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Toxicity and antifeedant activities of three non edible oils against *Epilachna dodecastigma* (Wied.) (Coleoptera: Coccinellidae)

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Abstract
Epilachna dodecastigma (Wied.) is an important and serious pest of many crops and causes considerable losses both the quality and quantity of the crops output. The present study was conducted to evaluate the efficacy of three non edible oils from neem, pongamia and rohituka plants against the adult of *Epilachna dodecastigma* Wied. in the laboratory conditions (28±2 ^{0}C and 75±5% RH). Four doses viz. 250, 200, 150 and 100 mg/ml along with control were used for the determination of adult mortality and the doses of 100, 75, 50 and 25 mg/ml along with control were used to determine the antifeedant activities. All the tested oils had toxic and antifeedant activities against epilachna beetle but rohituka oil was found the most toxic as compared to others. Rohituka oil showed the highest mortality (76.67%) in maximum dose (250 mg/ml) while neem oil showed the lowest average mortality (14.33%) in minimum dose (100 mg/ml) against adult beetles. Mortality percentages were also found directly proportional to the hour after treatments and dose dependent. The pongamia oil provided the highest antifeedant index (64.39%) but rohituka oil showed the lowest (11.15%). The lowest LD_{50} values of 260.66, 170.90 and 132.62 mg/ml at 24, 48 and 72 HATs, respectively also indicated the highly effective rohituka oil against the adults of *E. dodecastigma*. Based on the mortality percentages, LD_{50} values and antifeedant activities the order of the toxicity of the oils were found as rohituka > pongamia > neem oil. The results show the insecticidal potentiality of three non edible oil as a natual and cheaper source for the control of epilachna beetle.

Keywords: *Epilachna dodecastigma*, non edible oils, toxicity, antifeedant index

1. Introduction
Epilachna beetle or Hadda beetle, *Epilachna dodecastigma* (Wied.) is fairly common and the serious pests of vegetables in Bangladesh. Due to its infestation, substantial economic loss is occurring during every crop season that adversely affecting both quality and quantity of crop yields. Infestation primarily begins just after hatching of egg mass [1] and the grubs feed on the epidermal layer of leaves making concentric markings whereas the adults feed irregularly upon the upper surface of leaves. It is highly destructive at both adult and larval stages which feed on the epidermal tissues of leaves, flowers, and fruits by scrapping the chlorophyll content and cause huge yield loss [2]. Due to the attack by this notorius pest, the infested plant become skeletonized, gradually dry and drop down. Epilachna beetle completes their life cycle about twenty five days under local conditions and a number of generations can occur during a cropping period causing severe damage to the vegetable crop which may result in an economic loss to the farmer. From the economic point of view, *E. dodecastigma* bears immense significance as it can alone damage up to 80% of the host plants [3].
Synthetic insecticides are the only means of insect control in all crops including vegetables in Bangladesh. But chemical protection suffers so many serious drawbacks such as acute and chronic poisoning of applicators, farm workers, consumers, fishes, birds and other wild life animals etc. [4]. These ill effects have highlighted the development of new, safer and eco-friendly pest control measures. A number of researchers isolated and identified several chemical compounds from leaves and seeds of many plant species and screened out as insect feeding deterrents and growth inhibitors that could be used to repel, deter feeding, or limit reproduction and survival of various insect pest species including coccinellid beetles [5]. Talukder and Howse [6] have evaluated toxic effect of rohituka (*Aphanamixis polystachya*) extract on the red flour beetle and found highly toxic and antifeedant effect. Neem oil is widely used as a pesticide which has proved to cause antifeedant, repellent, growth inhibitory

properties, egg laying deterrent and impaired reproductive ability [7, 8]. Pongamia oil is widely used against a large number of insect pests such as shoot and fruit borers [9], aphids [10], white flies, plant hoppers, mites, leaf feeding beetles etc. [11]. However, use of neem oil against different insect pests is widely studied in our country but the study on the efficacy of oil of rohituka and pongamia is very few in Bangladesh. The present study was therefore, undertaken to evaluate the toxicity and antifeedant activity of rohituka, neem, pongamia oils against the adult of epilachna beetle, *Epilachna dodecastigma* (Wied.).

Materials and Methods

Collection and rearing of *E. dodecastigma*

A large number of adult beetles of *E. dodecastigma* were maintained in the laboratory to meet the supply of the test insect. For stock culture of the test insects, adult males and females were collected from severely infested sweet gourd and sponge gourd plants of HSTU campus. In the laboratory, the adult beetles were sexed and confined in pairs in the Petri dishes for mating and egg laying. Fresh and healthy leaves were supplied everyday to meet up the food requirements in the laboratory. After oviposition, adult beetles were transferred into glass jar (1000 ml) and the eggs were left undisturbed for hatching. Immediately after hatching the larvae were transferred to several glass jars (500 ml) and allowed to feed on fresh leaves. Ten larvae were maintained per glass jar. The newly emerged adults were again sexed and confined in pairs in the Petri dishes for mating and egg laying. The process was continued for the whole period of the study. Fresh sponge gourd leaves were supplied regularly to maintain stock culture.

Collection of oils and dose preparation

Three non edible oils namely rohituka (pithraj) (*Aphanamixis polystachya*), neem (*Azadirachta indica* A. Juss.) and pongamia (Karanja) (*Millettia pinnata*) oil were used as test materials. The oils were collected from the local market of Dinajpur town. Four doses of 250, 200, 150, and 100 mg/ml of three selected oils were prepared by diluting with acetone solvent for adult mortality and four doses of 100, 75, 50 and 25 mg/ml of three selected oils were prepared by diluting with same solvent for the antifeedant activity of the adult. Prior to conducting study, a pilot experiment was done to obtain the appropriate dose.

Insect bioassay test

i) Toxicity test

Direct toxicity test of three non edible oils against *E. dodecastigma* was conducted by dipping the adult in four concentrations just for a moment. Ten adult beetles was used in each treatment and each concentration and replicated thrice. Only solvent with equal number of insect were treated as control. Adult mortality was recorded at 24, 48, and 72 hours after treatment (HAT). The percentage of the bettle mortality was corrected by the Abbott's [12] formula.

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

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

ii) Antifeedant activity test

Antifeedant activity of three non edible oils was assayed against the adult of *E. dodecastigma* using leaf disc (6 cm). The experiment was done in Petri dishes (9 cm diameter). Moist cotton sheet covered with filter paper was kept at the bottom of each Petri dish in order to maintain optimum humidity and to keep the leaves fresh. The leaf disc was treated by dipping method with different concentration of each oil. Same ages adults were taken from stock culture and released singly per petridish to treated food. Fifteen replications of each treatment and each dose along with control treatments were maintained. Data on food consumption was measured by using square millimeter graph paper. The percentage of antifeedant index was calculated using the formula described by Karunaratne and Arukwatta [13].

AA% = (C-T /C+T) x 100
Where, C - Leaf area consumed in the control T - Leaf area consumed in the treatment

Statistical analysis

The data from the experiment was statistically analyzed by Completely Randomized Design (CRD) using MSTAT-C statistical software. The mean values were adjusted by Duncan's New Multiple Range Test (DMRT). The observed mortality data were subjected to probit analysis. All graphical work was done through excel program.

Results and Discussion

Probit analysis of adult *E. dodecastigma* for direct toxic effect

The probit analysis of the adult mortality at 24, 48 and 72 HATs using three botanical oils are presented in Tables 1. At 24 HAT, the LD_{50} values of rohituka (260.66 mg/ml), pongamia (342.56 mg/ml) and neem oil (443.54 mg/ml) indicated that rohituka oil was the most toxic and neem oil was the least toxic. The LD_{50} values of rohituka (170.90 and 132.62 mg/ml), pongamia (208.78 and 140.09 mg/ml) and neem (348.20 and 241.56 mg/ml) at 48 and 72 HAT repectively indicated that rohituka oil noted as most toxic followed by pongamia and neem oil. The chi-square values were insignificant at 5% level of probability of three non edible oils at different HATs and mortality data of the adults also did not show any heterogeneity.

Table 1: Probit analysis of three non edible oils against adult *E. dodecastigma* after 24, 48 and 72 HATs

Treatments (oils)	No. of insect used	LD_{50} values (mg/ml)	95% fiducially limits		χ^2 values with 2 df
			Lower	Upper	
24 HAT					
Neem	30	443.54	187.204	1050.857	0.634801
Pongamia	30	342.56	211.1468	555.7587	1.153246
Rohituka	30	260.66	205.5114	330.6143	1.066199
48 HAT					
Neem	30	348.20	180.5794	671.4194	2.439016
Pongamia	30	208.78	172.1019	253.2725	0.6161671

Rohituka	30	170.90	153.1502	190.7145	0.9182644
72 HAT					
Neem	30	241.56	168.4337	346.4262	1.088863
Pongamia	30	140.09	123.2913	159.1805	4.044356
Rohituka	30	132.62	119.4554	147.2531	1.264736

HAT = Hours after treatment. Values were based on four doses, three replications of 10 insects each. χ^2 = Goodness of fit. The tabulated value of χ^2 =5.99 with 2 df at 5% level.

From the above probit results it was observed that all the tested non edible oils would more or less effective against epilachna beetle but rohituka oil had the most effective followed by the pongamia and neem oil. The lowest LD_{50} values of rohituka oil showed the highest toxic effects against the adult beetles. The present finding agreed with Rahman *et al.* [14] where they found that the seed oils of *A. polystachya* was most toxic at 72 HAT (2.236%) and 96 HAT (1.91%) against *Alphitobius diaperinus* on wheat grains. Islam and Shahjahan [15] conducted experiments to evaluate the toxicity of five botanicals, rohituka (*Aphonamixis polystachya*), neem (*A. indica*), marigold (*T. erecta*), durba (*Cynodon doctylon*), castor (*R. communis*), and one insecticide (Malathion) against rice weevil (*S. oryzae* L.) and red flour beetle (*T. castaneum* Herbst.). They found that the rohituka extract was significant toxic effect against those insects.

Direct toxicity of three non edible oils against adult *E. dodecastigma*

Direct toxicity effect of three non-edible oils, doses and their interactions at different time intervals against the adult of *E. dodecastigma* are presented in the Tables 2. Average mortality percentage of epilachna beetle differed significantly among all the tested oils (P>0.05). The mortality percentage of the epilachna beetle at 24 hours after treatment indicated that rohituka oil showed the highest (20.67%), while neem oil performed the lowest mortality (16.00%) followed by pongamia . Similarly at 48 hours after treatment, rohituka oil also showed the highest (39.33%) while neem oil provided the lowest mortality (20.67%). After 72 hours of treatment rohituka also showed the highest (54.00%) but the lowest mortality (29.33%) implied in neem oil. The average mortality percentage of epilachna beetle at 24, 48 and 72 hours after treatment showed that rohituka oil observed the highest morality (38.00%), meanwhile neem oil referred the lowest mortality (22.00%). On the basis of insect mortality, the order of the toxic effect of three non edible oils against epilachna beetles were found as rohituka > pongamia > neem oil. Mortality of adult epilachna beetle was also differed significantly among all the doses at different time interval (Table 2). In 24, 48 and 72 HAT, the highest mortality was found (40.00%, 62.22% and 83.33% respectively) at the highest dose (100 mg/ml) whereas the lowest mortality (7.77%, 14.44% and 25.56% respectively) at the lowest dose (25 mg/ml). The average value indicated that the highest mortality (61.85%) was counted in 100 mg/ml and lowest mortality (15.92) was found in 25 mg/ml. The highest dose possessed the highest mortality while lowest dose possessed the lowest mortality. Interaction effects of non edible oils, doses, and time are presented in table 2. The result revealed that there was a significant different among the tested oils when applied against the adult of epilachna beetle (P>0.05). Average mortality indicated that the highest mortality (76.67%) was found in rohituka oil at maximum dose (250 mg/ml) whereas neem oil showed the lowest mortality (14.33%) at lowest dose (100 mg/ml), which was statistically different from all the oil at different dose level.

Table 2: Direct toxic effect of three non edible oils against adult *E. dodecastigma* at different HATs

Used botanical oils / doses (mg/ml)		**Insect mortality (%) in different time intervals**			**Average mortality (%)**
		24HAT	48HAT	72HAT	
Oil effects	Neem	16.00 b	20.67 c	29.33 c	22.00 c
	Pongamia	16.00 b	30.67 b	49.33 b	32.00 b
	Rohituka	20.67 a	39.33 a	54.00 a	38.00 a
	Control	0.00 c	0.00 d	0.00 d	0.00 d
	LSD	4.584	4.447	4.447	3.496
	CV (%)	35.01	19.73	13.48	15.29
	P-value	0.0716	0.001	0.001	0.001
Doses effects	Dose 1(100)	40.00 a	62.22 a	83.33 a	61.85 a
	Dose 2(75)	25.56 b	47.78 b	67.78 b	47.04 b
	Dose 3(50)	14.44 c	26.67 c	44.44 c	28.52 c
	Dose 4 (25)	7.778 d	14.44 d	25.56 d	15.92 d
	Control	0.00 e	0.00 e	0.00 e	0.00 e
	LSD	5.917	5.741	5.741	4.514
	CV (%)	35.01	19.73	13.48	15.29
	P-value	0.001	0.001	0.001	0.001
Interaction of oils and doses	Neem × Dose 1(250)	33.33 b	46.67 d	56.67 c	45.55 c
	Neem × Dose 2(200)	20.00 cde	23.33 efg	36.67 d	26.67 e
	Neem × Dose 3(150)	16.67 def	20.00 fg	30.00 de	22.22 ef
	Neem × Dose 4(100)	10.00 efg	13.00 g	20.00 e	14.33 f
	Control	0.00 g	0.00 h	0.00 f	0.00 g
	Pongamia × Dose1(250)	33.33 b	60.00 c	96.67 a	63.33 b
	Pongamia × Dose 2(200)	30.00 bc	50.00 d	73.33 b	51.11 c
	Pongamia × Dose 3(150)	10.00 efg	26.67 ef	46.67 c	27.78 de
	Pongamia × Dose 4(100)	6.667 fg	16.67 fg	30.00 de	17.78 f
	Control	0.00 g	0.00 h	0.00 f	0.00 g
	Rohituka × Dose 1(250)	53.33 a	80.00 a	96.67 a	76.67 a
	Rohituka × Dose 2(200)	26.67 bcd	70.00 b	93.33 a	63.33 b

	Rohituka × Dose 3(150)	16.67 def	33.33 e	56.67 c	35.55 d
	Rohituka × Dose 4(100)	6.676 fg	13.33 g	23.33 e	14.44 f
	Control	0.00 g	0.00 h	0.00 f	0.00 g
	LSD	10.25	9.943	9.943	7.818
	CV (%)	35.01	19.73	13.48	15.29
	P-value	0.0189	0.001	0.001	0.001

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

The tested three non edible oils showed toxic effect against epilachna beetle, but rohituka oil could be most effective. The present finding agreed with Rahman *et al.* [14] who found the lethal effects of *Aphanamixis polystachya, A. indica, Carthamus tinctorius, R. communis and Sesamum indicum* oils on *Alphitobius diaperinus.* The major active constituent of rohituka is aphanamixin, which is well known for toxic effects on insects [16]. Podder *et al.* [17] were assessed the effectiveness of rohituka seed extract, bishkatali leaf extract, neem oil, mehagony oil, and diazinon 60 EC for managing epilachna beetle, *Epilachna dodecastigma* (Wied.) where rohituka extracts showed significant toxic effect compared with other extraxts and oils.

Probit regression lines for adult epilachna beetle

The probit regression lines for the bio-efficacy of three non edible oils against adult epilachna beetle at 24, 48 and 72 HAT are presented in figures 1. The calculated probit regression equations for tested oils against epilachna beetle at 24 HAT were Y=3.708x-3.968, Y=2.899x-2.362 and Y=2.035x-0.439 for rohituka, pongamia and neem oils, respectively (Figure 1). Among the three lines, rohituka oil showed the highest but neem oil showed the lowest probit mortality. The regression equations of the probit regression lines for three oils were Y=5.205x-6.60, Y=3.146x-2.308 and Y=1.902x+0.127 against epilachna beetle at 48 HATs for rohituka, pongamia and neem oils respectively. Comparing the three oils, rohituka oil showed the highest probit mortality while neem oil showed the lowest. The regression equation of rohituka oil was Y=6.919x-9.651 and showed the highest probit mortality but neem oil (Y=2.136x-0.091) showed the lowest when the probit regression lines were compared at 72 HATs. The probit regression lines of three oils showed a clear linear relationship between probit mortality and their log doses and the regression lines become sleeper as doses increased.

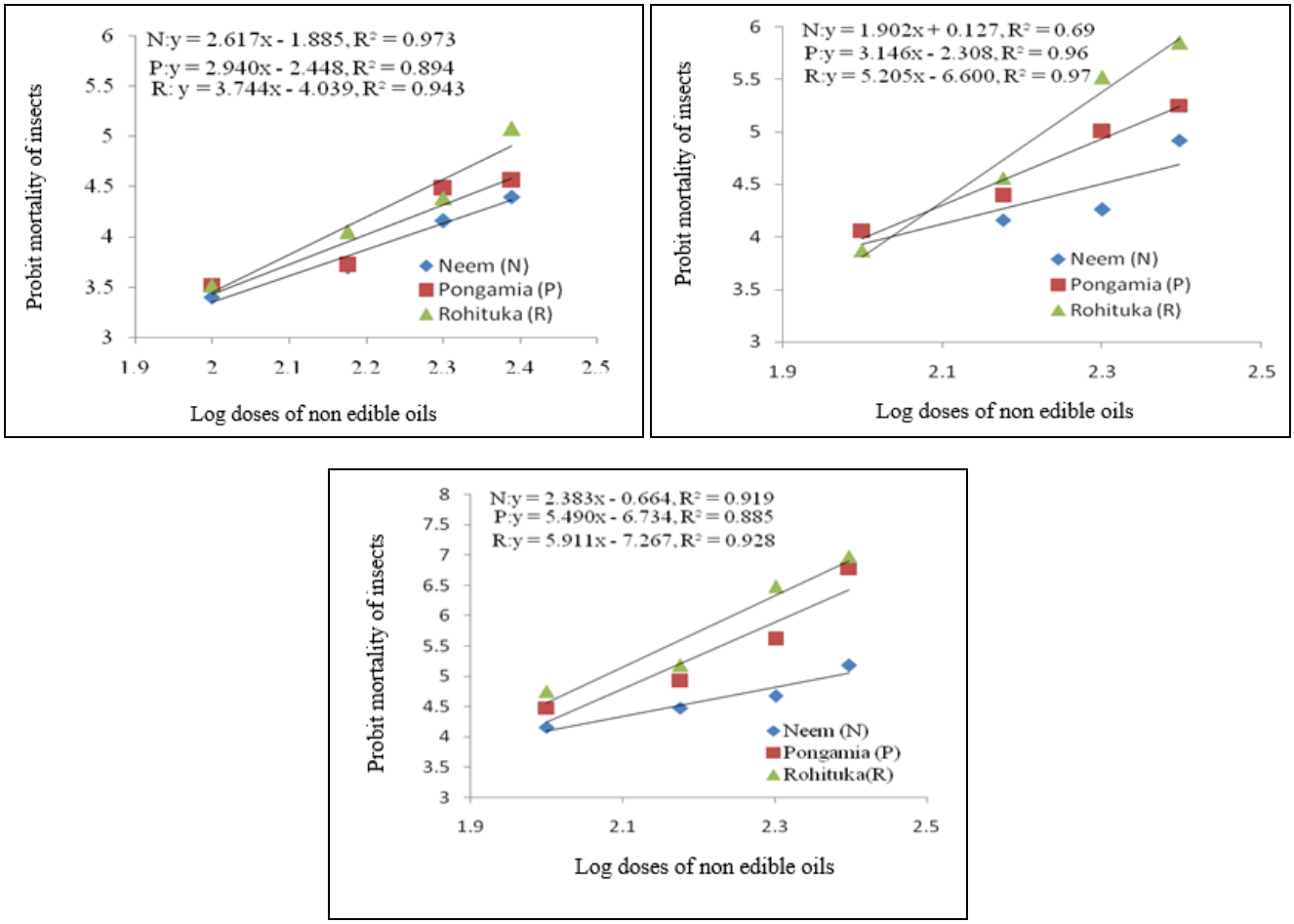

Fig 1: Relationship between probit mortality and log doses of three non edible oils on adult *E. dodecastigma* at 24 (up), 48 (middle) and 72 (below) HATs.

Antifeedant activity of oils against adult epilachna beetle, *E. dodecastigma*

The antifeedant effect of three non edible oils on adult epilachna beetle is presented in Table 3. The present result revealed that rohituka, pongamia and neem oils have antifeedant activity against adult epilachna beetle. Among the three oils pongamia oil showed the lowest leaf consumed area (74.61 mm^2) followed by neem (75.99 mm^2) and rohituka

(104.9 mm^2). On the basis of antifeedant index, it was found that pongamia oil possessed the highest (54.73%) and rohituka oil showed the lowest (35.55%) antifeedant index (Table 3). Among the four doses, the lowest leaf consumed area (41.04 mm^2) was observed in 100 mg/ml while the highest (171.1 mm^2 in the control. In view of antifeedant index, the highest (61.46%) was found in 100 mg/ml but the lowest (34.55%) antifeedant index was observed in 25 mg/ml. The interaction effect of tested oils and doses indicated that there was a significant difference in antifeedant activity against adult of epilachna beetle. The lowest leaf consumed area (37.00 mm^2) was observed in maximum dose (100 mg/ml) of pongamia oil while the highest (137.1 mm^2) in 25 mg/ml dose of rohituka oil. In antifeedant index, the highest antifeedant index (64.39%) was found in 100 mg/ml of pongamia oil but the lowest (11.15%) in 25 mg/ml of rohituka oil. In the control treatments the leaf consumed area was 171.1 mm^2.

From the above results it was observed that among the tested three oils would be effective to control adult epilachna beetle but pongamia oil will be the most effective. The present result agreed with the findings of Kumar *et al.* [18]. They observed that crude seed oil of *P. pinnata* showed the maximum growth reduction and antifeedancy against *Spodoptera litura*. Pongamia seed oil is known to contain active metabolites such as karanjin, pongamol, glabrin, and pinnatin etc. Karanjin is effective against large number of insects [19]. Phytol, 1, 2-Benzenedicarboxylic acid, di-iso-octyl ester, n-Hexadecanoic acid and 3, 7, 11, 15-Tetranethyl-2-hexadecen-l-ol compounds of *P. pinnata* are evaluated for their antifeedant properties against *S. litura* [20].

Table 3: Antifeedant activity of three non edible oils against adult of *E. dodecastigma* at 6 HAT

Used botanicals / doses (mg/ml)		Consumed leaf area (mm^2)	Antifeedant index (%)
Oil effects	Neem	75.99 c	53.53 a
	Pongamia	74.61 c	54.73 a
	Rohituka	104.9 b	35.55 b
	Control	171.1 a	-
	LSD	3.603	2.475
	CV (%)	13.14	14.32
	P-value	0.0	0.00
Dose effects	Dose 1(100)	41.04 e	61.46 a
	Dose 2(75)	55.67 d	52.89 b
	Dose 3(50)	70.31 c	42.84 d
	Dose 4 (25)	87.73 b	34.55 d
	Control	171.1 a	-
	LSD	4.651	2.858
	CV (%)	13.14	14.32
	P-value	0.0	0.001
Interaction effects of oils and doses	Neem × Dose 1(100)	41.00 gh	61.48 ab
	Neem × Dose 2(75)	48.93 fg	55.70 cde
	Neem × Dose 3(50)	55.93 ef	50.71 ef
	Neem × Dose 4(25)	63.00 de	46.23 f
	Pongamia × Dose1(100)	37.00 h	64.39 a
	Pongamia × Dose 2(75)	47.93 fg	56.23 cd
	Pongamia × Dose 3(50)	54.00 f	52.03 de
	Pongamia × Dose 4(25)	63.07 de	46.26 f
	Rohituka × Dose 1(100)	45.13 gh	58.52 bc
	Rohituka × Dose 2(75)	70.13 d	46.74 f
	Rohituka × Dose 3(50)	101.0 c	25.77 g
	Rohituka × Dose 4(25)	137.1 b	11.15 h
	Control	171.1 a	-
	LSD	8.056	4.949
	CV (%)	13.14	14.32
	P-value	0.0	0.00

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

Conclusion

Mortality percentage was also found directly proportional to the time after treatment. The order of the toxicity of three oils were rohituka>pongamia>neem. Non edible oils of rohituka, pongamia and neem showed antifeedant activity against epilachna beetle after 6 hour treatment. From the present research findings it can be concluded that rohituka oil was the most effective against the adult epilachna beetle.

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