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Study on the Evaluation of Toxicity of *Nerium indicum* (M.) Against Cabbage Butterfly, *Pieris brassicae* (L.) (Lepidoptera: Pieridae)

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ABSTRACT: Plant extracts contain many active compounds, which are tremendously fruitful for plant defence against several insect pests. This study is aimed to evaluate the leaf and bark extracts of *Nerium indicum* plant against cabbage butterfly, *Pieris brassicae*. The toxicity activity of leaf and bark extracts of *N. indicum* M. against second instar larvae of cabbage butterfly was carried out using methanol, hexane and distilled water as solvents by no choice method. Different concentrations of leaf and bark (100, 75, 50, 25 and 10%) were used in this study. The results revealed that among various solvents, methanol found to be significantly superior in extracting the toxic constituents than the hexane and distilled water. The highest concentration (100%) of methanol leaf extract used in this study, showed mortality of 40.0, 46.7 and 53.3 per cent after 24, 48 and 72 hours of exposure, respectively. whereas, at same concentration and time period, hexane and distilled water leaf extract showed 33.3, 35.0, 41.7 per cent and 25.0, 26.7, 28.3 per cent mortality, respectively. Among leaf extracts, methanol leaf extract at 100% concentration showed maximum per cent mortality 53.3% after 72 hours of exposure as compared to hexane and distilled water leaf extracts. However, among bark extracts, the maximum per cent mortality was recorded in methanol bark extracts (36.7%) at same concentration and time period. These results indicate that among other botanicals, *N. indicum* can also be used as one of the botanical in IPM and organic farming for managing cabbage butterfly.

Keywords: *Nerium indicum*, leaf and bark extracts, cabbage butterfly, Per cent mortality.

INTRODUCTION

Cole crops are one of the most important groups of vegetable crops which are widely grown and popular in almost all the regions of the country. The leafy vegetables especially cole crops make up a major portion of the diet of humans and are rich sources of vitamins (C, A, B1, B6, B9 and E), minerals, dietary fibre and Phytochemical (Dias, 2012). Among the various temperate vegetables produced in Jammu and Kashmir, the cole vegetable crops are the important ones that add higher revenue to the state (Shankar *et al.*, 2006). Cabbage is very convenient to grow both on large and small scale cultivation.

Cabbage, *Brassica oleracea var, capitata* belongs to brassicaceae family, is an important vegetable grown worldwide. It is one of the important cole crop grown mostly in winter season which occupy an important position in meeting dietary requirement of most of the people all over the world. It is cultivated on 0.31 million hectare with the total production of 6.87MT and average productivity of 22.1MT/ha. Insect pests are the major constraints for the cultivation and production of cole crops. Pajmon (1999) listed 38 insect pests that feed on the cole crops namely cabbage, cauliflower, broccoli, Brussels sprouts, collards, mustard, radish and turnip. However, in India, a total of 37 insect-pests have been reported to feed on cabbage (Lal, 1975). Among these insect pests, cabbage butterfly, *Pieris brassicae* (L.) (Lepidoptera: Pieridae) is one of the most destructive pest causing damage from seedling to vegetative and flowering stage (Lal and Ram 2004; Younas *et al.*, 2004) and also been found to be major pests of cabbage and cauliflower in India (Firake *et al.*, 2012). In India, this pest has been reported to be one of the serious pests in different regions of the country

(Rizvi *et al*., 2009) and distributed in Himalayas while in plains, it has been reported as major pest from Punjab, Haryana, West Bengal, Bihar, A.P., Orissa and Meghalaya (Hemchandra and Singh, 2005). Usually the management of this pest is insecticide oriented, but synthetic insecticides used to control infestation in the field conditions is becoming hazardous day by day specially with growing problems of the pest resistance, secondary pest outbreak, environment contamination and the side effects on the beneficial flora and fauna which have necessitated the development of newer control methods (Jainulabden and Prasad, 2004).

About 450 pest species of insects and mites have now developed resistance to one or more synthetic pesticides (Georghiou, 1986). So, the use of botanicals is one of the best alternatives for these hazardous pesticides. Plants being the richest source of organic chemicals have secondary plant metabolites which possess insecticidal properties. Insecticidal plants generally present a wide spectrum of control among insect pests, are relatively specific in their mode of action, degrade rapidly, usually have a low environmental impact and are fairly safe for non-target organisms (Sousa Neto *et al*., 2018). Plant products have proved to be useful in formulating sound pest management strategies (Kashyap *et al*., 1993). These are short lived in the environment, pose relatively low risk to non- target organisms and besides this, can be derived with minimal technology. A number of plant species like neem, lantana, eucalyptus, nerium, Pongamia, *Solanum nigrum* L. etc are known to possess insecticidal properties. The compound from these plants have a number of useful activities like toxicity, repellence, feeding and oviposition deterrence and insect growth regulator activity etc. (Mordue, 2004). Among these plant species, *Nerium indicum* M. is a small evergreen tree commonly known as kaner. Larvicidal activity of N. oleander due to the major components namely flavonoids, sterols and terpenes (Fouad *et al.*, 2015) and presence of a mixture of very toxic cardiac glycosides of cardenolides (Trease and Evans, 2002; Tiwari and Singh, 2004) has already been reported. This plant has also been reported for insect growth regulatory activity against *Anopheles stephensi* and *Culex quinquefasciatus* (Pushpalatha *et al*., 1995); ovicidal and adultcidal activity against *Anopheles stephensi* (Roni *et al*., 2013) and ovicidal and larvicidal properties (Kumar *et al.*, 2012). So, it is of utmost importance to explore its potential in the field of pest management. As such, further more work is needed to investigate the potential of this plant against cabbage butterfly.

MATERIALS AND METHODS

Maintenance of cabbage butterfly culture: Cabbage seeds variety Pride of India were sown on nursery beds at three intervals (10 days each) starting from September 19, 2018 in the vegetable field, Division of Vegetable Science and Floriculture of SKUAST-Jammu to raise the seedlings for transplanting. Four week old healthy cabbage seedlings were planted in pots having depth of 12 inches and a diameter of 18 inches, filled with soil and vermicompost in order to get the regular supply of leaves and kept at premises of Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu (SKUAST-J). Raised seedlings in fifteen pots were watered at regular intervals. These pots were later covered with nylon netting (10x9x7ft) and used to maintain the culture of cabbage butterfly. Some flowering plants of marigold and *Nasturtium sp* were also raised in the vicinity as nectar source. Cabbage seedlings were also planted in the field of Experimental Farm of Division of Entomology, SKUAST-Jammu in the flat bed method at a spacing of 16cm x 45cm in an area of 150 m^2. Three batches of cabbage seedlings were sown at 10 days interval starting from October 17 for regular supply of leaves for bioassay studies and collection of egg masses.

Rearing of test insect: The stock culture of *P. brassicae* was maintained in potted plants. For this purpose, egg clusters were collected from the cole crops in the field. The newly hatched larvae were reared in laboratory in glass jars (50x30cm) with their tops covered with muslin cloth to permit exchanges of gases and secured in position with the help of rubber band. Fresh healthy and tender leaves of cabbage were served as food to the larvae and the food was changed daily. The larvae were kept in rearing jars till adult formation and after emergence of adults, released for egg laying on cabbage plants raised earlier in pots under nylon netting. After emergence of larvae from eggs, the 2nd instar larvae were used for bioassay studies.

Collection of *N. indicum* leaves and bark for extraction:

The branches of *N. indicum* were cut and brought to the lab. The leaves were separated and bark was peeled off from the branches. Finally, the leaves and bark cut into small pieces and ground into a powder by making use of electric mixer. Plant parts used were leaves and bark using solvents methanol, hexane and distilled water.

Preparation of plant extracts: The extracts were prepared by mixing powdered leaves with three different solvents namely methanol, hexane and water in a ratio of 1:2 w/v (300 g in 600 ml of respective solvents). 300g of leaf powder was taken in the mixer (Model-Gx1Bajaj) and blended with 600ml of respective solvents for one minute at low speed. The extracts were placed on orbital shaker for half an hour at 120 rpm and the extract after shaking was filtered through double layered muslin cloth into beaker. After this, the extract was filtered through filter paper using a funnel. The remnants of the extract over the filter paper eluted with 50 ml of respective solvents. The final volume of leaf extracts was made to 500 ml. The extraction from bark has also been conducted in the same manner as discussed above. Finally, the extracts

so obtained were kept in a freezer at 4^0C until bioassay studies.

Toxic bioassay:

Evaluation of toxicity of *Nerium indicum* against cabbage butterfly by leaf dip method

Fresh cabbage leaves were collected and washed with tap water followed by preparation of 9cm diameter petriplate size leaf disc. These leaf discs were dried at room temperature under fan followed by dipping in desired concentrations of methanol, hexane and distilled water extracts for one minute and again dried. The control leaf discs were dipped in respective solvents for same period of time. The 2nd instar larvae were pre-starved for one hour and released on the treated leaves in each Petriplate (20 in each Petriplate) by using a soft camel hair brush. In order to maintain moisture, a moist filter paper was placed in each Petriplate under the leaf disc. The Petriplates containing the treated leaves and larvae were placed in an incubator 26±1°C. Each treatment including control was replicated three times. Data on larval mortality were recorded after 24, 48 and 72 hours and converted to per cent larval mortality as per the formula:

$$(\text{Mortality } \%) = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \times 100$$

Data Analysis. The experiment was conducted using completely randomized design (CRD). The data on per cent mortality were subjected to angular transformation. The data on per cent mortality of plant extracts at different treatments were analyzed using one way analysis of variance (ANOVA) at p=0.05 by OPStat.

RESULTS AND DISCUSSION

The results indicate that mortality values significantly increased depending on the increasing leaf and bark extract concentrations and with time of exposure. Considering leaf extracts, methanol leaf extract at 100% concentration was most effective treatment which recorded highest per cent mortality of 40.00, 46.67 and 53.33% after 24, 48 and 72 hours of treatment followed by leaf extract of hexane with mortality of 33.33, 35.00, 41.67% and distilled water extract having mortality to the tune of 25.00, 26.67 and 28.33% at same concentrations and time periods (Table 1). However among bark extracts, methanol bark extract again showed highest per cent mortality (31.67, 35.00 and 36.67%) followed by hexane (28.33, 33.33, 35.00%) and distilled water (20.00, 23.33 and 25.00%) after 24, 48 and 72 hours of exposure. Maximum cumulative mortality to the tune of 53.33 and 36.67% after 72 hours of exposure was observed at the highest botanical concentration (100%) for methanol leaf and bark extracts of *N. indicum* respectively (Table 2). Whereas, minimum cumulative mortality to the tune of 28.33 and 25.00% after 72 hours of exposure was observed at the highest botanical concentration (100%) for distilled water leaf and bark extracts of *N. indicum*. Overall, methanol leaf and bark extracts were found effective in comparison with hexane and water extracts.

In the present study methanol extract was found to be most effective. In contrast to our findings Ramya *et al*., (2008) evaluated larvicidal activity of *Catharan thusroseus* leaf extracts in crude methanol, petroleum ether, methanol fraction and ethyl acetate fraction against larvae of *Helicoverpa armigera* and they observed the highest larval mortality in ethyl acetate fraction followed by methanol fraction. This variation might be due to presence of high concentration of active compounds present in the leaves of plant, solvent used and test insect. Results of present study revealed that mortality was significantly increased with increasing concentrations and time period. Almost same findings has been reported by Moustafa *et al*. (2018) who evaluated seventy per cent hydroethanolic extracts of leaves, stems and flowers of *N.oleander* against the first instar larvae of *Pectinophora gossypiella* and recorded that the toxic effects of the extracts increased with increase in concentration and recorded the highest mortality, 80% at 20g/100g of diet. Experimental results of Ali *et al.* (2008) showed that *T. granarium* larvae showed 10% mortality after 72 hours when fed at 100mg/g of *N. oleander* leaf extract. Whereas, Satpathi and Ghatak (1990) found that methanol extracts of roots of *Nerium oleander* when applied topically resulted in 100% mortality of fourth instar larvae of *P. xylostella* in 12 to 24 hours after treatment. Semiz (2017) studied larvicidal activity of *N. oleander* crude aqueous leaf extract against pine processionary moth, *Thaumetopoea wilkinsoni* and recorded highest larvicidal activity with LD_{50} value of 322.5 and 190.0 ppm after 24 and 48 hours of exposure. However, Jemberie *et al*. (2017) evaluated chloroform, methanol and distilled water extracts of *Argemone mexicana* leaves against fourth instar larvae of Culex mosquito and recorded mortality to the tune of 80.0, 76.0 and 76.0% in chloroform, methanol and distilled water extracts of leaves after 96 hours of exposure time. This variation might be due to the feeding material, plant part used, larval instar used and exposure time. Javier *et al.* (2018) studied the potential of ethanolic extracts from five different plants *viz.*, *Lantana camara* Linnaeus, *Coleus amboinicus, Loureiro Alpinia pyramidata Blume*, and *Catharanthus roseus* Linn. as insecticide against second larval instar diamondback moth, *Plutella xylostella* Linn. Among the five plants, they found that L. camara was the most toxic against *P. xylostella* through topical application (LD50 = 99.17 µg/g larva).

Among bark extracts, methanol bark was more effective followed by hexane and distilled water bark extracts. The results are in conformity with Pradeshi and Zambare (2012), who studied the insecticidal activity of *N. indicum* bark extract against *Callosobruches chinensis* for their oviposition, adult emergence and mortality. They found that the number of eggs laid and adult emergence from the seeds treated with ethanol

extract of *N. indicum* bark were less than from seeds treated with methanol extract. These results are in agreement with Sinha, (2012) who studied the toxicity of *Albizia odoratissima* against 3rd instar larvae of *P. brassicae* and reported that after 72 hours of exposure methanolic extract of bark caused 87.5%. The variation might be due to plant material used. Similarly, Lokesh *et al*. (2010) found that the leaf extracts of *N.oleander* and *Trigonella foenum* resulted in mortality of mosquito's larvae of 50.0 and 30.0% at 3% concentration and the combination of the extracts increased the activity considerably to a high per cent. The toxicity of ethanol extracts of the leaves of *Citrullus colocynthis*, *Cannabis indica*, and *Artemisia argyi* against *Brevicoryne brassicae* were investigated by Ahmed *et al*. (2020) under laboratory conditions. They found that *A. argyi* proved to be the most toxic in both bioassay methods and caused maximum mortality, followed by *C. colocynthis* and *C. indica*, in a dose-dependent manner.

In the present study, among hexane and distilled water extracts, hexane was found to be effective followed by distilled water. In contrast to our findings Zewdu, (2010) studied toxic effects of deionized water, acetic acid, chloroform, toluene and hexane extracts of *Milletia ferruginea* against *Acyrthosiphon pisum* and reported that deionised water extract was more toxic (98% mortality) followed by acetic acid (89%) whereas, the chloroform, hexane and toluene extracts were the least toxic. The variation in mortality might be due to plant and test insect. Experimental results of Raveen *et al.* (2014) reported that extract of *Murraya koenigii* flower in hexane exhibited larva mortality of mosquitoes with LC_{50} value of 102.54 and 61.11ppm after 24 and 48 hours, respectively.

Table 1: Toxicity of *Nerium indicum* leaf extract against cabbage butterfly.

Conc. of leaf extract (%)	Mortality of larvae (%)								
	Methanol			Hexane			Distilled water		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
100	40.00 (39.19±1.69)	46.67 (43.07± 0.96)	53.33 (46.90±2.54)	33.33 (35.20±2.06)	35.00 (36.22±1.72)	41.67 (40.16±1.95)	25.00 (29.79±3.23)	26.67 (30.93±2.84)	28.33 (32.08±2.09)
75	35.00 (36.22±1.74)	38.33 (38.18± 2.63)	45.00 (42.10±1.67)	25.00 (29.91±1.92)	28.33 (32.00±2.86)	33.33 (35.24±1.02)	18.33 (25.18±2.40)	20.00 (26.44±2.08)	21.67 (27.70±1.14)
50	26.67 (30.98±2.21)	31.67 (34.13± 2.70)	36.67 (37.19±2.62)	18.33 (24.99±3.43)	21.67 (27.70±1.14)	25.00 (29.91±1.92)	8.33 (16.59±1.84)	11.67 (19.88±1.45)	11.67 (19.88±1.45)
25	5.00 (12.92± 0.00)	8.33 (16.59 ±1.84)	10.00 (14.04±2.85)	6.67 (14.75±1.84)	6.67 (14.75±1.84)	10.00 (18.04±2.85)	5.00 (12.92±0.00)	6.67 (14.75±1.84)	8.33 (16.59±1.84)
10	3.33 (8.61± 4.30)	6.67 (14.75± 1.84)	8.33 (16.59±1.84)	3.33 (8.61±4.30)	5.00 (12.92±0.00)	6.67 (14.75±1.84)	3.33 (8.61±4.30)	5.00 (10.45±5.46)	6.67 (14.75±1.84)
Control	1.67 (4.30±4.30)	3.33 (8.61± 4.30)	5.00 (12.92±0.00)	3.33 (8.61±4.30)	3.33 (8.61±4.30)	5.00 (12.92±0.00)	1.67 (4.30 ±4.30)	5.00 (12.92±0.000)	5.00 (12.92±0.000)
CD (P at 5%)	(8.68)	(8.08)	(6.68)	(9.84)	(7.46)	(5.69)	(9.58)	(8.78)	(4.85)
SE(m)	(2.82)	(2.59)	(2.14)	(3.16)	(2.40)	(1.83)	(3.07)	(2.82)	(1.56)

Data are mean ± S.E of three replications; Figures in parentheses are Angular transformed values.

Table 2: Toxicity of *Nerium indicum* bark extract against cabbage butterfly.

Conc. of leaf extract (%)	Mortality of larvae (%)								
	Methanol			Hexane			Distilled water		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
100	31.67 (34.13±2.70)	35.00 (36.22±1.74)	36.67 (37.24±0.99)	28.33 (32.00±2.86)	33.33 (35.15±2.72)	35.00 (36.14±3.08)	20.00 (26.44±2.08)	23.33 (28.84±1.14)	25.00 (29.91±1.92)
75	26.67 (31.06±1.07)	30.00 (33.15±1.81)	30.00 (33.15±1.81)	23.33 (28.65±3.08)	26.67 (30.93±2.84)	26.67 (30.93±2.84)	15.00 (22.59±2.35)	16.67 (24.04±1.26)	20.00 (26.44±2.08)
50	16.67 (24.04±1.26)	16.67 (24.04±1.26	18.33 (25.29±1.26)	13.33 (21.33±1.45)	13.33 (21.33±1.45)	13.33 (21.33±1.45)	6.67 (14.75±1.84)	6.67 (14.75±1.84)	6.67 (14.75±1.84)
25	8.33 (16.59±1.84)	8.33 (16.59±1.84)	8.33 (16.59±1.84)	5.00 (12.92±0.000)	6.67 (14.75±1.84)	8.33 (16.59±1.84)	3.33 (8.61±4.30)	5.00 (12.92±0.00)	6.67 (14.75±1.84)
10	3.33 (8.61±4.30)	5.00 (12.92±0.00)	6.67 (14.75±1.84)	1.67 (4.30±4.30)	5.00 (12.92±0.00)	6.67 (14.75±1.84)	3.33 (8.61±4.30)	3.33 (8.61±4.30)	5.00 (10.45±5.46)
Control	3.33 (8.61±4.30)	5.00 (12.92±0.00)	5.00 (12.92±0.00)	1.67 (4.30±4.30)	3.33 (8.61±4.30)	5.00 (10.45±5.46)	1.67 (4.305±4.30)	3.33 (8.61±4.30)	5.00 (10.45±5.46)
CD (P at 5%)	(9.04)	(4.27)	(4.51)	(9.59)	(7.99)	(9.53)	(10.55)	(8.37)	(10.97)
SE(m)	(2.90)	(1.37)	(1.45)	(3.08)	(2.56)	(3.06)	(3.39)	(2.69)	(3.52)

Data are mean ± S.E of three replications; Figures in parentheses are Angular transformed values.

Furthermore, a relatively high extract yield from *C. colocynthis* and *C. sativa* using ethanol as an extraction solvent was reported by Ahmed *et al*. (2019). From the analysis of above data, it shows that methanol leaf extract of *N. indicum* resulted in higher toxicity than hexane and distilled water leaf extracts. Mortality value increases with concentration of extract and also with exposure time. Similar kind of results were obtained from the studies of Sharma (2016), who reported that the methanol was significantly more effective in extracting the toxic constituents than other solvents namely chloroform and ethyl acetate whereas, plant extracts applied at highest concentration resulted in significantly higher toxicity to the third instar larvae of *P. brassicae* as compared to lower concentrations. However, Sharma and Gupta (2009) found that extract of *M. azedarach*, *N. indicum*, *A. indica* and *Ricinus communis* resulted in larval mortality of 19.6, 19.6, 18.5 and 8.9% of *P. brassicae*, respectively.

SUMMARY AND CONCLUSION

The results revealed that the per cent mortality in *N. indicum* leaf and bark extracts showed significant effect against second instar larvae of cabbage butterfly which were increased with the increase in the concentrations of extracts and time period. The results indicate that mortality values significantly increased depending on the increasing leaf and bark extract concentrations and exposure time. Among leaf extracts, methanol leaf extract at 100% concentration was the best treatment which recorded highest per cent mortality of 40.00, 46.67 and 53.33 after 24, 48 and 72 hours of treatment. However among bark extracts, again methanol bark extract proved as most effective which resulted in mortality of 31.67, 35.00 and 36.67% at respective concentrations. Maximum cumulative mortality of 53.33 and 36.67% after 72 hours of exposure was recorded at the highest concentration (100%) for methanol leaf and bark extracts of *N. indicum*. Whereas, minimum cumulative mortality of 28.33 and 25.00% after 72 hours of exposure was recorded at the highest concentration (100%) for distilled water leaf and bark extracts of *N. indicum*. The order of per cent mortality of different leaf and bark extracts against cabbage butterfly was methanol leaf extract >methanol bark extract >hexane leaf extract > hexane bark extract >distilled water leaf extract >distilled water bark extract. Since it is widely available and grows throughout the year, the prospect of using this locally available plant material as a potential source of insecticide can be considered. In conclusion, the results of the present study provide new data focusing on insecticidal activity of *N. indicum* extracts on cabbage butterfly. Such findings may encourage a further research to test *N. indicum* extracts effects against various insect pests of crops.

FUTURE SCOPE

This study will give evidence to support and suggest the usage of plant extracts/botanicals for management of cabbage butterfly and could also facilitate the new formulations thereby making them economically viable, eco-friendly and socially acceptable products.

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Conflict of Interest. None.

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