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Azadirachtin—A naturally occurring insect growth regulator

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Abstract. The well known and most useful property of the neem tree is the antifeedant property to insects expressed even in crude extracts. Azadirachtin has the highest biological activity and in addition to antifeedant property, it also produces developmental abnormalities in almost all insect orders. The chemical structure of azadirachtin has been determined unequivocally and the radio labelling opened the possibility of biochemical, metabolic and autoradiographic studies. The timing and titre of the two morphogenetic hormones are altered leading to loss of coordination of developmental events. This effect on morphogenetic hormones could be traced as the influence of azadirachtin on the neuroendocrine system which secrete the tropic hormones that eventually control the activity of corpora allata and prothoracic glands. Recent studies have shown that turnover of the neurosecretory material is poor in azadirachtin-treated insects leading to derangement of the hormonally controlled development.

Keywords. Azadirachtin; insect growth regulator; insect–plant relationship.

1. Introduction

The Indian neem tree, *Azadirachta indica* A Juss and its closely related China berry, *Melia azadirach* (Meliaceae) have been recognized since long for their unique properties. More than half a century ago, it was discovered that leaves of the neem tree contain chemicals strongly inhibiting feeding by the locusts that are polyphagous in nature (Chopra 1928; Volkonsky 1937). A plethora of studies have been made on the pesticidal properties, especially after the demonstration of strong antifeedant properties of the crude extracts of the neem seed kernels to locusts by Pradhan *et al* in 1962 and 1963 (for reviews see Gill 1972; Warthern 1979; Schmutterer 1981). A host of tetranortriterpenoids have been isolated from various parts of the neem tree and although all have not been tested for pesticidal properties, 3 compounds obtained from seeds are found to be active as feeding deterrants, toxicants and/or disruptants of growth and development against a variety of insect species and nematodes (Warthern 1979; Jacobson 1986). These compounds are meliantriol (Levie *et al* 1967), salannin (Henderson *et al* 1964) and azadirachtin (Butterworth and Morgan 1968, 1971). Azadirachtin has attracted worldwide attention not only as the most popular deterrent to insects but also as a promising growth regulator. The growing interest on the neem tree and azadirachtin in particular has not only led to detailed physiological and biochemical investigations on the action of this compound and development of pesticide formulations based on azadirachtin and neem oil but also to 3 international conferences exclusively on the neem tree. This review attempts to describe the biological effects of azadirachtin with special emphasis on its growth-disruptive action on insects.

2. Neem as the source of azadirachtin

The complicated structure of the molecule and the difficulties involved in the

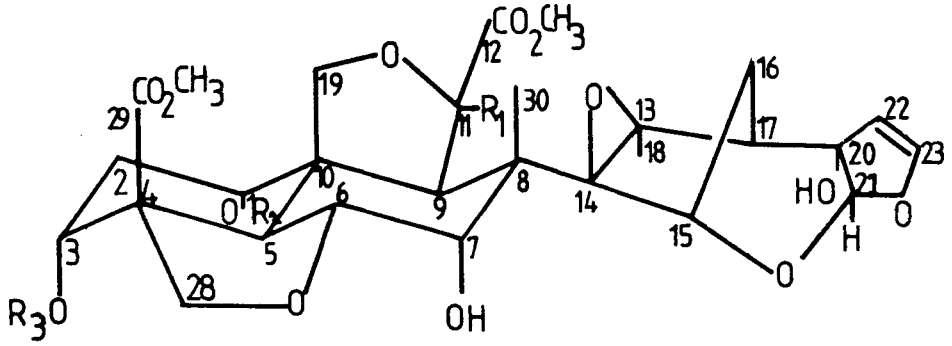
synthesis, leave the natural material as the only source of azadirachtin at present. Besides the 3 important tetranortriterpenoids already mentioned, other compounds isolated from the neem seed are β-sitosterol, fatty acids, flavanoids and pentanortriterpenoids (Jacobson 1986). Several investigators have estimated azadirachtin content of the neem seeds from various sources, mainly from Asia and Africa. The yield varies markedly, depending on the origin of the seed material, ranging from 0·2% of commercial Indian seed to 3·5% of seeds from Ghana (Morgan 1981). Using thin layer chromatography of ethanol extracts, Ermel *et al* (1984) found that the best yields were from seeds obtained from Togo (6·2%) and India (3·5%). Further determinations from a number of samples by high performance liquid chromatography technique (Ermel *et al* 1987) revealed that samples from Nicaragua and Indonesia have the highest content (average 4·8%) and that samples from Togo, India, Burma and Mauritius have 3·3–3·9% of azadirachtin. Besides the large variation among individual trees, factors like light, temperature and humidity influence the azadirachtin content of seed kernels and exposure to UV radiation leads to significant loss (65%) within 14 h (Ermel *et al* 1987).

3. Azadirachtins

From the 1930s onwards, crude extracts from various parts of the neem tree were studied for insecticidal properties but it was not until 1968 the first biologically active component was isolated in pure form. Butterworth and Morgan (1968) obtained the first samples of azadirachtin. The large number of functional groups and the sensitivity of azadirachtin to acids and bases posed problems of structural analysis, although initial studies by Butterworth *et al* (1972) revealed the key molecular fragments. A complete structural assignment was made by Zannao *et al* (1975) but certain doubts on the structure remained which were not consistent with nuclear magnetic resonance (NMR) data. However, an unequivocal determination of the structure was achieved by Broughton *et al* (1986) from a crystalline (detiglyolated dihydroderivative) of azadirachtin that was suitable for X-ray diffraction studies. On the basis of NMR data Kraus *et al* (1985) also proposed the structure. The most critical structural element is the epoxy group at position 13–14. Removal of this group ends up with inactive compounds. Pure azadirachtin is a clear, white, microcrystalline solid (mp 149°C). It has been demonstrated that several azadirachtins occur in neem seed, having the basic triterpenoid structure as common to all of them (Rembold *et al* 1984; Forster 1988). In the seed cake 4 major components (A–D) were found in a proportion of 1 azadirachtin D : 100 azadirachtin A : 50 azadirachtin B : 1 azadirachtin C. The structure of azadirachtin B has also been established recently (Rembold *et al* 1987b). Figure 1 shows the structure of azadirachtins A and B. Azadirachtins A and B differ in the position of tiglic acid, which is at C-1 for A and at C-3 for B. Another important difference in ring A substitution is the free OH-group of azadirachtin B in position 1. Azadirachtin A has been hydrogenated to dihydroazadirachtin A and also to its corresponding tritium labelled [22, 23 $-{}^3H_2$] dihydroazadirachtin A (Rembold *et al* 1984) opening up the possibility of studies on the biochemical mode of action.

4. Biological activity in insects

The neem tree is well known for its insect-repellent and antifeedant properties.



	R_1	R_2	R_3
azadirachtin A	OH	tig	ac
azadirachtin B	H	H	tig

Figure 1. Structure of azadirachtins A and B (Rembold *et al* 1987a).

Centuries before commercial insecticides were available, farmers in the Indian subcontinent used neem derivatives to protect agricultural crops from insect attack. Of recent knowledge is the discovery of the insect growth regulating and sterilizing property of some pure fractions. In the light of the knowledge on the biological activity of crude material and the advances made after the isolation of the biologically active components, it would be worthwhile to examine the biological effects of crude material also.

4.1 *Studies with crude extract and preparations*

When a water suspension of neem kernels as low as 0·05% was sprayed on different crops and offered to the desert locust, the pest preferred to starve to death rather than feed on the treated leaves (Pradhan *et al* 1962). The laboratory findings were confirmed under field conditions demonstrating the most potent antifeedant activity. Such studies were later extended to several insect species. For a comprehensive account of the pesticidal properties of neem, see reviews by Warthern (1979) and Jain (1983). A total of 123 species of insects, belonging to diverse insect orders viz., Coleoptera, Diptera, Heteroptera, Isoptera, Lepidoptera and Orthoptera besides 3 species of mites and 5 species of nematodes are known to be adversely affected by neem preparations (Jacobson 1986). The growth-regulating effects were identified in crude methanolic extracts of neem leaves on east African coffee bug, *Antestiopsis orbitalis bechuana* by Leuschner (1972) though effects on fecundity could not be demonstrated. Later studies by Steets and Schmutterer (1975) and Schultz (1981) on *Epilachna varivestis* clearly demonstrated the growth disruption and sterilizing effects of neem components.

Studies with crude extracts on diverse species of insects were made extensively in the Federal Republic of Germany, USA and India, with a view to use them under field conditions. Some of the observations on growth and development are outlined.

Ascher and Gsell (1981) reported that the methanolic extract of neem seed (20–30 mg/kg) disturbed metamorphosis of *E. varivestis*. Effects on metamorphosis of *E. varivestis* grubs were enhanced 6-fold by the addition of a synergist 'Tropital' to the methanolic extracts (Lange and Schmutterer 1982). Application of the inexpensive 'enriched neem seed extract' at the rate of 6–12 mg/kg resulted in 100% mortality of the larvae of *Plutella xylostella, Pieris brassicae* and *Leptinotarsa decimlineata* (Schmutterer 1984). Topical application of crude extracts of neem seed on *Dysdercus fasciatus* resulted in mortality/asymmetry of body after moulting/reduced fecundity of the emerging adults (Occhse 1982). Topical application of acetone extracts of neem seeds and leaves on *D. cingulatus* led to supernumerary moults. Addition of methanolic seed extracts to artificial larval rearing medium of Medfly, *Ceratitis capitata* led to prolongation of instar, low food intake, low rate of pupation and hatching finally to reduced adult population to 16% of control (Steffens and Schmutterer 1982). However, Schauer (1984) showed that aphids sprayed directly with neem extracts were not appreciably affected. Laboratory and greenhouse tests have shown that after consumption of leaves, treated with enriched and formulated neem seed extract, the fecundity of *L. decimlineata* greatly reduced and some were completely sterile (Schmutterer 1987). The aqueous extract had strong antifeedant activity than the formulated solvent extracts. Aqueous extracts and formulated extracts of an azeotropic solvent mixture and of butyl methyl ether were evaluated against mosquito larvae by Zebitz (1987). These extracts showed toxic, growth-regulating and sterilizing effects on *Anopheles, Culex* and *Aedes* sp depending on the dose.

A 3–5% extract of neem kernel and neem oil showed antifeedant property against *Heliothis armigera* under field condition and 1–5% extract has strong antifeedant reaction against *Spodoptera litura*, on tobacco nurseries (Joshi *et al* 1978). Neem oil extractive, although had very poor antifeedant activity than crude extracts of neem cake against locusts and *S. litura*, was an effective mosquito larvicide (Attri and Ravi Prasad 1980). Feeding of sorghum grain and stem treated with neem kernel suspension to *H. armigera* and *Chilo partellus* led to developmental deformities (Jotwani and Srivastava 1984). Parmar (1987) has listed the biological effects of various neem extracts and neem oil on several Indian insects. These include synergistic, ovicidal and ovipositional deterrent effects besides the growth-disrupting effects.

Analysis of these studies suggests that the growth-regulating effects of the neem extracts are very well pronounced even when the crude extracts are applied. However, the variations in the efficacy are mainly due to variations in the quantities of the active principles and standardization of the product. Though neem is rich in several biologically active chemicals, the antifeedant and growth-regulating activities are primarily due to azadirachtin. The actual concentration of this compound in a crude preparation or an enriched extract and its loss due to degradation after application would ultimately determine the effect. These studies are of great value from an applied viewpoint. However, the growth-disrupting activity attributed to neem seed extracts or azadirachtin could, in some cases, be due to their antifeeding action, as antifeedants can also cause developmental deviations. Several physiological and biochemical studies have been made with pure azadirachtin. These are discussed in detail.

4.2 *Antifeedant activity*

Following the earlier demonstration of the antifeedant activity of neem extracts to the desert locust, the component chiefly responsible for this activity was identified as azadirachtin (Butterworth and Morgan 1968, 1971) and later its activity was demonstrated on diverse groups of insects, notably lepidopteran larvae such as the diamond back moth *P. xylostella*, cabbage butterfly *P. brassicae*, the tobacco bud worm *H. virescens*, the greater wax moth *Galleria mellonella* and the fall army worm *S. frugiperda* (Nakanishi 1975; Ruscoe 1972; Zanno *et al* 1975; Warthern *et al* 1978). The antifeedant activity on Lepidoptera can be best exemplified by the observation of Redfern *et al* (1981) on fall army worm that control larvae were 17 times larger (84·7 mg) than the test larvae fed on a diet containing 0·2 ppm azadirachtin (5 mg) and 85 times larger than the larvae fed on a diet containing 1 ppm. Larvae transferred to an untreated diet were unable to overcome the effects of treatment. Similarly, newly hatched house crickets *Acheta domesticus*, when fed on a diet containing 1–25 ppm, had less weight and development than controls and the effect was proportional to the concentration of the antifeedant (Warthern and Uebel 1981). Contrary to the observation on *S. frugiperda*, the nymphs of the house cricket that were fed on treated diet for 6 weeks, when transferred to normal diet, developed normally.

Antifeedant activity of azadirachtin is not a universal phenomenon in insects and exceptions do occur. Azadirachtin is not a feeding inhibitor in case of *E. varivastis* and it had been amply demonstrated by Rembold *et al* (1980) that growth disruption could occur independent of feeding inhibition. Yet another novel example is the blood-sucking bug *Rhodnius prolixus* which exhibits a clear dose-dependent effect. The ED_{50} dose required for feeding inhibition is as high as 25 μg/ml of blood whereas that for moult inhibition is only 4×10^{-4} μg/ml (Garcia and Rembold 1984; Garcia *et al* 1984).

4.3 *Effect on food consumption and utilization*

To what extent azadirachtin would influence feeding and food utilization if administered through other routes, like injection, thereby avoiding the oral antifeedant action, in insects like the locusts which prefer to starve instead of feeding on azadirachtin-treated food? It was demonstrated that in the dose range of 1–8 μg of azadirachtin/g body weight, its injection caused dose-dependent reduction in body weight of final instar nymphs of the desert locust *Schistocerca gregaria* but even the highest dose did not cause absolute feeding inhibition (Rao and Subrahmanyam 1986). Such treatments significantly reduced the rate of feeding, growth and utilization of food to body mass. Similarly, a physiological dose of 2·5 μg/g injected into female migratory locust, *Locusta migratoria* did not cause starvation, though food consumption was reduced without significant loss or addition to body weight for one week post-treatment (Subrahmanyam *et al* 1989).

Such studies were also conducted on lepidopteran larvae by feeding sub-antifeedant concentrations added to food. Fagoonee (1984) allowed the cabbage web worm larvae to feed for 24 h on leaf discs treated with low concentrations of azadirachtin and showed that on the first day consumption index, larval body-

weight gain and growth index decreased with increasing concentrations, whereas the digestibility and efficiency of consumption of food to body mass were highest at highest concentration. Feeding subsequently on untreated food led to a rapid decline in food utilization efficiencies. Similar trend was also reported in case of *H. virescens* by Barnby and Klocke (1987). The antifeedant effects of azadirachtin are partly due to sensory detection and avoidance by insects (Schoonhoven and Jermy 1977) and partly due to centres that control feeding (Sieber and Rembold 1983).

4.4 *Growth-regulating activity*

Apart from the antifeedant effect, azadirachtin causes disorders in metamorphosis. It was Ruscoe (1972) who first demonstrated such an effect. Later these effects were reproduced in many insects species of several orders—on *L. decemlineata* and *E. varivastis* (Coleoptera) by Steets (1976) and Steets and Schmutterer (1975), on *Ephistia kuehniella* (Lepidoptera) and *Apis mellifera* (Hymenoptera) by Rembold *et al* (1980), on *D. koenigii* by Koul (1984) and *Bombyx mori* (Lepidoptera) by Koul *et al* (1987), on *L. migratoria* (Orthoptera) by Rembold and Sieber (1981) and Sieber and Rembold (1983), on *R. prolixus* (Heteroptera) by Garcia and Rembold (1984) and Garcia *et al* (1986). Typical disorders due to azadirachtin may be summarized as:

(i) Induction of moult inhibition and mortality in a dose-dependent manner.
(ii) Remarkable prolongation of instar duration accompanied by death or moult disruption. Locust fifth instar injected with a dose of 2 μg/g body weight may continue without adult moult for more than 60 days (normal intermoult period being 9 days), and *Rhodnius* bugs may survive beyond 5 months without moult.
(iii) Treated larvae remain in pharate condition unable to shed their old cuticle successfully. Weak ecdysial movements that could last for several hours accompanied by incomplete shedding.
(iv) Incomplete or depressed resorption of the exuvial fluid.
(v) Incomplete sclerotization and pigmentation of the new cuticle.
(vi) Unplasticization of wing lobes leading to either wingless adults (in bugs) or adults with crippled wings (in moths).
(vii) Severe deformities in head and thoracic appendages of the pupae of Holometabola.
(viii) Disruption of oogenesis when injected into young adults (eg., locusts) and inhibition of embryonic development when injected into adults at the end of vitellogenesis.

5. Physiological and biochemical studies

The developmental changes caused at moult and the inhibition of oogenesis due to azadirachtin treatment pose several questions regarding its mode of action. Many workers have proposed that it might be acting at the hormonal level. However, so far a complete answer could not be obtained despite studies in this direction. It has been adequately demonstrated that such growth-regulating effects are not a consequence of its antifeedant action since topical application and injection of even

a single dose of azadirachtin interferes with moulting programme of larvae and egg maturation of the adult insect (Rembold *et al* 1984).

5.1 *Influence on endocrine regulation of moulting*

Rembold and co-workers have pioneered the studies on the influence of azadirachtin on endocrine regulation of moulting, taking *L. migratoria* as the model. Sieber and Rembold (1983) demonstrated that the haemolymph ecdysteroid titre pattern is modified and the peak suppressed in 5th instar *L. migratoria* nymphs following a single injection of azadirachtin at the beginning of the instar. Such modification of ecdysteroid titre is closely correlated with morphogenetic effects. Similarly, Mordue *et al* (1986) showed that ecdysteroid levels can be drastically reduced, delayed and extended or not significantly affected by azadirachtin treatment of *L. migratoria* final instar nymphs depending upon the time of injection.

Koul *et al* (1987) observed two types of effects depending upon the time of injection of azadirachtin into fifth instar larvae of *B. mori*. When injected prior to release of prothoracicotropic hormone (PTTH) i.e. into 0–3-day-old larvae, defective pupae were formed, whereas injection into 4–6-day-old larvae led to complete failure of pupation. It was further demonstrated by tissue culture and ligation experiments that azadirachtin has no direct effect on PTTH and prothoracic gland secretion.

Studying the effects of azadirachtin on the Asiatic corn borer *Ostrinia furnacalis*, Min-Li and Shin-Foon (1987) demonstrated that the ecdysteroid titre of the treated fifth instar larvae did not raise above 20 mg/ml of haemolymph throughout the instar whereas that of the normal larvae reached a peak (326·5 ng/ml) on the 6th day after moult and dropped on the 7th day to 46 ng/ml.

Azadirachtin decreased the cold induced elevation of juvenile hormone titre in the body of chilled wax moth, *G. mellonella* final instar larvae but had no effect on the allatotropic activity of the brain. The ecdysteroid peak was higher and delayed by 24 h compared with the first ecdysteroid peak in controls (Malczewska *et al* 1988).

Ecdysteroid titres were too low for induction of ecdysis in the azadirachtin A-treated nymphs of *R. prolixus* (Garcia *et al* 1986). Ecdysone given orally (5 μg/ml) and juvenile hormone analogue (70 mg/insect) counteracted the ecdysis inhibition induced by azadirachtin (Garcia and Rembold 1984). Permanent larvae of the milk weed bug, *Oncopeltus fasciatus*, induced by azadirachtin doses show neither ecdysis nor apolysis and have a delayed and distinctly lower ecdysteroid peak (Dorn *et al* 1986).

5.2 *Influence on ovarian development*

The sterilizing effect of azadirachtin noticed in several insect orders brings out the fact that azadirachtin has a profound influence on the maturation of oocytes. Ecdysone and other ecdysteroids are synthesized in the cells of follicular epithelium at the end of oocyte maturation. Practically all the ovarian ecdysteroids are finally contained in the newly laid eggs and control cuticulogenesis during early development of embryo. After a single injection of azadirachtin into mature female locusts, follicle growth is inhibited. The length of the terminal oocyte remains only

1–2 mm compared to 6 mm in untreated females. Rembold and Sieber (1981) analysed the ovarian ecdysteroid levels of *L. migratoria* by radioimmunoassay (RIA) and showed that the ovarian ecdysteroid level in control increased near the end of vitellogenesis and reached a maximum within hours. Whereas injection of azadirachtin into females at the end of vitellogenesis (10–13 days after emergence) resulted only in very small amounts of moulting hormone in the ovaries. Ovaries of such insects were smaller and weigh only half that of controls. The number of mature oocytes was less, probably due to resorption.

Vitellogenesis is a process by which the fat bodies of the maturing adult insects synthesize specific proteins that are incorporated into the oocytes leading to their maturation. Juvenile hormone (JH) plays a vital role in this process (gonadotropic activity) and hence Rembold *et al* (1987c) analysed the haemolymph vitellogenins, JH as well as ecdysone levels of azadirachtin injected female or *L. migratoria*. These studies show that—(i) Azadirachtin significantly affects vitellogenin synthesis. Vitellogenin was detectable in females from day 6 after emergence, whereas in treated females it was absent until day 10 and appeared from day 14 onwards. (ii) In the control, a JH peak on day 8 precedes another on day 11, when ecdysone also reaches its maximum. These insects oviposit during days 13–15 and oviposition is succeeded by another JH peak at the start of the second gonadotropic cycle. Injection of 3 μg azadirachtin/g on day 4 after emergence leads to a completely different hormonal titre curves. The JH peak is seen only on day 15 and the ecdysone peak is seen on day 16 followed by oviposition on day 18. These observations clearly demonstrate the delay and mis-timising of the appearance of morphogenetic hormones leading to derangement of the gonadotropic cycle.

Does azadirachtin inhibit any of the enzyme systems involved in the biosynthesis of ecdysone? It was shown (Smith and Mitchell 1988) that azadirachtin inhibits in a dose-dependent fashion, the cytochrome P-450 dependent ecdysone-20 mono-oxygenase activity (an enzyme that converts ecdysone to its active metabolite 20-hydroxyecdysone) in homogenates of *Drosophila melanogaster* larvae, *A. aegypti* adult female abdomens or body or midgut of *Manduca sexta*. The concentrations required for 50% inhibition range from 1×10^{-4} to 4×10^{-4} M which are however an order higher than those required for moult inhibition. Accordingly, azadirachtin may not act at the level of this enzyme system.

A direct dependence of follicle cell differentiation and egg maturation on high JH titre has been already demonstrated for *L. migratoria*. However, synthesis of ecdysone by differentiated follicle cells is independent of JH or corpora allata and seems to be stimulated by neurohormones. Blockade of corpus allatum activity by azadirachtin hence seems unlikely. That azadirachtin has no direct influence on prothoracic gland activity has been already shown (Koul *et al* 1987).

The effect of azadirachtin on these two hormones could therefore be interpreted as an interference with neuroendocrine system which controls the ecdysone and JH synthesis. The tropic hormones viz., PTTH and allatotropic hormone (ATTH) secreted from brain are involved in such a regulation. Histological studies have been made to understand the neuroendocrine control mechanism in azadirachtin-treated insects (Rembold *et al* 1984; Rao and Subrahmanyam 1986; Subrahmanyam *et al* 1989). The nymphs of *L. migratoria* that fail to moult even 40 days after treatment as well as the female adults whose gonadotropic cycle was suppressed due to azadirachtin show remarkable accumulation of stainable neurosecretory product

in the corpus cardiacum, a gland that plays a vital role in the storage and release of neurohormones. Hence, this is considered as the first step in the mode of action or azadirachtin.

However, these studies do not give a complete answer to the precise mode of action unless the fate of azadirachtin in the insect body is studied in detail. With the availability of radioactive probe some advances have already been made.

5.3 *Fate of azadirachtin in insect body*

Using the tritum labelled (22, 23 $-{}^3H_2$) dihydroazadirachtin A, having the same biological activity as azadirachtin A it was shown by Rembold *et al* (1988) that a constant quantity of 0·4–0·5 μg of dihydroazadirachtin A/g body weight was recovered unchanged 5 days after injection of any physiologically effective doses (1·5–3 μg/g) into the female *L. migratoria*. It was remarkable to observe that Malpighian tubules account for 74% of dihydroazadirachtin A retained in the whole body. The site of its metabolism is not yet known but it is not degraded significantly by fat bodies and Malpighian tubules. In the Malpighian tubules it was localized in the basal and inner regions (Rembold *et al* 1988; Garcia *et al* 1989). It is hence likely that azadirachtin acts in its unchanged form through high affinity binding to organ-specific membrane receptors.

5.4 *Effect on tropic hormones*

Further studies (Rembold *et al* 1989; Subrahmanyam and Rembold 1989) reveal that azadirachtin concentrates more in the corpus cardiacum than in the brains and that while it does not penetrate the blood brain barrier, completely covers the corpus cardiacum gland structure. This suggests a strong possibility that while azadirachtin does not interfere with the neurosecretory activity of brain directly, may act on the functioning of the corpus cardiacum. Keeping this observation and the observation on the accumulation of neurosecretion in this gland in view, the turnover of the radio labelled (^{35}S-cystein) neurosecretory material was studied (Subrahmanyam *et al* 1989). In fact, it was shown that the turnover of the neurosecretion in the corpus cardiacum of azadirachtin-injected *L. migratoria* was very poor, leading to accumulation of the stainable neurosecretory material over a course of time. Hence, azadirachtin may influence the release mechanism of tropic factors leading to disruption of hormonally controlled processes like moulting and vitellogenesis.

6. Conclusion

Azadirachtin is one of the most thoroughly studied natural substances having potential as a pesticide of the future. Physiological and biochemical studies have yielded encouraging data on the possible mode of action. However, direct proof for the initial biochemical step, in the sequence of endocrinological events, affected by this molecule is yet to be identified and the exact interaction understood. Forthcoming investigations with new probes can certainly yield valuable information in this direction. In practice crude preparations and enriched

formulations, at least in the tropical countries, continue to be used for pest control in view of the ease of preparation and safety.

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