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EFFECTS OF TWO NEEM INSECTICIDE FORMULATIONS ON THE TROPHIC INTERACTIONS BETWEEN THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.) (LEPIDOPTERA: PLUTELLIDAE) AND ITS PARASITOIDS

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April 2002

DECLARATION BY CANDIDATE

This thesis is my original work and has not been presented for a degree in any other University

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I confirm that the work reported in this thesis was carried out by the candidate under my supervision. I have read and approved this thesis for examination.

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DEDICATION

To my mother, Ms. Angelica K. Nyakana; thank you.

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ABSTRACT

The diamondback moth (DBM), *Plutella xylostella* (L.) is a pest of Crucifers and, of worldwide importance. Reliance on chemical insecticide control is not a sustainable option therefore, new pest control initiatives emphasise the use of parasitoids and biorationals such as neem insecticides. Neemros® (a powder formulation) and Neemroc EC® (an oil formulation) are two neem insecticides that are registered in Kenya for use against vegetable pests, including the DBM. However, the much needed information on their impact on DBM parasitoids is lacking. The aim of this study was to assess the responses of DBM parasitoids to the neem formulations when exposed to them directly or through the plant or host larvae. The formulations were applied as foliar sprays using either a knapsack sprayer or a hand-held mist sprayer fitted with a cone nozzle.

In field experiments, conducted during 1999 at Juja (Kenya), Neemroc EC® checked DBM infestation but Neemros® was not as effective. Three parasitoid species namely *Diadegma mollipla* (Holmgren), *Oomyzus sokolowskii* (Kurdjumov) and *Cotesia plutellae* (Kurdjumov) were recovered. Parasitism by all three species did not differ significantly between Neemros® treated and control plots but was significantly lower in the Neemroc EC® treated plots during two weeks ($F= 3.09$, $d.f= 8, 36$; $P<0.05$). The individual parasitoid species also had different parasitism levels and also responded differently to the neem insecticide treatments.

Cause of mortality analysis in DBM cohorts revealed that larval parasitoids accounted for <1% of the total mortality during the experimental period while the neem formulations accounted for between 15-26% of the total mortality.

In laboratory tests undertaken with *D. mollipla,* parasitoid adults that had direct topical contact with the neem formulations showed comparable longevity to those exposed to water (control), while exposure to Karate®, a synthetic pyrethroid caused 100% mortality in less than six hours. There were no significant differences in the proportion of hosts parasitised by the neem- and water-sprayed adults ($F= 2.40$, d.f= 2, 75; $P>0.05$) and their foraging patterns were also similar (ANOVA, $P>0.05$). Also evaluated were the olfactory responses of *D. mollipla* to plants/plant-host complexes sprayed with the neem formulations. The parasitoids were significantly more attracted to odours from the Neemros®-sprayed plants/plant-host complexes than to a blank (clean air). In contrast, parasitoid response to odours from the Neemroc EC®-sprayed plants/plant-host complexes did not differ significantly from the blank. In choice tests between infested plants sprayed with water or a neem formulation, parasitoids showed no preference for odours from water-sprayed plants over those from Neemros®-sprayed plants but showed a preference for the odours from the water-sprayed plants over those from Neemroc EC®-sprayed plants. In host acceptance and suitability tests, parasitism rates in neem- and water-

sprayed hosts exposed to parasitoids at 24 hrs and 48 hrs after treatment were, with one exception, not significantly different. A high mortality level in the neem-treated parasitised hosts suggested their unsuitability for parasitoid development. It is concluded that the neem formulations may be relatively safe to adult DBM larval parasitoids at the doses used in the study. The establishment of plant refugia that would harbour untreated DBM larvae and, thus help to perpetuate the parasitoid population could offset the deleterious effects observed on parasitoid development.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AVRDC	Asian Vegetable Research and Development Centre
AZA	Azadirachtin
DBM	Diamondback moth
d.f	Degrees of freedom
IGR	Insect growth regulator(s)
KARI	Kenya Agricultural Research Institute
NKCP	Neem kernel cake powder
NSKE	Neem seed kernel extract(s)
NSO	Neem seed oil
SAS	Statistical Analysis System software
s.e.	Standard error
SNK	Student-Newmann-Keuls multiple range test
WAT	Week(s) after transplanting

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 GENERAL INTRODUCTION

Crucifers are important crops wherever they are cultivated but their production is severely constrained by the diamondback moth (DBM), *Plutella xylostella* (L.) and other pests. The development of insecticide-resistance in DBM populations has resulted in a more intensive and often inappropriate use of the chemical insecticides that are currently available for containing the pest (Osterman & Dreyer, 1995). This has given rise to concerns about the health risks posed by insecticide residues on vegetables and calls for the development of integrated pest management (IPM) strategies that farmers can use to combat the pest problem without compromising product quality.

Integrated pest management techniques seek to reduce pest-induced losses by managing the agronomic, biological and socio-economic components and interactions of the entire system. An understanding of how these different components function and interact with each other is crucial when developing an effective IPM programme. The conservation and enhancement of populations of natural enemies are cornerstones of successful IPM programmes (Stehr, 1975; Waage, 1989) as they reduce populations of primary pests, limit pest damage and keep secondary pests

below the economic threshold. Pesticide applications should therefore be kept to the minimum to ensure no or only minimal harm to natural enemies (Hassan, 1989; Gerling & Naranjo, 1998). Selective pesticides are therefore indispensable tools in IPM for achieving satisfactory control of pests (Schmutterer, 1997) without harming natural enemies.

Neem insecticides are promising candidates for pest management in many ecosystems, including cabbage. Considerable research has confirmed their bioefficacy against pests in a variety of crops (Schmutterer *et al.*, 1981; Schmutterer & Ascher, 1984, 1987; Schmutterer, 1995). Neem insecticides are typically a complex of several molecules, the most potent of which is azadirachtin. These insecticides are often competitively priced and, they do not leave potentially harmful residues on the produce. They are therefore a more acceptable alternative to the use of chemical insecticides on vegetables. Although neem's efficacy against the diamondback moth has been confirmed (Kirsch, 1987; Schmutterer, 1992; Isman, 1995), little is known about its effects and/or interactions with the natural enemies of this pest. Knowledge of the potential of natural enemies in regulating pest numbers and their susceptibility to insecticide treatments proposed for use in the field is important for developing a successful IPM programme (Gerling & Naranjo, 1998). Therefore, neem-based insecticides have to be evaluated for their effects on natural enemies and other ecologically important organisms present within the vegetable ecosystem.

The aim of this study was to investigate whether some neem formulations used for the management of the diamondback moth have any adverse effects on the parasitoids of this cabbage pest. The parasitoids are also a component of the IPM programme for diamondback moth. It is hoped that the results of the study will provide useful information that is necessary in developing cost-effective IPM programmes that assure a more compatible use of neem insecticides and parasitoids of the diamondback moth.

1.2 LITERATURE REVIEW

1.2.1 Status of pest management in vegetable based agrosystems

Rapid human population growth in the developing countries of the tropics and the subtropics has seen a huge increase in the demand for supplies of fresh vegetables and fruits. This has facilitated the almost year round cultivation of vegetables either on small-scale peri-urban gardens or large scale commercial farms to satisfy the demand. Vegetables are important supplementary sources of food and nutrition for many urban dwellers and one of the major sources of cash for small-scale farmers who often are the major producers.

Crucifers are one of the most important vegetables grown worldwide and the most important in Kenya. Their continuous cultivation has greatly encouraged the build-up of the associated pest complex of which the

diamondback moth [DBM] is the most notorious and the most difficult to control (Talekar & Shelton, 1993; Kibata, 1996). To ensure adequate yields and produce damage-free vegetables for the urban markets, farmers have been forced to apply ever more frequently increasing dosages and/or cocktails of chemical insecticides (Kibata, 1996). In Southeast Asia, where a similar problem prevails, DBM populations have developed resistance to most of the major classes of synthetic insecticides, thereby forcing farmers into pesticide dependency (Talekar & Shelton, 1993). Other insects previously listed as minor pests of Crucifers have gained major status largely because their natural enemies have been wiped out (AVRDC, 1992). This has led to the risk of the produce carrying insecticide residues beyond the maximum residue limits (MRLs), environmental contamination with insecticides and increased production costs.

An integrated management strategy for DBM control incorporating ecological, economic and sociological considerations remains the only option and is already being actively pursued in South-east Asia (AVRDC, 1992; Mengech *et al.,* 1995).

1.2.2 Economic Significance of Crucifers in Eastern Africa

Crucifers cultivated in the tropics include common cabbage (*Brassica oleracea* L. var. *capitata* L.), kale (*B. oleracea* L. var. *acephala* (DC) Alef.), Chinese cabbage (*B. chinensis* L.), cauliflower (*B. oleracea* L.

var. *botrytis* L.), broccoli (*B. oleracea* L. var. *italica* Plenck), rapeseed (*B. napus* L.), turnip (*B. oleracea* L. var. *rapa* L.) and radish (*Raphanus sativus* L.) (Tindall, 1983; Talekar & Shelton, 1993). In East Africa, cabbage and kale are the most important and are mostly grown by resource-limited farmers on relatively small gardens (Kibata, 1996). Nevertheless, annual production of these two crops in Kenya was just under 540,000 metric tonnes in 1998, with an estimated value of approximately $2 million (MALDM, 1998).

Cabbage is an important food crop rich in vitamins (A, the B complex, C, D) and minerals (iron, phosphorus, calcium) and low in calories (Tindall, 1983). In East and Southern Africa, DBM and aphids, [*Brevicoryne brassicae* (L.), *Myzus persicae* (Sulzer), and *Lipaphis erysimi* (Kalt.)] are key insect pests but other economically important pests include the cabbage webworm (*Hellula undalis* [Fabricius]) and the bagrada bug (*Bagrada hilaris* [Burmeister]) (Anon., 1995; Nyambo & Pekke, 1995; Oduor *et al.*, 1996; Varela *et al.*, 1999). Control measures are primarily targeted at DBM because it attacks (and if not checked, skeletonises 50-100% of) the crop from the seedling stage; aphids occur later in the season and infestations only become serious in the absence of rain.

1.2.3 The Diamondback moth - Life History, Pest Status and Management

1.2.3.1 Lifehistory

The diamondback moth (DBM), *P. xylostella* (Lepidoptera: Plutellidae) is a cosmopolitan pest that usually feeds and oviposits on members of the family Cruciferae. Members of the Cruciferae contain mustard oils and glucosinolates or their derivatives that are usually toxic to a large majority of generalist insect feeders but stimulate gustatory and oviposition responses in the DBM (Nayar & Thorsteinson, 1963; Taleker & Shelton, 1993; Verkerk & Wright, 1996). The DBM has also been recorded feeding and ovipositing on snowpeas (Leguminosae) in Kenya (B. Löhr, ICIPE, pers. comm.). It is widely believed that the DBM evolved in Europe probably around the Mediterranean region with the indigenous Crucifers (Talekar & Shelton, 1993), but Kfir (1998) is of the opinion that the species could have evolved in Southern Africa. Whatever its ecological origins, DBM's ability to tolerate a wide range of climatic conditions has enabled the species to establish itself in the temperate, subtropical and tropical regions of the world. It is especially serious in those regions where Crucifers are cultivated continuously and chemical insecticides are relied upon heavily for control.

The adult moth is about 8mm in length, grey-brown with a characteristic diamond pattern on its back from which it derives its name (Plate 1.1). The moths are commonly active towards dusk, when they mate and/or oviposit. Under captive conditions the female moth may live for up to 16 days during which she may lay more than 400 eggs. The pale yellow eggs are oviposited singly or in clusters of 2-4 along the leaf veins on the upper and/or lower leaf surfaces and have an incubation period of 2-4 days. First instar larvae mine into the leaf where they feed on the spongy mesophyll for two days and then emerge as second instars. The second, third and fourth instars each lasts for 2 days followed by a prepupal period of one day. Pupation may last for 7-11 days (Plate 1.2). Mating takes place soon after emergence and oviposition follows immediately, with peak oviposition occurring on the day of emergence. Development times are dependent on temperature but are shorter at higher temperatures (Shirai, 2000). The lifecycle may be completed in 20 days or less in warm climates (25-30°C) but is longer (25-40 days) at cooler temperatures (≤21°C) (Finch & Thompson, 1992; Keinmeesuke *et al.*, 1992; Wakisaka *et al.*, 1992; Shirai, 2000). Consequently there may be 15 or more generations per year in the tropics (Liu *et al.*, 1981; Keinmeesuke *et al.*, 1992; Varela *et al.*, 1999).

Plate 1.1: Diamondback moth adult

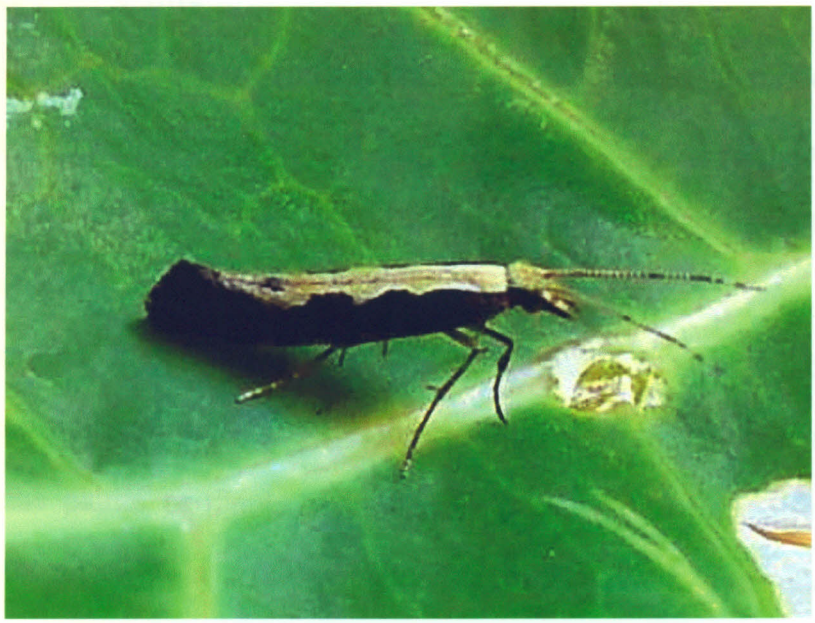
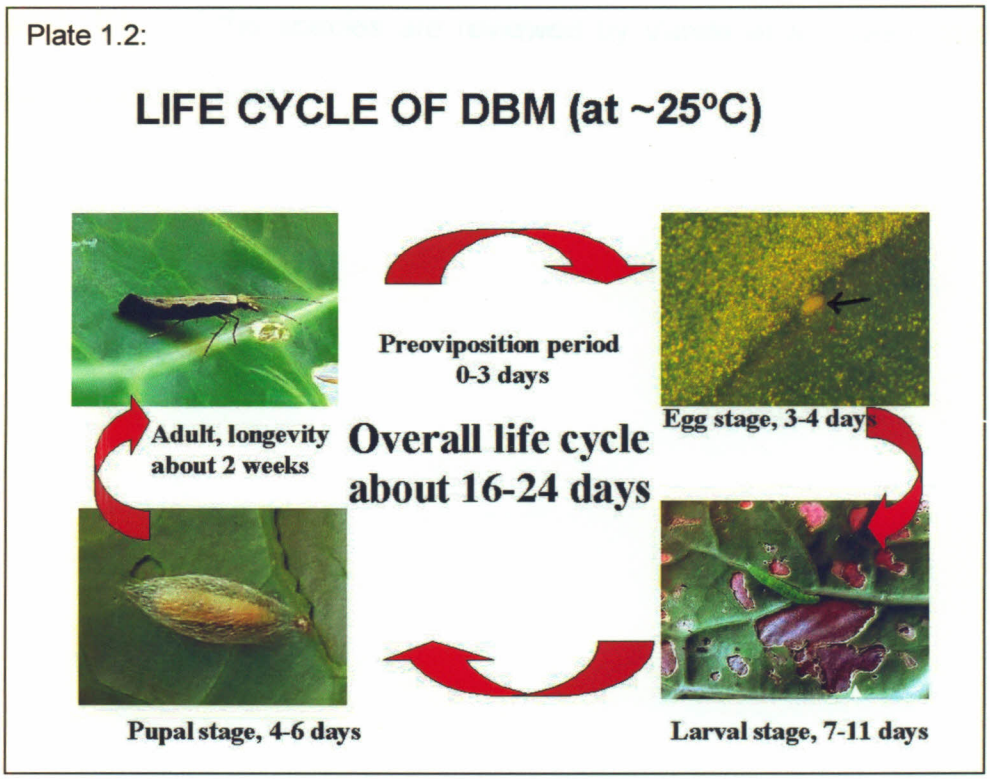


Plate 1.2:



1.2.3.2 Pest Status

All larval instars are destructive but the greatest damage is caused by the third and fourth instars (Plate 1.3). These eat away the lower tissues of the leaf and leave the upper epidermis intact, which gives the leaf the appearance of "windows". With time the "windows" tear and leave perforations in the leaves so affected (Plate 1.4). Early crop damage reduces the plant's photosynthetic capacity and yield, and may result in headless plants or plants with multiple undersized heads. Late damage may not affect yield but the perforated outer leaves lower the cosmetic quality and hence the market value of the crop. More aspects of the biology and pest status of the species are reviewed by Varela *et al.* (1999) and Talekar & Shelton (1993).

Plate 1.3: Diamondback moth larvae (arrows) feeding on cabbage leaf

Plate 1.4: Diamondback moth damage on cabbage [*note characteristic "windows" on leaves*]

1.2.3.3 Management

Management of DBM is heavily reliant on the use of chemical insecticides especially in the developing countries of the tropics (Tabashnik *et al.*, 1987). Worldwide, the annual cost for managing the pest is estimated at $1 billion (Talekar & Shelton, 1993). Intensive insecticide use has decimated DBM natural enemies (Idris & Grafius, 1993a) while DBM has developed resistance to most of the major classes of chemical insecticides (Liu *et al.,* 1981, 1982; Idris & Grafius, 1993b; Syed & Fauziah 1996). Botanicals and insect growth regulators (IGRs) are alternative control measures but it is possible that DBM may develop resistance to them too (Völlinger, 1995). There are already reports of resistance to some *B.t.* (*Bacillus thuringiensis*) formulations (Kirsch & Schmutterer, 1988; Tabashnik *et al.,* 1990, 1992a, 1992b) and IGRs (Perng *et al.,* 1988; Kobayashi *et al.,* 1992). Increasing production costs largely attributed to pest control of DBM have forced a reassessment of the management strategy for the species. The trend now is to adopt an integrated and ecologically compatible management package incorporating the use of microbial pesticides such as *B.t.,* botanicals such as neem insecticides, biological control and cultural methods such as intercropping with trap crops and minimising the use of chemical insecticides. The components of such an integrated control package should act interactively in suppressing the DBM population while slowing down the evolution of insecticide

resistance and conserving natural enemies (Tabashnik *et al.,* 1987). For instance, Liu *et al.* (1981) found that where insecticide pressure was low, DBM populations showed only low levels of resistance or none to synthetic pyrethroids.

1.2.4 Biological control of DBM

1.2.4.1 Parasitoids of DBM - a summary

The potential for biological control of DBM lies in the use of key parasitoids, which are important natural checks. About 40 parasitoids of DBM are known but only five show any real potential as biological control agents; these are *Diadegma* spp., *Cotesia plutellae* (Kurdjumov), *Oomyzus sokolowskii* (Kurdjumov), *Microplitis plutellae* (Muesebeck) and *Diadromus* spp. (Lim, 1986; Talekar & Shelton, 1993; Anon., 1995; Verkerk & Wright, 1996; AVRDC, 1997). Fitton & Walker (1992) reviewed the taxonomy of the hymenopterous parasitoids of DBM. *Diadegma* is an ichneumonid solitary endoparasitoid that preferentially attacks the second larval instar. *M. plutellae* and *C. plutellae* are braconid solitary endoparasitoids of subfamily Microgastrinae and prefer to attack the second and third larval instars of DBM. *C. plutellae* is the more predominant parasitoid in regions of higher temperatures (26-30°C) and could be the major biological control agent in such regions (Talekar, 1992). *Diadromus* spp. are pupal parasitoids in the ichneumonid subfamily Ichneumoninae; *Diadromus collaris* (Gravenhorst)

has been widely introduced in biological control efforts. *O. sokolowskii* is a gregarious primary and facultative hyperparasitoid that belongs to the superfamily Chalcidoidea. It is the only chalcidoid that shows any real potential for biocontrol of DBM despite its hyperparasitic tendencies. Other chalcidoids such as *Tetrastichus howardii* Olliff (also known as *T. ayyari* and *T. israeli*) and the egg parasitoids *Trichogramma* and *Trichogrammatoidea* (both of family Trichogrammatidae) also occur but show little potential as biocontrol agents for DBM (Fitton & Walker, 1992; Talekar & Shelton, 1993).

1.2.4.2 Diadegma spp. (Hymenoptera: Ichneumonidae, Campopleginae)

Wasps of the genus *Diadegma* are solitary endoparasitoids of DBM. Several species of *Diadegma* are known to occur but the taxonomic dilemma surrounding the genus suggests that many of the species are synonyms of each other. The genus includes the species *D. semiclausum* Hellén, *D. fenestrale* (Holmgren), *D. insulare* (Cresson), *and D. rapi* (Cameron) (Fitton & Walker, 1992). The species of *Diadegma* in sub-Saharan Africa has been identified as *Diadegma mollipla* (Holmgren) (Azidah *et al.,* 2000). *D. semiclausum* and *D. insulare* have shown the greatest potential for biological control in South-east Asia and the United States and Canada, respectively (Harcourt, 1986; Talekar, 1992).

Diadegma spp. attacks any of the four larval instars but more readily parasitises the second instar (Waage & Cherry, 1992). The parasitised host continues to feed and develop until pupation, when the developing parasitoid within it finally kills it and emerges to spin its own cocoon within that of the host. The developmental period is temperature dependent but is about 10-15 days at 25°C and the female parasitoid may live for more than 20 days when fed (Grafius, 1997).

Diadegma spp. is most effective at cooler temperatures (15-25°C) such as those characteristic of highland areas (Talekar & Shelton, 1993). Parasitism varies from less than 10% to over 80% in various parts of the world (Harcourt, 1986; Idris & Grafius, 1993b; Talekar & Shelton, 1993; Grafius, 1997). The parasitoid is very susceptible to chemical insecticides but readily establishes itself when chemical insecticides are applied infrequently.

1.2.5 Neem insecticides

Neem-based insecticides are promising candidates for pest management in many ecosystems, including cabbage. The insecticides are derived from the evergreen neem tree, *Azadirachta indica* A. Juss, which belongs to the Meliaceae (Mahogany) family. The tree is believed to have its origins in Southern and South-eastern Asia but today occurs throughout tropical and subtropical Asia, Africa, Australia, and Central and South

America (Schmutterer, 1990, 1995). The bark, leaves and seeds (drupes) all contain the active compounds found in neem insecticides but the seed kernels have the highest concentration of these, and are the main source. The most potent of these active compounds is Azadirachtin (aza), a steroid-like tetranortriterpenoid, which occurs as eleven isomers, but other chemical groups also occur (Schmutterer, 1990, 1995). This complex of active compounds in neem insecticides is responsible for the range of deleterious effects, which include killing, repellence, feeding and oviposition deterrence and growth disruption, against insects.

Azadirachtin's mode of action at the cellular and molecular level has been elucidated (Rembold *et al.*, 1984; Rembold, 1995). Within the insect body, azadirachtin is rapidly excreted and only a small fraction is retained. This fraction concentrates in the neurolemma of the brain and associated endocrine glands (the corpora allata and corpora cardiaca) and works by depressing the synthesis of neurohormones and their release into the haemolymph. The documented effects of neem insecticides (disrupted moulting, inhibited oogenesis, morphological defects) are a consequence of this disruption to the insect's neuroendocrinal controls.

Although neem insecticides have been used by farmers for pest control in Asia for centuries, their use has until recently been perceived as a 'backward practice' especially by the more affluent farmers in the region (Raheja, 1995). With increased awareness of IPM, consumer concerns

about pesticides residues in food and problems associated with excessive use of synthetic chemical pesticides, neem insecticides are increasingly regarded as an important input to IPM systems. Large-scale adoption of use of neem insecticides is however severely constrained by the limited availability of effective and standardised formulations that a farmer can rely on for pest control. As these become more readily available, neem insecticides are likely to become more widely adopted as viable alternatives to the use of synthetic chemical insecticides (Raheja, 1995).

1.2.6 Effects of neem formulations on natural enemies

Neem insecticides are known to adversely affect about 413 species/subspecies of insect pests in 15 orders (Schmutterer & Singh, 1995) but compared to synthetic insecticides are relatively harmless to their natural enemies, especially the adult stage (Schmutterer, 1990, 1997).

The antifeedant, anti-ovipositional, repellent, mortality and/or insect-growth regulating properties of neem insecticides have been demonstrated in various species of natural enemies. Spiders and mites are either not susceptible or are the least susceptible to neem insecticides [neem seed oil (NSO), neem seed kernel extracts (NSKE)] at concentrations ranging from 0.5-5% and azadirachtin-enriched products (Saxena *et al.*, 1984; Mansour *et al.*, 1987; Saxena, 1987). However, the development and the

fecundity of the predatory mite, *Phytoseiulus persimilis* (Athias-Henriot) was negatively influenced when its eggs were treated with various neem products.

No or only negligible mortality has been recorded in predators such as beetles (coccinellids, carabids), lacewings and ants exposed as adults to sprays of neem extracts (NSO, NSKE) or azadirachtin-enriched products (AZT-VR-K, MTB/H_2O-VR-K). Exceptions are syrphid flies which are more sensitive to the toxic effects of neem (Schmutterer, 1995). Studies in which larvae, pupae or nymphs have been exposed to direct sprays and/or residues of neem insecticides or through their food have recorded such adverse effects as increased mortality, disrupted development and morphogenetic defects. For example, Margosan-O (an oil-based neem formulation) applied to third instar nymphs of the heteropteran stinkbug, *Perillus bioculatus* (F.) caused delayed molting and morphogenetic defects (Hough-Goldstein & Keil, 1991). Larvae of the coccinellid, *Coccinella septempunctata* L. sprayed with Neemix, an enriched neem product, showed increased mortality and morphogenetic defects (Banken & Stark, 1997). In other parasitoid species, the adults are not killed when exposed to neem insecticides but their progeny are more susceptible especially when their hosts are fed on a diet containing the insecticides (Lamb & Saxena 1988; Raguraman & Singh, 1998).

With respect to the repellent and oviposition deterrent properties of neem insecticides, parasitoids are generally not deterred from attacking hosts treated with neem insecticides although their progeny may fail to develop or successfully emerge from such hosts. For example, NSKEs (2-3%) applied to egg masses did not deter the egg parasitoid, *Telenomus remus* Nixon from attacking its host (Joshi *et al.*, 1982) while *Bracon hebetor* Say, a gregarious larval parasitoid of Lepidoptera, parasitised host larvae treated with NSKEs (Raguraman & Singh, 1998). Saxena *et al.* (1981a) recorded doubled rates of parasitism by ichneumonid, braconid and encyrtid parasitoids in rice fields sprayed with NSO. The study by McCloskey *et al.* (1993) on the ichneumonid parasitoid *Erioborus (=Diadegma) terebrans* (Gravenhorst) showed that it readily attacked hosts fed on a diet containing azadirachtin but the emerging parasitoids had reduced pupal and adult weights, longer developmental periods prior to emergence and higher mortality, especially after emergence.

Disrupted development, low or no emergence and subsequent reduced vitality of parasitoids developing in hosts exposed to direct sprays of neem insecticides or through neem-treated diets has been reported for several species such as *Bracon brevicornis* Westm. (Srivastava *et al.*, 1997), *C. plutellae* (Loke *et al.*, 1992), *Goniozus triangulifer* Kieffer (Lamb & Saxena, 1988), *Tetrastichus howardii* (Olliff) (Lamb & Saxena, 1988; Fernandez *et al.*, 1992). In contrast, the development and emergence of

several aphid parasitoids, *Lysiphlebus testaceipes* (Cresson), *Aphelinus asychis* Walker, *Encarsia formosa* Gahan, *E. transversa* Smith, *Diaretiella rapae* (McIntosh) and *Ephedrus cerasicola* Stary were not disrupted when parasitised mummies were dipped in or sprayed with various neem products (Margosan-O, MeOH-NR, AZT, MTB) (Feldhege & Schmutterer, 1993; other citations by Schmutterer, 1995). Schneider and Madel (1991) (cited in Schmutterer, 1995) observed normal fecundity and activity in *Diadegma semiclausum* females that had developed in hosts previously treated with NSKE (0.1-5%).

NSO formulations apparently have more pronounced effects on the natural enemies than NSKE formulations, and alcoholic NSKEs appear to give stronger effects than the water-based extracts (Schmutterer, 1995, Srivastava *et al.*, 1997). Mixtures of neem products with other compounds may also increase the side effects on beneficial organisms than with the use of neem alone (Schmutterer, 1997). For example, higher mortality was recorded in the armyworm, *Spodoptera frugiperda* (J.E. Smith) and the mosquito, *Aedes togoi* (Theobald) when a mixture of a neem insecticide and *Bacillus thuringiensis* (*Bt*) was applied than with either component alone (Hellpap & Zebitz, 1986; cited in Schmutterer, 1995).

1.2.7 Pesticide-parasitoid interactions and implications for pesticide evaluations

Parasitoids function within a multitrophic context, using cues from different trophic levels of agroecosystems to locate and attack their hosts (Vet & Dicke, 1992). These cues are often chemical substances but may also be of a physical nature such as silk, body size or movement (Quicke, 1997). The chemical cues may derive from the host (=herbivore) such as volatiles from frass, or its food (the plant) such as the volatiles emitted during herbivore feeding. Thus, any disruption to the sources of these cues could impact negatively on the efficiency and evolutionary fitness of the parasitoid species. Disruption to the normal host-parasitoid synchrony may be caused by the adverse effects of chemical toxicants on adult female parasitoids, which may either be killed, or their searching ability is severely impaired. The pesticide may also selectively eliminate the host stage that would otherwise be available and susceptible to attack by searching females and thus result in the local emigration or local extinction of the natural enemies (Doutt *et al.*, 1976; Waage, 1989). Since parasitoids have specific rules that govern attraction to and residence time within a host patch (Godfray, 1994), changes in host distribution and/or density following pesticide applications could influence whether a parasitoid will be attracted to or arrested within a host patch (Waage, 1989).

The effects of pesticides on insects are a function of formulation of the pesticide, its toxicity and mode of exposure. Banken and Stark (1997) argued that a realistic assessment of the effects of a pesticide on natural enemies could only be made if they were exposed to the pesticide through the same routes of exposure as they would under field conditions. Natural enemies in the field are exposed to pesticides through two or more routes such as contact with direct sprays or indirectly through contact with surface residues and contact with or consumption of contaminated hosts/prey. The effects arising from such indirect contact and those that are expressed in the progeny of exposed natural enemies are often termed "sublethal effects".

The sublethal effects of pesticides that directly affect parasitoid functioning can be categorised into behavioural and physiological mechanisms (Elzen, 1989). Behavioural effects include alteration of foraging pattern or efficiency, disruption of sexual communication or lack of host recognition. Physiological effects manifest as altered reproduction, reduced longevity, egg viability and fitness. The fitness of a parasitoid population is maximised by the number of viable progeny that can be produced by each individual. This requires that ovipositing females not only maximise the number of hosts that they attack but also select high quality hosts. Such hosts are those capable of sustaining the development of parasitoid progeny and further ensuring parasitoid fitness by, for example,

giving rise to individuals with high fecundity. It is therefore important to take into consideration the sublethal effects of pesticides because they may ultimately result in reduced population numbers of the parasitoid or its effectiveness in suppressing host population numbers (Elzen, 1989).

For the new generation of insecticides such as botanicals and insect growth regulators (IGRs) that are slow acting and whose effects may be expressed several days and/or generations after initial exposure, the assessments should be extended to longer post-treatment periods and also to the subsequent generation. The need for such assessments was demonstrated by Rumpf *et al.,* (1997) who showed that within a 24-48 hr post-exposure period, the IGRs under evaluation could be classified as harmless but at a 120-360 hr post-exposure period, the IGRs were harmful. In contrast, the organophosphates (an example of conventional chemical insecticides) were acutely toxic within a 24 hr post-treatment period. Thus, the mode and period of exposure as well as sublethal effects in the target and subsequent generation must be taken into account when evaluating the effects of such biorational insecticides like neem-based products.

The effects of neem insecticides on insects include repellency, feeding and oviposition deterrence, disrupted development in addition to mortality (Schmutterer, 1995). Such effects have implications for parasitoid functioning and evolutionary success and their evaluation is best done within the context of trophic relations. Verkerk & Wright (1996) argued that

the effectiveness of an IPM package for DBM could be enhanced if trophic interactions were taken into consideration.

1.3 JUSTIFICATION AND RATIONALE OF THE STUDY

Sole reliance on chemical insecticide control of DBM is no longer viable because of the DBM's high capacity for developing insecticide resistance, spiralling costs of developing new insecticides, environmental risks posed by insecticide residues in the environment and near-zero tolerance for pesticide residues on food items in the markets. An ecologically compatible DBM management strategy that incorporates the use of parasitoids, biorational insecticides such as botanicals and rational use of synthetic chemical insecticides (Schmutterer, 1992; Taleker & Shelton, 1993; Verkerk & Wright, 1996) is urgently required.

The efficacy of neem insecticides against the DBM has been confirmed in a number of studies (Dreyer, 1987; Fagoonee, 1987; Schmutterer, 1992). So too has the efficacy of DBM larval parasitoids, particularly in North America and Southeast Asia (Ooi, 1992; Poelking, 1992; Talekar & Shelton, 1993). Incorporating neem insecticides and parasitoids into an IPM programme for the DBM in Eastern Africa requires information on the effects of neem insecticides on DBM larval parasitoids but only very limited information exists on this aspect. For instance, Schmutterer's reviews (1990, 1995, 1997) on the non-target effects of

neem insecticides include 17 citations on parasitoids out of which only three (McCloskey *et al.*, 1993; and Schneider & Madel, 1991; Klemm & Schmutterer, 1993 cited in Schmutterer, 1995) are specifically on DBM parasitoids.

Many studies assessing the non-target effects of neem insecticides have concluded that they are harmless to natural enemies, largely based on the International Organisation of Biological Control's [IOBC] protocols for judging insecticide safety (Hassan, 1989). The IOBC protocols were, however, designed to evaluate the effects of faster-acting chemical pesticides and may therefore be inappropriate for neem-based insecticides that have a more subtle effect on insect physiology and life history parameters.

The concentrations of the active ingredients in the raw materials from which neem insecticides are formulated differ from region to region. Additionally, different neem insecticide products are formulated differently. The azadirachtin content in commercial neem formulations is often standardised but since no universal standard exists, it is best to evaluate new formulations as and when they appear. Thus, the results of studies that assessed the non-target effects of neem insecticides using one neem product [or on organisms in a different region] are not readily applicable to a differently formulated product [or organisms in an entirely different region]. The goal of the present study was to investigate the effects of

Neemroc EC® and Neemros® on DBM larval parasitoids in Kenya. These neem insecticides are approved for use in Kenya against horticultural pests including the DBM. Field studies were conducted to obtain an overview of parasitism trends in the field under a neem insecticide application regime, and generate hypotheses for testing under more controlled laboratory conditions. The laboratory tests investigated specific properties of neem insecticides as they relate to parasitoid behaviour and functioning within the context of trophic interactions.

1.4 HYPOTHESES

1. Neem does not affect the level of field parasitism in DBM larval populations.
2. There is no difference in the proportion of hosts parasitised, host searching behaviour and survival of neem-treated and untreated parasitoids.
3. Parasitoid response to stimuli from neem-treated and untreated host plants is the same
4. There is no difference in the proportion of hosts parasitised in neem-treated and untreated hosts.
5. Neem has no effect on the survival, host searching behaviour, development and fitness of parasitoid progeny.

1.5 GENERAL OBJECTIVE

Assess the effects of two neem insecticide formulations on DBM parasitoids at different trophic levels in cabbage.

1.5.1 Specific Objectives

1. To evaluate the effect of neem insecticide applications on parasitism as a larval mortality factor in DBM populations under field conditions.
2. To assess the effects of the neem insecticides on the survival, parasitisation ability and host searching behaviour of *D. mollipla*.
3. To assess the [host-finding] response of *D. mollipla* to stimuli from neem-treated cabbage plants.
4. To establish whether *D. mollipla* attacks and develops normally in neem-treated DBM larvae (= host acceptance and suitability).
5. To assess the effects of the neem insecticides on the development and fitness (longevity, sex ratio, egg viability and parasitisation ability) of *D. mollipla* progeny.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 NEEM FORMULATIONS

The test neem formulations, Neemroc EC® and 'Neemros®, used in the study were obtained from Saroneem Ltd., Nairobi. Neemroc EC® is an emulsified oil [=neem seed oil, NSO] derived from neem seeds and contains the active ingredient azadirachtin at a concentration of 0.03% w/w. Neemros® is a neem kernel cake powder [NKCP] derived from neem seeds after oil extraction and, contains azadirachtin at a concentration of 0.5% w/w. Unless otherwise stated, the formulations were applied as foliar sprays at the field dose rate of 25gm/l of water for Neemros® (≈2.5% NKCP) and 15ml/l of water for Neemroc EC® (≈1.5% NSO) recommended for DBM control.

2.2 PLANT GROWING AND INSECT REARING TECHNIQUES

2.2.1 Plants

Cabbage seedlings (*B. oleracea* var. *capitata* cv Copenhagen Market) were raised in seed planters within a screened cage using recommended agronomic practices for brassica production (East African Seed Company). They were transplanted when four weeks old to plastic pots (15 cm diameter) filled with potting soil [2 parts soil: 1 part sand: 1 part

organic manure]. The plants were used in insect rearing and in the bioassays when six to eight weeks old. The leaves of such plants were tender enough to facilitate larval feeding* and the plants also had enough leaf biomass to sustain a large number of larvae to pupation.

2.2.2 Diamondback moth (DBM)

Adult moths were reared from field-collected fourth instar larvae and pupae and released into a clear Perspex rearing cage (measuring 20 x 20 x 40cm and fitted with mesh covered windows to facilitate ventilation) in the insectary. The cage contained a cabbage leaf (immersed in a water-filled glass vial) on which the moths oviposited. Moths were maintained on a 10% sugar solution impregnated in a piece of cotton wool which was changed after every 1-2 days. The moths were similarly provided with distilled water. The oviposition leaf was replaced with a fresh one after 48 hr to ensure a uniform age group of hosts. The eggs were disinfected in a 10% formaldehyde solution for 15 min, rinsed off thoroughly under running tap water for 30 min and held in a covered petri dish lined with moist filter paper until they hatched. The oviposition leaf bearing newly hatched first instar larvae was then placed on a cabbage plant to which the larvae migrated. The larvae were then reared to the adult stage to repeat the rearing cycle or used to rear the parasitoids as described in section 2.2.3.

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* it had been observed that young larvae tended to migrate from older plants probably because these had tougher leaves that were more difficult to feed upon.

Rearing of larvae to the adult stage was done at ambient insectary conditions (22-26°C) and a 14L:10D photoperiod.

2.2.3 *Diadegma mollipla*

D. mollipla was used as the model parasitoid species for the bioassays. This decision was based on the proposed utilisation of this species and/or *D. semiclausum* in a DBM-IPM programme being developed for Eastern Africa (B. Löhr, pers. comm.). Species of *Diadegma* have shown the highest parasitism rates on DBM (Alam, 1992; Ooi, 1992; Poelking, 1992; Mustata, 1992) and are the most promising candidates for biological control-based programmes for the DBM (Talekar & Shelton, 1993).

The laboratory-reared colony was initiated from field collected DBM fourth instar larvae, prepupae and early pupae [still green in colour]. These were maintained in the laboratory at ambient conditions (21-24°C; 5-20% R.H; 14L:10D photoperiod) until host [DBM] or parasitoid emergence. Dr. Mike Fitton of the Natural History Museum [London, England] identified the *Diadegma* species used in this study as *D. mollipla*. A cabbage plant heavily infested with insectary-reared second and early third instar DBM larvae (that is with up to 300 hosts) was exposed to mated *D. mollipla* females for 24-36 hours within a ventilated, clear Perspex cage (measuring 20 x 20 x 40cm). The parasitised hosts were placed onto two to three fresh

cabbage plants and reared to the pupal stage in the laboratory. Two to three parasitoid pupae were placed in a small homeopathic glass vial wherein the adults emerged. The *D. mollipla* adults were released into a Perspex cage at a ratio of 3 males : 1 female and used to perpetuate the colony as described above. The parasitoids were maintained on a 20% honey solution and distilled water. These were impregnated in cotton wool which was changed after every 1-2 days. The DBM and parasitoid colonies were regularly supplemented (approximately every 2-3 months) with field collected individuals so as to minimize a loss in fitness and genetic diversity under laboratory rearing conditions.

2.3 BIOASSAYS

The laboratory bioassays were conducted to investigate any lethal and sublethal effects of the neem formulations on an important parasitoid (*D. mollipla*) and, were done within the context of multitrophic interactions. Host larvae were used in the bioassays when they attained the second instar stage. Mated, naïve, three to five day-old *D. mollipla* females were used for the laboratory tests. A hand-held mist sprayer fitted with a cone nozzle was used to spray the neem formulations onto the test materials. The bioassays were conducted in the laboratory at ambient conditions of temperature (range 21-24°C) and relative humidity (range 5-20%). Details

on the various bioassays are outlined in the materials and methods section of each chapter.

2.4 DATA ANALYSIS

The Statistical Analysis System (SAS) for windows software (v6.12) was used for data analysis (SAS Institute, 1989-1996). The level of significance was set at $P<0.05$ for all tests. The Student-Newmann-Keuls (SNK) multiple range test was used to separate means where significance was indicated. Details of the specific statistical tests used are given in the materials and methods section of each chapter.

CHAPTER 3

IMPACT OF THE NEEM FORMULATIONS ON DBM LARVAL INFESTATION AND PARASITISM LEVELS UNDER FIELD CONDITIONS

3.1 INTRODUCTION

The diamondback moth is host to several parasitoid species (Fitton & Walker, 1992; Talekar & Shelton, 1993; Kfir, 1998) some of which effect substantial parasitism of DBM populations elsewhere (Harcourt, 1986; Poelking, 1992; Idris & Grafius, 1993a; Talekar & Shelton, 1993; Grafius, 1997; Liu *et al.*, 2000). Such levels of parasitism suggest that these parasitoids could be used for the control of DBM.

DBM populations in Kenya are predominantly parasitised by *Diadegma* sp., and *O. sokolowskii* (Oduor *et al.,* 1996), although there are other parasitoid species (Nyambo & Pekke, 1995). Larval parasitoids and the neem formulations, Neemroc EC® and Neemros®, are envisaged as components of an IPM package for DBM in Kenya but little is known about the compatibility of the two pest control options. Luck *et al.*, (1988) and Gerling & Naranjo (1998) stated that the potential of natural enemies in controlling a pest population and their susceptibility to insecticide treatments should be evaluated and taken into account when developing an IPM programme for a pest. Experimental methods and lifetables are two techniques that can be used to evaluate the impact of natural enemies on

the host/prey population. Experimental techniques quantify and compare the mortality caused by a natural enemy (such as parasitism levels) in relation to other factors such as insecticide treatments or infestation levels. They do not however account for other sources of mortality such as predation and diseases that act together with the natural enemy (Bellows *et al.,* 1992). The study reported here used the experimental approach to evaluate the effect of the neem formulations (Neemros® and Neemroc EC®) on the activity of DBM larval parasitoids in cabbage under field conditions.

Parasitoid activity was measured as percent parasitism, a parameter that has been widely used in studies assessing natural enemy efficacy and/or what impact insecticides have on the functioning of parasitoids over time (van Driesche, 1983; Mills, 1997). This measure is compared for plots that had natural enemies or were sprayed with insecticides and those in which natural enemies were excluded or remained unsprayed. To minimise the errors associated with this technique (van Driesche, 1983; Waage & Cherry, 1992), percent parasitism should be estimated on a host stage between that which is attacked and that from which the parasitoid emerges (Waage & Cherry, 1992; Mills, 1997). For DBM, this stage is the late fourth instar/prepupa if larval parasitism is being measured (Waage & Cherry, 1992).

3.2 MATERIALS AND METHODS

3.2.1 Experimental design

The study was conducted at the JKUAT Farm, Juja, Kenya, which lies at longitude 37°00' E, latitude 1°05' S and approximately 1525 m above sea level with a bimodal pattern of rainfall (856 mm per year). Three trials were established, the first in January 1999, the second in May 1999 and the third in August 1999 with Neemros® (NKCP) and Neemroc EC® (NSO) as treatments and, water-spray as a control. The first trial was laid out as a completely randomised design replicated five times while the second and third trials were completely randomised block design replicated six times [Appendices 1 & 2]. Four-week old cabbage seedlings were transplanted to field plots measuring 4.2 x 4.4 metres, at a spacing of 40 cm within rows and 60 cm between rows [trial one: 6x3 m plot size; 30 cm within and 60 cm between rows]. Two guard rows that were established around the entire block of land acted as a buffer against interference from neighbouring fields. Furrow irrigation was adopted to minimise interference to DBM establishment. Foliar applications of NKCP and NSO were made (using a hand-operated knapsack sprayer (Hardi, Kenya Ltd.) calibrated to deliver 100lt spray/ha) weekly starting when the DBM population established (at six and two weeks after transplanting [WAT] for the first and second trial, respectively) until the ninth WAT when the crop was due for harvest. No neem insecticide applications were made in the third trial

because the crop was not infested by DBM. Sampling was conducted prior to the neem applications in each week.

3.2.2 DBM infestation

Sampling started in the fourth and second WAT for the first and second and third trials, respectively, and continued up to the ninth WAT. Sampling during the third trial was halted in the seventh WAT because of lack of DBM infestation on the crop. DBM larvae were counted on ten randomly selected plants in each plot. To ensure that sampling was conducted evenly over the plot, each plot was partitioned into four quadrants and at least two plants selected from each, with at least one plant from each of the six innermost rows. The selected plants were tagged and used for the evaluation of infestation for the duration of each trial. DBM larval infestation in each plot in each week was measured as the total number of larvae recorded on the ten plants.

3.2.3 Larval parasitism

There was no natural occurrence of larval parasitoids during the first trial and therefore parasitism was monitored only for the second trial. Sampling was conducted weekly for five weeks, starting in the third WAT. Where possible, at least 7-8 late fourth instar larvae or recently pupated DBM (still green in colour) were collected from each quadrant totalling to a

sample of at least 30 DBM from each plot [DBM was not collected from the plants used to monitor DBM infestation]. The larvae from each quadrant were kept collectively in 150 ml plastic cups in the laboratory until either DBM or parasitoid adults emerged. Unemerged pupae were dissected to determine whether or not they had been parasitised. Percent parasitism for each week was determined as the proportion of hosts sampled that were parasitised.

3.2.4 Yield

This was determined in the tenth WAT. Mature cabbage heads were randomly selected from each plot (15/plot in trial one and 25/plot in trial two) and each assessed for damage and quality based on a modification of the classes defined by Dreyer (1987). Damage was scored as: 1= no feeding damage; 2= outer leaves slightly damaged; 3= moderate damage; 4= plant heavily damaged. The classes for damage were weighted with values of 1, 2, 3 and 4, respectively.

Quality was scored as: 1= no damage/high quality; 2= outer leaves damaged, head marketable after their removal/moderate quality; 3= head destroyed, not marketable/poor quality. The classes for quality were weighted with values of 3, 2 and 1, respectively.

For each plot, separate indices for damage and quality were calculated using the formula:

$$\frac{\text{weight for damage class [or quality] x number of cabbages in that class}}{\text{total number of cabbages harvested per plot}}$$

The indices were summed up to give an overall index for damage and for quality in the plot. After assessment, each cabbage head was weighed after removing the outermost 2-3 leaves and their pooled weight recorded for each plot.

Colour changes in the foliage of the crop receiving NSO sprays during trial two prompted an investigation into the chlorophyll content of the crop for the three treatments. The chlorophyll assays were undertaken on cabbage plants at 8 WAT, and were based on a modification of the method (and formulae) described by Dennison (1990). From each treatment plot, six leaves were taken from six plants (one /plant) and smaller pieces taken from this sample to get a second sample of 2 gm of leaf material. Chlorophyll was extracted by thoroughly crushing the leaf pieces in 10 ml of 90% methanol to which 0.5 gm of calcium carbonate had been added to prevent acidification (and therefore destruction) of the extracts. The extract was then centrifuged at 2000 rpm for 10 mins in two separate runs to obtain a clear pale green supernatant. Chlorophyll absorbency (at 653 and 666 nm wavelength) of the supernatant was determined in a

spectrophotometer against a blank (methanol). Chlorophyll content (ugChll/ml) was calculated using the formula:

2.57 x O.D666 + 23.6 x O.D653 [= Chll A + Chll B]

where O.D653 & O.D666 are absorbency at 653nm and 666nm wavelength.

3.2.5 Data analysis

Infestation data were log-transformed [$\log_{10}$ (x)] while percent parasitism were arcsine-transformed [1+ASIN(x)] prior to statistical analysis using Repeated Measures Analysis of Variance (PROC GLM, SAS Institute, 1990). One-way ANOVA with treatment as the independent variable was used to determine whether there were significant differences among the treatments in the weight, damage and quality of the harvested cabbage heads. The values for chlorophyll content were subjected to ANOVA to check for differences in chlorophyll content of leaves from cabbage plants receiving the treatment sprays. Where appropriate, the SNK multiple range test was used to separate means at $P<0.05$.

3.3 RESULTS

3.3.1 DBM infestation

In the first trial, treatment had no influence on the level of infestation within any one week ($F=1.06$, d.f=10, 16; $P>0.05$). Infestation levels were also not significantly different between the treatments ($F= 0.29$, d.f= 2, 12, $P>0.05$) but increased significantly over the weeks ($F= 13.63$ d.f= 5, 8; $P<0.01$). This trend was less defined in the NKCP and control plots than in the NSO plots (Table 3.1).

In the second trial, a significant interaction between treatment and time on infestation levels was recorded ($F= 2.41$, d.f= 14, 18, $P<0.05$) (Appendix 3). Infestation levels were also influenced separately by time ($F= 13.9$, d.f= 7, 9, $P<0.001$) and treatment ($F= 13.39$, d.f= 2, 15, $P<0.001$). Infestation in NSO plots decreased after two insecticide applications but was not significantly different from that in the NKCP and control plots during the second to fifth WAT. In the sixth WAT infestation in NSO plots was still lower but not significantly different from that in the NKCP plots; it was however significantly lower than that in the control plots. From the seventh to ninth WAT, NSO plots had significantly lower infestation levels than the NKCP and control plots. The lowest level of infestation in NSO plots was 13.3 ±1.3 larvae recorded during the ninth WAT and the highest infestation was 73.3 ± 26.0 larvae recorded in the sixth WAT. In contrast,

NKCP and control plots recorded a rise in infestation over the weeks but the levels were not significantly different between these treatments in any week (Table 3.1). For both treatments, infestation levels during the second to the fifth WAT were lower than during the sixth to eighth WAT. By the ninth WAT both treatments recorded a significant decrease in infestation, an observation that was also made for the NSO plots. The lowest level of infestation in NKCP plots was 35.0 ± 4.7 larvae recorded during the ninth WAT while the highest level was 123.7 ± 33.8 larvae recorded during the sixth WAT. In the control plots, infestation was lowest (37.8 ± 3.0 larvae) in the second WAT and highest (177.7 ± 15.9 larvae) in the sixth WAT.

Table 3.1: DBM larval infestation in cabbage receiving foliar applications of two neem insecticide formulations or water (control) at successive weeks after transplanting (WAT), 1999.

WAT	Mean (±s.e) larval numbers/10 plants Trial one			Mean (±s.e) larval numbers/10 plants Trial two		
	NKCP	NSO	Control	NKCP	NSO	Control
2	-	-	-	36.4 ± 4.6 (1.54)aB	46.3 ± 6.0 (1.6466)aA	37.8 ± 3.0 (1.5709)aC
3	-	-	-	52.2 ± 9.6 (1.6587)aAB	50.0 ± 9.0 (1.6566)aA	45.2 ± 7.1 (1.63)aC
4	18.8 ± 6.3 (1.1731)aB	15.8 ± 5.4 (1.0952)aC	10.4 ± 2.4 (0.9281)aB	55.8 ± 7.5 (1.7277)aAB	38.5 ± 6.3 (1.5527)aA	60.7 ± 9.7 (1.7561)aC
5	44.0 ± 13.0 (1.5684)aAB	30.0 ± 6.7 (1.441)aB	40.0 ± 13.2 (1.53)aA	40.8 ± 7.8 (1.5534)aB	29.2 ± 6.9 (1.3304)aAB	56.8 ± 5.4 (1.7442)aC
6	42.8 ± 15.6 (1.5205)aAB	24.0 ± 3.0 (1.3663)aB	33.8 ± 9.9 (1.4436)aA	123.7 ± 33.8 (2.0003)abA	73.3 ± 26.0 (1.74)bA	177.7 ± 15.9 (2.2402)aA
7	62.4 ± 17.4 (1.7301)aA	38.4 ± 7.8 (1.5431)aAB	37.4 ± 6.72 (1.5433)aA	107.5 ± 17.9 (2.0078)aA	47.5 ± 12.9 (1.6147)bA	143.3 ± 12.8 (2.1468)aA
8	52.8 ± 8.0 (1.7025)aA	59.6 ± 7.7 (1.7583)aA	54.8 ± 4.3 (1.7331)aA	89.9 ± 16.2 (1.9158)aA	28.2 ± 7.2 (1.3594)bAB	102.3 ± 17.0 (1.9778)aB
9	60.4 ± 7.0 (1.7677)aA	67.4 ± 6.7 (1.8204)aA	67.2 ± 8.2 (1.815)aA	35.0 ± 4.7 (1.528)aB	13.3 ± 1.3 (1.1144)bB	47.0 ± 4.8 (1.6614)aC

Log-transformed values presented within brackets; within a row, means compare treatments in one week and means with the same lowercase letter are not significantly different at $P<0.05$, SNK test. Within a column, means compare time intervals across a treatment and means with the same uppercase letter are not significantly different at $P<0.05$, SNK test.

3.3.2 Larval parasitism

There were significant differences in parasitism levels between the treatments ($F = 7.96$, $d.f = 2, 9$; $P<0.05$) and between the weeks ($F= 9.59$, $d.f= 4, 36$; $P<0.001$) (Table 3.2). A significant interaction for total larval parasitism (=by all parasitoid species) was recorded between time and treatment ($F= 3.09$, $d.f= 8, 36$; $P<0.05$) (Appendix 4). Parasitism levels were not significantly different between NKCP and control plots in any week. In the NSO plots however, parasitism levels during the fifth and eighth WAT (6.7±6.7% and 16.3±7.7%, respectively) were significantly lower than those in the NKCP (24.5±2.4% and 31.3±3.1%, respectively) and the control plots (22.0±3.4% and 33.6±1.9%, respectively).

In both NKCP and control plots, parasitism levels decreased from the third to fourth WAT and increased thereafter to a peak by the seventh WAT but started to decline thereafter. A similar trend was observed in the NSO plots although parasitism levels were much lower and the peak during the seventh WAT was less defined than in the other two treatments. In the NKCP plots, parasitism levels in the fourth WAT (16.1±2.2%) were significantly lower than in the seventh (33.8±5.6%) and eighth (31.3±3.1%) WAT but were otherwise not significantly different between the other weeks. In the control plots, parasitism levels in the fourth WAT (20.2±4.6%) were significantly lower than in the seventh WAT (37.1±4.7%) and were not significantly different between the other weeks. For both the NKCP and

control plots the lowest parasitism levels were in the fourth WAT (16.1±2.2% and 20.2±4.6%, respectively) and the highest in the seventh WAT (33.8±5.6% and 37.1±4.7%, respectively). In the NSO plots the highest parasitism levels were recorded in the third WAT (37.2±6.6%) and the lowest in the fifth WAT (6.7±6.7%).

Table 3.2: Percent parasitism of DBM larvae on cabbage receiving foliar applications of two neem insecticide formulations or water (control) at successive weeks after transplanting (WAT) 1999 - trial 2.

Treat.	3 WAT	4 WAT	5 WAT	7 WAT	8 WAT
NKCP	27.1±2.8%aAB (n=41)	16.1±2.2%aB (n=23)	24.5±2.4%aAB (n=57)	33.8±5.6%aA (n=98)	31.3±3.1%aA (n=83)
NSO	37.2±6.6%aA (n=54)	12.7±3.4%aB (n=18)	6.7±6.7%bB (n=4*)	24.0±4.5%aAB (n=26)	16.3±7.7%bAB (n=15)
Control	32.7±4.9%aAB (n=53)	20.2±4.6%aB (n=41)	22.0±3.4%aAB (n=73)	37.1±4.7%aA (n=107)	33.6±1.9%aAB (n=65)
C.V.%	*9.4*	*6.9*	*5.2*	*6.2*	*7.2*

Within a column, means compare treatments in a week and means with the same lowercase letter are not significantly different at $P<0.05$, SNK test. Within a row, means compare time intervals in a treatment and means with the same uppercase letter are not significantly different at $P<0.05$, SNK test. N= number of parasitised DBM in the sample collected. *Some plots were excluded due to the absence of DBM to sample.

Three larval parasitoids namely *Diadegma mollipla* (Holmgren), *Oomyzus sokolowskii* Kurdjumov and *Cotesia plutellae* Kurdjumov were recorded during the experimental period (Plate 3.1). Significant interactions for parasitism by each species were recorded for treatment, time and species ($F= 4.04$, $d.f= 16,184$, $P<0.001$), between treatment and time ($F= 3.96$, $d.f= 8, 120$; $P<0.001$), between species and time ($F= 28.46$, $d.f= 8, 120$; $P<0.001$) and between species and treatment ($F= 4.99$, $d.f= 4, 63$; $P<0.01$) (Appendix 5). Thus, variations in parasitism levels in the plots were attributable to the species, treatments and week of observation. For all the treatments, *D. mollipla* was the predominant parasitoid in the third WAT but its rate of parasitism declined thereafter and was less than 5% from the fifth WAT.

Plate 3.1 Larval parasitoids (females) of the diamondback moth

Plate 3.1 A: *Diadegma* spp. adult

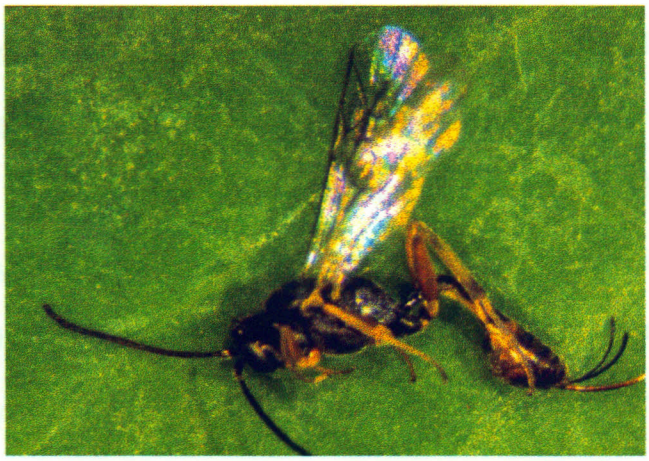
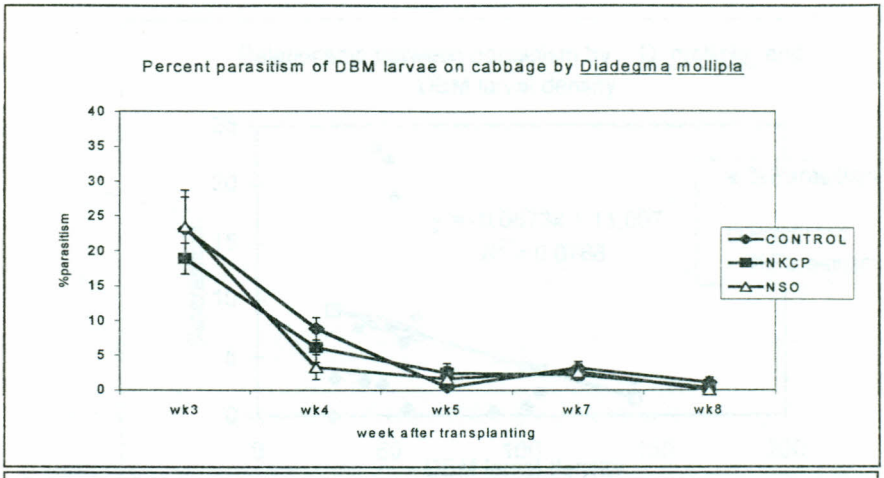


Plate 3.1B: *Oomyzus sokolowskii* adult
[*ovipositing into fourth instar DBM larva*]

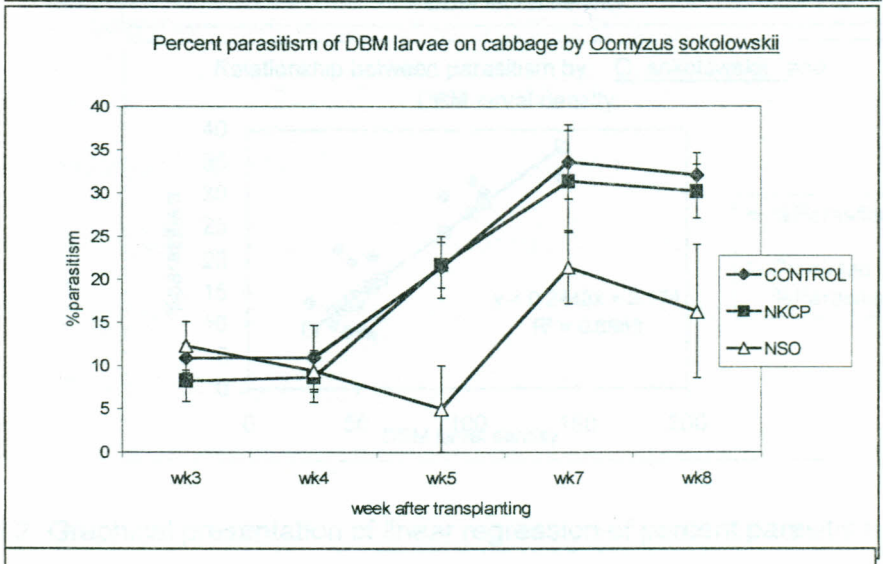
Plate 3.1C: *Cotesia* spp. adult

In any one week however, its rate of parasitism did not differ significantly between the treatments ($F= 0.69$, d.f= 2, 68; $P>0.05$) (Figure 3.1A). Parasitism rates for *O. sokolowskii* increased over the weeks and by the fifth WAT this species was the predominant parasitoid. The parasitism levels were also significantly different between the treatments ($F= 5.96$, d.f= 2, 67; $P<0.01$). In the NKCP and control plots, parasitism levels were not significantly different in any of the weeks but were significantly lower in the NSO plots from the fourth WAT (Figure 3.1B). Parasitism by *C. plutellae* was not significantly different between treatments ($F= 0.28$, d.f= 2, 67; $P>0.05$) but was very low throughout the period, accounting for less than 2% of the parasitised hosts in all the treatments (Figure 3.1C). *D. mollipla* was the predominant parasitoid in the third WAT but by the fourth WAT, its rate of parasitism had declined and was not significantly different from that by *O. sokolowskii*. From the fifth to the eighth WAT, *O. sokolowskii* had the highest levels of parasitism while parasitism by *D. mollipla* continued to decline and was not significantly different from that by *C. plutellae*. Regression of parasitism by each species against host density showed a significant (linear) relationship ($y= 0.245x + 2.174$, $r^2= 0.69$) ($F= 28.501$, d.f= 1, 13, $P<0.001$) for *O. sokolowskii* but not for *D. mollipla* ($y= -0.067x + 11.097$, $r^2= 0.08$) ($F= 1.081$, d.f= 1, 13, $P>0.05$) (Figure 3.2). A regression analysis was not done for *C. plutellae* because of its very low parasitism levels.

A



B



C

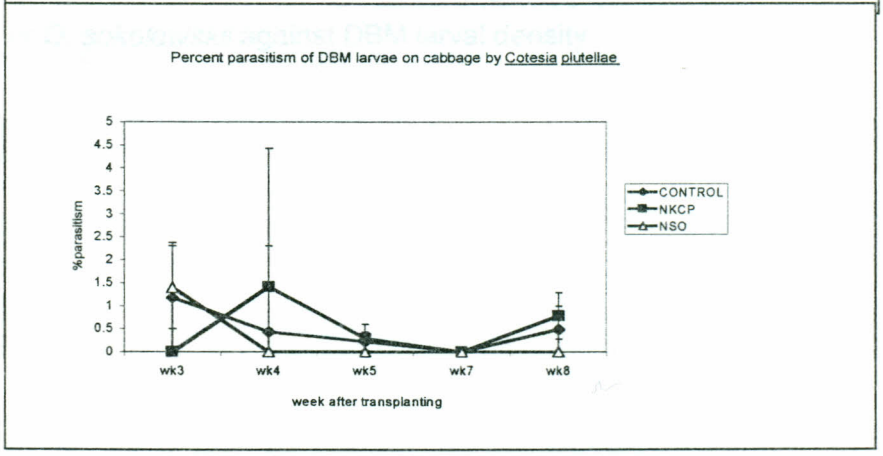


Figure 3.1: Field parasitism of DBM larvae by three parasitoid species in cabbage receiving foliar applications of two neem insecticide formulations or water (control). Error bars represent standard errors (s.e.).

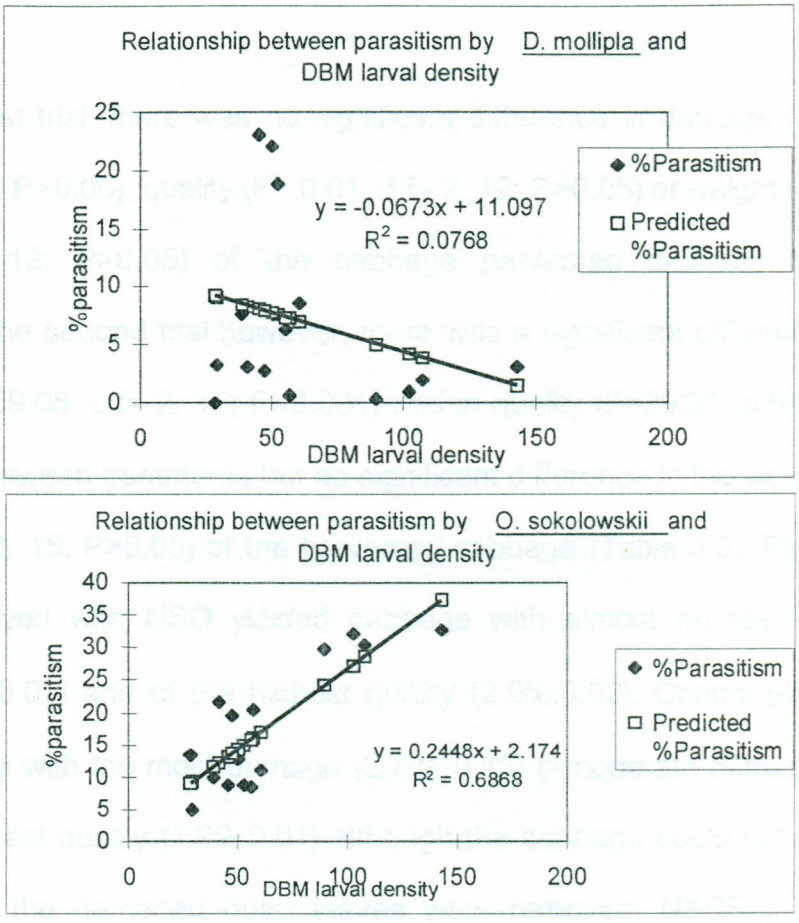


Figure 3.2: Graphical presentation of linear regression of percent parasitism by *D. mollipla* or *O. sokolowskii* against DBM larval density.

3.3.3 Yield

In the first trial, there was no significant difference in damage ($F= 0.17$, $d.f= 2, 12$; $P>0.05$), quality ($F= 0.01$, $d.f= 2, 12$; $P>0.05$) or weight ($F= 0.89$, $d.f= 2, 12$; $P>0.05$) of the cabbage harvested between the treatments. In the second trial however, there was a significant difference in damage ($F=59.08$, $d.f= 2, 15$; $P<0.001$) and in quality ($F=29.31$, $d.f= 2, 15$; $P<0.001$) between treatments but no significant difference in the weight ($F= 0.86$, $d.f= 2, 15$; $P>0.05$) of the harvested cabbage (Table 3.3). Plots that were sprayed with NSO yielded cabbage with almost no feeding damage (1.20±0.07) and of the highest quality (2.96±0.02). Control plots yielded cabbage with the most damage (2.78± 0.13) (≈moderate damage) and of the poorest quality (1.99±0.01) although the cabbage could still be marketed after the damaged outer leaves were removed. NKCP plots yielded cabbage with slightly damaged leaves (2.40±0.12) and were of moderate quality (2.29±0.16) (Plate 3.2).

Plate 3.2: Degree of DBM damage on cabbage plants in plots receiving sprays of either water (control) or one of two neem insecticide formulations.

↑
NKCP-protected cabbage

NSO-protected cabbage ↑

Water-sprayed (control) cabbage

Between the trials, NKCP-sprayed plots recorded no significant differences in weight ($F= 2.08$, $d.f= 1,9$; $P>0.05$), damage ($F= 3.92$, $d.f= 1,9$; $P>0.05$) or quality ($F= 0.01$, $d.f= 1,9$; $P>0.05$) of the crop. NSO-sprayed plots had significantly less damage ($F= 20.01$, $d.f= 1,9$; $P<0.01$) and higher crop quality ($F= 133.14$, $d.f= 1,9$; $P<0.001$) in trial two than in trial one but there was no significant difference in weight of harvested cabbage between the two trials ($F= 3.91$, $d.f= 1,9$; $P>0.05$). The control plots had significantly more damage ($F=9.74$, $d.f= 1,9$; $P<0.05$) and lower quality ($F= 11.99$, $d.f= 1,9$; $P<0.01$) in trial two than in trial one but there was no significant difference in weight of the harvested cabbage between the two trials ($F= 1.87$, $d.f= 1,9$; $P>0.05$).

Table 3.3: Weight, damage and quality of cabbage harvested from plots sprayed with two neem insecticides or water (control)

Treat.	TRIAL ONE (n=15)			TRIAL TWO (n=25)		
	Total weight (kg) Mean ± s.e.	Damage index Mean ± s.e.	Quality index Mean ± s.e.	Total weight (kg) Mean ± s.e.	Damage index Mean ± s.e.	Quality index Mean ± s.e.
NKCP	39.84 ± 2.25aA	2.03 ± 0.15aA	2.27 ± 0.09aA	43.56 ± 1.43aA	2.40 ± 0.12bA	2.29 ± 0.16bA
NSO	41.08 ± 1.66aA	1.99 ± 0.18aA	2.27 ± 0.06aB	48.13 ± 2.93aA	1.20 ± 0.07cB	2.96 ± 0.02aA
Control	41.36 ± 1.68aA	2.13 ± 0.17aB	2.25 ± 0.08aA	45.63 ± 2.47aA	2.78 ± 0.13aA	1.99 ± 0.01cB
Mean	*40.76*	*2.05*	*2.26*	*45.77*	*2.13*	*2.41*
C.V.%	*4.7*	*20.1*	*8.1*	*13.2*	*12.4*	*9.4*

Within a column means compare a parameter between treatments within the trial, and means with the same lowercase letter are not significantly different at $P<0.05$, SNK test. Within a row means compare a parameter within each treatment between trials and means with the same uppercase letter for the same parameter are not significantly different at $P<0.05$, SNK test. N= number of cabbage heads sampled.

During development, cabbage plants receiving the Neemroc EC® sprays (trial two) were observed to change colour from the bluish-green colour characteristic of the Copenhagen Market cabbage variety to a brilliant green colour with yellowish undertones. Chlorophyll analysis revealed that the change in colour did not signify a difference (ANOVA, d.f= 2, 15; P>0.05) in the chlorophyll content of the crop (Table 3.4).

Table 3.4: Mean (± s.e) chlorophyll content (µg/ml) of cabbage leaves at 8 WAT receiving sprays of two neem insecticide formulations or water (control)

Treatment	Chll A	Chll B	Chll AB
NKCP	3.28 ± 0.6a	20.67± 3.4a	23.96 ± 4.0a
NSO	4.93 ± 0.4a	31.83 ± 3.6a	36.76 ± 4.0a
Control	3.93 ± 0.6a	25.91 ± 4.7a	29.85 ± 5.3a
Mean	*4.05*	*26.14*	*30.19*

Within a column means compare treatments and means with the same letter are not significantly different at P<0.05, SNK test. Means of six replicates.

3.4 DISCUSSION

The apparent ineffectiveness of the two neem formulations in checking DBM infestation during the first trial was attributed to the fewer neem insecticide applications that were also started late. This was occasioned by the need to allow naturally occurring DBM populations to infest and establish on the crop and also encourage naturally occurring parasitoids to establish. In the second trial DBM populations were subjected to eight neem insecticide applications compared to the four applications made in the first trial. This difference in frequency was reflected in the greater effectiveness of the oil-based formulation (NSO, Neemroc EC®) in checking DBM populations and ensuring higher crop quality but the oil-free formulation (NKCP, Neemros®) was not as effective. The greater damage and lower quality of the crop in NKCP and control plots during trial two was attributed to the higher infestation pressure on the crop over this period than in trial one.

The fluctuations in parasitism levels within the Neemros® plots were similar to those in the control plots, and for both treatments, the highest parasitism levels were recorded later in the observational period. In contrast, parasitism in Neemroc EC® plots fluctuated differently from that in the Neemros® and control plots, and the highest parasitism levels were recorded at the beginning of the trial. Thus, in both the actual level of and trend in parasitism, the Neemros® and control plots were similar while the

Neemroc EC® plots differed from the two. With respect to the individual parasitoid species, Neemros® applications had no apparent adverse effects on the activity of the individual parasitoid species. The Neemroc EC® applications on the other hand resulted in somewhat lower parasitism levels by *O. sokolowskii* but had no apparent effect on the activity of either *D. mollipla* or *C. plutellae*. It should however be noted that the very low parasitism levels exhibited by these two species preclude more definite conclusions on the impact of the neem formulations on their activity.

Trends in the parasitism levels closely followed the trends observed for infestation levels. This was more evident in the Neemros® and control plots where as infestation increased so did the total parasitism; the trends were less defined in the Neemroc EC® plots where infestation was much lower. At the sixth WAT, a defined peak in infestation was observed while a similar peak was recorded in the seventh WAT for parasitism. As infestation pressure decreased from the seventh WAT, parasitism levels also showed a drop from the eighth WAT. These trends suggest a delayed density dependence effect on parasitism. Over this period, parasitism was predominantly by *O. sokolowskii*, which had a significant relationship with host density. The drop in pooled parasitism at the fourth WAT in the three treatments was probably due to the lowered parasitism by *D. mollipla* when *O. sokolowskii* activity was also still low. After the fourth WAT, *O. sokolowskii* activity increased and pooled parasitism did not fluctuate

widely. It is likely that the absence of competition from *D. mollipla*, which is known to be the intrinsically superior genus (Xu *et al.*, 2001), allowed *O. sokolowskii* to express greater activity. A significant linear relationship between host density and parasitism by *O. sokolowskii* suggests that the low infestation levels in Neemroc EC® plots could also have affected *O. sokolowskii* activity. It is also possible that the oil formulation had a direct adverse effect on *O. sokolowskii* activity. As observed by Schmutterer (1995), neem based products having a higher oil content have a stronger impact on non-target organisms than oil-free products. Defining which of these factors could have been responsible for the lowered parasitism levels in the Neemroc EC® plots would require experiments where more control can be exercised over host density. The differential response observed between the parasitoid species to individual neem formulations points to the need for monitoring the responses of individual species in studies of non-target effects of neem insecticides, rather than focusing on the total larval parasitism as a parameter. Despite lower parasitism levels in the Neemroc EC® plots, the reduced infestation pressure ensured better quality produce. Neemros® and the water-sprayed control plots had somewhat higher levels of parasitism. However, the poorer quality of produce resulting from the higher DBM infestation suggests that naturally occurring larval parasitoids on their own may not adequately check DBM infestation and protect the crop from significant damage. Total parasitism

(range 20-35%) for the three species was higher than the 3-8% level recorded by Oduor *et al.* (1996) at another site (KARI, Muguga) in Kenya. It was however much less than the rates of over 30% field parasitism that have been reported in North America and South-east Asia (Harcourt, 1986; Talekar 1992; Idris & Grafius, 1993a; Talekar & Shelton, 1993; Grafius, 1997; Liu *et al.*, 2000). *O. sokolowskii* and *C. plutellae* also occur in these regions as well as *D. insulare* or *D. semiclausum*, which predominate. These *Diadegma* species may be superior to *D. mollipla* and this could explain the higher parasitism rates in these regions.

Decreased infestation pressure with increasing crop maturity could be that mature leaves are not suitable for larval development or, that gravid DBM females have a selective preference for younger leaves. Such leaves would probably be more nutritious and offer more protection to larvae than cabbage heads whose compact leaves cannot be easily burrowed into. Dennill & Pretorius (1995) in South Africa made similar observations of reduced infestation pressure on mature cabbage.

Colour changes in the cabbage plants subjected to the neem seed oil treatments were also observed by Schmutterer (1992), who reported that such cabbage had smaller heads than normal although their quality was superior to that of untreated cabbage and would therefore fetch a higher market price. In the present study, the size and weight of Neemroc EC® treated cabbage was not different from that in the Neemros® and

control plots but its quality was superior and would have presumably fetched a higher market price. In conclusion, Neemroc EC® (the oil-based formulation) was more effective than Neemros® (the oil-free formulation) in checking DBM infestation and ensuring higher quality produce although there was an apparent trade-off in its effect on one of the parasitoid species.

CHAPTER 4

EVALUATION OF CAUSE-SPECIFIC MORTALITY OF DBM ON CABBAGE IN KENYA

4.1 INTRODUCTION

The diamondback moth, *P. xylostella* (L.) is the most serious pest of Crucifers in Eastern Africa (Nyambo & Pekke, 1995; Kibata, 1996). The use of synthetic chemical insecticides remains the most prevalent means for DBM control in Kenya (Kibata, 1996; Oduor *et al.*, 1996). Circumstantial evidence of DBM resistance to chemical insecticides in Kenya (Kibata, 1996) and more conclusive evidence of resistance in DBM populations in other parts of the world (Liu *et al.,* 1981, 1982; Idris & Grafius, 1993b; Syed & Fauziah, 1996) indicates that sole reliance on chemical insecticide control is not sustainable. This necessitates the adoption of a multifaceted approach to DBM control that incorporates other pest control options (Shelton *et al.*, 1996).

In Southeast Asia, where a similar DBM problem prevails, notable control of the DBM has been achieved through the use of larval parasitoids (Talekar, 1992; Sivapragasam *et al.*, 1996) as part of a DBM IPM programme. Such success is indicative of the potential of natural enemies especially parasitoids to check DBM populations. A number of DBM parasitoid species have been identified in East Africa (Nyambo & Pekke, 1995; Kibata, 1996) but their impact on DBM populations remains unclear.

The impact and role of natural enemies and other agents of mortality in the population dynamics of an insect can be assessed by use of lifetables (Bellows *et al.,* 1992). Lifetables are survival/mortality budgets that describe the number of individuals in a population surviving to specific ages or stages in their lifecycle. They enable the identification of factors in the environment that cause mortality in a defined population, the most important of these factors and which age groups of the population are most vulnerable to the individual or collective mortality factors (Bellows *et al.*, 1992; Elkinton, 1993). Lifetables can be used to estimate the specific contribution of each mortality factor to the total mortality in a generation or set of generations of the pest population (Bellows *et al.,* 1992). They are also useful for measuring the impact of a particular treatment on the mortality caused by natural enemies (Elkinton, 1993).

For example, van den Berg (1993) used the lifetable technique to demonstrate the importance of predators in checking populations of the African bollworm, *Helicoverpa armigera* Hübner, and the impact of different host plants of *H. armigera* on the efficacy of its predators. Songa (1999) used lifetables to assess the importance of indigenous parasitoids and an introduced parasitoid, *Cotesia flavipes* Cameron in the population dynamics of the stemborer, *Chilo partellus* (Swinhoe). Harcourt (1986), Sivapragasam *et al.* (1988). Keinmeesuke *et al.* (1992) and Wakisaka *et al.*

(1992) have also employed the lifetable technique to identify the major sources of natural mortality in DBM populations.

The objectives of this study were to assess the importance of larval parasitoids as a mortality factor of DBM during its development, and to evaluate the impact of neem insecticide treatments on the mortality caused by the larval parasitoids.

4.2 MATERIALS AND METHODS

DBM cohorts were initiated on potted cabbage plants. This was done by transferring laboratory reared first instar larvae onto the plants and allowing them to develop to the second instar stage (L2), whereupon their number on the plant was determined. Each cohort was then subjected to one of six treatments, namely 2.5% NKCP spray (Mnk), 1.5% NSO spray (Mns), naturally occurring larval and pupal parasitoids (Mp), 2.5% NKCP spray and parasitoids (Mnk+p), 1.5% NSO spray and parasitoids (Mns+p) and a control cohort (Minn) that was monitored in the laboratory and not exposed to parasitoids or sprays of the neem formulations. The control cohorts were assumed to suffer mortality due to innate/physiological factors only. The Mnk and Mns cohorts were placed in cages constructed of fine nylon netting (mesh size $\leq$0.5 mm) over a wooden frame (1.0x0.5x0.5 m) to eliminate parasitoid visits. The Mp, Mnk+p and Mns+p cohorts were placed on a raised platform but left exposed to the elements.

Except for the Minn cohorts, all other cohorts were placed in a field with a mixture of crops (onion, squash, cowpea) including cabbage; the cohorts were recovered from the field 7 days later when larvae pupated. A layer of insect glue was spread around the base of each pot to protect the cohorts from crawling predators. There was no rain during the periods that the experiments were conducted. Each treatment was replicated 4-5 times. Every three days, starting from the time that the cohorts were set up, the number of surviving individuals and their lifestage was recorded until all the individuals attained the adult stage.

The second instar stage was selected as the initial point for observations because it is at this stage that larvae exit the leaf mines that they created during the first instar stage and are therefore first exposed to possible insecticide sprays and attack by larval-pupal parasitoids. The third and fourth instar stages were combined because these are not always distinct from each other but are quite distinct from the L2 stage.

Data analysis:

Partial lifetables were constructed for each DBM cohort by determining the number of individuals (l_x) that reached the beginning of each lifestage (x). The number of individuals that died (d_x) within each stage was determined by subtraction of the l_x values of the preceding (x) and subsequent (x+1) stages. Mortality was partitioned into the following

causes: parasitism, neem insecticide, infection, and disappearance, which included all undetermined losses to predation, dispersal, abiotic factors and unspecified causes.

The multiple decrement lifetable method as described by Carey (1989) was adopted to calculate the probability of an individual dying from a specific cause in the presence of other causes. Conventional approaches for quantifying the impact of separate but contemporaneous factors are based on the calculation of marginal attack rates. This requires knowledge of the order in which the mortality factors acted on (= attacked) the population, failing which, assumptions are made about such internal competition among factors (Buonaccorsi & Elkinton, 1990; Bellows *et al.,* 1992). This was of particular relevance in the present study since neem insecticides are lethal to DBM larvae but slow-acting and it was not always possible to identify the actual cause of death. The multiple decrement lifetable method is based on observed deaths (not attacks), makes no assumptions about competition between mortality factors and also assumes that multiple causes of death act independently such that exposure to one cause of death does not imply increased vulnerability to other causes of death (Carey, 1989). The method involves the calculation of cause-specific probability of death from specified causes (aq_{ix}) in the presence of all causes, and thereafter the proportion of individuals that die in a stage due to a specific cause (ad_{ix}) and due to all causes (ad_x). From

these calculations one can determine at which stage the most deaths occurred, which factor resulted in the highest number of deaths and the proportion of individuals surviving to a specific stage. The method allows for analysis of how mortality changes when a specific cause of mortality (such as an insecticide, natural enemy) is either added or removed from the system.

4.3 RESULTS

The proportion of deaths from all causes combined and from specific causes at each life stage of DBM are given in Table 4.1. Mortality from all causes (ad_x) over the entire development period was highly significantly different among the treatments ($F= 14.91$; $d.f= 5, 21$; $P<0.001$). The cohorts that were not exposed to any extrinsic mortality factors (Minn) had the lowest total mortality (0.132) over the entire development period and this was significantly different from all the other treatments. Cohorts that were exposed to possible parasitoid attack without neem insecticide (Mp) had lower mortality (0.58) than the 'neem-exposed' cohorts but only significantly so from that in Mnk cohorts.

Table 4.1: Survival and deaths from specific causes and all causes combined in DBM cohorts subjected to different treatments

Treat	Lifestage	Prob. of surv. al_x	Prob. of death aq_x	All causes ad_x	Infect ad_{1x}	Ptoids ad_{2x}	Neem ad_{3x}	Disapp ad_{4x}
Minn	L2	1.000±0.0	0.027±0.01a[1]x[3]	0.027±0.01x	-	-	-	0.027±0.01a,x
	L3+4	0.973±0.01	0.052±0.01b,x	0.051±0.01x	0.038±0.0	-	-	0.043±0.01b,x
	Pupa	0.923±0.01	0.060±0.03b,x	0.055±0.03x	-	-	-	0.055±0.03a,x
	Adult	0.868±0.03	-	-	-	-	-	-
	L2-Adult	-	-	0.132 ± 0.03C[2]	0.008 ± 0.01A	-	-	0.126±0.03B
Mp	L2	1.000±0.0	0.303±0.14a,x	0.303±0.14x	0.000	0.000	-	0.303±0.14a,x
	L3+4	0.697±0.14	0.385±0.16ab,x	0.188±0.06x	0.013±0.01	0.007±0.01	-	0.168±0.05ab,x
	Pupa	0.509±0.18	0.110±0.07b,x	0.094±0.06x	0.002±0.0	0.000	-	0.092±0.06a,x
	Adult	0.415±0.14	-	-	-	-	-	-
	L2-Adult	-	-	0.585±0.14B	0.016±0.01A	0.007±0.01A	-	0.563±0.14A
Mnk	L2	1.000±0.0	0.573±0.08a,x	0.573±0.08x	0.000	-	0.070±0.04a,y	0.503±0.11a,x
	L3+4	0.427±0.08	0.857±0.07a,x	0.378±0.09x	0.002±0.0	-	0.172±0.04ab,x	0.204±0.05ab,y
	Pupa	0.049±0.03	0.582±0.16a,x	0.039±0.02y	0.000	-	0.024±0.01a,y	0.015±0.01a,y
	Adult	0.01±0.01	-	-	-	-	-	-
	L2-Adult	-	-	0.990±0.01A	0.002±0.0A	-	0.266±0.06A	0.722±0.06A

al_x is fraction surviving to stage x ; the fraction surviving in the first stage is set at 1.0; aq_x is probability of death from all causes in stage (x); $aq_{i,x}$ is probability of death from cause, i in stage x ; ad_x is fraction of deaths in stage x due to all causes; $ad_{i,x}$ is fraction of deaths in stage x due to cause i (Carey, 1989). Formulae for the calculation of terms are given in Appendix 6. [1]a, b, c; compares means of the parameter for the **same** lifestage **between** treatments. [2]A, B, C; compares means of "all causes" mortality **between** treatments. [3]x, y, z; compares means of the parameter **between** stages **within** a treatment. Means with the same letter are not significantly different at P<0.05, SNK test.

Table 4.1 (*continued*)

Treat	Lifestage	Prob. of surv. al_x	Prob. of death aq_x	All causes ad_x	Infect ad_{1x}	Ptoids ad_{2x}	Neem ad_{3x}	Disapp ad_{4x}
Mns	L2	1.000±0.0	0.349±0.13a[1]x[3]	0.349±0.13x	0.017±0.02	-	0.038±0.03a,x	0.294±0.13a,x
	L3+4	0.651±0.13	0.617±0.16a,x	0.324±0.06x	0.028±0.02	-	0.081±0.02b,x	0.215±0.04ab,x
	Pupa	0.327±0.14	0.182±0.10b,x	0.112±0.07x	0.000	-	0.026±0.03a,x	0.086±0.07a,x
	Adult	0.215±0.09	-	-	-	-	-	-
	L2-Adult	-	-	0.785±0.09AB[2]	0.045±0.04A	-	0.145±0.05A	0.595±0.13A
Mnk+p	L2	1.000±0.0	0.340±0.20a,x	0.340±0.2x	0.000	0.000	0.000a,y	0.340±0.2a,x
	L3+4	0.660±0.20	0.561±0.11a,x	0.311±0.06x	0.002±0.0	0.009±0.01	0.206±0.07a,x	0.094±0.05ab,x
	Pupa	0.349±0.16	0.387±0.11a,x	0.152±0.08x	0.000	0.000	0.000a,y	0.152±0.08a,x
	Adult	0.197±0.08	-	-	-	-	-	-
	L2-Adult	-	-	0.803±0.08AB	0.002±0.0A	0.009±0.01A	0.206±0.07A	0.586±0.16A
Mns+p	L2	1.000±0.0	0.441±0.21a,xy	0.441±0.21x	0.000	0.000	0.000a,y	0.441±0.21a,x
	L3+4	0.559±0.21	0.846±0.09a,x	0.434±0.11x	0.000	0.000	0.149±0.05ab,x	0.285±0.08a,x
	Pupa	0.125±0.10	0.128±0.13b,y	0.043±0.04x	0.000	0.000	0.000a,y	0.043±0.04a,x
	Adult	0.000	-	-	-	-	-	-
	L2-Adult	-	-	0.918±0.06AB	0.000A	0.000A	0.149±0.05A	0.769±0.10A

Mortality from all causes over the entire development period was not significantly different between the 'neem-exposed' cohorts. The caged Mnk cohorts had the highest total mortality (0.99) and was closer to mortality in the uncaged Mns+p cohorts (0.918) than in the uncaged Mnk+p cohorts (0.803); total mortality in Mns+p was higher than in Mns cohorts.

Total deaths due to infection, and where appropriate, due to parasitism and neem were in all cases not significantly different between the treatments (ANOVA, $P>0.05$). Total deaths due to unexplained causes (disappearance) were significantly lowest in the Minn cohorts but were not significantly different between the other treatments ($F= 4.23$, d.f= 5, 21, $P<0.01$). Parasitoids accounted for <1% of the total mortality recorded in the Mp, Mns+p and Mnk+p treatments. Only five records of parasitism due to *C. plutellae* were made; two in a parasitoid cohort and three in an 'NKCP+parasitoid' cohort. Infection induced mortality was also very low, generally <1% except in the Mp treatment where they averaged ≈1.6%. The larger proportion of total mortality was accounted for by the neem insecticides (where applicable) and disappearance. Total mortality due to NSO was more consistent at ≈15% while mortality due to NKCP was more variable (≈21-27%).

Neem induced mortality at the L2 and pupal stage was not significantly different between the treatments (ANOVA, $P>0.05$). At the L3+4 stage, deaths in the Mns cohorts were significantly lower than in the

Mnk+p cohorts but not significantly different from deaths in the Mnk and Mns+p cohorts ($F= 3.71$, $d.f= 3, 13$; $P<0.05$). The highest neem-induced mortality was recorded at the L3+4 stage. In the Mns cohorts, neem-induced mortality was not significantly different between lifestages but in the Mnk, Mnk+p and Mns+p cohorts, mortality at the L3+4 stage was significantly higher than that at the L2 and pupal stage (ANOVA, $P<0.05$).

Mortality due to disappearance was not significantly different between the treatments at the L2 ($F= 1.56$, $d.f= 5, 21$; $P>0.05$) and pupal stage ($F= 0.75$, $d.f= 5, 21$; $P>0.05$) but showed significant differences between treatments at the L3+4 stage ($F= 3.24$, $d.f= 5, 21$; $P<0.05$). Minn cohorts had the lowest L3+4 mortality due to disappearance (0.043); this was significantly different from that in Mns+p cohorts only. Increased mortality was recorded in the Mp and neem-exposed cohorts but the differences were not significant. Within treatments, mortality due to disappearance mostly occurred at the L2 stage. In the Mnk cohorts the L2 mortality due to disappearance was significantly higher than that recorded at the L3+4 and pupal stage ($F= 8.97$, $d.f= 2, 9$; $P<0.01$). In all the other treatments, there were no significant differences between the lifestages in the proportion of deaths due to disappearance.

Within treatments, the probability of death was greatest at the L3+4 stage than at the other stages in all the treatments but only with the Mns+p cohorts was the difference significant ($F= 5.6$, $d.f= 2,6$; $P<0.05$). For the

same lifestage between treatments, probability of death was not significantly different at the L2 stage ($F= 2.09$, d.f= 5, 21; $P>0.05$) between the treatments but showed significant differences at the L3+4 ($F= 6.78$, d.f= 5, 21; $P<0.001$) and pupal stage ($F= 3.87$, d.f= 5, 21; $P< 0.05$). At the L3+4 stage the probability was highest in the neem-exposed cohorts but it did not differ significantly from the Mp cohorts; the Minn cohorts did not differ significantly from the Mp cohorts but were significantly lower than the neem-exposed cohorts. At the pupal stage, the probability of death was highest in the Mnk cohorts; this was significantly higher than in all the other treatments except the Mnk+p treatment. No significant differences were recorded between the other treatments.

4.4 DISCUSSION

In the present study larval parasitoids had a negligible impact on DBM populations. This finding agrees with the observations of Kibata (1996) and Oduor *et al.,* (1996) that natural enemies in Kenya have only a negligible impact on DBM populations. This is however in contrast to the findings of Harcourt (1986) (in Canada), Keinmeesuke *et al.,* (1992) and Wakisaka *et al.,* (1992) (in Southeast Asia) who determined that parasitoids, as well as inclement weather, were an important cause of mortality in DBM populations. Their observations were however made on field populations where the host location cues are presumably stronger and

this may account for the differences. The low level of parasitism in this study could indicate low levels of parasitoid activity of the naturally occurring parasitoids at the time that the study was conducted. Alternatively, parasitised individuals may have migrated, otherwise disappeared or succumbed to the effects of the neem insecticides before evidence of parasitism appeared.

The addition of neem insecticides markedly increased mortality in the DBM cohorts such that only a maximum of 22% reached adulthood compared to 42% in the Mp cohorts and 87% in the Minn cohorts. It was observed that larvae that died as a result of the neem insecticides had shrivelled bodies that were easily dislodged from the plant. It is therefore possible that many larvae that were listed under 'disappearance' actually died as a result of the neem insecticide treatment. While this would cause an underestimation of neem-mortality [and overestimate disappearance] it would still be reflected in higher total mortality, as was observed in the neem-treated cohorts. The greater proportion of neem induced mortality in the L3+4 stage attests to the delayed action of neem-based insecticides.

It was also observed that the NKCP formulation sprays on drying left fine granular residues on the leaf surfaces. Thus, larvae feeding on such surfaces would take in more of the active ingredients and this may partly explain the higher mortality observed in the Mnk cohorts than in the exposed Mnk+p cohorts. Mnk+p cohorts were more exposed to the

elements and presumably the NKCP residues were either blown away or more quickly deactivated, thus causing less mortality. The observations made in the experiment on field parasitism where NKCP was less effective than NSO in checking DBM infestations would seem to lend some support to this hypothesis. Further, L2 mortality and the total mortality due to disappearance in the Mnk cohorts was rather high which suggests that NKCP-killed larvae that were not recovered could also have been included in this factor. The more exposed Mns+p cohorts apparently suffered higher mortality than in the Mns cohorts as indicated by an increase in disappearance-related mortality.

The main purpose of this study was to account for mortality caused by parasitoids and other individual natural factors, and the interactive effect of the neem insecticide treatments on the different mortality factors. However, the low levels of natural larval mortality attributable to parasitism (maximum = 0.9%) rendered it difficult to assess neem insecticide interactions with this mortality factor. This was perhaps due to the season being unfavourable for parasitoid activity and shows the need for follow-up studies when the parasitism levels are greater. The substantial proportion of 'disappearance' in the different treatments (56-77%) suggests the possible role of larval predators besides other causes. This should be further investigated in future studies.

CHAPTER 5

THE EFFECTS OF DIRECT EXPOSURE OF *DIADEGMA MOLLIPLA* TO NEEM INSECTICIDE SPRAYS ON ITS LONGEVITY AND FORAGING BEHAVIOUR

5.1 INTRODUCTION

Pesticides exert a wide range of lethal and sublethal effects on natural enemies. Lethal effects are often expressed as acute mortality while sublethal effects are usually chronic (=long-term) in nature and, are often expressed as a change in one or more of the affected insect's life history parameters such as longevity, parasitisation rates and behaviour. Changes in the life history parameters may affect the ability of parasitoids to regulate the density of their hosts (Croft, 1990). Lethal effects usually arise from direct topical contact with pesticide sprays or residues while sublethal effects may arise either from direct or indirect contact, for example, through feeding on contaminated prey or nectar (Croft, 1990; Ruberson *et al.*, 1998).

Extensive reviews by Schmutterer (1995, 1997) of the work done on the effects of neem insecticides indicate that these insecticides are not usually acutely toxic to adult insects but more often cause changes in their physiology and behaviour. For example, topical applications of neem oil (0.2% aza) onto adults of the desert locust, *Schistocerca gregaria* (Forskål)

did not effect mortality but flight ability was drastically reduced and even lost when the insects were treated during flight (Wilps *et al.*, 1992). Schmutterer and Wilps (1995) suggested that topical application of neem oil on locust and grasshopper nymphs could conceivably impair the ability of the treated insects to perceive pheromones and other volatiles since the oil destroyed the antennal receptors. In another study, males of the large milkweed bug, *Oncopeltus fasciatus* Dallas were unable to mate after topical application of azadirachtin (Dorn *et al.*, 1987). Saxena *et al.* (1993) also observed that females of the brown rice planthopper, *Nilaparvata lugens* (Stål) failed to produce normal courtship signals after topical treatment with neem oil and males could therefore not locate them. Neem insecticides have also been observed to induce feeding-deterrent and repellent behaviour (Fagoonee, 1984; Schmutterer & Ascher, 1987; Kaethner, 1992; Schmutterer, 1995).

Considerable information exists on the effects of neem insecticides on DBM populations (Dreyer, 1987; Fagoonee, 1987; Kirsch, 1987; Schmutterer, 1992; Isman, 1995), but hardly any is available on their effects on DBM natural enemies. Establishing the acute or chronic toxicity of neem insecticides to DBM parasitoids is necessary for determining the compatibility of these two promising DBM control options. The objectives of this study were to characterise the responses of *D. mollipla* after direct contact with sprays of the candidate neem formulations, specifically for the

effect on adult longevity, ability to parasitise DBM larvae and foraging patterns.

5.2 MATERIALS AND METHODS

5.2.1 Toxicity of the neem formulations to *D. mollipla* adults

Newly emerged parasitoid adults were apportioned into three Perspex treatment cages, about 30 wasps per cage [the total number of wasps tested in each treatment is indicated in Table 5.1]. The wasps were maintained on a 20% honey solution and distilled water as described in section 2.2.3. When the caged parasitoids were two days old, they were sprayed with 4-6ml of aqueous solutions of either NKCP (2.5%) or NSO (1.5%) or water (as a control) using a hand-held trigger sprayer. This volume was enough to ensure fine droplet coverage without drenching the cage and wasps. A separate batch of 40 insects was similarly sprayed with Karate® (1.75% EC, Twiga Agro-Chemical Industries, Nairobi; at the recommended field dose rate of 2ml/litre) which served as a chemical control. The wasps were held within the treated cages and therefore exposed to the neem insecticides through two routes: direct topical sprays and vapour and contact with residues on the walls of the cage. The number and sex of wasps dying in each treatment on each day was recorded until the last wasp had died. The LIFETEST procedure (SAS Institute, 1990), which calculates a Wilcoxon's chi-square statistic, was used to analyse

survivorship among the treatments. Because significant differences were indicated among the treatments, a t-test (PROC TTEST, SAS Institute, 1990) was applied to the means for survival rates of different treatment pairs to test for differences between them. The ANOVA procedure (PROC GLM, SAS Institute, 1990) was used to analyse for differences in mean longevity, with sex as an additional factor to the treatment; the SNK multiple range test was used to separate the treatment means where significance was indicated.

5.2.2 Parasitisation ability of *D. mollipla*

Two-day old naïve, mated and fed *D. mollipla* females were sprayed with 4-6 ml of aqueous extracts of either NKCP (2.5%) or NSO (1.5%) or water (as a control). After 24 hr each female was released into an 'oviposition cup' [300 ml clear plastic cup with a mesh-covered ventilation window inverted over a smaller cup] containing a cabbage leaf infested with 25-35 second instar DBM larvae and allowed to oviposit for 24 hours. At least 25 females were tested for each treatment. The parasitised DBM larvae were then left to develop for another 2 days and later dissected under a microscope (X160 magnification) for evidence of parasitism (parasitoid larvae). Parasitised DBM larvae were allowed to develop further before dissection so that the parasitoid eggs could hatch into larvae that were easier to detect. The proportion of DBM larvae parasitised by each

female was determined as a percentage. The percentages were arcsine-transformed to reduce dependence of the variance on the mean. One-way Analysis of Variance (ANOVA) (PROC GLM, SAS Institute, 1990) with treatment as a factor was used on the transformed values to test for significant differences in parasitisation ability among the treated females. Where significance was indicated, the SNK multiple range test was used to separate means at $P<0.05$.

5.2.3 Foraging behaviour of *D. mollipla* females

Two-day old naïve and mated parasitoid females were sprayed with 4-6 ml of either NKCP (2.5%), NSO (1.5%) or water (as a control). The parasitoids were used in the observations 24-36 hr after treatment. At least 20 parasitoids were tested for each treatment. A single parasitoid female was introduced into a clear Perspex cage (20x20x40 cm) containing a potted cabbage plant infested with approximately 100 second and early third instar DBM larvae. The parasitoid was observed continuously for 25 min while the absolute duration in seconds for each behavioural element (as described below) during the observation period was recorded using The Observer v3.0 edition software (Noldus, 1995). On each observation day, a fresh batch of unparasitised larvae was provided and both control and neem sprayed individuals were observed. Insects that did not initiate the foraging process within 10 min after the start of observation were

discarded. In preliminary observations, the behaviour of the parasitoid in the presence of hosts had been defined into six distinct elements and coded thus:

1. Searching ('srch') = walking with antennae bent downwards while tapping the surface or probing mines or eaten patches of leaf with the ovipositor;

2. Walking ('walk') = walk erratically with antennae held straight; no surface tapping;

3. Oviposition ('ovip') = wasp assumes the oviposition posture (ovipositor bent under the abdomen) and stings or attempts to sting a host;

4. Grooming ('groo') = legs and/or antennae involved in cleaning movements of one or more parts of the body;

5. Antennation ('avib') = wasp stationary but antennae were vibrating; and

6. Quiet ('rest') = wasp quiescent with no movement of antennae or general displacement of body.

In addition, the location of the wasp on the plant [= 'on'] or off the plant [= 'off', that is on the cage walls or floor] was recorded.

The percent duration for each element of behaviour and location was determined as a proportion of the total observation period (=25 min).

The percentages were arcsine-transformed and the data subjected to ANOVA (SAS Institute, 1990) to test for differences in duration of each parameter among the treatments.

5.3 RESULTS

5.3.1 Toxicity of the neem formulations to *D. mollipla* adults

Karate® sprayed wasps were unable to fly or walk within minutes after treatment and were all dead within six hours; they were therefore not included in the analysis. Highly significant differences in mean longevity among the different treatments were indicated by the chi-square (χ^2) statistic (Wilcoxon's χ^2=30.90, d.f = 5, P<0.001). The time estimate (and its associated 95% confidence intervals) at which at least 50% of males in each treatment would still be alive was shorter than for the female wasps (Table 5.1). Analysis using ANOVA, with sex as an additional factor to the treatment, revealed significant differences in mean longevity between the sexes (F=35.91, d.f =1, 269; P<0.001), with females living longer than males. There were however, no significant differences in the mean longevity of neem- or water-sprayed wasps of one sex (F=0.94, d.f = 2, 269; P>0.05) (Table 5.2). Survival rates at different time intervals for the various treatments were however not significantly different from each other (P>0.05) as shown in Figure 5.1; the *t*-statistics are given in Appendix 7.

Table 5.1: Summary statistics for time estimate at which at least 50% of *Diadegma mollipla* adults in each treatment would still be alive.

Treatment	Time (days)	95% confidence interval	
		Lower limit	Upper limit
NSO females	15.0	7.5	22.5
NKCP females	17.5	12.5	17.5
Control (water) females	17.5	7.5	22.5
NKCP males	7.5	7.5	12.5
NSO males	7.5	2.5	12.5
Control (water) males	7.5	7.5	12.5

Table 5.2: Mean longevity of *Diadegma mollipla* adults sprayed with one of two neem insecticide formulations or water

treatment	Male longevity (days) mean ± s.e	Female longevity (days) mean ± s.e
NSO 1.5%	9.5 ± 0.91aA (n = 53)	16.9 ± 1.71bB (n = 38)
NKCP 2.5%	10.4 ± 1.03aA (n = 47)	17.5 ± 1.80bB (n = 35)
WATER (control)	12.5 ± 1.05aA (n = 67)	17.1 ± 1.67bB (n = 35)
Mean	*10.9*	*17.3*
C.V.	*65.3*	

Within a column means compare treatment sprays and means with the same lowercase letter are not significantly different from each other at $P<0.05$, SNK test; within rows means compare sexes and means with the same uppercase letter are not significantly different from each other at $P<0.05$, SNK test. N = number of individuals tested.

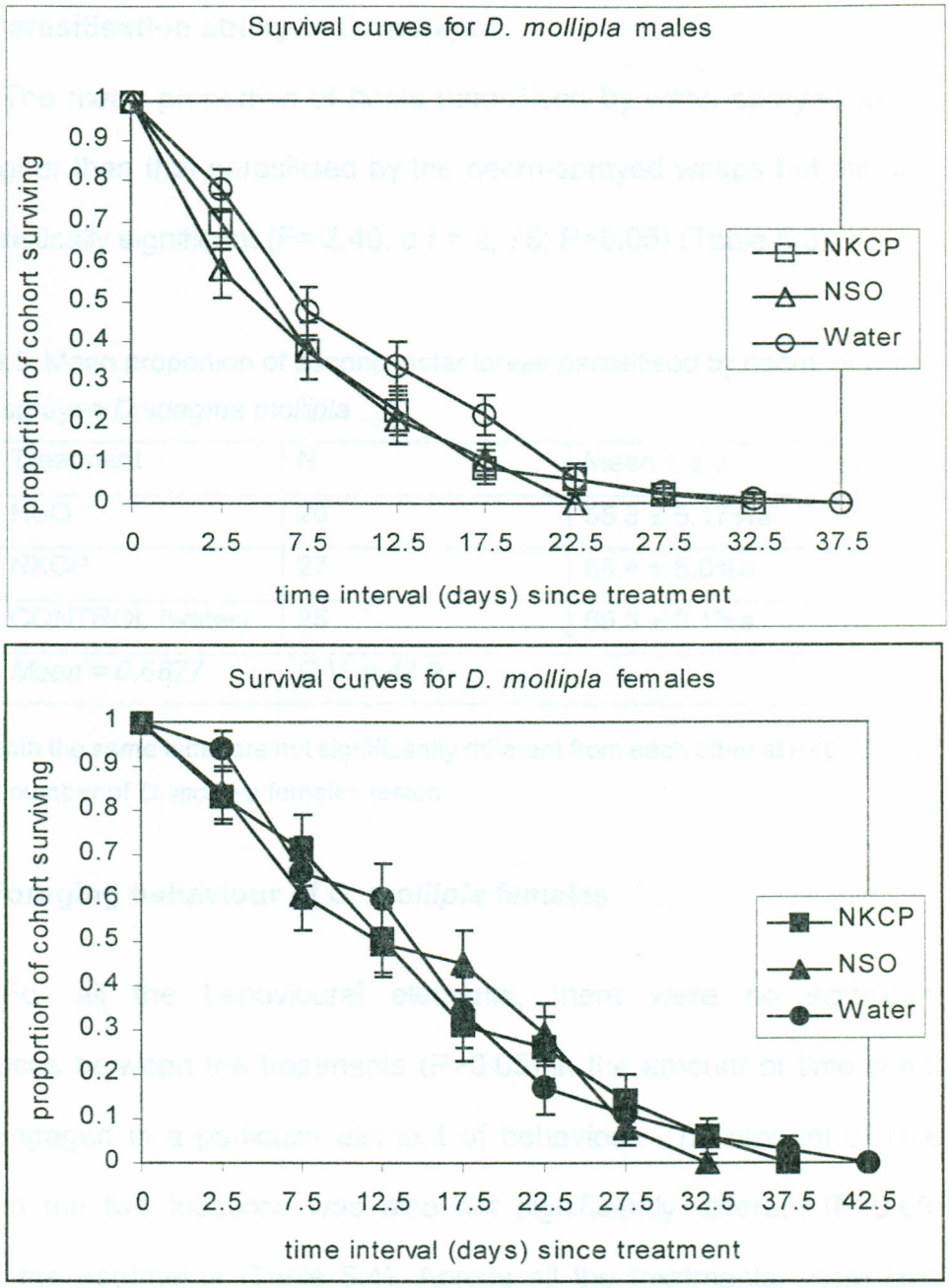


Figure 5.1: Rate of survival in *Diadegma mollipla* cohorts of adults sprayed with two neem insecticide formulations (NKCP, NSO) or water (control). Error bars are standard errors.

5.3.2 Parasitisation ability of *D. mollipla*

The mean proportion of hosts parasitised by water-sprayed wasps was higher than that parasitised by the neem-sprayed wasps but this was not statistically significant (F= 2.40, d.f = 2, 75; P>0.05) (Table 5.3).

Table 5.3: Mean proportion of second instar larvae parasitised by neem- or water-sprayed *Diadegma mollipla*

Treatment	N	Mean ± s.e.
NSO	26	55.3 ± 5.17%a
NKCP	27	58.4 ± 5.0%a
CONTROL (water)	25	69.3 ± 3.1%a
Mean = 0.6877	*C.V. = 42.9*	

Means with the same letter are not significantly different from each other at P<0.05, SNK test. N= number of *D. mollipla* females tested.

5.3.3 Foraging behaviour of *D. mollipla* females

For all the behavioural elements, there were no significant differences between the treatments (P>0.05) in the amount of time spent while engaged in a particular element of behaviour. The amount of time spent in the two locations was also not significantly different (P>0.05) among the treatments (Table 5.4). Across all the treatments, more time was spent on the infested plant than off it. Nevertheless, about 25% of the time available was spent off the plant, particularly on the cage floor, probably because of the presence of frass and hosts that had wriggled off the plant in an attempt to escape the foraging wasps.

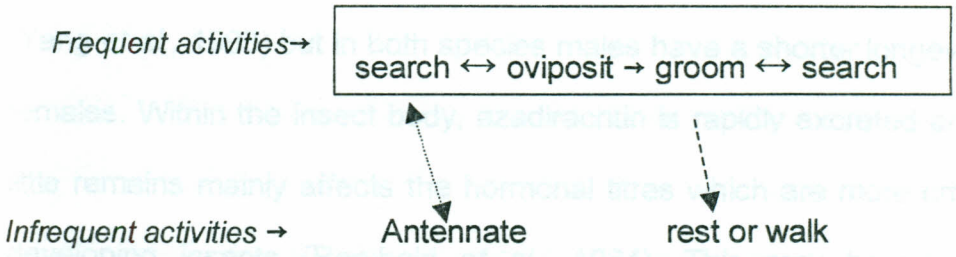
Table 5.4: Allocation of time (%) to different elements of foraging behaviour and hosts' location by *Diadegma mollipla* females treated with a neem insecticide or water (control) during a 25 min observation period.

Category	Parameter	% Duration (mean ± s.e.)			Statistics
		NKCP (n = 22)	NSO (n = 20)	Control (n = 26)	
Location	Off plant	25.9 ± 4.66	25.9 ± 5.68	34.9 ± 5.08	F= 1.15, df= 2, 65; ns
	On plant	74.1 ± 4.66	74.1 ± 5.68	65.2 ± 5.10	F= 0.99, df= 2, 65; ns
↑ Behavioural elements ↓	Search	38.4 ± 3.06	46.5 ± 3.88	44.9 ± 2.81	F= 1.72, df= 2, 65; ns
	Oviposit	28.7 ± 3.79	28.0 ± 2.48	30.7 ± 3.02	F= 0.22, df= 2, 65; ns
	Groom	23.4 ± 3.22	21.6 ± 3.05	17.6 ± 1.88	F= 1.30, df= 2, 65; ns
	Antennate	5.1 ± 1.26	1.6 ± 0.53	3.6 ± 1.60	F= 1.62, df= 2, 65; ns
	Rest	2.1 ± 0.44	0.6 ± 0.19	2.6 ± 1.28	F= 1.33, df= 2, 65; ns
	Walk	2.3 ± 1.32	1.8 ± 1.17	0.8 ± 0.57	F= 0.57, df= 2, 65; ns

For the category of location and behavioural elements total duration equals 100%. Within rows means compare the same parameter across treatments. NS = parameter is not significantly different between the treatments at $P<0.05$. N = number of *D. mollipla* females that were observed individually.

Search' and 'oviposition' were the predominant activities in all the three treatments. 'Oviposition' usually succeeded a bout of 'search'. 'Oviposition' time was nearly equal across the treatments while 'search' time was greater than 'oviposition' time. This was probably because oviposition in *D. mollipla* takes the form of a sting that lasts three to five seconds whereas searching is a more prolonged event. 'Search' was observed to comprise two components, namely antennal probing and ovipositor search/probes. Antennal probing was characterised by the wasp moving along the surface while continuously tapping with the antennae, and during ovipositor probes/search, the wasp used its ovipositor to probe leaf mines/windows [patches of eaten leaf with only the epidermis left intact]. The infested plants had plenty of 'windows' that induced the wasps to intensively search these areas for hosts. 'Groom' usually followed 'oviposit' or a long bout of 'search' and accounted for about 18-25% of the observation time. Only a small fraction of the time was spent antennating, resting or walking. Of these three elements, 'antennate' consumed the most time probably because it is an aspect of 'search'. Antennation was observed to mostly occur (albeit infrequently) after a long bout of searching without host encounter or when the wasp was first exposed to the hosts. Bouts of 'walk' and 'rest' were infrequent; 'rest' generally occurred towards the end of the observation period whereas 'walk' was more characteristic of

individuals that did not spend much time in 'search' or 'oviposit' behaviour. This general sequence of behaviour is illustrated in Figure 5.2.



Note: an explanation to the sequence is provided within the text.

Figure 5.2: General sequence of foraging behaviour of *Diadegma mollipla*

5.4 DISCUSSION

Mean days lived and survival (or mortality) rate are two parameters by which adult longevity can be assessed. Survival (or mortality) rate analysis enables more meaningful biological comparisons between treatments than mean longevity because it is not readily influenced by extraordinary events that occur. Such events include an extremely prolonged lifespan of a few individuals or the need to terminate experimental observations before all experimental units die off (Jervis & Copland, 1996). Topical and residual contact with the neem formulations did not cause acute toxicity in *D. mollipla* adults. This agrees with Schneider and Madel's (1991; cited by Schmutterer, 1995) finding that the survival in *D. semiclausum* adults exposed continuously to residues of

aqueous NSKE (0.1-5%) was unaffected. Haseeb *et al.,* (2000) observed that IGRs had low contact toxicity to adult *D. semiclausum.* Longevity in *D. mollipla* was shorter than in *D. semiclausum* (♂: 20 days, ♀: 24.7 days) (Yang *et al.,* 1993) but in both species males have a shorter longevity than females. Within the insect body, azadirachtin is rapidly excreted and what little remains mainly affects the hormonal titres which are more critical for developing insects (Rembold *et al.*, 1984). This may be why neem insecticides have only a weak or no contact insecticidal effect as opposed to conventional chemical insecticides that primarily act as nerve poisons. Saxena *et al.* (1981b) however recorded a reduction (>50%) in the adult longevity of *N. lugens* that had been sprayed with neem oil but the concentrations used (≥25%) were much higher than those used (1.5%) in the present study. Thus, the doses used and formulation are important considerations when evaluating the non-target effects of neem insecticides. The toxicity of Karate® (a chemical insecticide) to *D. mollipla* is consistent with the results obtained in other studies evaluating the toxicity of chemical insecticides to natural enemies (Kao & Tzeng, 1992; Idris & Grafius, 1993b; Rumpf *et al.,* 1997; Haseeb *et al.,* 2000).

Much of a parasitoid's adult life is spent foraging, that is, in the search for and attack of hosts. Thus, the longer a parasitoid lives the more progeny it is likely to leave behind and thereby ensure its evolutionary success (Jervis & Copland, 1996). Even in the absence of acute toxicity of

the neem formulations, the effects on foraging behaviour are important within the context of biological control. As stated by Waage (1989), the sublethal effects of pesticides on the host-finding behaviour of natural enemies may reduce their impact in much the same manner as the lethal effects. The results obtained on the parasitisation ability and foraging behaviour of neem-treated *D. mollipla* seem to indicate that the neem formulations did not have any apparent 'oviposition-deterring' effects that impair the foraging efficiency of the wasps.

Despite the parasitoids' direct exposure to the neem formulations through topical sprays and residues on the cage walls, no adverse effects were observed on parasitoid longevity or foraging behaviour including the ability to parasitise. It is concluded that the two neem formulations, Neemros® and Neemroc EC®, may be safe to *D. mollipla* adults at the doses used in the study.

CHAPTER 6

ACCEPTABILITY AND SUITABILITY OF NEEM-TREATED HOSTS BY *DIADEGMA MOLLIPLA*

6.1 INTRODUCTION

Much of a parasitoid's adult life is spent foraging, that is, in search for and attack of hosts. The foraging process comprises three phases, namely host habitat location, host location and host selection (van Alphen & Vet, 1986). Host selection can be further subdivided into host acceptance and host suitability (Doutt, 1964; Vinson, 1976). Parasitoids primarily rely on volatile olfactory cues to locate their hosts' habitat and the hosts (van Alphen & Jervis, 1996; Quicke, 1997). The olfactory cues are part of the range of infochemicals (information-conveying chemical molecules) that mediate insect behaviour (Nordlund, *et al.,* 1981; Vet & Dicke, 1992; Quicke, 1997). The infochemicals originate from the host (Noldus *et al.*, 1990) or micro-organisms associated with the host (Thibout *et al.,* 1993) and/or the host's food plant (Schuster & Starks, 1974; Shahjahan, 1974; Elzen *et al.*, 1984; Auger *et al.,* 1989; Turlings *et al.*, 1990). Parasitoids have however, adapted themselves to utilising these cues because they are reliable indicators of the host's presence (Vinson, 1976; Vet & Dicke, 1992). Host selection usually involves a number of tactile and/or chemical cues, with the final cue often being directly

associated with the host such as its surface texture, size, movement or the glue that attaches the host to the substrate (Arthur, 1981; Quicke, 1997). The cues used by a parasitoid elicit within it a series of directed responses that bring it closer to the host's habitat, help it identify the host as an oviposition site and determine whether or not it can successfully support the development of the parasitoid's progeny (Vinson, 1976; Vinson & Iwantsch, 1980).

As well as direct topical contact with insecticides, natural enemies and other arthropods may contact or be affected by insecticides through other trophic levels such as the plant or host/prey (Croft, 1990). For instance, females of the Colorado potato beetle, *Leptinotarsa decemlineata* Say did not feed on potato leaves treated with extracts of neem seed kernel and those that fed, had highly reduced fecundity (Schmutterer, 1987; Kaethner, 1992). Neem-based insecticides are known to have repellent and deterrent properties (Schmutterer, 1990). Dethier *et al.*, (1960) defined a repellent as a chemical that causes an organism to make oriented movements away from its source, and a deterrent as a chemical that inhibits feeding, mating or oviposition when in a place where an organism would, in its absence, feed, mate, or oviposit. These properties by their very nature could alter/mask the cues used by parasitoids during host habitat/host finding. On the other hand, they may alter the attributes that make hosts acceptable for attack by parasitoids and able to support

parasitoid development. The objectives of the present study were to evaluate whether Neemros® and Neemroc EC® applications on hosts/host-plant complexes interfere with the olfactory cues that are used by *D. mollipla* in host finding and selection. Also, to evaluate the suitability of treated hosts and measure some fitness parameters of the emerging parasitoid progeny in comparison to those of the progeny that developed in untreated hosts.

6.2 MATERIALS AND METHODS

6.2.1 Response to chemical cues in a Y-tube olfactometer

A glass Y-tube olfactometer (3.5 cm inner diameter) was used to assess the responses of naïve *D. mollipla* females to odours from different treatment categories. Airflow at the entry was set at 118 ml/min and at the exit at 526 ml/min. These settings ensured the most perfect lamina flow of air within the cross-section of the Y-tube (as shown by a plume of ammonium chloride crystals) while allowing the parasitoids to move through it. Each olfactometer arm was connected by a Teflon® tube (0.5 cm inner diameter) to a glass jar (2-ltr capacity) that was used for holding the source of test odours. Two pressure pumps (Air cadet vacuum/pressure station, Cole-Parmer Instrument Co., USA) were used to pump air into and out of the system while two flow meters (Cole-Parmer Instrument Co., USA) regulated the airflow. Additional Teflon® tubes

conveyed air from the inlet pressure pump through an activated charcoal filter where it was purified, then through one flow meter and into the separate treatment jars. The second flow meter was connected between the stem of the olfactometer and the second pump, which exhausted air out of the system. White Styrofoam boards (30cm high) screened the observation arena (Figure 6.1).

Cabbage plants with about six fully formed leaves (8-10 weeks old) that were growing in 200 ml perforated cups were used in the assays. Use of miniature plants was necessitated by the need to place whole plants in the jars (for the odour source) that could not accommodate a potted plant. Depending on the treatment being tested, the plants were either left uninfested or infested with 30 second to third instar DBM larvae, and these were in turn either sprayed (to near run-off) with one of the two neem formulations (NSO or NKCP) or water. The sprayed plants and plant-host complexes were used in the assays 24hrs later.

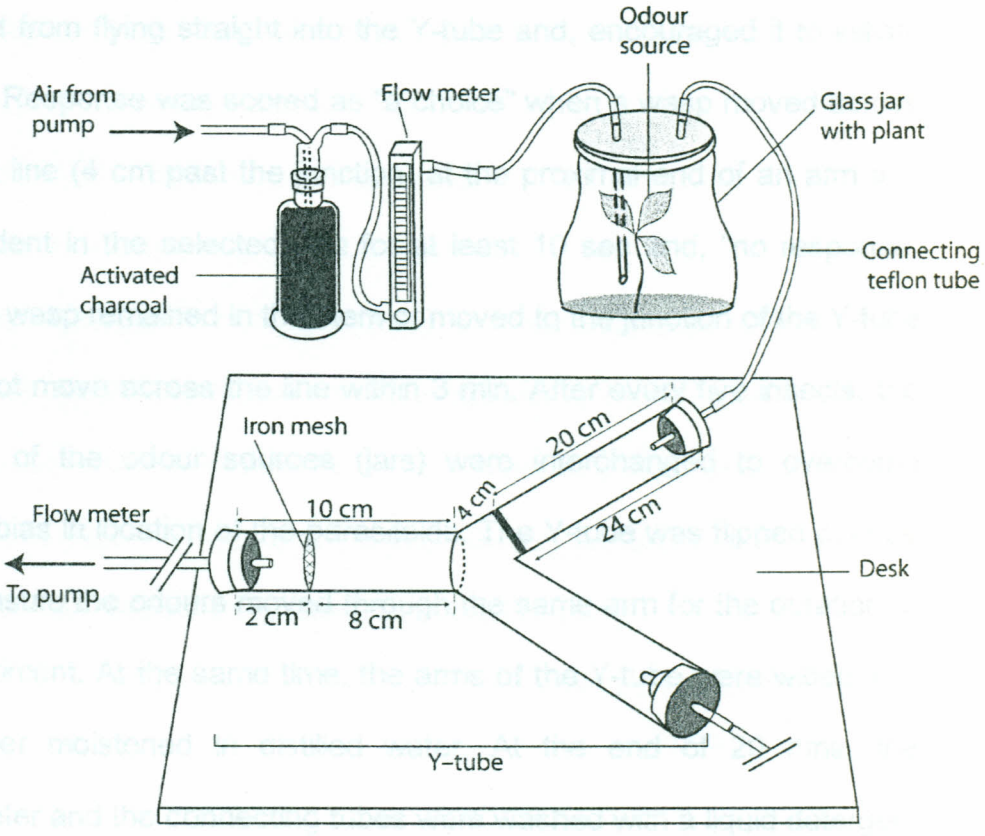


Figure 6.1: A schematic diagram of the Y-tube olfactometer

Three to five day old naïve mated female parasitoids were used. Each female was introduced individually into the olfactometer stem and observed continuously. A disc of iron mesh (mesh size 4 mm) in the stem of the olfactometer (placed 2 cm from the exit) prevented the introduced parasitoid from flying straight into the Y-tube and, encouraged it to initiate walking. Response was scored as "a choice" when a wasp moved across a pre-set line (4 cm past the junction) at the proximal end of an arm and was resident in the selected arm for at least 10 sec and, "no response" when the wasp remained in the stem or moved to the junction of the Y-tube but did not move across the line within 3 min. After every five insects, the positions of the odour sources (jars) were interchanged to overcome possible bias in location of the parasitoids. The Y-tube was flipped over as well to ensure the odours moved through the same arm for the duration of the experiment. At the same time, the arms of the Y-tube were wiped with filter paper moistened in distilled water. At the end of 20 runs, the olfactometer and the connecting tubes were washed with a liquid detergent (Teepol®, Shell Chemicals) and water and air-dried. At least 50 parasitoids were tested for each treatment.

The treatment options are given below. Tests against a blank (= a jar without a plant) were single choice tests conducted to characterise the response of the parasitoids to odours from cabbage plants left uninfested (plant) or infested with DBM larvae (plant-host complex), and then evaluate

if this response was changed by the application of the neem insecticides. Tests against an alternative treated plant were dual choice tests to determine whether wasps showed a preference of one odour source over another.

A- neem sprayed uninfested plant *vs.* blank

B- neem sprayed infested plant *vs.* blank

C- water sprayed uninfested plant *vs.* blank

D- water sprayed infested plant *vs.* blank

E- water sprayed infested plant *vs.* water sprayed uninfested plant

F- neem sprayed infested plant *vs.* water sprayed infested plant

Pooled data for all parasitoids in a treatment were analysed using the likelihood ratio chi-square (χ^2) test (= G-test) (PROC CATMOD, SAS, 1990) to test for significant differences in the choice of odour source made by the parasitoids. Parasitoids that made no choice were excluded from the analyses.

6.2.2 Host acceptance

Separate batches of 25-30 second instar DBM larvae were transferred to a leaf in an oviposition cup (described in 5.2.2) and sprayed with 4-6 ml of either NSO, NKCP or water (control). Twenty-four or forty-eight hours later, a single naïve and mated parasitoid (2-3 days old) or an experienced mated parasitoid (3-5 days old) was introduced into the cup and left to parasitise the larvae for 24 hrs. Experienced parasitoids were those that had parasitised hosts for a 24 hr period prior to the experiment; they were deprived of hosts for 12-18 hrs just before the experiment. At least 15 females were tested for each combination of treatment, parasitoid status (naïve or experienced) and time (24 hrs or 48 hrs). The hosts were subsequently dissected under a microscope (x160 magnification) and the percentage of larvae parasitised (= host acceptance; indicated by the presence of a parasitoid egg or young larvae) was determined for each parasitoid for the two time intervals for the various treatments.

The percentages were arcsine transformed and subjected to a three-way ANOVA (PROC ANOVA, SAS Institute, 1990) with treatment, parasitoid status and post-treatment exposure time as factors.

6.2.3 Host suitability and fitness of parasitoid progeny

Second instar DBM larvae were exposed to mated parasitoid females (3-5 days old) at a host: parasitoid ratio 20 : 1 for 24 hrs. The parasitised larvae were transferred to a young cabbage plant and an hour later, during which time they settled on the plant, they were sprayed with 10 ml of water (control) or one of the two neem formulations at a lethal or pre-determined sublethal dose. The lethal dose was that recommended for DBM control while the sub-lethal dose was taken as that which allowed development to the fourth instar in at least 50% of the test individuals. The doses used were 2.5% NKCP, 1.5% NKCP, 1.5% NSO, 1.0% NSO. Every two days, the number of surviving individuals, lifestage attained, day of adult emergence and sex ratio of the parasitoid progeny were recorded. For each parasitoid, the body length (BL), and head capsule width (HCW) were also measured. A ratio of BL : HCW gave an index of body size which is a good predictor of parasitoid fitness (Godfray, 1994; van Alphen & Jervis, 1996). Developmental duration to adult emergence, proportion of adult emergence and parasitoid size were determined and used to assess the impact of the different treatments on the suitability of the treated larvae to support parasitoid development, and their influence on fitness of the parasitoid progeny. Because of the high mortality of larvae treated with the neem insecticides, no meaningful statistical analyses could be done.

6.3 RESULTS

6.3.1 Response to chemical cues in a Y-tube olfactometer

The responses of *D. mollipla* females to odours in the single choice tests are presented in Figure 6.2; the associated statistics are shown in Table 6.1. In the single choice tests with the uninfested cabbage plants, the water-sprayed plant attracted a smaller proportion (42%) of *D. mollipla* females than the blank (54%) although the difference was not statistically significant. The water-sprayed infested plant however, attracted more parasitoids (57%) than the blank (37%).

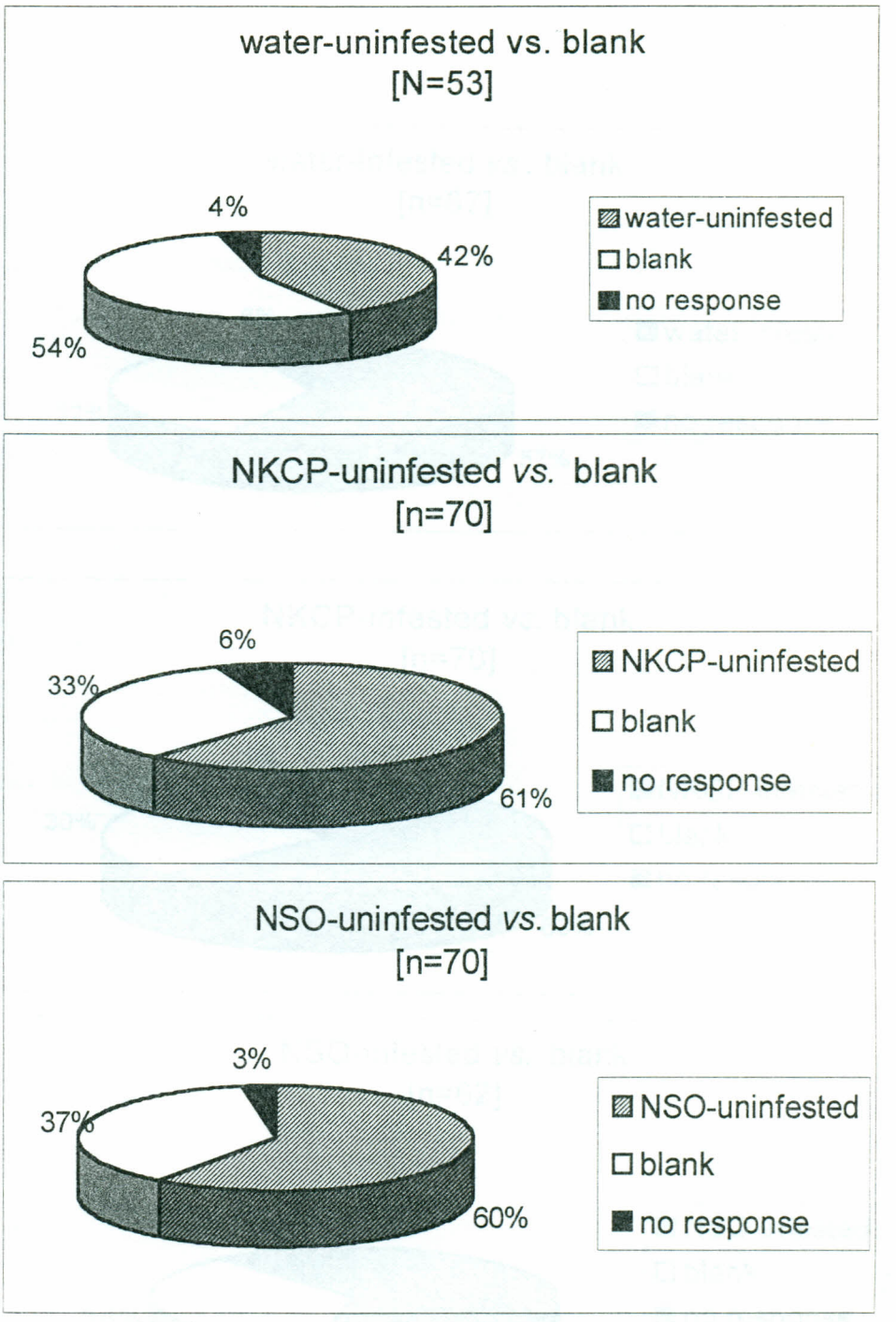


Figure 6.2: Response of naïve *Diadegma mollipla* females to odours from a plant or a plant-host complex sprayed with water or a neem insecticide in single choice tests. N= number of females tested. Numbers next to the pie sections indicate the percentages of parasitoids that made a choice for one of the two odour sources or did not respond.

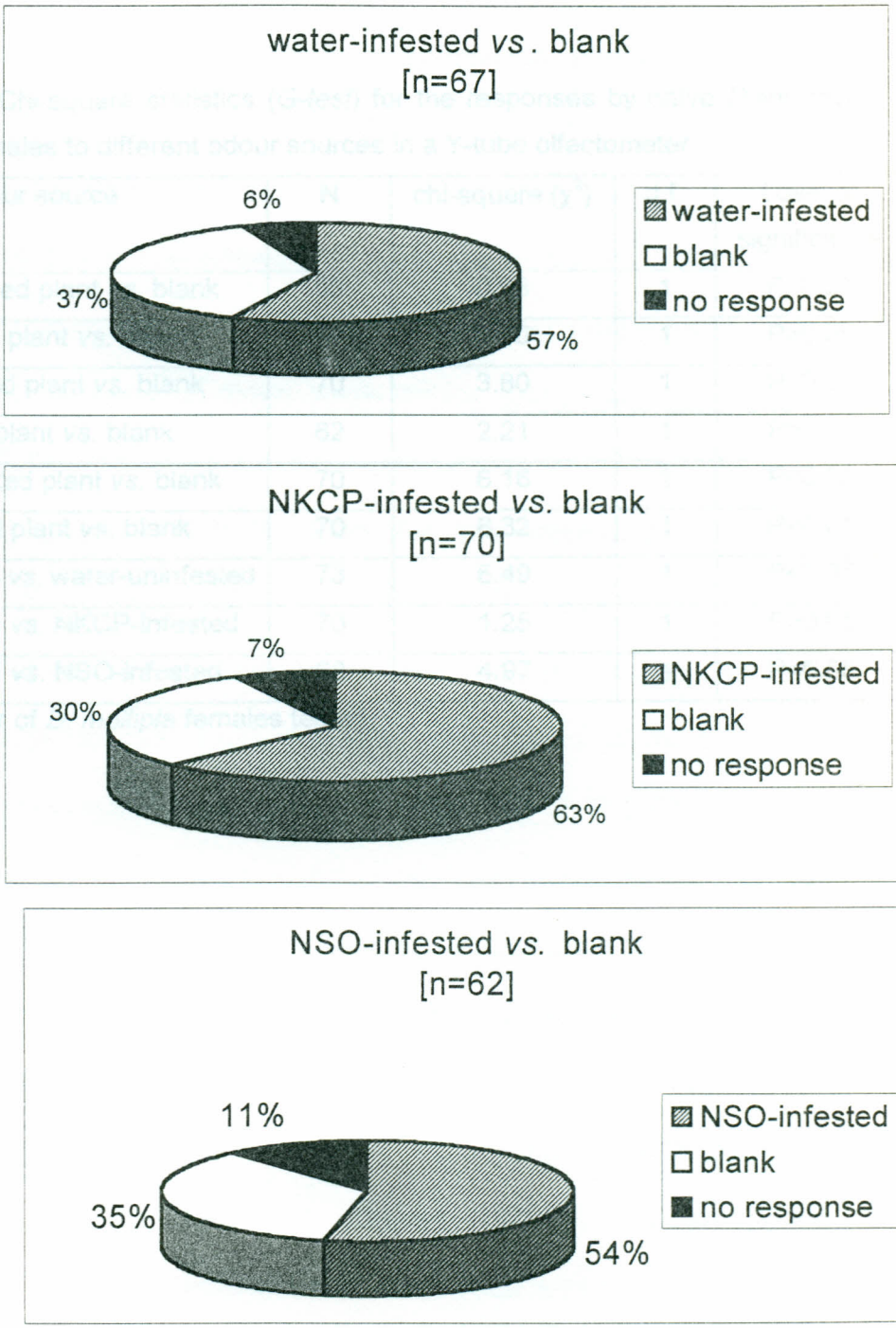


Figure 6.2 (*continued*)

Table 6.1: Chi-square statistics (*G-test*) for the responses by naïve *Diadegma mollipla* females to different odour sources in a Y-tube olfactometer.

Odour source	N	chi-square (χ^2)	d.f.	Level of significance
Water-uninfested plant *vs.* blank	53	0.96	1	P>0.05
Water-infested plant *vs.* blank	67	2.70	1	P>0.05
NSO-uninfested plant *vs.* blank	70	3.80	1	P=0.05
NSO-infested plant *vs.* blank	62	2.21	1	P>0.05
NKCP-uninfested plant *vs.* blank	70	6.16	1	P<0.05
NKCP-infested plant *vs.* blank	70	8.32	1	P<0.01
Water-infested *vs.* water-uninfested	73	6.49	1	P<0.05
Water-infested *vs.* NKCP-infested	70	1.25	1	P>0.05
Water-infested *vs.* NSO-infested	68	4.97	1	P<0.05

N = number of *D. mollipla* females tested.

In single choice tests with the NKCP-sprayed plants, whether uninfested or infested, a significantly higher proportion of parasitoids responded to the odours from the neem-sprayed plants than to the blank. In similar tests with the NSO treatment, the parasitoids' response to odours from the NSO-sprayed uninfested and infested plants did not differ significantly from their response to the blank. In these single choice tests, the proportion of *D. mollipla* adults responding to the neem-sprayed uninfested plants was significantly greater (60-61%) than for the water-sprayed (control) uninfested plants (42%) (χ^2 = 7.87, d.f= 2; P<0.05). When the same comparisons were made with the infested cabbage plants the neem-sprayed plants attracted nearly the same proportion of adults (54-63%) as did the water-sprayed plants (57%) (χ^2 = 1.583, d.f= 2, P>0.05).

In the dual choice tests, significantly more parasitoids (P<0.05) were attracted to odours from the water-sprayed infested plant (61%) than to the water-sprayed uninfested plant (33%). When offered a choice between odours from NKCP-sprayed infested plants and water-sprayed infested plants, parasitoid response to the two odour sources (40% and 53%, respectively) was not significantly different (P>0.05) between the two. (Figure 6.3). In the test between NSO and water spray, both on infested plants, a significantly higher proportion of parasitoids (62%) moved to the arm with odours from the water-sprayed plant than to the NSO-sprayed plant (35%) (P<0.05).

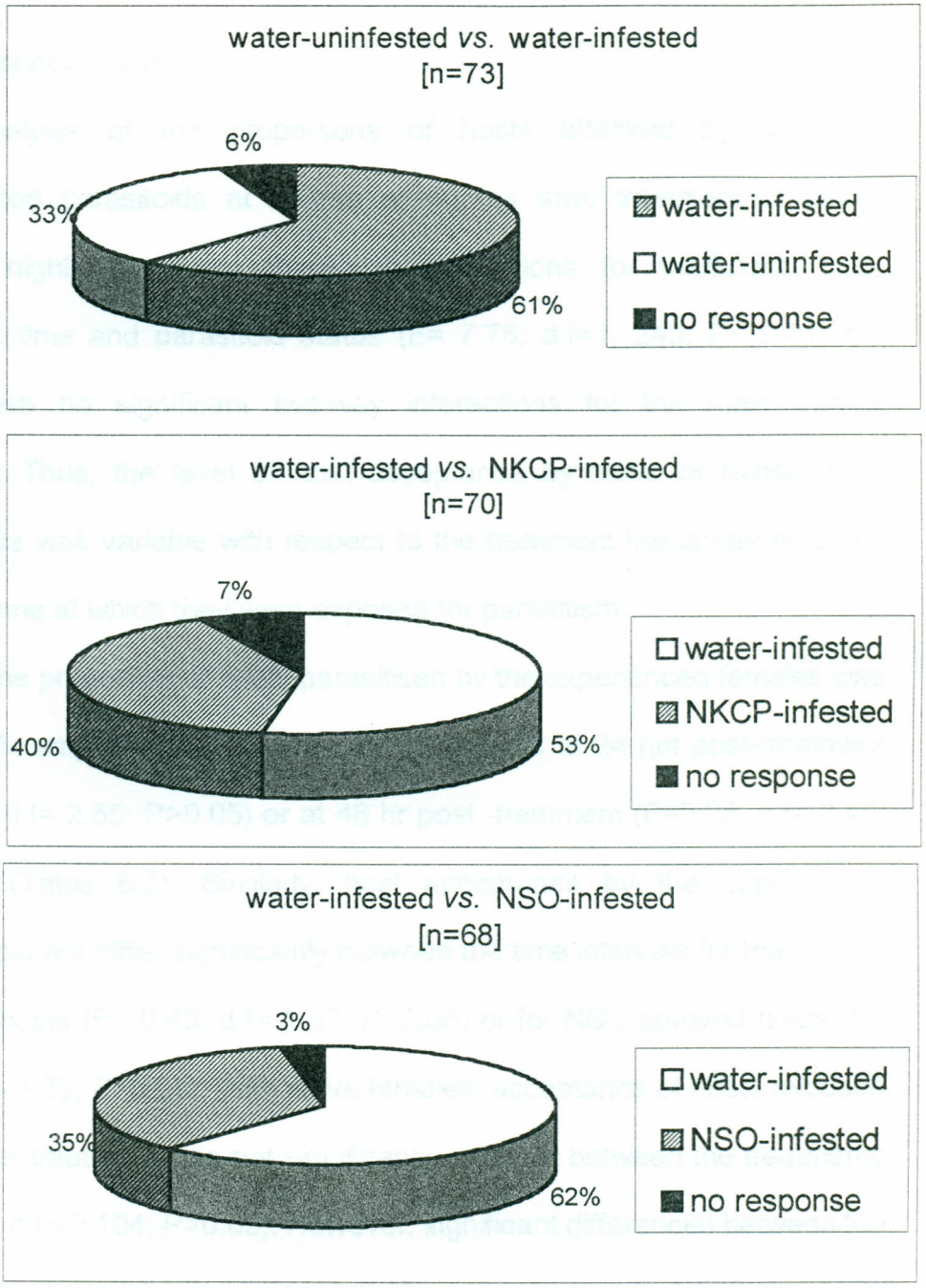


Figure 6.3: Response of naïve *Diadegma mollipla* females to odours from a plant-host complex sprayed with water or a neem insecticide in dual choice tests. N= number of females tested. Numbers next to the pie sections indicate the percentages of parasitoids that made a choice for one of the two odour sources or did not respond.

6.3.2 Host acceptance

Analysis of the proportions of hosts attacked by naïve or experienced parasitoids at 24 hrs or 48 hrs after treatment of larvae showed highly significant three-way interactions for treatment, post-exposure time and parasitoid status ($F= 7.78$; d.f=1, 245; $P< 0.01$) but there were no significant two-way interactions for the main effects ($P>0.05$). Thus, the level of host acceptance by naïve or experienced parasitoids was variable with respect to the treatment the larvae received and the time at which they were exposed for parasitism.

The proportion of hosts parasitised by the experienced females was not significantly different between the treatments at 24 hrs post-treatment ($F=1.39$, d.f= 2,55; $P>0.05$) or at 48 hr post -treatment ($F=0.26$, d.f= 2,49; $P>0.05$) (Table 6.2). Similarly, host acceptance by the experienced females did not differ significantly between the time intervals for the NKCP-sprayed hosts ($F= 0.49$, d.f= 1,32; $P>0.05$) or for NSO-sprayed hosts ($F= 2.55$, d.f= 1,32; $P>0.05$). With naïve females, acceptance of hosts exposed 24 hr after treatment was not significantly different between the treatments ($F= 0.45$; d.f= 2,104; $P>0.05$). However, significant differences between the treatments ($F= 4.80$, d.f= 2,91; $P<0.05$) were observed when hosts were exposed for parasitism 48 hrs after treatment. Host acceptance of NKCP-sprayed hosts was not significantly different from the water-sprayed control hosts but both were significantly higher than the NSO-sprayed hosts. The

proportion of NKCP-sprayed hosts parasitised by the naïve females after 24 hrs and 48 hrs was not significantly different ($F=0.51$, d.f= 1,63; $P>0.05$). The proportion of NSO-sprayed hosts parasitised after 24 hrs post-treatment was however, significantly higher ($F= 9.43$, d.f= 1,64; $P<0.01$) than that at 48 hrs after treatment.

Table 6.2: Percentage of neem- or water-sprayed DBM larvae parasitised by *Diadegma mollipla* at two post-treatment time intervals.

TREATMENT	NAÏVE FEMALES		EXPERIENCED FEMALES	
	24 hr	48 hr	24 hr	48 hr
NKCP	59.5±3.71%aA (N=34)	62.1±4.09%aA (N=31)	63.3±6.32%aA (N=18)	58.8±6.97%aA (N=16)
NSO	63.2±3.37%aA (N=38)	48.3±3.65%bB (N=28)	52.2±4.69%aA (N=19)	65.7±3.89%aA (N=15)
Control (water)	62.2±3.15%aA (N=35)		57.8±5.69%aA (N=21)	

Within columns, means compare treatments within a time interval for both types of parasitoid females and, means with the same lowercase letter are not significantly different at $P<0.05$, SNK test. Within rows, means compare time within a treatment for both types of parasitoid females and, means with the same uppercase letter are not significantly different at $P<0.05$, SNK test. N is number of parasitoids tested (=replicates).

6.3.3 Host suitability and fitness of parasitoid progeny

The high mortality levels in the neem insecticide treatments precluded statistical treatment of the data. Table 6.3 shows the proportion of parasitoids (pooled across replicates) that emerged in each treatment and the range of indices for body size of the emerged parasitoids.

Appendix 8 details the actual number of DBM larvae used in each replicate and the number of parasitoid progeny that emerged. The proportion of *D. mollipla* that emerged from hosts that were sprayed with the lethal (NKCP 2.5%, NSO 1.5%) and lower (= sublethal) doses of neem insecticides (NKCP 1.5%, NSO 1.0%) was extremely low compared to the proportion of parasitoids that emerged from the water-sprayed hosts. For the parasitoids that emerged, their sizes were seemingly comparable to those of parasitoids that had developed in the water-sprayed hosts.

Table 6.3: Effect of two neem insecticide formulations on the suitability of parasitised DBM larvae to support *Diadegma mollipla* development

Treatment	# DBM larvae tested*	% *D. mollipla* emerged*	# ♂:♀ *D. mollipla**	Range, body size index*	
				♂	♀
NKCP 2.5%	146	2.1	2 : 1	5.4	5.5
NKCP 1.5%	146	4.8	5 : 2	4.8-5.0	4.8
NSO 1.5%	201	2.5	4 : 1	6.0-6.3	5.4
NSO 1.0%	185	4.3	5 : 3	5.0-5.4	4.8-5.4
Water (control)	109	40.4	23 : 21	5.0-5.6	4.3-4.8

* = data pooled across replicates

6.4 DISCUSSION

Plant odours play an important role in insect communication in tritrophic systems (Vinson, 1976; Nordlund *et al.*, 1981). Intact cabbage plants release a blend of volatiles (isoprenoids, isothiocyanates, aldehydes and acetates) to which DBM adults respond and use to locate the host plants (Auger *et al.*, 1989; Pivnick *et al.*, 1994). It has also been amply

demonstrated that parasitoids show specific responses to volatile blends (synomones) from plants or substrates in which their hosts commonly occur and make use of the volatiles in host finding (Schuster & Starks, 1974; Shahjahan, 1974; Loke & Ashley, 1984; Auger *et al.,* 1989; Noldus *et al.*, 1990; Turlings *et al.,* 1990; Agelopoulos & Keller, 1994a; Ngi-Song *et al.*, 1996, 2000). Frass is also an important source of host finding cues (Elzen *et al.,* 1984; Loke & Ashley, 1984; Thibout *et al.,* 1993; Ngi-Song *et al.,* 1996). In the present study, *D. mollipla* females were unresponsive to odours from water-sprayed uninfested cabbage plants and showed a marked preference for odours from the water-sprayed infested cabbage plants when the two odour sources were presented together. When cabbage plants are infested with DBM, additional volatiles including methyl and allyl isothiocyanate are also released (Agelopoulos & Keller, 1994b; Pivnick *et al.,* 1994), and these are most likely used by DBM natural enemies in locating their hosts (Reddy *et al.,* 2002). Presumably the feeding action of the larval hosts released green leaf volatiles and other compounds that the parasitoids use in host location. A similar observation was made for *Cotesia rubecula* (Marshall) (Braconidae) which responded strongly to the altered volatile profile from host damaged cabbage but was apparently unresponsive to intact plants (Agelopoulos & Keller, 1994a). Similarly, Shiojiri *et al.,* (2000) observed that *C. plutellae* responded more strongly to infested cabbage plants than to uninfested artificially damaged

plants. The parasitoids also responded to damaged leaves but not to [isolated] larvae. Furthermore, *D. mollipla* response to odours from neem-sprayed plants was comparable to its response to odours from water-sprayed infested plants and stronger than the response to water-sprayed uninfested plants.

In the single choice tests with uninfested plants, the NKCP formulation resulted in a stronger attraction of the parasitoids to the plant volatiles than to the blank. In contrast, the parasitoids' response to odours from the NSO-sprayed uninfested plants was similar to the blank. Since the parasitoids did not respond to volatiles from uninfested plants, this suggests that parasitoids were able to detect and respond, albeit differently, to volatiles from the two neem formulations. In the single choice tests with infested plants, parasitoids were strongly attracted to odours from the NKCP-sprayed plants. This may have been due to the parasitoids responding to the additional volatile components released by larval feeding and, probably others from this neem formulation. However, with the NSO-sprayed plants, parasitoids did not show a marked attraction to volatiles from the treated plants. This may have been due to the presence of other volatile components in the neem oil formulation that contaminated the volatile blend from the infested plant, thus, interfering with the detection of the latter by the parasitoids. This was confirmed by the results from the dual choice tests with this formulation and water-sprayed infested plants,

which also suggest that the NSO formulation affected the foraging of the parasitoids negatively since they were significantly more attracted to volatiles from the water-sprayed infested plants. Parasitoids are able to discriminate between odours from different sources and/or from a host plant under various treatments (Turlings *et al.,* 1990; Ngi-Song *et al.,* 1996; Takabayashi *et al.,* 1998; Ngi-Song *et al.,* 2000) and 'select' the more preferred odour source. In the dual choice tests with the water- and NKCP-sprayed infested plants the parasitoids showed no clear preference between the two odour sources. This result and those of the single choice tests suggest that this formulation had no apparent adverse effect on the parasitoids foraging responses and may have even rendered the uninfested plants more attractive to the parasitoids. This could be an example of a plant-insecticide interaction that enhances natural enemy activity.

The aim of the host acceptance study was to determine whether neem insecticide-sprayed larvae elicited the appropriate host selection responses in the foraging parasitoids that would result in larval acceptance for parasitism. Acceptance levels of NKCP-sprayed hosts by naïve and experienced parasitoids were at either time interval not different from those of control hosts. At the 24 hrs interval, acceptance levels of NSO-sprayed hosts by naïve females were not different from the control and NKCP-sprayed hosts. However, at the 48 hrs interval hosts were not as

acceptable as in the other treatments. It is possible that in the NKCP treatments and in the NSO-24 hrs treatments there was no significant change in the cues that are emitted by the larvae or their by-products such as frass, and which are in turn utilised by the parasitoids in selecting hosts for attack. Auger *et al.,* (1989) found that DBM larval frass contained crucifer-derived disulphides to which *Diadromus pulchellus* Wesmael (Ichneumonidae) responded. Probably as time increased after treatment, the oil formulation caused a more significant change in the hosts' physiology that may have interfered with the production of cues that mediate host acceptance. The fact the experienced parasitoid females showed acceptance levels of the NSO-treated hosts after 48 hr that were comparable to the other treatments, may mean that experienced females are less discriminating probably due to a lower threshold of acceptability. This may have enabled them to 'decide' to oviposit in treated larvae in the absence of more suitable (untreated) hosts. Optimization models of oviposition behaviour predict that under conditions of limited host availability, even poor quality hosts including those already parasitised may be accepted for oviposition (Godfray, 1994; Quicke, 1997).

In the host acceptance study, parasitoids did not have to make a choice between neem insecticide- and water-sprayed larvae. It is therefore not possible to assess which group of hosts the parasitoids would have preferred to attack. A separate study measuring time to first oviposition and

level of parasitisation of both neem insecticide-treated and untreated hosts offered simultaneously to experienced females for parasitisation may help to elucidate the underlying reasons for the reduced host acceptance of NSO-48 hr hosts by naïve parasitoids.

The host suitability experiments showed that although the neem insecticide formulations do not deter adult parasitoids from foraging, they would adversely impact upon the parasitoid population's ability to successfully perpetuate itself. Verkerk & Wright (1993) suggested that the delayed action of neem insecticides on DBM larvae would allow parasitoids to develop within neem insecticide-treated hosts. The observations made in the present study do not support this and indicate that a healthy host is necessary for successful development of parasitoid progeny. Other researchers have also demonstrated that early host death due to infection or toxins such as insecticides invariably killed the parasitoids contained therein (Irabagon & Brooks, 1974; Outram, 1974; McNeil, 1975). Therefore, where the parasitoids are to be used in conjunction with neem insecticides, the establishment of plant refugia where the parasitoids can perpetuate their numbers would be advisable. Mitchell *et al.*, (1997) observed that cabbage interplanted with the more DBM-susceptible collard greens, *B. oleracea var. acephala* L. played an important role in maintaining *D. insulare* populations.

In conclusion, spray applications of Neemros® are unlikely to significantly impair the processes of host habitat location, host location and host selection by *D. mollipla* and may complement biological control programmes for the DBM. Neemroc EC® may result in undesirable effects on parasitoid foraging. Furthermore, effective applications of both formulations onto DBM larvae increases their mortality and therefore makes them unsuitable hosts for propagation of the parasitoid population.

CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 GENERAL DISCUSSION AND CONCLUSIONS

Insecticides are not confined to the target pests' trophic level on application but can move into and affect organisms at other trophic levels of the ecosystem (Croft, 1990). This ability to move into other trophic levels is especially pertinent to parasitoids because they utilise cues from plants or the host itself in host habitat and host location. Furthermore, successful development and maximised fitness parameters of their progeny is dependent on the quality of the hosts parasitised. Because of the complex trophic interactions between parasitoids and their environment a variety of bioassay techniques that take cognizance of among others, lifestage, sex, lifehistory parameters, routes of pesticide entry, anticipated uses and range of recommended rates of pesticide formulations, are required (Banken & Stark, 1997; Ruberson *et al.,* 1998).

In the present study, the effects of the neem formulations on DBM parasitoids were evaluated at three trophic levels: the plant (host location tests), the parasitoid (tests on longevity and foraging behaviour) and the host (host location, host acceptance and host suitability tests). The parasitoids were either exposed directly to the formulations (field tests and tests at the parasitoid trophic level) or indirectly through the hosts and host

plants (all other tests). Thus an attempt was made to encompass the major routes of toxicant contact with the parasitoid and the trophic interactions between the parasitoid and host/host plant.

In the field experiments, Neemroc EC® (the oil formulation) was more effective than Neemros® (the powder formulation) in checking DBM infestation. Furthermore, Neemroc EC® had a stronger and somewhat negative impact on parasitoid activity while Neemros® had no apparent effect. Pooled (= by all parasitoid species) parasitism in the Neemroc EC®-sprayed plots was significantly lower during two weeks out of the five weeks of observation but pooled parasitism in the Neemros®-sprayed plots did not differ from that in the water-sprayed (control) plots. Parasitism by *O. sokolowskii* was significantly lower in Neemroc EC® plots than in the Neemros® and control plots for most of the crop season but parasitism by *D. mollipla* was apparently unaffected by the Neemroc EC® sprays. Inspite of the effects on parasitoid activity, the Neemroc EC® formulation ensured better quality produce than was obtained in the Neemros® and control plots. This oil formulation may therefore be of greater value to farmers. The differential responses of the two predominant larval parasitoid species (*D. mollipla* and *O. sokolowskii*) to the individual neem formulations indicates the importance of monitoring individual key parasitoid species when assessing the non-target safety of neem insecticides.

Like the observations made by Kibata (1996) and Oduor *et al.* (1996), parasitoids were a negligible cause of DBM mortality during this study. The neem insecticide formulations are worthwhile inputs for DBM management since they significantly increased the total mortality suffered by the DBM cohorts. This finding is consistent with other studies that have demonstrated the efficacy of neem insecticides against the DBM (Dreyer, 1987; Schmutterer, 1992; Javaid *et al.,* 2000). Disappearance (due to unexplained causes, including possible neem-induced mortality and predation) accounted for the largest proportion of the total mortality. Because of the very low parasitoid-induced mortality, the effect of the neem insecticide formulations on the parasitoids as a cause of mortality could not be ascertained.

The neem formulations were not acutely toxic to *D. mollipla* adults and also had no effect on longevity, foraging pattern or parasitisation ability. Both the neem-treated and water-sprayed parasitoids spent most of the observed foraging time searching for and parasitising hosts.

The neem insecticide formulations were also tested for any sublethal effects on the responses of *D. mollipla* adults to cues used in host location and acceptance. Neem insecticide treatments have been reported to cause repellence in some insect species (Fagoonee, 1981; Saxena *et al.*, 1981a; Mansour & Ascher, 1984; Saxena & Rembold, 1984; Coudriet *et al.*, 1985; Jilani *et al.,* 1988) but very little information exists for

beneficial arthropods. In this study with *D. mollipla* no repellent effect towards *D. mollipla* was observed in tests with Neemros® formulation. In contrast, Neemroc EC® apparently contaminated the volatile cues from infested plants such that these were less preferable to the parasitoids than the volatile cues from infested plants sprayed with water. Unlike Neemros®, which had no effect on the acceptability of host larvae for parasitism, Neemroc EC® impaired the acceptability of treated host larvae over time. It is possible that the Neemroc EC® treatment had a greater effect on host physiology than Neemros® such that hosts sprayed by the former were less acceptable. A lower threshold for host acceptance in the experienced parasitoids due presumably to a higher egg load may explain the higher acceptance levels of Neemroc EC®-sprayed hosts than was seen for the naïve parasitoids. Host larvae sprayed with either of the neem formulations were however unsuitable for parasitoid development since they suffered high mortality and, along with them, the parasitoid progeny therein. Thus, the neem formulations may adversely affect the parasitoids' ability to perpetuate their numbers because of their toxicity to DBM larvae.

7.2 RECOMMENDATIONS AND SUGGESTIONS FOR FUTURE STUDY

1. It would be advisable in evaluations of non-target effects to compare the responses of individual parasitoid species to insecticides rather than relying on pooled parasitism. This would be especially important where

an individual parasitoid species is of particular significance in the population dynamics of a pest species.

2. Improvements to the Neemros® formulation and/or field dose may be warranted if it is to check DBM populations more effectively.
3. The formulations should also be evaluated for "non-target effects" on *O. sokolowskii* so as to ascertain whether reduced parasitism levels recorded in the field for this species were due to a direct toxicity of the oil formulation or a result of reduced infestation. Furthermore, incidence of this parasitoid species was higher than for *D. mollipla* in the fourth to eighth WAT and it may therefore be worthwhile establishing its actual potential as a biological control agent for the DBM.
4. To determine the actual importance of parasitoids in the population dynamics of DBM, attempts should be made to construct the partial lifetables using DBM populations on field plants in a typical crucifer-growing area and, over a longer period of time.
5. Ultimately, the selection of chemical insecticides, neem insecticides or natural enemies as the primary line of defence against DBM will depend on the demonstrated importance of each of these methods. The DBM is still susceptible to neem insecticides and the newer classes of insecticides (IGRs). However, their use will have to be carefully managed so as to avoid or delay the development of resistance to them as has been observed with synthetic chemical insecticides (Shelton *et*

al., 1996). This is where natural enemies are important and their conservation through the use of more selective insecticides is paramount. The present study has demonstrated a degree of safety of the neem products towards *D. mollipla*. The establishment of plant refugia, which would provide nutrition to the parasitoids and/or maintain a pool of host larvae that could support parasitoid development, could circumvent the observed detrimental effects on parasitoid progeny via the neem-treated hosts. The neem insecticide formulations may be further evaluated under field conditions and with other parasitoid species such as *O. sokolowskii* before final conclusions can be drawn regarding their safety to the important natural enemies of the DBM.

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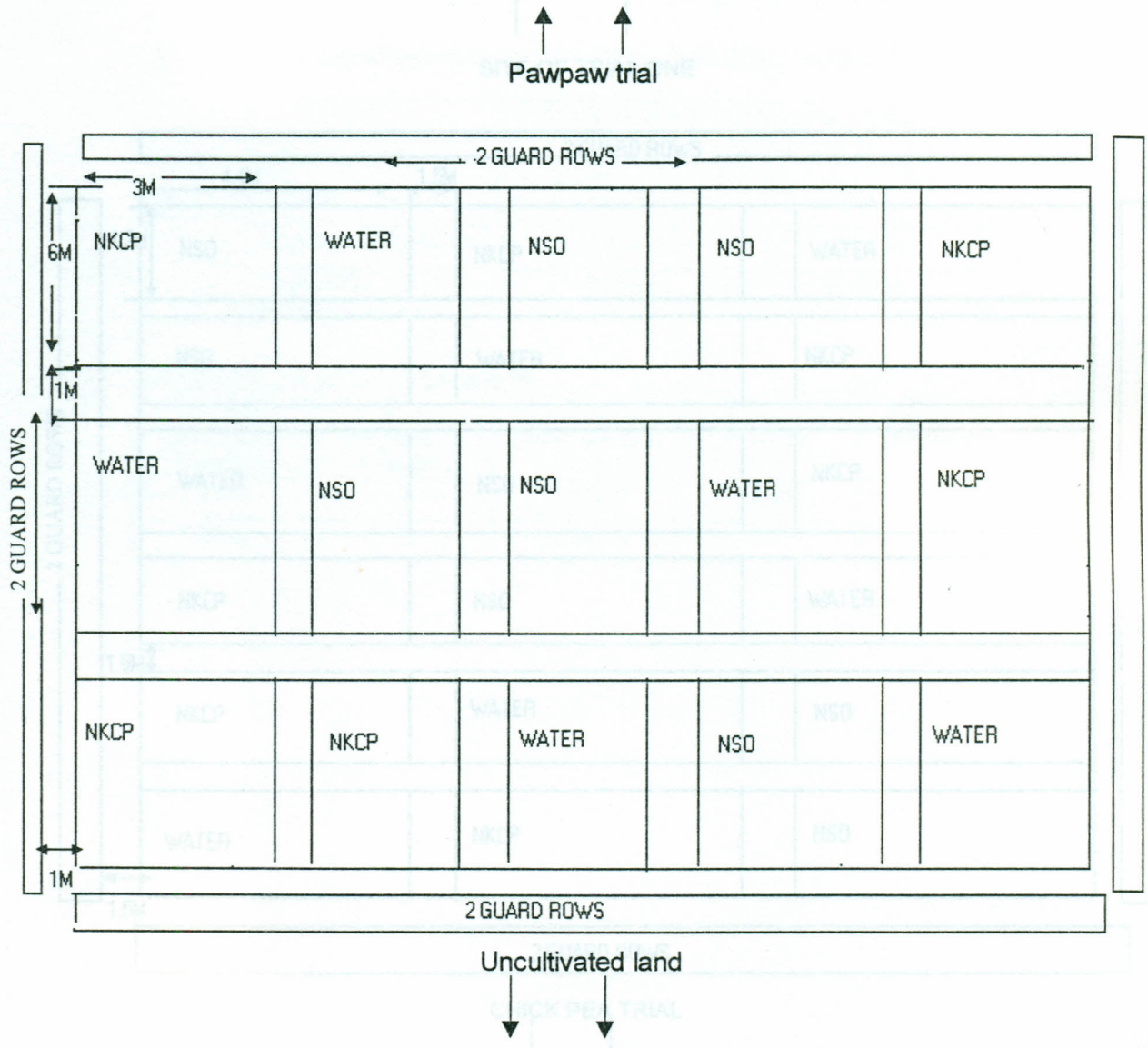
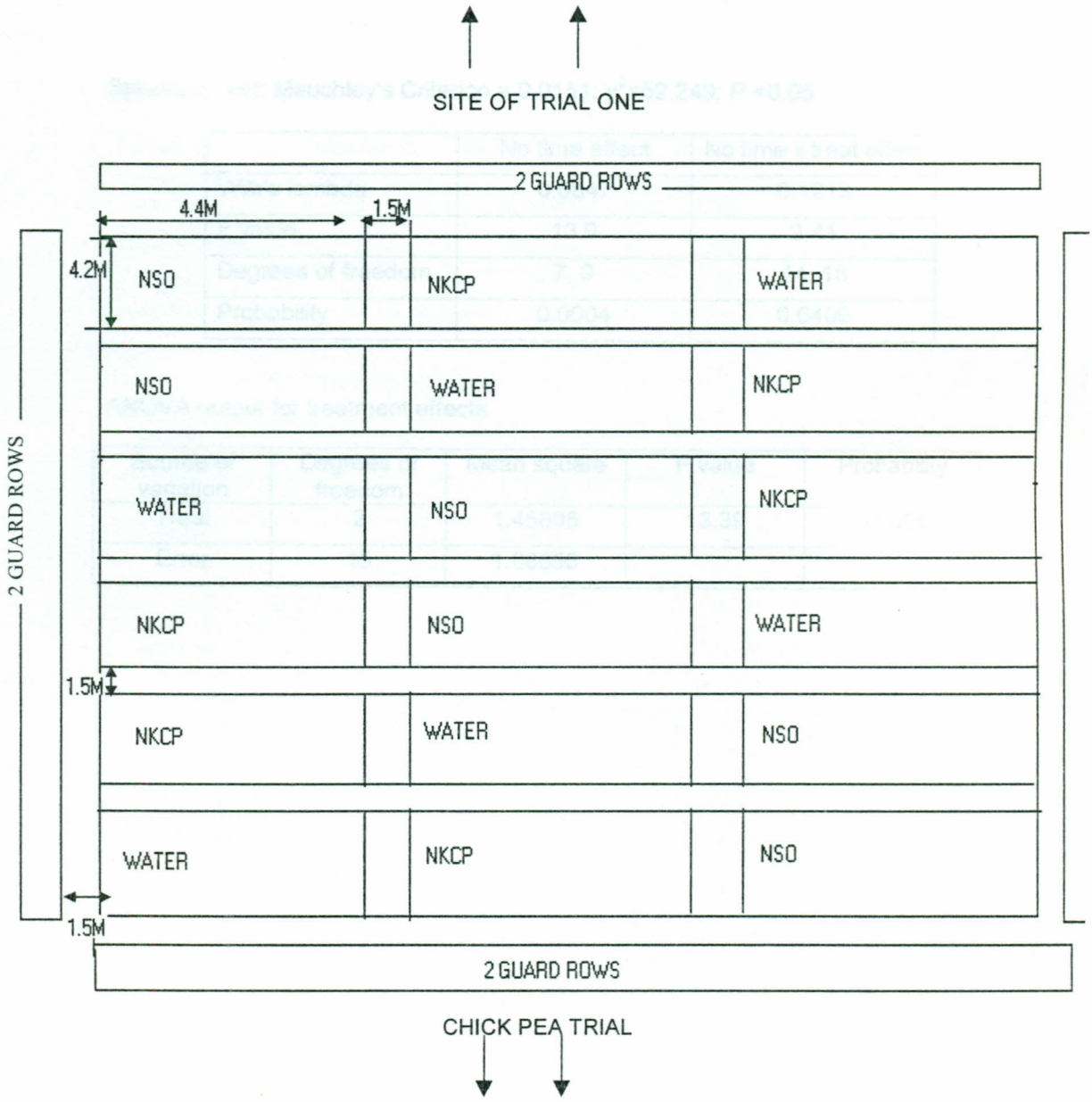
APPENDICES

APPENDIX 1: FIELD LAYOUT FOR TRIAL ONE [completely randomised design]



The right and left sides of the block were also bordered by uncultivated land. Plants were grown at a spacing of 30 cm within rows and 60 cm between rows. Alley spacing was set at 1 m.

APPENDIX 2: FIELD LAYOUT FOR TRIAL TWO [randomised complete block design]



The right and left sides of the block were bordered by uncultivated land. Plants were grown at a spacing of 40 cm within rows and 60 cm between rows. Alley spacing was set at 1.5 m.

APPENDIX 3: Multivariate output for Repeated Measures ANOVA on variation of DBM infestation in cabbage fields sprayed with the neem formulations – trial 2.

Sphericity test: Mauchley's Criterion = 0.0151; χ^2=52.249; P <0.05

	No time effect	No time x treat effect
Wilk's lambda	0.0847	0.1213
F value	13.9	2.41
Degrees of freedom	7, 9	14, 18
Probability	0.0004	0.0409

ANOVA output for treatment effects

Source of variation	Degrees of freedom	Mean square	F value	Probability
Treat	2	1.45805	13.39	0.0005
Error	15	1.08855		

APPENDIX 4: Univariate output for Repeated Measures ANOVA on variation of parasitism of DBM larvae in cabbage fields sprayed with the neem formulations – trial 2.

Sphericity test: Mauchley's Criterion = 0.1335; χ^2=14.932; *P* >0.05

Source of variation	Degrees of freedom	Mean Square	F value	Probability
Time (WAT)	4	0.0690	9.59	0.0002
Time x Treat	8	0.0223	3.09	0.0193
Error	36	0.0070	7.96	0.0072

ANOVA output for treatment effects

Source of variation	Degrees of freedom	Mean square	F value	Probability
Treat	2	0.1035	7.96	0.0279
Error	9	0.0130		

APPENDIX 5: Mulitivariate output for ANOVA on parasitism of DBM larvae by individual parasitoid species in cabbage fields sprayed with the neem formulations – trial 2.

Sphericity test: Mauchley's Criterion = 0.4974; χ^2=42.888; *P* <0.05

	No time effect	No time x treat effect	No time x species effect	No time x treat x species effect
Wilk's lambda	0.3452	0.6259	0.0862	0.3981
F value	28.45	3.96	28.46	4.04
Degrees of freedom	4, 60	8, 120	8, 120	16, 184
Probability	<0.0001	0.0003	<0.0001	<0.0001

ANOVA output for treatment and species effects

Source of variation	Degrees of freedom	Mean square	F value	Probability
Treat	2	0.0464	6.80	0.0021
Species	2	0.9556	140.08	<0.0001
Treat x Species	4	0.0341	4.99	0.0015
Error	63	0.0068		

APPENDIX 6: TERMS AND FORMULAE FOR CALCULATION OF CAUSE SPECIFIC PROBABILITY OF DEATH IN PRESENCE OF ALL CAUSES (CAREY, 1989).

al_x = fraction surviving to stage x ; the fraction surviving in the first stage is set at 1.0; fraction surviving in next stage, $al_{x+1} = al_x(1.0-aq_x)$;

aq_x = probability of death from all causes in stage (x) = $1.0 -[(K_x-D_x)/K_x]$; where K_x is number alive at beginning of stage and D_x is number of deaths in stage x.

$aq_{i,x}$ = is probability of death from cause, i in stage x , $=1.0[(K_x-D_{i,x})/K_x]$;

ad_x = is fraction of deaths in stage x due to all causes = al_x-al_{x+1};

$ad_{i,x}$ = is fraction of deaths in stage x due to cause i, $=al_x (aq_{i,x})$

APPENDIX 7: t-TEST STATISTICS FOR *D. MOLLIPLA* SURVIVAL RATES FOR DIFFERENT TREATMENT PAIRS.

Treatments	*t*-value	df	*P*
Contf vs. contm	0.3874	17	P>0.05
Contf vs. nkcpf	-0.1995	17	P>0.05
Contf vs. nkcpm	0.4518	16	P>0.05
Contf vs. nsof	-0.4533	16	P>0.05
Contf vs. nsom	0.0562	14	P>0.05
Contm vs. nkcpf	-0.5914	16	P>0.05
Contm vs. nkcpm	0.4518	16	P>0.05
Contm vs. nsof	-0.8410	15	P>0.05
Contm vs. nsom	-0.2889	13	P>0.05
Nkcpf vs. nkcpm	0.6516	15	P>0.05
Nkcpf vs. nsof	-0.2625	15	P>0.05
Nkcpf vs. nsom	0.2361	13	P>0.05
Nkcpm vs. nsof	-0.8939	14	P>0.05
Nkcpm vs. nsom	-0.3512	12	P>0.05
Nsof vs. nsom	0.4681	12	P>0.05

Contf= control female wasps; contm= control male wasps; nkcpf= NKCP female wasps; nkcpm= NKCP male wasps; nsof= NSO female wasps; nsom= NSO male wasps.

APPENDIX 8: ACTUAL NUMBERS OF DBM LARVAE TESTED IN EVALUATING THE EFFECT OF THE NKCP AND NSO FORMULATIONS ON THE SUITABILITY OF DBM LARVAE TO SUPPORT *D. MOLLIPLA* DEVELOPMENT.

treat	Replicate	# DBM larvae*	# ptoid progeny†	# ♂:♀
Water (control)	1	19	10	6:4
"	2	35	10	7:3
"	3	19	11	7:4
"	4	36	23	13:10
NSO 1.5%	1	22	1	1:0
"	2	43	0	-
"	3	23	0	-
"	4	20	2	1:1
"	5	40	0	-
"	6	53	2	2:0
NSO 1.0%	1	23	2	1:1
"	2	31	0	-
"	3	20	2	1:1
"	4	20	1	1:0
"	5	40	0	-
"	6	51	3	1:2
NKCP 2.5%	1	20	0	-
"	2	20	2	1:1
"	3	20	0	-
"	4	40	0	-
"	5	46	1	1:0
NKCP 1.5%	1	21	2	1:1
"	2	20	0	-
"	3	20	3	2:1
"	4	40	1	1:0
"	5	45	1	1:0

* = number of parasitised DBM larvae that were monitored; † = number of parasitoid progeny that emerged from the parasitised DBM larvae.