Evaluation of Neem (*Azadirachta indica* A. Juss) Extracts against American Bollworm, *Helicoverpa armigera* (Hubner)

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Methanol and chloroform:methanol (9:1) extracts of neem (*Azadirachta indica* A. Juss) seed kernels and green leaves were evaluated against American bollworm, *Helicoverpa armigera* (Hubner). Various concentrations of extracts were used to treat natural foods, *viz*. leaves and pods/bolls of chickpea, pigeonpea, and cotton. Treated foods were offered to early stage (neonate) and advanced stage (grown up) larvae of *H. armigera* for 48 h, and then, untreated food was offered for the rest of life. Chloroform:methanol (9:1) extracts of neem seed kernels and leaves showed better insecticidal properties than methanol extracts. However, neem seed kernels extract in chloroform:methanol (9:1) was the most promising in causing adverse morphogenic effects on various biological parameters of *H. armigera*. Early stage larvae were more sensitive to the exposure of neem extracts than advanced stage larvae.

Keywords: *Neem; seed kernels; chloroform:methanol; biology; Helicoverpa armigera*

INTRODUCTION

Environmentally safer pesticides are selectively toxic, do not bioaccumulate, and exhibit relatively short persistence in the environment. They are most desirable in the modern integrated pest management programs. Plant products (insecticides) appear to be ecofriendly because they have been found to be selective (Saxena *et al*., 1984; Stark *et al*., 1992) and pose less negative impacts to ecosystems than conventional insecticides (Stark, 1992).

Interest in plant products as insecticides has grown over the past 10 years as more and more pesticides are eliminated from use due to environmental and food safety problems (Koul *et al*., 1990; Schmutterer, 1990). The plant kingdom affords a rich storehouse of chemicals of diverse biological effects on insects. In recent years several plants with insecticidal properties have been identified (Grainge and Ahmad, 1988; Jood *et al*., 1993; Prakash and Rao, 1997) but neem, *Azadirachta indica* has received maximum attention of entomologists all over the world (Schmutterer, 1990; Stark and Walter, 1995). The extracts from neem tree were found to reduce or prevent insect feeding and also to adversely affect growth, development, and reproduction (Schmutterer, 1990; Mordue and Blackwell, 1993). Unlike synthetic chemicals, plant extracts from neem comprise a large variety of biologically active molecules which reduce the chances of developing pest resistance (Saxena, 1983).

Among the different insect pests, American bollworm, *Helicoverpa armigera* (Hubner) is recognized as an international pest because of its worldwide distribution and high damage potentials covering more than 182 species of plants (Manjunath *et al*., 1989). Total reliance on the application of synthetic insecticides to control *H. armigera* has not achieved the desired success, and induced resistance to several groups of chemicals has been one effect (Armes *et al*., 1992). Thus, attempts are being made to find alternate methods of its control. Within the broad ambit of integrated pest management, entomologists the world over are concentrating on the use of plant products, especially neem products, to tide over the menace of insect pests. The information regarding biological efficacy of neem against *H. armigera* is scanty. So, in view of its seriousness and polyphagous nature, it was considered worthwhile to study the biological efficacy/activity of neem (kernels and leaves) against *H. armigera* under laboratory conditions.

MATERIALS AND METHODS

Collection and Processing of Plant Parts. Fully ripe (yellow) neem fruits which had dropped to the ground from neem trees that were about 15 years old were collected in the months of July and August at the campus of CCS Haryana Agricultural University, Hisar. Fruits were depulped to obtain seeds. The seeds were shade-dried for about a week to facilitate decortication to obtain kernels (Singh, 1987). The kernels were further oven-dried at 40 °C for 24 h so that they could be easily broken into small pieces manually. Mature green leaves were also plucked from the trees selected for the collection of neem fruits. The leaves were also shade-dried and oven-dried similar to seeds/kernels. The processed plant parts were subjected to extraction in different solvents.

Extractions. The extraction in methanol was carried out as suggested by Meisner *et al*. (1983), and extraction in chloroform:methanol (9:1) was carried out as followed by Singh (1987). The plant material (500 g each of kernels and leaves) was soaked separately in 1 L of solvent for 48 h at room temperature in a stoppered round bottom flask and shaken after every 3 h for 5 min. The extracts were filtered, and filtrate was evaporated under vacuum on a water bath (30−35 °C) by distillation to obtain the crude extracts for biological studies. The crude extracts thus obtained were diluted in acetone (20% w/v) to prepare stock solution. The stock solution was used to prepare further concentrations in water containing emulsifier as followed by Jhansi (1988).

Biological Testing. Studies on the biological efficacy of neem against *H. armigera* were carried out in BOD incubators at 28 ± 2 °C and relative humidity 65−75%. The laboratory culture of insect was initially started by collecting grown up larvae of *H. armigera* from pigeonpea fields and then rearing them on pigeonpea pods under sterilized conditions until

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S0021-8561(97)00053-8 CCC: $14.00

Table 1. Effect of Neem (*A. indica*) Seed Kernel Extract (Methanol Extract) on the Different Biological Parameters of *H. armigera* When Fed on Treated Food during Early Stage[a]

stage of insect	no. of insects (n)	concentration of neem seed kernel extract 0.5%	2.5%	5.0%	7.5%	control (untreated)	CD ($P < 0.05$)
larval mortality (%)	100	24.0 (29.31)	50.0 (45.00)	70.0 (56.80)	88.0 (69.75)	9.0 (17.35)	(4.88)
larval duration (days)	100	18.8	22.1	23.6	27.6	17.7	0.8
larval weight (mg/larva)	10	420.7	401.3	382.0	360.5	445.8	26.5
prepupal mortality (%)	100	2.0 (5.17)	3.0 (7.75)	5.0 (12.92)	6.0 (14.02)	2.0 (5.17)	(1.80)
prepupal duration (days)	10	2.3	2.4	2.6	2.8	2.1	0.3
pupal duration (days)	10	12.0	13.6	16.4	*b*	11.4	1.2
adult emergence (%)	100	66.0 (54.33)	41.0 (39.82)	26.0 (30.64)	0.0 (0.01)	83.0 (65.67)	(4.51)
adult deformity (%)	100	2.0 (5.17)	2.0 (5.17)	3.0 (7.75)	*b*	2.0 (5.17)	(1.02)
adult longevity (days)							
(i) male moth	10	13.5	11.5	9.4	*b*	15.2	0.8
(ii) female moth	10	15.3	13.6	12.7	*b*	16.5	0.8
fecundity/female	10	532.6	482.0	446.6	*b*	556.8	38.2
egg fertility (%)	500	71.0 (57.43)	68.0 (55.57)	62.4 (52.19)	*b*	75.0 (60.02)	(4.02)

[a] Figures in parentheses are angular transformed values. CD denotes critical difference. 4−5-day old larvae were fed on neem seed kernels extract treated food for 48 h. [b] Observations could not be recorded as the insect did not reach the adult stage.

pupation. The adults emerged from pupae were fed on sucrose solution (10%) in oviposition chambers containing filter paper strips as oviposition substrates. The eggs laid took 3−4 days to hatch. The newly hatched (neonate) larvae were used for further rearing of the insect. In this way, the culture of *H. armigera* was maintained in laboratory. The insecticide-free food plants *viz*. pigeonpea (Manak), chickpea (Haryana Chana No. 1), and cotton (H974) grown at the research farm of CCS Haryana Agricultural University, Hisar, during different seasons were used for various biological experiments depending upon the time of neem extract preparations.

The methanol and chloroform:methanol (9:1) extracts were diluted in water using Teepol (0.2%) as emulsifier to prepare various concentrations. Early (4−5 days old) and advanced (10−11 days old) stage larvae of *H. armigera* were fed on foods treated with diluted extracts for 48 h as per the method of Meisner and Nemny (1992). The concentrations of extracts, *viz*. methanol (0.5%, 2.5%, 5.0%, and 7.5%) and chloroform: methanol (0.1, 0.5, and 1.0%) were selected on the basis of their effectiveness against a range of insect pests (Singh, 1993a). During handling of solvents, specifically chloroform, care was taken to avoid exposure through contact or inhalation. The methanol extract at 0.5%, 2.5%, 5.0% and 7.5% was sprayed on pigeonpea and chickpea leaves by hand automizer. Water with emulsifier (Teepol) served as control. The sprayed leaves were shade-dried for about 30−45 min to evaporate water. The pigeonpea leaves were then offered to early stage larvae and chickpea leaves to advanced stage larvae for 48 h. Thereafter larvae were restored to untreated foods (pods). The food was replenished on alternate days until pupation.

In order to evaluate the effectiveness of chloroform:methanol (9:1) extracts against early stage larvae, cotton leaves were sprayed with different concentrations, i.e., 0.1%, 0.5%, and 1.0% along with water and emulsifier as control by hand atomizer, while for advanced stage larvae, pigeonpea leaves were used. Sprayed leaves were shade-dried for about 30−45 min to evaporate water. These leaves were offered to larvae for 48 h, after which the food composing of cotton bolls for early stage larvae and pigeonpea pods for advanced stage larvae was supplied and replenished on alternate days till pupation. In each of five replicates 20 larvae were used to record larval mortality (%), larval duration (days), weight of full-grown larvae (mg/larva), prepupal mortality (%), prepupal duration (days), pupal duration (days), adult emergence (%), adult deformity (%), adult longevity (days), fecundity/female and egg fertility (%).

Statistical Analysis. The data were subjected to analysis of variance (ANOVA) in a completely randomized design to determine critical difference (CD) among treatments. The difference of two means between treatments exceeding CD value is significant (Panse and Sukhatme, 1978). The data (in %) were subjected to angular transformation because of binomial proportion (Snedecor and Cochran, 1968).

RESULTS

Effect of Neem Extracts on Larval Stage. The data indicate that there was a progressive increase in the mortality and life period of *H. armigera* larvae with the increase in concentration of neem extracts, when early and advanced stage larvae of *H. armigera* were fed on the food treated with neem seed kernels and leaves extracted in methanol and chloroform:methanol (9:1) at various concentrations (Tables 1−8). The mortality of early stage larvae of *H. armigera* ranged from 24.0% to 88.0%, when they were fed on food treated with different concentrations of methanol extract of neem seed kernels for 48 h. The mortality was only 9.0% in control (Table 1). All the concentrations were superior to the control in causing larval mortality, and the maximum (88.0%) mortality was observed at 7.5% concentration (Table 1). Chloroform:methanol (9:1) extract of neem seed kernels was found to be more effective than methanol extract. Maximum mortality (95.0%) of early stage larvae was observed at 1.0% concentration, and other lower concentrations were also significantly superior over control (Table 2). Feeding of early stage larvae for 48 h on food treated with different concentrations of neem leaves extract (methanol) caused 11.0%−70.0% larval mortality (Table 3), whereas feeding of larvae with chloroform:methanol (9:1) extract (neem leaves) caused 32.0%−72.0% larval mortality (Table 4). The results obtained with feeding of advanced stage larvae with neem seed kernel and leaf extracts (methanol and chloroform:methanol as solvents) showed a similar trend as observed for early stage larvae. However, the extracts against advanced stage larvae were less effective compared to the early stage larvae (Tables 5−8).

The data (Table 1) indicate that maximum prolonged larval duration (27.6 days) of *H. armigera* was observed at 7.5% concentration when early stage larvae were fed on food treated with neem seed kernels (methanol extract) as compared to control (17.7 days) and all the remaining concentrations also significantly prolonged the larval duration compared to the control. Feeding of early stage larvae with neem seed kernels (chloroform:methanol) extract prolonged the larval duration up to 27.4 days at 1.0% concentration compared to 17.8 days in control (Table 2). When early stage larvae were fed on neem leaf extract (methanol extract), the maximum larval duration (25.8 days) was observed at 7.5% concentration and all the concentrations significantly prolonged the larval duration as compared to control

Table 2. Effect of Neem (*A. indica*) Seed Kernel Extract (Chloroform:Methanol) on the Different Biological Parameters of *H. armigera* When Fed on Treated Food during Early Stage[a]

stage of insect	no. of insects (*n*)	concentration of neem seek kernel extract 0.1%	0.5%	1.0%	control (untreated)	CD ($P < 0.05$)
larval mortality (%)	100	43.0 (40.98)	70.0 (56.80)	95.0 (77.21)	8.0 (16.27)	(5.61)
larval duration (days)	100	20.6	22.9	27.4	17.8	0.6
larval weight (mg/larva)	30	406.3	378.2	*b*	453.4	32.4
prepupal mortality (%)	100	2.0 (5.17)	3.0 (7.75)	5.0 (11.44)	2.0 (5.17)	(1.74)
prepupal duration (days)	10	2.3	2.4	*c*	2.1	0.2
pupal duration (days)	20	14.7	16.8	*d*	11.5	1.2
adult emergence (%)	100	46.0 (42.71)	27.0 (31.28)	*d*	84.0 (66.44)	(5.28)
adult deformity (%)	100	2.0 (5.17)	5.0 (11.44)	*d*	2.0 (5.17)	(1.58)
adult longevity (days)						
(i) male moth	10	11.4	10.1	*d*	14.7	1.0
(ii) female moth	10	12.4	10.8	*d*	16.3	1.1
fecundity/female	10	534.0	485.0	*d*	592.0	39.6
egg fertility (%)	500	71.8 (57.94)	60.6 (51.13)	*d*	75.4 (60.29)	(4.06)

[a] Figures in parentheses are angular transformed values. CD denotes critical difference. 4−5-day old larvae were fed on neem seed kernels extract treated food for 48 h. [b] Observations could not be recorded as only 5% larva survived after the treatment. [c] Observations could not be recorded as all the individuals died in prepupal stage. [d] Observations could not be recorded as the insect did not reach the adult stage.

Table 3. Effect of Neem (*A. indica*) Leaf Extract (Methanol Extract) on the Different Biological Parameters of *H. armigera* When Fed on Treated Food during Early Stage[a]

stage of insect	no. of insects (*n*)	concentration of neem leaf extract 0.5%	2.5%	5.0%	7.5%	control (untreated)	CD ($P < 0.05$)
larval mortality (%)	100	11.0 (19.30)	35.0 (36.27)	55.0 (47.87)	70.0 (56.79)	9.0 (17.35)	(5.10)
larval duration (days)	100	18.4	20.1	22.1	25.8	17.7	0.5
larval weight (mg/larva)	30	431.2	408.5	393.2	382.4	445.8	32.4
prepupal mortality (%)	100	2.0 (5.17)	2.0 (5.17)	4.0 (10.33)	4.0 (10.33)	2.0 (5.17)	(1.89)
prepupal duration (days)	10	2.2	2.3	2.4	2.5	2.1	0.2
pupal duration (days)	10	11.6	12.3	15.2	16.6	11.4	1.3
adult emergence (%)	100	79.0 (62.74)	56.0 (48.45)	35.0 (36.27)	27.0 (31.28)	83.0 (65.67)	(4.58)
adult deformity (%)	100	2.0 (5.17)	2.0 (5.17)	2.0 (5.17)	4.0 (10.33)	2.0 (5.17)	(1.60)
adult longevity (days)							
(i) male moth	10	14.5	13.1	10.4	9.7	15.2	0.7
(ii) female moth	10	15.6	14.2	13.5	12.4	16.5	0.8
fecundity/female	10	543.4	502.0	468.2	448.0	556.8	35.4
egg fertility (%)	500	72.8 (58.58)	71.0 (57.44)	68.8 (56.05)	68.0 (55.57)	75.0 (60.02)	(3.42)

[a] 4−5-day old larvae were fed on neem leaves extract treated food for 48 h. Figures in parentheses are angular transformed values. CD denotes critical difference.

Table 4. Effect of Neem (*A. indica*) Leaf Extract (Chloroform:Methanol) on the Different Biological Parameters of *H. armigera* When Fed on Treated Food during Early Stage[a]

stage of insect	no. of insects (*n*)	concentration of neem leaf extract 0.1%	0.5%	1.0%	control (untreated)	CD ($P < 0.05$)
larval mortality (%)	100	32.0 (34.44)	53.0 (46.72)	72.0 (58.06)	8.0 (16.27)	(4.92)
larval duration (days)	100	20.4	21.7	24.4	17.8	0.6
larval weight (mg/larva)	25	415.6	390.0	358.2	453.4	29.2
prepupal mortality (%)	100	2.0 (5.17)	3.0 (7.75)	4.0 (10.33)	2.0 (5.17)	(1.60)
prepupal duration (days)	20	2.2	2.3	2.5	2.1	0.2
pupal duration (days)	20	13.3	14.4	15.5	11.5	1.1
adult emergence (%)	100	54.0 (47.30)	36.0 (36.87)	26.0 (30.63)	84.0 (66.44)	(4.65)
adult deformity (%)	100	2.0 (5.17)	3.0 (7.75)	4.0 (10.33)	2.0 (5.17)	(1.28)
adult longevity (days)						
(i) male moth	10	11.9	11.5	10.6	14.7	0.8
(ii) female moth	10	13.1	12.5	11.4	16.3	1.0
fecundity/female	10	545.4	510.0	495.0	592.0	42.6
egg fertility (%)	500	72.8 (58.58)	63.6 (52.90)	62.8 (52.43)	75.4 (60.29)	(3.96)

[a] 4−5-day old larvae were feed on neem leaf extract treated food for 48 h. Figures in parentheses are angular transformed values. CD denotes critical difference.

(Table 3). Feeding of early stage larvae of *H. armigera* with chloroform:methanol (9:1) extract of neem leaves prolonged larval duration up to 24.4 days at 1.0% concentration (Table 4). Maximum larval durations, *viz.* 21.8 and 23.0 days, were observed when advanced stage larvae were fed on food treated with neem seed kernels extracted in methanol and chloroform:methanol (9:1), respectively. The larval durations were 17.1 and 17.5 days in control (Tables 5 and 6). A similar trend was observed when advanced stage larvae were fed on neem leaves extracts but the results were not as encouraging as in case of neem seed kernel extracts (Tables 7 and 8).

The results on larval weight indicate that there was a decrease in weight of *H. armigera* larvae with the increase in concentration of various extracts (Tables 1−8). Feeding of early stage larvae with different concentrations of methanol extract of neem seed kernels and leaves indicates that reduction in larval weight at 0.5% concentration did not differ significantly with

Table 5. Effect of Neem (*A. indica*) Seed Kernel Extract (Methanol Extract) on the Different Biological Parameters of *H. armigera* When Fed on Treated Food during Advanced Stage[a]

stage of insect	no. of insects (*n*)	concentration of neem seed kernel extract 0.5%	2.5%	5.0%	7.5%	control (untreated)	CD ($P < 0.05$)
larval mortality (%)	100	9.0 (17.35)	19.0 (25.81)	34.0 (35.65)	46.0 (42.71)	6.0 (14.10)	(4.38)
larval duration (days)	100	17.6	18.7	20.4	21.8	17.1	0.5
larval weight (mg/larva)	50	442.2	430.5	410.2	392.4	460.2	35.4
prepupal mortality (%)	100	2.0 (5.17)	2.0 (5.17)	3.0 (7.75)	3.0 (7.75)	2.0 (5.17)	(NS)
prepupal duration (days)	50	2.3	2.4	2.4	2.5	2.2	NS
pupal duration (days)	50	10.7	11.6	13.8	15.5	10.2	1.4
adult emergence (%)	100	80.0 (63.44)	70.0 (56.80)	60.0 (50.78)	49.0 (44.44)	85.0 (66.44)	(4.20)
adult deformity (%)	100	2.0 (5.17)	2.0 (5.17)	3.0 (7.75)	3.0 (7.75)	2.0 (5.17)	(NS)
adult longevity (days)							
(i) male moth	20	14.7	14.6	10.5	9.9	15.4	0.9
(ii) female moth	20	17.0	16.8	14.5	12.4	17.7	1.0
fecundity/female	20	592.4	586.2	543.4	506.4	614.0	38.8
egg fertility (%)	500	69.6 (56.55)	68.0 (55.57)	65.6 (54.10)	62.0 (51.95)	72.8 (58.58)	(3.92)

[a] 10−11-day old larvae were fed on neem seed kernel extract treated food for 48 h. Figures in parentheses are angular transformed values. CD denotes critical difference. NS denotes nonsignificant.

Table 6. Effect of Neem (*A. indica*) Seed Kernel Extract (Chloroform:Methanol Extract) on the Different Biological Parameters of *H. armigera* When Fed on Treated Food during Advanced Stage[a]

stage of insect	no. of insects (*n*)	concentration of neem seed kernel extract 0.1%	0.5%	1.0%	control (untreated)	CD ($P < 0.05$)
larval mortality (%)	100	10.0 (18.43)	37.0 (37.45)	51.0 (45.48)	7.0 (15.18)	(4.50)
larval duration (days)	100	18.1	19.9	23.0	17.5	0.8
larval weight (mg/larva)	45	430.1	402.6	382.8	450.2	32.4
prepupal mortality (%)	100	2.0 (5.17)	2.0 (5.17)	3.0 (7.75)	2.0 (5.17)	(NS)
prepupal duration (days)	45	2.2 2.4	2.4 2.2	NS		
pupal duration (days)	45	11.6	13.5	15.2	11.2	1.3
adult emergence (%)	100	74.0 (59.35)	53.0 (46.72)	40.0 (39.23)	86.0 (68.05)	(5.02)
adult deformity (%)	100	2.0 (5.17)	3.0 (7.75)	4.0 (10.33)	2.0 (5.17)	(1.02)
adult longevity (days)						
(i) male moth	15	14.0	12.6	10.6	14.9	1.2
(ii) female moth	15	15.6	13.2	11.5	16.6	1.1
fecundity/female	15	515.8	480.0	430.0	532.4	34.7
egg fertility (%)	500	72.4 (58.33)	68.2 (55.68)	63.2 (52.67)	76.6 (61.08)	(4.06)

[a] 10−11-day old larvae were fed on neem seed kernel extract treated food for 48 h. Figures in parentheses are angular transformed values. CD denotes critical difference. NS denotes nonsignificant.

control (Table 1). Similarly, the weight of advanced stage larvae fed on food treated with neem seed kernel and leaf extracts (methanol) at 0.5% and 2.5% concentrations also did not differ significantly with control (Table 5), thereby indicating a poor effect on weight of advanced stage larvae than early stage larvae. Larval weight of *H. armigera* at 0.1% concentration in chloroform:methanol (9:1) extract (neem seed kernels and leaves) also did not differ significantly with control in advanced stage larvae (Table 6). Observations could not be recorded at 1.0% concentration in chloroform:methanol (9:1) extract, when early stage larvae were fed on food treated with neem seed kernel extracts due to very high (95.0%) larval mortality (Table 2).

Effect of Neem Extracts on Prepupal and Pupal Stages. The perusal of data presented in Table 1 indicates that maximum prepupal mortality (6.0%) of *H. armigera* was observed at 7.5% concentration when early stage larvae were fed on food sprayed with neem seed kernels extract (methanol extract). Prepupal mortality did not differ significantly with control at lowest concentration (0.5%). Feeding of early stage larvae with chloroform:methanol (9:1) extract (neem seed kernels) indicates that maximum prepupal mortality (5.0%) was observed at 1.0% concentration and all the individuals died in prepupal stage, while the lowest concentration (0.1%) did not differ significantly with control (Table 2). Feeding of larvae during early stage on methanol extract of neem leaves at different concentrations caused maximum prepupal mortality up to 4.0%, and the mortality at lower concentrations (0.5 and 2.5%) was nonsignificant with control (Table 3). Feeding of early stage larvae with neem leaves extracted in chloroform:methanol (9:1) caused maximum (4.0%) mortality at 1.0% concentration, and the lowest concentration (0.1%) did not differ significantly as compared to control (Table 4). Maximum prolongation of prepupal duration (2.8 days) was observed at 7.5% concentration when early stage larvae were fed on methanol extract (neem seed kernels). The lower concentrations (0.5% and 2.5%) did not prolong the prepupal duration significantly compared to the control (Table 1). When early stage larvae were fed on food treated with chloroform: methanol (9:1) extract (neem seed kernels) for 48 h, observations at 1.0% concentration could not be recorded because all of the population died in prepupal stage (Table 2). Maximum prepupal duration (2.5 days) was observed at 7.5% concentration when early stage larvae were fed on treated food with neem leaf extract (methanol) for 48 h at different concentrations as compared to 2.1 days in control (Table 3), and the lower doses (0.5% and 2.5%) did not prolong the prepupal duration significantly ($P < 0.05$) as compared to control. Similarly, the lower concentrations (0.1% and 0.5%) in chloroform: methanol (9:1) extract (neem leaves) did not prolong the prepupal duration significantly ($P < 0.05$) compared with control (Table 4). Feeding of advanced stage larvae with different concentrations of neem seed kernels and leaves extracted in methanol and chloroform:methanol (9:1) also did not show marked influence on the mortality and duration of prepupae (Tables 5−8).

Pupal duration of *H. armigera* was prolonged pro-

Table 7. Effect of Neem (*A. indica*) Leaf Extract (Methanol Extract) on the Different Biological Parameters of *H. armigera* When Fed on Treated Food during Advanced Stage[a]

stage of insect	no. of insects (*n*)	concentration of neem leaf extract 0.5%	2.5%	5.0%	7.5%	control (untreated)	CD ($P < 0.05$)
larval mortality (%)	100	8.0 (16.27)	10.0 (18.43)	18.0 (25.01)	27.0 (31.30)	6.0 (14.10)	(4.24)
larval duration (days)	100	17.5	18.1	20.1	20.3	17.1	0.4
larval weight (mg/larva)	70	432.2	422.3	410.8	402.7	445.8	30.8
prepual mortality (%)	100	2.0 (5.17)	3.0 (5.17)	2.0 (5.17)	3.0 (7.75)	2.0 (5.17)	(NS)
prepupal duration (days)	50	2.3	2.3	2.3	2.4	2.2	NS
pupal duration (days)	50	10.5	10.8	12.6	13.4	10.2	1.4
adult emergence (%)	100	82.0 (64.91)	79.0 (62.74)	72.0 (58.06)	64.0 (53.14)	85.0 (67.24)	(4.10)
adult deformity (%)	100	2.0 (5.17)	2.0 (5.17)	2.0 (5.17)	2.0 (5.17)	2.0 (5.17)	(NS)
adult longevity (days)							
(i) male moth	20	15.0	14.8	12.5	11.4	15.4	1.0
(ii) female moth	20	17.3	16.9	15.4	13.6	17.7	1.1
fecundity/female	20	612.0	593.0	561.8	529.0	614.0	30.4
egg fertility (%)	500	70.4 (57.06)	69.8 (56.67)	68.0 (55.56)	64.4 (53.38)	72.8 (58.58)	(3.98)

[a] 10−11-day old larvae were fed on neem leaf extract treated food for 48 h. Figures in parentheses are angular transformed values. CD denotes critical difference. NS denotes non-significant.

Table 8. Effect of Neem (*A. indica*) Leaf Extract (Chloroform:Methanol Extract) on the Different Biological Parameters of *H. armigera* When Fed on Treated Food during Advanced Stage[a]

stage of insect	no. of insects (*n*)	concentration of neem leaves extract 0.1%	0.5%	1.0%	control (untreated)	CD ($P < 0.05$)
larval mortaility (%)	100	14.0 (21.92)	27.0 (31.30)	38.0 (38.05)	7.0 (15.18)	(4.18)
larval duration (days)	100	17.9	19.6	21.5	17.5	0.7
larval weight (mg/larva)	60	432.8	417.6	402.8	450.2	30.6
prepupal mortality (%)	100	2.0 (5.17)	2.0 (5.17)	2.0 (5.17)	2.0 (5.17)	(NS)
prepupal duration (days)	45	2.2	2.3	2.4	2.2	NS
pupal duration (days)	45	11.5	12.6	13.4	11.2	1.2
adult emergence (%)	100	78.0 (62.04)	63.0 (52.54)	51.0 (45.57)	86.0 (68.05)	(4.94)
adult deformity (%)	100	2.0 (5.17)	2.0 (5.17)	2.0 (5.17)	2.0 (5.17)	(NS)
adult longevity (days)						
(i) male moth	15	14.6	12.7	11.5	14.9	1.1
(ii) female moth	15	15.5	13.8	12.7	16.6	1.2
fecundity/female	15	528.2	498.0	460.0	532.4	39.2
egg fertility (%)	500	74.0 (59.36)	70.8 (57.30)	67.0 (54.96)	76.6 (61.09)	(4.02)

[a] 10−11-day old larvae were fed on neem leaves extract food for 48 h. Figures in parentheses are angular transformed values. CD denotes critical difference. NS denotes nonsignificant.

gressively with the increase in concentration of neem seed kernels and leaves extracted in methanol and chloroform:methanol (Tables 1−8). Pupal duration of *H. armigera* when early stage larvae were fed on food treated with methanol extract (neem seed kernels) at 7.5% concentration could not be recorded because the insect did not reach the adult stage (Table 1). Maximum pupal duration (16.4 days) during early stage larval feeding was observed at 5.0% concentration in methanol extract (neem seed kernels), and all of the concentrations significantly increased the pupal duration compared to the control (Table 1). Feeding of early stage larvae with chloroform:methanol (9:1) extract (neem seed kernels) also caused a significant increase in pupal duration with the increase in concentration and similarly at highest concentration (1.0%), observations could not be recorded because the insect did not reach the adult stage (Table 2). Feeding of early stage larvae on food treated with neem leaves extract (methanol) at different concentrations did not prolong the pupal duration at lower concentrations (0.5% and 2.5%) significantly as compared to control (Table 3) but in the case of chloroform:methanol (9:1) extract, all the concentrations significantly prolonged the pupal duration as compared to control (Table 4). Feeding of advanced stage larvae on food treated with neem seed kernels extract did not prolong the pupal duration of *H. armigera* at 0.5% and 2.5% concentrations in methanol extract (Table 5) and at 0.1% in chloroform:methanol (9:1) extract (Table 6) which did not differ significantly with control. When advanced stage larvae were fed on neem leaves extracted in methanol, the pupal duration did not differ significantly ($P < 0.05$) and 0.5% and 2.5% concentrations (Table 7), while in chloroform:methanol (9:1) extract the pupal duration of *H. armigera* did not differ significantly with control at 0.1% concentration (Table 8).

Effect of Neem Extracts on Adult Stage. The perusal of the data (Tables 1−8) indicate that with the increase in concentration of extracts of kernels and leaves (methanol and chloroform:methanol extracts), there were decreases in adult emergence, longevity of male and female moths, and fecundity and egg fertility; however, there was a slight increase in adult deformity with increase in concentration in both types of extracts and plant materials. When early stage larvae were fed on food treated with methanol extract of neem seed kernels for 48 h, the adult emergence at 7.5% concentration was recorded to be zero compared to 83.0% in control (Table 1), and all the concentrations significantly ($P < 0.05$) reduced the adult emergence compared to control. In chloroform:methanol (9:1) extract (neem seed kernels) at 1.0% concentration, the population did not reach the adult stage because all of the individuals died up to prepupal stage (Table 2). Feeding of larvae on food treated with neem leaf extract showed a similar trend as in case of neem seed kernels extract on the adult emergence, but the extracts were not as effective as in latter (Tables 3 and 4). The adult emergence of advanced stage larval feeding was also at a low level compared to early stage larval feeding (Tables 5−8). During early stage larval feeding, the observations on adult longevity and fecundity and egg fertility could not be recorded in neem seed kernel extract (methanol

extract) at 7.5% concentration because the population did not reach the adult stage (Table 1). Similarly, in chloroform:methanol (9:1) extract also at 1.0% concentration, observations could not be recorded because the population did not reach the adult stage and all of the individuals died before the prepupal stage (Table 2). The data on adult longevity indicate that longevity of male and female moths was significantly reduced at all concentrations compared to control in methanol and chloroform:methanol (9:1) extracts of neem seed kernels and leaves at early stage larval feeding (Tables 1−4). However, feeding of advanced stage larvae on food treated with neem extracts did not reduce the longevity of moths at lowest concentrations and also did not differ significantly with control (Tables 5−8). Similar results were observed with regard to fecundity and egg fertility in both types of extracts.

From these results it can be inferred that with the use of extracts from neem seed kernels and leaves, all the biological parameters were adversely affected. The larval duration, larval mortality, prepupal duration, prepupal mortality, and pupal duration increased with the increase in concentration of extracts. However, the larval weight, adult emergence, adult longevity, and fecundity and egg fertility were found to decrease with the increase in concentration of extracts. These observations were made in case of early as well as advanced stage larval feeding, though the effect was more pronounced in the former stage. The variations were narrow in advanced stage and also the lowest concentration did not differ significantly with control in all the parameters. Chloroform:methanol (9:1) extract was more effective than methanol extract. Neem seed kernel extract depicted more efficacy than the leaf extract.

DISCUSSION

The data presented in Tables 1−8 indicate that different biological parameters of *H. armigera* were influenced by the neem extracts to varying levels. Prolonged larval period and mortality of the insect in various stages indicated antifeeding and insecticidal properties of neem extracts. The differences in the efficacy of methanol and chloroform:methanol (9:1) extracts could be attributed to the phenomenon of polarity. Methanol extract was less effective than chloroform:methanol extract. Methanol being highly polar (less polar than water) would have also extracted many inactive polar substances, such as sugars, tannins, and others, thus diluting the active principle of extract (methanol extract). In chloroform:methanol (9:1) extract, the chloroform's lower polarity precluded the extraction of these inactive substances. Hence, chloroform:methanol (9:1) had a broader spectrum of polarity, extracting a wider range of active substances, thus increasing their effectiveness at lower concentrations. Jhansi and Singh (1996) also observed higher efficacy of chloroform neem seed kernel extract compared to methanol extract against *H. armigera*. The growth-disrupting properties of neem extracts were reported to increase 50-fold depending upon the type of solvents used (Feuerhake and Schmutterer, 1982). The results of the present investigations further show that neem seed kernel extract had more insecticidal properties than neem leaf extract against *H. armigera*. This could be attributed to the higher content of azadirachtin in neem seed kernels compared to the leaves (Singh, 1987). The presence of azadirachtin in neem seed kernel extract was reported to interfere with neuroendocrine system of insects by reducing and delaying the titers of morphogenic hormones, *viz*. ecdysone and juvenile hormones (Singh, 1993b). In the present investigations, early stage larvae seem to be affected more adversely by the feeding of neem extracts through treated food than advanced stage larvae. Similar observations were recorded by Gujar and Mehrotra (1983) against *Spodoptera litura*, in which feeding of last stage larvae with 1 and 5 μg of azadirachtin-treated castor leaves had no adverse effect on larvae or subsequent stages. Therefore, it may be concluded that early stage larvae are more sensitive to neem extracts than advanced stage larvae, thereby leading to age specific differences in impact on biological parameters.

Chloroform:methanol (9:1) extract of neem seed kernels was found to exhibit more insecticidal property, which might be due to the higher azadirachtin concentration as observed by Jhansi (1988) and Singh (1993b). Barnby and Klocke (1987) reported that by addition of azadirachtin (0.03 ppm) in an artificial diet of *H. virescens* caused a significant reduction in weight gained by larvae. Similarly, the pupal period of *S. litura* was extended by 2−4 days (13−15 days) compared to control (11 days) when pupae were obtained from azadirachtin-injected grown-up larvae (Mohan Raj, 1990). Schmutterer *et al*. (1983) also observed that different fractions of neem seed extracts fed to *Mythimna separata* exhibited high pupal mortality and lower adult emergence. This indicated the effectiveness of neem extracts against several noctuids including *H. armigera*. However, the effectiveness of neem products against *H. armigera* is dependent on the type of solvents used for extraction, plant parts used (kernels and leaves), and the stage at which the insect is treated.

ACKNOWLEDGMENT

We are thankful to Dr. J. S. Naresh, Professor and Head, Department of Entomology, and Dr. O. P. Malik, Professor of Chemistry, CCS Haryana Agricultural University, for providing laboratory facilities.

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Received for review January 24, 1997. Revised manuscript received June 3, 1997. Accepted June 4, 1997.[⊗]

JF9700539

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1997.