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POTENCY OF NATURAL OILS AGAINST THE GRUB OF EPILACHNA BEETLE ON BITTER GOURD

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ABSTRACT

The experiment was conducted in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during June to October, 2019. The effectiveness of four botanical oils of black cumin (*Nigella sativa*), sesame (*Sesamum indicum*), castor (*Ricinus communis*) and neem (*Azadirachta indica*) were assayed for toxic and repellent effects against the third instar grub of *Epilachna duodecastigma* (Wied.) on bitter gourd. The result revealed that all the tested oils had promising insecticidal effect against the grub. However, among the treatments, castor oil performed the highest toxicity of LD_{50} value as 6.4mg/ml against the larva after 72 hours exposure. Similarly, castor oil at 10% showed the highest (78.3%) repellency while sesame oil at 2.5% provided the lowest (45.5%) against the grub of epilachna beetle. Based on efficacy, castor oil might be used in integration with other IPM components against the 3rd instar grub of epilachna beetle.

Keywords: Botanical oils, *Epilachna duodecastigma*, repellency, toxicity

Introduction

Among the vegetable pests, epilachna beetle*, Epilachna duodecastigma* (Wied.) is considered as one of the serious arthropod pests of cucurbits in Bangladesh (Khan *et al*. 2000). It is widely distributed in South and East Asia, Australia, America and the West Indies (Hossain *et al*. 2009). Epilachna beetles are common throughout the country and cause a considerable damage to crops (Islam*, et al*. 2011). Both adults and grubs scrap the chlorophyll in between the veins, skeletonize the leaves, which present a lace like appearance, later dry up and fall from the plants (Endo *et al.* 2004). The grubs are much more destructive and voracious than the adult beetles (Tiwari and Yadav 2008).

At present, pest control measures are solely relies on synthetic insecticides but suffers so many serious drawbacks (Hossain *et al*. 2014). Their extensive use causes numerous problems, as acute and chronic poisoning of applicators, consumers, fishes, birds and others wild life etc. (Rohani *et al*. 2001). Hence, researchers all over the world are now trying to adopt alternatives to protect crop from insect infestation (Isman 2006). Botanicals become promising to control pests by offering several advantages in comparing to chemicals (Kedia *et al*. 2015). Nowadays, natural products are eco-friendly and used against many pests (Wang *et al*. 2012). It has been reported that essential plant oils demonstrated high insecticidal activity against several insect pests (Khater 2012). So, an attempt was made to find out the larvicidal efficacy of black cumin, sesame, castor and neem oils as biopesticides against the epilachna beetle.

Materials and Methods

Insect culture: The beetles were collected from the infested cucurbit plants of the University central farm. Stock culture of large number of *E. duodecastigma* was maintained in Petri dishes (150 mm) at the laboratory (30 ± 5^{o}C, 75±10% RH) during June to October, 2019. Healthy fresh leaves of bitter gourd were collected and placed in the Petri dishes and changed regularly. Moist cotton was used in the cut end of the leaf petiole to prevent the leaves from wilting. Pairing of male and female beetles (1:1) was maintained in the Petri dishes for mating. After oviposition, the adults were replaced into another Petri dish and the eggs were kept undisturbed. Immediately after hatching, the grubs were transferred in several Petri dishes and reared till adult emergence. The newly emerged adults were again sexed and confined in pairs in Petri dishes for mating and egg laying. The process was continued to maintain a series of stock culture.

Treatments: The botanical oils of black cumin (*Nigella sativa*), sesame (*Sesamum indicum*), castor (*Ricinus communis*) and neem (*Azadirachta indica*) were collected from the local market. Four doses viz. 10.0, 7.5, 5.0 and 2.5% of selected botanical oils were prepared by diluting in water as solvent. Test solutions were emulsified separately by using one drop of tween-20 with the help of micro pipette (Single channel micro pipette, by Dragon lap China, model: C40038142). Pilot experiments were done prior to select the appropriate doses.

Toxicity test: Insect bioassays were performed in the laboratory conditions to determine the direct toxic and repellent effects of oils against 3rd instar grub. Fresh young leaves of bitter gourd were collected from the University experimental field. Ten third instar grubs of *E. duodecastigma* were taken in a Petri dish with food and sprayed separately of each concentration of each treatment by a hand sprayer. Three replications were maintained for each dose. Only water with equal number of insects was treated as control. Insect mortality was recorded up to the 72 hours at 6 hours intervals after treatment. The percent of the mortality was corrected by the following Abbott's (1987) formula.

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

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

Repellency test: The repellency test was conducted according to the method as described by Talukder and Howse (1995) with a slight modification. For this, leaves were cut into 5cm discs and dipped separately in each treatments of each concentration while another dipped in water as control treatment. The treated leaves were then air cured for 20 minutes under electric fan. Then two leaves were placed in a Petri dish (150mm) facing ventral surface upward. Ten individuals of third instar grub of *E. duodecastigma* were introduced in between the two treated leaves in the Petri dish and closed with respective lids. Number of grubs on each treated and untreated leaves was counted at two hour intervals up to 12th hour. Three replications were maintained for each treatment. The data were expressed as percentage repulsion (PR) by the following formula: [PR (%) = (Nc-50) × 2]. Where, Nc = the percentage of insects present in the control half. Positive (+) values expressed repellency while negative (-) attractancy.

Statistical analysis: The data were statistically analyzed using Completely Randomized Design (CRD). The mean values of the treatments were adjusted by Duncan's New Multiple Range Test (DMRT). The observed mortality were corrected by Abbott's (1987) formula and subjected to probit analysis.

Results

Toxicity effects of botanical oils differed significantly among the treatments at different times after intervals (Table 1). Among the treatments, the highest mortality (53.3%) was observed at 10% castor oil while the lowest (6.6%) at 2.5% sesame oil (Table 1). But minimum mortality (4.4%) was recorded in the untreated control treatment. The probit mortality of LD_{50} values, $\chi 2$ values and their 95% fiducially limits at 24, 48 and 72 HAT against the 3rd instar grub of all the treatments are highlighted in Table 2. Among the treatments, the highest LD_{50} values were calculated as 66.2 mg/ml at 24 HAT for black cumin oil while 44.6 and 27.6 mg/ml at 48 and 72 HAT, respectively for sesame oil. Conversely, the lowest LD_{50} values were calculated as 25.1, 9.7, and 6.4 mg/ml at 24, 48 and 72 HAT, respectively for castor oil. Comparing the LD_{50} values, the order of toxicity of oils was found as castor > neem > black cumin > sesame at 24, 48 and 72 HAT (Table 2). Repellent effects of different treatments on the 3rd instar grub differed at different HAT (Table 3). Among the treatments, significantly the highest (78.3%) repellency effect was found at 10% castor oil while the lowest (45.5%) at 2.5% sesame oil.

Discussion

Present results evident that the tested oils had profound toxic effects against the 3rd instar grub of *E. duodecastigma*. Mortality of the tested oils was observed doses and time exposure dependent. Present outcomes are similar to the observations as stated of Karmakar and Bhole (2001). They observed the efficacy and persistent toxicity of some neem products and found 2% of neem oil and resulted 100% mortality on the *E. duodecastigma*. Our findings are in close proximity with that of Mala *et al*. (2012). They experienced that plant based insecticide effectively reduced epilachna beetle in field conditions. Our findings are also comparable with Araya and Emana (2009) who cited that the botanicals have good impact in controlling coleopterons beetle. Again, these results are in line with those of Shanmugapriyan and Kingsly (2001) who reported that neem oil at 0.25, 0.5 and 1.5% effective on *E. vigintioctopunctata* larvae.

Table 1. Direct toxic effect of botanical oils and doses against 3rd instar grub of epilachna beetle at different hours after treatment (HAT)

Treatments (oils)	Dose (%)	24HAT	48HAT	72HAT	Average
Black cumin	10	16.6 ce	30.0 c	40.0 c	28.9 cd
	7.5	13.3 df	23.3 cd	26.6 df	21.1 de
	5	10.0 eg	13.3 ef	23.3 eg	15.5 ef
	2.5	6.6 fg	10.0 f	13.3 gi	10.0 f-h
Sesame	10	13.3 df	20.0 de	30.0 ce	21.1 de
	7.5	10.0 eg	13.3 ef	20.0 eh	14.4 eg
	5	6.6 fg	10.0 f	16.6 fi	11.1 fh
	2.5	3.3 g	6.6 f	10.0 hi	6.6 gh
Castor	10	33.3 a	53.3 a	73.3 a	53.3 a
	7.5	26.6 ab	46.6 ab	56.6 b	43.3 b
	5	23.3 bc	26.6 cd	40.0 c	29.9 c
	2.5	13.3 df	20.0 de	26.6 df	20.0 e
Neem	10	26.6 ab	43.3 b	63.3 ab	44.4 b
	7.5	20.0 bd	30.0 c	36.6 cd	28.9 cd
	5	16.6 ce	23.3 cd	26.6 df	22.2 ce
	2.5	10.0 eg	13.3 ef	20.0 eh	14.4 eg
Control	-	3.3 g	3.3 f	6.6 i	4.4 h
CV (%)		39.2	26.8	23.9	22.3

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

Table 2. Probit analyses of treated botanical oils against the third instar grub of epilachna beetle at 24, 48 and 72 HAT

Treatments (oils)	No. of insect used	LD_{50} (mg/ml)	95% confidence limits		χ^2 values with 2 df
			Lower	Upper	
			24 HAT		
Black cumin	30	66.2	3.3	1315.1	8.9
Sesam	30	31.0	5.6	169.5	0.2
Castor	30	25.1	5.6	111.8	0.1
Neem	30	40.2	4.7	337.5	5.8
			48 HAT		
Black cumin	30	24.1	7.1	81.6	0.5
Sesame	30	44.6	4.8	414.2	0.1
Castor	30	9.7	6.2	15.0	0.8
Neem	30	14.9	7.1	31.0	0.1
			72 HAT		
Black cumin	30	18.1	7.6	43.2	0.4
Sesame	30	27.6	7.1	106.6	0.2
Castor	30	6.4	4.9	8.2	0.7
Neem	30	9.7	6.7	13.9	3.1

Values were based on four concentrations, three replications of 10 insects of each, χ^2 = Goodness of fit, The tabulated value of χ^2 is 5.99 (df = 2 at 5% level).

Table 3. Repellent effect of botanical oils and doses against 3rd instar larva of epilachna beetle at different HAT

Treatments (oils)	Doses (%)	Repellency (%) at different times of intervals						Average repellency
		2 HAT	4 HAT	6 HAT	8 HAT	10 HAT	12 HAT	
Black cumin	10.0	86.6 a	80.0 a	73.3 ab	66.6 ab	66.6 ac	70.0 ae	73.8 ab
	7.5	83.3 a	73.3 ab	73.3 ab	66.6 ab	50.0 cf	50.0 eh	66.1 ad
	5.0	73.3 ac	70.0 ac	60.0 ac	50.0 bc	36.6 ef	33.3 h	53.8 de
	2.5	60.0 bd	56.6 be	46.6 cd	46.6 bc	40.0 df	33.3 h	47.2 ef
Sesame	10.0	70.0 ae	56.6 be	63.3 bc	66.6 ab	70.0 ac	63.3 be	65.0 ad
	7.5	60.0 bd	53.3 ce	60.0 ac	56.6 ac	46.6 cf	56.6 dg	55.5 de
	5.0	50.0 d	50.0 df	56.6 ac	63.3 ac	60.0 ad	66.6 ae	57.7 ce
	2.5	53.3 cd	46.6 ef	53.3 bd	50.0 bc	33.3 f	36.67 gh	45.5 ef
Castor	10.0	76.6 ab	70.0 ac	80.0 a	76.6 a	80.0 ab	86.6 a	78.3 a
	7.5	66.6 ad	63.3 ae	63.3 ac	63.3 ac	63.3 ac	70.0 ae	65.0 ad
	5.0	60.0 bd	66.6 ad	60.0 ac	70.0 ab	56.67 be	60.0 cf	62.2 bd
	2.5	50.0 d	50.0 df	56.6ac	63.3 ac	60.0 ad	66.6 ae	57.7 ce
Neem	10.0	80.0 ab	80.0 a	66.6 ac	66.6 ab	83.3 a	80.0 ac	76.1 ab
	7.5	80.0 ab	66.6 ad	76.6 ab	63.3 ac	76.6 ab	73.3 ad	72.7 ab
	5.0	70.0 ad	70.0 ac	80.0 a	63.3 ac	70.00 ac	76.6 ad	71.6 ac
	2.5	70.0 ad	53.3 cf	66.6 ac	63.3 ac	70.0 ac	83.3 ab	67.7 ad
CV (%)		17.2	14.7	20.0	21.7	20.8	19.2	12.4

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

The LD_{50} values of tested oils have also been calculated to compare the efficacy. From the probit results it was observed that all the tested oils would more or less effective against the 3rd instar grub of *E. duodecastigma* but castor oil had the most effective followed by the neem oil. The lowest LD_{50} values of castor oil showed the highest toxic effects. Our findings are comparable with those of Ruhul *et al*. (2020). They tested oils of rohituka, pongamia and neem and found that rohituka were very effective against the *E. duodecastigma* provided the lowest LD_{50} values of 260.66 mg/ml at 24 hour after treatment. Again, Sharma and Saxena (2012) found that 1.0% concentration of *Eucalyptus globulus* leaf extracts gave 80.0 to 93.3% larval mortality of epilachna beetle. Saxena and Sharma (2007) also tested insecticidal activity of different plant extracts against third instar larvae of epilachna beetle and found good results. Our results are in line with Podder *et al.* (2013). They assessed the effectiveness of botanicals for managing *E. duodecastigma* where neem oil showed significant toxic effect. Present results are clearly demonstrated the toxic effects tested plant oils which possess remarkable larvicidal action at 10% concentration.

Outcomes also revealed that trialed biopesticides diversely repelled the grub of epilachna beetle. This indicated that the pest repellent properties of tested treatments were not distributed uniformly. Also, the repellency rate decreased with increasing of exposure times. The results of the present study are comparable with Ali *et al*. (2011) who opined that neem oil strongly repelled the cucumber beetle. Again, our results are in close proximity with those of Araya and Emana (2009). They experienced that botanicals have good impact in controlling coleopteran beetle's upto 67%. Researchers, nowadays, addressed that numerous plant products having a series of pest control properties such as insecticidal, antifeedant, repellent, growth inhibitory, chitin synthesise but eco-friendly and attracted in attention of pest control program (Swaminathan *et al.* 2010). Our results are in line with the aforesaid researchers.

Tested botanical oils used in the present study had direct toxic and repellant effect on grub of *E. duodecastigma*. However, among the tested oils, castor showed the highest toxic and repellent effects against the grub. Based on efficacy, castor oil might be used in integration with other IPM components as natural, safe, available and easily processible.

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