International Journal of Agricultural Research

ISSN 1816-4897

Academic Journals Inc.

www.academicjournals.com

International Journal of Agricultural Research 6 (4): 335-346, 2011
ISSN 1816-4897 / DOI: 10.3923/ijar.2011.335.346
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Neem Based Insecticides Interaction with Development and Fecundity of Red Cotton Bug, *Dysdercus cingulatus* Fab.

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ABSTRACT

The red cotton bug, *Dysdercus cingulatus*, an important polyphagous pest, causes heavy loss to cotton and lady's finger (bhindi) crops which badly affects the economy of poor farmers. In the present study, the impact of different concentrations of neem based insecticides (NBIs) on coupling, moulting, development, hemocyte, fecundity and egg-hatching of *D. cingulatus* was evaluated. Various concentrations (0.25, 0.5, 1.0 and 2.5%) and doses (2.5 to 20 μL) of NBIs viz. neemark, multineem and neemazal were prepared by diluting them in acetone were applied topically on the dorsum of the body of nymphs and adults and eggs of different age groups. NBIs causes developmental abnormalities such as prolongation of nymphal periods, ecdysial stasis, formation of adultoids and adults with varied degrees of wing deformities. The delay in moulting of treated nymphs is found concentration, dose and stage dependent. The application of NBIs on cephalic and thoracic regions and on ventral surface of insects showed more sensitivity than on abdominal region and dorsal surface. The NBIs application interrupted the coupling and cause lessening of fecundity and egg-hatching also. Among aforesaid NBIs, neemazal treatment was somewhat more effective. In addition, significant reduction in Total Hemocyte Count (THC) and deformities in hemocyte morphology were also observed in NBIs treated insects. It is assumed that the phenomena like metamorphic developments, coupling and fecundity are controlled by Juvenile Hormone (JH) via regulating the release of allatotropic factor from the brain and all these effects of NBIs are stage specific and concentration and dose dependent. NBIs induced changes in hemocyte contour might be also regulated by the brain hormone.

Key words: Adultoids, ecdysial-stasis, eco-friendly, 20-hydroxyecdysone, juvenile hormone, *Azadirachta indica*

INTRODUCTION

The synthetic pesticides have been a menace to human health and are hazardous to our environment causing phytotoxicity, pollution, development of insecticide resistance and negative impact on non-target organisms (Ogbuewu *et al*., 2011; Boursier *et al*., 2011; Jadeja *et al*., 2011). Considering all these aspects, many chemical insecticides have been barred in most of the advanced countries. Now-a-days, eco-friendly methods are being adopted that are rather precise for the target species without harming the other organisms to a great extent. The neem tree *Azadirachta indica*, thus, has emerged as a most important source of eco-friendly insecticide. Neem contains various

biological functions viz. feeding and oviposition deterrent, repellent, sterilant, larvicidal, growth regulatory agent and ovicidal (Singh, 1996; Kumar *et al*., 2003; Tiwari *et al*., 2006; Khan *et al*., 2007; Sadeghian and Mortazaienezhad, 2007; Gunasekaran *et al*., 2009; Depieri and Martinez, 2010) and showing many effective qualities for insect pest control. More than 300 insect species have now been tested with neem (Singh, 1996). The much varied physiological effects of NBIs on various insect species at different doses are toxicity, change in hemocyte profile, prolongation of larval-pupal durations and developmental deformities (Saxena *et al*., 1981a, b; Singh, 1996; Medina *et al*., 2004; Efil *et al*., 2005; Pandey *et al*., 2008b; Sharma *et al*., 2010). It is reported (Koul, 1996) that developmental aberrations caused by azadirachtin are due to the modulation of ecdysteroid titres. Tiwari *et al*. (2006) proposed that azadirachtin blocks release of neurosecretory material from the corpora cardiaca and others reported (Salehzadeh *et al*., 2003) anti-mitotic effect of the neem-terpenoid azadirachtin on cultured insect cells. Impact of azadirachtin on alterations of acetyl-cholinesterase and electrical activity in the nervous system has been also reported by Shafeek *et al*. (2004) and Senthil *et al*. (2008). The studies related with azadirachtin effects, thus, reveal that the endocrine system alone may not be entirely the target of this compound; it has direct effects on a whole variety of tissues and organs as suggested by Koul (1996). Most of the studies regarding NBIs effect on insects are not unanimous and are curbed to few instars of their life only. Exceptionally, a very few studies have been made during entire postembryonic development. It was, therefore, thought enviable to explore this aspect throughout nymphal-adult transformation to link biological changes with the developmental measures and hemocyte morphology.

MATERIALS AND METHODS

Insect culture: The insect species employed in the present study is the red cotton bug, *Dysdercus cingulatus* (Fabricius) belonging to order Hemiptera. Its life cycle includes five nymphal instars and one imaginal instar. The various nymphal instars and adult insects collected from okra fields in the vicinity of Gyanpur town were reared in glass jars (4.5×3.5 inches) and glass troughs (8×4 inches) in a BOD incubator maintained at 28±1°C, 70% RH and 16L: 8 D photoperiod. The mouth of the glass jars and glass troughs were covered with muslin cloth pieces bound with rubber band or wax coated cotton threads. The insects were fed on soaked cotton seeds and water was provided in glass vials plugged with small cotton balls. The containers, cotton seeds and water were changed twice a week. A strip of folded blotting paper was kept in culture jars to provide spaces for hiding and egg laying and to facilitate insect's movements. The cream colored oval eggs deposited in heaps were separated and kept in fresh glass jars. The eggs hatched into first instar nymphs in 6-9 days. There are five nymphal instars of which the first lasts for 7-9 days and each of the remaining instars take 4-5 days to moult into the next stage. The ultimate (fifth) instar nymphs take 6 days to become adults and thus a total of 35-40 days were required from egg to adult. One generation of insect culture was acclimatized at aforesaid controlled condition. Newly emerged V instar nymphs or adults were regarded as 0 h old V instar nymphs or adults and different ages (viz. 24, 48, 72, 96 and so on) of these two instars were reckoned from 0 h stage. Different stage and age group insects from the said lab culture were used. Research work was carried out during the year 2003-2004 at Department of Zoology, K.N. Govt. P.G. College, Gyanpur, Bhadohi, India.

Neem based insecticides (NBIs): Neem based insesticides viz. neemark (neem oil based E.C. containing azadirachtin 0.03% EC 300 PPM minimum, West Coast Herbochem Ltd. Mumbai,

India), multineem (seed extract containing 0.03% azadirachtin; Multiplex Fertilizers Pvt. Ltd., Bangalore, India) and neemazal T/S (azadirachtin 1%, other limonoids 3%, oil fatty acids glycerol esters 46.3%, polyethylene monosorbitol oleate 49.7%; EID Parry, India Ltd.) procured from city markets of Varanasi and Allahabad were used in present study.

Neem based insecticides treatment: Various concentrations of NBIs (0.25, 0.5, 1.0 and 2.5%) were prepared by diluting them in acetone and different doses were applied topically with the help of a glass micropipette on the dorsum of the body of nymphs and adults and eggs of different age groups. Various age group insects were treated with required concentrations and doses of NBIs whereas control insects received a treatment with acetone only. The effects of above treatments on survival, sensitivity, hemocyte morphology moulting, coupling, degrees of development, wing shape, fecundity and egg hatching of test insects were observed.

Hemocyte study: The fresh hemolymph samples from control and NBIs treated insects were collected individually by cutting the antennae on a slide and mixed well with anti-coagulant. A thin uniform smear of hemolymph was spread on the slide by rubbing the edge of an inclined slide backward. Stock solution of Giemsa stain was prepared by employing the method of Yeager (1945). For total hemocyte count (THC), the hemolymph was drawn into a thoma blood-cell pipette up to its graduated mark of 0.5 and diluted up to the 11th mark with Tauber-Yeager's fluid (Tauber and Yeager, 1935). The number of circulating hemocytes per cubic millimetre (mm^3) was calculated using the formula of Jones (1962). The methods of smear formation, staining, THC and hemocyte categorization were similar to those applied earlier (Tiwari *et al.*, 2006; Pandey *et al.*, 2010).

Statistical analysis: The Impact of NBIs on survival, moulting, coupling, fecundity and egg hatching of test and control insects were observed and experiment was repeated three times. Data from thirty insect were taken from each category and analysed statistically using Students't' test and presented as Mean±SD.

RESULTS

The interactions of NBIs in different concentrations and doses with sensitivity, moulting, coupling, fecundity, egg hatching and hemocyte morphology of *D. cingulatus* were described here briefly.

NBIs interaction with sensitivity and morphogenetic development: The application of NBIs on cephalic and thoracic regions and on ventral surface of insects showed more sensitivity than on abdominal region and dorsal surface. Immediately after treatment with a lower dose, the insects showed a swift movement hither and thither but their movement is immediately stopped for a while before getting them normal at higher doses. The different concentrations and doses of three NBIs used in present study affect the morphogenetic development and each NBI effect is stage and age specific. Among three NBIs, neemazal treatment was much effective than neemark and multineem. While 5 μL dose of neemark at 0.5% concentration inhibited the moulting of late III instar nymphs into IV instar and caused their mortality after surviving for 3 days in majority of the treated insects, it also led into the death of those IV instars which emerged from treated III instars after 3 days of survival. Application of the said dose and concentration on late IV nymphal instar though prolonged their survival period up to 9 days showing ecdysial stasis (Fig. 1a) but could not avert

Fig. 1: (A-J) Showing effect of NBIs on morphogenetic development of red cotton bug, *Dysdercus cingulatus* (a) IV nymphal instar showing complete ecdysial stasis following application of 5 μL dose of 0.5 % neemark. (b) an adult bug with less deformed left hindwing (arrow) emerging from 3 day old V instar nymph treated with 5 μL dose of 0.5% neemark. (c) adult bug with deformed right forewing (arrow) emerging from 3 day old V instar nymph treated with 10 μL dose of 0.5% neemark. (d) An adultoid with little larger wings and sunken abdomen as adult and black abdominal spots as nymphal characters following application of 15 μL dose of 0.5% neemark on 3 day old V instar nymph (e) An adultoid with a little larger forewing (thin arrows) and left hindwing (thick arrow) after application of 5 μL dose of 0.5% neemazal on 3 day old V instar nymph. (f) The adult bug with much reduced and deformed right forewing (arrow) and without right hindwing emerging from 3 day old V instar nymph treated with 10 μL dose of 0.5% multineem. (g) The adult bug with much deformed right forewing (thin arrow) and exuviae attached to posterior body end (thick arrow) emerging from 3 day old V instar nymph treated with 10 μL dose of 0.5% neemark. (h) An adultoid with exuviae at posterior body end (thin arrow) and small wing (thick arrow) as nymphal character and disappearance of black abdominal spots as adult characters following application of 8 μL dose of 0.25% neemazal on 3 day old V instar nymph. (i) A normal V instar nymph. (j) A normal adult

their mortality after this duration whereas a dose of 30 μL at the same concentration caused immediate mortality. The application of 5μL dose of 0.5% neemark on 3 day old V instar nymph resulted adult bug with less deformed left hind wing (Fig. 1b). Treatment of 10 μL dose of 0.5% neemark on 3 day old V instar nymph yielded adult bug with deformed right forewing (Fig. 1c) but when 3 day old V instar nymphs were treated with 15μL dose of 0.5% neemark, it formed adultoids with little larger wings and sunken abdomen as adult and black abdominal spots as nymphal characters (Fig. 1d). The 5 μL dose of 0.5% neemazal application on 3 day old V instar nymphs resulted adultoids with a little larger forewing and left hindwing (Fig. 1e) but while treatment of 3 day old V instar nymphs with 10 μL dose of 0.5% multineem formed adult bug with much reduced and deformed right forewing and without right hindwing (Fig 1f). Treatment of 3 day old V instar nymph with 10 μL dose of 0.5% neemark resulted adult bug with much deformed right forewing and exuviae attached to posterior body end (Fig. 1g) but 8 μL dose of 0.25% neemazal on 3 day old V instar nymph formed adultoid with exuviae at posterior body end and small wing as nymphal character and disappearance of black abdominal spots as adult characters (Fig.1h). There was no molting deformity in acetone treated respective control insects and all produced nymphs (Fig. 1i) and adults (Fig. 1j).

NBIs interaction with coupling, fecundity and egg hatching: The copulation occurs on the second day of the adult emergence in control insects and continues up to few hours before egg laying. It has been normally observed that the females have six egg cycles, the first taking 7-8 days and remaining ones 4-5 days each. In case of newly emerged adults, a considerable reduction in coupling period (only for ½ h) was observed if the four day old virgin females were treated with NBIs and allowed to couple with normal males. It was further observed that the total number of eggs laid during the first egg laying cycle and their hatchability were very much reduced in experimental insects. The 1% concentration and 10 µL dose of neemark proved fatal for adult bugs but a lesser concentration of 0.5% interrupted the continued and prolonged coupling resulting in a loss of fecundity and hatchability of eggs. Application of 0.5% concentration of neemark with 5 µL dose on 3 day old V instar nymphs could not stop the moulting but episodic coupling, little deformity in wings and reduction in fecundity and egg-hatching % were recorded. Similarly, equal concentration of same NBI with 10 µL dose caused episodic coupling, much deformity in wings and heavy reduction in fecundity and egg-hatching percentage. The 0.5% concentration of neemark with 20 µL dose was lethal but 15 µL dose caused only 40% mortality and 60% adultoids. Moulting deformities, episodic coupling, reduction in fecundity and decrease in egg-hatching percentage were also observed in multineem treated insects. In case of neemazal treated insects, even less concentration (0.25%) and dose (10 µL) was effectual than 0.5% concentration and 10 µL dose of neemark and multineem individually (Table 1). Likewise, various concentrations (0.5 and 1%) and doses (10 and 20 µL) of NBIs application on different age groups of adult caused episodic coupling, mortality, reduction in fecundity and sharp decline in hatching percentage and these effects were age specific (Table 2). Similarly, topical application of 2.5 and 0.5% concentration and 10µL dose of NBIs caused decrease in egg-hatching percentage. In contrast to 90% egg hatching in control, only 15, 16 and 5% eggs were hatched when 4 day old eggs were treated with of 2.5% concentration and 10 µL dose of neemark, multineem and neemazal respectively. Interestingly, eggs of 0.5% (10 µL) NBIs treated bugs were much sensitive to NBIs than eggs from normal (untreated) bugs. Among three NBIs, neemazal treatment was much effective than neemark and multineem (Table 3).

Table 1: Impact of topical application of azadirachtin containing NBIs on three day old fifth instar nymphs of *D. cingulatus*

		NBIs impact on			
NBIs/Conc. (%)	Volume (µL)	Moulting	Coupling	Fecundity	Hatching (%)
Neemark/0.5	5	took place, not much deformed wings	episodic	56±4	83
Neemark/0.5	10	took place, wings more deformed	episodic	44±5	77
Neemark/0.5	15	60% became adultoids, 40% could not stay alive	-	-	-
Neemark/0.5	20	died	-	-	-
Multineem/0.5	5	took place, right forewing very small, no right hindwing, left forewing comparatively larger with normal hindwing	episodic	66±4	84
Neemazal/0.25	10	68% became adultoids, 32% died as nymphs	-	-	-
Neemazal/0.5	2.5	Moulting delayed for 48hr days, abnormal, wings fully deformed	-	-	-
Neemazal/0.5	5	66 % became adultoids, 34% died as nymphs	-	-	-
Neemazal/0.5	10	took place, wings fully deformed	-	-	-
Neemazal/0.5	15	all died as nymphs		-	-
Respective control		took place, normal	normal	106±6	93

- = Insect died prior to adult stage. Values represent Mean±SD for 30 insects

Table 2: Impact of topical application of azdirachtin containing NBIs on adult *D. cingulatus* egg-hatching

			Effects on		
Adult insect age	NBI/Conc. (%)	Dose (µL)	Coupling	Fecundity	Hatching (%)
A_0	Neemark/0.5	10	episodic	47±6	30
A_0	Neemark/1.0	10	died prior to coupling	-	-
A_3	Neemark/1.0	20	died prior to coupling	-	-
A_0	Multineem/1	10	died prior to coupling	-	-
A_3	Multineem/1	20	died prior to coupling	-	-
A_0	Neemazal/0.5	10	55% died preceding to coupling, 45 % mated	33±5	26
A_0	Neemazal/0.5	20	died prior to coupling	-	-
A_2	Neemazal/1.0	20	died prior to coupling	-	-
Respective control			took place, normal	105±7	93

-: Adult Insect died prior to coupling and egg laying, therefore, no fecundity and egg-hatching. Values represent Mean±SD for 30 insects

Table 3: Impact of topical application of azdirachtin containing NBIs on *D. cingulatus* eggs

Insect stage	NBI/Conc. (%)	Dose (µL)	Effects on egg-hatching (%)
4 day old eggs	Neemark/2.5	10	15% eggs hatched, rest shrivelled and died
4 day old eggs from o.5% neemark	Neemark/0.5	10	All shrivelled and died (10 µL) treated bugs
4 day old eggs	Multineem/2.5	10	16% eggs hatched and rest shrivelled and died
4 day old eggs from 0.5% multineem (5 µL) treated bugs	Multineem/0.5	10	All shrivelled and died
4 day old eggs	Neemazal/2.5	10	5% eggs hatched and rest shrivelled and died
4 day old eggs from 0.5% neemazal (10 µL) treated bugs	Neemazal/0.5	10	All shrivelled and died
Respective control	-	-	93

Values represent Mean±SD for 30 insects

NBIs interaction with THC and hemocyte morphology: Significant reduction in THC was observed in all NBIs treated nymphs and adults in comparison to control insects (data not shown). Five types of hemocyte have been recognized in both V instar nymphs and adults. They are prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), adipohaemocytes (ADs) and oenocytoids (OEs) (Fig. 2A-F). Control insect GR showed distinct cytoplasmic granules (Fig. 2F) and application of 5 µL dose of 2.5% neemark on 3 day old V instar nymph caused breakdown of GRs (Fig. 2G) some GRs devoid of plasma membrane (Fig. 2H). In treated insects, mostly PLs and GRs are seen to participate in capsule formation by fusing with each other. While 5µL dose of 0.5% neemazal on 3 day old V instar nymph resulted PL without pseudopods (Fig. 2I) and PLs lost their pseudopods, their consistency and became transparent after application of 5 µL dose of 2.5% multineem on 3 day old V instar nymph (Fig. 2J), it also resulted PLs without pseudopods (Fig. 2K). Vacuolization of the cells mostly of GRs and PLs occurred frequently was observed in hemolymph smear of neemazal treated (5 µL dose of 0.5%) 3 day old V instar nymph (Fig. 2L). A large number of nodules (arrows) seen in the blood smear following 5 µL dose of 2.5 neemark on 3 day old V instar nymph (Fig. 2M). Application of 5µL dose of 0.5% neemazal on 1 day old adult bug induced aggregation of hemocytes (Fig. 2N). The 5µL dose of 0.5% neemazal on 3 day old V instar nymph caused GRs with many peripheral vesicles/vacuoles (Fig. 2O) and GRs nuclei digitations leading ultimately into disintegration of cells (Fig. 2P). Similarly broken hemocytes, devoid of plasma membrane (Fig. 2Q) and disintegrating GRs with lysed cellular contents (Fig. 2R) were seen when in 3 day old nymphs were treated with 5 µL dose of 2.5% neemark.

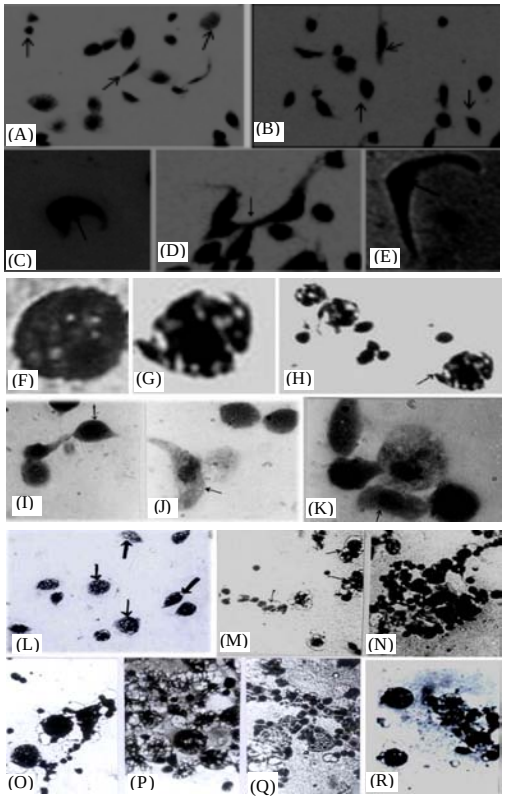

Fig. 2: A-R Showing impact of topical application of NBIs on hemocyte morphology of *D. cingulatus* (A) different types of hemocytes of control insect x 675. (B) PLs morpho-types in control insect x 675. (C) control PL x 1000. (D) control insect PL showing mitotic division (arrow). x 675. (E) PL variant with a much elongated pseudopod. x 1500. (F) control insect GR with distinct cytoplasmic granules.x 1000. (G) broken GR (arrow) devoid of complete plasma membrane following 5µL dose of 2.5% neemark on 3 day old V instar nymph.x 1000. (H) half broken GRs without plasma membrane following 5 µL dose of 2.5% multineem on 3 day old V instar nymph. x 1000. (I) PL (arrow) without pseudopods following 5 µL dose of 0.5% neemazal on 3 day old V instar nymph. x 1000. (J) PL (arrow) seen with losing cellular compactness following 5 µL dose of 2.5% multineem on 3 day old V instar nymph. x 1500. (K) PL (arrow) without pseudopods following 5 µL dose of 2.5% multineem on 3 day old V instar nymph. x 1500. (L) much vacuolisation of PLs (thick arrows) and GRs (thin arrows) following 5 µL dose of 0.5% neemazal on 3 day old V instar nymph. x 675. (M) A large number of nodules (arrows) seen in the blood smear following 5 µL dose of 2.5 neemark on 3 day old V instar nymph. x 675. (N) hemocyte aggregation following application of 5 µL dose of 0.5% neemazal on 1 day old adult bug. x 675. (O) GRs with many peripheral vesicles/vacuoles following 5 µL dose of 0.5% neemazal on 3 day old V instar nymph.x 1000. (P) GRs nuclei showing digitations leading ultimately into disintegration of cells following 5 µL dose of 0.5% neemazal on 3 day old nymph. x 1000. (Q) broken and disintegrating GRs following 5 µL dose of 2.5% neemark on 3 day old nymph. x 1000. (R) disintegration of cells following 5 µL dose of 2.5% neemark on 3 day old V instar nymph. x 1000

DISCUSSION

It has been observed that the sensitivity of the insects in present investigation against NBIs varies greatly depending upon region and surface of the body. The NBIs applied topically on ventral surface of the cephalo-thoracic region showed more sensitivity than if they were applied on dorsal surface and abdominal region. This variation in their response seems to lie in the fact that the NBIs could have acted directly upon central nervous system lying on the ventral side and ultimately on the brain which lies at the anterior end of the body. A large number of developmental aberrations caused by NBIs treatments observed in present study are in conformity with those of Schmutterer and Ascher (1984), Schmutterer (1988), Singh (1996), Khan and Kumar (2003), Tiwari *et al.* (2006), Al-Fifi (2006), Gunasekaran *et al.* (2009), Depieri and Martinez (2010) and Sharma *et al.* (2010). The factors for these developmental aberrations are reported to lie in the endocrine system and the metamorphosis hormones secreted by them which support the finding of Sieber and Rembold (1983), Schluter *et al.* (1985), Mordue *et al.* (1986) and Koul and Isman (1991).

NBI induced prolongation of *D. cingulatus* nymphal period without moulting to next instar or ecdysial stasis seem to be caused by a change in intrinsic JH titre as reported by Khan and Kumar (2003) in *Earias vitella*. Production of adultoids or deformed adults by V instar nymphs treated with NBIs, showed their juvenilising effects probably by acting at the level of Corpus Allatum (CA). A similar effect was found by Katiyar and Srivastava (1982) in *Callistemon lanceolatus* oil treated nymphs of *D. koenigii* also supports present findings. This indicated their juvenoid or JH-mimick behaviour. In the present study, the failures of hatching of multineem treated eggs indicated its ovicidal effects and substantiate the finding of Sharma *et al.* (2010).

Interruptions in coupling and loss of fecundity have also been observed in NBIs treated V instar nymphs and newly emerged adults. The role of CA or its hormone (JH) in regulation of coupling and egg maturation has been reported earlier by Srivastava and Tiwari (1978) and Tiwari and Srivastava (1979). It is, therefore, assumed that the reductions in coupling period and in egg number are caused by interruption of JH release. The decline in CA hormone decreases the rate of vitellogenin accumulation in the haemolymph, on one hand and its incorporation in the developing oocytes, on the other, with the help of brain hormone.

Further, the lesser percentage of egg hatching in newly emerged adults in comparison to V instar nymphs following topical application of these NBIs is explained on the basis of the fact that since the oogenesis starts in adult bugs, the insecticides are more effective in this developmental stage rather than the latter one. Present findings are thus in contradicted the reports of Tanzubil and McCaffery (1990) and Medina *et al.* (2004) who did not find the effect of azadirachtin on the percentages of egg-hatch. Since NBIs contains numerous active ingredients to affect the embryonic development of eggs hence, present observations are in contradiction with these authors. Effects of neem products on reproduction have been observed earlier in insects belonging to various orders viz Orthoptera, Hemiptera, Hymenoptera, Lepidoptera and Diptera (Schmutterer, 1995; Tiwari *et al.*, 2006). Vinuela *et al.* (1999) and Sharma *et al.* (2010) also found inhibition in egg laying/hatching after azadirachtin containing insecticides application and thus our observations are in agreement with these authors.

The hemocyte response to biological agents as well as toxins has been studied in many insects and the resulting phenomena are phagocytosis, encapsulation and distortion of cell contour or cellular disintegration (Wago and Kitano, 1985). The drastic reduction in THC in present investigation following NBI treatment is also reported by Saxena and Tikku (1990) in *D. koenigii*,

Azambuja *et al.* (1991) in *R. prolixus* and Sharma *et al.* (2003) in *S. litura* supports present findings. The decrease in hemocyte count may be attributed to the formation of nodules comprising groups of hemocytes or to the inhibition of endocrine glands and their secretions substantiate the finding of Tiwari *et al.* (2006) where decrease in THC and variation in hemocyte profile was observed in *D. koenigii*. The damages thus caused to PRs, PLs and GRs as well as the loss of pseudopodial processes of PLs suggested that the defence reactions in present insect have been reduced following NBI application. Furthermore, the changes in hemocyte morphology leading into cell death following NBIs treatments in present study have also been reported earlier by Saxena and Tikku (1990), Sharma *et al.* (2003), Tiwari *et al.* (2006) and Pandey *et al.* (2008b). It is assumed that metamorphic developments, coupling and fecundity are controlled by JH via regulating the release of allatotropic factor from the brain and all these effects of NBIs are stage specific and concentration and dose (volume applied topically) dependent. Since Mini and Prabhu (1986), Mala *et al.* (1987), Tiwari and Shukla (2000), Pandey and Tiwari (2005) and Pandey *et al.* (2008a) reported that the stresses like starvation, chilling, heating, injury etc., exert their effects directly upon neurosecretory cells of the brain.

CONCLUSION

On the basis of present experiment with NBIs, it is suggested that the phenomena like metamorphic developments, coupling and fecundity are controlled by JH via regulating the release of allatotropic factor from the brain and all these effects of NBIs are stage specific and concentration and dose (volume applied topically) dependent. Since a number of workers reported that the stresses like starvation, chilling, heating, injury etc. exert their effects directly upon neurosecretory cells of the brain, it is postulated that cell damages/deaths induced by these physical factors as well as NBIs application in present investigation are probably regulated by the brain hormone.

ACKNOWLEDGMENTS

The authors are thankful to Prof. G. C. Maurya, Principal of the College for laboratory facilities.

REFERENCES

Al-Fifi, Z.I.A., 2006. Molting inhibitory and lethal effects of azadirachtin on the desert locust, *Schistocerca gregaria* (Forskal). J. Entomol., 3: 312-318.

Azambuja, P.D., E.S. Garcia, N.A. Ratcliffe and J.D. Warthen, 1991. Immune-depression in *Rhodnius prolixus* induced by the growth inhibitor azadirachtin. J. Insect Physiol., 37: 771-777.

Boursier, C.M., D. Bosco, A. Coulibaly and M. Negre, 2011. Are traditional neem extract preparations as efficient as a commercial formulation of azadirachtin A. Crop Protec., (In Press), 10.1016/j.cropro.2010.11.022

Depieri, R.A. and S.S. Martinez, 2010. Reduced survival and infestation of coffee borer, Hypothenemus hampei (Ferrari) (Coleoptera: Scolytidae), on coffee fruits, in response to neem sprays in laboratory. Neotrop. Entomol., 39: 632-637.

Efil, L., O. Inanc and N.Y. Erdal, 2005. Effects of a commercial neem insecticide ((Neem AzalTM-T/S) on early and late developmental stages of the beet armyworm, *Spodoptera exiqua* (Hubner) (Lepidoptera: Noctuidae). Pak. J. Biol. Sci., 8: 520-526.

Gunasekaran, K., T. Vijayakumar and M. Kalyanasundaram, 2009. Larvicidal and emergence inhibitory activities of NeemAzal T/S 1.2 per cent EC against vectors of malaria, filariasis and dengue. Indian J. Med. Res., 130: 138-145.

Jadeja, G.C., R.C. Maheshwari and S.N. Naik, 2011. Extraction of natural insecticide azadirachtin from neem (*Azadirachta indica* A. Juss) seed kernels using pressurized hot solvent. J. Supercrit. Fluids, (In Press), 10.1016/j.supflu.2011.01.004

Jones, J.C., 1962. Current concepts concerning insect haemocytes. Am. J. Zool., 2: 209-246.

Katiyar, R.L. and K.P. Srivastava, 1982. An essential oil from Australian bottlebrush, *Callistemon lanceolatus* (Myrtaceae) with juvenoid properties against the red cotton bug, *Dysdercus koenigii* Fabr. (Heteroptera : Pyrrhocoridae). Entomon, 7: 463-468.

Khan, I.A. and K. Kumar, 2003. Heterochrony in cotton bollworm, *Earias vitella* (Fab.) following ethoxyprecocene administration. Indian J. Exp. Biol., 41: 328-335.

Khan, M., M.A. Hossain and M.S. Islam, 2007. Effects of neem leaf dust and a commercial formulation of a neem compound on the longevity, fecundity and ovarian development of the melon fly, *Bactrocera cucurbitae* (Coquillett) and the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). Pak. J. Biol. Sci., 10: 3656-3661.

Koul, O. and M.B. Isman, 1991. Effects of azadirachtin on the dietary utilization and development of the variegated cutworm, *Peridroma saucia*. J. Insect Physiol., 37: 591-598.

Koul, O., 1996. Mode of Action of Azadirachtin in Insects. In: Neem, Randhawa, N.S. and B.S. Parmar (Eds.). New Age International Pvt. Ltd. Publishers, India, pp: 160-170.

Kumar, A.R.V., H.C. Jayadevi, H.J. Ashoka and K. Chandrashekara, 2003. Azadirachtin use efficacy in commercial neem formulations. Curr. Sci., 84: 1459-1465.

Mala, J., F. Sehnal, A.K. Kumaran and N.A. Granger, 1987. Effect of starvation, chilling and injury on endocrine glands function in *Galleria mellonella*. Arch. Insect Biochem. Physiol., 4: 113-128.

Medina, P., F. Budia, P.D. Estal and E. Vinuela, 2004. Influence of azadirachtin, a botanical insecticide on *Chrysoperla carnea* (Stephens). Reproduction, toxicity and ultrastructural approach. J. Econ. Entomol., 97: 43-50.

Mini, A. and V.K.K. Prabhu, 1986. Significance of critical developmental stage on starvation induced endocrine mediated precocious metamorphosis in *Oryctes rhinoceros* (Coleoptera:Scarabaeidae). Proc. Indian Acad. Sci. (Anim. Sci.), 95: 379-385.

Mordue, A.T., K.A. Envans and M. Charlet, 1986. Azadirachtin, ecdysteroids and ecdysis in *Locusta migratoria*. Comp. Biochem. Physiol., 85: 297-301.

Ogbuewu, I.P., V.U. Odoemenam, H.O. Obikaonu, M.N. Opara and O.O. Emenalom *et al*., 2011. The growing importance of neem (*Azadirachta indica* A. juss) in agriculture, industry, medicine and environment: A review. Res. J. Med. Plant, 5: 230-245.

Pandey, J.P. and R.K. Tiwari, 2005. Changes in haemocyte counts and fecundity following haemolymph withdrawals in red cotton bug, *Dysdercus koenigii* fabr. J. Exp. Zool. India, 8: 85-92.

Pandey, J.P., R.K Tiwari and D. Kumar, 2008a. Reduction in hemocyte mediated immune response in *Danais chrysippus* following treatment with neem based insecticides. J. Entomol., 5: 200-206.

Pandey, J.P., R.K. Tiwari and D. Kumar, 2008b. Temperature and ganglionectomy stresses affect haemocyte counts in plain tiger butterfly, *Danais chrysippus* L. (Lepidoptera: Nymphalidae). J. Entomol., 5: 113-121.

Pandey, J.P., P.K. Mishra, D. Kumar, B.M.K. Singh and B.C. Prasad, 2010. Effect of temperature on hemocytic immune responses of tropical tasar silkworm, *Antheraea mylitta* D. Res. J. Immunol., 3: 169-177.

Sadeghian, M.M. and F. Mortazaienezhad, 2007. Investigation of compounds from azadirachta indica (Neem). Asian J. Plant Sci., 6: 444-445.

Salehzadeh, A., A. Akhkha, W. Cushley, R.L.P. Adams, J.R. Kusel and R.H.C. Strang, 2003. The antimitotic effect of the neem terpenoid azadirachtin on cultured insect cells. Insect Biochem. Mol. Biol., 33: 681-689.

Saxena, R.C., N.J. Liquido and H.D. Justo, 1981a. Neem Oil a Potential Antifeedant for the Control of Rice Brown Hopper, *Nilaparvata lugens*. In: Natural Pesticides from the Neem Tree (*Azadirachta indica* A. Juss), Schmutter, H., K.R.S. Ascher and H. Rembold (Eds.). Agency for Technological Co-operation, Berlin, Germany, pp: 177-188.

Saxena, R.C., G.P. Wald baver, N.J. Liquido and B.C. Puma, 1981b. Effect of Neem Oil on the Rice Leaf Folder, (*Naphalocrocis medinalis*). In: Natural Pesticides from the Neem Tree (*Azadirachta indica* A. Juss), Schmutter, H., K.R.S. Ascher and H. Rembold (Eds.). Agency for Technological Co-operation, Berlin, Germany, pp: 157-170.

Saxena, B.P. and K. Tikku, 1990. Effect of plumbagin on haemocytes of *Dysdercus koenigii* F. Proc. Indian Acad. Sci. (Anim. Sci.), 99: 119-124.

Schluter, U., H.J. Bidmon and S. Grewe, 1985. Azadirachtin affects growth and endocrine events in larvae of the tobacco hornworm *Manduca sexta*. J. Insect Physiol., 31: 773-777.

Schmutterer, H. and K.R.S. Ascher, 1984. Natural pesticides from the neem tree and other tropical plants. Proceedings of the 2nd International Neem Conference, (INC'1984), Rauischholzhausen, pp: 587-587.

Schmutterer, H., 1988. Potential of azadirachtin-containing pesticides for integrated pest control in developing and industrialized countries. J. Insect Physiol., 34: 713-719.

Schmutterer, H., 1995. Side-Effects on Beneficial and Other Ecologically Important Non-Target Organisms. In: The Neem Tree, Schmutterer, H. (Ed.). VCH, Weinhein, Germany, pp: 495-517.

Senthil, N.S., M.Y. Choi, H.Y. Seo, C.H. Paik, K. Kalaivani and J.D. Kim, 2008. Effect of azadirachtin on acetylcholinesterase (AChE) activity and histology of the brown planthopper Nilaparvata lugens (Stal). Ecotoxicol. Environ. Saf., 70: 244-250.

Shafeek, A., R.P.J. Prasanthi, G.H. Reddy, C.S. Chetty and G.R. Reddy, 2004. Alterations in acetylcholinesterase and electrical activity in the nervous system of cockroach exposed to the neem derivative, azadirachtin. Ecotoxicol. Environ. Safety, 59: 205-208.

Sharma, P.R., O.P. Sharma and B.P. Saxena, 2003. Effect of neem gold on haemocytes of the tobacco armyworm. Curr. Sci., 84: 690-695.

Sharma, T., A. Qamar and A.M. Khan, 2010. Evaluation of neem (*Azadirachta indica*) extracts against the eggs and adults of *Dysdercus cingulatus* (Fabricius). World Applied Sci. J., 9: 398-402.

Sieber, K.P. and H. Rembold, 1983. The effects of azadirachtin on the endocrine control of moulting in *Locusta migratoria* (L.). J. Insect Physiol., 29: 523-527.

Singh, R.P., 1996. Bioactivity Against Pests. In: Neem, Randhawa, N.S. and B.S. Parmar (Eds.). New Age International Pvt. Ltd. Publishers, India, pp: 146-159.

Srivastava, K.P. and R.K. Tiwari, 1978. Effect of cardiacectomy and aorta severance on moulting and egg maturation in the red cotton bug, *Dysdercus koenigii*. Indian J. Exp. Biol., 16: 997-999.

Tanzubil, P.B. and A.R. McCaffery, 1990. Effect of azadirachtin on reproduction in the African armyworm (*Spodoptera exempta*). Entomol. Exp. Appl., 57: 115-121.

Tauber, O.E. and J.F. Yeager, 1935. On the total haemolymph (blood) counts of insects. I. orthoptera, odonata, hemiptera and homoptera. Ann. Entomol. Soc. Am., 28: 229-240.

Tiwari, R.K. and K.P. Srivastava, 1979. Corpus allatum in relation to egg maturation in the red cotton bug, *Dysdercus koenigii* (Heteroptera). Acta Entomol. Bohemoslov., 76: 175-180.

Tiwari, R.K. and R.S. Shukla, 2000. Effect of certain stresses and 20-hydroxyedysone injection on total haemocyte count in lemon-butterfly, *Papilio demoleus*. L. (Lepidoptera). Proc. Nat. Acad. Sci. India, 70: 243-254.

Tiwari, R.K., J.P. Pandey and D. Kumar, 2006. Effects of neem based insecticides on metamorphosis, haemocytes count and reproductive behavior in red cotton bug, *Dysdercus koenigii* fabr (Heteroptera: Pyrrhocoridae). Entomonology, 31: 267-275.

Vinuela, E., A. Adan, G. Smagghe, M. Gonzalez and M.P. Medina *et al.*, 1999. Laboratory effects of ingestion of azadirachtin by two pests (*Ceratitis capitata* and *Spodoptera exigua*) and three natural enemies (*Chrysoperla carnea*, *Opius concolor* and *Podisus maculiventris*). Biocontrol. Sci. Technol., 10: 165-178.

Wago, H. and H. Kitano, 1985. Morphologial and functional characterization of the larval haemocytes of the cabbage white butterfly, *Pieris rapae crucivora*. Applied Entomol. Zool., 20: 1-7.

Yeager, J.F., 1945. The blood picture of the southern armyworm (*Prodenia eridania*). J. Agric. Res., 71: 1-40.