



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

BS Chandel, Hemraj Singh Sengar

Biopesticides and Toxicological Laboratory, Department of Zoology, DBS PG College, affiliated to CSJM University, Kanpur, Uttar Pradesh, India

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°C 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 EC₅₀ values i.e. Azadirone > Bioneem > NLE > Neemarin > Neemgold > Econeem > DNSKP > Azadirachtin > Neemazal > Nimbecidine > NSKE and the order of the protectivity on the based on the relative Ec₅₀ 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 Azadirone 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 Triebkorn, 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 Meliaceae 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 Bhatnagar 1996, 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±2° 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 59x21x18 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±2° 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 grubs 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 products (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
<i>Neem</i> seed kernel Extracts.	Alcohol	Benzene+emulsified +H ₂ O
<i>Neem</i> leaf Extracts.	Alcohol	Benzene+emulsified +H ₂ O
Deoiled <i>Neem</i> Seed kernel powder suspension	Alcohol	Benzene+emulsified +H ₂ O
Neem Based Insecticides		
Azadirachtin	—	Benzene+emulsified +H ₂ O
Azadiron	—	Benzene+emulsified +H ₂ O
Bioneem	—	Benzene+emulsified +H ₂ O
Econeem	—	Benzene+emulsified +H ₂ O
Neemazal	—	Benzene+emulsified +H ₂ O
Neemgold	—	Benzene+emulsified +H ₂ O
Neemarin	—	Benzene+emulsified +H ₂ O
Nimbecidine	—	Benzene+emulsified +H ₂ O

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, azadiron and neemgold were tested under laboratory against third instar starved groups 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 figure 1 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 EC₅₀ value (less than 0.50) and rest seven showed less repellent effect to the larvae of *A. lugens proxima*. On the basis of relative EC₅₀ 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) > Nimbecidine (1.31) > Neemazal (1.28) >> NSKE (1.00) and the order of the protectivity on the based on the relative Ec₅₀ 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 X² was found non- significant heterogeneous at P=0.05, Y=Probit Repellency, X=Log Concentration X 10². D.F.=Degree of Freedom, EC₅₀= Conc. Calculated at given 50% Repellency and Heterogeneity=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, Ketkar 1976, Jotwani and Srivastava. 1981 and 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 bioactivity of certain plant products are reported by sevrsal 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.

6. Acknowledgement

The authors are thankful to Dr. R.K Singh, Principal, D.B.S College, Kanpur for providing the necessary facilities. Prof. N.D.Pandey, Retd., Head, Division of Entomology, C S Azad University of Agriculture and Technology, Kanpur for rendering their support and help for the completion of this work.

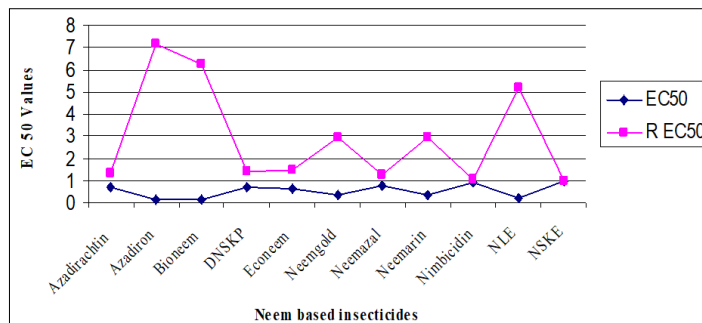


Fig 1: Calculation of log conc./Probit Repellency Regression graph on *A. lugens proxima*

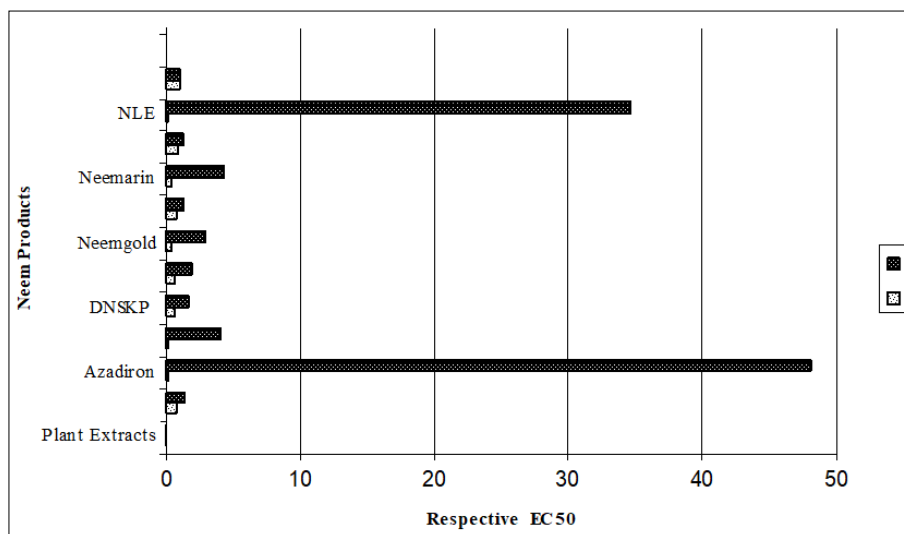


Fig 2: Calculation of log conc./Probit Repellency Regression column on *Athalia lugens proxima*.

7. Reference

- Abbott WS. A method of computing the effectiveness of insecticides. *J. Economic Entomology*. 1925; 18:265-267.
- Abdul Kareem RC, Saxena MEM, Boncodin V Krishnasamy, Seshu DV. Neem as seed treatment for rice before sowing: effects on two homopterous insects and seedling vigor. *J. Econ. Entomol*, 1989; 82:1219-1223.
- Ali SI, Singh OP, Misra US. Effectiveness of plant oils against pulse beetle, *Callosobruchus chinensis* Linn. *Indian J Entomol*, 1983; 45:6-9.
- Attri BS, Prasad GR. Studies on the pesticide value of neem oil byproducts. *Pestology*, 1980; 4:16-20.
- Baltazar MT, Dinis-Oliveira RJ, de Lourdes Bastos M, Tsatsakis AM, Duarte JA, Carvalho F *et al.* Pesticides exposure as etiological factors of Parkinson's disease and other neurodegenerative diseases-A mechanistic approach. *Toxicology Letters*, 2014; 230:85-103.
- Brar KS, Arora SK, Ghai TR. Efficacy of neem oil, dipel and insecticides for the control of cotton jassid and fruit borer on okara. *Pestology*. 1994; 18(2):14 -16.
- Chandele AG. Pesticides - Past, Present and Future. *Pestology*. 2003; 27(12):13-16.
- Chandel BS, Chauhan RRS, Kumar A. Phagodeterrent efficacy of rhizome extract of sweetflag, *Acorus, calamus* against *Tribolium castaneum*, *Indian J Ent*. 2001; 63(1):8-10.
- Chandel BS, Sachan N, Chauhan RRS, Yadav S. Bioefficacy of plant extracts for the management of red cotton bug, *Dysdercus koenigii* Fab, *India J Ent*. 2005; 67(1):89-90.
- Chandel BS, Arti Singh. Repellent biopotency of *Cichorium intybus*, *Inula racemosa*, *Tagetes minuta* and *Mantisalca duriaeri* aquash formulations against pulse beetle, *Callosobruchus chinensis* Linn. *Coleoptera: Bruchidae. Life Science Bulletin*. 2016; 13(2):65-68.
- Chandel BS. Evaluation of repellent potentials of *Cichorium intybus*, *Inula racemosa*, *Tagetes minuta* and *Chrysanthemum cinerariaefolium* against pulse beetle, *Callosobruchus chinensis* Linn. *Coleoptera: Bruchidae International Journal of Entomology Research*. 2017; 2(6):88-93.
- Chandel BS. Biopotency of *Acorus calamus*, *Wthania somnifera* and *Azadirachta indica* in suppressing oviposition and adult emergence, caused by of *Callosobruchus chinensis* L infesting stored chickpea seeds in U.P. *International Journal of Zoology Studies*. 2017; 2(6):161-166.
- Chowdhury M. Incidence of saw fly, *Athalia lugens proxima* Klug as influenced by level of irrigation and fertilizers on mustard. *J Plant Prot Sci*. 2009; 1:80-82.
- Guyton K, Loomis D, Grosse Y, El Ghissassi F, Brenbrahim-Tallaa L, Guha N *et al.* Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *Lancet Oncology*. 2015; 2045(15):70134-8.
- Kausik B, Ishita C, Ranajit KB, Uday B. Biological activities and medicinal properties of neem *Azadirachta indica*. *Current Science*. 2002; 82(11):1336-1345.
- Khan MA, Gumbs FA. Repellent effect of *Ackee Blighia sapida* Koenig component fruit parts against stored product insect pests. *Journal of Tropical Agriculture*, 2003; 80:19-27.
- Lowery DT, MJ Smirle. Toxicity of insecticides to obliquebanded leafroller, *Choristoneura rosaceana*, larvae and adults exposed previously to neem seed oil. *Entomol. Exp. Appl*. 2000; 95:201-207.
- Huen K, Bradman A, Harley K, Yousefi P, Barr DB, Eskenazi B *et al.* Organophosphate pesticide levels in blood and urine of women and newborns living in an agricultural community. *Environmental Research*, 2012; 117:8-16.
- Isman MB. The role of botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entemology*, 2006; 51:45-66.
- Jilani G, Saxena RC. Repellent and feeding deterrent effects of turmeric oil, sweetflag oil, neem oil, and a neem-based insecticide against lesser grain borer *Coleoptera: Bostrychidae J Econ. Entomol*, 1990; 83:629-634.

21. Jilani G, Saxena RC, Rueda BP. Repellent and growth-inhibiting effects of turmeric oil, sweet flag oil, neem oil, and 'Margosan-O' on red flour beetle Coleoptera: Tenebrionidae. *J Econ. Entomol.* 1988; 81:1226-1230.
22. Joshi BG, Ramaprasad G, Nageswara Rao S. Neem seed kernel suspension as an antifeedant for Spodoptera Litura in a planted flue-cured Virginia tobacco crop. *Phytoparasitica.* 1984; 12(1):3-12.
23. Jotwani MG, Sircar P. Neem seed as a protectant against stored grain pests infesting wheat seed. *Indian J Entomol.* 1965; 27:199-202.
24. Jotwani MG, Sircar P. Neem as a protectant against bruchid *Callosobruchus maculatus* Fabricius infesting some leguminous seeds. *Indian J Entomol.* 1967; 29:21-24.
25. Jotwani MG, Srivastava KP. Neem insecticide of the future. I. As protectant against stored grain pests. *Pesticides Bombay.* 1981; 15:19-23.
26. Kavitha VS, Revathi N, Kingsley S. Bioefficacy of neem derivatives against IV instar larvae of *Leucinodes orbonalis* Guenee Lepidoptera: Pyralidae. *J Applied Zoological Res.* 2008; 19(2):183-185.
27. Ketkar CM. Utilization of neem *Azadirachta indica* Juss and its byproducts. Directorate of Non-edible Oils & Soap Industry, Khadi & Village Industries Commission, Bombay, India, 1976, 234.
28. Köhler HR, Triebkorn R. Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond? *Science.* 2013; 341:759-765.
29. Koureas M, Tsakalof A, Tsatsakis A, Hadjichritodoulou C. Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. *Toxicology Letters.* 2012; 201:155-168.
30. Murugesan N, Murugesan T. Efficacy of some plant products against spotted leaf beetle Hadda beetle, *Henosepilachna vigintioctopunctata* (F.) in brinjal. *J Biopesticides.* 2008; 1(1):67-69.
31. Patel GP, Jhala RC. Oviposition deterrent, antifeedant and larvicidal action of neem-based formulations on sawfly, *Athalia lugens proxima* Klug Tenthredinidae: Hymenoptera infesting radish, *Raphanus sativus* Linnaeus. *Pestology.* 1999; 23:68-71.
32. Patil SP, Pokharkar RN. Bionomics and control of *Athalia proxima* Klug. *Res J Mahatma Phule Agril Uni.*, 1973; 4:44-50.
33. Patnaik NC, Panda N, Bhuyan K, Mishra BK. Development aberrations and mortality of the mustard sawfly larvae, *Athalia proxima* Klug by neem oil. *Neem Newsl.* 1987; 4:18-19.
34. Pascual N, Marco MP, Belles X. Azadirachtin induced imaginal moult deficiencies in *Tenebrio molitor* L. Coleoptera: Tenebrionidae. *J Stored Prod. Res.*, 1990; 26:53-57.
35. Rahim M. Biological activity of azadirachtin-enriched neem kernel extracts against *Rhyzopertha dominica* F. Coleoptera: Bostrichidae in stored wheat. *J Stored Prod. Res.*, 1997; 34:123-128.
36. Rajendran S, Sriranjini V. Plant products as fumigants for stored product insect control. *Journal of Stored Product Research.* 2008; 44:126-135.
37. Revathi N, Kingsley S. Neem derivatives as a larvicide against country bean pest, *Pericallia ricini* F. Lepidoptera: Arctidae. *J Entomological Res.* 2008; 32(1):27-29.
38. Ruchi T, Amit KV, Sandip C, Kuldeep D, Shoor VS. Neem *azadirachta indica* and its potential for safeguarding health of animals and humans: a review. *Journal of Biological Sciences.* 2014; 14(2):110-123.
39. Salako EA. A review of neem biopesticide utilization and challenges in central Nigeria. *African Journal of Biotechnology.* 2008; 7:4758-4764.
40. Shanmugapriyan R, Dhanalakshmi V. Biological effects of neem on the pupation of *Henosepilachna vigintioctopunctata* Fab on Bittergourd. *Journal of Entomology and Zoology Studies.* 2015; 3(1):78-81.
41. Sarup P, Srivastava VS. Observations on the damage of neem *Azadirachta indica* A. Juss seed kernel in storage by various pest and efficacy of the damaged kernel as an antifeedant against the desert locust, *Schistocerca gregaria* F. *Indian J Entomol.* 1971; 33:228-230.
42. Shanmugapriyan R, Kingsley S. Bioefficacy of neem oil on larvae of bittergourd beetle *Epilachna vigintioctopunctata* Coccinellidae: Coleoptera. *J Ecotoxicology and Environmental Monitoring.* 2001; 11(3):215-219.
43. Schmutterer H. Potential of azadirachtin-containing pesticides for integrated pest control in developing and industrial countries. *J Insect Physiol.* 1988; 34:713-719.
44. Sukhirun N, Pluempanupat W, Bullangpoti V, Koul O. Bioefficacy of *Alpinia galanga* Zingiberaceae rhizome extracts, (E)-p-acetoxycinnamyl alcohol, and (E)-p-coumaryl alcohol ethyl ether against *Bactrocera dorsalis* Diptera: Tephritidae and the impact on detoxification enzyme activities. *Journal of Economic Entomology.* 2011; 104(5):1534-1540.
45. Thara S, Kingsley S, Revathi N. Effect of neem extracts on the bhendi pest *Earias vittella* Lepidoptera: Noctuidae. *J Experimental Zoology India.* 2008; 11(1):93-94.
46. Thangapandian K, Muthusamy M, Vimal P. Efficacy of the plant products *Acorus calamus*, *Piper longum*, and *Econeem* against the Larvae of Tobacco Caterpillar *Spodoptera litura* Fab. Lepidoptera: Noctuidae. *Journal of Research in Biology.* 2011; 7:490-493.
47. Trisyono A, Whalon ME. Toxicity of neem applied alone and in combination with *Bacillus thuringiensis* Colorado potato beetle Coleoptera: Chrysomelidae. *J Econ. Entomol.* 2000; 92:1281-1288.
48. Wang A, Cockburn M, Ly T, Bronstein JM, Ritz B. The association between ambient exposure to organophosphates and Parkinson's disease risk. *Occupational Environmental Medicine.* 2014; 7:275-281.
49. Yadav SK, S Patel. Bio activity of some plant extracts against larvae of *Spodoptera litura* Fab and *Athalia proxima lugens* Klug under laboratory conditions. *Journal of Entomology and Zoology Studies.* 2017; 5(2):1430-1433.

50. Yuan Y, Chen C, Zheng C, Wang X, Yang G, Wang Q *et al.* Residue of chlorpyrifos and cypermethrin in vegetables and probabilistic exposure assessment for consumers in Zhejiang Province, China. *Food Control*, 2014; 36:63-68.
51. Zehrer W. The effect of the traditional preservatives used in northern Togo and of neem oil for control of storage pests, In H. Schmutterer and K.R.S. Ascher, eds. *Natural Pesticides from the Neem Tree Azadirachta indica A. Juss and Other Tropical Plants*. Proc. 2nd Int. Neem Conf., Rauschholzhausen, Germany, 1983-1994, 453-460.
52. Zeringue Jr, Bhatnagar D. Neem and control of aflatoxin contamination, In R.P. Singh, M.S. Chari, A.K. Raheja, and W. Kraus, eds. *Neem and Environment*, Proc. World Neem Conf., Bangalore, India, 1993. Oxford & IBH Publ. Co. New Delhi, India, 1996; 2:713-727.