

KENYATTA UNIVERSITY

**The effect of Neem-based pesticides
(*Azadirachta indica* A. Juss.) on the natural
enemies of the Cabbage aphid and
Diamondback moth.**

By

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A thesis submitted in Partial Fulfillment of the Requirement for the Award of a
Master of Science (MSc.) Degree in Agricultural Entomology of Kenyatta
University.

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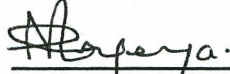
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*The effect of
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DECLARATIONS

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



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DEDICATION

With gratitude, I dedicate this thesis foremost to my loving wife Becky, our daughter Dione and son Barry who are a constant joy and challenge and the greatest gift God has given us.

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Glory and honour to Jesus Christ my Lord for availing to me the opportunity, the grace and the enabling environment for pursuing these studies. May his name be praised.

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ABSTRACT

The effect of pesticides from the Neem tree (*Azadirachta indica* A. Juss) on the natural enemies of cabbage aphid and Diamondback moth was investigated. The test on the effect of Neem products on the reproductive potential of *Diaeretiella rapae* (an aphid parasitoid) was also carried out. Results showed that the parasitoid survival was significantly higher after the application of Neem powder (0.5% AZA) and enriched Neem oil (0.5% AZA) treatments than the Dimethoate treatment. Neem powder positively influenced survival 3 days and 6 days after the treatment applications, though not significantly. Neem treatments caused significantly higher first generation emergence from the treated parasitoids than those treated with Dimethoate but lower than the control treatment. Similar results on the number of parasitoids with deformed wings were recorded.

Observations on the treatment effect on the parasitoid survival in aphid mummies showed no significant differences for *D. rapae*, *Alloxysta* sp. and *Pachyneuron* sp. seven days after the treatment applications. However, Neem treatments recorded significantly higher differences for *Pachyneuron* sp. and for the total parasitoid emergence, fourteen days after the treatment applications. Results on Neem repellency against *D. rapae*, showed that Neem powder was the least repellent treatment.

A study on the effect of Neem on cabbage aphid and DBM natural enemies was carried out in the field and in the laboratory. Results showed that Neem

powder seemed to enhance the emergence of *Diadegma sp.*, *O. sokolowskii*, *D. rapae* and *Alloxysta sp.*, though not significantly. Significantly lower syrphid fly numbers were recorded after the Neem treatment but there were no significant differences recorded for ladybird beetles after the treatment applications.

CHAPTER ONE

1:0: GENERAL INTRODUCTION AND LITERATURE

REVIEW.

1:1: General introduction.

The decline in per capita land availability for human habitation and crop production in Kenya poses a significant problem to the maintenance of the population–food–nutrition balance. Intensive vegetable production promises to be the appropriate enterprise in alleviating the inadequacies of nutritional security, employment generation, poverty reduction and increase in farm incomes (Chadha *et al.*, 1994).

Cabbage (*Brassica oleracea* L. var. *capitata* L.) is one of the important vegetable crops in Kenya. Both small and large-scale farmers practice its cultivation. The crop grows best at moderately cool temperatures (i.e. 16–20°C), and growth rate and head formation is reduced at temperatures above 25°C. (Anon, 1989). Cabbages are sensitive to drought, about 500mm of rainfall per production period is considered optimal. With sufficient water, cabbage can be cultivated on nearly all types of soils, at altitude ranges of between 800m to above 2200m above sea level (Anon, 1989).

In Kenya, the total hectare under cabbage by 1996, spread over seven provinces (i.e. Central, Eastern, Western, Nairobi, Rift valley, Coast and

Nyanza) was 16,176, with an approximate production of 258,460 metric tones per year valued at K £ 75,706,626 (Anon, 1996). Most suitable cabbage growing areas are the high altitude districts of Nakuru (2310-3050m), Bomet (2350-2800m), Kisii (1900-2211m), Nandi (1900-2400m), Kakamega (1500-1700m), Meru (2300-2500m) and Nyandarua (2400-3000m) meters above sea level (Jaetzold and Schmidt, 1973).

Cabbage is eaten cooked or raw in salads almost throughout the entire country. It provides relatively high nutritional value of 1.6g proteins, 0.3g fat, 55mg calcium, 31 mg Iron and various types of vitamins (Tindall, 1993).

Studies carried out in Kenya have identified insect pests as one of the main causes of low cabbage yield, with the prevalence of a pest complex that includes diamondback moth (*Plutella xylostella* (L)) and the cabbage aphid (*Brevicoryne brassicae* (L)) (Madumadu, *et al.*, 1991; KARI / ODA, 1994; Seif, 1995).

1:2:Literature review

1:2:1:Diamondback moth (DBM) (*Plutella xylostella* (L))(Lepidoptera: Yponomeutidae)

DBM is a serious pest of Brassica. It is currently resistant to nearly all classes of insecticides (Talekar and Griggs, 1986). The biology of this pest varies considerably depending on the prevailing climatic conditions. The duration from egg to adult takes 23 and 16 days at 20°C and 25°C respectively (Yamada and Kawasaki, 1993). The egg incubation period ranges from 3 to 6 days depending on the temperature (Abraham and Padmanabhan, 1968).

First instar larva mines into leaf where it stays for two days. The second, third and fourth instar stages each takes 2 to 3 days while the pre-pupal stage lasts 1 day. The total larval period ranges between 10 and 15 days depending on temperature conditions. It is the third and fourth larval instars that cause leaf windowing and leaf serration (Plates: 3 and 4). Pupation in a silken cocoon takes between 7 and 11 days (Abraham and Padmanabhan, 1968). Adults are grey moths with longevity of between 3 to 11 days (Plate 5).

1:2:2: DBM natural enemies

There is overwhelming evidence that parasitoids and predators play an important role in population dynamics of DBM (Ulliyet, 1947; Yamada and Yamaguchi, 1985; Lim, 1986; Waterhouse and Norris, 1987). The effective contributions of parasitoids are particularly evident in parts of Canada, Europe and USA., where the infestations of DBM are generally low. In outlining the situation in the USA, DBM was pointed out as an example of a potentially serious pest which is held in suppression naturally by parasitoids (Muggeridge, 1939; Sutherland, 1966). *Diadegma* species is one of the larval parasitoids of DBM, however its nomenclature and identification problems have compounded its prevailing taxonomic difficulties. Several species of *Diadegma* (Hymenoptera: Ichneumonidae) have been reported which could turn out to be synonyms of *Diadegma semiclausum* Hellen. The various species recorded include *Diadegma fenestrata* (Holmgren), *Diadegma insulare* (Cresson), *Diadegma leontinae* (Brethes), *Diadegma rapi* (Cameron), and *Diadegma varuna* (Gupta) and *Diadegma xylostellae*

(Kusigemati). Most species of *Diadegma* are relatively host specific (Fitton and Walker, 1990). Little attention has been paid to the habitat requirements of *Diadegma* adults of the subfamily Campopleginae, to which *Diadegma* belongs. However, they have been observed feeding from flowers (Fitton and Jervis, Personal communication.) The availability of suitable flowers in and around crops could therefore influence the activity levels of parasitoids (Fitton and Walker, 1990).

Cotesia plutellae L. (formerly: *Apanteles plutellae*) (Hymenoptera: Braconidae) belongs to the large braconid subfamily microgastrinae. Eighteen species of Microgastrid braconids are recorded from DBM in literature. Many of these are probably misidentifications of *C. plutellae* that need to be re-examined and investigated further.

Oomyzus sokolowskii Kurdj. (Hymenoptera: Eulophidae) is a primary parasitoid, as well as a facultative hyperparasitoid. It is the only chalcidoid to have shown any real potential for biological control of DBM. The rates of parasitism in countries where it has been introduced have been as high as 89–100% with up to 10 % levels of hyperparasitism on *C. plutellae* (Cock, 1985). Waterhouse and Noris (1987) have expressed the opinion that this chalcidoid might still prove to be an effective biological control agent. May and Hassel (1981) pointed out that facultative hyperparasitoids could interact in the same way as two competing primary parasites and not adversely affect the system. Reported DBM predators include spiders, coccinellid beetles, pentatomid bugs, *Phytoseilus* mites, chrysopids and *Ophionea* beetle

(Nemoto *et al.*, 1985).

Vos (1953) introduced *D. semiclausum* Hellen from New Zealand to Indonesia, which became established as a biological control agent around Pacet, West Java. Firm establishment was however, achieved during the period 1971-75 with parasitism rates of well beyond 60% (Sudarwohadi and Eveleens 1977). In Taiwan, In addition to *C. plutellae* , the newly introduced *D. semiclausum* has become established in crucifera-growing areas in the highlands and there has been considerably less DBM damage (Talekar, 1990). In Zambia it was claimed that a combination of the newly established *C. plutellae* and *Diadromus collaris* L. (Hymenoptera: Ichneumonidae) along with endemic *O. sokolowskii* have provided a 80% reduction in DBM damage (Yaseen, 1978).

Seasonality studies conducted in several regions in Kenya recorded three DBM larval parasitoids: *C. plutellae*, *Diadegma* sp. and *O. sokolowskii* (Oduor *et al.*, 1996; Magenya, 1997).

1:2:3: The cabbage aphid (*Brevicoryne brassicae* L. (Homoptera: Aphididae)

This is a pest of cabbage, cauliflower, brussel sprouts and kales. It is also an important vector of several viruses. Adults occur in colonies, are medium sized, 1.6-2.8 mm in length and either winged or wingless, grey-green or dull mid-green in colour and are covered with a fine waxy mealy powder (Plate: 6). The adult life span is 8.1 days at 30°C and 28.2 days at 10°C, with four

nymphal instars and 39 generations per year depending on temperature (Akinlosotu, 1977). They cause damage by direct feeding and by viral transmission that results in leaf curls, discoloration and stunted growth (Plate: 7). In heavy infestations, copious amounts of honeydew are produced on which sooty mold grows. The conventional pesticide treatment against aphids includes Dimethoate. However, Straka (1976) reported that insecticidal applications against the aphid are not necessary at predator : prey ratio of 1:100, since at this ratio natural enemies effectively keep down the aphid population to below the threshold economic damage.

1:2:3:1: Natural enemies of *B. brassicae*

1:2:3:2: Primary parasitoids

Most aphid primary parasitoids are hymenopterans (Ichneumonid: Aphidiidae) with an adult size ranging from one to several millimeters long. The two common genera are: *Diaeretiella* Stary and *Lysiphlebus* Forster. Oviposition in these two parasitoids occurs soon after emergence of the female irrespective of mating or food availability (Stary, 1970). Males usually emerge earlier than females under similar conditions. Emergence from mummies is via a circular hole that bears a lid. Females mate only once while males can mate with several females. Sex ratio in the field favours the females but is rather variable due to environmental conditions (Stary, 1970). Reproductive capacity is variable and it can amount to several hundred eggs per female, but all the eggs are not successfully laid, nor is the full supply of eggs spent (Cloutier *et al.*, 1981).

Direct effect of parasitization on the host depends on the developmental stage of the host. The parasitoid egg in aphid host does not seem to have direct injurious effects except for behavioural reactions of the host. First instar larva diffuses a cytolytic excretion into the host's haemolymph, which has a detrimental effect on some of the host tissues. Second and third instar larvae feed orally on haemolymph, while fourth instar larvae actively destroys the remaining tissues ultimately killing the host (Campbell and Mackauer, 1975; Cloutier and Mackauer, 1979). Before completing the development, larva spins a cocoon inside or under the empty aphid skin and at this stage aphid skin becomes indurate developing into a typical mummy (Tremblay, 1964; Cloutier, *et al.*, 1981).

The developmental rate of a parasitoid species is influenced by temperature in a linear way and may take two to several weeks. Minimal survival occurs without water and food. Availability of food (mostly aphid honeydew), favourable temperature and humidity conditions extends longevity to between 2-3 weeks (Broussal, 1964; Cloutier *et al.*, 1981). Parasitoid developmental rate depends on the parasitised aphid instar (Tremblay, 1964).

Parasitoid action is one of the factors that contribute to the control of aphid populations. Stry (1970) distinguished several effects of aphid parasitoids. That parasitism kills the host aphid gradually, because the parasitoid needs some time for development before consuming the food source completely. However, parasitoid action does not stop aphids acting as vectors. Parasitism in aphid colonies can substantially reduce population, but does not prevent aphid migrations. Parasitoid action is usually accompanied by disturbance of

the aphid colony i.e. a sufficiently strong ovipositor action, whether successful or not may dislodge the aphids which then run down or fall off. However, it should be noted that parasitoid action by itself does not cause complete eradication of aphid populations.

1:2:4: Cabbage pest management

Most attempts at controlling cabbage pests have focused on the use of synthetic chemicals such as Endrin 0.25%, Dimethoate 0.5 kg a.i./ha., Lindane 0.075% (Henderson, 1957; Abraham and Padmanabhan, 1968; Ho and Ng, 1970; Bohlen, 1973; Straka, 1976; Gupta and Sharma, 1971; Sachan and Srivastava, 1975), and use of second generation pyrethroids and the biopesticide: *Bacillus thuringiensis* var. *Kustakis* (B.t.).

The increasing awareness of the problems of flagrant use of chemical pesticides such as: environmental pollution, deleterious effects on non-target organisms, resistance, resurgence of pests, secondary pest outbreaks, escalating production and application costs, have all affected the vitality and profitability of cabbage production as an enterprise, (Tauber *et al.*, 1985). This therefore leaves the option of integrated control. Where techniques are harmonized into a compatible multi-faceted and flexible evolving system, geared at maintaining pest populations, at levels below those causing economic injury (Smith and Reynolds, 1966). Pest management in cabbage crop is presently therefore viewed as an ecological process, that involves the depression and regulation of pest populations, into which pesticide and

biological control fit as distinct but often interacting mortality factors (Waage, 1989). One of the tools of integrated control is the application of effective pesticides that do not impair the pests' biological control agents. Biological control agents that cause a suppressive effect on the cabbage pest complex include parasitoids, predators and pathogens. This study looks at the complex interactions of Neem-based pesticides on host populations and their biological control agents.

1:2:5: Use of selective pesticides

Pesticides may affect pest populations indirectly by killing their natural enemies (Waage, 1989). The depression of natural enemy population often results in an increase in pest numbers, implying that the 'irreplaceable mortality' (Southwood, 1985) i.e. net pesticide effect, may be substantially less than their direct kill effect would suggest. Debach and Bartlett (1951) reported that adverse effects of chemical control treatments on natural enemy populations in citrus occurred in three ways: namely direct toxicity, repellent action and elimination of beneficial insects.

Pesticide impact on beneficial insects is better examined not only on direct mortality but also on sub-lethal doses that cause reduced population numbers, thus hampering their effectiveness in suppressing the host. Other effects include changes in foraging pattern, disruption of sexual communication / lack of host recognition. Physiological effects would include altered reproduction, reduced longevity and egg viability / fitness (Moriarty, 1969).

Although herbivores and carnivores consume foods that are nutritionally different, nutrient requirements of insects appear to be qualitatively similar (Mullin and Croft, 1985). Fatty acid composition of dipteran and hymenopteran parasitoids is a duplicate of their host insects (Bracken and Barlow, 1967; Thomson and Barlow, 1974). Interaction studies among three trophic levels have revealed that nutrient deficiencies in the herbivores diet may seriously affect entomophage development (Zohdy, 1976). It is clear that plant toxins are major disrupters of entomophage fitness (Barbosa *et al.*, 1982; Price *et al.* 1980), and this is due to the passive accumulation of toxins in a herbivore's non—essential tissues. This benefits the herbivore at the expense of the carnivore, since phytochemicals are often more toxic to the potential entomophage than to the protected host (Duffy, 1980).

Therefore natural enemies should be protected by using less disturbing practices or developing strains of natural enemies that are more tolerant to a wide family of pesticides (Huffaker, 1985).

1:2:6: Neem

Neem, *Azadirachta indica* Juss. (Rutales:Meliaceae), is a fast growing evergreen, drought resistant tree that grows to a height of 15–20m. It has olive like glabrous fruits, yellow when mature, and the seed endocarp encloses one or two elongated seed kernels (Schmutterer, 1995).

Extracts of Neem fruits, seed kernels, twigs, stem and root barks have been shown to possess insect antifeedant, insect growth disrupting, nematicidal

and fungicidal properties (Schmutterer and Ascher, 1987; Randhawa and Parmar, 1993).

The compounds isolated from Neem seed extracts with feeding inhibitory properties for insects are: meliantriol (Lavie *et al.*, 1967), azadirachtin (Butterworth and Morgan, 1971) and Salannin (Warthen, 1978). Apart from its antifeedant effect, azadirachtin causes disorders in metamorphosis in some insects (Ruscoe, 1972), growth inhibitions, malformations, mortality, and reduced fecundity (Steets, 1975; Schmutterer and Rembold, 1980).

Neem constituents are composed of biologically related euphol and triucalol triterpenoids found in Meliaceae (Lavie and Levy, 1968; Dryer, 1986).

Triterpenoids are suggested to be the precursors of limonoids (Meliacins) occurring in Meliaceae and Rutales (Dryer, 1986). The Limonoids in Neem are related to basic compounds such as azadirone, amoorastatin, vepinin, vilasinin, gedunin, nimbin, nimbolinin and salannin groups, many of which have been isolated and shown to possess various degrees of insecticidal properties (Lavie and Levy, 1971; Schwinger *et al.*, 1984).

1:2:6:1: Effect of Neem on pests

The kernel extracts and pure compounds isolated from the Neem seed have shown diverse biological effects against insects (Singh, 1993). Insects evaluated with Neem for antifeedant activity show varying degree of sensitivity to various extracts and pure compounds irrespective of the order or the family of the insect. For example, 0.001% concentration of Neem seed kernel suspension (NSKS) caused absolute feeding deterrence against the desert

locust, *S. gregaria* while 0.05% concentration was needed for the same effect on the migratory locust (Singh, 1993).

Heyde *et al.*, (1984) found in assays using three species of plant and leaf hoppers on rice, that settling by the brown plant hopper, *Nilaparvata lugens* (Stal) and the white backed plant hopper, *Sogatella furcifera* (Horvath), was progressively reduced by increasing concentrations of Neem oil (1-50%) on the plants. At concentrations of 20% and 50%, very few or no planthopper settled on the treated plants and if they did settle, they were restless whereas adults of the green leafhopper *Niphotetlix virescens* were not or only slightly repelled, even by application of 50% Neem oil.

Serra and Schmutterer (1993) in a number of trials against the whitefly (*Bemisia tabaci* (Gennadius)) on tomato, recorded a strong reduction in egg laying in plants treated with 4-5% NSKE's. This antioviposition effect was stronger than that of synthetic pesticide Buprofezin. Neem oil volatiles significantly reduced egg-laying of the leafhopper *Amrasca devastans* on cotton leaves in the laboratory (Saxena and Basit, 1982).

1:2:6:2: Effect of Neem on natural enemies

Joshi *et al.* (1982) studied the influence of 2 % aqueous extract (suspension) of Neem seed kernels on *Telenomus remus* L. an important egg-parasitoid of *Spodoptera litura* L. in India. When treatment was made prior to egg laying, emergence of adult wasps was normal but their life span was shorter than that of the controls. In contrast, spraying with Neem after oviposition of the

parasitoid prolonged the life of the wasps and led to the production of more eggs than in untreated controls. Li *et al.* (1986) compared the toxicity of *Bacillus thuringiensis* and Neem oil products with other insecticides and came to the conclusion that products based on *B. thuringiensis* and Neem oil were the least detrimental to parasitoids.

A higher population of the parasitoid *Diadegma semiclausum* was found in 'Biosol' - treated cabbage plots than in the control plots (Chandramohan and Nanjan, 1990). Biosol is a Neem-oil-based commercial product in India. Laboratory trials (Schneider and Madel, 1992) showed no adverse effects on *D. semiclausum* adults after exposure to residues of aqueous NSKE (0.1–5 %) in cages for 3 days or throughout their lifetime. The life span of the wasps exposed to Neem was somewhat prolonged but the difference between the treated individuals and controls was statistically not significant. Females of *D. semiclausum*, deriving from the specimen that developed in the Neem-treated *Plutella xylostella* larva, did not show reduced fecundity or activity (vitality).

Tewari and Moorthy (1985) investigated in the laboratory the effect of Neem oil (+acetone +Triton X-100) on the degree of larval parasitization of the phytophagous coccinellid *Epilachna vigintioctopunctata* by the eulophid *Pediobius foveolatus*. When the beetle larvae were exposed to the adult parasitoids immediately after the topical treatment with Neem oil (0.075 % and 0.05 %), the parasitization rate was strongly reduced, but the exposure one day later led to no reduction.

Osman and Bradley (1993) explained the high mortality of larvae and morphogenetic defects of adult parasitoids developed from hosts treated with NSKE as mainly due to the effects of active ingredients on metamorphosis of

the parasitoids. Neem oil at 2.5% reduced the emergence rate of the braconid *C. plutellae* adults when pupal cocoons were sprayed in the laboratory, and a higher concentration (10 %) prevented emergence completely (Loke *et al.*, 1992).

1:3: Justification

Neem-based pesticides are a potential alternative to the synthetic chemicals that cause pest resistance, health risks and environmental degradation. In the attempt to address the current interest of developing and adopting safe and efficient methods of managing agricultural pests, the need to achieve sustainable and cost effective Integrated Pest Management (IPM) strategies can not be over emphasised. In view of these, the importance of understanding the interaction between the products of Neem, DBM, cabbage aphid and their natural enemies' population dynamics is necessary as an avenue of providing the biological basis for determining the appropriate management strategies and tactics for the economic suppression of these pest species. The information on the effects of Neem-based pesticides on the natural enemies of these two listed pests would therefore be invaluable in the judicious use of Neem pesticides, as part of an IPM package for cabbage.

1:4: Hypotheses

- 1 Application of Neem-based pesticides has no effect on parasitoids within host pest tissues.
- 2 The tested Neem-based treatments have no effect on parasitoids and predators of the cabbage aphid and Diamondback moth.

1:5: Objectives

The objectives of this study were:

1. To study the effect of Neem-based pesticides on parasitoid reproductive potential.
2. To determine the effect of Neem treatment on parasitoid survival in parasitized cabbage aphid.
3. To determine the repellency of Neem against cabbage aphid parasitoids.
4. To determine the effect of Neem-based pesticides on DBM and cabbage aphid parasitization and predation in comparison with standard recommended chemical methods.

1:6: General materials and methods

1:6:1: Study site

The field study site was at Kisii, KARI-Center, located between latitudes 0° and 2° S. and longitude 35°30' E and 36° E (Figure: 1). The site is classified within Agro-Ecological Zone (AEZ): Upper Midlands 1 (UM 1). With an altitude of 1680m above sea level, mean annual temperatures of between 18°C–22°C, annual average rainfall of 1400–2100mm, well drained deep reddish brown, friable clay with thick humic top soil, i.e. humic cambisols (Jaetzold and Schmidt, 1983). Figure: 1 shows the meteorological data recorded during the sampling period.

Laboratory studies were conducted at the International Center of Insect Physiology and Ecology (ICIPE) Kasarani, Nairobi.

1:6:2: Field collection of insects

Unparasitised cabbage aphids and aphid mummies were collected from the fields in Kisii located in SouthWest Kenya and at Kasarani in Central Kenya. The two were reared separately in cages (Plate: 1). at room temperatures ranging between 19.5–26°C. with relative humidity of 67-70%.

1:6:3: Handling of the insects

Most of the actively flying insects were handled using an aspirator and insect counts were made using the hand-held tally counter (Plate: 2)

Figure 1: Map showing the location of Kisii in Kenya

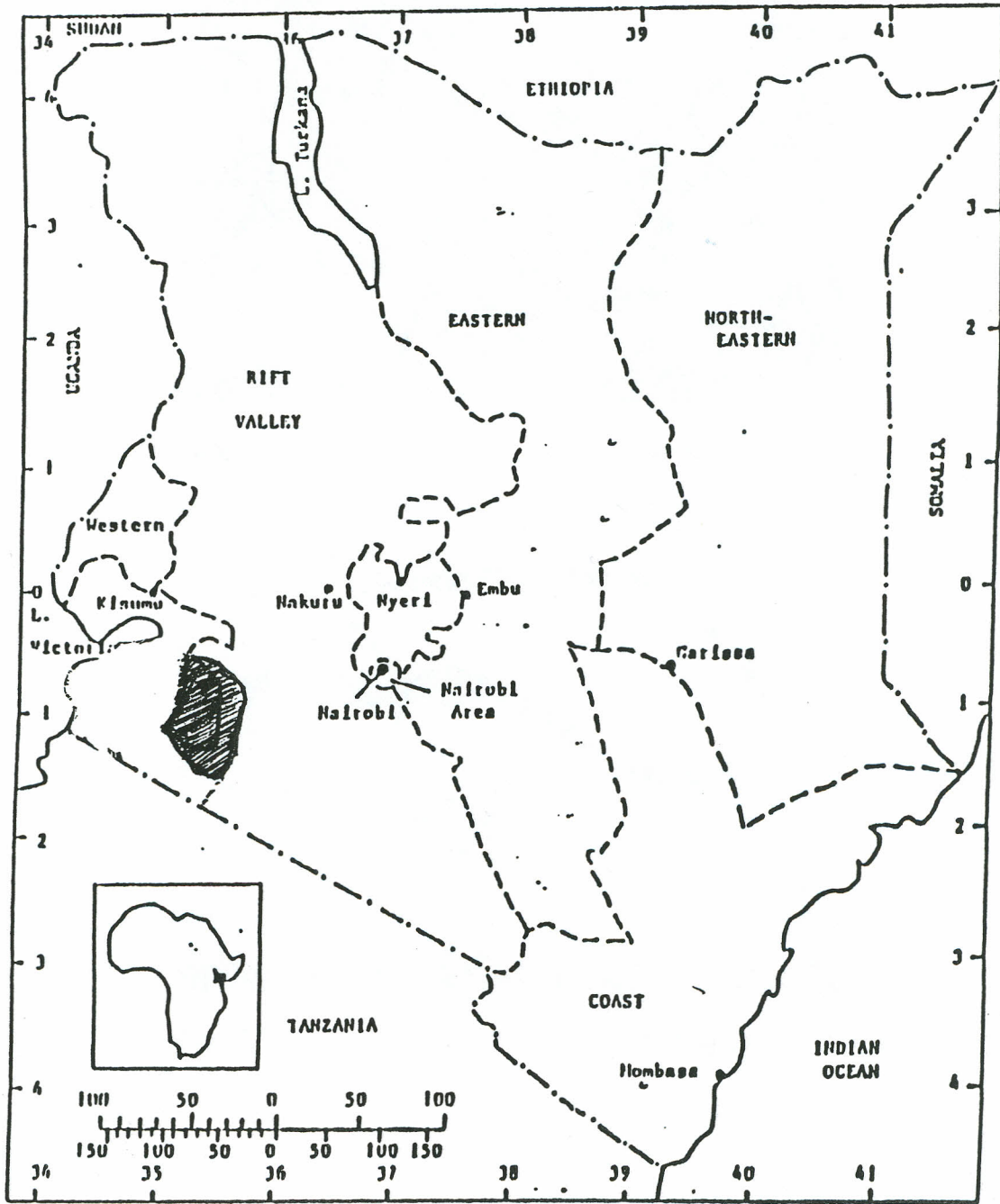




Plate 1: Rearing cages for aphids and their parasitoids.

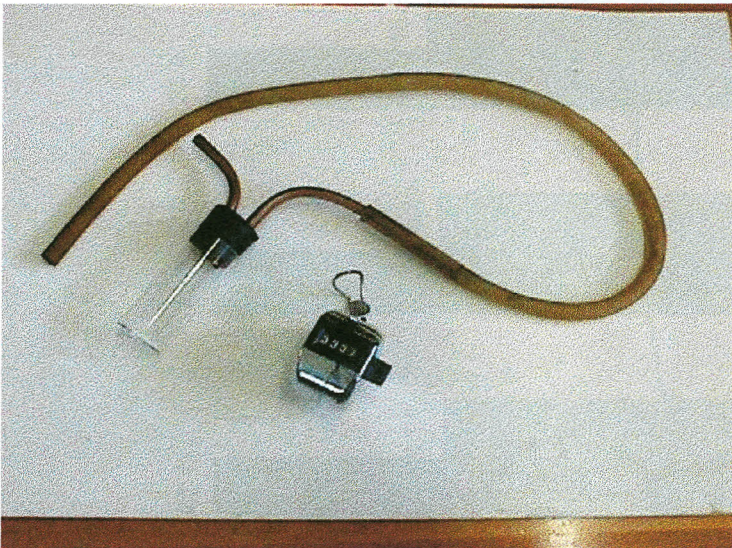
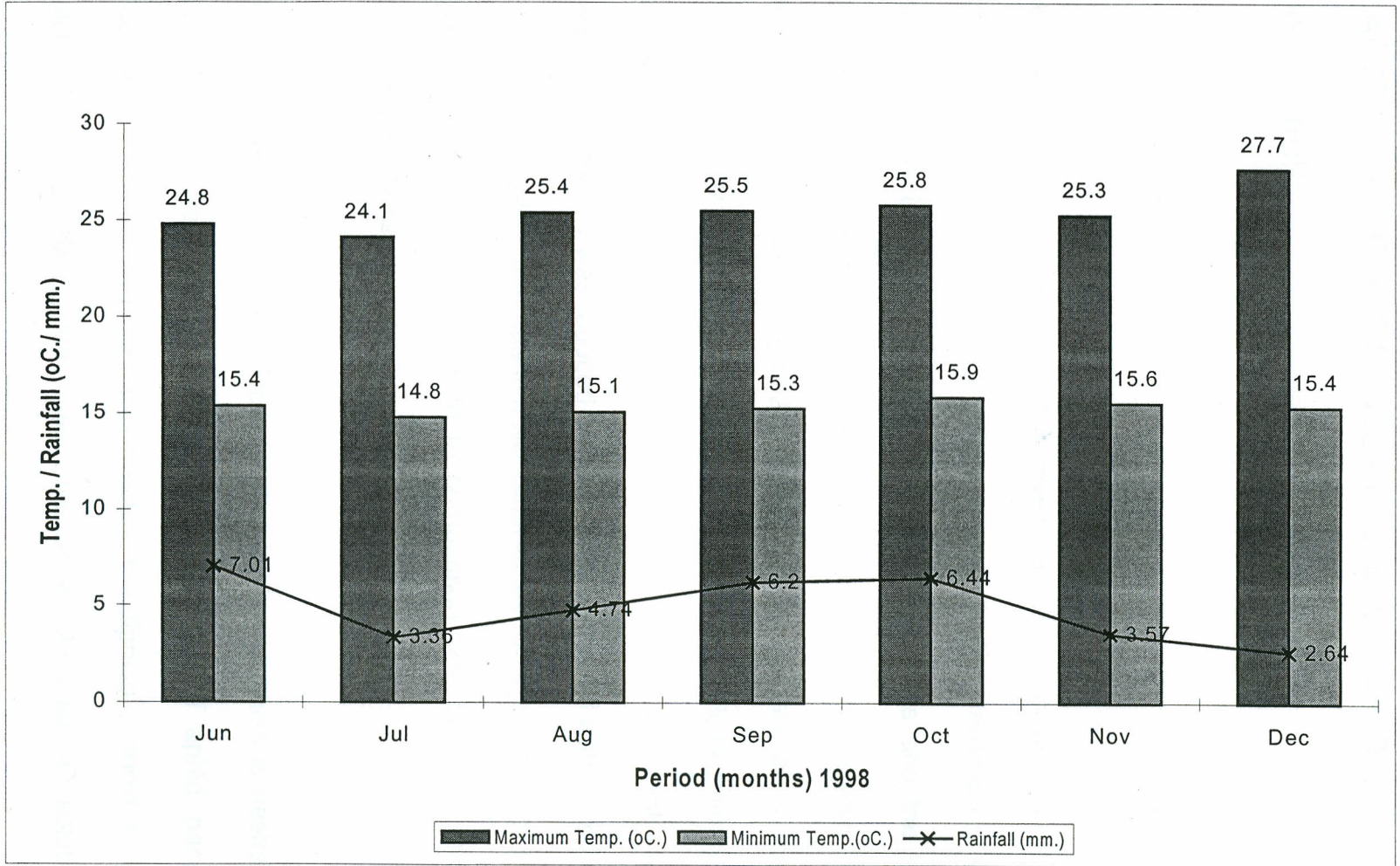


Plate 2: The aspirator used in handling insects, tally counter. is beside it.

Figure 2: Meteorological data for the sampling period in Kisii.



CHAPTER TWO

2:0: LABORATORY STUDIES OF NEEM-BASED PESTICIDES ON PARASITISATION

2:1: Introduction

Quantitative analysis of the effect of a given substance on the reproductive capacity can be carried out in many different ways. Topical treatment of known test concentrations is usually applied on to the parasitoids and the latter's survival and multiplication on host observed (Lamb and Saxena, 1988). Beitzen-Heineke and Hofman (1992) investigated in the laboratory the side effects of AZT-V-NR (a product of Neem) on the endoparasitic tachnid fly *Drino inconspicua*, and reported that adult flies in glass cages were not harmed. However, their fecundity was reduced by 18.5 % as compared with the control. This study investigated the quantitative differences of the effect of topical application of Neem-based pesticide in comparison with the recommended pesticides on the laboratory reared parasitoids.

Information on the behaviour of Neem treated aphid mummies is desirable in understanding the treatment effects at various stages of aphid parasitoid development. Hoelmer *et al.* (1990) reported that the dipping of aphid mummies parasitised by *Lysiphlebus testaceipes* Cresson in Margosan-O solution (a

product of Neem), did not affect the parasitoids' emergence. However, in the case of *Eretmocerus californicus* (Aphelinidae), the emergence of the parasitoid was reduced by more than 50 % as compared with the untreated control. In this study, the effect of topical treatment of aphid mummies with Neem-based pesticides in comparison with recommended pesticides was investigated.

Deterrence is a chemosensory phenomenon. It is apparent from the diversity of insects and their ranges of feeding habits that the impact of deterrent compounds and their capacity varies with every insect species (Barneys and Chapman, 1987). This study investigated the responses of the cabbage aphid parasitoid *D. rapae* to host searching within a Neem treated environment, in comparison to other conventional pesticides.

2:2: Materials and methods

2:2:1: The effect of Neem on reproductive potential of the parasitoids

Unattacked first instar aphids were transferred from the naturally infested plants with a moistened fine camel-hair-brush. Large aphids were gradually picked up with a pair of fine curved forceps, while allowing them a fraction of a second to withdraw their stylets.

A continuous supply of young and tender Copenhagen market cabbage plants was assured by potting seedlings in a screen house. 0.5kg of sterilised soil mixed with 20mg of DAP fertilizer per plant pot was used to raise the seedlings.

Sowing was done every two weeks to ensure a constant supply of young actively growing plant tissues.

Three cages measuring 1 m³ were each used to enclose two potted plants. Good quality monofilament nylon of mesh denier size 210 μ m was used to cover the sides of the cage. This material ensured adequate lighting and ventilation and served in 'aphid proofing'.

Twelve three weeks old potted cabbage plants were each put separately in twelve square cages measuring 45cm³. The cages were made of stiff 8mm wire covered on all sides by a fine netting material. Thirty unattacked aphids from the rearing cages were transferred to each of these caged plants. Twelve parasitoids (*D. rapae*) of 1:1 female to male sex ratios were released into each of the cages. Twenty percent (20%) honey solution was sprinkled on the leaves daily, as food source for the adult parasitoids. Using a hand-held sprayer, test treatments were applied by making six rapid presses while directing the spray nozzles into the cages (i.e. releasing almost 6ml of the treatment into each cage).

The surviving parasitoid counts were made daily for six days after the treatment applications. Treatments were: Dimethoate, control, Neem powder aqueous extract, and Neem oil). Neem cake powder water extract (NCP-WE) was prepared by weighing 25 g of powder into a container and one liter of water added. The mixture was stirred thoroughly and left to stand for 12 hours. Neem oil extract was prepared by dissolving 10 ml of Neem oil in one liter of water and

stirred.

Dimethoate was prepared by mixing 30 ml of the chemical in 20 liters of water.

All dead adult parasitoids were removed from the cages.

Rearing and observations of the aphids on the caged plants continued for twenty days. This was carried out at average temperatures of 21°C (minimum) and 26.5°C (maximum).

Counts of the emerged first generation parasitoids from adults that had been exposed to the treatments were made. The specimens were observed under the microscope and the number with crippled wings recorded.

Analysis of variance was performed using the general linear model (GLM) to determine any significant differences between the treatments. Mean comparisons between the treatments for each of the various categories was performed using the Student–Newman–Keuls (SNK) test (SAS Institute, 1987).

2:2:2: The effect of Neem on the survival of parasitized hosts

Three hundred and sixty (360) naturally parasitised mummies were collected from eighteen randomly selected plants (i.e. twenty mummies per plant) from an unsprayed field. Thirty mummies were separately placed in each of the treatment petri–dishes namely Neem powder aqueous extract, Neem oil, Dimethoate, and water as the control. The treatments were applied using a hand–held syringe sprayer at the rates of approximately 6ml per petri–dish. Each treatment was replicated three times.

Two counts of parasitoids were made i.e. seven days and fourteen days after the treatment applications. Specimens were later identified at the ICIPE biosystematics unit.

95% confidence limits for the means was calculated. Mean comparisons between the treatments for each of the insect species and the total emergence were performed using the Tukey's test (Spjotvoll and Stoline, 1973).

2:2:3: The effect of Neem extract repellency on *D. rapae*.

The bioassays were conducted using a version of a sensitive Y-shaped olfactometer designed to evaluate the repellent activities of the constituents of two traditional protectants against maize weevils *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) (Hassanali *et al.*, 1990). It was constructed of glass consisting of three compartments A, B and C, connected by a 6-mm glass tube. The three compartments were fitted with ground glass joints i.e. D, E and F each with a glass stopper of the appropriate size. Stoppers for E and F had narrow grooves along their lengths to allow airflow into the olfactometer during bioassays (Figure 2).

Prior to the introduction of the test material, air was suctioned out at G using an aspirator at a flow rate of 1.5ml/min. All odorous air from the olfactometer to the aspirator pump was carried out through a tygon tube 8mm in diameter. This pump ensured no saturation of the tubes by the test material. The test materials: Neem oil (i.e. with 0.5 % AZA, 5 kg / ha.), Neem cake powder (with 0.5 % AZA at 2.5kg / ha.) *B.t.* (at the rate of 10 g / 20 liters, 500g / ha.) and Dimethoate (at

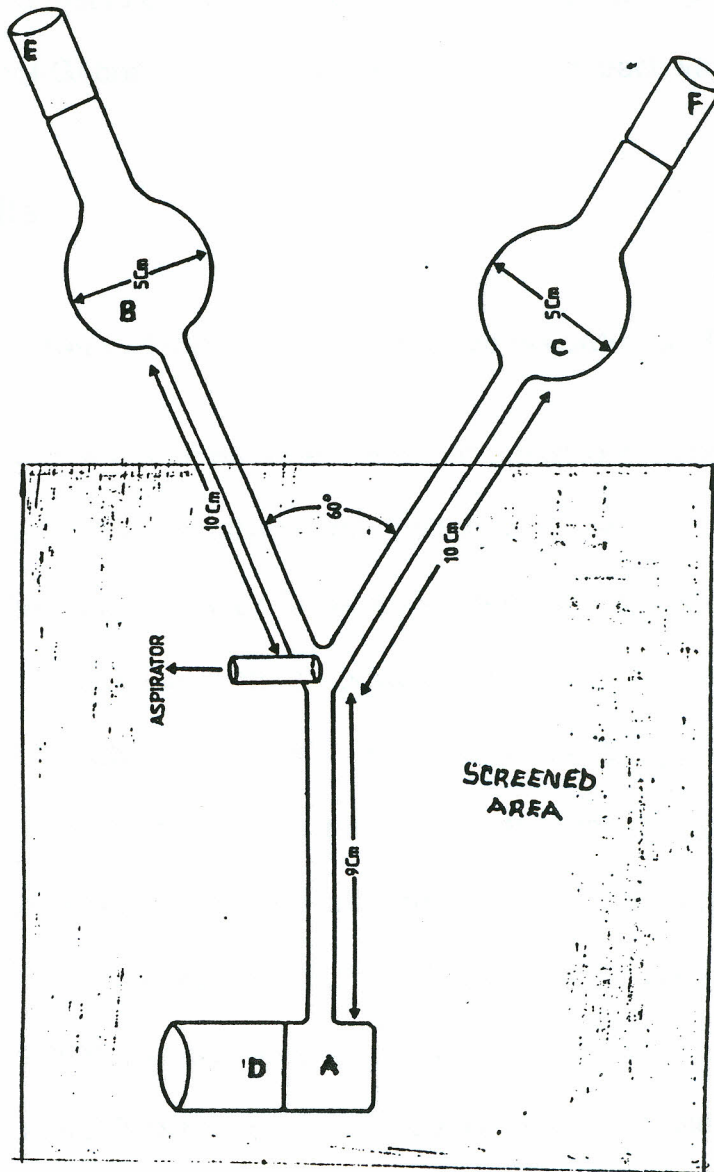
a rate of 335g / ha.) were each separately applied using a hand held sprayer onto a cabbage leaf that was infested with cabbage aphid (*B. brassicae*). The treated leaf was wrapped up in a muscilin cloth and carefully introduced into compartment B. An infested leaf with the same pests was wrapped the same way and introduced into compartment C to serve as the control.

(30) thirty randomly selected parasitoids (*D. rapae*) of mixed sexes and age were aspirated into a glass vial from the rearing cage and introduced into compartment A. Advantage was taken of the parasitoids being positively phototactic and hence half of the tubes connecting to compartments B and C (Figure 2) was covered with a black cloth to ensure complete darkness, while compartments B and C were directed towards the light source (open window). Light induced the parasitoids to leave compartment A towards the junction where the insects made their choice.

Each assay procedure was repeated 5 times, during which data on the number of insects in control arm (N_c), treated arm (N_t) and those that did not make a choice was recorded. The olfactometer was later rinsed with acetone and dried at 100°C, and the assay run a second time after interchanging the treatment and control arms in order to avoid bias.

Each assay run used untreated insects and all runs were done between 11.00am and 3.00pm during the peak parasitoid's activity.

Figure 3: Diagram of the olfactometer used in the bioassay



Chi-square test was applied in testing for treatment independence and independence of insect choice between the test materials and the control. Catmod Procedure from the SAS package was used to calculate the analysis of variance (SAS Institute, 1987). Treatment mean separations was performed by the Ryan-Einot-Gabriel-Welsch multiple range test for each variable.

2:3: Results

2:3:1: The effect of Neem on reproductive potential of the parasitoids

Table 1 shows the comparative effect of treatment applications on adult parasitoids and the resulting first generation parasitoid emergence. All parasitoids died one day after Dimethoate treatment, and this was significantly lower than for the control, Neem powder and Neem oil treatments (8.333 ± 0.667 , 7.667 ± 0.667 and 6.667 ± 0.667 respectively). Mean number of parasitoids for the Neem powder treatment was significantly different from the Dimethoate treatment three days after the treatment application, and this was higher than that obtained from Neem oil and the control treatments but not significantly different at 5% level of significance (3.333 ± 0.66) compared to 2.667 ± 0.66 and 2.000 ± 0.66 respectively. Only two parasitoids from the Neem powder treatment and 1 parasitoid in the control treatment survived six days after the treatment applications.

The first generation parasitoid emergence from the attacked hosts recorded significant differences among the treatments. Parasitoid emergences in the control treatment were significantly higher (13.667 ± 1.13) at 5% level of significance, followed by Neem powder and Neem oil treatments (7.000 ± 1.13 and 6.333 ± 1.13 respectively). There were no parasitoids that emerged from the Dimethoate treatment.

The control treatment recorded significantly higher mean number of parasitoids without wing defects (13.333 ± 0.87) as compared to those from the Neem oil and Neem powder treatments, but the two treatments were not significantly different from each other (5.333 ± 0.87 and 2.333 ± 0.87 respectively). However, the mean number of parasitoids without wing defects from the Neem oil treatment was significantly different from that recorded from the Dimethoate treatment (5.333 ± 0.87 and 0.000 ± 0.87).

Table 1: Mean number of *Diaeretiella rapae* observed after treatment applications in cages

Treatment	Survival of adult parasitoids				
	1 day after treatments	3 days after treatments	6 days after treatments	Number that emerged after host attack.	Number without deformed wings
Control (water)	8.333 ± 0.667a	2.000 + 0.66ab	1	13.667 ± 1.13a	13.333 ± 0.87a
Dimethoate	0.000b	0.000b	0	0.000c	0.000c
Neem oil 0.5% a.i.	6.667 + 0.667a	2.667 + 0.66ab	0	6.333 ± 1.13b	5.333 ± 0.87b
Neem powder 0.5% a.i.	7.667 + 0.667a	3.333 + 0.66a	2	7.000 ± 1.13b	2.333 ± 0.87bc

Means within the same column followed by the same letter are not significantly different at 5% significance level.

The fourth column data records the actual numbers, statistical analysis in this column was not done due to insufficient data.

2:3:2: Effect of Neem on the survival of parasitized hosts

Table 2 shows the mean number of aphid parasitoids that emerged from the treated field collected aphid mummies. No significant differences were recorded seven days after the control, Dimethoate, Neem oil and Neem powder treatment applications. *D. rapae* mean numbers were 0.667 ± 0.9 , 0.000 ± 0.9 , 0.667 ± 0.9 and 0.000 ± 0.9 for the above treatments respectively; *Alloxysta sp.* had 4.000 ± 1.28 , 1.000 ± 1.28 , 4.0000 ± 1.28 and 4.333 ± 1.28 mean numbers respectively; while *Pachyneuron sp.* had 5.000 ± 1.08 , 0.667 ± 1.08 , 5.000 ± 1.08 , 4.667 ± 1.08 mean numbers respectively. There were no significant differences in the number of *D. rapae* and *Alloxysta sp.* among the treatments fourteen days after the applications, but significant differences were recorded in the mean number of *Pachyneuron sp.* (1.333 ± 1.52) for the Dimethoate treatment. Dimethoate treatment recorded significantly lower number of the total cumulative parasitoid emergence as compared to the other three treatments (2.333 ± 1.23 , 13.667 ± 1.23 , 16.333 ± 1.23 and 16.667 ± 1.23 - for Dimethoate, Neem oil, control and Neem powder respectively).

Table 2: Mean number of aphid parasitoids that emerged from the treated mummies

Treatment	Number of parasitoids						Total number of emerged parasitoids
	Seven days after treatment			Fourteen days after treatment			
	A	B	C	A	B	C	
Control	0.667 ± 0.9a	4.000 ± 1.28a	5.000 ± 1.08a	0.667a	4.667a	11.000 ± 1.52a	16.333 ± 1.23a
Dimethoate	0.000a	1.000 ± 1.28a	0.667 ± 1.08a	0.000a	4.333a	1.333 ± 1.52b	2.333 ± 1.23b
Neem oil	0.667 ± 0.9a	4.000 ± 1.28a	5.000 ± 1.08a	0.667a	4.000a	10.667 ± 1.52a	13.667 ± 1.23a
Neem powder	0.000a	4.333 ± 1.28a	4.667 ± 1.08a	0.000a	1.000a	12.333 ± 1.52a	16.667 ± 1.23a

Means within the same column followed by the same letter are not significantly different at 5 % significant level.

A: *D rapae* . B: *Alloxysta sp.* C: *Pachyneuron sp.*

2:3:3: The effect of Neem extract repellency on *Diaeretiella rapae*

The choice of each test material by the parasitoids was independent of each other ($\chi^2 = 6.18$, $df = 3$ NS) (Table 3). Similarly, the choice of the treatment arm or the control arm by the parasitoids was independent of each other ($\chi^2 = 823.43$, $df = 1$ N.S.). Neem powder had the highest mean number of parasitoids (13.400 ± 0.400). These were significantly different from those recorded after Dimethoate treatment, followed by *B.t.* and Neem oil treatments (13.100 ± 0.674 and 10.800 ± 0.975 respectively), while the controls were not significantly different (15.200 ± 1.272 , 14.800 ± 0.489 , 14.700 ± 1.012 and 14.700 ± 0.761 respectively).

Table 3: Repellency effect of different Treatments on *Diaeretiella rapae*

Treatment	Level of action	
	Chemical Mean \pm SE	Control Mean \pm SE
Neem oil	10.800 \pm 0.975 ab	15.200 \pm 1.272a
Neem powder	13.400 \pm 0.400a	14.800 \pm 0.489a
Dimethoate	9.200 \pm 0.727b	14.700 \pm 1.012a
<i>Bacillus thuringiensis</i>	13.100 \pm 0.674a	14.700 \pm 0.761a

Means in the same column from the same level of action followed by the same letter do not differ significantly (95% significance level).

2:4: Discussions

The results clearly show that all the three treatments had effect on the *D. rapae* reproductive potential. Neem based-pesticides had no effect on the survival of parasitoids day one after topical treatment application as compared to the 100% mortality recorded in the Dimethoate treatment. These results are consistent with those reported by Lamb and Saxena, (1988) who showed that *Tetrastichus howardi*, (Olliff.), an endoparasitic wasp of a leafroller *Marasmia patnalis* Bradley survived for twelve days after exposure to a solution of Neem seed bitters, as compared to parasitoids treated with synthetic Monocrotophos, which died within four hours of the treatment.

However, parasitoid counts of the third day after the treatment application showed that Neem powder mean survival was higher than the control and that of the Neem oil treatment, though not significantly. This implied that Neem powder caused the least effect on the parasitoid survival. This observation was in line with the work by Schneider and Madel (1992) who reported that exposure of aqueous NSKE (0.1-5%) to *D. Semiclausum* in cages for 3 days showed no adverse effects. The NSKE treatment seemed to prolong the wasp's life span, though not significantly. Sixth day parasitoid counts agreed with the higher survival shown previously by the Neem powder treatment.

The results showed that Neem-based pesticides significantly affected the number of the parasitoids (initial young) emerging from the attacked hosts. A higher number of parasitoids emerged from the cages treated with Neem powder water extract as compared to Neem oil though the difference was not

statistically significant. However, this contrasted with the high parasitoid number without wing defects in the Neem oil treatment compared to the Neem powder treatment.

The higher number of parasitoids with deformed wings in the Neem powder aqueous water extract treatment cages was probably as a result of the reported observation that Neem oil contains approximately 10-20% of the active principles of the seed kernel. This does not compare to the Neem seed kernel water extracts (NSKWs) which have a cocktail of numerous compounds (Vollinger, 1995). The hypothesized implication of azadirachtin agent concentration in Neem oil, is that it could cause higher toxicity, resulting in lower parasitoid emergence, while in Neem powder, the numerous metamorphosis inhibiting constituents may have no acute toxicity, but could have long term synergistic or antagonistic effects (Vollinger, 1995).

Future studies should address these interacting effects of the various active insecticidal constituents of the Neem tree.

The results showed that Neem-based pesticides have no significant effect on the total number of parasitoid emergence as compared to Dimethoate treatment that had significantly lower emergence. These results are corroborated by previous work by Hoelmer *et al.* (1990) who reported that aphid mummies parasitized by *Lysiphlebus testaceipes* Cresson dipped in a solution of Margosan-O (a Neem-product) did not affect the emergence of the parasitoids. Schauer (1985) similarly reported that the aphid parasitoid *D. rapae* developed normally after parasitized nymphs or mummies of *Myzus persicae* were sprayed

with various Neem products such as MeOH-NR (0.1%), AZT (0.05%) and MTB (0.01%).

However, it is noted that the concentration of the Neem product is a contributory factor to its effect on parasitoid emergence. Loke *et al.* (1992) reported that Neem oil at 2.5% reduced the emergence rate of the braconid *Cotesia plutellae* L. adults when pupal cocoons were sprayed in the laboratory. A higher concentration (10%) prevented emergence completely. Fernandez *et al.* (1992) reported similar results where when pupae of striped stemborer of rice *Chilo suppressalis* Walker parasitized by *Tetrastichus howardi* (Olliff) were dipped for 30 seconds in 3% Neem oil, 5% aqueous NSKE, 50% EC Neem oil, 0.41% Brodan and water. The synthetic product (Brodan) caused 100% mortality, while the Neem oil-based product treatments resulted in the emergence of very few short-lived adults.

Besides the level of Neem concentration, the relative sensitivity of each insect is probably species specific. There were significant differences among the treatment effects fourteen days after the treatment application for *Pachyneuron* sp. Dimethoate treatment recorded significantly reduced emergence. This is in line with what has been reported by Hoelmer *et al.* (1990) that dipping *B. tabaci* puparia in Morgasan-O did not affect the emergence of *Encarsia formosa* Gehan and *Encarsia transversa* (Timberlake). However, for *Eretmocerus californicus* Howard, emergence of the parasitoid was reduced by more than 50%.

The high numbers of *Pachyneuron* sp. counted on the fourteenth day as compared to counts made seven days after the treatment were because

Pachyneuron sp. (Pteromalidae) is a hyperparasitoid that took longer to emerge as compared to the primary parasitoids.

The results showed that Neem powder had the least effect on the emergence rates of *Pachyneuron sp.*, which is a disadvantage as far as classical biological control is concerned. However, although hyperparasites have in the context of applied ecology been viewed as being harmful to the primary parasites, (Stary, 1970), more recent arguments have tended to dismiss such generalizations. Luck *et al.* (1981) and Van den Bosch *et al.* (1979) in their research on the Walnut-aphid complex arrived at the conclusion that the hyperparasite *Aphidencyrthus sp.* seemed to enhance control of the Walnut aphid by preventing extreme fluctuations in the primary parasite and host aphid populations.

Since in this study, only one dose level per treatment was used, further investigations should include different Neem oil and Neem powder dosages to ascertain the least lethal parasitoid dosage that is effective against the pest.

All the treatments demonstrated relative repellency against *D. rapae*. Dimethoate was the most potent repellent treatment in olfactometric assays. Suggesting that its application could reduce the effectiveness of *D. rapae* in host searching as compared to the Neem-based treatments. Nevertheless, the duration of the relative potency of the Neem-based pesticides' repellency remains to be demonstrated. Neem oil repelled parasitoids more than the Neem powder though not significantly.

Studies by Saxena *et al.* (1981) and Heyde *et al.* (1984) on Neem oil repellency, showed that rice plants treated with an ultra-low-volume (ULV) spray of 30%

Neem oil were found to be less attractive to *Nilaparvata lugens* (Stal) and *Sogatella furcifera* (Horvath) than the control plants. The oil repelled plant hoppers even without body contact indicating olfactory repellency. This agrees with the conclusions reported by Serra, (1992), that higher oil level concentrations in formulations increase their side effects on parasitoids.

Barneys and Chapman, (1987) explains that the different properties of any compound are perceived by a parasitoid's contact chemoreceptors, particularly by those on mouthparts. In some insects each neuron in a sensillum is maximally sensitive to different classes of chemical compounds, while in others, one single cell responds to a particular chemical compound resulting in rejection. The reason for the differences in mean repellency of Neem oil (10.800 ± 0.975) as compared to that of Neem powder (13.400 ± 0.400), could be due to the effect of the other Neem constituents in the Neem powder other than those that directly stimulate the individual sensory cells. These observations on Neem powder are consistent with previous work by Saxena *et al.* (1987), which showed that spraying Neem seed bitters on rice plants did not discourage the settling response of rice planthoppers and leafhoppers. The length of time that the Neem oil remains potent as a repellent agent could be further investigated. Previous work by Lowery and Isman (1993), reported that feeding deterrence in the greenhouse of 1% and 2% Neem oil against the Strawberry aphid *Chaetosiphon fragaetolii* (Cockerell) disappeared after 12 and 24 hours respectively.

CHAPTER THREE

3:0: THE EFFECT OF FIELD APPLICATION OF NEEM-BASED PESTICIDES ON PARASITISATION

3:1: Introduction

Neem products are medium-to broad-spectrum materials, which are used against phytophagous and other insect pests. Information on side effects on predators, parasitoids, pathogens and other beneficial organisms is desirable to enable an assessment of the compatibility of these products with the ecosystem. Schmutterer (1995) reported that the laboratory application of Neem products (i.e. when used at the same concentration as in the field), leads to stronger side effects on beneficial organisms than in the field where they are often not hit by sprays and where rapid degradation of active ingredients occurs. This study investigated the field application effects of Neem on beneficial insects.

3:2: Materials and methods

3:2:1: Experimental design

Cabbage was grown on 25 plots each measuring 5m x 5m. Treatments were randomly assigned in five plots replicated five times in a randomized complete block design. A local spacing of 60cm x 60cm was used with one seedling per planting hole, giving a total of 81 plants per plot.

3:2:2: Seedling

A raised well prepared seed-bed of loose, friable soil measuring 1m x 3m was made. Well-decayed manure and Diammonium phosphate fertilizer was applied at the rate of 3g per square meter and thoroughly mixed with soil and leveled with a wooden plank. Seeds were sown in rows, 5cm apart and lightly covered with soil. Watering was carried out regularly with a garden hose.

Before the uprooting of the seedlings, the seedbed was thoroughly watered in order to help keep intact the soil around the roots and thus reduce root damage. One month old seedlings i.e. about 10cm were transplanted using a basal fertilizer (DAP)(18.5 % N + 48 % P₂ O₃) at a rate of two teaspoonfuls (approx. 20g per planting hole. Recommended agronomic practices such as weed free plots, top-dressing once with Calcium Ammonium Nitrate were carried out.

3:2:3: Treatments:

- i.) Enriched Neem oil (Neemroc combi) with 0.5% AZA, 5 kg / ha.
- ii) Neem cake powder extract treatment (NCPE) (Neemros) with 0.5 % AZA at 2.5 kg / ha.
- iii) Control plot sprayed with water
- iv) Dimethoate treatment at an application rate of 335g /ha.
- v) Dipel treatment (*Bacillus thuringiensis*) applied at the rate of 10g /20 liters of water 500g /ha.

Neem cake powder water extract (NCP–WE) was prepared by weighing 25 g of powder into a container and one liter of water added. The mixture was stirred thoroughly and left to stand for 12 hours. Necessary volumes for field trial were prepared with this same ratio.

Neem oil extract was prepared by dissolving 10ml of Neem oil in one liter of water and stirred.

Dimethoate was prepared by mixing 30 ml of the chemical in 20 liters of water.

Dipel treatment was prepared by mixing 10g of commercially prepared *Bacillus thuringiensis* in 20 liters of water

Treatments were applied using a knapsack sprayer at the rates of between 150-200 ml per 25m x 5m experimental plot.

3:2:4: Sampling methods

Sampling before treatment applications

A: Ten (10) DBM larvae (Plate 3) of third or fourth instar stages were randomly collected from each experimental plot, put on leaf pieces and placed in petri-dishes and taken to the laboratory for rearing and observation.

B: Thirty (30) adult aphids i.e. six aphids (Plate: 6) from each of the five randomly selected plants, were collected from each plot and placed on a leaf piece in a petri–dish and taken to the laboratory for observation. Moistened filter papers were placed in the petri–dish to prevent drying of the leaf. Number of emerging parasitoids from the laboratory-reared aphids were counted and recorded.

Sampling after treatment applications.

Four and fourteen days after the treatment applications samplings were conducted as explained above:

During each sampling period all DBM and cabbage aphid predators on each sample plant were counted and recorded and five specimens of each taken to the laboratory for rearing and observation.

Treatment application and sampling schedule was as follows:

Treatments were applied after every 16 (sixteen) days i.e. 14th.September, 1998, 29th. September, 1998 and 20th. October, 1998.

First sampling was done on 8th.Sept. 1998

Second sampling: 17th.Sept.1998 (i.e. four days after first treatment)

Third sampling: 27th. Sept. 1998 (i.e. fourteen days after first treatment)

Fourth sampling: 6th. Oct. 1998 (i.e. four days after second treatment)

Fifth sampling: 13th. Oct. 1998 (fourteen days after second treatment)

Sixth sampling: 25th Oct. 1998 (four days after third treatment)

First sampling activity targeted the effects of the quick acting treatments (i.e. Dimethoate and *Bacillus thuringiensis*), while the second sampling targeted the slow acting treatments such as the Neem-based pesticides.

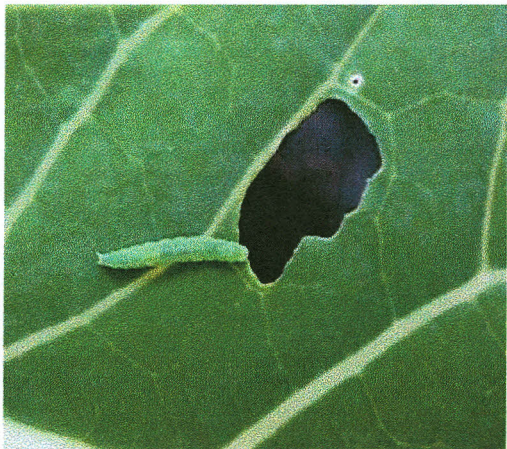


Plate 3:DBM larvae



Plate 4 : DBM damage on a cabbage plant.



Plate 5: DBM adult.



Plate 6: Aphids and aphid mummies on a cabbage leaf.

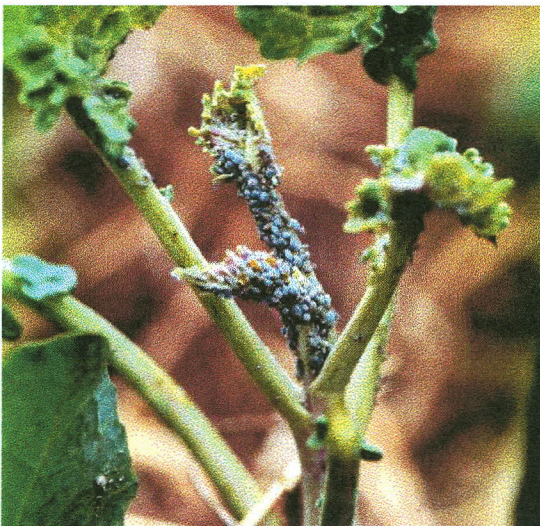


Plate 7: A cabbage shoot damaged by aphids.

3:2:5: Statistical analysis

Repeated measures analysis of variance using SAS procedure (SAS Institute, 1987) was used to compare differences between the treatment effects on parasitisation and predation of DBM and aphids in the field, reproductive impairment and emergence of parasitoids from parasitized aphids. Mean separation was carried out using Student–Newman–Keuls test.

3:3:Results

3:3:1: DBM natural enemies

Figures 4 and 5 show the different field treatment effects on percent *Diadegma* spp. and *O. sokolowskii* emergences. Percentages of *Diadegma* spp. for the control treatment during the first treatments were higher than that of Neem powder, Neem oil, Dimethoate and *B.t.* (4 and 2, 2, 2 and 0 respectively). Control treatment numbers of *O. sokolowskii* during the same period differed from the rest, while Neem powder and Neem oil numbers were lower than Dimethoate treatment but higher than *B.t.* treatment numbers (82, and 38, 30, and 52 and 18 respectively). In contrast, *Diadegma* spp. percentages of the second sampling fourteen days after the first treatment applications recorded differences, in the following order starting with the highest: Neem powder, control, Neem oil, Dimethoate and *B.t.* (8, 6, 2, 0, and 0 respectively). *O. sokolowskii*, during the same sampling period recorded numbers that were not

different for the control and Neem powder treatments (60 and 54 insects respectively). However, these numbers differed from the Neem oil and Dimethoate treatments (38 and 40 respectively).

Higher percentages of *Diadegma spp.* for the control and *B.t.* treatments (10 and 4 respectively) were recorded during the first sampling, four days after the second treatment applications, while percentages of Neem powder and Dimethoate treatments were not different (2 and 2 respectively). *O. sokolowskii* numbers during the same period recorded 52 insects each for Neem oil and Neem powder treatments, which were significantly lower than the control treatment (140) but higher than the *B.t.* and Dimethoate treatments (22 and 0 respectively).

Fourteen days after the second treatment applications, *Diadegma spp.* recorded higher Neem powder percentage (8), followed by Neem oil and the control (4 and 4 respectively), *B.t.* and Dimethoate (2 and 0 respectively). For *O. sokolowskii*, Neem powder and Neem oil numbers were different (44 and 40 respectively). These were however, lower than the numbers of the control and Dimethoate treatments (60 and 72 respectively). By the third treatment applications, a trend of lower parasitoid numbers for Neem powder and Neem oil after first sampling had emerged especially for *Diadegma spp.*, which increased after the second sampling i.e. fourteen days after the treatment applications.

Figure 4: Treatment effect on percent *Diadegma spp.*

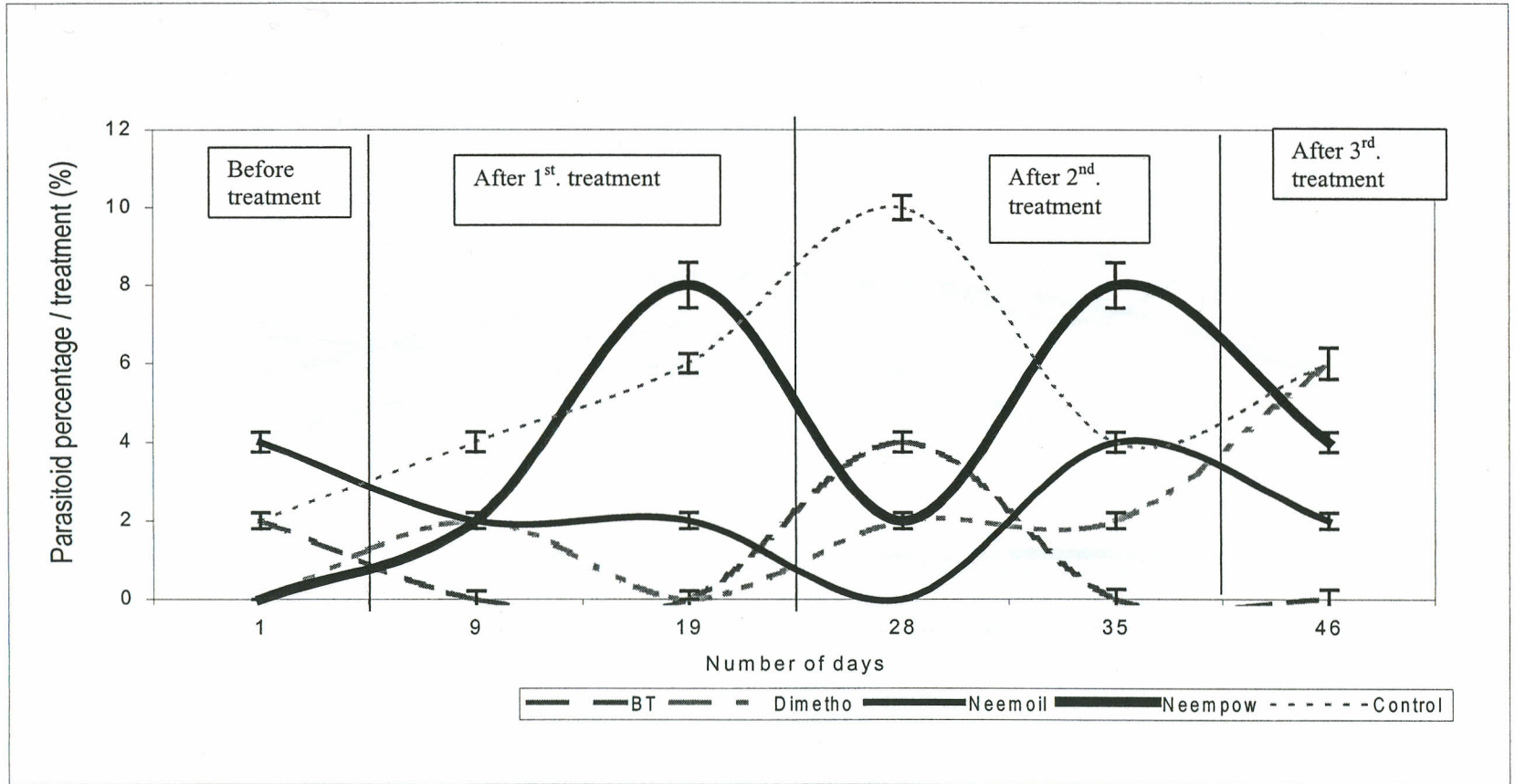
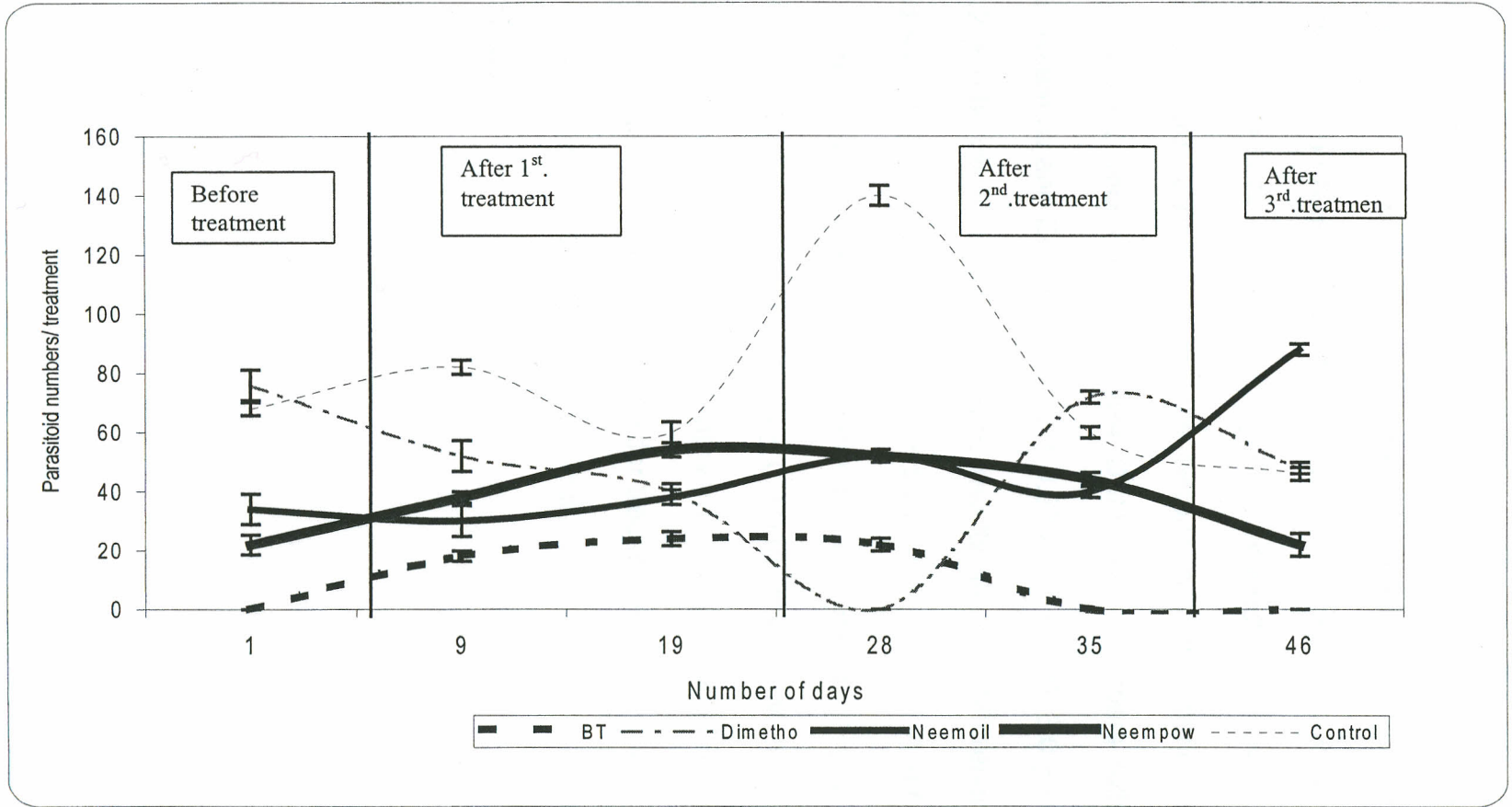


Figure 5: Treatment effect on *O. sokolowskii*.



The results showed no significant differences in the mean number of DBM before and after the first treatment applications (Table 4). During the first sampling period after the first treatment applications, Dimethoate, Control, Neem oil, *B.t.* and Neem powder mean parasitoid numbers were: 7.0 ± 1.095 , 5.2 ± 1.77 , 3.2 ± 0.74 , 1.8 ± 1.2 , $0.2 \pm 0.2.8$ respectively. However, significant differences were recorded during the second sampling period where Neem powder and *B.t.* recorded the lowest DBM mean numbers followed by the control, Neem oil and Dimethoate (0.8 ± 0.583 , 1.6 ± 0.66 , 3.0 ± 0.8 , 3.2 ± 0.4 , and 5.2 ± 0.86 respectively). However, results showed differences during the second sampling period, i.e. Neem powder (0.8 ± 0.583) and *B.t.* (2.0 ± 0.55) mean numbers were significantly different from Dimethoate (8.2 ± 1.24), the control (7.0 ± 1.79), and Neem oil (5.4 ± 0.87) treatments. Mean numbers of the emerging DBM adults of *B.t.* treatment were however not significantly different from those of Neem oil and Neem powder treatments. There were no significant differences among the treatments four days after the third treatment applications.

Table 4: Comparison of the treatment effect on the mean number of emerging DBM adults

Treatment	Sampling periods					After third treatment N = 10 Mean±S. E.
	Percentages					
	Before treatment N = 10 Mean ± S. E.	After first treatment N = 10 Mean ±S. E.	After second treatment N = 10 Mean ±S. E.	After second treatment N = 10 Mean ±S. E.	After third treatment N = 10 Mean±S. E.	
0	1	2	3	4	5	
<i>Bacillus thuringiensis</i>	5.6 ± 0.979a	1.8 ± 1.2 a	1.6 ± 0.66a	3.0 ± 2.32a	2.0 ± 0.55a	0.6 ± 0.6a
Control	8.0 ± 1.224a	5.2 ± 1.77a	3.0 ± 0.8 ab	7.2 ± 0.86a	7.0 ± 1.79b	4.8 ± 1.59a
Neem oil	5.8 ± 1.74a	3.2 ± 0.74a	3.2 ± 0.4ab	3.6 ± 0.51a	5.4 ± 0.87ab	5.2 ± 1.16a
Neem powder	0.0 ± 0.0a	0.2 ± 0.2a	0.8 ± 0.583a	0.2 ± 0.2a	0.8 ± 0.583a	0.4 ± 0.245a
Dimethoate	1.8 ± 0.66a	7.0 ± 1.095a	5.2 ± 0.86b	3.2 ± 1.625a	8.2 ± 1.24b	2.6 ± 0.93a

Means in the same column followed by the same letter do not differ significantly

(P > 0.05, Student–Newman–Keuls test (SNK))

3:3:2: Cabbage aphid parasitoids

Figure 6 shows the treatment effect on percent emergence of *D. rapae*. Percentage parasitoid emergence from the first sampling period, four days after the first treatment applications recorded no significant differences between *B.t.*, Neem oil and Neem powder treatments (8, 8 and 7 respectively). However, these percentages differed from Dimethoate treatment, which had 1% emergence. Similar results were realized at the fourteenth day sampling period, where zero emergence was recorded for the Dimethoate treatment. Control, Neem powder and Neem oil treatments (7, 7 and 4) differed from Dimethoate and *B.t.* treatments which had zero emergence each at the second sampling period four days after the second treatment applications. At the fourteenth day after the second treatment applications, control and Neem oil percentages (17 and 15) differed from Neem powder and *B.t.* (5 and 5), while Dimethoate recorded no emergence. Emergence from Neem powder treatment increased significantly on the fourth day after the third treatment applications (12), in contrast to the Neem oil (3%) and *B.t.* (2) and Dimethoate (0) emergence (Table 5).

Results in figure 7 show percent *Alloxysta sp.* emergence after the treatment applications. Percent emergences after the first treatment applications were high for Neem powder treatment (11), which differed from the control, *B.t.* and Neem oil (7, 7 and 5). No parasitoids emerged from the plots treated with Dimethoate. Similar results were obtained during the fourteenth day sampling period where 9 parasitoids emerged from the Neem powder treatment, differing

from those obtained from the control (6) and *B.t.* (4) and Neem oil (2). Percentages of the first sampling period, four days after the second treatment applications, Neem powder and the control treatments recorded 27 and 26 emergences which differed from the Neem oil treatment (13), while Dimethoate and *B.t.* treatments recorded zero emergence. The percentage emergence during the second sampling period after the second treatment applications differed among the treatments though not significantly (control, Neem oil, Neem powder, *B.t.* and Dimethoate as 12, 7, 5, 3, and 1 respectively). Percent emergences after the first sampling period, four days after the third treatment applications for the control, Neem oil and Neem powder did not differ significantly (11, 8 and 5 respectively); however, these differed from *B.t.* (2) and Dimethoate (0) treatments.

Figure 6: Treatment effect on percent *D. rapae*.

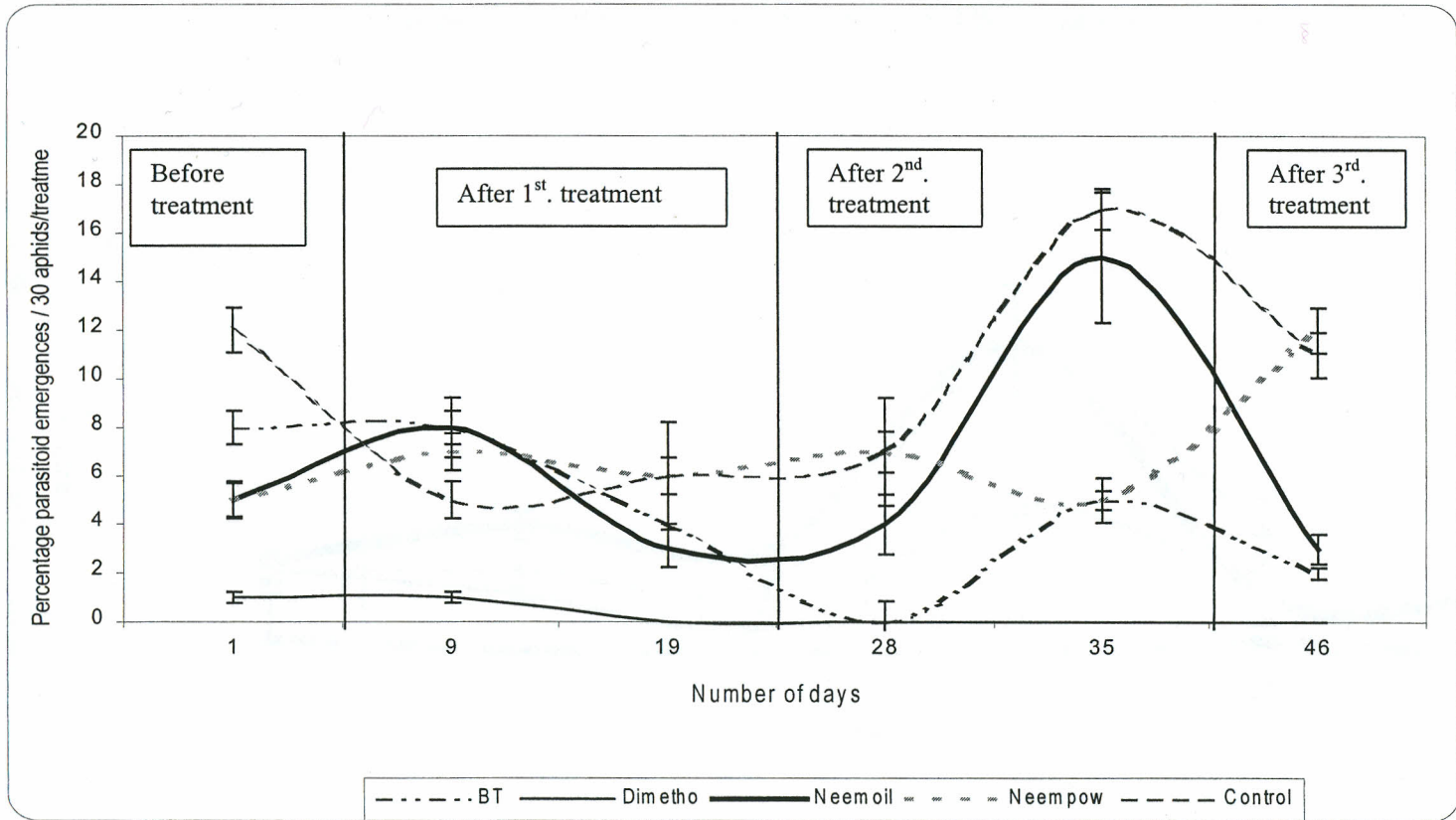


Figure 7: Treatment effect on percent *Alloxysta* sp.

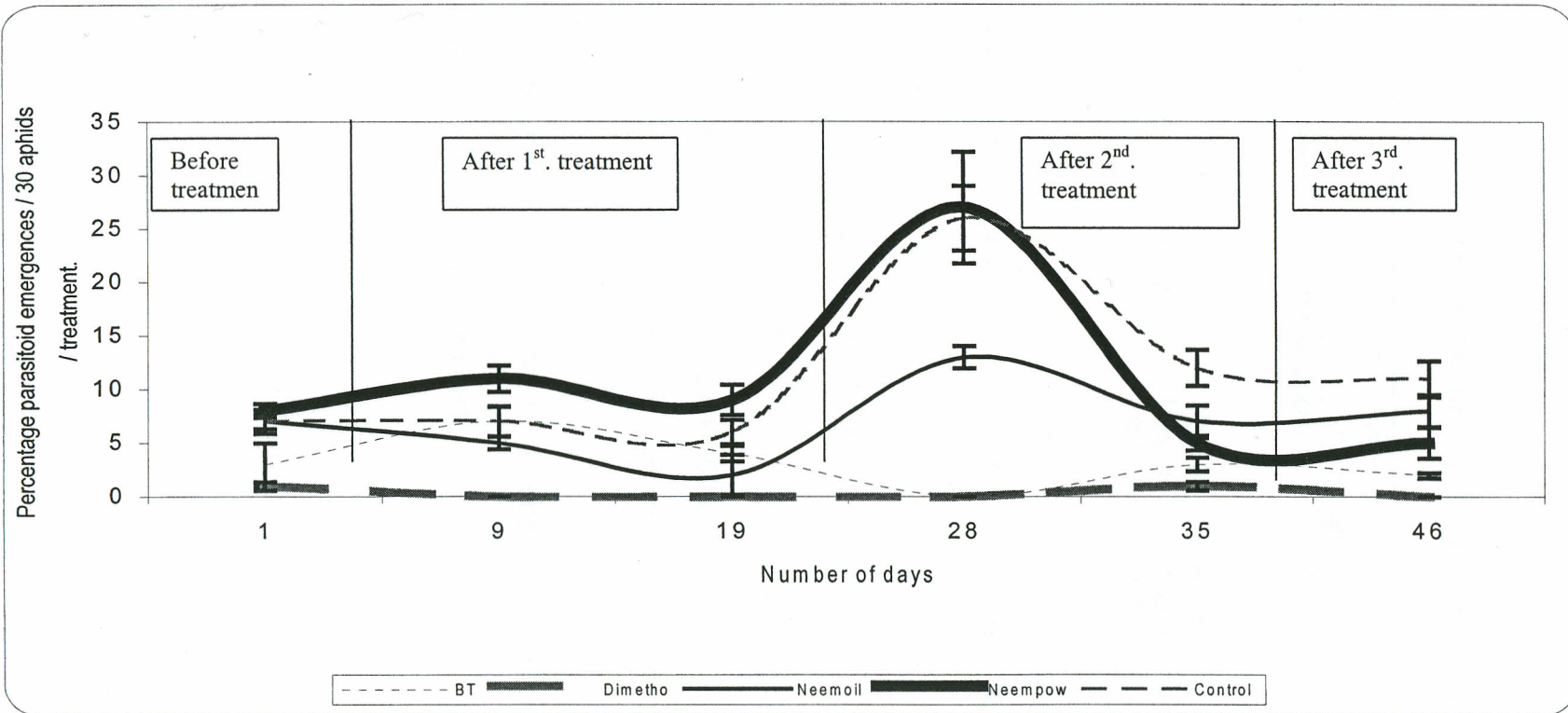


Table 5: Comparison of the treatment effect on *Diaeretiella rapae*

Treatment	Sampling periods (Percentage)					
	Before treatment	After first treatment		After second treatment		After third treatment
	N = 30 Mean \pm S. E.	N = 30 Mean \pm S.E.	N = 30 Mean \pm S.E.	N = 30 Mean \pm S.E.	N = 30 Mean \pm S.E.	N = 30 Mean \pm S.E.
	0	1	2	3	4	5
<i>Bacillus thuringiensis</i>	2.4 \pm 0.678ab	2.4 \pm 1.21 a	1.2 \pm 0.8a	0.0 \pm 0.0a	1.4 \pm 0.87a	0.6 \pm 0.4c
Dimethoate	0.0 \pm 0.0a	0.2 \pm 0.2a	0.0 \pm 0.0 a	0.2 \pm 0.2a	0.2 \pm 0.2a	0.6 \pm 0.40c
Neem oil	1.6 \pm 0.748ab	2.4 \pm 0.68a	0.8 \pm 0.8a	1.2 \pm 1.2a	4.4 \pm 2.69a	0.8 \pm 0.6c
Neem powder	1.6 \pm 0.678ab	2.2 \pm 0.74a	1.8 \pm 0.74a	2.2 \pm 2.2a	1.4 \pm 0.872a	3.6 \pm 0.927a
Control	3.6 \pm 0.93ab	1.6 \pm 0.245a	2.4 \pm 0.927a	2.2 \pm 1.114a	5.0 \pm 3.38a	3.2 \pm 1.5ab

Means in the same column followed by the same letter do not differ significantly

($P > 0.05$, Student–Newman–Keuls (SNK) test.)

3:3:3: Cabbage aphid predators

Table 7 and shows the results of the treatment effects on the sampled Syrphid fly (*Ischiodon egyptians* (Wiedemann) mean numbers. At four days after the first treatment application, *B.t* and Dimethoate treatments recorded higher mean insect numbers (1.4 ± 0.75 and 1.2 ± 0.58), but lower than the control treatment (3.6 ± 1.08) respectively. Syrphid fly mean numbers for Neem powder (0.6 ± 0.25) and Neem oil (0.2 ± 0.2) treatments were significantly lower than the control (3.6 ± 1.08) treatment. Fourteen days after the first treatment applications, *B.t.*, Neem oil Dimethoate, and control showed no significant differences in mean syrphid fly numbers (13.4 ± 5.7 , 5.4 ± 2.82 , 3.8 ± 2.8 and 3.2 ± 2.33 respectively), however, these treatments differed significantly from the Neem powder treatment (2.6 ± 1.78). Of these four treatments, insect mean numbers from the *B.t.* were higher than for Neem oil, while these two treatments differed from Dimethoate, the control and Neem powder treatments (Figure 8). Neem powder, Neem oil and Dimethoate syrphid fly mean numbers on the fourth day after the second treatment applications did not differ significantly. However, they were significantly lower than the control (0.8 ± 0.5 , 0.6 ± 0.4 , 0.0 ± 0.0 and 5.4 ± 1.5 respectively). Similar results were realised fourteen days after the treatments, while at the fourth day after the third treatment applications the mean numbers of syrphids were very low.

Results of the treatment effects on the ladybird beetle numbers (Figure. 9), recorded insect mean numbers of Neem powder (4) and Neem oil (4), which were higher than for the control and *B.t.* treatments (2 and 2 respectively), and

the Dimethoate treatment (0), during the second sampling, fourteen days after the first treatment applications. Neem oil and *B.t.* (one insect each) treatments recorded higher coccinellids than the Dimethoate treatment (nil count) but lower than the Neem powder and the control treatments (2 and 3 respectively) during the first sampling period four days after the second treatment applications.

Table 6 : Comparison of the treatment effect on the mean numbers of Syrphid fly

Treatment	Number of flies(larvae) / treatment					
	Before treatment	After first treatment		After second treatment		After third treatment
	N = 50 Mean \pm S. E.	N = 50 Mean \pm S. E.	N = 50 Mean \pm S. E.	N = 50 Mean \pm S. E.	N = 50 Mean \pm S. E.	N = 50 Mean \pm S. E.
	0	1	2	3	4	5
<i>Bacillus thuringiensis</i>	4.0 \pm 1.64a	1.4 \pm 0.75 ab	13.4 \pm 5.7a	1.8 \pm 0.58b	2.8 \pm 0.74a	0.8 \pm 0.38a
Dimethoate	3.8 \pm 1.02a	1.2 \pm 0.58ab	3.8 \pm 2.8 a	0.0 \pm 0.0b	0.2 \pm 0.2b	0.2 \pm 0.2a
Neem oil	2.8 \pm 01.019a	0.2 \pm 0.2b	5.4 \pm 2.82a	0.6 \pm 0.4b	0.2 \pm 0.2b	0.8 \pm 0.59a
Neem powder	2.0 \pm 0.632a	0.6 \pm 0.25b	2.6 \pm 1.78b	0.8 \pm 0.58b	0.2 \pm 0.2b	0.4 \pm 0.4a
Control	4.2 \pm 1.11a	3.6 \pm 1.08a	3.2 \pm 2.33a	5.4 \pm 1.5a	3.0 \pm 1.224a	1.8 \pm 0.97a

Means in the same column followed by the same letter do not differ significantly

(P> 0.05, Student–Newman–Keuls (SNK) test.

Figure 8: Treatment effect on syrphid fly larvae numbers

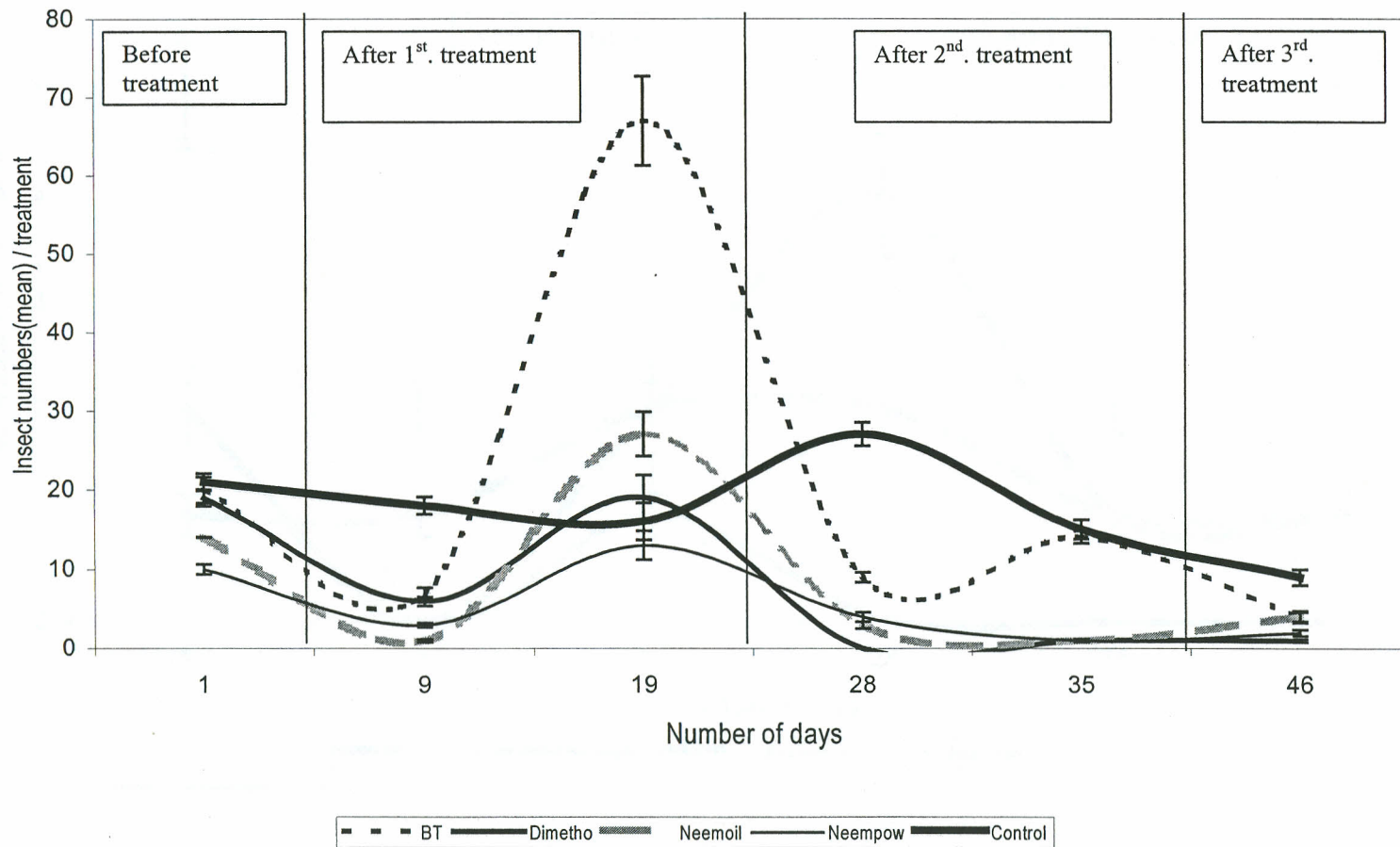
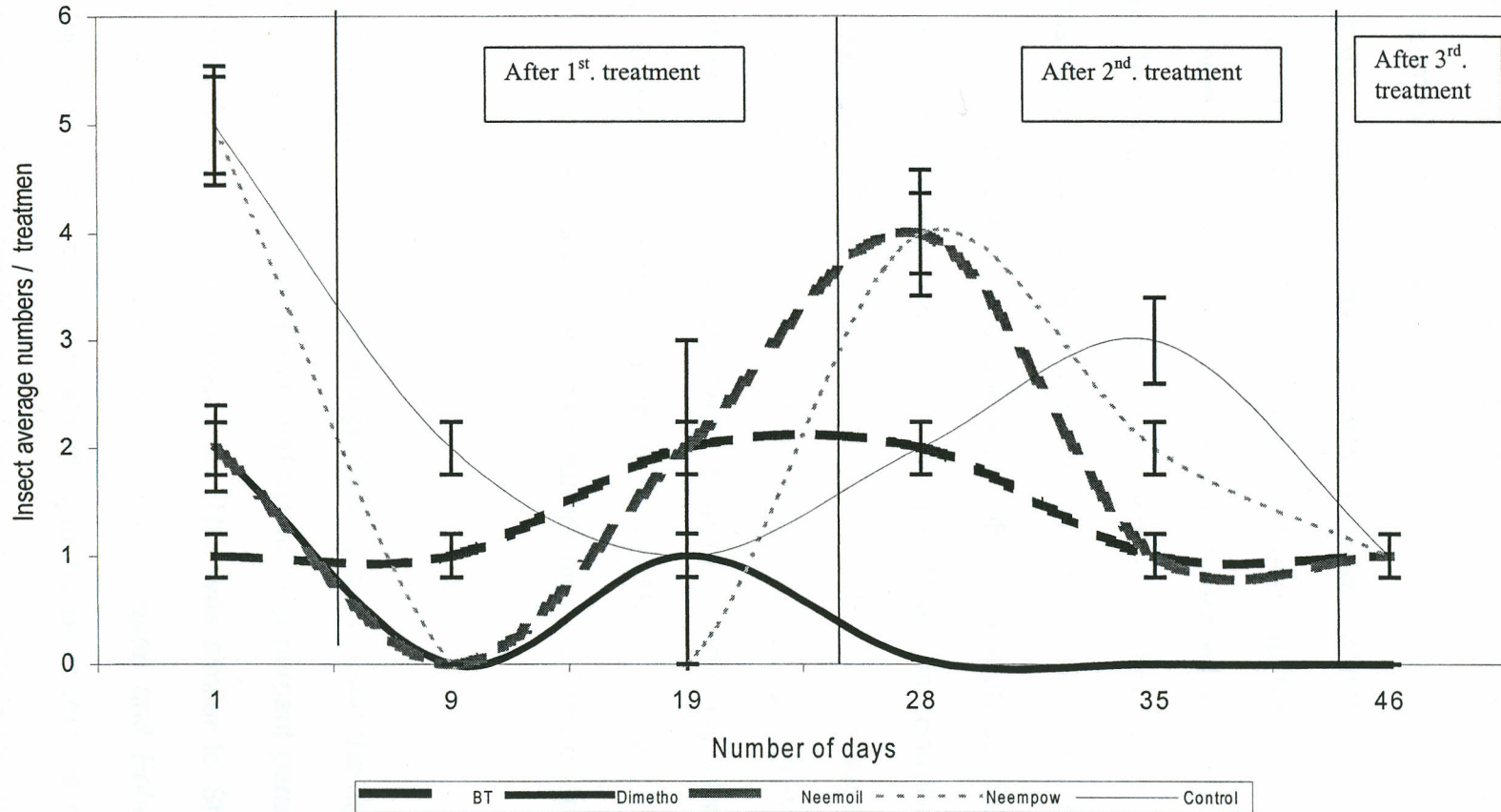


Figure 9: Treatment effect on Ladybird beetle numbers



3:4: Discussion

The results clearly showed that Neem-based pesticides had less adverse effect on DBM parasitoid emergences compared to *B.t.* and Dimethoate treatments. Neem powder treatment resulted in having higher parasitoid emergences than the control treatment. This was consistent with Schneider and Madel (1992) findings who reported that *D. semiclausum* exposed to aqueous NSKE (0.1-5%) for three days showed no adverse effects, and that it seemed to prolong the wasp's life span. This has practical implications since corroborative data on DBM emergences showed that at the fourteenth day after applications, Neem powder and *B.t.* significantly recorded the least DBM emergences. Several studies have reported similar findings on the effect of NSKE in controlling *P. xylostella* (Adhikary, 1981; Dryer, 1986; Schmutterer, 1992). Percentages for the solitary *Diadegma spp.* and the number of the gregarious *O. sokolowskii* were higher fourteen days after the treatment than the fourth day sampling period; possibly because of the delayed effect of the Neem activity. Future studies should address the effect of Neem on fecundity and longevity of specific parasitoids.

Cabbage aphid parasitoids' results showed that Dimethoate caused the highest mortality while Neem powder treatment recorded the highest percent parasitoid emergences for both *D. rapae* and *Alloxysta sp.* This was similar to Shauer (1985) findings who reported that aphid parasitoids *D. rapae* and *Ephedrus cerasicola* developed normally after parasitised nymphs or mummies of *M. persicae* were sprayed with various Neem products such as MeOH-NR (0.1%),

AZT (0.005%) and MTB (0.01%).

Neem-based pesticide treatments resulted in having low syrphid fly numbers. The effect on ladybird beetle numbers was however, minimal. These results were consistent with those reported by Shauer (1985) who after applying 100 ppm of a Neem product MTB/H₂O-VR-K resulted in having the death of most of the treated syrphid flies. Most of the deaths occurred at the end of the pupal stage. The flies' development was apparently normal in their pupal skin, but could not emerge. This was probably because of the higher titer of ecdysteroids at the end of the pupal stage caused by the Neem treatment (Bidmon *et al.*, 1987). Studies on the ladybird beetle *Coccinella septempunctata* by Bigler (1988), reported that spraying NSKE (2%) and Neem oil (3%) had no adverse effects on the fecundity and on the beetle's fitness. Similarly, feeding the second instar larvae with the Neem treated aphids resulted in having no damage but direct topical spraying of the fourth instar larvae led to significant mortality. Future research should include susceptibility studies of the various life stages of syrphid flies and the ladybird beetles to Neem-based pesticides.

CHAPTER FOUR

4:0:SUMMARY, GENERAL DISCUSSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

1 Studies on the effect of Neem on parasitoid reproductive potential were conducted under laboratory conditions. Dimethoate treatment caused 100% parasitoid mortality one day after the treatment applications. This was significantly different from the control, Neem powder and Neem oil treatments. Neem powder recorded the highest survivals up to the sixth day, and this seemed to enhance survival, though not significantly. Enhancement of the parasitoid survival has practical contributions in the promotion of biological control agents as part of IPM strategies. Future research should address the determination of the appropriate dosages that would effectively suppress pest populations without causing detrimental effects on the natural enemies.

The number of the first generation parasitoid emergences from hosts that had been exposed to the treated parasitoids recorded significant differences among treatments. Emergences from Neem powder and Neem oil treated hosts were significantly lower than those recorded on the control but higher than those from Dimethoate treatment. However, the number of parasitoids with wing deformities was higher in the Neem powder treatment than in the Neem oil treatment. Future research should determine the interacting factors of the various synergistic or antagonistic active constituents of Neem and how they affect mortality, morphogenesis / and fecundity.

2 Studies on the effect of Neem on *D. rapae* and *Alloxysta sp.* survival in aphid mummies recorded no significant differences among the treatments, while Dimethoate treatment recorded significantly lower emergences for the hyperparasitoid *Pachyneuron sp.*, than for Neem powder Neem oil and the control treatments. Dimethoate treatment, similarly, recorded significantly lower cumulative parasitoid emergences from the same mummies. Future studies should address the implications of Neem powder enhancement of the hyperparasitoid *Pachyneuron sp.* population in the context of classical biological control. It is necessary that further information on the hyperparasitoid *Pachyneuron sp.* be obtained.

3 Tests to determine the repellency of Neem powder against *D. rapae* showed that Neem powder treatment was the least repellent, while Dimethoate was significantly the most repellent treatment. Repellency studies are necessary since host suitability for parasitoids and predators is dependent on nutrient components. These factors may be repellants, phagostimulants, or deterrents, and it is the balance of these factors that determines the final acceptability of food (Bernays and Simpson, 1982). Further research should be carried out to determine the duration of Neem powder potency against DBM and cabbage aphid predators. This information is necessary for designing the appropriate treatment application methods.

4 Studies on the effect of Neem-based pesticides on DBM and cabbage aphid natural enemies were carried out in the field. Results showed that Neem powder treatment was effective in suppressing DBM population while at the same time it enhanced the emergence of *Diadegma spp.* and *O. sokolowskii* from the field sampled DBM larvae. Similar results were obtained with cabbage aphid parasitoids: *D. rapae* and *Alloxysta sp.* However, the effectiveness of Neem-based pesticides in suppressing aphid populations was not confirmed. In most cases, Dimethoate caused 100% mortality for both the host and the natural enemy. Neem powder and Neem oil treatments, on the other hand caused marked reduction in syrphid fly and ladybird beetle populations. Future studies should aim at developing methodologies that will determine the effect of Neem-based pesticides on percent parasitism/percent mortality, especially in cases where a complex of natural enemies are involved. Secondly, the design of life tables would be valuable tools for identifying points in time where natural enemy survivorship is high while host life stage is vulnerable.

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