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Insectifuge effect of neem, *Azadirachta indica* A. Juss and their derivatives against sawfly, *Athalia lugens proxima* Klug. (Hymenoptera: Tenthredinidae)

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Abstract
Experiment were carried to assess insectifuge activity of neem and their derivatives against third instars larvae of *Athalia lugens proxima* (Klug.) under laboratory conditions. The *Neem* seed kernel extracts (NSKE), Neem leaf extracts (NLE), Deoiled *Neem* seed kernel powder suspension (c) and *neem* based insecticide viz azadirachtin, bioneem, econeem, neemazal, neemarin, nimbecidine, azadirone and neemgold were tested by dry-film technique. The spraying of the insecticidal preparation was done in glass petridishes (10cm diameter) by potters spray tower, using 1.0 ml. of solution per petridish. Five concentrations were tested in three replications, along with over control (Benzene + emulsified water). Ten third instars larvae of *A. lugens proxima* were released inside each pair of petridishes and allow remaining there up to two hours. After which, they were transferred to the fresh petridishes containing fresh mustard leaves for feeding under control conditions (27 + 2 ºC temp. 75 + 5% relative humidity). The insectifuge biopotency was assessed and result was arranged in the following descending order on the basis of their respective relative EC_{50} values i.e. Azadiron > Bioneem > NLE > Neemarin > Neemgold > Econeem > DNSKP > Azadirachtin> Neemazal > Nimbicidine > NSKE and the order of the protectivity on the based on the relative Ec_{50} values are as*:* 0.1359 > 0.1531 > 0.1833 > 0.3207 > 0.3208 > 0.6397 > 0.6868 > 0.7124 > 0.7421 > 0.9025 > 0.9554 times least protective, respectively. as Azadiron taken as unit. This paper mainly investigated effect of neem-based formulation third instars larvae of *Athalia lugens proxima* (Klug.).

Keywords: Azadirachtin, bioneem, *Athalia lugens proxima,* nimbecidine, azadirone. NSKE and NSKE

1. Introduction
India is the second largest producer of rapeseed-mustard in the world after China. Rapeseed and mustard are most important oilseed crops viz; toria, brown sarson, yellow sarson, gobhi sarson, raya or Indian mustard, black mustard, rai and taramira of our country. Major bottleneck in its production is reduction of yield due to pest attacks. The major concerns in enhancing and stabilizing the yield of rapeseed mustard is the incidence of insect and diseases, which are causing the damage to crop at different stages and responsible for huge yield losses to a extent ranging from 10 to 90 per cent.

Mustard sawfly, *Athalia lugens proxima* Klug (Hymenoptera: Tenthredinidae) has become a serious pest of cruciferous crop in oriental region (Patil and Pokharkar 1973, Patnaik 1983, Patel and Jhala 1999, Yadav and Patel 2017) [32, 33, 31, 49]. There are five larval instars, and the pupation takes place in the soil. The larvae alone are destructive and feed from the margin of the leaf towards centre. The larvae infested on young leaves, and skeletonise them. The larvae also feed on the epidermis of the tender shoots, flowers and fruits (Chowdhury 2009) [13].

There are several synthetic insecticides viz; carbaryl, endosulfan, phosalone, etc. employed in the management of pest on mustard crops bearing direct adverse effects on humans, wildlife, aquatic life and the environment at large (Sukhirun *et al.,* 2011; Huen *et al.,* 2012; Koureas *et al.,* 2012; Köhler and Triebskorn, 2013; Baltazar *et al.,* 2014; Wang *et al.,* 2014; Yuan *et al.,* 2014; Guyton *et al.,* 2015) [44, 19, 28, 29, 48, 50, 14]. However, there are concerns about the use of pesticides, because of their negative effects on the environment and human health. These pesticides are also expensive and out of reach to the poor farmers. Therefore, there is a need to develop alternative methods of pest management.

Among so many plants investigated, the Indian tree, *Azadirachta indica* A.Juss from the Meliacea family, known as margosa has long been recognized for its properties both against insects and in improving human health (Rahim 1997, Trisyono and Whalon. 2000, Chandele

2003, Murugesan and Murugesh, 2008, Salako 2008 Baltazar *et al*. 2001) [35, 47, 7, 30, 39, 5]. Neem extracts make the ideal insect control for farmers worldwide by providing a safe, inexpensive and very effective insect control for both ends of the agricultural spectrum. Biopesticides made from neem are biodegradable, non-toxic, eco-friendly and have no residual effect on agriculture produce.This concept has the bearing upon the present investigation that evaluates the bioefficacy of neem derivatives on okra fruit borer, *Helicoverpa armigera* Hubner. Some plants are known to contain bioactive metabolites, which show antifeedant, repellent and toxic effects on a wide range of insect pests (Chandel *et al*. 2001, Rajendran and Sriranjini 2008, Ruchi *et al*. 2014, Shanmugapriyan and Dhanalakshmi, 2015,) [8, 36, 38, 40]. Many plants can protect themselves against insects by producing their own chemical defences that are toxic or repellent (Ali *et al.* 1993, Chandel and Singh 2016, and Chandel, 2017) [3, 10, 11]. The consideration for the use of extracts of plants origin is that they are easily biodegradable, effective on some pests and considered safe in pest control operations as they minimize pesticide residues, ensure safety of the consumers of the treated grains and the environment. Further, the production of organic extracts of plant origin for pest control may be easier and less expensive than the synthesis of some complex chemical. They possess many of the attributes of an ideal biological control agent, including broad host range, high virulence, host seeking capability, ease of mass production, recycling ability, non- hazardous to environment, etc. (Sarup and Srivastava. 1971, Chandel *et al.* 2001, Chandel *et al.* 2005, Chandel *et al*. 2017, Thangapandian *et al.* 2011) [41, 8, 9, 11, 46].

Neem Pesticide is a natural product, absolutely non-toxic, 100% biodegradable and environmentally friendly in nature (Attri and Prasad. 1980, Schmutterer, 1988, Abdul Kareem, 1989, Pascual, 1990) [4, 43, 2, 34]. If required, it can be mixed with other synthetic pesticides. Gradually, the ratio of Neem content in the mixture can be increased and synthetics reduced till you reach a stage where synthetics become redundant. Neem consists of several compounds hence development of resistance is impossible. Neem does not destroy natural enemies of pests thereby allowing these natural enemies to keep a check on the pest population (Zehrer 1984, Joshi *et al.*1984, Zeringue and Bhatnagar1996, Lowery and Smirle. 2000, Kausik *et al.* 2002) [51, 22, 52, 15, 17]. Neem also has a systemic action and seedlings can absorb and accumulate the neem compounds to make the whole plant pest resistant. Neem is harmless to non-target and beneficial organisms like pollinators, honey bees, mammals and other vertebrates. Neem has a broad spectrum of action active on many species of pests.

2. Materials and Methods

The present study was conducted in the post graduate Department of Zoology, Entomology, Biopesticides and Toxicological Laboratory, D.B.S. College, affiliated to CSJM University, Kanpur, India. The laboratory culture of *H. armigera* was initiated from the eggs collected from fields at C.S. Azad university of Agriculture and Technology, Kanpur. As recommended by Chandel and Singh (2001), the insects were reared in the laboratory at 28 ± 2º C on a diet of okra. The collected eggs were placed in a well ventilated plastic container and okra fruits were provided to newly hatched larvae. The laboratory reared third instar larvae were used for the present investigation to evaluate the insecticidal efficacy of neem derivatives.

2.1 Mass culturing of pulse beetle

The larvae of *A. lugens proxima* were obtained from the experimental farms of CSAzad University of Agriculture and Technology, Kanpur and maintained in the laboratory on natural diets. The collected larvae were kept for at least 5 days in the laboratory to check, whether or not, there are any other infections before using them for experiments.Mustard sawfly, *Athalia lugens proxima* (Klug.) required for the study were mass reared on mustard leaves in the laboratory. The mass culturing was initiated by confining 10-20 grubs of mustard sawfly in the plastic containers of 59 x 21 x 18 cm having green mustard leaves which were then covered with muslin cloth and secured tightly with rubber band. Mass culturing of *A.lugens proxima.* was done at 28 ± 2º C temperature in the plastic container and observed daily.

2.2 Procurement of raw plant materials: In the present investigation three neem plant materials were collected while their derivatives were obtained from local market and used for their repellent effectiveness against third instar larvae of *Athalia lugens proxima* (Klug.) in laboratory trials.

2.3 Preparation of powder: Fresh collected green plant parts (leaves and seeds etc) were washed with distilled water and kept in the laboratory for 7 days for air drying followed by one day sun drying before making powder. Electric grinder was used to have coarse powder then these were passed through a 60-mesh sieve to get fine powder. Powders were kept in polythene bags at room temperature and properly sealed to prevent quality loss (Chandel and Singh, 2016)

2.4 Preparation of botanical extracts: For the extraction, Soxhlet Apparatus was used; about 20g powder of each category of powder were extracted with 300 ml of different solvents (n-hexane, acetone, methanol, petroleum ether and distilled water). Extraction of each category of powder were done in about 12 hrs. After soxhlet extraction, the material was run on rotary evaporator. The extracts were concentrated on rotary evaporator by removing the excess solvent under vacuum. After evaporation of solvent with rotary evaporator the remaining extracted material was kept on water bath for removing remaining solvent from the extracts. The extracts were stored at 4°C prior to application.

2.5 Apparatus used for experiment: Small plastic jars (capacity 200 ml) were used for the experiment, there was one set of two jars joined by clear plastic pipe of 1cm diameter at an angle of 180 degree for each replication. One jar of each set was provided with 10 g of grains given the name 'A' while the other jar was kept empty and given the name **'**B**'**. In jar 'A**'**, the grains treated with extracts were placed, while the jar B remained empty. The jars used for experiment were disinfected with alcohol.

2.6 Preparation of Stock Solution: For stock solution, 50ml. extract in each case was taken into reagent bottles and 50ml. benzene was added in it to dissolve the constituents of the materials. The mouth of the bottles were stopper with airtight corks after which, these bottles containing the solutions were kept in refrigerator. The alcoholic extracts of *Neem* seed kernel extracts (NSKE) and Neem leaf extracts (NLE),

Deoiled *Neem* seed kernel powder suspension (DNSKPS) and *neem* based insecticide viz azadirachtin, bioneem, econeem, neemazal, neemarin, nimbecidine, azadirone and neemgold were tested under laboratory against third instar starved groubs of *Athalia lugens proxima* Klug., which is noxious insect pest of cruciferous vegetables and crops. The details of which, are described as under:-

2.7 The Insecticidal Formulations: Five concentrations of neem peoducts (0.25, 0.5, 1.0, 1.5, 2.0 percent) and were used for experiments on repellent tests in the laboratory conditions. The different concentrations of the herbal extracts were prepared from the stock solution using benzene as solvent and Triton X-100 as emulsifier. The level of solvent and emulsifier were kept constant.

Table 1: Detailed List of Neem & Neem Based Insecticides

Botanical name	Solvent	Concentration with
Neem seed kernel Extracts.	Alcohol	Benzene+emulsified +H_2O
Neem leaf Extracts.	Alcohol	Benzene+emulsified +H_2O
Deoiled *Neem* Seed kernel powder suspension	Alcohol	Benzene+emulsified +H_2O
Neem Based Insecticides		
Azadirachtin	_	Benzene+emulsified +H_2O
Azadiron	_	Benzene+emulsified +H_2O
Bioneem	_	Benzene+emulsified +H_2O
Econeem	_	Benzene+emulsified +H_2O
Neemazal	_	Benzene+emulsified+H_2O
Neemgold	_	Benzene+emulsified +H_2O
Neemarin	_	Benzene+emulsified +H_2O
Nimbicidine	_	Benzene+emulsified +H_2O

Table 2: Formulations of Extracts

Concentration (%)	Amount of Stock Solution (ml)	Amount of Benzene (ml)	Amount of Emulsifiable Water (ml)	Total Amount (ml)
0.25	2.50	22.50	475.00	500.00
0.50	5.00	20.00	475.00	500.00
1.00	10.00	15.00	475.00	500.00
1.50	15.00	10.00	475.00	500.00
2.00	20.00	5.00	475.00	500.00

2.8 Field Collection and culture of Pulse Beetle: Adults of *Athalia lugens proxima* (Klug.) was drawn from laboratory mass cultures reared in glass jars at ambient laboratory temperature. The *Athalia lugens proxima* (Klug.) used for experiment were third instars larvae were used for experiment and fed on mustard leaves.

3. Experimental Protocol

The alcoholic extracts of *Neem* seed kernel extracts (NSKE) and Neem leaf extracts (NLE), Deoiled *Neem* seed kernel powder suspension (DNSKPS) and *neem* based insecticide viz azadirachtin, bioneem, econeem, neemazal, neemarin, nimbecidine, azadirone and neemgold were tested under laboratory against third instar starved groubs of *Athalia lugens proxima* Klug., which is noxious insect pest of cruciferous vegetables and crops. For testing the repellent effect the mustard leaves were used as food against the third instar larvae of *Athalia lugens proxima* (Klug.) treated with different concentrations of eleven *neem* extracts and *neem* based insecticides. The treated foods were kept in jar (23cm x 10cm) on moist filter paper. Then third instar, 24 hours starved *A. lugens proxima* larvae were released in each jar. In each set of extract and one control was introduced, where the leaves pieces were dipped in Benzene + emulsified water only. After four hours of the release of larvae the data was collected on the number of larvae reached at each treated food. Three replication of treatment were made. The repellent effect of all the extracts was judged by counting the number of larvae after 4 hours, present on the treated leaf in each treatment and the percentage of repellency were adjudged over control.All the values were calculated as per Abbott formula (Abbott W.S. 1925) [1].

4. Result and Discussions

The data depicted in table 4 and figure1 and 2 indicated that all the neem extracts and neem based insecticides have proved to more or less repulsive against the *Athalia lugens proxima*. It is seen that all the neem extracts and neem based insecticides have proved to more or less repulsive against the larvae of *Athalia lugens proxima*. Among all selected neem extracts and neem based insecticides, only three plant extracts gave promising repellency with minimum EC_{50} value (less than 0.50) and rest seven showed less repellent effect to the larvae of *A. lugens proxima*. On the basis of relative EC_{50} values order of merit can be arranged in following descending manner: Azadiron (48.15) > NLE (34.77) > Bioneem (4.03) > Neemgold (2.93) > Econeem (1.92) > DNSKP (1.66) > Azadirachtin (1.38) > Nimbicidine (1.31) > Neemazal (1.28) > > NSKE (1.00) and the order of the protectivity on the based on the relative Ec_{50} values are as*:* 0.1359 > 0.1531 > 0.1833 > 0.3207 > 0.3208 > 0.6397 > 0.6868 > 0.7124 > 0.7421 > 0.9025 > 0.9554 times least protective, respectively. as NSKE taken as unit. There is every reason to believe that the use of neem materials for pest control will increase both in developing as well as developed countries. But as far as stored product pests are concerned, with increased use of neem based insecticides cannot be negated.

Table 4: Calculation of log conc./Probit Repellency Regression column on *Athalia lugens proxima*.

Plant Extracts	Het.*	X^2	Regression Equation	Respective EC_{50}	Fiducial Limit	Relative EC_{50}
Azadirachtin	3	1.23	Y = 1.23x + 2.75	0.7124	M_1 = 2.0266 M_2 = 1.6557	1.38
Azadiron	3	1.07	Y = 1.07x + 2.70	0.1359	M_1 = 2.3256 M_2 = 1.9392	48.15
Bioneem	3	0.90	Y = 0.90x + 3.21	0.1531	M_1 = 2.3866 M_2 = 0.1286	4.03
DNSKP	3	0.75	Y = 0.75x + 4.11	0.6868	M_1 = 2.1368 M_2 = 2.1278	1.66
Econeem	3	1.19	Y = 1.19x + 2.84	0.6397	M_1= 1.9744 M_2 = 1.6385	1.92
Neemgold	3	0.87	Y = 0.87x + 4.94	0.3208	M_1= 2.0696 M_2 = 0.1369	2.93
Neemazal	3	0.87	Y = 0.87x + 4.94	0.7421	M_1= 1.6003 M_2 = 0.0336	1.28
Neemarin	3	1.52	Y = 2.10x + 1.36	0.3208	M_1= 1.5144 M_2 = 0.4061	4.33
Nimbicidin	3	1.34	Y = 0.73x + 4.56	0.9025	M_1= 1.1678 M_2 = 0.0321	1.31
NLE	3	0.88	Y = 0.84x + 3.84	0.1833	M_1= 1.7533 M_2 = 1.0066	34.67
NSKE	3	0.62	Y = 0.62x + 4.15	0.9554	M_1= 2.1869 M_2 = 1.0881	1.00

In case of X^2 was found non- significant heterogeneous at P=0.05, Y=Probit Repellency, X=Log Concentration X $10^{2,}$ D.F.=Degree of Freedom, EC_{50}= Conc. Calculated at given 50% Repellency and Heterogenecity=Het.*

In the support of above findings the following scientist tested different concentrations of neem materials for stored product management. (Jotwani and Sircar 1965, Jotwani and Sircar 1967, Ketkar1976, Jotwani and Srivastava. 1981and Isman, 2006) [23, 27, 25, 19]. Larvicidal effect of neem oil was also earlier reported by Brar *et al*. (1994) and Thara *et al*. (2008) on *Earias vittella*, Shanmugapriyan and Kingsley (2001) and Murugesan and Murugesh (2008) on *Epilachna vigintioctopunctata*, Revathi and Kingsley (2008) on *Pericallia ricini* and Kavitha *et al.* (2008) on *Leucinodes orbonalis* [6, 45, 42, 30, 37, 26]. Repellent bioacitivity of certain plant products are reported by sevsral entomologist (Jilani *et al*. 1988, Jilani and Saxena. 1990, Khan and Gumbs, 2003) [21, 20, 16]. This consideration was behind the present study to evaluate the effect of *neem* extracts and neem derivatives on 3rd instar larvae of *Athalia lugens proxima*.

5. Conclusion

The findings of the present investigations indicate that botanical derivatives might be useful as insect control agents for commercial use. Among ten plant extract, only Azadiron (48.15) showed highest repellency followed by NLE (34.77) and Neemarin 94.33) times repellent than NSKE (1.00) against larvae of *Athalia lugens proxima*. All the extracts tested were effective to some degree of repellency reducing the feeding and destruction rates. More studies on major biochemical constituents responsible for repellent activity to the test insect on mustard against larvae of *Athalia lugens proxima*. need to be investigated.

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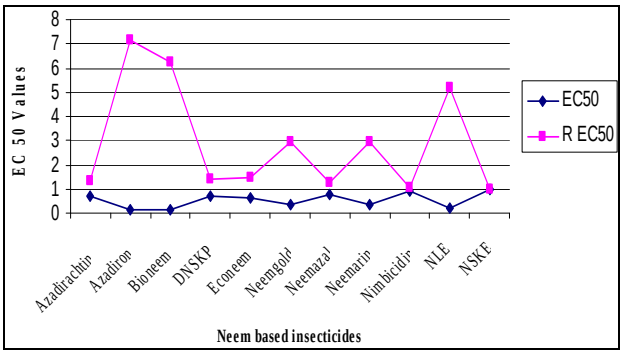

Fig 1: Calculation of Conc. / Probit Repellency Regression Graph on a. Lugens Proxima

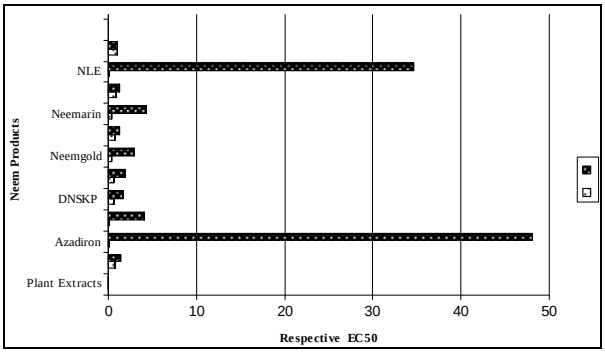

Fig 2: Calculation of Log Conc. /Probit Repellency Regression Columu on Athalia Lugens Proxima

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