EFFECTS OF NEEM EXTRACTS ON THE FEEDING, SURVIVAL, LONGEVITY AND FECUNDITY OF AFRICAN BOLLWORM, *Helicoverpa armigera* (HUBNER) (LEPIDOPTERA: NOCTUIDAE) ON COTTON

BY

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ABBREVIATIONS

ABW	African Bollworm
Aza.	Azadirachtin
CABI	Center for Agricultural and Bioscience International
CPB	Colorado Potato Beetle
CPPC	The Caribbean Plant Protection Commission
EARO	Ethiopian Agricultural Research Organization
E.C.	Emulsifible Concentrate
EIAR	Ethiopian Institute of Agricultural Research
HAT	Hours After Treatment
IPM	Integrated Pest Management
LPI	Larval Pupal Intermediates
MOA	Ministry of Agriculture
MSFD	Ministry of State Farm Development

ABSTRACT

The effects of neem oil (Nimbecidine 0.03% Aza) and water extracts of neem seed and leaf were studied each at three different concentration levels on various developmental stages of African bollworm both under laboratory and field conditions at Werer Agricultural Research Center. The laboratory study comprised of square dip and larval immersion experiments, both replicated three times using Completely Randomized Design. Whereas the field experiment was conducted using a Randomized Complete Block Design in three replications to verify neem extracts as a botanical insecticide under field condition. Mean mortality of Helicoverpa armigera larvae ranged from 4.67-8.00 in square dip experiment and 3.67-6.00 in larval immersion experiment. While control mortality was 0.33 & 1.00 in square dip and larval immersion experiment, respectively. Lower number of damaged squares, ranging from 1.00-4.67 were observed in square dip experiment from different concentration levels of Nimbecidine and water extracts of neem seed and leaf. Significantly lower mean weight of artificial diet consumption (0.18-0.68 & 0.43-0.78g) was recorded at 6 & 9 DAT, respectively from various concentration levels of Nimbecidine and water extracts of neem seed and leaf in larval immersion experiment. Moreover, higher percent weight loss of ABW larvae, lower pupal weight, relatively extended duration at the larval stage, higher number of larval-pupal intermediates, lower number of eggs of which the majority are non-fertile as compared to the control were recorded from the various concentration levels of water extracts of neem seed and leaf and Nimbecidine in square dip and larval immersion experiment. However, results of field experiment have indicated that Nimbecidine and water extracts of neem seed and leaf have non-significant effect on the survival of ABW larvae, damaged flowers, squares and bolls in all rounds of spray application and in combined analysis. Moreover, no yield advantage was obtained from neem extracts.

1. INTRODUCTION

In Ethiopia cotton is the most important fiber crop grown for fiber and oil. It is grown under irrigated and rainfed conditions by the State Farms and private commercial farmers (EARO, 2001). Despite its importance, the production and productivity of cotton is greatly affected by biotic and abiotic factors. Insect pests are one of the most important factors affecting the production of cotton both in quality and quantity.

Survey results revealed that more than 68 species of insect and mite pests have been recorded on cotton in major cotton growing areas of Ethiopia (Alemayehu and Ababu, 1985). The cotton aphid, *Aphis gossypii* (Glover); the cotton white fly, *Bemisia tabaci* (Gennadius)*;* African bollworm, *Helicoverpa armigera* (Hubner)*;* Pink bollworm, *Pectinophora gosspiella* (Saunders)*;* Sudan bollworm, *Diaparopsis watersi* (Roths)*;* Spiny bollworm, *Erias insulana* (Boisduval); the cotton jassid, *Empoasca lybica* (de Berg)*;* and Red spider mite, *Teranychuus cinnabrinus* (Boisduval) constitute the major pest species of cotton (Tsedeke, 1982; Alemayehu and Ababu, 1985; Woktole, 1996). Among these, the bollworms (African, Pink, Sudan, and Spiny) caused 36-60% yield loss in which *H. armigera* is the dominant bollworm species (Tsedeke, 1982; Waktole, 1996).

African bollworm is a polyphagous insect attacking a wide range of crop including legumes, sorghum, cotton, tomato, pepper, sunflower, safflower, flax and niger seed. *Hibiscus spp, Baleria spp, Guziotia scabra, Amarantus spp* and *Gynandropsis gynandra* were recorded as alternative hosts (Tebkew *et al.*, 2002; Tsedeke, 1982; Waktole, 1996).

For decades, wide ranges of different insecticides and acaricides have been used for the control of cotton insect and mite pests in general and African bollworm in particular. These include chlorinated hydrocarbons, organophosphates, carbamates and synthetic pyrethroids (Tsedeke, 1982).

Ethiopia's annual pesticide purchase exceeds 3000 metric tones, estimated at nearly USD 20 million of which insecticides constitute about 71% of the total annual purchase. About 63.5% of all pesticides in Ministry of State Farm Development (MSFD) are used for the control of cotton pests (Tsedeke, 1997).

Synthetic pesticides have been in use for more than 50 years and have resulted in fast, economical and effective pest control. But, their excessive use has adverse effects such as pesticide resistance, resurgence of new pests, side effects on non-target organisms, and other environmental risks (Raguraman and Singh, 1999).

Though currently endosulfan is the major insecticide used for the control of cotton bollworm in almost all commercial farms in Ethiopia, a study by Geremew & Surachate (2005) has clearly indicated that *H. armigera* population from Arbaminch area has shown development of resistance to endosulfan. They also reported that populations from other locations have shown signs of resistance to endosulfan. Geremew & Surachate (2005) have finally suggested that the application of endosulfan must be limited to one time per cropping season in order to prolong the active life of the insecticide. Moreover, they have

emphasized designing of alternative control methods and /or screening of alternative insecticides for future use.

The use of more biodegradable pest control materials with greater selectivity might help avoid the disadvantage caused by the use of synthetic pesticides (Raguraman and Singh, 1999). To this end, recently an interest in alternatives to synthetic pesticides has greatly increased (Stark *et al.*, 1992).

Azadirachtin a tetrano triterpenoid, is the most promising insecticidal compound found in neem, *Azadirachta indica* A.Juss seeds and leaves (Butterworth and Morgan, 1968; Spollen and Isman, 1996). Among the wider range of plants, neem, derivatives have shown great potential in controlling insect pests.

More than 450 species of insects have been tested with neem products in the world and 413 of these are susceptible to neem used at different concentrations (Tebkew *et al.*, 2002). It has been reported by various scientists that neem products have several biological effects on insects including antifeedant, insect growth regulator, repellency and they are also less toxic to natural enemies of insect pests in contrary to synthetic pesticides. Moreover, they are safer to adult stages of numerous beneficial insects and eggs of many predators (Zehnder and Warthen, 1988; Saxena, 1989).

For the last three to four decades various researchers in the Crop Protection Department of Cotton Research Project have been mainly engaged in insecticide screening research activities along with some preliminary research on cultural and biological control measures. However, research in the area of botanical control measures targeting *H. armigera* on cotton remained untouched, which needs special focus in order to increase the number of component control measures that will be utilized in the Integrated Pest Management Program of *H. armigera* on cotton.

Therefore, this study was proposed with the following objectives:

General objective: To evaluate the effect of neem extracts from different plant parts (seeds and leaves) on *Helicoverpa armigera.*

Specific objectives:

1. To evaluate the effect of neem extracts on the survival, longevity and fecundity of African bollworm.
2. To investigate the influence of neem extracts on the feeding and growth of African bollworm.
3. To verify neem extracts as a botanical pesticide under field condition.

2. LITERATURE REVIEW

2.1 Cotton

The genus *Gossypium* contains 49 species distributed throughout most tropical and subtropical regions of the world. Centers of diversity include northwestern Australia, northeastern Africa, the Arabian Peninsula, and western and northern Mexico. Four of these 49 species are domesticated: two Old World diploids *Gossypium herbaceum* and *Gossypium arboreum*, and two New World tetraploids, *Gossypium hirsutum* and *Gossypium barbadanse.* The genus contains an amazing amount of diversity, ranging from herbaceous perennials to small trees (Smith and Cothren, 1999).

Cotton is one of the most important oil seed and fiber crops grown in more than 70 countries in the world, and plays an important role in the global economy. No crop computes with it in the potential of value added and processing. Cotton seed was crushed for cooking oil and a cake used for livestock feed. The crop helps farmers to earn a reasonable and reliable income from the sale of seed cotton (Munro, 1987).

2.1.1 Cotton production

Cotton is generally regarded as a tropical crop, but two-thirds of the world production comes from north of latitude 30^{o}N, where the three major producers, USA, the former USSR and China are located. Small quantities of cotton are grown north of 40^{o}N in Bulgaria, Russia, China and Korea, but the summers are too short for anything but varieties, which mature very quickly. About 10% of the total crop comes from the

southern hemisphere, ripening in May to July, while the remaining 25% comes from the northern tropics up to 30^0N, mostly ripening in December to February. Outside the tropical belt, temperature rather than rainfall determines the cropping cycle, and north of 30^0N crops can only grow in the summer months, ripening in September to November (Munro, 1987).

The primitive cultivated forms of species of *Gossypium herbaceum* (L), race acerifolium is found in Ethiopia. The old perennial cultivated species are grown in the back yards of few farmers in the South and South Western parts of the country (Bedada, 1982). The only variety widely grown in Ethiopia by small farmers and large-scale producers across the country is *Gossypium hirsutum* (L).

In Ethiopia, cotton production has a long history. Cotton fiber was used to produce different clothes by traditional weavers and large textile factories. It is also important in fetching foreign currency by exporting. Cotton seed is an important raw material for oil factories and the by-product of the oil factories is used as feed for livestock (MOA, 2003).

Cotton is produced commercially by the state farms and private farmers in different agro-ecologies ranging in altitude from 300 to 1800 m.a.s.l. (EARO, 2001; MOA, 2003). The major cotton growing areas include Awash Rift Valley (Upper, Middle and Lower), Southern Rift Valley (Arbaminch, Sille, Abaya, Woyto and Omorate), Gambella in the

South West, and in the Northwest Ethiopia (Belles, Humera and Metema) (MOA, 2003). The total area under cotton production is not known exactly, however the area of cotton growing in 1993/94 was estimated to be 75,900 ha out of which 56,000 ha are held by the peasant sector, 8,500 ha by private farmers and 11,400 ha by State Farms (USAID, 1994). A report by Ministry of Agriculture indicated that around 100, 000 hectare is held by small scale farmers and investors and currently there are five government State Farms (Tandaho, Middle Awash, Upper Awash, North Omo and Abobo) engaged in cotton production (MOA, 2003).

The national yield average is very low, only 11q/ha. The average yield ranged from 20-30 q/ha on irrigated farms, 15-20 under rainfed condition while the potential in research farms is 35-40 q/ha (EARO, 2000; MOA, 2003).

2.1.2 Insects and mite pests of cotton

In Ethiopia, insect and mite pests are major constraints in the production of fiber crops, particularly cotton. So far more than 68 species of insects and mites were recorded on cotton. Of these bollworms (*Helicoverpa. armigera* (Hubner), *Pectinophora gossypiella* (Saunders), *Erias insulana* (Boisduval) *and Diparopsis watersi* (Roths)) and Aphids, *Aphis gossypii* (Glover) are the major pests of cotton (Alemayehu and Ababu, 1985). However, since 1980/81-crop season the cotton whitefly, *Bemisia tabaci* (Gennadius) became the key pest of cotton especially in the Awash Valley. Though cotton jassids, *Empoasca lybica* (de Berg) once became important cotton pest, the use of resistant

variety reduced its status to a minor pest. The red spider mite, *Tetranychus cinnabarinus* (Boisduval) can be major pest of late planted cotton. Though easily controlled by seed dressing insecticides, flea beetles are important seedling pests (Tsedeke, 1982). Currently cotton production in North Western part of Ethiopia (Humera & Metema) is under great risk due to high infestation of flea beetles.

2.1.3 Control of insect pests of cotton

About 60% of the seasonal fluctuation of the cotton yields has been traced to damage by pests, which unless controlled would in many years reduce the cotton yield below the economic break, even mark and thus make cotton production unprofitable (Munro, 1987). These pests must be controlled with a high degree of efficiency by the most economic methods at our disposal. Cultural control (fertilizer application, tilling or cultivating the soil, destruction of crop residues and weeds, variation in the time of planting and harvesting, watering and flooding), varietal resistance, natural control agents (biological control agents: parasitoids, predators and pathogens), sterilization of pests by irradiation and chemicals, use of insect attractants and chemical control of cotton pests are some of the major available control measures. Among these control methods cotton producers chiefly depend on chemical control methods for it clearly works best though it has its own disadvantages (Ripper and George, 1965).

2.2 African bollworm, *Helicoverpa armigera*

Helicoverpa armigera is a highly polyphagous pest of many economically significant

crops in portions of Africa, Asia, Australia (including Oceania) and Europe (King, 1994). Because of the number of crops that this pest affects, it has many common names: scarce bordered straw worm, corn earworm, African cotton bollworm, American bollworm, and tomato fruit worm (Zhang, 1994; Begemann and Schoeman, 1999).

2.2.1 Biology and life cycle of *Helicoverpa armigera*

Adults emerge from the ground in the spring between dusk and mid night, climb vertical structures, and dry their wings for a period of 2 or more hours (King, 1994; CAB, 2003). In order to mate and lay eggs, adults typically feed on nectar. In particular, amino acids and sugars are key components of the adult diet (King, 1994; CAB, 2003). Mating occurs 1-4 days after emergence during cool, humid conditions and ceases during warm, dry conditions (King, 1994; Fowler and Lakin, 2001).

King (1994) reviewed several adult longevity studies and reported a range in adult life span of 5 to 36 days. According to Geremew and Surachate (2003) recorded adult longevity was 7.4 days. Adult longevity depends on several factors including pupal weight, food (nectar) supply, food quality (sucrose content), temperature, water availability, disease pressure, and predator activity (King, 1994).

A female may produce a maximum of 4394 eggs, but on average a female will produce 730-1,702 eggs (King, 1994; Fowler and Lakin, 2001). Eggs can be laid over 10 to 23 days (King, 1994). Oviposition begins 2-6 days after emergence, and egg laying often

occurs at night (Fowler and Lakin, 2001; CAB, 2003). Moths tend to lay eggs singly, on or near floral structures. Peak egg laying typically occurs prior to or during host flower production (King, 1994). Incubation lasts 3-14 days, depending on temperature (King, 1994; Fowler and Lakin 2001; CAB, 2003). Eggs hatch in about 3 days at 25°C, but at lower temperatures, hatching may take up to 11 days (CAB, 2003). Geremew and Surachate (2003) reported egg development period of 2-3 days.

Larvae may complete up to 7 instars, though generally there are between 5 and 7 instars (King, 1994; Fowler and Lakin, 2001). The time required to complete each larval stage varies considerably depending on host plant, temperature and other factors. In laboratory studies, the complete larval period (all instars combined) lasted between 12-36 days (Bhatt and Patel, 2001; Fowler and Lakin, 2001). During summer, larval development is completed in 14-18 days, while it may take up to 21 days in fall (CAB, 2003). First generation larvae require more time to develop (24-36 days) than the second or third generations, which are typically completed between 16-30 days and 19-26 days, respectively (CAB, 2003).

First instar larvae have a high mortality rate, most likely caused by larval movement or predators (Kyi and Zalucki, 1991). The prepupal stage lasts 1-4 days, and during this time larval activity decreases (King, 1994). Geremew and Surachate (2003) studied the biology and partial ecological life table of *Helicoverpa armigera* and reported 12-16 days of total larval period (1-6 instar).Larval development depends primarily on temperature

and secondarily on host nutritional quality (King, 1994; CAB, 2003). Before feeding on their host plant, newly hatched larvae typically consume all or part of their egg shells; larvae may then feed on leaf surfaces or floral structures, moving about the plant for a short distance before selecting a preferred feeding spot (King, 1994).

Helicoverpa armigera is particularly damaging to crops because larvae can move from plant to plant, particularly when food is scarce (King, 1994). Late-instar larvae are more damaging to the host plant due to their attraction to "full buds" (Mabbett *et al.,* 1980). "Antagonism" and "cannabalism" have been observed among older larvae on corn in situations where several eggs were deposited (King, 1994).

Once feeding is completed, larvae move between 2.5-17.5 cm below the soil surface to pupate depending on soil moisture, organic matter on the surface, and other factors (King, 1994). Depending on temperature, the pupal stage lasts between 6-33 days, unless the insect goes into diapause, in which case pupation may require several months. Geremew and Surachate (2003) reported 7-12 days of pupal development period and 48% of the pupae emerged within 9 days.

Total longevity (from egg to adult death) is 30-40 days with females generally living 2-3 days longer than males (King, 1994). Bhatt and Patel (2001) recorded a slightly longer life span of about 51 days for males and 54 days for females. Rochester (2002) reported a life span 35-75 days from egg to adult.

2.2.2 Effects of climate on *Helicoverpa armigera*

Generally, strong winds, heavy rains, or extremes in temperature negatively affect *Helicoverpa armigera* populations. Heavy rainfall and winds can decrease the population at the egg and larval stages (Karmawati and Kardinan, 1995; Fowler and Lakin, 2001). Dry seasons can adversely affect pupal development. High humidity can lead to fungal attack (Karmawati and Kardinan, 1995).

Extremely high temperatures have a negative effect on *H. armigera* (Tripathy *et al*., 1999). The optimum temperature for development from 1st instar larva to adult was 33.9°C (Twine, 1978). However, Twine (1978) reported optimal survival temperatures of 27°C for pupae and 24°C for larvae. In a laboratory study, high temperatures (above 37°C) caused pupal dormancy (Nibouche, 1998).

2.2.3 Occurrence of *Helicoverpa armigera* generations

Because *Helicoverpa armigera* exhibits overlapping generations, it can be difficult to determine the number of completed generations, but typically 2-5 generations are achieved in subtropical and temperate regions, and up to 11 generations can occur under optimal conditions, particularly in tropical areas (King, 1994; Fowler and Lakin, 2001).

In Australia, up to 7 generations can be completed in warmer regions of the country (Kirkpatrick, 1962). If larvae do not diapause, approximately 4 or 5 generations can be completed from late-September to early April, and 1-2 generations can be completed in

winter (Kirkpatrick, 1962).

In China, *H. armigera* completes 3-4 generations annually (Xiao *et al*., 2002). In eastern New Zealand coastal regions, a more temperate climate where the average summer temperature is 23.5°C, 2-3 generations are completed (Cameron *et al*., 2001). Temperature and availability of suitable host plants are the most important factors influencing the seasonality, number of generations, and the size of *H. armigera* populations (King, 1994). Population size is also influenced by the size of the previous generation, timing of adult emergence, timing of migrant arrival, and climatic conditions (King, 1994). Population size in fall serves as an indicator of the size of the spring population (Begemann and Schoeman, 1999).

2.2.4 Distribution of *Helicoverpa armigera*

Helicoverpa armigera is found in the Palearctic, Oriental, Ethiopian, and Austalian zoogeographical provinces, south of a line at approximately 52°N (IIE, 1993). This range occupied by the species includes tropical, dry, and temperate climates. The currently reported global distribution of *H. armigera* suggests that the pest may be most closely associated with deserts and xeric shrublands, Mediterranean scrub, temperate broadleaf and mixed forests, tropical and subtropical grasslands, savannas, and shrublands and tropical and subtropical moist broadleaf forest (CAB, 2000).

2.2.5 Host ranges of *Helicoverpa armigera*

African bollworm breeds on a very large number of host plants. Many of these are cultivated plants and include *Gossypium spp*. (cotton), *Betula nigra* (dura), *Lupinus bingenensis* (lubia), *Zea mays* (maize), *Lycopersicon esculentum* (tomato), *Capsicum annum* (sweet pepper), *Pisum sativum* (peas), various beans including *Abelmoschus esculentus* ('Fasulia'), *Phaseolus lunatus* (the haricot bean), cucurbits, *Medicago sativa* (alfalfa), *Citrus spp.* (citrus), *Arachis hypogaea* (groundnut), *Cajanus cajan* (pigeon pea), sunn hemp, *Nicotiana tabacum* (tobacco) and *Hibiscus esculentus* ('bamia'). The alternative hosts are various shrubs and herbaceous plants, including weeds such as 'Tabar' (Morning glory), *Ipomea cordofana*, *Acalypha segentalis* (Euphorbiaceae), *Malvastrum tricuspidatum, Nicandra physaloides, Sonchus oleraceus, Xanthium pungens, Portulaca oleracea, Tridax procumbens, Hibiscus spp, Baleria spp, Guziotia scabra, Amarantus spp, Gynandropsis gynandra*, (Tsedeke, 1982; Ripper and George, 1965; Waktole, 1996).

Tagetes erecta (African marigold) has been used as a trap crop in tomato and *Hibiscus subdariffa* (red ambadi) has been used as an intercrop in cotton to help manage *H. armigera* pest populations (Bantewad and Sarode, 2000).

2.2.6 Host preference of *Helicoverpa armigera*

Host preferences, including artificial diet, by *H. armigera* have been studied in a laboratory setting. Pigeon pea was found to be the most "suitable," followed by an

artificial diet, maize, sorghum, red ambadi, cowpea and marigold (Bantewad and Sarode, 2000). *H. armigera* prefers particular host plants and appears to follow a hierarchy in food choice when a preferred host is unavailable (Jallow and Matsumura, 2001). From laboratory studies, tobacco, maize and sunflower were categorized as most preferred hosts; soybean, cotton and lucerne were categorized as intermediate hosts; and cabbage, pigweed and linseed were least preferred (Firempong and Zalucki, 1990).

In other food preference studies, maize has been ranked as a highly preferred host while cowpea has been ranked low (Jallow and Zalucki, 1998). Feeding studies on neem have identified it as an unsuitable host plant for *H. armigera* (Ma *et al*., 2000a). *Vitis vinifera* (Grapevine) has also been identified as an unsuitable host, though an isolated case notes *H. armigera* feeding on this plant (Voros, 1996).

2.2.7 Economic importance and type of injury due to *Helicoverpa armigera*

Helicoverpa armigera is a severe economic pest in most places where it occurs (Mabbett *et al*., 1980; Bhatnagar and Khurana, 1992; CABI/EPPO, 1997; Agusti *et al*., 1999). It is an important pest of cotton, particularly in Australia and China (King, 1994). All parts of the cotton plant are vulnerable to attack. Cotton yields were reduced by 50-60% by *H. armigera* each year from 1980-1990 in China (Xiao *et al*., 2002).

In Queensland Australia, *H. armigera* damage accounted for 7% yield loss in cotton in spite of pest control costs of $800/ha in 1998 (Sequeira, 2001). In Andhra Pradesh region

of India, *H. armigera* reduced yields of seed cotton from 436 kg/ha in 1986-87 to 168 kg/ha in 1987-88 (Sekhar *et al*., 1996; Loganathan *et al*., 1999). Significant tomato crop loss also occurred in Burkina Faso, India and New Zealand, particularly in unsprayed or late season varieties (Tewari and Prasado Rao, 1987; Bouchard *et al*., 1992; Cameron *et al*., 2001). Pigeon pea and chickpea are severely damaged in India, where losses up to 90-100% in the 1992/93 and 1997/98 growing seasons have been reported. Worldwide, annual losses from this pest on chickpea are approximately 10%, equaling $300 million dollars (Mulimani and Sudheendra, 2002).

The African bollworm feeds on the reproductive parts (flowers, buds and bolls) of the cotton plant. Attack due to this pest results in reduced production both in quality and quantity. In years of short growing seasons, infestation of this pest results in reduced yield; in most seasons infestations reduced the quality of cotton and on rain fed cotton can some times destroy the whole crop. This pest can damage all stages of the plant. The leaves can be eaten, the growing points damaged or even destroyed by the young larvae, buds of all sizes and flowers are pierced and fall even if the damage is only slight. The larvae perforate the bolls and eat their contents at all stages of development, causing the young bolls to shed and older bolls, more than 20 mm in size to rot and deteriorate (Ripper and George, 1965).

Overall, the pest affects economies by reducing yields, lowering crop values, and causing market loss from quarantine restrictions (Fowler and Lakin, 2001). The pest is listed by

the European and Mediterranean Plant Protection Organization as an A2 quarantine pest and is considered a quarantine pest by the Caribbean Plant Protection Commission (CPPC) and the country of Brazil (EPPO, 2000).

2.2.8 Control of *Helicoverpa* species

Helicoverpa can breed on a very wide range of plant species, including many cultivated crops like maize, *Zea mays*; sorghum, *Sorghum bicolor*; tobacco, *Nicotina tabacum*; groundnuts, *Arachis hypogea*; cowpeas, V*igna unguiculata*; dolichos beans, *Lablab niger*; pigeon pea, *Cajanus cajan* and chick peas, *Cicer arietinum* are indeed often more attractive to this bollworm than cotton itself. These alternative host plants allow the bollworm to maintain or increase its number prior to attacking cotton or may divert the attack from cotton, depending on the relative acreage, timing and attraction of the cotton crop and the host plants. These factors have been used in retrospect to explain why *Helicoverpa* is a major serious pest in some regions than others, and why some seasons are worse than others for bollworm attack (Munro, 1987).

The use of other crops to divert egg laying from cotton is an attractive proposition, but it has not been universally successful and evidently needs careful timing if it is to be of value and not a danger to the cotton crop. Where the trap crop has to be sacrificed once its object has been achieved, this method is unlikely to be adopted by peasant farmers; but in the case of maize and sorghum this may not be necessary. In these crops the losses from bollworms attack are relatively slight, and survival of larvae is lower than it is in

cotton (Munro, 1987).

Strains that can flower prolifically over a long period can compensate for bollworm losses early in the season by producing a late crop after the first sever attack is over; such varieties have been selected for this character in South Africa (Kerkhoven, 1963). At the other extreme the developments in short season cotton in the United States avoid among other things late season attacks by boll weevils and bollworms (Watson, 1980).

Varietal differences in resistance to *Helicoverpa* species are known to exist in cotton, but they have not been considered large enough to be worth exploiting (Munro, 1987).

Attempts at biological control by release of the egg parasite, *Tricogramma luteum*, in South Africa were unsuccessful, as a high rate of parasitism was never attained (Parsons and Ulyett, 1936). Destruction of the moths by poison baits and in light tarp has been tried, but so far they have proved ineffective as a control measure, though useful in recording population change. Native predators and parasites feed extensively on *Helicoverpa* eggs and small larvae. Outbreaks of these pests are more commonly the result of insecticide treatment for other pests, which eliminate their natural enemies (Munro, 1987).

Control is most often in the form of chemical sprays, but *H. armigera* has developed resistance to many insecticides (Mabbett *et al*., 1980; Maelzer and Zalucki, 2000).

Although resistance has been documented to many insecticides, chemical control is still the primary tool used to manage these insects (Clower *et al*, 1987). Insecticide resistance management plans have been implemented in Midsouth and Texas (Plapp *et al*., 1987) to preserve insecticide efficacy against these pests. The major components of those plans are the use of agronomic practices that promote an early- maturing crop, practice rotation among insecticide classes during the season, and timely insecticide application (Munro, 1987).

2.2.9 Botanical control of insect pests

Botanical pesticides are substance of plant origin and extracted from different parts like seed, flower, leaves, stem, rhizomes, bulbs and roots. They may be crude preparation of plant parts ground to produce a dust powder or emulsion that may be used either directly or after dilution in carriers such as clay, talk, diatomaceous earth or water preparation dusts are known to be made from pyrethrum daisy flowers, cube roots (rotenone), sabadilla seeds, ryania stems or neem leaves, fruits and barks. These extracted chemicals may repel the pest insects, deter them from feeding and oviposition on the plants, and disrupt the normal developmental stages, besides acting as synergists in combination with other conventional insecticides (Ahmed and Stoll, 1996).

More than 2400 plant species around the world are known to possess pest control properties. The plant species identified belong to 235 plant families differing greatly in the pests they control or are alleged to control, the type of pest control activity they

exhibit, and their complementary uses. Using these plant species, about 2402 pests (including animal diseases) have been controlled (Ahmed and Stoll, 1996; Vyas *et al.*, 1999).

Much before the advent of synthetic organic insecticides, neem, pyrethrum, rotenone, nicotine, ryania, sabadilla and number of other lesser-known botanical pesticides were used to protect agricultural crops from ravages of insect pest in different parts of the world. However, after the advent of modern insecticide their roll in agricultural production dramatically declined, particularly in the developing countries (Dhaliwal and Arora, 2000).

2.2.9.1 Neem, *Azadirachta indica*

Neem is the member of mahogany family, Meliaceae known by the botanical name *Azadirachta indica* A.Juss. The tree is evergreen that grow up to 30m and 2.5m in grith. It is capable of regrowing from both pollarding and coppicing fastly for it is severed by a root system large enough to feed a full-grown tree. Though neem is native to Indian sub-continuant, recently it is distributed to more than 50 countries in the world (Asia, Africa, and Central America). It adapts well in climates ranging from semi-arid to semi-humid and thrives even in places with less than 500mm of rainfall per year. Neem grows equally well on poor, shallow, sandy or stony ground. Four to five years old trees can bear fruits, on average giving 30-50 kg of fruit per tree. All parts of the tree contain the effective ingredients, but are most highly concentrated in the seeds (NRC, 1992).

“Neem protects itself from a multitude of pests with a multitude of pesticidal ingredients”. The main chemical composition of neem is a mixture of 3 or 4 related compounds, which belongs to a general class of natural products called “triterpenses” specially “limonoids”. At least nine limonoids have been discovered which have the ability to block insect growth, affecting mainly species that includes some of the most destructive pests of agriculture (NRC, 1992).

Though, new limonoids are still being discovered in neem; Azadirachtin, salanin, meliantriol, and nimbin are the most known and significant components. Among the discovered limonoids, azadiracthin is the major agent battling insects. It is responsible for 90% of the effect on most pests even though it doesn’t kill insects immediately. The mechanism employed by azadiracthin are repelling and disrupting the growth and reproduction of insects. Azadiracthin is structurally similar to insect hormones called “Ecdysones” which are involved in controlling the process of metamorphosis as insects pass from larva to pupa to adult (NRC, 1992).

2.2.9.1.1 Effects of neem on insect pests and beneficials

The insect repellent (Pradham and Jotwani, 1971) and growth disrupting (Jilian *et al.*, 1988; Redfern *et al.*, 1984; Zehnder and Warthen, 1988) effects of neem seed extracts, particularly the neem seed chemical azadiracthin, are now well established and have been studied in many insect pest groups (Warthen and Uebel, 1982).

Purthi (1937) reported the repellent action of neem leaves and cake against insect pests of stored wheat. According to Jotwani and Sircat (1965), neem seed powder mixed with wheat grain provided protection from insect pests for 9-12 moths. A study by Jilani and Malik (1973) revealed that water and ethanol extracts of leaves and seeds of neem repelled the red flower beetles, *Tribolium castaneum* (Herbst); the khpra beetle, *Trogoderma granarium* (Everts); and the lesser grain borer, *Rhyzopertha dominica* (F). Extracts of neem are effective mosquito larvicides (Attri and Prasad, 1980) and inhibitors of metamorphosis of the Mediterranean fruit fly, *Ceratitis capitat*a (Wiedemenn) (Steffens and Schmutterer, 1982).

Gaaboub and Hayes (1984) investigated the effect of azadiracthin against face fly, *Musca autumnalis* (De *Geer*). When third instars were treated larval and pupal mortality increased, egg production and hatching reduced from the adults emerged. Zehnder and Warthen (1988) have reported morphogenetic defects in Colorado Potato Beetle (CPB) larvae, reduced fecundity in adults, increasing feeding inhibition, and mortality in both larvae and adults treated with the extracts of neem seed kernel.

A study on brown plant hopper, *Nilaparvata lugens*, a destructive rice pest, both under the laboratory and field condition on rice plants treated with neem oil, has shown a marked decrease in the survival and population increase (Saxena and Khan, 1985). Also, the first generation male progeny of brown plant-hopper parents caged produced few viable gametes.

Babu *et al.* (2000) reported synergistic effect of methanolic neem seed extract in cotton. Even though, the effectiveness of neem seed kernel extract alone was not high, it has delayed the metamorphosis and decreased the fecundity of African bollworm.

A study conducted by Debre Ziet Agricultural Research Center to evaluate the efficacy of some plant species in controlling *Helicoverpa armigera* in chick pea revealed that crude neem extracts collected from Melka Werer has significantly reduced percent pod damage (Tebkew *et al.*, 2002).

Another study on chick pea revealed, significantly lower pod damage due to *H. armigera* after being treated with neem seed kernel extracts as compared to the untreated plots (Sahgal and Ujagir, 1990). Similarly, the same pest was effectively controlled by neem extracts at 5 and 6%. Hence, it was concluded that neem seed kernel extracts could be used instead of highly toxic synthetic insecticides owing to its safety to beneficial insects and its affordable price (Sadawarte and Sarode, 1997).

Moreover, Lingappa *et al.* (2000) obtained high reduction in damage by *H. armigera* to fruiting bodies of cotton by using a neem based formulation containing 0.3% azadirachtin alone or in combination with *Bacillus thuringiensis* or nuclear polyhydrosis virus. Similar result was obtained on upland cotton in Australia by Ma *et al.* (2000b). As opposed to the synthetic insecticides, the botanicals used were found to be safer to the predators

including the coccinellids, chrysopids, Araneae, and Hemiptera.

Dhawan and Simloler (1995) have compared neem product of RD-9 Repin (1 and 2%), Neemark (0.5 and 0.75%) and Neemrich 20 EC (0.1 and 0.15%) with quinalphos (0.2%) as standard for their control action against young larvae of *H. armigera* in cotton. The highest concentration of Repin and Neemrich were more effective than Neemark in the crop spraying experiment (mortality levels of 70, 70, and 66.7%, respectively), but less effective than Quinalphos (mortality of 100%).

Abnormal adult emergency and death during the pre pupal stage increased with increasing neem suspension concentration in an experiment where the pupation site of *H. armigera* was treated by 0.5-6% neem seed powder on a W/W basis (Gupta *et al.*, 1998).

Botanicals decrease the chance of the occurrence of resistance due to their wide mode of actions. Neem leaf extracts adversely affect the gonadal weight, fecundity rate, egg fertility, and chitin content of *H. armigera* (Sharma *et al.*, 1999).

Botanicals are friendly to the natural enemies of *H. armigera* predators (lady bird beetle, *Mallada signatus* (Schneider)) feed on *H. armigera* treated with azadirachitin, the duration of the larval stage was extended which in turn increased the number of *H. armigera* consumed per individual predators (NRC, 1992; Ma *et al.*, 2000b).

3. MATERIALS AND METHOD

3.1 Description of the Study Area

The study was conducted under laboratory and field conditions of Werer Agricultural Research Center (WARC), Ethiopian Institute of Agricultural Research (EIAR). WARC is located 278 km to the East of Addis Ababa at an altitude of 750 m.a.s.l, latitude of $9^0$16'N and longitude of 40^0 9'E. The study area is characterized by having chromic vertisol and alluvial soil types, mean annual rainfall of 540mm and mean maximum and minimum temperatures of 34.4^0c and 19.6^0c, respectively.

3.2 Artificial Diet Preparation

Artificial diet was prepared following the procedure of Taekle and Jenson (1985) modified from Shorey and Hale (1965) and Raulston and Lindgren (1969). Agar was boiled in 500ml water by stirring frequently and was allowed to cool to 50-60^0c. Simultaneously, 125 g soybean flour, 2.5ml formalin and 750ml water were mixed in a blender jar and blended for 2-3 minutes. The boiled agar was poured into the blender and blending was continued for 1-2 minutes. Yeast, ascorbic acid, casein salt, methyl paraben, benlate and vitamin stock solution (Appendix 2) were mixed one by one, while blending to ensure complete mixing and the final blending was made for 2 minutes. The prepared diet was immediately poured into plastic cups and allowed to solidify at room temperature and kept in refrigerator until used. The vitamin stock solution was prepared by weighing and dissolving all the components (Appendix 3) with distilled water in a volumetric flask, tightly closed and was kept in the refrigerator for further use.

Ingredients of artificial diet

Boiling agar

Blending artificial diet

Artificial diet poured into plastic cups

Plate 1. Brief processes of artificial diet preparation.

3.3 Rearing African Bollworm, *Helicoverpa armigera*

Older instar ABW larvae were collected from unsprayed cotton production field at Werer Agricultural Research Center. The collected larvae were reared on artificial diet (Raulston and Lindgen, 1969). Pupae and the adults were reared. Pupae were collected each morning and transferred to plastic pots embedded with soil. Pairs of male and female emerged adults were placed in adult rearing cages made up of cheese cloth and

they were supplied with adult diet which was prepared from 5g sugar and 200ml water (Geremew and Surachate, 2003). One or two plastic cups plugged with cotton wool immersed in adult diet solution were kept in each adult rearing cage so that the adults could easily suck the sugar solution. Young cotton plants or branches of old cotton plant were kept in the adult rearing cages for the purpose of oviposition and support of the adults. The experiment was conducted using third instar larvae of the second generation.

H. armigera larvae on artificial diet

H. armigera pupae in plastic pots

Adult rearing cages

H. armigera adult on top and cotton branches inside

Plate 2. Laboratory rearing of *H. armigera.*

3.4 Treatment Preparation

Undamaged ripe neem fruits (barriers), which are yellow and fresh leaves were gathered from neem trees at Werer Agricultural Research Center and Middle Awash Agricultural Development Enterprise. The outer layer of the pulp was removed from the seeds. After cleaning, the seeds and leaves were spread out separately under shade on cloth to dry for a few days. The dried seeds and leaves were stored in well–aerated sacks. Clean neem seeds and a bulk of dried leaves were ground using electric grinder until a fine powder was obtained. The powder was sieved using test sieves in order to remove larger particles.

Then three different concentration levels (2.5, 5 and 10%) of water extracts of neem seed and leaf were prepared following the next procedure. Two and half, five and ten gram neem seed and leaf powder were separately weighted and each was mixed with 100 ml tap water. Then five gram soap powder was added to dissolve the active substances in the neem powder and to make them stick to the plant surface. The resulting solutions were repeatedly stirred manually for several times and were left to stand for 24 hours. After thorough stirring, the solutions were repeatedly filtered through fine gauze in order to remove larger particles and obtain clear liquid.

Three different rates (manufacturers' and two higher) of Nimbecidine (0.03% Aza) were prepared. The manufacturers' rate solution was prepared by mixing 1:1000 ratio of Nimbecidine (ml) to tap water (ml).Whereas the first (NH_1) and the second higher rate

(NH_2) solutions of Nimbecidine were prepared by mixing 1.25 :1000 and 1.50 :1000 ratio of Nimbecidine (ml) to tap water (ml), respectively.

Depulping neem berry

Washing the depulped neem

Drying neem seed under shade

Neem seed powder

Drying neem leaf under shade

Neem leaf powder

Plate 3. Processing of neem seed and leaf.

Crude water extract of neem seed

Filtering crude extract of neem seed

Crude water extract of neem leaf

Filtering crude extract of neem leaf

Plate 4. Preparation of water extracts of neem seed and leaf.

3.5 Laboratory Experiments on *Helicoverpa armigera*

Laboratory experiments were conducted using the square dip and larval immersion methods/techniques (Geremew and Surachate, 2003). In square dip experiment cotton squares were dipped into individual treatments for 20 seconds and then allowed to dry for 60 minutes before they were supplied to *H. armigera* larvae. Whereas in the larval immersion experiment, unlike the square dip experiment the larvae themselves were

dipped into individual treatments for 20 seconds and then supplied with artificial diet when they start to crawl.

3.5.1 Square dip experiment

The purpose of this experiment was to contaminate the fruiting bodies (squares) of cotton plant with extracts of neem and thereby evaluate the effect of neem extracts on *H. armigera* either through repellency or affecting internal organs after being consumed.

The design in this experiment was CRD with three replications. A total of 30 larvae (ten in each replication) were tested in each treatment. Medium sized cotton squares were collected from unsprayed cotton fields. A total of 30 equal sized squares were dipped into each treatment for 20 seconds. The dipped squares were transferred to paper padded trays for air-drying. Weight of each larva was recorded before treatment application using sensitive balance. After 60 minutes of drying, a single square was placed into plastic cup together with one weighed 3rd instar larva.

Data on the number of damaged squares was taken in 24, 48 & 72 hours after treatment application. After 72 hours, the survived larvae were transferred to artificial diet. Weight of larvae 3, 6 & 9 days after treatment application was recorded using sensitive balance and survival of the larvae was assed every day. The pupae and the adults were reared following the method stated by Geremew and Surachate (2003).

The pupae were collected each morning and transferred on to plastic pots embedded with soil after data on the weight and date of pupation were recorded. Data on the normality and the date of emergence were taken for the emerged adults and a couple of them were placed in separate cages. The adults were supplied with adult diet made up of 5g sugar and 200ml water. Plastic cups plugged with cotton wool soaked into sugar solution were placed in each adult rearing cage so that the adults could suck the sugar solution easily. The adult rearing cages were inspected every morning and data on the number of eggs laid per female and date of adult death were recorded. Eggs were counted using hand lens and their fertility was checked by thoroughly following the hatching of larvae.

Plate 5. Air drying of dipped squares.

3.5.2 Larval immersion experiment

The purpose of larval immersion experiment was to evaluate the contact effect of extracts of neem on *H. armigera.*The design in this experiment was CRD with three replications. Like in the square dip experiment, a total of 30 larvae (ten in each replication) were

tested in each treatment. Third instar larvae were weighed before treatment application. The larvae were immersed into the respective treatments for 20 seconds and then transferred to paper-padded tray in order to remove excessive liquid from the body of the larvae. There after, each larva was transferred into separate plastic cups containing artificial diet.

Data on the weight of the supplied artificial diet and the amount consumed within 3, 6 and 9 days after treatment application (DAT) were recorded. After three days the larvae were supplied with fresh diet as per their requirement. The pupae and the larvae were reared following the method stated by Geremew and Surachate (2003) as in the square dip experiment. Data on the survival, feeding, growth, longevity, fecundity and fertility were recorded in the same way as in the square dip experiment.

A

B

Plate 6. Partial view of larval immersion experiment. A) Dipping the larvae and allowing them to crawl on paper, B) Larvae kept in plastic cups after dipping & air drying.

3.6 Field Trial

The purpose of the field trial was to verify the effects of neem extracts on *H. armigera* under field condition. The cotton variety Deltapine 90 was planted in mid May 2006 on a plot size of 54 m^2. The trial was conducted using the randomized complete block design (RCBD) with three replications. All agronomic practices recommended for the area were followed. Two rounds of carbosulfan 25% ULV were applied late in the cropping season after the bolls have fully opened in order to control aphid infestation. A total of three rounds of spray were applied using knapsack sprayer based on natural infestation when the economic threshold level (8 first to third instar larvae/100 plants) was attained.

Data on pre and post spray (3, 5, 7 and 10 days) counts of ABW egg & larvae, damaged flowers, squares & bolls, non-target and beneficial insects were recorded from 15 predetermined plants per plot. At harvest; boll number per plant, stand count per plot and number of scars per plant were recorded. Finally seed cotton yield was harvested and weighed.

View of the experimental plots

Pest assessment during field trial

Field spray application of extracts

Field spray application of extracts

Plate 7. Partial view of the field trial, pest assessment and application.

3.7 Statistical Analysis

Both data from laboratory experiment (square dip and larval immersion) and field trial were subjected to Analysis of Variance with General Linear Model (GLM) procedures using the SAS software (SAS, 1999-2001) and means for the significance were separated by Tukey's Studentized Range (HSD) test at $P \leq 0.05$.

In field experiment, the data in all rounds of spray application were considered for pre and post spray counts of ABW larvae whereas only data in two rounds of spray application were considered for pre and post spray counts of damaged squares and bolls (first and second round for damaged squares, second and third round for damaged bolls). This was done, because, the first and second round spray application have coincided with the pick square formation period of the cotton while the second and the third rounds of spray application have respectively coincided with the boll formation and boll opening period of the experimental cotton plant. Only the first round spray application was considered for pre and post spray counts of damaged flowers, because the pick flowering period coincided only with the first round spray application.

4. RESULTS

4.1 The Effects of Neem Extracts on *Helicoverpa armigera* in Laboratory

4.1.1 The effect on the survival of larvae

In square dip experiment a highly significant difference (P<0.0001) was recorded among the treatments for mean mortality of ABW larvae. Statistically significant mean mortalities were observed from larvae allowed to feed on squares dipped in endosulfan 35% EC and all concentration levels of water extracts of neem seed and leaf, except 10% NLE. Hundred percent mean mortality was due to the effect of endosulfan 35% EC followed by 5, 2.5 & 10% NSE with their respective mean mortalities of 8, 7 & 7 larvae. Among seed extracts, 5% NSE caused a comparable mortality (8) to endosulfan 35% EC. Recorded mean mortality for Nimbecidine ranged from 0.67-2.00. The lowest mean mortality (o.33) was recorded from the control treatment (Table 1).

In larval immersion experiment, significant differences (P<0.0001) were recorded among mean mortalities of ABW larvae immersed in different treatment concentrations. Endosulfan 35% EC has resulted in 100% mortality of larvae, while 10% NSE and 10% NLE caused mean mortality of 6.00 & 6.00, respectively. Mean mortalities from Nimbecidine were very low and ranged from 1.00-3.30, which is comparable to water treatment (Table 1).

Table 1. Mean number of *Helicoverpa armigera* larvae died in square dip and larval immersion experiments, WARC 2006.

Treatments	Mean number of larvae died in	
	square dip experiment	larval immersion experiment
2.5% NSE	7.00 ± 2.00ab	2.67 ± 0.58bc
5% NSE	8.00 ± 1.15ab	3.67 ± 0.67bc
10% NSE	7.00 ± 0.00 ab	6.00 ± 1.00ab
2.5% NLE	6.67 ± 1.45ab	0.00 ± 0.00c
5% NLE	6.00 ± 0.58abc	1.00 ± 0.58c
10% NLE	4.67 ± 0.58bcd	6.00 ± 1.73ab
NM	1.00 ± 0.58d	1.00 ± 0.58c
NH_1	0.67 ± 0.67d	1.33 ± 0.67c
NH_2	2.00 ± 0.58cd	3.33 ± 0.88cd
Control	0.33 ± 0.33d	1.00 ± 0.58dc
Endosulfan 35% EC	10.00 ± 0.00a	10.00 ± 0.00a

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate. Note: Mean number of larvae tested = 10

4.1.2 The effect on the feeding of larvae

At 24 hours after treatment (HAT), mean number of damaged squares was high (7.33) for the control treatment. All concentration levels of neem seed and leaf extracts & Nimbecidine (NM) resulted in lower damage of cotton squares. At 48 HAT, only 5% NSE resulted in lower damage. However, at 72 HAT all concentration levels of neem seed extract have resulted in reduced square damage compared to the control treatment.

As the exposure time of treated cotton squares to ABW larvae increased, only neem seed extract at the three concentration levels (2.5, 5 & 10%) were found effective in reducing the number of damaged squares. But, neem leaf extracts and Nimbecidine at various concentration levels were found ineffective in reducing the damage to cotton squares (Table 2).

At 3 days after treatment (DAT), the lowest mean weight of artificial diet consumed by larva was recorded from 10% NSE. At 6 DAT, lower diet consumption was recorded from 5% NSE, 10% NSE, 2.5% NLE, NH_1 & NH_2. Only 5 & 10% NSE have significantly reduced the amount of diet consumed at 9 DAT. Diet consumption was significantly lower for 10 & 5% NSE at 6 & 9 days after larval immersion. The highest percent diet was consumed by larvae immersed in Nimbecidine (manufacturers' rate) and the lowest from 10 & 5% NSE as compared to the control. As concentration of NSE increased percent feeding decreased (Table 3).

Table 2. Mean number of *Helicoverpa armigera* damaged squares within 24, 48 and 72 hours after treatment application in square dip experiment, WARC 2006.

Treatments	No. squares consumed within		
	24 HAT	48 HAT	72 HAT
2.5% NSE	1.00 ± 0.58b	3.67 ± 0.67ab	4.67 ± 0.67b
5% NSE	1.33 ± 0.88b	3.33 ± 1.33b	3.67 ± 1.20b
10% NSE	1.67 ± 0.88b	4.00 ± 1.00ab	4.33 ± 0.88b
2.5% NLE	1.67 ± 0.88b	4.87 ± 1.33ab	6.33 ± 0.67ab
5% NLE	1.67 ± 0.88b	5.00 ± 1.00ab	7.33 ± 0.88ab
10% NLE	1.33 ± 0.67b	4.00 ± 0.58ab	6.33 ± 0.33ab
NM	3.33 ± 0.88b	5.00 ± 1.15ab	7.00 ± 0.00ab
NH_1	4.67 ± 0.88ab	6.00 ± 1.53ab	6.67 ± 0.67ab
NH_2	4.33 ± 0.33ab	6.67 ± 0.33ab	7.33 ± 1.20ab
Control	7.33 ± 0.33a	9.00 ± 0.58a	10.00 ± 0.00a

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate.Note: Mean number of squares supplied = 10

Table 3. Mean weight of artificial diet consumed per *Helicoverpa armigera* larva within 3, 6 and 9 days after treatment application in larval immersion experiment, WARC 2006.

Treatments	Weight of diet (g) consumed within		
	3 DAT	6 DAT	9 DAT
2.5% NSE	0.24 ± 0.03a	0.85 ± 0.19ab	3.06 ± 0.20a
5% NSE	0.18 ± 0.02ab	0.20 ± 0.05 c	0.78 ± 0.13dc
10% NSE	0.11 ± 0.01b	0.18 ± 0.06c	0.43 ± 0.13d
2.5% NLE	0.22 ± 0.02ab	0.68 ± 0.03b	2.30 ± 0.14abcd
5% NLE	0.28 ± 0.03a	0.86 ± 0.01ab	2.37 ± 0.10ab
10% NLE	0.21 ± 0.00a	0.72 ± 0.09ab	2.36 ± 0.42abc
NM	0.21 ± 0.06ab	1.00 ± 0.08ab	3.18 ± 0.41a
NH_1	0.25 ± 0.02a	0.63 ± 0.08bc	3.00 ± 0.17ab
NH_2	0.22 ± 0.03ab	0.63 ± 0.05bc	1.41 ± 0.25bcd
Control	0.22 ± 0.02ab	1.14 ± 0.04a	3.06 ± 0.36ab

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate.

4.1.3 The effect of neem extracts on the growth of *Helicoverpa armigera*

4.1.3.1 The effect on the weight of larvae

In square dip experiment there were highly significant differences (P<0.0001) among the effects of the treatments on mean weight of *H. armigera* larva at 3, 6 and 9 DAT. The highest percent weight losses of larva were recorded from 5 & 10% NSE at 3, 6 & 9 DAT. On the contrary, significantly higher weight gain was observed from the control treatment. However, percent weight loss due to Nimbecidine treatment was very low. Neem seed extracts at various concentration levels better reduced the weight of larvae as compared to leaf extracts and Nimbecidine. Generally percent weight loss was high at 9 DAT followed by 6 & 3 DAT (Table 4).

In larval immersion experiment mean weight of ABW larvae 3, 6 & 9 DAT have clearly revealed that there was highly significant difference (P<0.0001) among treatments. At 3 DAT, 10% NSE & 10% NLE have resulted in significantly lower mean weight of ABW larvae as compared to the control (49.51 mg). However, a significantly highest mean weight of 66.88 mg was recorded from those larvae dipped in 5% NLE. At 6 DAT, all concentration levels of water extracts of neem seed have resulted in significantly lower mean weights of ABW larvae as compared to the control treatment. At 9 DAT, 5 & 10% NSE have resulted in significantly lower mean weight of ABW larvae as compared to mean weight of 334.11 mg recorded from larvae dipped in water. The percentage weight losses (at 3, 6 & 9 DAT) were relatively higher for the three concentration levels of neem seed extract (Table 5).

Table 4. Mean weight (mg) and percent weight losses viz control treatment of *Helicoverpa armigera* larva at 3, 6 and 9 days after treatment application in square dip experiment, WARC 2006.

Treatments	Initial Weight	Weight at 3 DAT	% weight loss	Weight at 6 DAT	% weight loss	Weight at 9 DAT	% weight loss
2.5% NSE	7.33 ± 1.75a	8.31 ± 2.24c	75.14	25.02 ± 4.28b	79.99	72.11 ± 14.07b	74.85
5% NSE	6.05 ± 1.18a	6.09 ± 0.90c	81.91	16.28 ± 4.24b	87.07	26.60 ± 6.35b	90.72
10% NSE	6.65 ± 0.88a	5.88 ± 1.11c	82.54	12.16 ± 2.84b	90.35	18.72 ± 5.17b	93.47
2.5% NLE	5.01 ± 0.46a	13.81± 2.61c	58.98	39.28 ± 9.49b	68.81	112.16 ± 24.06b	60.88
5% NLE	4.41 ± 0.15a	11.85 ± 0.44c	64.80	26.82 ± 3.57b	78.71	68.29 ± 18.64b	76.18
10% NLE	5.28 ± 0.27a	10.59 ± 1.97c	68.54	28.77 ± 10.61b	77.16	67.31 ± 18.06b	76.65
NM	4.79 ± 0.43a	29.62 ± 2.97ab	12.03	103.16 ± 10.26a	18.09	255.66 ± 26.39a	10.82
NH_1	3.98 ± 0.13a	30.12 ± 3.02ab	10.54	119.48 ± 17.55a	5.14	311.38 ± 45.71a	- 8.61
NH_2	4.88 ± 0.56a	23.32 ± 1.15b	30.74	87.70 ± 5.16a	30.37	222.46 ± 7.65a	22.40
Control	5.18 ± 0.13a	33.67 ± 1.31a	-	125.95 ± 4.70a	-	286.69 ± 12.43a	-

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate.

Table 5. Mean weight (mg) and percent weight losses viz control treatment of *Helicoverpa armigera* larva at 3, 6 and 9 days after treatment application in larval immersion experiment, WARC 2006.

Treatments	Initial Weight	Weight at 3 DAT	% weight loss	Weight at 6 DAT	% weight loss	Weight at 9 DAT	% weight loss
2.5% NSE	7.42 ± 0.26b	30.24 ± 1.22cde	38.92	92.71 ± 8.65bcd	55.45	262.86 ± 10.22ab	21.33
5% NSE	7.43 ± 0.48b	30.51± 2.95cde	38.34	74.62 ± 15.07cd	64.14	198.81 ± 41.48bc	40.50
10% NSE	9.86 ± 0.54ab	27.88 ± 2.80de	43.69	59.68 ± 10.03d	71.32	125.61 ± 16.70c	62.40
2.5% NLE	7.30 ± 1.17b	51.22 ± 6.18ab	-3.45	191.31 ± 9.10ab	8.06	368.09 ± 18.71a	-10.17
5% NLE	10.71± 0.42a	66.88 ± 7.82a	-35.08	202.00 ± 26.72a	2.92	344.34 ± 23.84a	-3.06
10% NLE	7.80 ± 0.49ab	10.87 ± 2.02e	78.04	112.82 ± 6.67abcd	45.78	286.61 ± 28.66ab	14.22
NM	8.56 ± 0.65ab	44.61 ± 3.21bcd	9.90	171.84 ± 15.15abc	17.42	350.74 ± 17.78a	-4.98
NH_1	6.91±0.69b	31.09 ± 1.54bcde	37.20	141.08 ± 6.67abcd	32.20	374.30 ± 29.74a	12.03
NH_2	8.84 ± 0.18ab	29.80 ± 3.66cde	39.81	146.17 ± 12.71abcd	29.75	373.63 ± 30.87a	-11.83
Control	8.16 ± 0.37ab	49.51 ± 2.12abc	-	208.08 ± 14.80a	-	334.11 ± 16.49a	-

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate.

4.1.3.2 The effect on the weight of pupae

In square dip experiment significantly lower pupal weights (197 & 196.67 mg) were obtained from 5 & 10% NSE, respectively as compared to pupal weight (258.64 mg) from the control treatment. Treating larvae in different concentration levels of Nimbecidine has resulted in positive effect and weight gain of 1-3% was observed (Table 6).

In larval immersion experiment, there were highly significant differences ($P<0.0001$) among mean pupal weights of ABW larvae dipped in different treatment concentrations. Statistically lower pupal weights were recorded from 5 & 10% NSE as compared to pupal weight of 250.36 mg recorded from the control treatment. Neem seed extract showed stronger effect, and reduced pupal weight by 11.85, 16.43 and 22.12 percent when dipped in 2.5, 5 and 10% concentration levels, respectively. The Nimbecidine treatment has shown positive effect as compared to water treated control (Table 6).

Table 6. Mean weight of *Helicoverpa armigera* pupa on the date of pupation in square dip and larval immersion experiments, WARC 2006.

Treatments	Weight (mg) of pupae in square dip experiment	Weight loss (%)	Weight (mg) of pupae in larval immersion experiment	Weight loss (%)
2.5% NSE	238.52 ± 2.20ab	7.78	220.69 ± 6.69bcd	11.85
5% NSE	197.50 ± 3.12b	23.64	209.22 ± 7.01cd	16.43
10% NSE	196.67 ± 2.85b	23.96	194.97 ± 10.36d	22.12
2.5% NLE	244.78 ± 8.74ab	5.36	252.42 ± 3.30ab	-0.82
5% NLE	238.44 ± 39.38ab	7.81	238.24 ± 6.87abc	4.84
10% NLE	220.46 ± 10.16ab	14.76	225.89 ± 3.95bcd	9.77
NM	262.11 ± 3.21a	-1.34	247.00 ± 5.92ab	1.34
NH_1	266.84 ± 17.31a	-3.17	263.70 ± 5.96a	-5.33
NH_2	261.34 ± 5.27a	-1.04	261.49 ± 8.54a	-4.44
Control	258.64 ± 5.59a		250.36 ± 6.91ab	

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate

4.1.4 The effect of neem extracts on the development of *Helicoverpa armigera*

4.1.4 .1 The effect on larval development time

In square dip experiment, the majority of the survived larvae have completed their larval period within 21-30 days after hatching. Very extended larval period (30-35 days) was recorded form larvae dipped in all concentration levels of water extracts of neem seed and leaf. However, hundred percent of the control larvae pupated within 21-25 days after hatching (Table 7).

In larval immersion experiment, the majority of the survived larvae completed their larval period within 17-25 days after hatching. Larvae dipped in 2.5% NSE, 10% NSE, 10% NLE & NH2 have completed their larval period within 26-30 days, while 16.67% of larvae dipped in 10% NSE have completed in 31-35 days after hatching which is far more extended (Table 8).

Table 7. Mean number of days required to complete larval period of *Helicoverpa armigera* in square dip experiment, WARC 2006.

	No. of individuals completed their larval period within _____ days							
	17-20	%	21-25	%	26-30	%	31-35	**%**
Treatment								
2.5% NSE	0	0.00	4	44.44	3	33.33	2	22.22
5% NSE	0	0.00	3	50.00	2	66.67	1	16.67
10% NSE	0	0.00	3	33.33	3	33.33	3	33.33
2.5% NLE	0	0.00	6	60.00	2	20.00	2	20.00
5% NLE	0	0.00	3	25.00	4	33.33	5	41.67
10% NLE	0	0.00	7	41.18	6	35.29	4	23.53
NM	10	37.04	16	59.26	1	3.70	0	0.00
NH_1	11	39.29	15	53.57	2	7.14	0	0.00
NH_2	4	16.67	20	83.33	0	0.00	0	0.00
Control	0	0.00	29	100.00	0	0.00	0	0.00

NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate.

Table 8. Mean number of days required to complete larval period of *Helicoverpa armigera* in larval immersion experiment, WARC 2006.

Treatment	No. of individuals completed their larval period within_____ days									
	≤16	%	17-20	%	21-25	%	26-30	%	31-35	%
2.5% NSE	0	0.00	13	59.09	5	22.73	4	18.18	0	0.00
5% NSE	0	0.00	11	57.89	8	42.10	0	0.00	0	0.00
10% NSE	0	0.00	5	41.67	4	33.33	1	8.33	2	16.67
2.5% NLE	3	10.00	25	83.33	2	6.67	0	0.00	0	0.00
5% NLE	6	22.22	18	66.67	3	11.11	0	0.00	0	0.00
10% NLE	0	0.00	5	41.67	3	25.00	4	33.33	0	0.00
NM	4	14.81	21	77.78	2	7.41	0	0.00	0	0.00
NH_1	2	7.69	21	80.77	3	11.54	0	0.00	0	0.00
NH_2	0	0.00	13	65.00	4	20.00	3	15.00	0	0.00
Control	6	22.22	18	66.67	3	11.11	0	0.00	0	0.00

NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate.

4.1.4.2 The effect on pupal development time

In square dip and larval immersion experiments, there was non-significant difference between treatments and the control ($P< 0.05$) in the number of days required to complete the pupal period of *H. armigera.* Significant difference was observed only between 10% NSE and NM in larval immersion experiment (Table 9).

Table 9. Mean number of days required to complete pupal period of *Helicoverpa armigera* in square dip and larval immersion experiment, WARC 2006.

Treatments	Mean number of days required in	
	square dip experiment	larval immersion experiment
2.5% NSE	9.53 ± 0.03a	9.57 ± 0.43ab
5% NSE	No adult emerged	9.59 ± 0.30ab
10% NSE	9.53 ± 0.03a	10.33 ± 0.33a
2.5% NLE	9.83 ± 0.17a	9.50 ± 0.59ab
5% NLE	9.40 ± 0.21a	9.70 ± 0.09ab
10% NLE	9.53 ± 0.37a	9.84 ± 0.10ab
NM	9.55 ± 0.10a	9.05 ± 0.18b
NH_1	9.51 ± 0.20a	9.41 ± 0.29ab
NH_2	9.73 ± 0.31a	9.45 ± 0.15ab
Control	9.42 ± 0.24a	9.29 ± 0.06ab

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE= water extract of neem seed, NLE=water extract of neem leaf, NM= manufacturers' rate Nimbecidine solution, NH_1= Nimbecidine solution at 25% more than manufactures' rate, NH_2= Nimbecidine solution at 50% more than manufactures' rate.

4.1.4.3 The effect on adult emergence

In square dip experiment, some of the larvae allowed to feed on squares dipped in 2.5% NSE, 5% NSE, 5% NLE & 10% NLE have changed into larval-pupal intermediates (Plate 8). But no such intermediates were observed among larvae fed on squares dipped in other treatments including the control. Comparatively lower number of larvae fed on squares dipped in various concentration levels of water extracts of neem seed and leaf have got a chance to reach to the adult stage. Furthermore, some adult abnormalities were recorded from 2.5% NSE, 2.5% NLE, 10% NLE, NH1, & NH_2 (Table 10).

In larval immersion experiment, some of the larvae dipped in 10% NSE & NH_2 were changed into larval-pupal intermediates (Plate 8). However, no such intermediates were observed from those larvae dipped in other treatments. Five and ten percent neem seed extracts have impaired adult emergence significantly. Comparatively lower number of those larvae dipped in 10% NSE, 5% NSE &10% NLE have reached the adult stage (Table 10).

Table 10. The effects of Nimbecidine and water extracts of neem seed and leaf on the development of *Helicoverpa armigera* in square dip and larval immersion experiment, WARC, 2006

Treatment	Square dip experiment			Larval immersion experiment		
		Adults emerged			Adults emerged	
	No. of LPI	Healthy	Abnormal	No. of LPI	Healthy	Abnormal
2.5%NSE	1	7	1	0	9	8
5%NSE	1	0	0	0	10	0
10%NSE	0	8	0	3	3	1
2.5%NLE	0	8	1	0	18	8
5%NLE	4	6	0	0	22	1
10%NLE	1	14	1	0	10	1
NM	0	26	0	0	21	1
NH_1	0	24	3	0	21	2
NH_2	0	20	1	2	12	3
Control	0	20	0	0	24	1

LPI = Larval-pupal Intermediate, NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate.

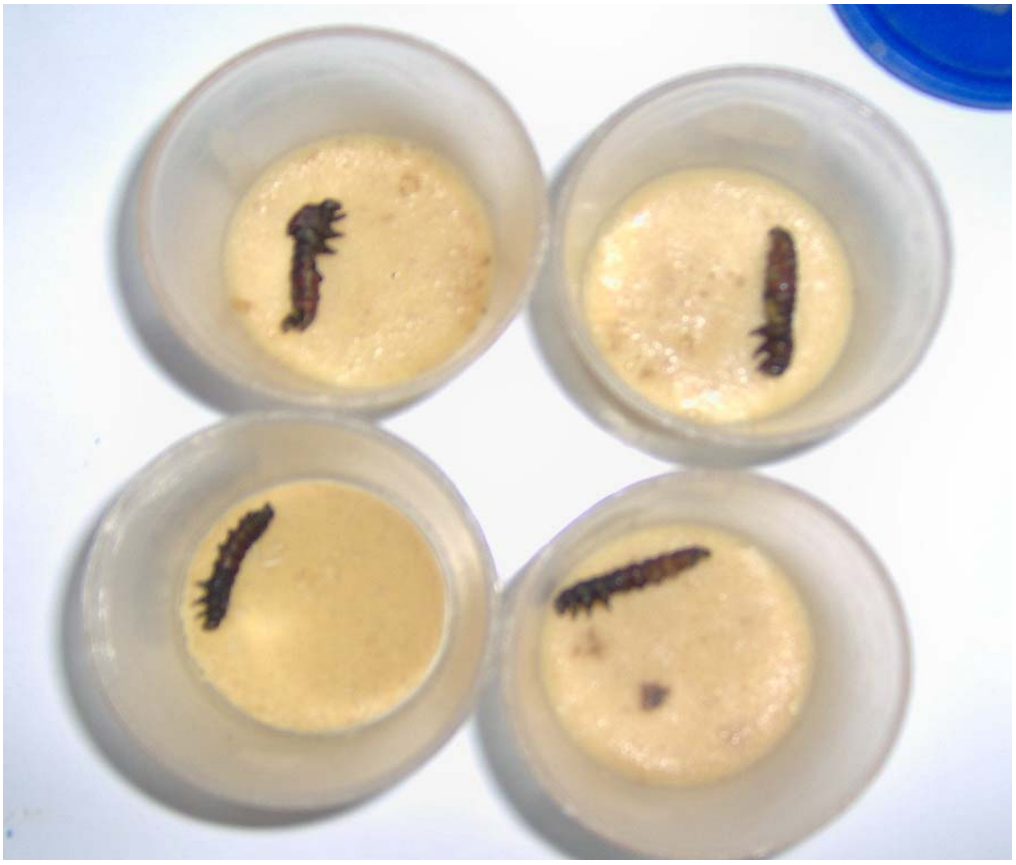

Plate 8. Larval-pupal intermediates observed in laboratory experiment.

4.1.5 The effect on adult longevity

In square dip experiment, there was a significant difference ($P < 0.05$) among treatments for mean number of days adult *H. armigera* survived. Significantly lower mean adult longevities of 1.83, 1.97, 3.07 & 3.42 days were recorded from 10% NLE, 2.5% NLE, NH_2 & NM, respectively as compared to adult longevity of 7.98 days recorded from control treatment (Table 11).

In larval immersion experiment, significantly lower adult longevity of 2.03 days was recorded from adults developed from larvae dipped in 5% NSE. Adult longevity of control treatment was 4.74 days (Table 11).

Table 11. Mean number of days *Helicoverpa armigera* adults survived in square dip and larval immersion experiment, WARC 2006.

Treatments	Number of days adults survived in_____	
	square dip experiment	larval immersion experiment
2.5% NSE	4.00 ± 0.33ab	3.61 ± 0.44ab
5% NSE	No adult emerged	2.03 ± 0.61b
10% NSE	3.50 ± 0.29ab	2.50 ± 0.76ab
2.5% NLE	1.97 ± 0.50b	3.15 ± 0.23ab
5% NLE	6.25 ± 1.92ab	4.00 ± 0.78ab
10% NLE	1.83 ± 0.60b	3.34 ± 0.38ab
NM	3.42 ± 0.21b	4.33 ± 0.17ab
NH_1	3.79 ± 0.95ab	3.49 ± 0.38ab
NH_2	3.07 ± 0.35b	3.39 ± 0.53ab
Control	7.98 ± 1.17a	4.74 ± 0.42a

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate

4.1.6 The effect on fecundity and fertility of *Helicoverpa armigera*

In square dip experiment, very low mean number of eggs was recorded from adults developed from larvae allowed to feed on squares dipped in 2.5% NSE, 2.5% NLE, 5% NLE, NM & NH_2, whereas the highest number of eggs was obtained from adults developed from larvae allowed to feed on squares dipped in NH_1 followed by the control. In addition to the relatively lower number of eggs recorded from adults developed from larvae allowed to feed on 2.5% NSE, 2.5% NLE, 5% NLE and NH_2, low proportion of them were found fertile (Table 12).

In larval immersion experiment, 5% NSE, 5% NLE, NH_1 & NH_2 have relatively resulted in lower mean number of eggs. Whereas the highest number was obtained from 2.5% NLE followed by control treatment, NM & 2.5% NSE. Hundred percent of the eggs obtained from adults emerged from larvae dipped in 2.5% NSE, NH_1 & NH_2 were found non-fertile followed by 5% NLE. However, comparatively higher percent fertile eggs of 97.1 and 78.9 were due to the effects of 2.5% NLE & control treatment, respectively (Table 12).

Table 12. Mean number and percent fertile eggs per *Helicoverpa armigera* female in square dip and larval immersion experiment, WARC 2006.

Treatments	Mean No. of eggs in square dip experiment	Fertile eggs (%)	Mean No. of eggs in larval immersion experiment	Fertile eggs (%)
2.5% NSE	3.0	0.0	66.0	0.0
5% NSE	NAE	-	0.0	-
10% NSE	NAE	-	NFA	-
2.5% NLE	15.0	0.0	149.3	97.1
5% NLE	13.0	34.6	26.6	29.5
10% NLE	0.0	-	NFA	-
NM	63.1	89.5	76.0	74.8
NH_1	205.0	0.4	34.1	0.0
NH_2	14.7	52.5	17.8	0.0
Control	191.0	89.2	86.8	78.9

NAE = no adults emerged, NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate.

4.2 The Effect of Neem Extracts on *Helicoverpa armigera* under Field Condition

4.2.1 The effect on larval infestation

None of the treatments have resulted in a significantly lower number of *H. armigera* larval infestation in all rounds of spray application as compared to control treatment in all days of data record (Table 13, 14 & 15).

Table 13. Pre and post spray counts of *Helicoverpa armigera* larvae per plot in first round spray application in field experiment, Werer 2006.

Treatment	Pre-spray count	Post - spray count			
		3 DAS	5 DAS	7 DAS	10 DAS
2.5% NSE	13.7 ± 2.03a	14.3 ± 2.40a	23.3 ± 4.33a	17.3 ± 4.91a	9.7 ± 1.33a
5% NSE	15.0 ± 1.73a	15.7 ± 1.45a	16.3 ± 1.76ab	14.3 ± 1.33a	4.0 ± 1.53ab
10% NSE	20.0 ± 4.04a	15.0 ± 5.29a	17.7 ± 4.70ab	15.3 ± 4.37a	4.0 ± 0.58ab
2.5% NLE	18.0 ± 0.58a	13.0 ± 0.00a	13.3 ± 1.45ab	11.7 ± 2.67a	4.0 ± 1.73ab
5% NLE	17.7 ± 4.48a	15.7 ± 1.76a	23.0 ± 6.11a	14.7 ± 3.48a	4.7 ± 2.19ab
10% NLE	21.7 ± 6.39a	14.3 ± 1.45a	15.0 ± 5.51ab	17.0 ± 3.21a	4.7 ± 0.88ab
NM	14.3 ± 2.33a	14.0 ± 1.15a	18.0 ± 2.89ab	13.3 ± 1.45a	5.7 ± 1.76ab
NH_1	19.3 ± 4.67a	17.7 ± 5.36a	19.0 ± 3.51ab	15.3 ± 6.39a	3.0 ± 0.58b
NH_2	17.3 ± 3.28a	16.0 ± 3.46a	18.7 ± 3.84ab	18.3 ± 3.71a	4.3 ± 1.33ab
Control	13.3 ± 5.84a	14.7 ± 2.85a	14.3 ± 3.28ab	13.3 ± 3.76a	2.3 ± 0.33b
Endosulfan 35% EC	16.0 ± 3.21a	5.0 ± 1.15a	1.3 ± 0.33b	1.0 ± 0.58a	0.3 ± 0.33b

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE= water extract of neem seed, NLE=water extract of neem leaf, NM= manufacturers' rate Nimbecidine solution, NH_1= Nimbecidine solution at 25% more than manufactures' rate, NH_2= Nimbecidine solution at 50% more than manufactures' rate.

Table 14. Pre and post spray counts of *Helicoverpa armigera* larvae per plot in second round spray application in field experiment, Werer 2006.

Treatment	Pre-spray count	Post- spray count			
		3 DAS	5 DAS	7 DAS	10 DAS
2.5% NSE	2.7 ± 1.20a	2.3 ± 1.20a	3.7 ± 2.19a	5.0 ± 1.00a	5.0 ± 2.08a
5% NSE	1.3 ± 0.33a	2.0 ± 0.58a	1.7 ± 0.88a	3.3 ± 1.86a	2.0 ± 2.00a
10% NSE	1.7 ± 0.33a	3.7 ± 1.33a	3.3 ± 1.86a	2.7 ± 0.67a	2.3 ± 0.33a
2.5%NLE	1.3 ± 0.33a	3.7 ± 0.67a	3.7 ± 0.67a	3.3 ± 0.33a	2.7 ± 0.33a
5%NLE	1.7 ± 0.67a	2.7 ± 0.33a	4.7 ± 2.19a	5.0 ± 1.15a	3.7 ± 0.88a
10% NLE	1.7 ± 0.67a	4.3 ± 1.45a	5.7 ± 1.76a	3.7 ± 0.67a	4.3 ± 1.67a
NM	3.0 ± 0.58a	4.3 ± 0.88a	7.0 ± 1.00a	5.0 ± 3.00a	4.0 ± 2.45a
NH_1	2.0 ± 1.00a	2.3 ± 1.33a	5.0 ± 1.53a	4.3 ± 0.33a	3.7 ± 1.20a
NH_2	1.0 ± 0.00a	2.3 ± 0.88a	5.3 ± 0.88a	5.3 ± 1.20a	3.7 ±1.20a
Control	1.0 ± 0.00a	2.0 ± 1.15a	4.0 ± 1.73a	5.7 ± 2.33a	4.7 ± 0.67a
Endosulfan 35%EC	2.0 ± 0.58a	1.3 ± 1.33a	1.0 ± 0.00a	0.3 ± 0.33a	0.0 ± 0.00a

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate.

Table 15. Pre and post spray counts of *Helicoverpa armigera* larvae per plot in third round spray application in field experiment, Werer 2006.

Treatment	Pre-spray count	Post- spray count			
		3 DAS	5 DAS	7 DAS	10 DAS
2.5% NSE	1.0 ± 0.00a	0.3 ± 0.33a	2.0 ± 0.00a	1.0 ± 1.00a	0.7 ± 0.67a
5% NSE	0.7 ± 0.33a	0.3 ± 0.33a	0.3 ± 0.33a	1.0 ± 0.58a	4.0 ± 1.73a
10% NSE	1.0 ± 0.00a	1.0 ± 0.58a	1.0 ± 1.00a	1.3 ± 0.88a	4.0 ± 1.15a
2.5% NLE	1.0 ± 0.00a	1.0 ± 0.58a	0.7 ± 0.67a	1.7 ± 0.67a	4.3 ± 1.86a
5% NLE	1.0 ± 0.00a	1.3 ± 0.88a	1.3 ± 0.67a	3.0 ± 1.00a	2.0 ± 2.00a
10% NLE	1.0 ± 0.00a	1.7 ± 0.67a	2.0 ± 0.58a	1.3 ± 0.88a	5.3 ± 0.88a
NM	1.0 ± 0.00a	0.7 ± 0.33a	0.7 ± 0.67a	0.0 ± 0.00a	1.0 ± 1.00a
NH_1	1.0 ± 0.00a	0.0 ± 0.00a	0.3 ± 0.33a	1.3 ± 0.67a	2.0 ± 1.53a
NH_2	1.0 ± 0.00a	1.0 ± 0.58a	0.3 ± 0.33a	0.0 ± 0.00a	1.0 ± 0.58a
Control	1.0 ± 0.00a	0.3 ± 0.33a	0.7 ± 0.67a	1.3 ± 1.33a	3.7 ± 1.76a
Endosulfan 35%EC	0.7 ± 0.33a	0.0 ± 0.00a	0.0 ± 0.00a	1.3 ± 0.67a	1.3 ± 0.33a

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate.

4.2.2 The effect on the number of *Helicoverpa armigera* damaged flowers

In first round spray application, all treatments including endosulfan 35% EC didn't significantly reduced the damage caused by *H. armigera* larvae on cotton flowers on all days of post spray count (Table 16).

Table 16. Pre and post spray counts of damaged flowers per plot in first round spray application in field experiment, Werer 2006.

Treatments	Pre-spray count	Post- spray count			
		3DAS	5DAS	7DAS	10DAS
2.5% NSE	4.0±1.53a	1.3±0.33a	3.7±0.88a	6.0±2.00a	5.7±1.45a
5% NSE	0.3±0.33a	1.0±0.58a	2.3±0.88a	6.3±0.33a	3.7±1.33a
10% NSE	1.0±0.58a	0.3±0.33a	6.0±1.15a	6.3±1.86a	3.7±0.88a
2.5% NLE	2.3±0.88a	1.0±0.58a	1.7±0.67a	3.7±1.76a	5.3±2.85a
5% NLE	3.0±1.73a	0.0±0.00a	2.0±1.00a	4.0±1.00a	7.7±1.45a
10% NLE	7.0±3.06a	0.3±0.33a	7.7±4.41a	6.3±1.45a	5.3±1.45a
NM	0.7±0.33a	0.7±0.67a	1.3±0.88a	4.7±1.67a	4.0±1.53a
NH_1	4.0±2.00a	0.3±0.33a	2.7±1.76a	3.7±1.45a	2.7±0.33a
NH_2	2.3±0.67a	0.7±0.33a	2.7±2.19a	4.0±1.53a	3.0±2.00a
Control	2.3±1.20a	0.3±0.33a	3.3±2.33a	5.0±2.31a	5.0±0.58a
Endosulfan 35%EC	1.7±0.88a	0.3±0.33a	0.7±0.33a	0.3±0.33a	0.0±0.00a

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate.

4.2.3 The effect on the number of *Helicoverpa armigera* damaged squares

None of the treatments have significantly reduced the number of damaged squares due to *H. armigera* larvae in the first and second round spray application on all days of post spray data records (Table 17 & 18).

Table 17. Pre and post spray counts of damaged squares per plot in first round spray application in field experiment, Werer 2006.

Treatments	Pre-spray count	Post- spray count			
		3DAS	5DAS	7DAS	10DAS
2.5% NSE	9.0 ± 2.52a	11.7 ± 0.88ab	30.7 ± 3.18a	32.0 ± 4.73a	40.3 ± 9.84a
5% NSE	7.3 ± 2.03a	7.7 ± 2.60ab	18.0 ± 4.58ab	23.7 ± 2.60ab	23.3 ± 2.40ab
10% NSE	4.7± 1.20a	11.0 ± 3.79ab	23.7 ± 3.84a	32.3 ± 6.748a	40.7 ± 7.75a
2.5% NLE	7.0 ± 1.73a	6.0 ± 2.52ab	16.0 ± 3.21ab	22.0 ± 2.65ab	33.0 ± 5.69ab
5% NLE	6.3 ± 0.33a	12.3 ± 0.88ab	27.7 ± 8.95a	30.3 ± 5.46a	42.7 ± 6.01a
10% NLE	18.0 ± 5.770a	9.3 ± 2.03ab	29.0 ± 4.93a	32.0 ± 6.56a	36.3 ± 4.98a
NM	7.3 ± 2.91a	12.0 ± 3.61ab	26.0 ± 3.79a	29.3 ± 1.76a	27.0 ± 4.51ab
NH_1	16.0± 6.08a	7.3 ± 1.76ab	27.0 ± 4.58a	30.3 ± 5.49a	37.7 ± 4.91a
NH_2	6.7 ± 0.33a	15.0 ± 0.58a	27.0 ± 4.36a	31.0 ± 7.09a	36.3 ± 6.39a
Control	8.3 ± 3.28a	9.3 ± 1.76ab	17.3 ± 4.33ab	21.0 ± 6.00ab	27.0 ± 7.23ab
Endosulfan 35%EC	8.3 ± 2.19a	2.3 ± 0.33b	1.7 ± 0.88b	1.7 ± 0.88b	3.0 ± 2.00b

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate.

Table 18. Pre and post spray counts of damaged squares per plot in second round spray application in field experiment, Werer 2006.

Treatments	Pre-spray count	Post- spray count			
		3DAS	5DAS	7DAS	10DAS
2.5% NSE	6.0 ± 1.15a	4.3 ± 1.86a	5.3 ± 2.96a	3.0 ± 1.53a	4.3±1.76a
5% NSE	4.0 ± 1.15a	3.7 ± 0.33a	1.3 ± 0.67a	1.0 ± 1.00a	1.0 ± 0.58a
10% NSE	0.7 ± 0.67a	2.0 ± 0.00a	2.3 ± 0.67a	1.0 ± 0.58a	2.0 ± 0.58a
2.5% NLE	1.7 ± 1.20a	1.3 ± 0.88a	2.3 ± 0.67a	4.0 ± 1.00a	3.3 ± 0.33a
5% NLE	2.7 ± 0.33a	1.0 ± 1.00a	4.0 ± 1.15a	5.3 ± 0.67a	3.7 ± 2.03a
10% NLE	5.3 ± 2.60a	4.7 ± 1.45a	5.3 ± 1.76a	2.0 ± 1.00a	3.3 ± 1.20a
NM	5.0 ± 3.06a	3.7 ± 3.18a	8.3 ± 3.48a	3.3 ± 0.88a	3.0 ± 2.52a
NH_1	2.0 ± 0.58a	1.7 ± 0.67a	2.3 ± 0.88a	4.0 ± 1.00a	4.7 ± 0.88a
NH_2	2.0 ± 1.53a	1.7 ± 0.67a	2.7 ± 1.20a	2.7 ± 0.33a	3.3 ± 0.67a
Control	2.0 ± 1.00a	2.3 ± 1.20a	1.7 ± 0.67a	5.3±2.03a	4.7 ± 0.67a
Endosulfan 35%EC	2.3 ± 0.88a	0.7 ± 0.33a	0.0 ± 0.00a	0.3 ± 0.33a	0.3 ± 0.33a

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate.

4.2.4 The effect on the number of *Helicoverpa armigera* damaged bolls

None of the treatments, including the standard insecticide, endosulfan 35% EC have significantly reduced the damage on cotton bolls caused by ABW larvae as compared to water treatment at all days of post spray data records in second and third round spray application (Table 19 & 20).

Table19. Pre and post spray counts of damaged bolls per plant in second round spray application in field experiment, Werer 2006.

Treatments	Pre-spray count	Post- spray count			
		3DAS	5DAS	7DAS	10DAS
2.5% NSE	5.3 ± 1.86a	2.3± 0.33a	5.3±0.88a	2.7 ± 0.67a	4.0 ± 0.58a
5% NSE	2.7 ± 0.88a	2.3 ± 0.67a	3.0 ± 1.15a	1.7 ± 1.20a	4.0 ± 1.53a
10% NSE	2.0 ± 0.58a	4.0 ± 1.00a	1.7 ± 0.33a	1.3 ± 0.67a	4.0 ± 2.31a
2.5% NLE	2.3 ± 0.88a	2.0 ± 0.58a	2.7 ± 0.88a	2.0 ± 0.58a	5.3 ± 1.76a
5% NLE	5.3 ± 0.88a	5.3 ± 1.45a	3.0 ± 1.15a	2.3 ± 0.88a	5.0 ± 0.00a
10% NLE	3.0 ± 1.73a	1.7 ± 1.20a	2.3 ± 0.88a	1.7 ± 0.67a	5.0 ± 1.15a
NM	2.0 ± 0.58a	3.7 ± 0.67a	2.0 ± 0.58a	2.0 ± 0.58a	5.7 ± 0.88a
NH_1	3.3 ± 0.88a	2.3 ± 1.45a	2.0 ± 1.00a	2.3 ± 1.33a	4.0 ± 1.00a
NH_2	2.0 ± 1.00a	1.7 ± 1.20a	3.3 ± 0.33a	4.7 ± 2.19a	6.3 ± 0.33a
Control	3.3 ± 1.33a	3.0 ± 1.15a	1.3 ± 0.33a	2.7 ± 0.88a	3.7 ± 1.76a
Endosulfan 35%EC	1.3 ± 0.67a	1.0 ± 0.00a	1.7 ± 0.88a	0.7 ± 0.67a	1.3 ± 1.33a

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate.

Table 20. Pre and post spray counts of damaged bolls per plant in third round spray application in field experiment, Werer 2006

Treatments	Pre - spray count	Post - spray count			
		3DAS	5DAS	7DAS	10DAS
2.5% NSE	4.3 ± 2.60a	2.7 ± 0.88a	7.0 ± 1.53a	2.7 ± 0.88a	4.0 ± 1.00a
5% NSE	4.0 ± 2.00a	4.0 ± 2.31a	3.3 ± 0.88a	3.0 ± 1.53a	4.0 ± 1.15a
10% NSE	2.7 ± 1.45a	2.7 ± 1.20a	3.0 ± 0.58a	4.0 ± 1.15a	2.7 ± 0.33a
2.5% NLE	3.0 ± 1.15a	6.7 ± 1.86a	4.0 ± 1.00a	3.3 ± 0.88a	7.0 ± 1.00a
5% NLE	1.3 ± 0.88a	2.3 ± 0.67a	4.3 ± 0.33a	2.0 ± 1.00a	6.7 ± 2.16a
10% NLE	2.0 ± 1.00a	2.3 ± 1.45a	3.0 ± 0.58a	5.0 ± 1.00a	2.7 ± 0.33a
NM	3.0 ± 2.08a	3.3 ± 1.20a	1.3 ± 0.33a	1.7 ± 1.20a	3.7 ± 2.67a
NH_1	3.3 ± 0.67a	4.0 ± 1.00a	2.3 ± 1.45a	3.0 ± 1.00a	5.3 ± 0.88a
NH_2	6.7 ± 3.67a	5.3 ± 2.19a	3.7 ± 2.67a	3.0 ± 1.15a	6.0 ± 2.65a
Control	3.7 ± 2.33a	2.3 ± 1.20a	3.3 ± 1.86a	1.7 ± 0.33a	5.3 ± 1.76a
Endosulfan 35%EC	1.3 ± 0.67a	0.3 ± 0.33a	1.7 ± 1.20a	2.7 ± 1.67a	0.7 ± 0.33a

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate.

4.2.5 The effect on the number of bolls, scars and seed cotton yield

None of the plots treated with water extracts of neem seed and leaf and Nimbecidine at different concentration levels have given significantly higher number of bolls per plant as compared to those treated with water. However, endosulfan treated plots have yielded significantly higher number of bolls while non-significant difference was observed among treatments for the number of scars per plant at harvest (Table 21).

Finally, records on seed cotton yield have indicated that none of the treatments, except the standard insecticide, endosulfan 35% EC have resulted in statistically higher yield. Endosulfan 35% EC resulted in 37.90 q/ha which is significantly higher compared to control treatment (Table 21).

Table 21. Mean number of bolls & scars per plant at harvest and seed cotton yield in field experiment, Werer 2006.

Treatments	Boll number	Number of scars	Seed cotton yield (q/ha)
2.5% NSE	319.0 ± 4.73ab	362.7 ± 19.20a	33.00 ± 1.81ab
5% NSE	321.3 ± 14.44ab	302.7 ± 28.18a	33.20 ± 0.67ab
10% NSE	301.7 ± 4.91ab	324.7 ± 23.69a	31.84 ± 0.77b
2.5% NLE	285.3 ± 2.91b	339.7 ± 24.13a	33.13 ± 1.16ab
5% NLE	285.7 ± 13.981b	351.3 ± 33.80a	31.60 ± 1.08b
10% NLE	286.0 ± 9.50b	332.3 ± 32.64a	32.30 ± 0.85ab
NM	324.3 ± 22.36ab	329.7 ± 29.48a	31.30 ± 1.21b
NH_1	289.3 ± 32.30b	339.0 ± 43.00a	31.97 ± 1.19b
NH_2	296.7 ± 26.74ab	303.0 ± 19.60a	31.97 ± 1.48b
Control	291.0 ± 1.46b	317.7 ± 21.53a	31.80 ± 1.83b
Endosulfan 35%EC	381.3 ± 19.06a	297.3 ± 10.73a	37.90 ± 0.84a

Means followed by the same letter within a column are not significantly different from each other at $P<0.05$, Tukey's Studentized Range (HSD) Test. NSE = water extract of neem seed, NLE = water extract of neem leaf, NM = manufacturers' rate Nimbecidine solution, NH_1 = Nimbecidine solution at 25% more than manufacturers' rate, NH_2 = Nimbecidine solution at 50% more than manufacturers' rate.

5. DISCUSSION

Larval mortality was very high in square dip study than in the larval immersion experiment. Among the different concentrations of neem seed & leaf extracts, seed extracts showed higher efficacy as compared to leaf extracts in both studies. The high mean mortality obtained in neem seed extract concentrations (7-8) in the square dip experiment could be due to repeated intake of treated squares.

The low mean mortality recorded in larval immersion study (0-6) could be due to the short exposure time (20 seconds) resulted in the minimum absorption of the active ingredient that might be required to give effect on treated larvae. This clearly shows the low cuticular penetration of the active ingredient, which further indicates the need for repeated application of extracts to get good control of *Helicoverpa armigera*. Thus, it is very important to increase exposure time or repeat spray application to get maximum effect of the neem extracts. Recorded mean control mortality in both studies was very low (0.3-1).

Generally, this study indicated that neem extracts have larvicidal activity. The high survival rate observed in both studies in the control treatment was in agreement with the findings of Anani *et al.* (2004), which states that survival was significantly higher in the control than in the neem treatment for *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Attri and Prasad (1980) reported larvicidal effect of neem on mosquito. Gaaboub and Hayes (1984)

investigated the effect of azadiracthin against face fly, *Musca autumnalis* (De *Geer*). When third instars were treated larval and pupal mortality was observed. Similarly, Zehnder and Warthen (1988) have reported increased larval mortality in Colorado Potato Beetle (CPB) larvae after being treated with the extracts of neem seed kernel.

The study on the feeding of *H. armigera* larvae clearly indicated the antifeedant effect of neem extracts except the 0.03% oil. The antifeedant effect of neem seed extract was greater than the neem leaf extract and the neem leaf extract was greater than Nimbecidine. Results of the study are in agreement with the findings of Barnby & Klocke (1987) on *Heliothis virescens* (Fabricius) (Lep. Noctuidae). According to Barnby & Klocke (1987) in *H. virescens* azadirachtin-rich diets lead to decreased feeding and weight gain, as well as biomass conversion rates. Antifeedant effects on stem borers have also been reported by Arnason (1985) and Juan & Sans (2000) for *Ostrinia nubilalis.* According to Aldhous (1992), within the azadirachtin molecule, the hydroxy furan fragment causes andifeedant effects widely among the target species. Schmutterer (1990) and Ascher *et al.* (1992) defined primary and secondary antifeedant effects of azadirachtin. Primary effects include the process of chemoreception by the organism (e.g. sensory organs on mouthparts that stimulate the organism to begin feeding) whereas secondary processes are effects such as gut motility disorders.

Purthi (1937) reported the repellent action of neem leaves and cake against insect pests of stored wheat. According to Jotwani and Sircat (1965), neem seed powder mixed with

wheat grain provided protection from insect pests for 9-12 months. A study by Jilani and Malik (1973) revealed that water and ethanol extracts of leaves and seeds of neem repelled the red flower beetles, *Tribolium castaneum* (Herbst); the khpra beetle, *Trogoderma granarium* (Everts); and the lesser grain borer, *Rhyzopertha dominica* (F).

In general, water extracts of neem seed and leaf have significantly reduced the rate of growth and development of *H. armigera* larvae as compared to the control. The repelling action of neem on *H. armigera* larvae from feeding on dipped cotton squares coupled with its delaying effect on metamorphosis might have resulted in reduced rate of growth and development, which is clearly manifested by reduced larval weight gain.

Anani *et al*. (2004) reported larval weight decrease of 26–48% for both *Sesamia calamistis* and *Eldana saccharina* after being treated with neem products. Lower weight gain rates due to neem were also reported by Haasler (1984), Meisner *et al*. (1987), Melamed-Madjar *et al*. (1989) and Isman (1993) for *Manduca sexta* (L.) (Lep. Sphingidae); the gypsy moth, *Mantria dispar* (L.) (Lepidoptera: Lymantriidae); the European corn borer, *Ostrinia nubilalis*, *Sesamia nonagroides* and several noctuids, respectively.

Nimbecidine and water extracts of neem seed and leaf extended the larval period of *H. armigera*. This extension of the larval period will in turn result in prolonged generation time thereby decreases the number of *H. armegera* generations that will develop/ build up in a certain season. The lesser the number of generation per season, the lesser the crop

damage and the cost of control will be.

Babu *et al.* (2000) reported synergistic effect of methanolic neem seed kernel extract in cotton. Even though the effectiveness of neem seed kernel extract alone was not high, it has delayed the metamorphosis of African bollworm. Haasler (1984) reported extended life cycles due to neem for *M. sexta*. Meisner *et al*. (1987), Melamed-Madjar *et al*. (1989) and Isman (1993) have also reported similar results for the gypsy moth, *M. dispar*; the European cornborer, *O. nubilalis*; *S. nonagroides* and several noctuids, respectively. Anani *et al*. (2004) reported for both *Sesamia calamistis* and *Eladna saccharina*, intrinsic rates of increase (rm) and net reproductive rates (Ro) were highest in the control and smaller in the neem oil treatments. For *S. calamistis*, the control yielded a significantly shorter generation time (G) than the highest oil concentration, whereas, for *E. saccharina*, G was similar in the oil treatments but significantly shorter in the control. In this study however both in square dip and larval immersion experiments larval growth period was extended for 6-10 days when treated in higher concentrations of NSE, NLE and Nimbecidine.

Botanicals are friendly to the natural enemies of *Helicoverpa armigera* predators (lady bird beetle, *Mallada signatus* (Schneider) fed on *H. armigera* treated with azadirachitin, the duration of the larval stage was extended which in turn increased the number of *H. armigera* consumed per individual predators (NRC, 1992; Ma *et al.*, 2000b). The current study also recorded extended larval growth period, which is in full agreement with the

findings of Ma *et al*. (2000b). Therefore, using neem seed extracts at different concentrations, prolonged larval development period, but decreased feeding potential of the larvae, which cause direct damage to the crop.

Neem oil and water extracts of neem seed and leaf didn't affect the duration of pupal period. In the study of Geremew and Surachate (2004) pupal stage lasted 9- 12 days without treatment. The current study also recorded similar results, indicating the low efficacy of neem extracts on pupal duration.

The botanical insecticide Nimbecidine and water extracts of neem seed and leaf have interfered with the normal life cycle of of ABW at different growth stages. Meisner *et al*. (1987) and Melamed-Madjar *et al*. (1989) reported reduced immature survival rates and persistent effects of neem for *O. nubilalis* and *S. nonagrioides*, respectively. Gupta *et al.* (1998) recorded abnormal adult emergence and death during the pre pupal stage and increased with increasing neem suspension concentration in an experiment where the pupation site of *Helicoverpa armigera* was treated by 0.5-6% neem seed powder on a W/W basis.

Significantly lower adult longevity was recorded from 5% NSE, 2.5% NLE, 10% NLE, NM and NH_2. These lower adult longevity periods recorded from neem extracts are below the minimum number of days required for the female to mate. Therefore, neem extracts have lowered the longevity of adult ABW below that is required to give the first

batch of eggs. As stated by Zehnder and Warthen (1988) treating with neem seed kernel extracts reduced fecundity and increased adult mortality of Colorado Potato Beetle (CPB). Geremew and Surachate (2003) reported average adult longevity of 7.4 days which is in full agreement with the adult longevity of 7.98 days recorded from the control treatment in square dip experiment. However, the average adult longevity of the control treatment in the larval immersion experiment declined to 4.74 days due to unknown reason.

Very low mean number of eggs was recorded from adults developed from larvae allowed to feed on squares dipped in various concentration levels of neem oil and water extracts of neem seed and leaf. Moreover, the majority of these eggs were found non-fertile. It was reported that neem leaf extracts adversely affect the gonadal weight, fecundity rate, egg fertility, and chitin content of *Helicoverpa armigera* (Sharma *et al*, 1999). Babu *et al.* (2000) reported synergistic effect of methanolic neem seed kernel extract in cotton. Even though the effectiveness of neem seed kernel extract alone was not high, it has decreased the fecundity of *H. armigera*. Anani *et al.* (2004) has also reported that neem treatments have reduced *Sesamia calamistis* and *Eldana saccharina* fecundity by 30 and 46%, respectively. For both species, egg viability decreased with neem concentration. Reduction in fecundity was also found for *Dacus dorsalis* (Hendel) (Dip. Tephritidae) (Sombatsiri & Tigvattanont, 1983). Teshome (1992) reported that neem seed powder reduced oviposition, egg hatching and adult progeny emergence of the Adzuki Bean Beetl, *Callosobruchus chinensis.* Schulz & Schu¨ter (1983) showed changes in the

ooplasm and in vitellogenesis, which is necessary for oocyte maturity, resulting in egg sterility.

Gaaboub and Hayes (1984) investigated the effect of azadiracthin against face fly, *Musca autumnalis.*When third instars were treated, reduced egg production and hatching from the adults emerged were observed. Zehnder and Warthen (1988) have reported reduced fecundity in CPB when treated with the extracts of neem seed kernel.

In field experiment, in all rounds of spray application none of the treatments have reduced the larval infestation during the post-spray application periods. However, Dhawan and Simloler (1995) have reported different result in their trial on oil products of neem in cotton. They have compared neem product of RD-9 Repin (1and 2%), Neemark (0.5 and 0.75%) and Neemrich 20 EC (0.1 and 0.15%) with quinalphos (0.2%) as standard for their control action against young larvae of *H. armigera* in cotton. Accordingly, the highest concentration of Repin and Neemrich were more effective than Neemark in the crop spraying experiment with mortality levels of 70, 70, and 66.7%, respectively, but less effective than Quinalphos (mortality of 100%).

Similarly in all rounds of spray application none of the treatments have reduced the damage on fruiting bodies of cotton due to *H. armigera* larvae. However, Lingappa *et al.* (2000) obtained high reduction in damage by *H. armigera* to fruiting bodies of cotton by using a neem based formulation containing 0.3% azadirachtin alone or in combination

with *Bacillus thuringiensis* or nuclear polyhydrosis virus. Similar result was also obtained on upland cotton in Australia by Ma *et al.* (2000b) and as opposed to the synthetic insecticides, the botanicals used were found to be safer to the predators including the coccinellids, chrysopids, Araneae, and Hemiptera.

Moreover, a study conducted by Debre Ziet Agricultural Research Center to evaluate the efficacy of some plants species in controlling *H. armigera* in chickpea revealed that crude neem extracts collected from Melka Werer has significantly reduced percent pod damage (Tebkew *et al.*, 2002). Another study on chickpea revealed, significantly lower pod damage due to *H. armigera* after being treated with neem seed kernel extracts as compared to the untreated plots (Sahgal and Ujagir, 1990). Similarly, the same pest was effectively controlled by neem extracts at 5 and 6%. Hence, it was concluded that neem seed kernel extracts could be used instead of highly toxic synthetic insecticides owing to its safety to beneficial insects and its affordable price (Sadawarte and Sarode, 1997).

The failure of Nimbecidine and water extracts of neem seed and leaf to control *H. armigera* infestation and damage on cotton under field condition could be attributed to the high temperature prevailing in the study area, which might have enhanced the rapid degradation of the active ingredients of neem in general and azadirachtin in particular. Azadirachtin, the major active ingredient in neem is photo and thermo labile. It easily degrades under light and high temperature condition.

6. CONCLUSION AND RECOMMENDATIONS

Under laboratory condition in square dip experiment, water extract of neem seed at different concentration levels have negatively affected the survival of *Helicoverpa armigera* larvae, reduced the damage on fruiting bodies of cotton, regulated the growth and development and reduced the fecundity and fertility *of H. armigera*.Though water extract of neem leaf at different concentration levels have shown better results, it was found weaker than extract of neem seed. Neem oil (Nimbecidine 0.03% Aza.) was relatively weaker than water extracts of neem seed and leaf. It has only shown promising results on adult longevity and fertility.

In larval immersion experiment, neem seed extract have shown almost similar trend in their effect on *H. armigera* as that of the square dip experiment. But, larval mortality was very low in larval immersion than in the square dip experiment. While the leaf extract was relatively weaker in its effect. Only 10% NLE was found effective in reducing the number of pupae and percent adults emerged. The higher concentration level (NH_2) of Nimbecidine has better affected *H. armigera* at various developmental stages than the lower rates.

However, the effect of Nimbecidine and water extracts of neem seed and leaf was very poor under field condition. Moreover, no seed cotton yield advantage was obtained from neem extracts treated plots as compared to the control plot.

In summary, water extracts of neem seed and leaf and Nimbecidine had different effects on the various developmental stages of *H. armigera* (egg, larva, pupa and adult). Therefore, it is the cumulative effect of neem on these different developmental stages that shouldn't be neglected.

Therefore, before further popularization of neem extracts as suitable control option against *H. armigera*, especially in IPM, it is of paramount importance to execute detailed studies on the time of application, methods of spray solution preparation and the composition of the spray solution which will help in improving the persistence of the active ingredients of neem under high temperature conditions. It is also more appropriate to develop techniques of active ingredient extraction, stabilization and storage as it is more vulnerable to environmental changes.

Finally, neem with these dozens of effects on *H. armigera* is the most appropriate botanical pesticide that should get due attention in the process of IPM development for cotton and other export commodities, especially vegetable crops for it is safer to the user and doesn't leave hazardous residues on the economic yield and the environment.

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APPENDICES

Appendix 1. List of treatments included in laboratory and field experiments

SN	Treatments	Description
1	2.5% NSE	2.5% water extract of neem seed
2	5% NSE	5% water extract of neem seed
3	10% NSE	10% water extract of neem seed
4	2.5% NLE	2.5% water extract of neem leaf
5	5% NLE	5% water extract of neem leaf
6	10% NLE	10% water extract of neem leaf
7	NM	Manufacturers' rate Nimbecidine solution
8	NH_1	Nimbecidine solution at 25% more than manufactures' rate
9	NH_2	Nimbecidine solution at 50% more than manufactures' rate
10	Control/water	Tap water
11	Endosulfan 35%EC	The standard synthetic insecticide at field rate

Appendix 2. Composition of artificial diet used for mass rearing of *Helicoverpa armigera* larvae.

Ingredients	Specifications	Quantity
Soybean bean flour	Roughly blended	125g
Agar	Biological grade powder	12.5g
Yeast	Bread or brewers	10g
Ascorbic acid	L-ascorbic acid AR	3g
Casein salt	Casein from bovine milk	5g
Methyl paraben	Methyl 4-hydroxy benzoate	2.5g
Benlate(Benzomyl)	Fungicide, 50% WP	1g
Formalin	Formaldehyde 40%	2.5ml
Vitamin stock solution	Prepared as described in Appendix table 3	10ml
Water	Tap water	750ml

Source: Pest Management Journal of Ethiopia Vol. 9, 2005

Appendix 3. Composition of vitamin stock solution for artificial diet preparation

Ingredients	Specifications	Quantity

Niacin	Nicotinic acid	6g
Calcium salt	Calcium penthothenic acid	6g
Insitol	Myo insitol	5g
Riboflavin	Vitamin B1	3g
Thiamin	Vitamin B1	1.5g
Pyridoxin	Vitamin B6 hydrochloride	1.5g
Folic acid	Pure, AR	1.5g
Vitamin B12	Vitamin B12 hydrochloride	20mg
Water	Distilled	1000ml

Source: Geremew Terefe, PhD Dissertation

Appendix 4. Meteorological data of Werer Agricultural Research Center for the months of May- September, 2006.

Date	Temperature(°c)										Relative Humidity (%)					Rainfall (mm)				
	May		June		July		August		September		May	June	July	Aug.	Sept.	May	June	July	Aug.	Sept.
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max										
1	22	39.2	28	39.5	22	38.4	21	29.8	20.5	31.2	49	32	48	69	74	-	-	-	0.3	-
2	21	38.5	28	40	26	38.6	21	34	22.5	34.5	44	31	43	62	56	-	-	-	-	-
3	22	38.3	27	39.3	25.5	39.2	21.5	36	21.0	35	43	34	46	53	57	-	-	-	15.6	-
4	23	34.2	26	39.3	22	35	20	30.5	21.0	33	58	34	56	81	62	2.3	-	-	6.6	-
5	21	35.5	26	39.6	23	36.6	20	30	20.5	34.5	59	33	44	79	61	0.4	0.2	1.4	-	-
6	23	32.5	26	39.8	22	36.1	21.5	34	23.0	35.5	64	33	50	65	42	0.8	-	-	6.9	-
7	20	36.8	24	40.6	25	34.5	20.5	31.2	23.0	33.5	47	33	52	73	59	-	-	-	4.7	11
8	21	38.3	27	40	22.5	37.4	20.6	26.2	18.5	27	39	32	44	84	84	-	-	47	4.3	-
9	21	37.7	27	39.6	21	35	21	31	20.5	35	45	37	58	72	62	-	-	-	-	-
10	20	38	27	38	22	36.7	22.5	33.6	22.5	35	44	40	55	56	54	0.4	1	17.8	-	-
11	20	38.5	23	37.5	21	34	23.5	33.5	21.0	35	41	52	62	67	55	-	-	8	6.9	1.2
12	21	39	23.5	37.6	21	35.3	21	34.5	21.0	34.3	41	44	59	64	49	-	2.9	32.3	12	1.2
13	20	38	22	36.5	20.5	32	20.5	28	23.0	35.2	51	54	70	79	55	0.3	-	7	0.5	-
14	20	39	24.5	38.4	20.5	35	20.5	33	21.5	36.5	37	43	64	58	54	-	-	-	-	17
15	20.5	39	24.5	39	22	35	21	34.5	21.5	36.5	37	38	58	59	43	-	-	-	0.2	-
16	21	39.5	24.5	39.2	23.5	35.8	20.5	28.5	23	35.3	36	38	52	78	52	-	-	-	-	-
17	21	39	25	38.8	25.5	36.5	22	35.6	24.5	36	41	39	45	58	43	-	-	-	3.6	-

Appendix 4. Cont'd

Date	Temperature(°c)										Relative Humidity (%)					Rainfall (mm)				
	May		June		July		August		September		May	June	July	Aug.	Sept.	May	June	July	Aug.	Sept.
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max										
18	21	38	24	39.	23.5	36	20	27	23	34	38	39	50	79	52	-	-	28.3	1.2	-
19	19.5	38	23.5	37.8	20.5	35	20.5	34.5	22	35.3	39	46	60	61	53	-	-	-	-	-
20	20	38.2	25.5	39.5	21	36.1	21.5	34.9	22.5	35.4	36	42	49	56	53	-	-	-	-	-
21	20	37	24.5	38.5	23	37.5	25.5	36.7	23	35.5	38	41	47	48	50	-	0.6	-	16	-
22	20.5	38.3	22.5	39.5	22.5	37	20.5	32.7	20.5	36.2	36	43	47	67	57	-	-	3.5	-	-
23	21	38.5	24.5	39.3	20.5	36	22	33	22.5	36.5	39	42	57	70	51	-	-	-	-	1
24	20	38.5	27	39.7	21.5	35.8	23.5	35.5	21	34.5	34	35	53	60	55	-	-	16.6	-	-
25	22	38.8	25	39	20.5	32.3	21.5	36	22	35.8	45	38	65	53	49	-	-	7.5	4.5	-
26	22.5	38.8	27	38.4	20.2	30	20	32.5	22	34	44	40	78	75	48	-	-	0.6	-	1.4
27	23	39	26	39	20	30.5	21	33	21	33.7	35	40	66	69	55	-	-	4	2.5	-
28	21	40.1	26	40	21	30.2	22	33	21	35	31	41	69	69	55	-	-	2.7	0.1	-
29	23.5	39.2	25	39.5	25	31.5	22	37	20	35.5	35	45	77	55	49	-	-	-	3.5	-
30	22.5	39.6	23.5	38.2	23.5	34.2	22	35.6	19	35	34	47	53	53	54	-	1.8	7.2	7.5	-
31	26	40.5			20.5	33	20	31.8			32		65	76		-		2.4	0.6	
Total	660	1184	757	1170	688.2	1082	660.6	1017	648	1039	1292	1153	1742	2048	1662	4.2	6.5	186	97.5	33.8
Mean	21.1	38.0	25.3	39.0	22.2	35.1	21.3	32.7	21.7	34.6	42	40	56	66	55					

- =No rainfall

Appendix 5. Temperature (^{0}c) data of Werer Agricultural Research Center

Entomology Laboratory for the months of August, September and October 2006.

Date	August		September		October	
	9:00AM	3:00PM	9:00AM	3:00PM	9:00AM	3:00PM
1	26.00	27.00	26.00	29.00	30.00	31.00
2	27.00	27.50	28.00	29.90	29.90	31.90
3	27.00	28.00	29.00	31.00	29.90	30.00
4	26.00	27.00	27.90	30.00	29.00	30.00
5	26.00	27.50	28.90	31.50	29.50	30.50
6	27.00	28.00	28.90	31.00	29.90	30.50
7	27.00	27.50	28.90	30.00	30.50	31.90
8	26.90	27.00	27.00	27.00	30.50	31.90
9	26.00	27.00	27.50	29.00	30.00	30.90
10	27.00	29.00	28.00	30.00	30.00	30.50
11	27.00	28.00	28.50	31.00	29.00	29.90
12	27.00	29.00	27.90	30.90	30.00	30.50
13	25.00	27.00	28.90	31.90	28.90	29.90
14	26.00	28.00	30.00	32.50	28.90	29.90
15	26.50	29.00	28.90	30.90	28.50	29.50
16	26.00	28.00	29.00	32.00	28.90	29.50
17	27.50	29.90	29.50	30.00	28.90	29.00
18	25.90	27.00	29.50	31.00	28.50	29.00
19	27.00	29.90	29.50	31.50	28.90	29.00
20	27.00	27.00	28.90	30.90	28.90	29.50
21	28.50	31.50	30.00	30.90	28.50	29.90
22	28.90	31.00	30.00	31.00	26.90	30.00
23	27.50	29.90	29.90	30.50	27.00	29.50
24	28.00	30.00	30.50	30.90	27.90	29.00
25	28.90	31.00	29.90	30.50	28.50	28.00
26	28.00	30.00	28.90	30.00	27.50	27.50
27	28.50	29.90	29.90	30.50	27.90	28.00
28	28.50	29.00	29.50	31.00	26.00	27.50
29	28.00	31.00	29.90	30.00	26.00	27.00
30	29.00	30.50	29.90	30.50	27.00	27.50
31	27.00	30.00			25.50	27.00
Total	841.60	892.1	869.10	916.80	887.30	915.70
Mean	27.15	28.78	28.97	30.56	28.62	29.54

DEDICATED

TO

My Father - Ato Wondafrash Gossa

My Mother - W/o Yabeshawerk Tesfa

&

My Wife - W/o Wegayehu Bogale