against insect damage is of serious concern (Haq et al. 2005).

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Currently, various types of preventive and curative control measures are practiced to protect this insect pest. Among them, chemical pesticides and fumigation with chemicals are the common method and has been used for a long time, but has serious drawbacks. Indiscriminate and abandoned use of these chemicals causes great environmental hazards due to their persistent nature, increased risk of neurotoxic, carcinogenic, teratogenic and mutagenic effects in non-target animals (Bakkali et al. 2008, Ayaz et al. 2010). To protect stored grains from insect infestation exploration of other alternatives becomes quite essential. In this regard, pesticides formulated with indigenous plant extracts are in practice as a safer alternative and have become part of leading research all over the world (Silva et al. 2002, Clemente et al. 2003).

Plants make up a rich source of many secondary metabolites, alkaloids, bioactive compounds that might act toxic on the insect physiological system and kill those (Kim et al. 2005). Recent studies have demonstrated the insecticidal properties of a chemical that derived from plants are active against specific target species are biodegradable and potentially suitable for use in integrated pest management program (Tare et al. 2004). Plant extracts contain compounds that show ovicidal, repellent, antifeedant and toxic effects in insects (Isman 2006). Numerous studies conducted on the toxicity effects of neem, thorn apple, castor and custard apple leaf extracts against the adult stages of red flour beetle (Isman 2006, Zahir et al. 2010, Joel 2015) but a few research works have been focused on the efficacies of plant extracts directly on the larval mortality of red flour beetle (Yasir et al. 2012, Bachchu et al. 2017). Hence, the present study was, therefore, aimed to investigate the efficacy of the four extracts obtained from thorn apple, neem, custard apple and castor plant against the 5th instar larvae of *T. castaneum.*

MATERIALS AND METHODS

The present study on the evaluation of toxicity effect of four indigenous plant extracts against the 5th instar larvae of red flour beetle, *T. castaneum* was conducted in the laboratory conditions (30 ±2ºC, 70 ±5% RH).

Collection and preparation of food medium for test insect: Healthy wheat grain, *Triticum aestivum* was purchased from the local market of Dinajpur town. Then the grain was made into flour in a grinding machine. This flour was then used as afood medium for the rearing of the red flour beetle. For the preparation of foodmedium, astandard mixture of whole flour with powdered dry yeast in a ratio of 19:1 (Park 1962, Zyromska-Rudzka 1966) was used. Before use, the food medium was sterilized at 60ºC temperature for 6 hours in an oven. After sterilization, food was not used until recovering its moisture contents (Mondal 1984). Both flour and yeast were passed through the micrometer sieve and then were mixed thoroughly for homogeneous mixing.

Collection and rearing of *Tribolium castaneum*: The adult beetles were collected from naturally infested wheat flour from the local market of Dinajpur town. Beetles were reared in a glass beaker (500 ml) with the prepared food medium. Five hundred adults in each beaker were introduced with 500 g of food. The beakers were then kept in an incubator at 30 ±0.5ºC temperature without light and humidity control. The cultures were checked in regular intervals and eggs along with larvae were separated to increase the population properly. For the easy movement of the adults and to avoid the cannibalism of eggs, a crumpled filter paper was placed inside the beaker. The beakers were covered with pieces of muslin cloth tightly fixed with the help of rubber bands to avoid the possible escape of the beetles. New cultures were set up each alternate week for the continuation of the culture throughout the experimental period.

Collection of eggs and determination of larval instars: For egg collection, about 500 beetles were placed in a beaker containing prepared standard food medium. The beaker was covered with a cloth and kept in an incubator at 30ºC. In the regular intervals, the eggs were collected by sieving the food medium with the help of two separate sieves of 500 and 250 µm mesh separating the adults and eggs, respectively following the methods of Khan and Selman (1981). Mainly 250 µm sieves were used for egg collection. The collected eggs were kept in 50 mm diameter glass Petri dishes and incubated at the same temperature (30ºC). Eggs were hatched after 3 – 5 days and the newly hatched larvae were collected with a fine camel hair brush and then shift to the fresh food medium for culture. The larval instars were determined by counting the number of exuviate (larval skin) deposited in the food medium (Mondal 1984). The 2nd day larvae were found as first instar larvae while the second, third, fourth, fifth, and sixth instar larvae were found from the larval culture on 3rd, 6th, 9th, 12th, and 16th day after hatching, respectively. The larval culture was also maintained in an incubator in the temperature at 30 ±0.5ºC without light and humidity control. The food medium was replaced every 3 days interval to avoid conditioning by the larvae.

Collection and preparation of plant extract: Fresh mature plant leaves of custard apple (*Annona reticulata*), neem (*Azadirachta indica*), castor (*Ricinus communis*) and thorn apple (*Datura stramonium*) were collected from the HSTU campus, Dinajpur, Bangladesh. The collected leaves were dried separately in shade followed by sun dried for 4 hours. They were then made powder separately by an electric grinder (Nova Blackberry Blender, AD999, Bangladesh) in the laboratory. The leaves powder was passed through a 60-mesh sieve to get a very fine powder. The powdered materials were subjected to extract preparation. Methanol was used as a solvent for extract preparation. A hundred grams of every plant powders were taken separately in a 500 ml glass made conical flask and mixed with 300 ml of methanol solvent (Bachchu et al. 2013). The mixture was stirred for 30 minutes in a magnetic stirrer (600 rpm) and left to stand for 72 hours with shaking at several intervals. After that, the mixture was filtered through a filter paper (Whatman no. 1) and was allowed to evaporate with the help of a rotary evaporator (Lab Tech EV311H Rotary Evaporator, Manufactured in China). The extracts were preserved in tightly corked glass vials in a refrigerator for further experimental use.

Bioassay test (mortality test): For larval bioassay, residual film method was used to toxicity test of different plant extracts (Busvine 1971). The crude extracts were weighted in the electronic balance and dissolved in methanol solvent for making desired concentrations. Five concentrations (7.07, 3.54, 1.77, 0.88, 0.44 mg/cm^2) were made with each plant extracts against larvae. Prior to conducting the study, a pilot experiment was done to obtain the appropriate dose. Then 1 ml plant extract of each dose was dropped separately on Petri dishes (60 mm) with the help of a pipette, covering uniformly the whole area of the Petri dish internally. Petri dishes were kept open to evaporate the solvents fully. Five pairs of 5th instar larvae were released in each Petri dish. Only methanol solvent was used for the control treatment. Three replications were made for each dose of all the treatments including control. Petri dishes were then kept without food in the laboratory and larval mortality was recorded at 12, 24, 36, 48, 60 and 72 hours after treatments. The percentage of mortality was corrected using Abbott’s formula (Abbott 1987).

$$P = \frac{p' - C}{100 - C} \times 100$$

Where,

P= Percentage of corrected mortality

P'= Observed mortality (%)

C= Mortality (%) at control.

Statistical analysis: The data were statistically analyzed by MSTATC program. The significance of the mean difference was tested by DMRT. The observed mortality was also subjected to probit analysis.

RESULTS AND DISCUSSION

Toxicity effect of different plant extracts against 5th instar larvae of the red flour beetle: Average mortality percentage of red flour beetle at different hours after treatment indicated that castor plant extract possessed the highest (mortality, 45.77%) toxic effect and thorn apple plant extract possessed the lowest (mortality, 25.14%) toxic effect (Table 1). There were a significantly different (p<0.01) among the toxicity of four plant extracts applied against 5th instar larvae of *T. castaneum.* Mortality percentages were directly proportional to the time after treatment. The orders of toxicity effect of four plant extracts were found as: castor > custard apple > neem> thorn apple. The mortality percentage of beetle also differed significantly among all the concentration levels at a different time intervals (Table 1). The highest mortality (55.00%) was found at the maximum concentration (7.07 mg/cm^2) of plant extract. It was also observed that average mortality percentages were directly proportional to the level of concentration of different plant extracts. The Interaction effect of plant, dose and time is presented in Table 2. Mortality percentages of different plant extracts of different dose level at different hours were found statistically significant (P< 0.001). The highest mortality was recorded in castor (mortality 96.67%) at the dose of 1.77, 3.54 and 7.07 mg/cm^2 followed by neem and thorn apple (mortality 96.3%) of the dose of 7.07 mg/cm^2 at 72 HATs respectively which was not statistically different (Table 2). No larval mortality was recorded up to 72 HATs in the untreated control.

Extracts of four plants of castor, neem, thorn apple and custard apple were applied to determine their possible toxic effects against the 5th instar larvae of *T. castaneum.* Results from the present study indicates that all the tested botanical extracts had toxic effects against the 5th instar larvae in laboratory conditions but extract ofcastor was proved comparatively more effective and caused 96.67% larval mortality of *T.castaneum* at 1.77% concentration after 72 hours exposure time (Table 2). The finding of the current study was close to those of Bachchu et al. (2017) in where they found 100% and 96.67 % mortalities of 6th instar larvae of *T. castaneum* with extract of *A. reticulata* and *R. cummunis* after 72 hours of treatment application under laboratory conditions (30 ± 0.5 ºC temperature). Our findings were also similar to Singh and Kaur (2016) who found 72% mortality against *Musca domestica* with methanol extract of *R. communis*. A slight difference may be due to different insect species.Castor bean contains thealkaloid ricinin, the polyphenolic molecule epicatechin and fatty acids in their leaves (Zahir et al. 2012) all of which have insecticidal properties. The biological activity of castor plant extracts might be attributed to its alkaloid contents such as saponins, lectins, trypsin inhibitor etc. which caused mortality to the larvae. Mortality of the tested extracts were concentrations and exposure time dependent under laboratory conditions.A significant level of success of potential suppression of the larval population as reported by various researchers with different botanicals including *R. communis*, *A. reticulata*,*A. indica* and *D. stramonium* (Babarinde et al. 2008b**,** Okonkwo and Okoye 1992,Babarinde et al. 2011**,** Tripathi et al. 2000**,** Lale and Alaga 2001, Neghaban et al. 2007,Babarinde and Ogunkeyede 2008, Babarinde and Adeyemo 2010. The result of this study indicates that all levels of tested plant extracts at 0.44% to 7.07 % concentrations had shown sufficient insecticidal effects (Table 2).Though all plant extracts showed potential but castor plant extract exerted promising toxicity by applying the highest concentration (7.07%) against the 5th instar larvae of *T. castaneum*. *Ricinus communis* has been reported to have insecticidal properties against different species of insects (Babarinde et al. 2008b, Okonkwo and Okoye 1992, Babu et al. 1989). Several formulations of its products have been experimented. Babarinde et al. (2008b) reported the bioactivity of *R. communis* aqueous extract against termites, *Nasutitermes* species. Okonkwo and Okoye (1992) reported the efficacy of *R. communis* leaf powder against *C. maculatus*. Babu et al. (1989) reported the efficacy of *Ricinus* oil against *C. chinensis*, while Salas and Hernandez (1985) reported its efficacy against *Acanthoscelides obtectus*.

Table 1. Toxicity effect of different plant extracts and their concentrations against 5th instar larvae of *Tribolium castaneum* at different HATs

Plant extract used	Percentage of larval mortality at different time intervals						
	12HAT	24HAT	36HAT	48HAT	60HAT	72HAT	Aver.mort.
Neem	4.44 c	5.00 d	13.33 c	24.44 c	40.86 c	63.58 b	25.28 c
Thorn apple	7.77 bc	8.88 c	11.67 c	21.11 c	41.54 c	59.88 b	25.14 c
Custard apple	12.78 a	26.67 a	38.33 b	49.26 b	54.17 b	63.49 b	40.78 b
Castor	11.1 ab	17.78 b	43.33 a	58.64 a	66.17 a	77.59 a	45.77 a
LSD	3.351	3.869	3.620	5.538	5.762	5.048	2.740
CV (%)	55.38	39.59	20.25	21.54	16.96	11.39	11.94
Doses (mg/cm^2)							
7.07	18.33 a	29.17 a	44.17 a	62.04 a	80.14 a	96.12 a	55.00 a
3.54	14.17 b	22.50 b	38.33 b	56.30 a	72.20 b	93.35 a	49.48 b
1.77	10.83 b	16.67 c	32.50 c	47.78 b	62.85 c	78.14 b	41.46 c
0.88	6.667 c	11.67 d	24.17 d	34.72 c	50.00 d	68.71 c	32.65 d
0.44	4.167 c	7.500 d	20.83 d	29.35 c	38.94 e	60.50 d	26.88 e
Control	0.00 d	0.00 e	0.00 e	0.00 d	0.00 f	0.00 e	0.00 f
LSD	4.104	4.739	4.433	6.782	7.057	6.183	3.356
CV (%)	55.38	39.59	20.25	21.54	16.96	11.39	11.94

HAT = Hour after treatment. Within column values followed by different letter(s) are significantly different at 5% level of probability by DMRT.

Probit analysis for direct toxic effect of different plant extracts against 5th instar larvae of the red flour beetle: The result of the probit analysis for the estimation of LD_{50} values and their 95% fiducially limits at 12, 24, 36, 48, 60 and 72 HAT of different plant extracts against 5th instar larvae of red flour beetle are presented in Table 3. The LD_{50} values at 12 and 24 HAT indicated that thorn apple (45.60 and 5.38 mg/cm^2) plant extract was the most toxic effect. On the other hand, LD_{50} values at 36, 48, 60 and 72 HAT indicated that castor (1.57, 0.61, 0.34 and 0.010 mg/cm^2) plant extract was the more toxic effect The chi-square values were insignificant at 5% level of probability of different plant extracts at different HAT and mortality data did not show any heterogeneity.

The reduction of larval population by using the leaf extracts of *R. communis* is also similar to the previous findings of Basheer (2014). He reported that the castor leaf extract obtained as the best with the mortality of the larvae of *Anopheles arabiensis* was 96% after 24 hours with an LC_{50} at 0.390 mg/l, 100% mortality was observed after 48 hours with LC_{50} at 0.284 mg/l. Collavino et al. (2006) reported that castor bean leaf powder is effective against male moth larvae, *Plodia interpunctella* HBN (Lepidoptera: Phycitinae).

Probit regression lines for mortality effect of different plant extracts against 5th instar larvae of red flour beetle: The probit regression lines for the effect of different plant extracts of against *T. castaneum* at 12,

Table 2. Toxicity effect of different plant extracts of different doses against 5th instar larvae of *Tribolium castaneum* at different HATs (interaction of plant, dose and time)

Plant extracts used	Doses	Percentage of larval mortality at different time intervals						
		12HAT	24HAT	36HAT	48HAT	60HAT	72HAT	Aver. mort.
Neem	7.07	10.0 cde	13.3 def	20.00 fg	37.78 e-h	67.41 bc	96.30 a	40.80 de
	3.54	6.66 def	6.66 efg	20.00 fg	34.0 e-i	60.37 cd	88.89 a	36.11 ef
	1.77	3.33 ef	3.33 fg	13.33 g	30.7 f-j	49.63 def	70.37 cd	28.46 g
	0.88	3.33 ef	3.33 fg	13.33 g	27.4 g-k	39.26 fg	66.67 cd	25.56 gh
	0.44	3.33 ef	3.33 fg	13.33 g	16.67 jk	28.52 g	59.26 de	20.74 h
	0	0.00 f	0.00 g	0.00 h	0.00 l	0.00 h	0.00 g	0.00 i
Thorn apple	7.07	13.33 cd	16.67 de	20.00 fg	34.4 e-i	68.15 bc	96.30 a	41.48 de
	3.54	10.0 cde	13.3 def	13.33 g	34.0 e-i	56.67 cde	92.59 a	36.67 def
	1.77	10.0 cde	10.0 efg	13.33 g	24.07 h-k	50.00 def	74.08 bc	30.25 fg
	0.88	6.66 def	6.66 efg	13.33 g	20.37 ijk	42.59 efg	51.85 ef	23.58 gh
	0.44	6.66 def	6.66 efg	10.00 g	13.70 k	31.85 g	44.44 f	18.89 h
	0	0.00 f	0.00 g	0.00 h	0.00 l	0.00 h	0.00 g	0.00 i
Custard apple	7.07	26.67 a	50.00 a	63.33 b	85.93 a	91.67 a	95.24 a	68.81 a
	3.54	23.33 ab	43.33 ab	56.6 bc	67.41 bc	79.17 ab	95.24 a	60.86 b
	1.77	16.67 bc	36.67 b	50.00 c	57.04 cd	62.50 cd	71.43 cd	49.05 c
	0.88	10.0 cde	23.33 cd	33.3 de	42.59 d-g	50.00 def	66.67 cd	37.66 de
	0.44	0.00 f	6.66 efg	26.6 ef	42.59 d-g	41.67 efg	52.38 ef	28.33 g
	0	0.00 f	0.00 g	0.00 h	0.00 l	0.00 h	0.00 g	0.00 i
Castor	7.07	23.33 ab	36.67 b	73.33 a	90.00 a	93.33 a	96.67 a	68.89 a
	3.54	16.67 bc	26.67 c	63.33 b	89.63 a	92.59 a	96.67 a	64.26 ab
	1.77	13.33 cd	16.67 de	53.33 c	79.26 ab	89.26 a	96.67 a	58.09 b
	0.88	6.66 def	13.3 def	36.67 d	48.52 de	68.15 bc	89.63 a	43.83 cd
	0.44	6.66 def	13.3 def	33.33de	44.44 def	53.71 c-f	85.93 ab	39.57 de
	0	0.00 f	0.00 g	0.00 h	0.00 l	0.00 h	0.00 g	0.00 i
LSD		8.208	9.478	8.866	13.56	14.11	12.37	6.712
CV (%)		55.38	39.59	20.25	21.54	16.96	11.39	11.94

HAT = Hour after treatment. Within column values followed by different letter(s) are significantly different at 5% level of probability by DMRT.

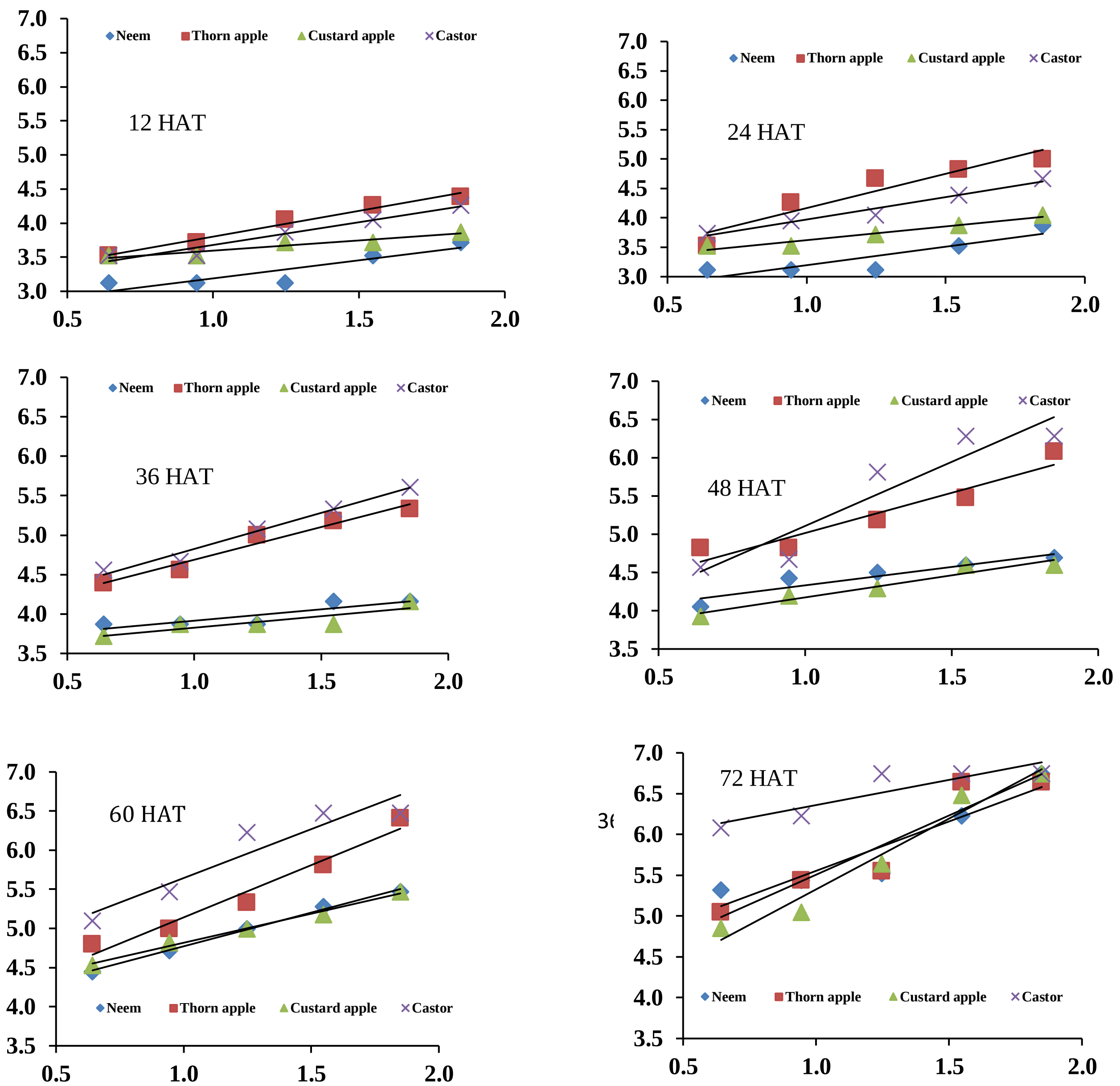

Figure 1. Relationship between probit mortality and log doses of plant extracts on red flour beetle, *Tribolium castaneum*at 12, 24, 36, 48, 60 and 72 HATs

24, 36, 48, 60 and 72 HAT are shown in Figure 1. The rate of mortality of the red flour beetle showed positive correlations with the doses in all cases. Comparing among four lines, the regression line for thorn apple extract showed the highest probit mortality and neem plant extract showed the lowest probit mortality at 12 and 24 HATs. But at 36, 48, 60 and 72 HATs, comparing among the four lines, the regression line for castor plant extract showed the highest mortality effect whereas neem plant extract showed the lowest mortality effect. The probit regression lines for the effects of different plant extracts against red flour beetle showed a clear relationship between probit mortality and their doses and the probit regression lines become sleeperas doses increased because the 5th instar larvae were treated with more toxins for the same period at higher doses.

Novel innovative research illustrated that diverse plant products have been tried by several researchers with a good degree of success against *T castaneum* (Bachchu et al. 2017, Rehman et al. 2018, Mamun et al. 2009). In the present study at 36, 48, 60 and 72 HATs comparingamong the four lines, the regression line for castor plant

Table 3. Relative toxicity (by probit analysis) of different plant extracts treated against5th instar larvae of *Tribolium castaneum* after 12, 24, 36, 48, 60 and 72HATs

Plant extracts used	No. of larvae used	LD_{50} values (mg/cm^2)	95% fiducial limits		χ^2 values at 3 df
			Lower	Upper	
		12 HAT			
Neem	30	1242.25	0.12	$1.21E^{+07}$	0.34
Thorn apple	30	45.60	1.73	1195.19	0.13
Custard apple	30	60150.67	$1.56E^{-06}$	$2.30E^{+15}$	0.10
Castor	30	100.92	2.27	4479.55	0.19
		24 HAT			
Neem	30	327.04	0.93	114506.1	0.61
Thorn apple	30	5.38	2.75	10.54	2.11
Custard apple	30	959.88	0.13	6798599	0.10
Castor	30	25.87	3.09	216.41	0.55
		36 HAT			
Neem	30	4413.47	$9.80E^{-04}$	$1.98E^{+10}$	0.28
Thorn apple	30	2.40	1.31	4.39	0.55
Custard apple	30	8282.26	$3.30E^{-04}$	$2.07E^{+11}$	0.29
Castor	30	1.57	0.92	2.66	0.32
		48 HAT			
Neem	30	28.45	1.13	711.10	0.49
Thorn apple	30	0.97	0.57	1.66	1.95
Custard apple	30	30.03	1.86	483.57	0.41
Castor	30	0.61	0.37	1.01	2.32
		60 HAT			
Neem	30	1.85	1.06	3.22	0.13
Thorn apple	30	0.79	0.48	1.29	0.79
Custard apple	30	1.77	0.94	3.32	0.06
Castor	30	0.34	0.16	0.71	2.03
		72 HAT			
Neem	30	0.36	0.16	0.80	2.01
Thorn apple	30	0.45	0.25	0.83	2.34
Custard apple	30	0.64	0.43	0.97	1.09
Castor	30	0.01	$5.09E^{-05}$	2.15	0.45

HAT = Hour after treatment, Values were based on five concentrations, three replications of 10 insects each.
χ^2 = Goodness of fit, The tabulated value of $\chi^2$is 7.815 (d.f = 3 at 5% level

extract showed the highest mortality effect whereas neem plant extract showed the lowest mortality effect (Figure 1). The Ricin is a protein toxin that is extracted from the castor bean (*Ricinus communis*). It is poisonous if inhaled, injected, or ingested, acting as a toxin by the inhibition of protein synthesis. Deaths caused by the ingestion of castor oil plant seeds are rare (Aplin and Eliseo 1997). The Ricin may be the main properties of castor oil that control the stored beetle.

CONCLUSION

Botanical extracts used in the present study had a direct toxic effect on the 5th instar larvae of *T. castaneum*. Among the tested plants, castor extracts showed the highest toxic effect. The larvicidal potential of indigenous plant extracts against *T. castaneum* has good prospects. The result of the present study suggests that castor plant extracts possess compounds with high bioactive properties that can be used as agents of biocontrol in designing and developing new insecticides. It has also recommended further study on other biological agents for the innovation of environment friendly insecticides.

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