Toxic Effects of Neem (*Azadirachta indica*) Based Insecticides on Feeding, Oviposition, Larval Mortality and Flight Behaviour of Cabbage Butterfly (*Pieris brassicae* Linn.) at Ladakh Zanskar

Showket A Dar[1*], Zaffar Bashir[2], Sajad H Mir[3], Mudasir Hassan[4], Tashi Dolkar[5], Tashi Dawa[6], Umer-Bin-Farook[7], Sabreena Ashraf[8], Shafiyah Rashid[9]

[1]Department of Entomology-KVK-Kargil, SKUAST-K, Shalimar Srinagar
[2]Center of Research for Development, University of Kashmir, Srinagar
[3,7,8,9]Department of Entomology-FOA Wadura, Sopore, SKUAST-K, Srinagar
[4]Department of Agriculture, Govt. India, Jammu and Kashmir
[5]Department of Home Science, KVK-Kargil, Ladakh
[6]Programme Assistant, KVK-Kargil-II (Ladakh)

[*]Corresponding Author: showketdar43@gmail.com

Abstract
The present experiment was aimed at the ecofriendly management of *Pieris brassicae*. Total of five Neem based insecticide were evaluated against various behavioral parameters of *Pieris brassicae* at KVK-Kargil Zanskar during 2019 to 2020. Neem based products such as Organeem (5000ppm @1-2 ml/liter), Neem oil (0.25% EC @ 20 mg azadirachtin/liter), Astha killer (1000ppm @1ml/liter), killer 15(1000ppm @1ml/liter)and killer 30 (1000ppm @1ml/liter) were evaluated at standard field rates for oviposition deterrence, feeding inhibition, larval mortality and adult movement. Standard concentrations were tested under filed and laboratory conditions against *P. brassicae* starting from first larval egglaying till adult. Significant ($P < 0.01$) differences were recorded in oviposition deterrence and mortality on both treated and untreated control under both experiments. Conducting *Chi-square test* thecumulative larval feedingwas found significant.The egg count per leaf oviposited was lower in treated plants compared to untreated, and asignificant differences were detected among the neem insecticides. Feeding inhibition and larval mortality were significantly at 1st instar stage. Similarly, the treated plants showed deterrence to larval feeding and larvae dropped from the leaf, resulting in minimal damage. Larval survivals were reduced significantly under treated plots compared to control.Effects of pesticides on adult movements were recorded in open field conditions immediately after treatments and variation were noted. Taking this into account we concluded that all neem-based biopesticides have considerable effect on many behavioral characteristics of *P.brassicae* under cold arid conditions of Ladakh.

Keywords: *Pieris brassicae,* neem based insecticide, biopesticides, Ladakh

Introduction

In Union territory of Ladakh, the Zanskar valley is one of the coldest arid inhabited highlands in the world. It has an altitudinal range of 3,500 m-6,478 m AMSL, remotest and least accessible land with minimum annual rainfall and mean temperature range from -30 to 28°C.Among many vegetables grown, the White Cabbage is one of the major vegetable produced and consumed round year. India is second producer of cabbage after Japan.India produces 184.4 million tonnes of vegetables cultivated on the total area of 10.26 Million Hectares. Vegetables shear highest contribution of 59-61% of total horticulture crop production over last five years. Cabbage was cultivated on an area of 339 thousand hectares produced approximately 19037 thousand million tonnes (HSD, 2017-2018). Among many constraints vegetable production faces in Ladakh region, insect pest are the biggest threat. *Pieris brassicae* is a most common pest of family Brassicae (cabbage, mustard), brussels sprouts, cauliflower, kohlrabi, rape, swede and turnip.The agriculture techniques, methods and crop production (quality and quantity) are different from rest of the country and farm holding under vegetables production is smaller than 0.2 acre in the region (Stobdan *et al.*, 2017).Due to multiple side effects of synthetic pesticides the development of new safer and eco-friendly pesticides have forced scientists and industrialist to take immediate measures to protect yield losses besides to save environment. Plants are rich sources of secondary metabolites, and extracts and oils from various plants have pesticidal effects. Among these botanical plants the Neem tree (*A.indica*) have been found to be one of the excellent alternatives to synthetic chemicals for the management of insect pest. Neem act as pesticides due to various properties such as toxicity, repellence, feeding inhibition, oviposition deterrence, prolong or inhibiting life cycle, retard growth regulation and flight (RRR) interruption of *Pieris brassicae*. The azadirachtin is a steroid-like tetranortriterpenoid induce antifeeding and toxic effects in larvae of *P.brassicae* (RRR). Higher doses of neem biopesticides work as contact insecticides and cause direct killing of larvae; further it influence the process of hormone synthesis and produce ecdysone-type effects in Lepidopteran larvae. Turcani (2001) sprayed cabbage leaves with neem seed kernel (NSK) suspension and recorded a reduced damage by *P.brassicae* larvae at all stages of their growth.Further, the organic agriculture systems increase and boast the diversity of other beneficial organisms, irrespective number of disease it suffers under natural conditions (Tlak *et al.*, 2010; 2014a, 2014b). Commercially available biorationals (pheromones and attractants) are effective against various insects' pests and have shown the huge impact in insect pest control (e.g. fruit fly). Therefore, summarizing the role of organic insecticides, it is confirmed that there is no alternate way except organic system that is too much compatible with the environment for prolong life and food sustainability (Mir and Ahmad 2017; Mir and Mir 2015a, 2015b). therefore, taking the role of biopesticides into consideration for yield loss reduction, various studies have showed a significant impact on insect control besides being safer to environment; therefore, aim of the current investigation was to study the effects of neem based insecticides on *P.brassicae* behaviour and its management in Zanskar Ladakh.

Material and methods

Experiment was conducted in fieldat three locations and at laboratory of KVK-Zanskar under normal temperature, RH with photoperiod. The *P. brassicae* adults, 1st, 2nd, 3rd, 4th and 5thinstars were collected from field at different intervals of crop growth. Five commercial Neem based products of standard formulations were sprayed at standard field rates with a time gape of 15 day interval. Spraying started from 2nd week of May and last till 1st week of July (1st generation of *P. brassicae*). The cabbage seedlings were sown under green house in the plots of area 4 x 2.5 ft. For studying feeding, oviposition, larval movement, mortality and oviposition parameters under laboratory conditions we took five pots planted with cabbage seedlings. The pots were placed in laboratory and caged, and later adult pairs of *P. brassicae* were inserted. Normal recommended package of practice were followed for raising plants in laboratory as well as in fields. The pest completes two generations in year (1st generation from 2nd week May- 1st week July; 2nd generation from 3rd week July-2nd week September). To evaluate various effects of Neem based insecticides on *P. brassicae*, five formulations were made and water as spray was taken as control. All the six treatments were test under field and laboratory experiments for various toxicological parameters. Spraying was done every 15 days interval and fields were monitored continuously during the study periods; however the effect on adult movement were evaluated under field conditions only. Data on 1st generation were recorded and residual effect of pesticides if any on 2ndgeneration populations of *P. brassicae* were not considered, so excluded from current study. Analysis of data was done using excel and R software.

Results and discussion

We observed that proportion of eggs laid on the lower side of leaves were significantly different for different treatments used under both experimental conditions. Under caged laboratory conditions the ovipositions were lesser than under open conditions due to various reasons (**Table 1**), such as quick photo-degradation of chemicals by intense UV rays and chemical dissipation by afternoon winds. Whileas as under control treatments, in laboratory as well in filed, the oviposition were comparatively higher.Comparing bothexperimental conditions, significantly ($P<0.001$) lesser number of eggs were deposited when organeem were sprayed, compared to all other treatments. Significant differences ($P<0.001$)were detected between Organeem and Neem oil treatments, when compared with other treatments; and treatments were ranked in terms of oviposition deterrence as: Organeem>Neem oil>Astha killer> killer 30> killer 15. Significantly ($P<0.001$) more leaf area was consumed under control compared to other treatments in both experiments. The effect of insecticides on 1st instar stage were more compared to later instars as shown by curves (1 to 4) and regression equations.

The direct contact Organeem and neem oil caused higher larval mortality under field conditions compared to other treatments (**Table 1**) and laboratory conditions.Under different treatment applied on larvae of *P.brassicae*, various effects on adults were observedstarting at the time of adult oviposition, especially in their flight distance, movement and landing. Immediately after treatment application, the approximate distance traveled and movement types and landing on vegetation (desirable and undesirable) were found to be disturbed compared to normal behaviour. We observed that *P. brassicae* adults normally orient their flights from field to fields on brassicae plants. On non-host plants their landing during morning hours is not observed especially during their peak oviposition periods. Under no treatment condition, minimum straight path distance traveled was 200 meters. Later followed by their movement to pastures, non-host plants and water channels to warm and get energy under full sun light. But maximum of flight time and orientation were in cabbage fields under normal conditions and untreated plots compared to treatments plots. During our study period, F1-Hybrid Cabbage S-92 was more susceptible to damage compared to Red cabbage varieties (**Figure 1**). The distance travelled over 40 plots of F-1 Hybrid cabbage S-92 were straight, uninterrupted, continuous and slow under no treatment. The slow movement over the F1-Hybrid Cabbage S-92 plots indicates that the adult female prefer this variety for oviposition and subsequent feeding by larvae, a principle of host preference and suitability.Therefore, based on our visual observations the flight movements and distance travelled weredisturbed when a plot were sprayed and is noted as shown in **Table 1**. However, more study is needed to determine the exact behavioral changes brought about by neem based insecticides.

Pieris brassicae is present throughout the Ladakh region in agricultural and horticultural area, meadows, sandy deserts and parklands. Eggs look like large ova pale yellow, turning darker within 3-4 hours of being oviposited. Larvae have four moultings and five instar stages, light yellow in colour with brown head and soft bodies. The 2nd instar larval movement is slow with localized damage (**Figure 2**); while as 3rd instar stage is most active and observed to eat voraciously and cause significant damage to cabbage (**Figure 3**). During the 4th instar stage, the activity and feeding rate is reduced and followed by final 5th instar stage (**Figure 4**). During all instar stages maximum of the food is consumed in order to support full development, otherwise larvae dies quickly (compared to temperate areas) before pupation under harsh dry environmental conditions of Ladakh region. We found that *P.brassicae* habitat consists of large, open spaces, farm and vegetable gardens, lawns and meadows. Walls, fences, tree trunks and other fruit crops (during flowering periods) are also visited for landing, mate searching and sucking nectar (apricot, apple, etc). The most favorite host plants are members of family brassicae, adult female butterfly oviposit on under surface of leaves in clusters (10-95 eggs/cluster).Pre oviposition periods lasts for 3-8 days, however mating season is even longer under cold arid conditions (>10 days). Before oviposition females use its forelegs to drum on surface to test surface landing, mating and oviposition suitability as an important suitable host selection factor. Egg-laying occurs 2-8 days after copulation, oviposit 6-8 times in day in small clusters, and mate 20 days after previous mating event.We found that females rely on visual cues like colour plants, to decide where to lay eggs. Females land on green vegetations (**Figure 5**) during day time especially from morning to afternoon hours (10 am-1.00 pm). Females spent almost 85% of their time over cabbage and related brassicae plants. The females choose thistles for nectar (**Figure 6**) and landing too. However, other plants were also observed to be landed by *P. brassicae* but thistle plants preferred because of plant tallness, and nectar content that is easily available to butterflies due to their long tongue. Alfalfa is other dominant plants in Zanskar; but its nectar content is exhausted by many species of flies and bees. Adult movement over other weeds and ornamental flowers were also observed, but data were not recorded. None of the plants were found to act as oviposition site, except cabbage. The eggs were deposited on undersurface of leaves away from leaf center towards the apex and near to leaf edges. Lower leaves were preferred for oviposition and hatching took place at same place around 10-12 hours after, depending on the environmental conditions. Upon hatching the larvae cause a lot of damages to leaves by eating, drying, skeletonization and defecation (**Figure 3**) on leaving keeping only mid vain visible (**Figure 8**). The damage by first instars (1^{st}-3^{rd}) is localized and less compared to advanced stages (**Figure 1, 2, 7-9**). All instar stages were found to start feeding from apical margin of leaf and sides; however in many plants oviposition was found in middle of leaf on under side; therefore first instar stages start feeding from the middle of leaf due to which leaf look transparent. First (1^{st}) instar larval mortality and feeding inhibition under laboratory and field conditions are represented in **Figure 10, 11, 12 & 13** respectively.

Table 1: Mean effect of various neem based pesticides on oviposition, feeding inhibition, larval mortality and straight path flight and movement types of *P. brassicae* under cold arid conditions of Ladakh.

Treatment	Oviposition (eggs/cm^2)		Feeding inhibition (%) (Area/minute)		Larval mortality (larvae died/week)		Adult Flight(movement type, straight path distance travelled in meters)	
	Lab	Field	Lab	Field	Lab	Field	Laby	Field
Organeem	10	14	50	48	65	88	-	10 (random)
Neem oil	16	21	35	33	43	56	-	25 (random)
Astha killer	32	46	10	9	12	13	-	36 (random)
Killer 30	33	44	12	7	11	12	-	40 (random)
Killer 15	34	40	11	6.8	9.7	10	-	45 (random)
Water	40	52	-	-	5	4	-	-
Significance	**N=6; df.=5; P<0.001)P<0.001)P<0.001)**							

Figure 1: Red cabbage variety comperatively resisatnt *to P. brassicae* damage (no damage, no egg laying observed)

Figure 2: 2nd instar larave of *P. brassicae* started feeding on cabbage lower leaf margin

Figure 3: 3rd instar larave of *P. brassicae* finished cabbage lower leaf margin

Figure 4: 5th instar defecation and feeding inside the leaf worls of cabbage.

Figure 5: Adult female cabbage buttefly landing on thistel plant during full sunlight (4-5 minutes spent in warming)

Figure 6: Adult female cabbage buttefly nectaring on thistel plant; however no egg laying on it was observed (1-2 minutes spent)

Figure 7: 1st and 2nd instar mortality after pesticide treatment; survived 3rd instar obsrved moving for safe surface on margin

Figure 8: 2nd instar larave feeding and leaving behind leaf veins visible.

Figure 9: 2nd instar larave feeding from margin insdies on both sides of leaf.

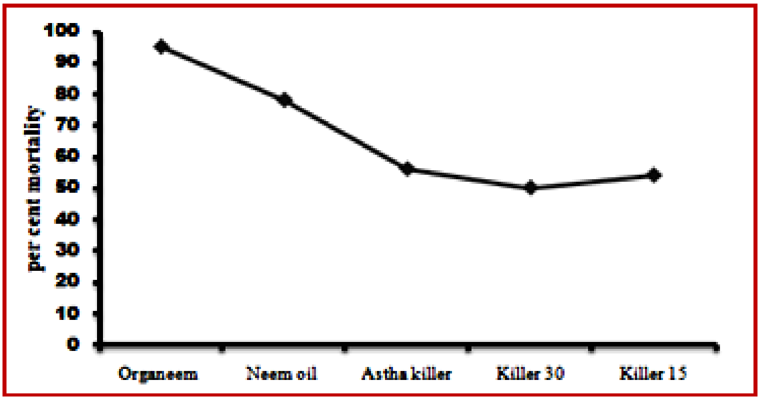

Figure 10: 1st instar larval mortality of *P. brassicae* under filed conditions

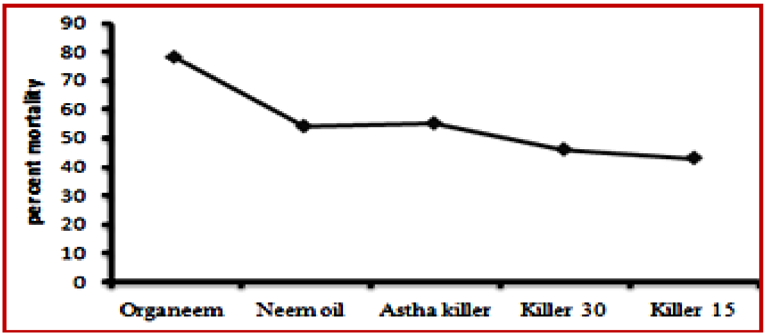

Figure 11: 1st instar larval mortality of *P. brassicae* under laboratory conditions

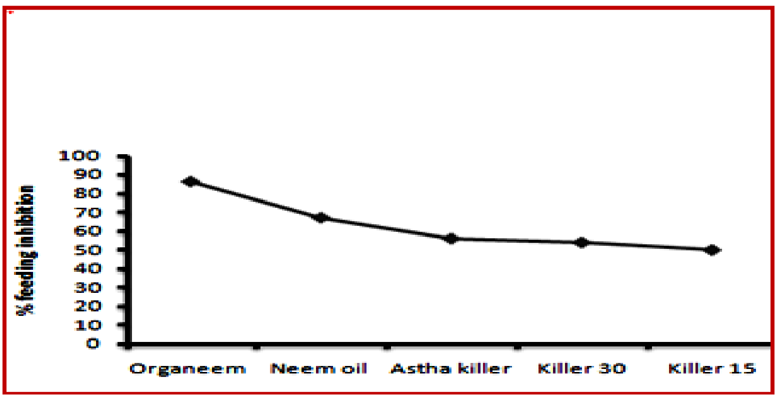

Figure 12: Feeding inhibition of 1st instar larvae of *P. brassicae* under laboratory conditions

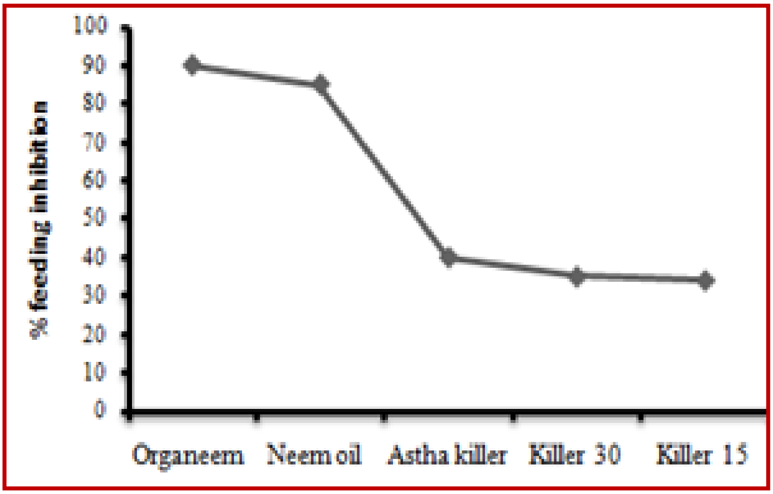

Figure 13: Feeding inhibition of 1st instar larvae of *P. brassicae* under field conditions

Acknowledgement: We are thankful to SKUAST-K, for providing many facilities to compile this research article

References

Gajger, T., Jurković, I., Koščević, M., Laklija A. I., Ševar, M 2014a: Prevalence and distribution patterns of seven different honeybee viruses in diseased colonies: a case study from Croatia. ***Apidologie***. **45 (6):** 701-706. doi: 10.1007/s13592-014-0287-0.

Gajger, T., Jurković, I., Koščević, M., Laklija A. I., Ševar, M. (2014b: Prevalence of *Cacoxenus indagator* larvas in Osmia spp. artificial nests setteld in Croatia. Book of abstracts: ***ApiEco Flora and Biodiversity International Symposium***. 6-7. November Rome, Italy, 73-73.

Horticulture Statistics Division. 2018. Department of agriculture, cooperation and farmers welfare. Ministry of agriculture and farmers welfare Government of India. Horticulture statistics at Glance. Government of India, **PDES-256 (E) CP**, 500-2018-(DSK-III). 458pp

Mir S. H, Ahmad S. B. 2017. Field Evaluation of various dispensers for methyl eugenol in india, an attractant of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). ***Journal of the Kansas Entomological Society***. **90 (3):** 189-193

Mir SH, Mir GM. (2015). Evaluation of neem seed kernel extract for the management of melon fly, Bactroceracucurbitae Coquillett in cucumber (Cucumis sativus L.). ***Biopesticides International***. **11 (1):** 73-77.

Mir SH, Mir GM. (2015). Lekking behaviour and male-male rivalry in the melon fly *Bactrocera cucurbitae* (Coquillett)(Diptera: Tephritidae). ***Journal of Insect Behavior***. **29 (4):** 379-384

Stobdan, T., Angmo, S., Angchok, D., Paljor, E., Dawa, T., Tsetan, T., Chaurasia, O. 2017. Vegetable Production Scenario in Trans-Himalayan Leh Ladakh Region, India. ***Defence Life Science Journal***, ***3*****(1):** 85-92. https://doi.org/10.14429/dlsj. 3.11661

Tlak Gajger, I., Z. Tomljanović, Z. Petrinec (2010): Monitoring health status of Croatian honey bee colonies and possible reasons for winter losses. ***J. Apicul. Res*****. 49(1):** 107-108. doi.org/10.3896/IBRA.1.49.1.19

Turcani M. (2001). The preliminary results of trails conducted with neem and combinations of neem and *Bacillus thuringiensis var Kurstaki* in gypsy moth (*Lymantria dispar* L) control in Slovaka. In Metspalu, L & Mitt, S. (eds): Practice oriented results on the use of plant extracts and pheromones in pest management. ***Tartu***, pp. 120-123