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Insect Feeding Inhibitors
and Growth Regulators

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AZADIRACHTA INDICA: A SOURCE OF INSECT FEEDING
INHIBITORS AND GROWTH REGULATORS

By J. D. Warthen, Jr.^{1/}

ABSTRACT

Nontoxic, environmentally sound methods of pest control are of prime importance. For this reason neem tree components have been suggested as insect feeding inhibitors and growth regulators for 16 years. The activity of neem components upon Acari, Insecta, and Nematoda has been compiled. Some of these components, such as meliantriol, azadirachtin, and salannin, have been obtained in pure form. The structures of these triterpenoids along with the structures of other untested triterpenoids from neem have been incorporated into this review. All of these structures can serve as model compounds for the synthesis of commercially feasible insect feeding inhibitors and growth regulators to control larval and adult forms of stored-grain pests, termites, grasshoppers, locusts, and nematodes.

KEYWORDS: Azadirachta indica, neem, insect feeding inhibitors, growth regulators, meliantriol, azadirachtin, salannin, stored-grain pests.

INTRODUCTION

The search for environmentally sound methods for controlling insect pests is being carried out in many laboratories. It is a search of great importance when one considers the alternatives: pollution, lack of species specificity, and development of insect resistance. One area of investigation in this search is the examination of plants for secondary metabolites that may have insect-repelling, insecticidal, antihormonal, or antifeeding characteristics (39). Such materials could serve as models for the synthesis of substances to be used with other agents in biologically sound integrated pest management systems.

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Feeding inhibition of pest insects is of utmost importance to the long-range research goals of this laboratory. The terms used to describe feeding inhibition (antifeedant activity) seem to fall into three categories: the repellent which actively repels the insect before contact, the suppressant which suppresses feeding by the insect after contact, and the deterrent which deters feeding after making contact. Some classify the last two terms as rejectants (9). Others do not consider antifeedants as repellents because they believe that insects are not driven away by antifeedants (102). However, there seem to be two types of repellents: olfactory and gustatory. Olfactory repellents in the vapor phase stimulate olfactory receptors and drive the insect away from the treated material. Gustatory repellents act upon receptors, which are not normally sensitive to vapors but are sensitive to feeding (68). Thus, a gustatory repellent could easily be called an antifeedant. All these terms, however, are used interchangeably (50).

The neem tree, Azadirachta indica A. Juss.,^{2/} is a source of insect feeding inhibitors and growth regulators belonging to the order of Meliaceae. It grows 40 to 60 feet tall in the arid parts (71) of India and Burma, and was introduced into the arid regions of Africa during the last century as an ornamental avenue tree. It grows in poor, dry soil and tolerates heat well, but it will not tolerate excessive cold or frost. The many common names for neem in different languages indicate that it is found in many countries (29, 43, 73).

DISCUSSION

The uses of neem are mentioned in the earliest Sanskrit medical writings (72). However, it was Pradhan et al. (66) who first reported on the repellent properties (now called suppressant) of neem for desert locust adults after using a 0.001 percent aqueous suspension of ground neem kernels. In 1962, field tests were carried out in Delhi, India, where a 0.1 percent aqueous ground neem kernel suspension was sprayed on different crops at 300 to 600 liters per hectare. Although locusts collected on the treated crops, no feeding was observed (63). This effect lasted for 2 to 3 weeks. Thus, neem kernels provided a far more potent weapon than strong insecticides to Delhi farmers, because locusts were able to consume insecticide-treated crops before succumbing (64). No phytotoxicity from the neem suspension was observed at concentrations as high as 0.5 percent sprayed on cabbage, tomato, peas, onion, cucurbits, wheat, tobacco, rose castor, litchi, fig, citrus, pomegranate, and mango (66).

Table 1 lists the insects, nematodes, and a mite upon which neem and some of its components have been tested and indicates the modes of action--feeding inhibition, growth regulation, toxicity, and aphicidal activity--for each species. In addition to these activities, two other neem components, nimbin and nimbidin, have been found to have antiviral activity against potato virus X(PVS) (89, 98), vaccinia virus, and fowl-pox virus (74). Neem has also been

^{2/} Also referred to as Melia azadirachta L., M. indica Brandis, Margosa tree, or Indian lilac.

found to stabilize residues of pyrethrins for longer activity (1) and to have a synergistic action with custard apple against Callosobruchus chinensis (Lucas), Rhyzopertha dominica (F.), and Musca domestica nebulosa F. (69).

Three feeding inhibitory triterpenoids have been isolated from neem. They are meliantriol (36) (fig. 1), salannin (20, 21, 101) (fig. 4b), and azadirachtin (5, 6, 7, 100, 104) (fig. 3e). Insects and nematodes tested with one or more of these compounds are indicated in table 1. Recent studies with these triterpenoids indicate good feeding inhibition with azadirachtin or salannin against the striped cucumber beetle (77) and the Japanese beetle (33) in laboratory and field tests. Salannin also acts as a feeding inhibitor for California red scale (48) and Locusta (4), while azadirachtin is a feeding inhibitor at 0.35 parts per million for fall armyworm (100) and a growth regulator (chitin inhibition) for the large milkweed bug (75).

The insect growth-regulating activity of neem is probably due to azadirachtin, since it is structurally similar to insect ecdysones (51). Robbins et al. (78) showed that ingestion of ecdysone analogs caused inhibition of insect growth and development in the tobacco hornworm. This type of activity is just one added benefit to the feeding inhibition activity possessed by neem.

Another benefit is the translocation in plants of azadirachtin and possibly other components of neem extracts. Young bean plants grown in soil that was treated with azadirachtin showed little damage by desert locust, and bean seedlings grown from seeds soaked in azadirachtin solutions were protected against damage by desert locust adults for 1 week after germination in cage feeding tests (15). Systemic uptake without phytotoxicity has also been demonstrated in wheat, barley, rice, sugarcane, grass, tomato, cotton, chrysanthemums, and the small spindle tree (73).

Table 1.--Arthropods and nematodes evaluated with neem (Azadirachta indica) for feeding inhibition (FI) and growth regulation (GR)^{1/}

Scientific name	Common name	Activity ^{2/}	Reference
ARTHROPODA			
Acari			
+ <u>Panonychus citri</u> (McGregor)	citrus red mite	FI, (-)FI [†]	23, 48
Insecta			
<u>Coleoptera</u>			
*+ <u>Acalymma vittatum</u> (F.)	striped cucumber beetle	FI	77
<u>Anthrenus flavipes</u> LeConte	furniture carpet beetle	FI	29
<u>Aulacophora foveicollis</u> L.	red pumpkin beetle	FI	8, 63, 64, 67, 73
<u>Callosobruchus chinensis</u> (Lucas)	a seed weevil	FI	29
<u>C. maculatus</u> (F.)	cowpea weevil, pulse beetle	FI	27, 63, 64, 81
<u>Carabidae</u> -Fam.	ground beetle in tobacco	FI	29
<u>Carpophilus hemipterus</u> (L.)	driedfruit beetle	FI	23
<u>Cryptolestes pusillus</u> (Schönherr)	flat grain beetle	FI	29, 32, 81
* <u>Epilachna varivestis</u> Mulsant	Mexican bean beetle	GR	93, 95
<u>Lasioderma serricorne</u> (F.)	cigarette beetle	FI	81
<u>Latheticus oryzae</u> Waterhouse	longheaded flour beetle	FI	81
* <u>Leptinotarsa decemlineata</u> (Say)	Colorado potato beetle	FI, GR	82, 94
<u>Oryzaephilus surinamensis</u> (L.)	sawtoothed grain beetle	(-)FI	81
*+ <u>Popillia japonica</u> Newman	Japanese beetle	FI	33, 34
<u>Rhyzopertha dominica</u> (F.)	lesser grain borer	FI	16, 24, 26, 64, 73, 81
<u>Scarabaeidae</u> -Fam.	chafer grub in tobacco	FI	29
<u>Sitophilus oryzae</u> (L.)	rice weevil	FI	26, 63, 64, 68, 73, 81
<u>Stegobium paniceum</u> (L.)	drugstore beetle	FI	29
<u>Tribolium castaneum</u> (Herbst)	red flour beetle	FI	24, 63, 68, 73, 81
<u>T. confusum</u> Jacquelin duVal	confused flour beetle	FI	29
<u>Trogoderma granarium</u> Everts	khapra beetle	FI	16, 24, 26, 63, 64, 73, 80, 81

<u>Diptera</u>			
*† <u>Aedes aegypti</u> (L.)	(-)FI	yellowfever mosquito	83
<u>Anopheles</u> spp.	GR	—	73
<u>Atherigona soccata</u> Rondani	FI	sorghum shoot fly	28
<u>Hydrellia</u>	(-)FI	whorl maggot	29
<u>Musca</u>	GR	—	73
† <u>Musca domestica</u> L.	FI	house fly	101
<u>Stomoxys</u>	GR	—	73
<u>Hemiptera (Heteroptera)</u>			
<u>Antestiopsis orbitalis</u> bechuana (Kirk)	GR	coffee bug, shield bug	38, 51, 101
* <u>Dysdercus suturellus</u> (Herrich-Schäffer)	GR	cotton stainer	73, 79
* <u>Oncopeltus fasciatus</u> (Dallas)	GR	large milkweed bug	75
<u>Piesma quadratum</u> Fieb	GR	beet leaf bug	93
<u>Urentius hystricellus</u> (Richter)	FI	Bringle lace wing	29, 73
<u>Hemiptera (Homoptera)</u>			
<u>Aleurothrix floccosus</u> (Maskell)	FI	wooly whitefly	23
† <u>Aonidiella aurantii</u> (Maskell)	FI	California red scale	23, 48
<u>A. citrina</u> (Coquillett)	FI	yellow scale	23
—	FI	aphids	29
<u>Aphis gossypii</u> Glover	FI	melon aphid, cotton aphid	29, 73
<u>A. umbrellae</u> (Boerner)	FI	—	73
<u>Brevicoryne brassicae</u> (L.)	Aphicidal	cabbage aphid	29
<u>Cicadellidae</u>	FI	cicadellids	29
* <u>Myzus persicae</u> (Sulzer)	(-)FI	green peach aphid	9
<u>Parasaissetia nigra</u> (Nietner)	FI	nigra scale	29, 73
<u>Planococcus citri</u> (Risso)	(-)FI	citrus mealybug	23
<u>Rhopalosiphum nymphaeae</u> (L.)	Aphicidal	waterlily aphid, singhara aphid	17
<u>Isoptera</u>			
<u>Microtermes</u> sp.	Toxic	—	29
* <u>Reticulitermes santonensis</u>	(-)FI	Mediterranean moist wood termite	6
* <u>Reticulitermes</u> sp.	FI	—	104

Table 1 - continued.

Scientific name	Common name	Activity	Reference
<u>Lepidoptera</u>			
<u>Achaea janata</u> (L.)	croton caterpillar	FI	73
<u>Agrotis ipsilon</u> (Hufnagel)	black cutworm	FI	73
<u>Amsacta moorei</u> (Butler)	hairy caterpillar	FI	62,73
<u>Atteva fabriciella</u> Swederus	—	FI	73
<u>Boarmia (Ascotis) selenaria</u> (Denis & Schiffermüller)	giant looper	FI, GR	41
—	climbing cutworm	Toxic	29
<u>Corcyra cephalonica</u> (Stainton)	rice moth	FI	32,81
—	cotton boll worm	Toxic	29
<u>Diacrisia obliqua</u> (Walker)	—	FI	73
<u>Earias insulana</u> (Boisduval)	spiny bollworm	FI	40
<u>Ephestia cautella</u> (Walker)	almond moth	FI	81
<u>Euproctis fraterna</u> (Moore)	—	FI	28
<u>E. laniata</u> Hampson	castor hairy caterpillar	FI	2,3,4,5,67
<u>Eupterote mollifera</u> Walker	—	FI	73
* <u>Galleria mellonella</u> (L.)	greater wax moth	FI	104
* <u>Heliothis virescens</u> (F.)	tobacco budworm	FI, GR	79,104
* <u>Hypsipyla grandella</u> (Zeller)	—	FI	82
<u>Indarbela quadrinotata</u> (Walker)	bark eating caterpillar	Toxic	29
—	late stem borer	Toxic	29
—	leaf roller	Toxic	29
<u>Lymantria dispar</u> (L.)	gypsy moth	GR	91
* <u>Mamestra brassicae</u> (L.)	—	(-)FI	82
<u>Nephantis serinopa</u> Meyrick	—	FI	28
* <u>Pieris brassicae</u> (L.)	cabbage moth, cabbage white	GR	73,79,82,104
<u>Plusia peponis</u> (F.)	—	FI	73
* <u>Plutella xylostella</u> (L.)	diamondback moth	FI, GR	73,79,93,104
<u>Sitotroga cerealella</u> (Olivier)	Angoumois grain moth, paddy moth	FI	29
<u>Spodoptera exigua</u> (Hübner)	—	FI	73
† <u>S. frugiperda</u> (J. E. Smith)	fall armyworm	FI, (-)FI [†]	76,100
<u>S. litura</u> (F.)	tobacco caterpillar	FI	25,63,64,65,67
<u>Utetheisa pulchella</u> (L.)	—	FI	64,73

Orthoptera

<u>Acrida exatana</u> Walker	—	FI	64, 65, 73
<u>Chrotogonus trachypterus</u> Blanchard	surface grasshopper, toka	FI	29
<u>Chrotoicetes terminifera</u>	—	FI	73
+ <u>Locusta</u>	—	FI	4
<u>Locusta migratoria</u> (L.)	migratory locust	FI	63, 64, 67, 73, 90
—	North American grasshoppers	(-)FI	49
<u>Poecillocerus pictus</u>	Ak grasshopper	FI	63, 64, 67
*++ <u>Schistocerca gregaria</u> Forsk	desert locust	FI	5, 6, 7, 15, 17, 35, 36, 63, 64, 66, 67, 73, 81, 90, 104
NEMATODA			
<u>Hoplolaimus indicus</u> Sher., 1963	—	GR	30
<u>Meloidogyne incognita</u> (Kofoid & White, 1919) Chitwood, 1949	—	GR	30
* <u>Pratylenchus brachyurus</u> (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941	—	FI	11, 15, 73
<u>Rotylenchulus reniformis</u> Linford & Oliveira, 1940	—	GR	11, 30, 99
<u>Tylenchorhynchus brassicae</u> Siddiqi, 1961	—	GR	30

1/ * Tested with azadirachtin (101).

+ Tested with salannin (20, 21).

++ Tested with meliantriol (36).

2/ (-) = negative response.

The structures of components of neem related to the three feeding inhibitors, meliantriol, azadirachtin, and salannin appear in figures 2, 3, and 4. These related components have not been tested for feeding inhibition or growth regulation. However, since they possess structures similar to the three mentioned feeding inhibitors, it is likely that they would possess some activity.

The biosynthetic pathway to these compounds involves cyclization of squalene and rearrangement to give (20S)-tirucallol or (20R)-euphol. These epimers are precursors of limonoids. (20S)-Tirucallol is the precursor of the meliane limonoid, meliantriol, in figure 1. Skeletal rearrangement of (20R)-euphol to the hypothetical intermediate apo-euphol will then give rise to the meliacin limonoids in figures 2, 3, and 4. Those compounds from neem which have the A, B, and C rings intact, and thus retain the apo-euphol basic structure, appear in figure 2 and belong to the gedunin group. Biosynthetic oxidation of apo-euphol leads to intermediate precursors (fig. 3) and then to the c-seco meliacin limonoids (fig. 4) (103).

There are also a number of other ill-defined crude materials from neem such as nimbidin T (63), thionimone (8, 30, 73), nimatone (72), nimbidol (86), and nimbidin (44, 45, 72, 84, 86) which have appeared in the literature with mention of insect feeding inhibition and growth regulation. Mention of hydrolysis products from these materials such as neo-nimbidin, nimbidic acid (45, 46, 86), nimbidinic acid, neonimbidin (86), and nimbidinin (44) also appears, but most of these materials have not been tested. Other components which have been isolated from neem are β -sitosterol (3, 70), 24-methylenecycloartanol (70), fatty acids (92), and the flavanoids: quercetin-2-galactoside (3, 96), kaempferol-3-glucoside (47, 96), and myricetin-3'-L-arabinoside (96).

The use of neem and its products as a source of insect feeding inhibitors and growth regulators is naturally dependent upon its toxicity and impact on the environment. Neem may be nontoxic to warm-blooded animals--the neem fruit is a favorite food for birds and neem twigs have long been used as chewing sticks to prevent tooth infection (51). However, neem products do have various pharmacological actions (86) which must be considered.

Figure 1.--Meliantriol (36).

a $R_1 = H, R_2 = 2H$ azadirone (35, 37)

b $R_1 = H, R_2 = O$ azadiradione (31, 35, 37, 87)

c $R_1 = OH, R_2 = O$ 17- β -hydroxy-azadiradione (31, 85)

d 17-*epi*-azadiradione (31)

e epoxyazadiradione (nimbinin) (10, 35, 37, 46, 53, 56,
58, 59, 84, 86, 88, 105)

Figure 2.--Gedunin group.

f R = COCH₃ gedunin (13, 35, 37)

g R = H 7-deacetoxy-7-α-hydroxygedunin (35, 37)

h vepinin (59)

i meldenin (10)

Figure 2.--Gedunin group (con.).

- a $R_1 = R_2 = R_3 = H, R_4 = 2H$ vilasinin (61)
b $R_1 = R_2 = R_3 = H, R_4 = O$ nimbidinin (45, 46)
c $R_1 = R_2 = COCH_3, R_3 = COCH=CHPh, R_4 = 2H$ nimbolin A (13)

d nimbolin B (13)

e azadirachtin (5, 6, 7, 100, 104)

Figure 3.--Precursors of C-seco meliacins.

a nimbolide (12, 14)

b salannin (20, 21)

c nimbin (12, 19, 22, 42, 52, 53, 54, 55,
56, 57, 60, 72, 84, 98, 105)

Figure 4.--C-seco meliacins.

Although neem is susceptible to attack by some insect pests (table 2) (29), it seems to offer an effective way of controlling larval and/or adult forms of stored-grain pests, termites, grasshoppers, locusts, and nematodes. Since the neem tree grows abundantly (71), its ground parts could be used until a commercial synthetic feeding inhibitor is available. Compounds similar in structure to those in figures 1-4 should be synthesized, but, of course, they must be simple enough to make them commercially feasible as insect feeding inhibitors and growth regulators.

Table 2.--Pests of neem

Scientific name	Common name	Reference
Acari		
<u>Calepiterimerus azadirachta</u>	eriphyid mite	97
Insecta		
<u>Coleoptera</u>		
<u>Araecerus fasciculatus</u> (De Geer)	coffee bean weevil,	97
	anthribid fruit borer	
<u>Cryptocephalus ovulus</u> Suffr.	—	97
<u>Holotrichia consanguinea</u> Blanchard	white grub beetle	18
<u>H. insularis</u> Brenske	white grub beetle	18
<u>H. serrata</u> (F.)	white grub beetle	18
<u>Hemiptera (Heteroptera)</u>		
<u>Helopeltis antonii</u> Signoret	mirid bug	97
<u>Hemiptera (Homoptera)</u>		
<u>Pulvinaria maxima</u> Green	mealy scale	97
<u>Lepidoptera</u>		
<u>Laspeyresia aurantianna</u> Collar	—	97
<u>Orthoptera</u>		
<u>Orthacris simulans</u>	—	97

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LITERATURE CITED

- (1) Ahmed, S. M., M. R. Gupta, and H. M. Bhavanagary. 1976. Stabilization of pyrethrins for prolonged residual toxicity. Part II: Development of new formulations. *Pyrethrum Post* 13: 119-123.
- (2) Babu, T. H., and Y. P. Beri. 1969. Efficacy of neem (Azadirachta indica) seed extracts in different solvents as a deterrent to the larvae of Euproctis lunata. *Andhra Agric. J.* 16: 107-111.
- (3) Basak, S. P., and D. P. Chakraborty. 1968. Chemical investigation of Azadirachta indica leaf (M. azadirachta). *Indian Chem. Soc.* 45: 466-467.
- (4) Bernays, E. A. 1978. Personal communication.
- (5) Butterworth, J. H., and E. D. Morgan. 1968. Isolation of a substance that suppresses feeding in locusts. *Chem. Commun.*, p. 23-24.
- (6) Butterworth, J. H., and E. D. Morgan. 1971. Investigation of the locust feeding inhibition of the seeds of the neem tree, Azadirachta indica. *J. Insect Physiol.* 17: 969-977.
- (7) Butterworth, J. H., E. D. Morgan, and G. R. Percy. 1972. The structure of azadirachtin; the functional groups. *J. Chem. Soc. Perkin Trans. I*: 2445-2450.
- (8) Chakravorty, D. P., G. C. Ghosh, and S. P. Dhua. 1969. Repellent properties of thionimone on red pumpkin beetle Aulacophora foveicollis L. *Technology* 6: 48-49.
- (9) Chapman, R. F. 1974. The chemical inhibition of feeding by phytophagous insects: a review. *Bull. Entomol. Res.* 64: 339-363.
- (10) Connolly, T. D., K. L. Handa, and R. McCrindle. 1968. Further constituents of nim oil: the constitution of meldonin. *Tetrahedron Lett.* No. 4: 437-440.
- (11) Egunjob, O. A., and S. O. Afolami. 1976. Effects of neem (Azadirachta indica) leaf extracts on populations of Pratylenchus brachyurus and on the growth and yield of maize. *Nematologica* 22: 125-132.

- (12) Ekong, D. E. U. 1967. Chemistry of the meliacins (limonoids). The structure of nimbolide, a new meliacin from Azadirachta indica. Chem. Commun., p. 808.
- (13) Ekong, D. E. U., C. O. Fakunle, A. K. Fasina, and J. I. Okogun. 1969. The meliacins (limonoids). Nimbolin A and B, two new meliacin cinnamates from Azadirachta indica L. and Melia azedarach L. Chem. Commun., p. 1166-1167.
- (14) Ekong, D. E. U., S. A. Ibiyemi, and E. O. Olagbemi. 1971. The meliacins (limonoids). Biosynthesis of nimbolide in the leaves of Azadirachta indica. Chem. Commun., p. 1117-1118.
- (15) Gill, J. S., and C. T. Lewis. 1971. Systemic action of an insect feeding deterrent. Nature (London) 232: 402-403.
- (16) Girish, G. K., and S. K. Jain. 1974. Studies on the efficacy of neem seed kernel powder against stored grain pests. Bull. Grain Technol. 12: 226-228.
- (17) Goyal, R. S., K. C. Gulati, P. Sarup, M. A. Kidwai, and D. S. Singh. 1971. Biological activity of various alcohol extractives and isolates of neem (Azadirachta indica) seed cake against Rhopalosiphum nymphaeae (Linn.) and Schistocerca gregaria Forsk. Indian J. Entomol. 33: 67-71.
- (18) Gupta, K. M. 1973. Neem leaves attract white grub beetles. Indian J. Entomol. 35: 276.
- (19) Harris, M., R. Henderson, R. McCrindle, K. H. Overton, and D. W. Turner. 1968. Tetranortriterpenoids - VIII. The constitution and stereochemistry of nimbin. Tetrahedron 24: 1517-1523.
- (20) Henderson, R., R. McCrindle, A. Melera, and K. H. Overton. 1968. Tetranortriterpenoids - IX. The constitution and stereochemistry of salannin. Tetrahedron 24: 1525-1528.
- (21) Henderson, R., R. McCrindle, and K. H. Overton. 1964. Salannin. Tetrahedron Lett. No. 52: 3969-3974.
- (22) Henderson, R., R. McCrindle, K. H. Overton, M. Harris, and D. W. Turner. 1963. The constitution of nimbin. Proc. Chem. Soc., p. 269-270.
- (23) Jacobson, M., D. K. Reed, M. M. Crystal, D. S. Moreno, and E. L. Soderstrom. 1978. Chemistry and biological activity of insect feeding deterrents from certain weed and crop plants. Entomol. Exp. Appl. (In press.)
- (24) Jilani, G., and M. M. Malik. 1973. Studies on neem plant as repellent against stored grain insects. Pakistan J. Sci. Ind. Res. 16: 251-254.
- (25) Joshi, B. G., and G. Ramaprasad. 1975. Neem kernel as an antifeedant against the tobacco caterpillar (Spodoptera litura F.). Phytoparasitica 3: 59-61.

- (26) Jotwani, M. G., and P. Sircar. 1965. Neem seed as a protectant against stored grain pests infesting wheat seed. *Indian J. Entomol.* 27: 160-164.
- (27) Jotwani, M. G., and P. Sircar. 1967. Neem seed as a protectant against bruchid Callosobruchus maculatus (Fabricius) infesting some leguminous seeds. *Indian J. Entomol.* 29: 21-24.
- (28) Jotwani, M. G., S. Sadakathulla, M. S. Venugopal, and T. R. Subramaniam. 1974. Efficacy of two organotin compounds and neem extract against the sorghum shoot fly. *Phytoparasitica* 2: 127-129.
- (29) Ketkar, S. C. M. 1976. Utilization of neem (Azadirachta indica Juss.) and its by-products. 1st Ed., 234 p.; Nana Dengle Sadhana Press, Poona (India).
- (30) Khan, M. W., M. M. Alam, A. M. Khan, and S. K. Saxena. 1974. Effect of water soluble fractions of oil-cakes and bitter principles of neem on some fungi and nematodes. *Acta Botan. Indica* 2: 120-128.
- (31) Kraus, W., and R. Cramer. 1978. 17-Epi-azadiradion und 17- β -Hydroxy-azadiradion, zwei neue Inhaltsstoffe aus Azadirachta indica A. Juss. *Tetrahedron Lett.* No. 27: 2395-2398.
- (32) Krishnamurti, B., and D. S. Rao. 1950. Some important insect pests of stored grains and their control. *Entomol. Ser. Bull.*, No. 14, 93 p.; Bangalore (India).
- (33) Ladd, T. L., Jr. 1968. Personal communication.
- (34) Ladd, T. L., Jr., M. Jacobson, and C. R. Buriff. 1978. Japanese beetles: extracts from neem tree seeds as feeding deterrents. *J. Econ. Entomol.* 71: 810-813.
- (35) Lavie, D., and M. K. Jain. 1967. Tetranortriterpenoids from Melia azadirachta L. *Chem. Commun.*, p. 278-280.
- (36) Lavie, D., M. K. Jain, and S. R. Shpan-Gabrielith. 1967. A locust phagorepellent from two Melia species. *Chem. Commun.*, p. 910-911.
- (37) Lavie, D., E. C. Levy, and M. K. Jain. 1971. Limonoids of biogenetic interest from Melia azadirachta L. *Tetrahedron* 27: 3927-3939.
- (38) Leuschner, K. 1972. Effect of an unknown plant substance on a shield bug. *Naturwissenschaften* 59: 217-218.
- (39) Meinwald, J., G. D. Prestwich, K. Nakanishi, and I. Kubo. 1978. Chemical ecology: studies from East Africa. Interdisciplinary studies at the ICIPE laboratories in Nairobi are yielding exciting chemical results. *Science* 199: 1167-1173.

- (40) Meisner, J., M. Kehat, M. Zur, and C. Elizik. 1978. Response of Earias insulana Boisd. larvae to neem (Azadirachta indica A. Juss.) kernel extract. *Phytoparasitica* 6: 85-88.
- (41) Meisner, J., M. Wysoki, and K. R. S. Ascher. 1976. The residual effect of some products from neem (Azadirachta indica A. Juss.) seeds upon larvae of Boarmia (Ascotis) selenaria Schiff. in laboratory trials. *Phytoparasitica* 4: 185-192.
- (42) Mitra, C. R. 1957. On the constitution of nimbin. *J. Sci. Ind. Res.* 16B: 477-478.
- (43) Mitra, C. R. 1963. Neem monograph. 190 p. Indian Central Seeds Committee, Hyderabad, India.
- (44) Mitra, C. R. 1970. Studies on certain plant (inclusive of neem) extracts and isolates having pesticidal properties--final report. PL 480, Project No. A7-Ent-60, Progr. Rep.
- (45) Mitra, C. R., H. S. Garg, and G. N. Pandey. 1970. Constituents of Melia indica - II. Nimbidic acid and nimbidinin. *Tetrahedron Lett.* No. 32: 2761-2764.
- (46) Mitra, C. R., H. S. Garg, and G. N. Pandey. 1971. Identification of nimbidic acid and nimbidinin from Azadirachta indica. *Phytochemistry* 10: 857-864.
- (47) Mitra, C. R., P. N. Rao, S. Bhattacharji, and S. Siddiqui. 1947. Chemical examination of nim blossoms (Melia azadirachta Flora) *J. Sci. Ind. Res.* VIB: 19-24.
- (48) Moreno, D. S., and L. K. Tanigoshi. 1978. Personal communication.
- (49) Mulkern, G. B. 1971. Effect of desert locust repellent on grasshoppers. *Proc. North. Cent. Branch, Entomol. Soc. Am.* 26: 84.
- (50) Munakata, K. 1977. Insect feeding deterrents in plants. *In* H. H. Shorey and J. J. McKelvey, Jr. (eds.), *Chemical control of insect behavior*, p. 93-102. John Wiley & Sons, New York.
- (51) Nakanishi, K. 1975. Structure of the insect antifeedant azadirachtin. *Recent Adv. Phytochem.* 9: 283-298.
- (52) Narasimhan, N. S. 1959. Konstitution des Nimbins. I. Natur der Funktionellen Gruppen. *Chem. Ber.* 92: 769-775.
- (53) Narayanan, C. R., and R. V. Pachapurkar. 1965. Ring D in nimbin. *Tetrahedron Lett.* No. 48: 4333-4336.

- (54) Narayanan, C. R., and R. V. Pachapurkar. 1966. The structure of nimbinic acid. *Tetrahedron Lett.* No. 6: 553-557.
- (55) Narayanan, C. R., R. V. Pachapurkar, S. K. Pradhan, V. R. Shah, and N. S. Narasimhan. 1964. Stereochemistry of nimbin. *Chem. & Ind.*, p. 324.
- (56) Narayanan, C. R., R. V. Pachapurkar, S. K. Pradhan, V. R. Shah, and N. S. Narasimhan. 1964. Structure of nimbin. *Chem. & Ind.*, p. 322-324.
- (57) Narayanan, C. R., R. V. Pachapurkar, S. K. Pradhan, V. R. Shah, and N. S. Narasimhan. 1964. Structure of nimbin. *Indian J. Chem.* 2: 108-113.
- (58) Narayanan, C. R., R. V. Pachapurkar, and (in part) B. M. Sawant. 1967. Nimbinin: a new tetranortriterpenoid. *Tetrahedron Lett.* No. 37: 3563-3565.
- (59) Narayanan, C. R., R. V. Pachapurkar, B. M. Sawant, and M. S. Wadia. 1969. Vepinin, a new constituent of neem oil. *Indian J. Chem.* 7: 187.
- (60) Narayanan, C. R., S. K. Pradhan, R. V. Pachapurkar, and N. S. Narasimhan. 1962. The molecular formula of nimbin. *Chem. & Ind.*, p. 1283.
- (61) Pachapurkar, R. V., P. M. Kornule, and C. R. Narayanan. 1974. A new hexacyclic tetranortriterpenoid. *Chem. Lett.* 4: 357-358.
- (62) Patel, H. K., V. C. Patel, M. S. Chari, J. C. Patel, and J. R. Patel. 1968. Neem seed paste suspension - a sure deterrent to hairy caterpillar (Amsacta moorei But.). *Madras Agric. J.* 55: 509-510.
- (63) Pradhan, S., and M. G. Jotwani. 1968. Neem as an insect deterrent. *Chem. Age India* 19: 756-760.
- (64) Pradhan, S., and M. G. Jotwani. 1971. Neem kernel as antifeedant for locust. *Sneha-Sandesh* 13: 1-5.
- (65) Pradhan, S., and M. G. Jotwani. 1971. Repeated confirmation of our discovery of antifeedant property of neem kernel. *Entomol. Newlett.* 1: 75-77.
- (66) Pradhan, S., M. G. Jotwani, and B. K. Rai. 1962. The neem seed deterrent to locusts. *Indian Farming* 12: 7-11.
- (67) Pradhan, S., M. G. Jotwanni, and B. K. Rai. 1963. The repellent properties of some neem products. *Bull. Reg. Res. Lab. Jammu (India)* 1: 149-151.

- (68) Qadri, S. S. H. 1973. Some new indigenous plant repellents for storage pests. *Pesticides (India)* 7: 18-19, 22.
- (69) Qadri, S. S. H., and B. Rao. 1977. Effect of combining plant seed extracts against household insects. *Pesticides (India)* 11: 21-23.
- (70) Quasim, C., and N. L. Dutta. 1970. Chemical investigation of Azadirachta indica. *Indian J. Appl. Chem.* 33: 384-386.
- (71) Radwanski, S. 1977. Neem tree. 1: Commercial potential, characteristics and distribution. *World Crops & Livestock* 29: 62-63, 65-66.
- (72) Radwanski, S. 1977. Neem tree. 2: Uses and potential uses. *World Crops & Livestock* 29: 111-113.
- (73) Radwanski, S. 1977. Neem tree. 3: Further uses and potential uses. *World Crops & Livestock* 29: 167-168.
- (74) Rai, A., and M. S. Sethi. 1972. Screening of some plants for their activity against vaccinia and fowl-pox viruses. *Indian J. Anim. Sci.* 42: 1066-1070.
- (75) Redfern, R., J. D. Warthen, Jr., G. D. Mills, Jr., and E. C. Uebel. 1978. Large milkweed bug: molting inhibitory effects of azadirachtin. (In process.)
- (76) Redfern, R. 1978. Personal communication.
- (77) Reed, D. K. 1978. Personal communication.
- (78) Robbins, W., J. N. Kaplanis, M. J. Thompson, T. J. Shortino, S. F. Cohen, and J. C. Joyner. 1968. Ecdysones and analogs: effects on development and reproduction of insects. *Science* 161: 1158-1160.
- (79) Ruscoe, C. N. E. 1972. Growth disruption effects of an insect anti-feedant. *Nature (New Biol.)* 236: 159-160.
- (80) Saramma, P. U., and A. N. Verma. 1971. Efficacy of some plant products and magnesium carbonate as protectants of wheat seed against attack of Trogoderma granarium. *Bull. Grain Technol.* 9: 207-210.
- (81) Sarup, P., and U. S. Srivastava. 1972. Observations on the damage of neem (Azadirachta indica A. Juss.) seed kernel in storage by various pests and efficacy of the damaged kernel as an antifeedant against the desert locust, Schistocerca gregaria Forsk. *Indian J. Entomol.* 33: 228-320.
- (82) Schoonhoven, L. M., and T. Jermy. 1977. A behavioral and electrophysiological analysis of insect feeding deterrents. In N. R. McFarlane (ed.), *Crop protection agents, their biological evaluation*, p. 133-146. Academic Press, New York.

- (83) Schreck, C. E., and D. E. Weidhaas. 1978. Private communication.
- (84) Siddiqui, S. 1942. A note on the isolation of three new bitter principles from the nim oil. *Current Sci.* 11: 278-279.
- (85) Siddiqui, S., S. Fuchs, J. Lücke, and W. Voelter. 1978. Struktur eines neuen Naturstoffes aus Melia azadirachta Linn.: 17-Hydroxy-azadiradion. *Tetrahedron Lett.* No. 7: 611-612.
- (86) Siddiqui, S., and C. R. Mitra. 1945. Utilization of nim oil and its bitter constituents (nimbidin series) in the pharmaceutical industry. *J. Sci. Ind. Res.* 4: 5-10.
- (87) Siddiqui, S., T. N. Waheed, J. Lücke, and W. Voelter. 1975. Zur Struktur einer Substanz aus dem Fruchtfleisch von Melia azadirachta Linn. *Z. Naturforsch.* 30B: 961-964.
- (88) Siddiqui, S., T. N. Waheed, J. Lücke, and W. Voelter. 1975. Isolierung und Strukturidentifizierung eines Naturstoffs aus dem Fruchtfleisch von Melia azadirachta Linn. *Chem. Ztg.* 99: 504-506.
- (89) Singh, R. 1971. Inactivation of potato virus X by plant extracts. *Phytopath. Medit.* 10: 211-213.
- (90) Sinha, N. P., and K. C. Gulati. 1963. Neem seed cake (Azadirachta indica) as a source of pest control chemicals. *Bull. Regional Res. Lab. Jammu (India)* 1: 176-177.
- (91) Skatulla, N., and J. Meisner. 1975. Labor-Versuche mit Neem-Samenextrakt zur Bekämpfung des Schwammspinners, Lymantria dispar L. *Anz. Schädlingssk., Pflanzenschutz., Umweltschutz* 48: 38-40.
- (92) Skellon, J. H., S. Thorburn, J. Spence, and S. N. Chatterjee. 1962. The fatty acids of neem oil and their reduction products. *J. Sci. Food Agric.* 13: 639-643.
- (93) Steets, R. 1975. Die Wirkung von Rohextrakten aus den Meliaceen Azadirachta indica und Melia azederach auf verschiedene Insektenarten. *Z. Angew. Entomol.* 77: 306-312.
- (94) Steets, R. 1976. Zur Wirkung eines gereinigten Extraktes aus Früchten von Azadirachta indica A. Juss. auf Leptinotarsa decemlineata Say (Coleoptera, Chrysomelidae). *Z. Angew. Entomol.* 82: 169-176.

- (95) Steets, R., and H. Schmutterer. 1975. The effect of azadirachtin on the longevity and reproduction of Epilachna varivestis Muls. (Coleoptera, Coccinellidae). Z. Pflazenkr. Pflanzenschutz 82: 176-179
- (96) Subramanian, S. S., and A. G. R. Nair. 1972. Melicitrin - a new myricetin glycoside from the flowers of Melia azadirachta. Indian J. Chem. 10: 452.
- (97) Uthamasamy, S., P. V. S. Rao, M. Mohanasundaram, and T. R. Subramaniam. 1973. A note on some new pests attacking the neem tree (Azadirachta indica) in Tamil Nadu. Sci. Cult. 39: 399-400.
- (98) Verma, V. S. 1974. Chemical compounds from Azadirachta indica as inhibitors of potato virus X. Acta Microbiol. Polon. Ser. B. 6: 9-13.
- (99) Verma, V. S., and S. K. Prasad. 1970. The reniform nematode, Rotylenchulus reniformis. II. Studies on control. Indian J. Entomol. 32: 68-73.
- (100) Warthen, J. D., Jr., R. E. Redfern, E. C. Uebel, and G. D. Mills, Jr. 1978. An antifeedant for fall armyworm larvae from neem seeds. USDA Sci. & Educ. Adm. Agric. Res. Results, Northeast. Ser., No. 1, 9 p. Beltsville, Md.
- (101) Warthen, J. D., Jr., E. C. Uebel, S. R. Dutky, W. R. Lusby, and H. Finegold. 1978. Adult house fly feeding deterrent from neem seeds. USDA Sci. & Educ. Adm. Agric. Res. Results, Northeast. Ser., No. 2, 11 p. Beltsville, Md.
- (102) Wright, D. P., Jr. 1967. Antifeedants. In W. W. Kilgore and R. L. Doutt (eds.), Pest control, p. 287-293. Academic Press, New York.
- (103) Zanno, P. R. 1974. I. The structure of azadirachtin, a potent insect phagorepellent and systemic growth disruptor from Azadirachta indica. Ph.D. diss., 159 p. Columbia Univ., New York.
- (104) Zanno, P. R., E. Miura, K. Nakanishi, and D. L. Elder. 1975. Structure of the insect phagorepellent azadirachtin. Applications of PRFT/CWD carbon-13 nuclear magnetic resonance. J. Am. Chem. Soc. 97: 1975-1977.
- (105) Ziffer, H., and U. Weiss. 1966. Absolute stereochemistry of nimbin. "Complex" optical rotary dispersion of pyronimbic acid. J. Org. Chem. 31: 2691.

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