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Lethal and toxic effects of some botanicals against the larvae of *Tribolium castaneum* (Herbst)

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ABSTRACT

A study was carried out on the lethal and toxic effects of neem, thorn apple, custard apple and castor plant extracts at 12, 24, 36, 48, 60 and 72 hour after treatment (HAT) interval against the 3rd and 4th instar larvae of red flour beetle, *Tribolium castaneum* (Herbst) in the ambient laboratory conditions. Five doses viz., 1.77, 0.88, 0.44, 0.22, 0.11 and 3.54, 1.77, 0.88, 0.44, 0.22 along with control were made with methanol solvent for the 3rd and 4th inatar larvae, respectively. Larval mortality of castor plant extract showed the highest toxic effect in both the larval instar (3rd and 4th.) (average mortality 47.60% and 47.75%, respectively) whereas the lowest toxicity was found in the thorn apple leaf extracts (average mortality 20.14% and 30.26%, respectively). The larval mortality was significantly differed among all the concentrations of the plant extracts applied. The highest mortality of 96.67% was recorded at the concentration of 1.77 mg/cm^2 in both 3rd and 4th larval instar at 72 HAT in castor plant extract. No larval mortality was observed in untreated control up to 72 HATs. Mortality percentage was also observed directly proportional to the level of concentrations of plant extracts and to the exposure period.

Keywords: Insect mortality, laboratory, larvae, plant extract, Red flour beetle

INTRODUCTION

Stored food pests are responsible for commercial loss every year. The global post-harvest grain losses caused by insect pest damage and other bio-agents ranged from 10 to 40% (Papachristos and Stamopoulos 2002). Safe storage of grains and food products against insect damage is the serious concern (Haq et al. 2005). It has been estimated that about 9% grain production of the world is lost to post harvest insect and mite's infestation (Rahman et al. 2009). About 600 species of beetle pests cause quantitative and qualitative losses of agricultural store products (Rajendran and Sriranjini 2008). These insect pests are the main responsible of grain losses during post harvest storage, particularly in the tropical countries (Talukder 2006). It was estimated that more than one-third of the food products are loss to various pests during post-harvest storage and are the most serious problem, specifically in the developing countries like Bangladesh (Talukder et al. 2004, Tripathi et al. 2009).

Among the storage pests, red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is one of the most destructive pests of stored products and 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, Mishra et al. 2012). Flour beetles get their name because they most commonly infest flour and other grains. They feed on dockage, fines and grain dust. Infestation also cause contamination, odor, mold and heat-damage problems that reduce the

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quality of the grain and may make it unfit for processing into feed for humans and animals (Mondal 1994). During the last few decades fumigants mainly phosphine and methyl bromide played a key role in the control of several stored grain pests but methyl bromide residues have been found to persist in certain fumigated food products (Norman 2000). However, application of chemicals has led to a number of severe problems such as environmental deterioration due to chemical residues, insect resistance against these repeatedly used chemicals, deterioration of food grains due to residues and affect to the non-target organisms in the surroundings (Grunwald et al. 2014). Considering the health hazards and ecological changes has forced the researchers to find the new ways of stored grains insect pests' management. In recent years, a lot of research has been done to searching environmental safe compounds for controlling of store grain pest.

Botanicals products have been successfully exploited as insecticides, insect repellents and insect antifeedants (Bachchu et al. 2017, Ara 2015). These botanical products are safe to use for human beings and have minimum effects on the ecosystem. There are a large number of studies conducted on the toxicity effects of neem, dhutara, castor and custard apple plant extracts against the adult stages of stored grain pests like red flour beetle (Zahir et al. 2010, Joel 2015) but research works have been focused with the efficacies of plant extracts directly on the larval mortality of red flour beetle are suprisingly very few (Yasir et al. 2012, Bachchu et al 2017, Kismot et al. 2021). Therefore, the present study was undertaken to evaluate the lethal and toxic effect of thorn apple, neem, custard apple and castor plant extracts against the 3rd and 4th instar larvae of *Tribolium castaneum* under laboratory conditions.

MATERIALS AND METHODS

Location of the study: The present study on the lethal and toxic effect of four indigenous plant extracts against the 3rd and 4th instar larvae of red flour beetle, *Tribolium castaneum* (Herbst) was conducted in the laboratory, Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during the period of February to July 2016.

Collection and preparation of food medium: Healthy wheat grain, *Triticum aestivum* was purchased from the local market of Dinajpur town. After purchased the grain was made flour in a grinding machine. This flour was then used for food medium to rearing of red flour beetle. Standard mixture of whole wheat flour with powdered dry yeast in a ratio of 19:1 (Park and Frank 1948, Park 1962, Zyromska-Rudzka 1966) was used as food medium. Food medium was sterilized at 60 ^{0}c temperature for 6 hours in an oven, before use. After sterilization food was not used until at least 15 days to recover its moisture contents (Khan and Selman 1981, Mondal 1984). In the micrometer sieve, both flour and yeast were passed through and then were mixed thoroughly for homogeneous mixing.

Rearing of *Tribolium castaneum* and collection of eggs: The adults of *Tribolium castaneum* were collected from naturally infested wheat flour from the local market of Dinajpur. Beetles were reared in glass beaker (500 ml) with the food medium. Then the beakers were kept in an incubator at 30 ± 0.5ºC temperature without light and humidity control. About 500 adults in each beaker were introduced with 500 g of food, respectively. The cultures were checked in regular intervals and eggs along with larvae were separated to increase the population properly. A crumpled filter paper was placed inside the beaker for the easy movement of the adults and to avoid the cannibalism of eggs. The beaker was covered with a cloth and kept in an incubator at 30 ± 0.5ºC for egg collection. In regular interval, the eggs were collected by sieving the food medium by two sieves of 500 and 250 mesh separating the adults and eggs, respectively following the methods of Khan and Selman (1981). For egg collection, mainly 250µm sieves were used. The collected eggs were kept in 50 mm in diameter Petri dishes and incubated at the same temperature (30 ± 0.5^{0}C).

Determination of larval instars: The 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. Larval culture was also maintained in an incubator in the temperature at 30^{0} c ± 0.5^{0} c without light and humidity control. The food medium was replaced by three days interval to avoid conditioning by the larvae.

Preparation of plant extract: The fresh plant leaves of custard apple (*Annona reticulata*), neem (*Azadirachta indica*), castor (*Ricinus communis*) and thorn apple (*Datura stramonium*) were collected from the HSTU campus and the nearest of Dinajpur. After collection, they were dried in shade followed by one day sun dried for 4 hours. Powders were made separately by an electric grinder in the laboratory. For extract preparation, 100 g of every plant powders were taken separately in a 500 ml conical flask and mixed with 300 ml of methanol. The mixture was stirred for 30 minutes and allowed to shaking in the shaker machine for 24 hours. Then the mixture was filtered through a filter paper (Whatman no. 1) and allowed to evaporate the solvents in the vacuum rotary evaporator. After evaporation, crude extracts were found which were preserved in tightly corked vials and stored in a normal refrigerator for further experimental use.

Preparation of doses: Five doses viz., 1.77, 0.88, 0.44, 0.22, 0.11 and 3.54, 1.77, 0.88, 0.44, 0.22, along with control were made with methanol solvent for the 3rd and 4th instars larvae, respectively. Prior to conducting study, pilot experiment was done to obtain the appropriate dose in different larval instars.

Bioassay test (mortality test): Residual film method (Busvine 1971) was used to toxicity test. For larval bioassay, 1 ml plant extract of each dose was dropped separately on Petri dishes (60 mm) with the help of pipette, covering uniformly the whole area of the Petri dish internally. The Petri dishes were then kept open for sometimes to evaporate the solvents fully. Ten larvae of 3rd and 4th instars were released in each Petri dish separately. Only methanol solvent was used for the control treatment. Three replications were made for each dose of all the treatments. The 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).

$$P = \frac{\acute{P} - C}{100 - C} \times 100$$

Where,
P = Percentage of corrected mortality
P' = Observed mortality (%)
C = Mortality (%) at control

Statistical analysis: The experiment was conducted using CRD. The collected data were statistically analyzed by MSTAT-C computer program. The significance of the mean difference was tested by DMRT. The observed mortality was also subjected to probit analysis.

RESULTS AND DISCUSSION

Lethal effect of different plant extracts against 3rd and 4th instars larvae of red flour beetle: Lethal effect at 12, 24, 36, 48, 60 and 72 HATs of different plant extracts against 3rd and 4th instars larvae of red flour beetle are presented in Table 1. The LD_{50} values at 12 HAT indicated that castor (142.51 mg/cm^2) plant extract was the most toxic followed by thorn apple (1133.78 mg/cm^2) plant extract while custard apple (1556.61 mg/cm^2) plant extract was the least toxic among the treatments against 3rd instar larvae. On the other hand, LD_{50} values at 24 HAT indicated that thorn apple (2.07 mg/cm^2) plant extract was the more toxic effect followed by castor (3.52 mg/cm^2) whereas custard apple plant extract showed the least toxic. Similarly, castor plant extract was also maintained its highest toxicity (0.16, 0.12, 0.0002, and 0.0014 mg/cm^2) at 36, 48, 60 and 72 HATs among all other plant extract against 3rd instar larvae. Among the treatments, LD_{50} values at 12, 24 and 48 HATs indicated that castor (55.67, 2.61 and 0.18 mg/cm^2) plant extract was the most toxic effect among the treatments against 4th instar larvae. On the other hand, LD_{50} values at 36, 60 and 72 HATs indicated that thorn apple (0.61, 0.05, and 0.033 mg/cm^2) plant extract was the more toxic effect against 4th instar larvae. The chi-square values were insignificant at 5% level of probability of different plant extracts at different HAT and mortality of custard apple did not show any heterogeneity.

Results from the present study indicated that all the tested plant extracts would be effective to control the larvae of *Tribolium castaneum* but castor plant extract will be most effective. This result is in agreement with those results reported by Bachchu et al. (2017) and Ara et al. (2021). Bachchu et al. (2017) reported that LD_{50} values (77.75, 1.50, 0.32, 0.0096, 0.0093 and 1.72E-07 mg/cm^2) of castor plant extracts against 6th instar larvae of red flour beetle at 12, 24, 36, 48, 60 and 72 HATs, respectively reveled the most toxic followed by custard apple (443.13 mg/cm^2) plant extract while thorn apple plant extract (2464.44 mg/cm^2) was the least toxic. Ara et al. (2021) found that LD_{50} values at 36, 48, 60 and 72 HATs indicated castor (1.57, 0.61, 0.34 and 0.010 mg/cm^2) plant extract was the more toxic effect against 5th instar larvae of red floor beetle. The present results is also in agreement with results reported by Ramos-Lopez et al. (2012) who evaluated the effect of ingested ricin oil, ricinin and hexanic, acetatoethylic and methanolic extracts from 16 to 24,000 ppm on first instar *Spodoptera frugiperda* larva. All treatments with ricinin (560 ppm) and acetatoethylic extracts (1600 ppm) from *R. communis* seeds had reduced weight of the pupae by 21.6% to 4.9% respectively. The present investigation is coincided with the findings of Basheer (2014) who 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.

Table 1. Relative toxicity (probit analysis) of different plant extracts treated against 3rd and 4th instar larvae of *Tribolium castaneum* after 12, 24, 36, 48, 60 and 72 HATs

Used extracts	No of larvae used	LD_{50} values (mg/cm^2)		95% fiducially limits				χ^2 values with 3 df	
				3rd instar		4th instar			
		3rd instar	4th instar	Lower	Upper	Lower	Upper	3rd instar	4th instar
				12HAT					
Neem	30	1331.77	11123.9	0.0011	$1.53E^{+09}$	$4.89E^{-05}$	$2.52E^{+12}$	0.91	0.31
Thorn apple	30	1133.78	$1.43E^{+11}$	0.0010	$1.17E^{+09}$	$3.36E^{-20}$	$6.11E^{+37}$	0.40	0.51
Custard apple	30	1556.61	30390.2	$8.98E^{-04}$	$2.69E^{+09}$	$7.62E^{-07}$	$1.21E^{+15}$	0.40	0.10
Castor	30	142.51	55.67	0.045	448081.4	0.44	6949.28	0.15	0.21
				24HAT					
Neem	30	523.62	152.25	0.01	$2.69E^{+07}$	0.21	105386.6	0.28	0.17
Thorn apple	30	2.07	18.29	0.58	7.31	1.19	280.65	0.90	0.41
Custard apple	30	15053.2	32.41	$3.79E^{-07}$	$5.96E^{+14}$	1.25	840.02	0.11	0.80
Castor	30	3.52	2.61	0.099	125.17	0.48	14.05	0.09	0.11
				36HAT					
Neem	30	7.06	8.87	0.37	133.01	1.17	66.99	0.18	0.39
Thorn apple	30	0.59	0.61	0.28	1.24	0.23	1.61	0.26	0.62
Custard apple	30	430.16	4.55	0.0047	$3922E^{+07}$	1.43	14.46	0.06	0.21
Castor	30	0.16	0.90	0.057	0.49	0.47	1.72	0.07	0.39
				48HAT					
Neem	30	0.61	1.13	0.35	1.07	0.61	2.08	0.70	1.39
Thorn apple	30	0.13	0.29	0.056	0.32	0.09	0.92	0.10	2.17
Custard apple	30	242.17	0.89	0.0049	$1.18E^{+07}$	0.46	1.72	0.12	0.03
Castor	30	0.12	0.18	0.031	0.50	0.03	1.11	0.05	0.29
				60HAT					
Neem	30	0.29	0.69	0.16	0.52	0.45	1.07	0.38	1.44
Thorn apple	30	0.009	0.05	0.033	0.29	0.004	0.71	0.19	0.13
Custard apple	30	1.31	0.36	0.41	4.18	0.08	1.52	0.13	2.07
Castor	30	0.0002	0.10	$1.55E^{-06}$	5.77	0.03	0.34	0.09	0.30
				72HAT					
Neem	30	0.0246	0.28	0.0024	0.24	0.15	0.51	0.11	1.54
Thorn apple	30	0.0364	0.033	0.0050	0.26	0.002	0.47	0.87	0.19
Custard apple	30	0.35	0.15	0.18	0.67	0.050	0.45	0.42	2.72
Castor	30	0.0014	0.041	$1.46E^{-06}$	1.41	0.005	0.29	0.37	0.73

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 χ^2 is 7.815 (d. f = 3 at 5% level).

Toxicity effect of different plant extracts against 3rd and 4th instar larvae of red flour beetle: From the table 2 it was observed that castor plant extract showed the highest larval mortality of 74.72 and 74.12% at 72 HAT against 3rd and 4th instar larvae, respectively. On the other hand the lowest larval mortality was recorded 44.14% in thorn apple at 72 HAT against 3rd instar larvae where incase of 4th instar larvae the lowest mortality was recorded in neem plant extracts (58.03%) at 72 HAT. On the basis of average mortality, the order (highest to lowest) of toxicity effect of four plant extracts against the 3rd instar larvae of red flour beetle were found as: castor > custard apple > neem > thorn apple. But incase of 4th instar larvae, the order of toxicity was found as castor > custard apple > thorn apple > neem. The mortality percentages of four plant extracts were directly proportional to the time after treatment.

Toxicity effect of different concentration of plant extracts against 3rd and 4th instars larvae of red flour beetle: The from the Table 3 it was revealed that the highest and lowest mortality (15.00 and 5.83%) was recorded at the concentration of 1.77 and 0.11 mg/cm^2 of plant extract at 12 HAT against 3rd instar larvae. On the other hand, the highest and lowest mortality (14.17 and 6.66%) was recorded at the concentration of 3.54 and 0.22 mg/cm^2 of plant extract at 12 HAT against 4th instar larvae. Larval mortality percentage was increased proportional to the level of concentrations. At 72 HAT, the highest larval mortality was recorded 86.19 and 95.46 % at the concentration of 1.77 and 3.54 mg/cm^2 against 3rd and 4th instars larvae, respectively. But the lowest larval mortality (62.69 and 64.17%) was recorded at the concentration of 0.11 and 0.22 mg/cm^2 against 3rd and 4th instars larvae, respectively at 72 HAT. There was no insect mortality was noticed at control treatments up to 72 HAT against both the 3rd and 4th instars larvae.

Table 2. Toxicity effect of different plant extracts against 3rd and 4th instars larvae of *Tribolium castaneum* at different HAT (interaction effects of plant extracts and time)

Plant extracts used	Percentage of larval mortality at different time intervals						Average mortality
	12HAT	24HAT	36HAT	48 HAT	60HAT	72 HAT	
3rd instar larvae							
Neem	3.88 c	5.00 d	23.33 c	37.78 b	46.67 c	66.48 b	30.52 c
Thorn apple	6.11 bc	8.88 c	12.22 d	16.67 c	32.78 d	44.14 c	20.14 d
Custard apple	8.88 b	27.22 b	39.44 b	54.44 a	57.33 b	63.59 b	41.82 b
Castor	12.78 a	32.78 a	49.38 a	51.08 a	64.86 a	74.72 a	47.60 a
LSD	3.159	3.443	4.111	3.72	4.263	4.830	1.94
CV (%)	59.55	27.81	19.72	13.89	12.62	11.58	8.30
4th instar larvae							
Neem	3.88 c	13.33 c	22.22 b	39.01 b	45.06 b	58.03 b	30.26 c
Thorn apple	7.77 b	13.33 c	24.44 b	41.67 b	46.85 b	61.73 b	32.63 c
Custard apple	5.00 bc	18.33 b	44.44 a	51.05 a	62.97 a	69.14 a	41.82 b
Castor	16.67 a	35.56 a	41.67 a	52.47 a	66.02 a	74.14 a	47.75 a
LSD	3.257	4.104	4.104	3.662	4.717	6.814	2.553
CV (%)	58.31	30.41	18.45	11.86	12.75	15.46	10.00

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

Table 3. Toxicity effect of plant extracts of different concentrations against 3rd and 4th instars larvae of *Tribolium castaneum* at different HAT.

Doses (mg/cm^2)	Percentage of larval mortality at different time intervals						Average mortality
	12HAT	24 HAT	36 HAT	48 HAT	60 HAT	72 HAT	
3rd instar larvae							
1.77	15.00 a	30.83 a	47.96 a	59.95 a	73.61 a	86.19 a	52.26 a
0.88	10.00 b	25.00 b	43.98 a	56.41 a	68.10 b	80.90 ab	47.40 b
0.44	8.33 b	20.00 c	36.39 b	50.28 b	61.64 c	75.05 b	41.95 c
0.22	8.33 b	19.17 c	32.04 b	40.09 c	53.54 d	68.56 c	36.96 d
0.11	5.83 b	15.83 c	26.20 c	33.22 d	45.56 e	62.69 c	31.56 e
Control	0.00 c	0.00 d	0.00 d	0.00 e	0.00 f	0.00 d	0.00 f
LSD (0.05)	3.869	4.217	5.035	4.560	5.222	5.915	2.387
CV (%)	59.55	27.81	19.72	13.89	12.62	11.58	8.30
4th instar larvae							
3.54	14.17 a	35.83 a	56.67 a	72.04 a	82.73 a	95.46 a	59.48 a
1.77	11.6 ab	26.67 b	44.17 b	62.32b	71.95 b	84.45 b	50.20 b
0.88	9.16 bc	21.6 bc	38.33 c	51.02c	66.48 b	78.98 bc	44.28 c
0.44	8.33 bc	20.00 c	33.33 c	49.35c	59.17 c	71.48 cd	40.28 d
0.22	6.66 c	16.67 c	26.67d	41.57d	51.02 d	64.17 d	34.46 e
Control	0.00 d	0.00 d	0.00 e	0.00 e	0.00 e	0.00 e	0.00 f
LSD (0.05)	3.989	5.027	5.027	4.485	5.777	8.345	3.127
CV (%)	58.31	30.41	18.45	11.86	12.75	15.46	10.00

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

From the table 4 it was found that castor plant extracts showed the highest average percent larval mortality (66.11%) at the highest dose (1.77 mg/cm^2) which was statistical different from all other plant extract at different concentration level against 3rd instar larvae and mortality percent was dose dependent in where the highest larval mortality (96.67%) was recorded in castor plant extracts followed by custard apple (91.07%) and neem (89.26%) plant extracts at the dose of 1.77 mg/cm^2 at 72 HAT which was statistical identical. No larval mortality was noticed at control treatments up to 72 HATs against the 3rd instar larvae.

Table 4. Toxicity effect of different plant extracts and their doses against 3rd 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						Average mortality
		12HAT	24HAT	36HAT	48HAT	60HAT	72HAT	
Neem	1.77	10.0 bcd	10.0 hi	36.6 fgh	63.3 cde	73.3 abc	89.26 ab	47.10 e
	0.88	3.33 de	6.66 hij	33.3 ghi	56.6 ef	66.67 cd	85.9 abc	42.10 fg
	0.44	3.33 de	6.66 hij	30.0 hij	50.0 f	60.00 de	82.59 bc	38.77 gh
	0.22	3.33 de	3.33 ij	23.3 ijk	33.3 g	46.67 f	75.56 cd	30.93 i
	0.11	3.33 de	3.33 ij	16.6 kl	23.3 h	33.33 hi	65.56 de	24.26 j
	0.00	0.00 e	0.00 j	0.00 m	0.00 i	0.00 j	0.00 h	0.00 l
Thorn apple	1.77	10.0 bcd	13.3 gh	20.0 jkl	23.33 h	51.85 ef	67.78 d	31.05 i
	0.88	10.0 bcd	13.3 gh	16.67 kl	23.33 h	44.81 fg	64.45 de	28.77 i
	0.44	6.66 cde	10.0 hi	13.33 kl	23.33 h	41.4 fgh	53.33 ef	24.69 j
	0.22	6.66 cde	10.0 hi	13.33 kl	16.67 h	34.4 ghi	46.67 f	21.30 j
	0.11	3.33 de	6.66 hij	10.00 lm	13.33 h	24.07 i	32.59 g	15.00 k
	0.00	0.00 e	0.00 j	0.00 m	0.00 i	0.00 j	0.00 h	0.00 l
Custard apple	1.77	16.67 ab	53.33 a	63.3 abc	80.00 a	84.26 ab	91.07 ab	64.78 ab
	0.88	10.0 bcd	36.6 cd	56.67 cd	76.67 ab	80.09 ab	81.55 bc	56.94 c
	0.44	10.0 bcd	26.67 ef	43.3 efg	66.67 cd	67.59 cd	72.62 cd	47.81 e
	0.22	10.0 bcd	26.67 ef	40.0 fgh	56.67 ef	59.72 de	67.86 d	43.49 f
	0.11	6.66 cde	20.0 fg	33.3 ghi	46.67 f	52.31 ef	68.45 d	37.91 h
	0.00	0.00 e	0.00 j	0.00 m	0.00 i	0.00 j	0.00 h	0.00 l
Castor	1.77	23.33 a	46.6 ab	71.85 a	73.1 abc	85.00 a	96.67 a	66.11 a
	0.88	16.67 ab	43.3 bc	69.26 ab	68.9 bcd	80.83 ab	91.67 ab	61.79 b
	0.44	13.33 bc	36.6 cd	58.8 bcd	61.1 de	77.5 abc	91.67 ab	56.53 c
	0.22	13.33 bc	36.6 cd	51.48 de	53.7 ef	73.3 abc	84.1 abc	52.11 d
	0.11	10.0 bcd	33.3 de	44.81 ef	49.5 f	72.5 bc	84.1 abc	49.06 de
	0.00	0.00 e	0.00 j	0.00 m	0.00 i	0.00 j	0.00 h	0.00 l
	LSD (0.05)	7.739	8.433	10.07	9.121	10.44	11.83	3.464
	CV (%)	59.55	27.81	19.72	13.89	12.62	11.58	8.30

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

From the table 5 it was obtained that castor plant extracts showed the highest average percent larval mortality (68.18%) at the highest dose (1.77 mg/cm^2) which was statistical different ($p< 0.01$). from all other plant extract at different concentration level against 4th instar larvae and mortality percent was dose dependent in where the highest larval mortality (96.67%) was recorded in castor plant extracts followed by thorn apple (96.30%) and neem (96.30%) plant extracts at the dose of 3.54 mg/cm^2 at 72 HAT which was statistical identical. No larval mortality was noticed at control treatments up to 72 HATs against the 4th instar larvae.

The present findings are in confirmation with Ara et al (2021) and Bachchu et al (2017) where their attempted was larvicidal efficacy of four plant extracts against 5th and 6th instar larvae of red floor beetle. They found that castor plant extracts was the most toxic against both the 5th and 6th inatar larvae of red floor beetle with larval mortality was recorded 96.67% at 72 HAT. Our findings are also similar to Singh and Kaur (2016) who found 72% mortality against *Musca domestica* with methanol extract of *R. communis*. This is a little bit difference which may be due to insect species. Castor bean contains the alkaloid 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 (Zahir et al. 2012).

Table 5. Toxicity effect of different plant extracts and their doses against 4th instar larvae of *Tribolium castaneum* at different HAT (interaction of plant, dose and time)

Plant extracts used	Doses	Percentage of larval mortality at different time intervals						
		12HAT	24HAT	36HAT	48HAT	60HAT	72HAT	Average mortality
Neem	3.54	6.66 def	23.33 def	40.00 cde	65.5 bcd	85.56 ab	96.30 a	52.90 cd
	1.77	6.66 def	20.00 efg	33.33 efg	61.8 cde	60.37 efg	77.7 abc	43.33 fgh
	0.88	3.33 ef	13.33 fg	23.33 ghi	37.78 hi	53.33 fgh	70.3 cde	33.58 ij
	0.44	3.33 ef	13.33 fg	20.00 hi	37.78 hi	42.59 h	55.56 ef	28.76 jk
	0.22	3.33 ef	10.00 gh	16.67 hi	31.11 i	28.52 i	48.15 f	22.96 k
	0	0.00 f	0.00 h	0.00 j	0.00 j	0.00 j	0.00 g	0.00 l
Thorn apple	3.54	13.33 cd	30.0 cde	46.67 bcd	66.6 bcd	75.1 bcd	96.30 a	54.69 cd
	1.77	10.00 cde	16.67 fg	36.67 def	56.67 def	53.33 fgh	74.8 bcd	41.36 gh
	0.88	10.00 cde	13.33 fg	26.67 fgh	50.00 fg	53.33 fgh	71.4 cde	37.47 hi
	0.44	6.667 def	10.00 gh	23.33 ghi	43.33 gh	49.63 gh	71.1 cde	34.01 ij
	0.22	6.667 def	10.00 gh	13.33 i	33.33 i	49.63 gh	56.6 def	28.27 jk
	0	0.00 f	0.00 h	0.00 j	0.00 j	0.00 j	0.00 g	0.00 l
Custard apple	3.54	6.66 def	36.67 bc	70.00 a	81.85 a	85.19 ab	92.59 ab	62.16 ab
	1.77	6.66 def	23.33 def	53.33 b	60.37 def	81.4 abc	88.8 abc	52.35 cde
	0.88	6.66 def	20.00 efg	53.33 b	57.04 def	77.78 bc	81.4 abc	49.38 def
	0.44	6.66 def	16.67 fg	46.67 bcd	57.04 def	70.3 cde	77.7 abc	45.86 efg
	0.22	3.33 ef	13.33 fg	43.3 bcde	50.00 fg	62.97 def	74.0 bce	41.17 gh
	0	0.00 f	0.00 h	0.00 j	0.00 j	0.00 j	0.00 g	0.00 l
Castor	3.54	30.00 a	53.33 a	70.00 a	74.08 ab	85.00 ab	96.67 a	68.18 a
	1.77	23.33 ab	46.67 ab	53.33 b	70.37 bc	92.59 a	96.30 a	63.77 a
	0.88	16.67 bc	40.00 bc	50.00 bc	59.26 def	81.4 abc	92.59 ab	56.67 bc
	0.44	16.67 bc	40.00 bc	43.3 bcde	59.26 def	74.0 bcd	81.4 abc	52.47 cde
	0.22	13.33 cd	33.33 cd	33.33 efg	51.85 efg	62.96 def	77.7 abc	45.43 fg
	0	0.00 f	0.00 h	0.00 j	0.00 j	0.00 j	0.00 g	0.00 l
	LSD (0.05)	7.977	10.05	10.05	8.970	11.55	16.69	6.255
	CV (%)	58.31	30.41	18.45	11.86	12.75	15.46	10.00

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

CONCLUSION

It is concluded that the botanicals used in the present study had different level of toxic effect on 3rd and 4th larval instar of *T. castaneum*. Among the tested plants, castor extracts showed the highest toxic effect. High mortality percentage depends on high dose of plant extract and time. The larvicideal potential of indigenous plant extracts against *T. castaneum* has good prospects. Moreover, additional studies have required to developed appropriate formulation and application method of plant based pesticides against stored product pests.

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