Effects of Azadirachtin and the natural pesticides Spinosad and Avermectin on the leafminer *Liriomyza sativae* (Diptera: Agromyzidae) and its parasitoids on tomatoes under protected cultivation in the humid tropics

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Summary

Among the economic important *Liriomyza* spp., *Liriomyza sativae* causes substantial damage to tomatoes for instance infestation strongly reduces the photosynthetic activities resulting in high yield losses. The studies were conducted to investigate the effects of biopesticides (NeemAzal®-U and NeemAzal®-T/S) and biorational pesticides (Spinosad and Abamectin) on *L. sativae* and its two parasitoids, *Opius* (*Opiothorax*) *chromatomyiae* and *Neochrysocharis formosa* both under laboratory and greenhouse conditions.

All tested NeemAzal®-U (17% Azadirachtin) concentrations applied as solutions to the substrate of potted tomatoes had very low effects on oviposition and egg hatch. However, strong systemic effects were observed in different larval stages attaining mortalities up to 100% when a high dosage of soil drenching solution (3.0 g NeemAzal®-U/lw) was implemented. The early instar (L1) larvae were found to be most susceptible to all dosages tested. Significant efficacy of NeemAzal®-U could be measured up to one week post-application. Leafminer prepupae moving to pupate in treated soil suffered from a very high mortality which resulted in only very few adults emerging even when the lowest NeemAzal®-U concentration of 0.75 g/lw was used. The direct effects of soil treatment were elucidated with high mortality values subsequent to instant soil treatment of prepupae and pupae reared on untreated plants.

NeemAzal®-T/S (1% Azadirachtin) was applied on aerial plants parts with five increasing concentrations (1 ml, 3 ml, 5 ml, 7 ml and 10 ml/lw), and different ages of residues. Irrespective of the residual age of the topical application, no significant effects of NeemAzal®-T/S was found on oviposition and egg hatch. However, NeemAzal®-T/S strongly induced immature mortality at higher dosage rates. The L1 and L2 larvae were found to be most susceptible. The larval mortality reached up 100% and completely inhibited adult eclosion. Irrespective of NeemAzal®-T/S concentrations its efficiency in terms of induced larval mortality or inhibition of adult eclosion decreased much faster in greenhouses than in an air conditioned environment. Nevertheless, the results suggest that NeemAzal®-T/S applied topically has a high potential to control *L. sativae* in netted greenhouses in the humid tropics.

In comparative study, NeemAzal®-T/S and Success® caused no effects on oviposition and egg hatch compared to untreated control (water treatment).

Irrespective of tested dosages, Abamectin strongly reduced egg deposition and severely affected embryonic development. All three pesticides severely affected the survival of immature stages (i.e. L1, L2 and L3) of *L. sativae*, with mortality up to 100% for fresh (one day old) residues and adult eclosions were completely stopped. Success® and Abamectin had a longer persistency (up to 14 days) both under laboratory and greenhouse conditions compared to NeemAzal®-T/S, the activity of which decreased significantly with residual age under greenhouse conditions.

The successes of adult emergence of *O. chromatomyiae* from all concentrations of NeemAzal®-U drenched soil against parasitized larvae/prepupae of *L. sativae* were slightly affected compared to untreated control. In contrast, adult emergence of *L. sativae* encountering NeemAzal®-U only directly from drenched soil in the prepupal stage which has not been exposed to the parasitoids was strongly reduced. However, adult emergence of *O. chromatomyiae* in L3 of *L. sativae* was strongly affected from topical application of NeemAzal®-T/S, Success® and Abamectin. In a further study, NeemAzal®-T/S revealed no detrimental effect on the adult emergence of *N. formosa* developed in L2 of *L. sativae* within leafminer mines in the leaves. In contrast, Success® and Abamectin strongly reduced *N. formosa* emergence when applied at different immature developmental stages of *N. formosa.*

Keywords: *Liriomyza sativae*, *Opius* (*Opiothorax*) *chromatomyiae*, *Neochrysocharis formosa*, Neem, Spinosad, Abamectin

Zusammenfassung

Unter den wirtschaftliche bedeutenden *Liriomyza* –Arten verursacht *Liriomyza sativae* substantielle Schäden an Tomaten insbesondere durch die Reduktion der photosynthetischen Leistung der Pflanze durch die minierende Lebensweise der Larven.

Alle geprüften Aufwandmengen von NeemAzal®-U (17% Azadirachtin), die an die Wurzeln getopfter Tomaten über das Substrat verabreicht wurden, hatten nur sehr geringe Einflüsse auf die Eiablage der adulten Minierfliegen und den Schlupf der Larven aus den Eiern. Ausgeprägte systemische Effekte konnten aber gegenüber den Larvalstadien beobachtet werden. Es wurden Mortalitätsraten bis zu 100% mit den höchsten getesteten Dosierungen von NeemAzal®-U (3.0 g/lw) erreicht. Das erste Lavenstadium (L1) reagierte am empfindlichsten auf alle geprüften Dosierungen. Das Neem Präparat zeigte noch eine signifikante Wirkung auf die Minierfliegen, wenn die Pflanzen erst eine Woche nach der Behandlung besiedelt wurden. Für Praepuppen der Minierfliegen, die nach Behandlungen noch zur Verpuppung in das Substrat abwanderten, ergab sich dort eine sehr hohe Mortalitätsrate, so dass in der Regel nur wenige Adulte selbst nach Anwendung der geringsten Aufwandmengen von NeemAzal®-U (0.75 g/lw) schlüpften. Zudem beeinflusste NeemAzal®-U intensiv direkt das Puppenstadium, was anhand von hohen Sterberaten bei Substratbehandlungen, die erst nach Abwanderung von Praepuppen aus vorher unbehandelten Pflanzen erfolgten, nachgewiesen werden konnte.

NeemAzal®-T/S (1% Azadirachtin) wurde auf die oberirdischen Pflanzenteile der Tomaten mittels Sprühapplikation aufgebracht. Dabei wurden fünf Verdünnungsstufen (1 ml, 3 ml, 5 ml, 7 ml and 10 ml/lw), und unterschiedlich alte Beläge auf ihre Wirksamkeit hin überprüft. Unabhängig vom Alter der Beläge ergaben sich keine Auswirkungen von NeemAzal®-T/S auf Eiablage und Larvenschlupf. Bei den Larven- und Puppenstadien wurden jedoch hohe Mortalitätsraten induziert. Wie bei den Bodenbehandlungen reagierten auch hier L1 and L2 Stadien besonders empfindlich. Die Mortalität der Larven erreichte zum Teil 100% und die Entwicklung von Adultstadien wurde vollständig unterbunden. In allen Verdünnungsstufen nahm die Effizienz von NeemAzal®-T/S bei Betrachtung der Larvalmortalität als Parameter mit der Zeit

im Gewächshaus wesentlich schneller ab als in der geschützten und kontrollierten Umgebung der Labor- und Zuchträume. Trotzdem lässt sich aus den Ergebnissen folgern, dass topikale Behandlungen mit NeemAzal®-T/S ein hohes Potential zur Kontrolle von *L. sativae* in Netzhäusern unter den klimatischen Bedingungen der feuchten Tropen haben.

In einer vergleichenden Versuchsreihe mit den sogenannten Biopestiziden NeemAzal®-T/S, Success® und Abamectin konnten nur bei Anwendung von Abamectin negative Effekte auf die Eiablage, die Embryonalentwicklung und den Larvenschlupf festgestellt werden. Alle drei Präparate schädigten hingegen die Larvalstadien (L1, L2 and L3) von *L. sativae* erheblich mit Mortalitätsraten bis zu 100% bei frischen (einen Tag alt) Spritzbelägen, und der Schlupf adulter Fliegen wurde vollständig unterbunden. Success® and Abamectin waren deutlich persistenter (bis zu 14 Tage), sowohl im Labor als auch im Gewächshaus, als NeemAzal®-T/S, dessen Aktivität mit Alterung der Beläge signifikant abnahm, insbeondere unter Gewächshausbedingungen.

Die Schlupfrate adulter *O. chromatomyiae* wurde im Vergleich zu unbehandelten Varianten nur geringfügig reduziert, wenn Substrat mi NeemAzal®-U behandelt wurde nachdem die parasitierten Praepupppen von *L. sativae* den Boden aufgesucht hatten. Im Gegensatz dazu verhinderte eine entsprechende Behandlung den Schlupf unparasitierter *L. sativae* extrem. Die Entwicklung von *O. chromatomyiae* wurde massiv unterbunden, wenn Blätter der Tomate mit minierenden parasitierten L3 Stadien des Wirtes mit NeemAzal®-T/S, Success® oder Abamectin behandelt wurden. Demgegenüber ergaben sich mit mit NeemAzal®-T/S nur geringfügige Schädigungen des Parasitoiden *N. formosa,* der sich in in L2 Stadien von *L. sativae* entwickelte. Success® and Abamectin jedoch schädigten auch bei diesem Parasitoiden alle larvalen Entwiclungsstadien*.*

Stichworte: *Liriomyza sativae*, *Opius* (*Opiothorax*) *chromatomyiae*, *Neochrysocharis formosa*, NeemAzal®-U, NeemAzal®-T/S, Bodenbehandlung, Spinosad, Abamectin

Contents

[1]Hossain MB, Poehling H–M, Thöming G and Borgemeister C. Effects of soil application of Neem (NeemAzal®-U) on different life stages of *Liriomyza sativae* (Dip.: Agromyzidae) on tomatoes in the humid tropics. Submitted to Crop Protection.

[2]Hossain MB, Poehling H–M. Effects of topical application of Azadirachtin (commercial product: NeemAzal®-T/S) on different immature life stages of the leafminer *Liriomyza sativae* (Dip.: Agromyzidae) on tomatoes in the humid tropics. Submitted to Phytoparasitica.

[3]Hossain MB and Poehling H–M. A comparative study of the residual effects of Azadirachtin, Spinosad and Avermectin on *Liriomyza sativae* (Dip.: Agromyzidae) on tomatoes. To be submitted to Pest Management Science.

[4] Hossain MB and Poehling H-M. Side effects of Azadirachtin, Spinosad and Avermectin on *Opius* (*Opiothorax*) *chromatomyiae* (Hymenoptera: Braconidae) and *Neochrysocharis formosa* (Hymenoptera: Eulophidae), two endo-larval parasitoids of *Liriomyza sativae* (Diptera: Agromyzidae) under laboratory conditions. Submitted to Journal of Applied Entomology.

Abbreviations

AIT	Asian Institute of Technology
ANOVA	Analysis of variance
AZA	Azadirachtin
d.f	Degree of freedom
Exp	Experiment
F	Statistical F-value
g/lw	Grams per litre water
h	Hours
IPM	Integrated Pest Management
L:D	Relation of light to darkness
L1	First instar larvae
L2	Second instar larvae
L3	Third instar larvae
HSD	Honestly significant difference
ml/lw	Mili-litres per litre water
P	Statistical probability value
SAS	Statistical analysis system
SE	Standard error
wt/vol	Weight to volume
Ø	Diameter

1   General Introduction

The cultivated tomato, *Lycopersicon esculentum* (Mill) member of the family Solanaceae is one of the most popular and wide grown vegetable food crops that has achieved prominence and popularity largely in the past century (Tigchelaar, 1991). It is believed to have been domesticated in Mexico (FAO, 2000). Tomato varieties in Europe and Asia developed from seeds were introduced by Spanish and Portuguese merchants during the mid 16th century. Thereafter, in less than a century tomato has become a major world food crop (FAO, 2000). Despite the tomato's nutritional importance as a source of vitamins A and C, its consumption per capita is approximately four times as high in developed countries as in developing ones (Tigchelaar, 1991). In Thailand, tomatoes are grown in all provinces of the country but the major production areas are found in the central and north-eastern regions. They are consumed as fresh fruits and, in addition, processed for export as canned fruits, concentrated juice, dried fruits, and generating export incomes of over one billion Thai Baht annually (Anonymous, 2005). In 2002, world production of tomatoes was estimated at 108 million metric tons of which 2.42 million metric tons were produced in Thailand (FAO, 2004).

Numerous species of insect pests, mites as well as other pathogens cause severe economic losses in tomato production. Within the pests complex of tomatoes, leafminers *Liriomyza* spp. (Diptera: Agromyzidae) cause substantial damage in tomato production (Waterhouse and Norris, 1987). The genus *Liriomyza* was first documented in 1894, contains more than 300 species, with 23 species economically important (Spencer 1973). Leafminers, particularly *Liriomyza sativae* Blanchard*, Liriomyza trifolii* Burgess *and Liriomyza huidobrensis* Blanchard are among the most destructive poly-phytophagus pests of vegetables and ornamental plants worldwide (Spencer, 1973; Parrella, 1987; Spencer, 1990; Zhao, 2002;). Recently *Liriomyza sativae* invaded in many Asian countries including Thailand (Martinez, 1994), Indonesia (Prijono et al., 2004), China (Chen et al., 2003), and Japan (Abe and Kawahara, 2001). Being extremely polyphagous, *L. sativae* feeds on wide range of host plants for instance Solanaceae, Leguminosae, Cucurbitaceae and Asteraceae (CAB International, 2001).

The damage of *L. sativae* is related to feeding punctures of adults and serpentine mines produced by feeding of larvae in the mesophyll tissues of leaves (Spencer 1973) (Figure 1.1). Both lead to high losses of photosynthetic activities in tomato (Johnson et al., 1983). Yield losses can reach up to 70% due to *L. sativae* invasion (Waterhouse and Norris, 1987).

A- Feeding punctures made by adult female leafminer

B- Feeding mines made by larvae

Figure 1.1 Feeding damage caused by *Liriomyza sativae*

Nearly 100% leafminer control is necessary to produce cosmetically marketable crops (Sher et al., 2000). Currently, this high level of control has traditionally been achieved by the predominant plant protection strategy in vegetables production in Asia using synthetic pesticides. For instance, between 1980 and 1999 the amount of pesticides imported to Thailand has drastically increased from 9,855 to 33,969 tons, at an annual growth rate of 6.7% (Anonymous, 2005). The frequent use of pesticides resulted in emerging problems such as pesticide resistance of multiple pest species (Talekar and Shelton, 1993; Williams and Dennehy, 1996; Ferguson, 2004) and pest resurgence, detrimental effect on natural enemies, contamination of water sources as well as direct health hazards to both farmers and consumers (Saha, 1993). Though leafminers are an increasing pest problem in tropical and sub-tropical climates, only few investigations have been conducted on their integrated control in vegetables and especially on tomatoes (CAB International, 2001). In order to

evade the detrimental effects of heavy use of synthetic broad spectrum pesticides, integrated pest management (IPM) strategies, mainly based on biological control measures need to be developed and implemented against *L. sativae*. Therefore, suitable management strategies against *L. sativae* are urgently needed with reduced insecticide load, use of more safe, selective and environmentally friendly pesticides and biological control as a main alternative (Kang, 1996; Chen et al., 2003).

Parasitoids are the key group of natural enemies of leafminers (Parella, 1987; Kang, 1996). It is evident that indigenous natural enemy communities of *Liriomyza* spp. particularly parasitoids can regulate leafminers in pesticides free areas (Murphy and LaSalle, 1999). Leafminers parasitoids have been widely investigated and evaluated in many countries in commercial greenhouses planted with vegetables as well as ornamental plants particularly tomatoes and chrysanthemum in Europe and North America (Chen et al., 2001). In Asia 41 species of parasitoids of *Liriomyza* in 4 different families were recorded (Chien and Ku, 1998; Lin and Wang, 1992; Murphy and LaSalle, 1999). However, in Thailand only 6 species of *Liriomyza* parasitoids have been recorded i.e. *Asecodes* sp. nr. *notandus* (Silvestri) (Hymenoptera: Eulophidae), *Hemiptarsenus variconis* (Girault) (Hymenoptera: Eulophidae); *Cirrospilus ambiguous* Hansson & LaSalle (Hymenoptera: Eulophidae); *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae); *Quadrastichus* sp. nr *Liriomyzae* (Hymenoptera: Eulophidae); and *Opius dissitus* (Muesebeck (Hymenoptera: Braconidae) (Petcharat, et al., 2002). So far no comprehensive investigations have been done on parasitoids and their efficacies on *Liriomyza* spp in Thailand. In the year 2002 and 2005, two parasitoids *Opius (Opiothorax) chromatomyiae* Belokobylskij & Wharton sp. n. (Hymenoptera: Braconidae) and *Neochrysocharis formosa* Westwood (Hymenoptera: Eulophidae) (larval-pupal and larval, respectively) have been recorded from *L. sativae* larvae/pupae in the study area (Figure 1.2), Asian Institute of Technology (AIT), Thailand. Moreover, the *Opius (Opiothorax) chromatomyiae* Belokobylskij & Wharton sp. n. (Hymenoptera: Braconidae) was not reported in Thailand in the past. Presently, no information is available on their potential to combat *L. sativae* outbreaks and the impact of pesticides on these parasitoids.

Figure 1.2 Parasitoids of *L. sativae* A) *O. chromatomyiae* and B) *N. formosa*

In recent years, as alternative more IPM compatible pesticides those from natural sources such as plants or microorganisms are discussed and tend to replace synthetic products. Such biopesticides i.e. Azadirachtin from the Neem tree *Azadirachta indica* A. Juss (Tedeschi et al., 2001), Spinosyns (Spinosad) or Avermectins (Abamectin) from soil microorganisms (Jones et al., 2005, Weintraub, 2001) are described to efficiently control different important pests but with considerably less risk to farmers and consumers, shorter persistency in the environment, lower risk for leaching and lower impact on non-target organisms than most conventionally used synthetic insecticides in the Asian vegetable crop systems.

Neem (*Azadirachta indica* A. Juss) is native to India (Roxburgh, 1874) from where it has spread out to many Asian and African countries as well as Australia and South America (Srivastava et al., 1997). In recent years, the bioactivity of Neem against insect pests has been particularly investigated in detail (Schmutterer 1990; Singh, 1993). Large numbers of insect pests from different orders have been shown to exhibit different levels of susceptibility to Neem seed extracts, or the most active constituent Azadirachtin (AZA) (Schmutterer and Singh, 1995). Azadirachtin, a mixture of several structurally related tetranortriterpenoids has attracted the greatest attention in recent years (Govindachari et al., 1992) for modern pest control strategies. Mostly, three kinds of reaction are found: alteration of behavior leading to repellent and/or antifeedant effects, disruption of insect development by inhibiting the release of prothoracicotropic hormones and allatotropins and sterilant effects of females caused mainly by alterations of ecdysteroid and juvenile hormone in the target organism (Schmutterer, 1988; Mordue and Blackwell, 1993; Mordue (Luntz) et

al., 1998; James, 2003). Plant feeding insects can be contaminated with Neem topically and systemically by taking up the ingredient with the food source. Hence, both soil and foliar applications of Neem can have strong effects on insets pest (Warthen 1979; Larew et al., 1985; Schmutterer 1990; Mordue et al., 1998). However, equally important, Neem extract are often described to have minimal toxicity to non-target organisms such as parasitoids, predators, and pollinators (Lowery and Isman 1995; Naumann and Isman, 1996; Raguraman and Singh, 1999). Another important feature of Neem is the rapid degradation in the environment (Isman, 1999) improving the situation with residues. However, on the other hand the short persistence of Neem especially when applied in field crops due to degradation by sunlight, UV radiation, rainfall and high acidity on treated leaves of plants is a major drawback for the farmers (Schmutterer, 1988; Johnson et al., 2003).

Spinosad is a newly established microbial-derived insecticide with active ingredients isolated from the aerobe fermentation of the soil bacterium *Saccharopolyspora spinosa* Mertz and Yao (Actinomycetales) (Boek, et al., 1994, Sparks et al., 2001). Commercial formulations of Spinosad are a mixture of the two most active naturally occurring secondary metabolites spinosyns A and D (Sparks et al., 2001). Spinosad has a novel and unique mode of action with species-specific activity initially causing involuntary muscle contractions and tremors by exciting neurons in the central nervous system. After prolonged periods of spinosyn-induced hyper excitation, insects become paralyzed, apparently due to neuromuscular fatigue (Salgado, 1998). Spinosad has applied to over 200 different crops and is currently labeled only for control of Lepidoptera and certain Thysanoptera (Dow, 1997; Bret et al., 1997; T Thompson et al., 2000). Spinosyns and spinosoids have some broad spectrum activity and efficacy has been reported against some other insects in the orders of Coleoptera, Diptera, Homoptera, Hymenoptera, Isoptera, Orthoptera, Siphonaptera, as well as mites (Salgado et al., 1997). Spinosad's non-phytotoxicity has already been discussed and it exhibits wide margins of safety to beneficial insects as well as related organisms (Schoonover and Larson, 1995; Liu et al., 1999), e.g. Jones et al. (2005) found Spinosad harmless for *Amblyseius cucumeris*, and moderate toxic to *Orius insidiosus* both important biological control agents of the western flower thrips *Frankliniella occidentalis*

and highly toxic *to Encarsia formosa* the biocontrol parasitoid of the common white fly *Trialeurodes vaporariorum*.

Abamectin is consisting of a mixture of 80% Avermectin B_{1a} and 20% Avermectin B_{1b} (macrocyclic lactones) fermented from the soil actinomycete, *Streptomyces avermitilis* Burg. and commercially available for killing insects, mites and nematodes (Putter et al., 1981; Leibee, 1988; Fisher and Mrozik, 1989). In recent years, Abamectin has been considered an outstanding chemical against leafminer flies. It is not only highly effective but it derives from a biological source which reveals it as a bio-rational pesticide that can be used environmentally friendly in integrated pest management programs (Dybas, 1989). Exposure of insects to avermectin results in increased mortality (Wolfenbarger et al., 1985, Bull, 1986), reduced feeding (Pienkowski and Mehring, 1983), disrupted development (Wright, 1984, Robertson, 1985) and various reproductive effects including damaged ovaries (Glancey et al., 1982), reduced mating (Bariola, 1984, Cochran, 1985), reduced fecundity (Bariola, 1984, Beach and Todd 1985, Reed et al., 1985) and reduced fertility (Beach and Todd, 1985). Abamectin rapidly degrades on the plant surface (Bull et al., 1984). Schuster and Everett (1983) reported that Abamectin was effective against *Liriomyza trifolii* Burgess on tomato. Although Abamectin has been shown to be harmful to many parasitoids i.e. *Gronotoma micromorpha* it is still far less toxic than Chlorpyriphos (Prijono et al., 2004). Weintraub (2001) reported a relatively lower detrimental effect of Abamectin on *Diglyphus isaea* a parasitoid of leafminer *Liriomyza huidobrensis* than Cyromazine.

The studies described here were conducted to evaluate the systemic properties and persistency effects of Neem products using a water solvent formulation (NeemAzal®-U) especially developed for soil treatments. For comparison topical applications with residual performance of aqueous solutions of NeemAzal®-T/S, Success® and Abamectin against *L. sativae* were investigated. Moreover, the impacts of these pesticides on two endo-parasitoids *Opius* (*Opiothorax*) *chromatomyiae* and *Neochrysocharis formosa* of *L. sativae* were studied. The aim is to adapt the use of Neem, Spinosad and Abamectin for the Integrated Pest Management (IPM) of the leafminer *L. sativae* in tomato production under protected cultivation in the humid tropics. To determine the effects of these biopesticides against development of immatures (both foliar and soil-inhabiting

life stages) of *L. sativae*, experiments were carried out in small scale in laboratory under controlled conditions as well as in large scale under practical greenhouse conditions. Several parameters such as pesticides concentrations, time of application, treatment of larvae-dwelling and pupa-dwelling life stages of parasitoids were evaluated to assess the most effective rates and to discriminate between systemic and direct contact effects in choice and no choice options.

In detail the main objectives of Chapter 2 were to assess the potential systemic and persistency effects of the Neem based bio-pesticide NeemAzal®-U (17% Azadirachtin) against *L. sativae* under air conditioned laboratory vs greenhouses conditions. In these studies, NeemAzal®-U was tested against both foliar and soil inhabiting life stages of *L. sativae* and the susceptibility of different life stages of *L. sativae* to NeemAzal®-U were determined.

In Chapter 3, the residual and direct toxicity effects of topical application of aqueous solutions of NeemAzal®-T/S (1% Azadirachtin) against foliar inhabiting life stages of *L. sativae* were investigated under laboratory and greenhouses conditions. Moreover, the most vulnerable immatures life stadia of *L. sativae* to NeemAzal®-T/S were determined.

Chapter 4 presents a comparative study of botanical pesticides: NeemAzal®-T/S (1% Azadirachtin) and two biorational novel pesticides Success® (12% Spinosad, Spinosyns A and D) and Abamectin (1.8% EC, Avermectin) for the management of *L sativae*. The study was conducted to determine the susceptibility of this notorious pest to these pesticides on tomatoes in laboratory and greenhouse conditions. Effects of these products were tested on the survival of foliar-inhabiting life stages of *L. sativae* i.e., egg and larvae, as well as their development to adults. In these studies, NeemAzal®-T/S, Success® and Abamectin products were tested as topical application including determination of their residual toxicities.

In Chapter 5, the impact of NeemAzal®-U, 17% Azadirachtin and NeemAzal®-T/S, 1% Azadirachtin as well as Success® (12% Spinosad, SC) and Abamectin (1.8% EC, Avermectin) on two endo-parasitoids *Opius (Opiothorax) chromatomyiae* Belokobylskij & Wharton sp. n. (Hymenoptera: Braconidae) and *Neochrysocharis formosa* Westwood (Hymenoptera: Eulophidae) of *L. sativae*, were investigated. The experiments were arranged to differentiate between

effects of direct contamination only by diffusion through the host cuticle and combined diffusion and ingestion toxicities of these pesticides.

The study was carried out at the Asian Institute of Technology (AIT), a peri-urban area of greater Bangkok, Thailand during 2002-2005. The study was sponsored by the German Research Foundation (DFG) in the frame of the DFG Research Group FOR 431. It was part of a larger project which aims to establish sustainable and environmentally friendly vegetable production systems under protected cultivation in the humid tropics.

2 Effects of soil application of Neem (NeemAzal[®]-U) on different life stages of *Liriomyza sativae* (Dip.: Agromyzidae) on tomatoes in the humid tropics[1]

2.1   Introduction

Various species of leafminers cause severe damage to vegetable and ornamental field crops. Three *Liriomyza* species: *L. sativae, L. trifolii* and *L. huidobrensis* are reported to pose a worldwide threat to horticultural field crops (Webb et al., 1983; Murphy and LaSalle, 1999). *L. sativae* is a polyphagous herbivore and a serious pest, which attacks a wide array of vegetable and ornamental crops (Parrella, 1987; Spencer, 1990; Zhao, 2002). In tomatoes *L. sativae* can cause losses up to 70% (Waterhouse and Norris, 1987). In the latest years, the focal pest control strategy in vegetables in Asia with intensive use of synthetic pesticides has resulted in multiple problems such as the development of pesticide resistant strains (Talekar and Shelton, 1993; Williams and Dennehy, 1996), pesticide-induced resurgence of insects pests, adverse effects on non-target organisms, namely parasitoids and predators, contamination of water sources and direct health hazards to both farmers and consumers (Raguraman and Singh, 1999).

L. sativae and other leafminer species are primarily controlled with chemical insecticides such as Permethrin, Fenvalerate, Methamidophos, Chlorpyrifos, and Abamectin (Tryon and Poe, 1979; Johnson et al., 1980; Leibee, 1988; Weintraub, 2001) and the development of resistant strains is already described (e.g. Spencer, 1990; Ferguson, 2004). Moreover, the use of non selective broad-spectrum insecticides to control lepidopteran pests such as *Heliothis zea* (Boddie) and *Keiferia lycopersciella* Walsingham resulted in increasing densities of *L. sativae* consequent to the destruction of the agromyzid's effective natural enemies (Johnson et al., 1980).

In temperate areas as well as warmer regions like the Mediterranean region vegetables are often grown in greenhouses or under plastic films. There leafminers can be controlled by inundative releases of parasitoids such as *Dagnusa* spp. or *Diglyphus* spp. (Albajes and Sekeroglu, 2000). In the humid

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[1]based on Hossain MB, Poehling H–M, Thöming G and Borgemeister C. Effects of soil application of Neem (NeemAzal[®]-U) on different life stages of *Liriomyza sativae* (Dip.: Agromyzidae) on tomatoes in the humid tropics. Submitted to Crop Protection.

tropics however, during periods of extremely favourable climatic conditions for development of the pest, the beneficial organisms solely cannot suppress leafminer development under critical thresholds even in protected environments. Combinations of natural enemies with temporary applied fast acting insecticides can be a convenient solution but such ingredients should fulfill important conditions: complete environmental degradability, low human toxicity, easy and cheap production as well as partial selectivity to various beneficial organisms and low risk of selecting pest biotypes. The so-called "green" insecticides or biopesticides are suitable candidates for sound IPM tactics. Of special interest are the Neem products extracted from seeds or leaves of the Neem tree *Azadirachta indica* A. Juss categorized as broad spectrum but IPM compatible insecticides and alternatives to the synthetics.

Over 400 insect pests have been shown to exhibit varying degrees of susceptibility to Neem seed extracts, or the most active constituent Azadirachtin (AZA) (Schmutterer and Singh, 1995). Two kinds of reaction are mostly found: alteration of behavior leading to repellent and/or antifeedant effects and modification of insect development by inhibiting the release of prothoracicotropic hormones and allatotropins (Mordue and Blackwell, 1993; Gonzales et al. 1999). Antifeedant activity has been described for Neem for several species of different insect orders, including Orthoptera (Attri, 1975; Simmonds and Blaney, 1996), Coleoptera (Saradamma et al., 1977; Trisyono and Whalon, 1999), Lepidoptera (Mordue and Blackwell, 1993; Tang et al., 2000), and Diptera (Warthen, 1979; Su and Mulla, 1998). Growth regulating activity was described for a wide variety of phytophagous insects (Warthen, 1979; Rembold et al., 1982; Schmutterer, 1988; Mordue and Blackwell, 1993; James, 2003).

Leafminers such as *L. trifolii* and *L. sativae* are sensitive to Neem treatments expressed in lower fecundity and longevity of the adults (Parkman and Pienkowaski, 1990; Azam et al., 2003) or increased mortality of the larval stages (Webb et al., 1983), which is typical for many Dipteran and Lepidopteran species (Hashem et al., 1998; Hassan, 1998). Most often, Neem products are applied as foliar sprays to control leafminers. Despite the efficacy of foliar application of Neem, major drawbacks are addressed: The fast biodegradability of Neem is simultaneously an attractive advantage in domestic areas but a

hindering drawback where the shorter persistence lowers the efficacy in field applications. Johnson et al., (2003) reported that Azadirachtin sprayed as a thin film on the leaf surface was only effective for 2.5 days if exposed to sunlight. Schmutterer (1988) stated that UV-light, rainfall and perhaps high acidity on treated surfaces of plants cause a fast degradation or the loss of active material sprayed on the foliage. Moreover, it has been addressed in a number of studies that topical applications of Azadirachtin solutions with direct contamination of plant dwelling organism can pose a risk to non-target beneficials such as parasitoids and predators (Schulz et al., 1997; Krishnaya and Grewal, 2002). Application strategies with high efficiency against the target pests, with reliable persistence but minimal effects on non-target organisms would be desirable. Thus, soil application such as seed dressing or plant substrate treatments could be advantageous providing that systemic translocation of the active ingredient is possible. Root uptake, acropetal translocation and systemic effects of Neem compounds have been studied with different pests such as spider mites (Sundaram et al., 1995) or most recently the Western flower thrips *Frankliniella occidentalis* (Thoeming et al., 2003). Regarding leafminers a first study with *L. huidobrensis* demonstrated the systemic potential of Neem for our target (Weintraub and Horowitz, 1997).

The studies described here were conducted to evaluate the systemic properties of the Neem product NeemAzal using a water based formulation (NeemAzal®-U) especially developed for hydroponics and soil treatments. The aim is to adapt the use of Neem for the integrated control (IPM) of the leafminer *L. sativae* in tomato production under protected cultivation in the humid tropics. To determine the systemic effects of Neem on oviposition, development of immatures and soil-inhabiting life stages, experiments were carried out as small scale and laboratory trials under controlled conditions but also as large scale under practical greenhouse conditions. Several parameters such as applied AZA concentrations, time of application, treatment of pupae dwelling in the soil were evaluated to assess the most effective rates and timing of application as well as to discriminate between systemic and direct contact effects.

2.2   Materials and Methods

General frame

The project was part of an interdisciplinary research program of the German Research Foundation (FOR 431) entitled "Protected cultivation - an approach to sustainable vegetable production in the humid tropics". The experiments were conducted in air conditioned laboratory rooms and greenhouses of 6 m x 6 m each (plastic roof, side walls covered with 40-mesh net) at the Asian Institute of Technology (AIT) located in a peri-urban area of Bangkok, Thailand. All experiments were repeated two times within the following time periods: Exp.1 (March-April, 2003), Exp. 2 (February– May, 2004), Exp. 3 (June– July, 2003), and Exp. 4 (August –September, 2003).

Insects and plant sources

The *L. sativae* strain used in the experiments was selected in July 2002 from tomato plants (v. King Kong II) grown in the greenhouses complex at AIT. Thereafter, a stock culture of *L. sativae* was continuously reared on the same tomato variety in cages placed in air conditioned rooms at 29 ± 1 $^{\circ}$C, 60-65% RH and 16:8 or photophase [L:D]. Synchronized adults were obtained by placing two day old adults (males and females) on young potted tomato plants for 6 hours. After oviposition, the adults were removed. This short oviposition time ensured uniformly age of eggs and subsequent larvae, pupae and thereafter emerged adults.

Locations and conditions

First series of experiments were established in air conditioned rooms at 29 ± 1 $^{\circ}$C, 60-65% RH and 16:8 h [L:D]. Acrylic cages (65 cm x 61 cm x 61 cm) with upper side and two perforated side holes (25 cm Ø) covered by 78-mesh net to allow ventilation were used. Thirty-five day old tomato plants grown in pots (8 cm high and 10 cm Ø) containing 180 g of a clay loam substrate composed of silt, sand and clay (39.2, 29.9 and 30.9%, respectively) and organic mater 27.9% were used in all experiments. The pots were watered twice a day with 50 ml tap water per pot, which satisfies the substrate saturation capacity. Second series were run in greenhouses (see above). In the first trial, the pots size, plants age and used amount of substrate per pot were parallel to the climate room experiments. The pots were watered three times a day with 50 ml tap water per pot just to reach the saturation capacity of the substrate. In the

second trial, forty-five day old potted (15 cm high x 20 cm Ø, filled with 1 kg substrate) tomato plants were used in the experiments. The day-night temperature ranged from 24.4-34 $^{\circ}$C with relative humidity from 65 - 70%, respectively. Mean daily temperature and RH were maintained throughout the experimental period. Plants were watered manually with 250 ml irrigation water applied in the morning and evening mixed with fertilizers at local recommended dosages.

Neem

Powdered NeemAzal$^{®}$-U (Trifolio–M GmbH, Germany) containing 17% Azadirachtin was used for all experiments. Application solutions with dosage rates of 0.75, 1.5, 2.25 and 3.0 g/lw NeemAzal$^{®}$-U equivalent to 0.0125, 0.025, 0.038 and 0.05%, respectively Azadirachtin were produced by dissolving the respective amount of NeemAzal$^{®}$-U powder in tap water and stirring for 30 minutes at room temperature. As a control, a blank formulation containing all carrier substances but without AZA was used at a concentration of 3.0 g/lw water of the blank substances (Figure 2.1). All concentrations were prepared immediately prior to use.
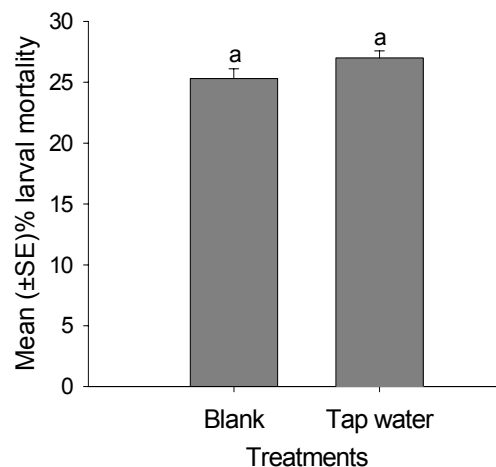

Figure 2.1 Mean (±SE) percentage of larval mortality of *L. sativae* per leaf treated either with NeemAzal$^{®}$-U blank formulation (3.0 g/lw) or tap water. Columns marked with the common letter are not significantly different (*P* > 0.05, ANOVA, Tukey's HSD multiple mean comparisons)

Experiments

Exp. 1. Systemic and dosage effects, and persistency of NeemAzal®-U under laboratory conditions (climate rooms)

To evaluate (i) systemic properties of NeemAzal®-U, (ii) dosage dependence and (iii) persistence effects as well, pot soil was drenched with 50 ml of the above mentioned NeemAzal®-U dilutions and blank formulation resulting in concentrations of 0.21, 0.42, 0.63 and 0.83 g NeemAzal®-U/kg substrate. Treatments were performed 5, 4, 3, 2 and 1 day prior to introducing the leafminers. Plants were arranged in a completely randomised design and at day 0 one-day-old adult leafminers of both sexes (100/cage) from the stock culture were released for a period of 24 h. Different treatments were randomly arranged in the same cages to offer the leafminers the choice for oviposition on treated and untreated plants. Ten replications were run but split to two time periods so that a total of 25 plants per treatment could be evaluated. Thereafter, all adult leafminers were removed from the cages using an aspirator. Upon adult removal from the cages, the eggs were counted using a stereo-microscope with substage lighting. After 48 h, the eggs were checked again to record the number of hatched ones. From 72 h onwards, the plants were inspected daily under the microscope to determine larval mortality until the larvae dropped for pupation. Larvae that dropped from the foliage were collected in plastic bags. Then pupae were transferred to petri dishes (9 cm Ø) and retained until adult emergence.

Exp. 2. Systemic effects of NeemAzal®-U at different dosage rates and persistency under greenhouse conditions

First trial: Plants infested with L1

Even aged L1 on potted grown tomato plants were achieved as described above. After removal of the adults, the plants were transferred to another insect free greenhouse with similar environment and the plants exposed under open conditions. Each treatment was replicated 5 times. The numbers of L1 were counted 48 hours after infestation and afterwards pots were drenched with NeemAzal®-U solution following the above-mentioned concentrations (50 ml per 0.18 kg substrate per pot). Watering was not applied to the plants 12 h before soil drenching. Twenty-four hours after application of NeemAzal®-U solutions, the drenched plants were inspected daily for four consecutive days under a

stereo-microscope. The procedures of larval mortality counting, pupae collection and rearing of pupae until adult emergence were alike as in Exp. 1.

Second trial: Persistence effects

Potted tomato plants (1 kg substrate/pot) placed in the greenhouse and the substrate drenched with different amounts of NeemAzal®-U (see above), 250 ml per pot resulted in 0.18; 0.38; 0.57 and 0.75 g NeemAzal®-U per kg substrate and 0.75 g blank substances/kg substrate. Treatments were timed 7, 6, 5, 4, 3, 2 and 1 d prior to leafminers' introduction. Four NeemAzal®-U dosages and a blank control were used and five replications per treatment per date were performed thus totaling to 175 plants arranged in a completely randomised design. At day 0, approximately 5000 one-day-old even aged adult leafminers of both sexes were released. Thus, the females were given a choice between treated and non-treated plants for oviposition. Therefore, 48 h after the release, the adults were removed and plants carrying synchronized larvae were transferred to another identical insect free greenhouse. An infested middle leaf from each plant was tagged. Five days later tagged leaves were excised and checked in the laboratory for dead larvae. Prepupae were collected in plastic bags for pupation. The initial number of larvae was calculated from the sum of prepupae, pupae and dead larvae. Surviving prepupae were reared to pupa and adult eclosion like in Exp. 1.

Exp. 3. Effects on different immature developmental stages of *L. sativae* under laboratory conditions

To obtain different stages of leafminers plants were exposed to mature females for 6 hours for oviposition. Thereafter, plants were removed from the cages and arranged into 4 sets (25 plants/set) for different developmental stages (eggs, L1, L2 and L3), each placed in different insect prove cages (ten replications). The group of egg containing plants was drenched immediately after the counting of the eggs with the different NeemAzal®-U concentrations (5 plants for each treatment) as described above. Similarly, 2, 4 and 5 days after infestation the plants carrying L1, L2 and L3 leafminer stages were drenched, respectively. The initial number (before treatment) of each stage per plant was counted before NeemAzal®-U drenching. Different larval instars were distinguished by stereo-microscope with micrometer scale. Petitt (1990) distinguished different larval instars of *L. sativae* by measuring the length of the cephalopharyngeal

skeleton, L1 (0.058-0.111 mm), L2 (0.123-0.173 mm) and L3 (0.196-0.249 mm). In each treated larval stage, mortality was recorded 1 day after drenching. Dead larvae were marked by placing a small black dot on leaflets on every sampling day. In the case of alive larvae, leaflets were checked daily until prepupae dropped for pupation or died on the foliage. Surviving prepupae were reared to pupae and afterwards adult emergence noted (see Exp. 1).

Exp. 4. Direct effects on soil-inhabiting life stages

First trial

Uniform aged L1 were achieved as described above. Late L1 were counted and afterwards plants excised to the soil level and immersed with the lower stem end individually in glass vials (9.5 cm high and 2 cm Ø) filled with tap water. The vials were placed on top of pots filled with soil to insure a dropping site for the emerging prepupae. Pots and plants were covered with fitting plexi glass cylinders (30 cm high and 10 cm Ø) so that emerging adults could be trapped and counted. The top of the cylinders and additional ventilation holes at the side of the cylinders were covered with nylon tissue (pore size $\approx$ 64 μm) for ventilation. Four days after infestation (36 h prior to larval dropping to the soil for pupation), pots were drenched with NeemAzal®-U solution (concentration and blank see above). Plant vials were removed from the cylinders after all prepupae had left the foliage and hatching adults were monitored. Percentages of adult emergence were calculated based on number of larvae initially that should have dropped from the foliage. The experiment was replicated ten times.

Second trial

In the second trial, the entire procedures of the experiment were similar to the first one, however, NeemAzal®-U solutions were applied later after 5.5 days of plant infestation immediately before late L3 larvae or prepupae started dropping to the soil for pupation.

Statistical procedures

Data with percentage mortality were subjected to HOVTEST = LEVENE option of SAS to account for homogeneity of variance and normality. In case of non-homogeneity, percent values were transformed using arcsine–square-root (arcsine√) transformation and insect count values were transformed by square-root (√) transformation before running an ANOVA. The interaction effects in addition to single factor effects were evaluated in factorial experiments. In case

of no significant interactions the data were pooled. Where significant F values were obtained ($P < 0.05$), treatments means were separated using Tukey's test. All statistical analysis was performed using the GLM procedure in SAS (2002).

2.3   Results

Exp. 1. Systemic, dosages and persistency effects of NeemAzal®-U under laboratory conditions (climate rooms)

Interactions (days*treatments) of oviposition and egg hatch were not significant and the data were pooled. The effects of all NeemAzal®-U treatments on egg laying were not significant ($F = 0.70$; df = 4, 249; $P > 0.05$). Therefore, neither preference nor avoidance of treated plants was obvious. The proportion of hatched eggs was not affected ($F = 2.36$; df = 4, 249; $P > 0.05$) by NeemAzal®-U concentrations. On average, about 99% of the treated eggs hatched (Table 2.1). The interactions between days and treatments were significant where larval mortality prevailed. All four concentrations of NeemAzal®-U resulted in significantly higher larval mortality than the untreated control ($F = 8250.11$; df = 4, 249; $P < 0.0001$) and significant differences on larval mortality were recorded among different NeemAzal®-U concentrations (Table 2.2). Mortality ranged from 36.04% (NeemAzal®-U 5 days before release, 0.75 g/lw) to 100% (NeemAzal®-U 1 day before release, 2.25 and 3.0 g/lw). Adult eclosion was significantly affected by all tested NeemAzal®-U concentrations ($F = 6646.51$; df = 4, 249; $P < 0.0001$) (Figure 2.2). No adults eclosed from any of the pupae which developed on plants treated with 2.25 and 3.0 g/lw NeemAzal®-U and only very few from plants treated with 0.75 and 1.5 g/lw.

Table 2.1 Effects of NeemAzal®-U on oviposition and egg hatch of *L. sativae* on drenched plants under laboratory conditions

NeemAzal®-U (g/lw)	Eggs/leaf	Egg hatched (%)
Blank	33.04±2.44a	99.78±0.24a
0.75	32.20±2.40a	99.50±0.55a
1.5	32.72±2.56a	99.51±0.48a
2.25	32.54±2.14a	98.86±0.67a
3.0	31.90±2.37a	98.70±0.82a

Mean (±SE) numbers followed by same letter are not significantly different (P > 0.05, ANOVA, Tukey's HSD test).

Table 2.2 Effects of NeemAzal®-U on larval mortality of *L. sativae* with different timing of soil drenching (persistence effect) under laboratory conditions

NeemAzal®-U (g/lw)	Mean (SE) % mortality (1…5: days of NeemAzal®-U application before leafminer infestation)				
	1	2	3	4	5
Blank	1.17±0.81aA	0.68±0.68aA	0.84±0.57aA	0.93±0.62aA	0.71±0.48aA
0.75	74.20±0.63bA	64.17±0.84bB	51.74±1.01bC	48.55±1.12bC	36.04±2.69bD
1.5	81.59±0.54cA	81.35±0.85cA	79.37±1.60cA	65.22±0.53cB	63.21±0.81cB
2.25	100±0dA	97.61±0.87dA	89.43±0.74dB	80.64±0.75dC	73.74±0.80dD
3.0	100±0dA	99.06±0.69dA	97.27±0.49eB	88.86±0.71eC	80.44±0.51eD

Mean (±SE) numbers followed by same letter are not significantly different: upper case letters within a row, lower case letters within a column (P > 0.05, ANOVA, Tukey's HSD test).
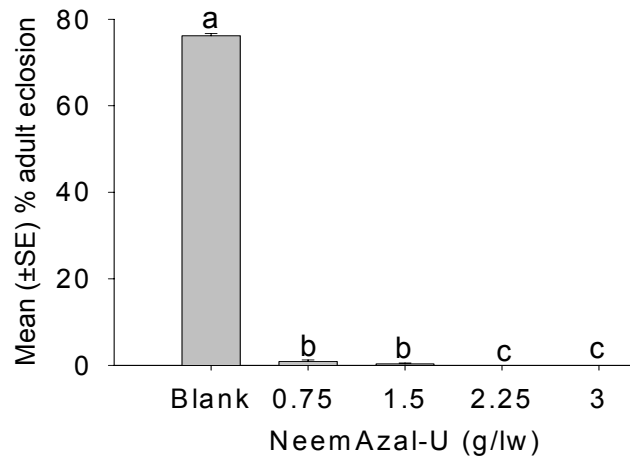
Figure 2.2 Mean (±SE) percentage adult emergence of *L. sativae* from pupae collected from plants drenched with different dosage rates of NeemAzal®-U (0.75, 1.5, 2.25 and 3.0 g/lw water (Climate room experiment). Columns marked with common letters are not significantly different (*P* > 0.05, ANOVA, Tukey's HSD multiple mean comparisons)

Exp.2. Systemic effects of NeemAzal®-U at different dosage rates and persistency in the greenhouse

First trial: Plants infested with L1

Significant interactions were observed between sampling days*treatments. Generally, larval mortality increased with increasing concentrations of NeemAzal®-U in the soil. All NeemAzal®-U treatments significantly increased larval mortality compared to the control and significant differences in larval mortality were recorded among NeemAzal®-U treatments (F = 1309.54; df = 4, 199; P < 0.0001) (Table 2.3). Time to death varied among treatments. With the highest NeemAzal®-U concentration of 3.0 g/lw, one day after treatment, nearly 68% of final mortality of 93.4% was attained compared to only 8% of the final morality of 26% with a 0.75 g NeemAzal®-U/lw. Strong and significant effects of NeemAzal®-U were observed for pupal development (F = 378.47; df = 4, 49; P < 0.0001). Only those pupae from plants receiving the lowest dosage of 0.75 g/lw NeemAzal®-U allowed adult hatching compared to a complete inhibition of emergence from pupae developed from 1.5, 2.25 and 3.0 g/lw treated plants (Table 2.4).

Table 2.3 Effects of NeemAzal[®]-U on larvae of *L. sativae:* soil drenching after establishment of L1 on leaves under greenhouse conditions

NeemAzal[®]-U (g/lw)	Mean (±SE) accumulated larval mortality on four consecutive sampling days			
	1	2	3	4
Blank	0.40±0.27aA	0.40±0.27aA	0.40±0.27aA	1.99±0.64aA
0.75	2.08±0.86abA	7.95±1.07bB	13.88±1.29bC	26.21±1.52bD
1.5	3.64±1.30bA	8.17±0.83bB	15.82±1.11bC	35.78±0.87cD
2.25	42.64±1.14cA	68.34±2.37cB	75.96±1.74cBC	81.10±0.77dC
3.0	58.27±1.54dA	82.33±1.19dB	88.71±1.32dC	93.37±2.11eD

Mean (±SE) numbers followed by same letter are not significantly different: upper case letters within a row, lower case letters within a column (P > 0.05, ANOVA, Tukey's HSD test).

Table 2.4 Effects of soil drenching of NeemAzal[®]-U on adult emergence of *L. sativae* larvae under greenhouse conditions

NeemAzal[®]-U (g/lw)	Adult emergence (%)
Blank	80.52±0.98a
0.75	6.12±1.87b
1.5	0±0c
2.25	0±0c
3.0	0±0c

Mean (±SE) numbers followed by same letter are not significantly different (P > 0.05, ANOVA, Tukey's HSD test).

Second trial: Persistence effects

Larval density did not differ between NeemAzal[®]-U drenched and control plants (F = 0.13; df = 4, 349; P = 0.97) (Table 2.5). However, all concentrations of NeemAzal[®]-U resulted in significantly higher larval mortality compared to control treatments (F = 3166.54; df = 4, 349; P < 0.0001) and significant differences of larval mortality were found among different NeemAzal[®]-U concentrations within all time treatments (Table 2.6). Across all persistency (time of treatment) groups, the highest larval mortality was recorded in 3.0 g/lw NeemAzal[®]-U treated plants. Efficiency of NeemAzal[®]-U was reduced with the increasing time

span between treatment and infestation ($F = 323.68$; df = 6, 349; $P < 0.0001$) but even a pre-application period of seven days resulted in mortalities extending between 40% to 70% in the 1.5 g/lw and 3.0 g/lw treatments, respectively. No significant differences were detected in adult emergence with respect to time ($F = 1.82$; df = 6, 349; $P > 0.10$). Hence, data were pooled over all time treatments and then compared. All NeemAzal®-U concentrations significantly affected adult emergence (Figure 2.3).

Table 2.5 Systemic effects of NeemAzal®-U on larval density of *L. sativae* per leaf under greenhouse conditions

NeemAzal®-U (g/lw)	Mean (SE) larval density per leaf
Blank	30.00±1.45a
0.75	30.30±1.34a
1.5	28.21±1.20a
2.25	27.87±1.19a
3.0	27.71±1.13a

Mean (±SE) numbers followed by same letter are not significantly different ($P > 0.05$, ANOVA, Tukey's HSD test).

Table 2.6 Effects of NeemAzal®-U on larval mortality of *L. sativae* with differently timed soil drenching treatments (persistence effect) under greenhouse conditions

NeemAzal®-U	Mean (SE) % mortality (1…7: days of NeemAzal®-U application before leafminer infestation)						
(g/lw)	1	2	3	4	5	6	7
Blank	1.20±0.89aA	1.52±0.66aA	1.46±0.97aA	2.08±0.97aA	2.53±1.14aA	1.87±1.26aA	1.65±0.87aA
0.75	72.37±2.29bA	64.59±4.04bA	52.52±2.47bB	47.38±2.52bB	34.67±1.22bBC	10.39±1.52bC	9.38±1.95bC
1.5	82.76±1.51cA	79.42±2.10cA	78±1.58cA	64.02±1.78cB	61.74±0.64cBC	57.70±1.19cC	40.55±1.54cD
2.25	99.26±0.37dA	95.41±1.79dA	91.78±0.97dA	78.86±0.90dB	70.96±2.88dC	68.69±1.32dC	65.34±1.49dC
3.0	99.15±0.62dA	99±0.66dA	96.58±1.53dA	84.26±1.96dB	78.48±0.55eC	69.89±1.27dC	69.50±0.94dC

Mean (±SE) numbers followed by same letter are not significantly different: upper case letters within a row, lower case letters within a column ($P > 0.05$, ANOVA, Tukey's HSD test).
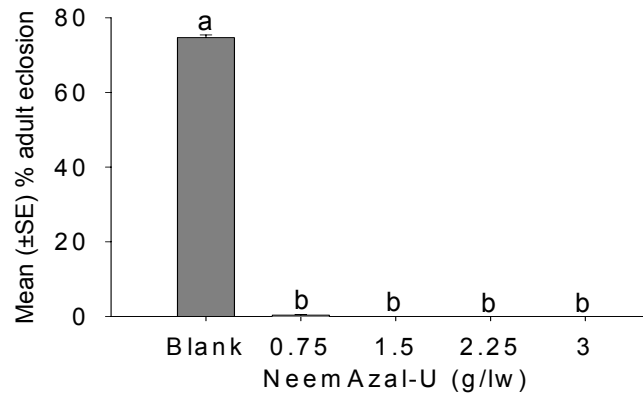
Figure 2.3 Mean (±SE) percentage adult emergence of *L. sativae* from pupae collected from plants drenched with different dosage rates of NeemAzal[®]-U (0.75, 1.5, 2.25 and 3.0 g/lw water (Greenhouse experiment). Columns marked with common letters are not significantly different (*P* > 0.05, ANOVA, Tukey's HSD multiple mean comparisons)

Exp. 3. Effects on different immature developmental stages of L. sativae

Application of NeemAzal[®]-U at 0.75, 1.5, 2.25 and 3.0 g/lw to plants carrying eggs did not significantly affect the emergence of first instar larvae after soil drenching (F = 0.13; df = 4, 49; P = 0.97) compared to untreated control but significant differences were detected in larval mortality (F = 2569.7; df = 4, 49; P < 0.0001) (Table 2.7). Larval mortality increased with increasing NeemAzal[®]-U concentration and significant differences were obtained between the individual developmental stages, L1 (F = 1332.70; df = 4, 49; P < 0.0001), L2 (F = 273.79; df = 4, 49; P < 0.0001) and L3 (F = 266.11; df = 4, 49; P < 0.0001). Mortalities ranged from 24.17% to 98.73% for L1, 7.60% to 60.39% for L2 and 6.43% to 58.66% for L3, respectively, when evaluating low (0.75 g/lw) to high (3.0 g/lw) NeemAzal[®]-U concentrations (Table 2.7). When inspecting adult emergence, significant differences were observed among treatments, eggs (F = 1651.05; df = 4, 49; P < 0.0001), L1 (F = 428.68; df = 4, 49; P < 0.0001), L2 (F = 1051.02; df = 4, 49; P < 0.0001) and L3 (F = 412.68; df = 4, 49; P < 0.0001) (Table 2.8). Overall all NeemAzal[®]-U treatments inhibited adult emergence from the pupae independent of treated stage.

Table 2.7 Effects of NeemAzal®-U after soil drenching on different immature developmental stages of *L. sativae*

NeemAzal®-U (g/lw)	Mean (±SE) % mortality				
	Eggs	Larval mortality hatched from treated eggs	L1	L2	L3
Blank	1.45±0.59	1.33±0.72a	1.06±0.71a	1.54±0.84a	2.18±1.11a
0.75	1.05±0.56	74.10±0.88b	24.17±1.37b	7.60±1.39a	6.43±1.56a
1.5	1.40±0.73	87.37±1.42c	73.67±1.14c	46.63±2.16b	25.88±2.08b
2.25	1.58±0.81	100±0d	93.34±1.61d	57.94±2.50c	55.99±1.82c
3.0	1.87±0.86	100±0d	98.73±0.90e	60.39±1.66c	58.66±1.43c

Mean (±SE) numbers within a column followed by same letter are not significantly different ($P > 0.05$, ANOVA, Tukey's HSD test).

Table 2.8 Effects of NeemAzal®-U after soil drenching on adult emergence from different treated immature stages of *L. sativae*

NeemAzal®-U (g/lw)	Mean (± SE) % adult emergence from different treated immature stages			
	Eggs	L1	L2	L3
Blank	77.81±1.92a	76.36±3.63a	78.19±0.52a	77.72±1.91a
0.75	0±0b	0.60±0.61b	4.52±2.32b	13.63±3.02b
1.5	0±0b	0±0b	0±0b	0.95±0.95c
2.25	0±0b	0±0b	0±0b	0±0c
3.0	0±0b	0±0b	0±0b	0±0c

Mean (±SE) numbers within a column followed by the same letter are not significantly different ($P > 0.05$, ANOVA, Tukey's HSD test).

Exp. 4. Direct effects on soil-inhabiting life stages
First trial

Mortalities of pupae caused by 2.25 and 3.0 g/lw NeemAzal®-U were significantly higher compared to control treatment, and to the lower concentrations of 0.75 and 1.5 g NeemAzal®-U/lw ($F = 5.57$; df = 4, 49; $P < 0.0001$) (Figure 2.4A). However, the overall mortality was rather low with 21.9%, 22.5%, 37.9% and 39.4% for 0.75, 1.5, 2.25 and 3.0 g NeemAzal®-U/lw treatments, respectively.
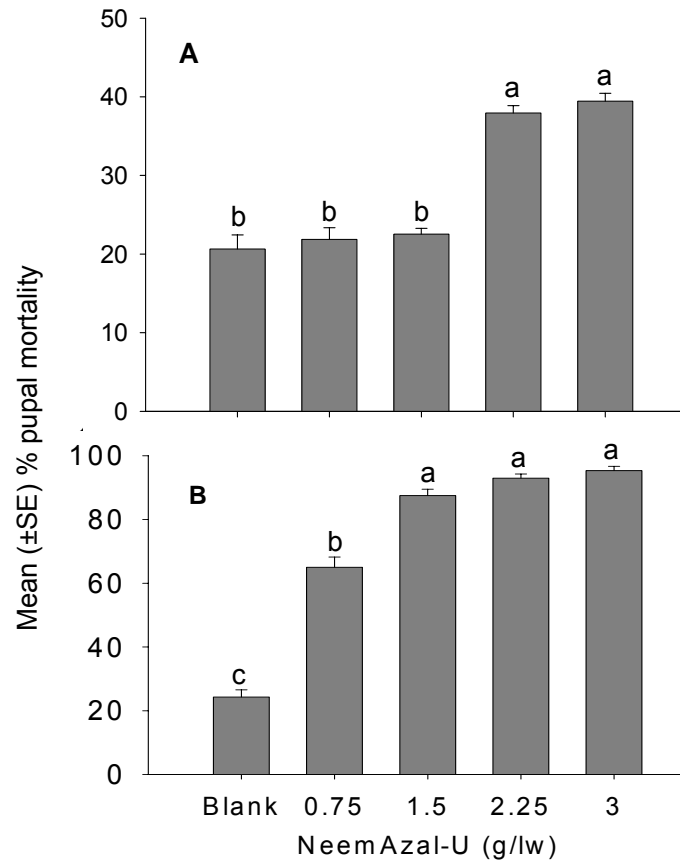
Figure 2.4 Mean (±SE) percentage mortality of *L. sativae* pupae caused by different dosage rates of NeemAzal[®]–U 17% Azadirachtin (0.75, 1.5, 2.25 and 3.0 g/lw) applied to soil 36 hrs (A) or immediately (B) before late 3rd instar from untreated plants started to drop for pupation. Columns marked with same letter are not significantly different (*P* > 0.05, ANOVA, Tukey's HSD multiple mean comparisons)

Second trial

Suppression of pupal development to adult emergence was more pronounced when soil drenching occurred immediately before prepupae started dropping to the soil for pupation. Mortalities of pupae in the soil treated with 0.75, 1.5, 2.25 and 3.0 g NeemAzal[®]-U/lw were significantly higher than untreated control (*F* = 193.23; df = 4, 49; *P* < 0.0001). Strong contact effect of NeemAzal[®]-U on soil inhabiting life stages of *L. sativae* was evidenced by the mortalities of 65.0%, 87.5%, 93.0% and 95.3% with the deployment of 0.75, 1.5, 2.25 and 3.0 g NeemAzal[®]-U/lw, respectively (Figure 2.4B).

2.4   Discussion

This study presents the first report of Neem products (NeemAzal®-U) applied as soil drench to control *L. sativae* in the humid tropics. The outcomes from the various laboratory and greenhouses experiments showed clear systemic properties of NeemAzal®-U with strong effects on different life stages of *L. sativae.* The dual faces of this efficacy are positively related to the soil application of the Azadirachtin concentration and negatively correlated to the time span between treatment and infestation.

Oviposition

Neem extracts have been found to deter oviposition of many crop pests (Singh and Srivastava, 1983; Raguraman, 1987; Schmutterer, 1990; Isman 1996). Most of these experiments were performed with spray applications of Neem to aerial plant parts. Webb et al. (1983) reported in choice experiments that *L. trifolii* laid fewer eggs after immersing bean leaves in a solution derived from dry Neem seed kernels. In our soil application experiments, no such effects on *L. sativae* oviposition were found. Thus, even the drenching with high NeemAzal®-U concentration solutions (e.g. 3.0 g NeemAzal®-U/lw) did not inhibit adult females from oviposition. These findings corroborate with results from Larew et al. (1985) who similarly reported that high concentration of Neem with 200 ml 0.4% crude Neem extract applied to the substrate of chrysanthemum plants in 10-cm plastic square pots had no effect on *L. trifolii* oviposition. Moreover, Weintraub and Horowitz (1997) noted no significant effects on oviposition of *L. huidobrensis* on bean plants (*Phaseolus vulgaris*) when Neemix-45 (4.5% Azadirachtin, produced by W. R. Grace & Co., Conn., Colombia MD, USA) was drenched at 1 ppm, 5 ppm, 10 ppm and 25 ppm to a substrate of peat moss, vermiculite, sand of 1:1:1.

Egg hatch

Even high concentrations of Neem did not affect egg hatch after soil drenching of NeemAzal®-U. Screening the literature, no comparable studies with leafminers were found to uphold this data, but similar observations were reported with other insect species. Von Elling et al. (2002) working with the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Hom.: Aleyrodidae) could not find any influence on egg hatch even after spraying of NeemAzal-TS on aerial parts of plants. Eggs may strongly be protected by their impermeable

membrane or egg chorion which may inhibit the penetration of NeemAzal®-U into the egg.

Larval mortality

The studies have demonstrated that the active ingredient of NeemAzal®-U is relative fast taken up by the root system and translocated to the leafminer feeding sites, thus bearing the credit of being of high efficiency to combat *L. sativae* larvae. Throughout the observation period, all concentrations (0.75, 1.5, 2.25 and 3.0 g/lw) of NeemAzal®-U induced a significant higher mortality of immature stages of *L. sativae* compared to untreated control plants. Mortality ranged from 9.4% to 100% when concentrations of NeemAzal®-U solutions increased from 0.75, 1.5, 2.25 and 3.0 g/lw, respectively. Parallel results could be retrieved from other studies. Weintraub and Horowitz (1997) obtained 40.3%, 48.6% and 84.4% larval mortality of *L. huidobrensis* from Neemix-45 (4.5% Azadirachtin) when 5 ppm, 10 ppm and 25 ppm Azadirachtin were drenched to substrate of bean plants, respectively. Moreover, Meisner et al. (1986) reported a 95.4% reduction in pupal numbers when plant substrates were treated with 1% aqueous Neem extract (methanolic and ethanolic extracts) before exposure to *L. trifolii*. Meadow et al. (2000) found that soil drench of potted cabbage plants with 100 ml of NeemAzal-T (Trifolio-M, GmbH, Germany) diluted to a concentration of 0.01% Azadirachtin caused 90% larval mortality of the cabbage moth *Mamestra brassicae* and Thoeming et al. (2003) observed strong systemic effects with larval mortalities up to 90% after soil application of a NeemAzal-T/S on the western flower thrips *Frankliniella occidentalis*. Multiple studies proved that Azadirachtin acts as an insect growth regulator due to inhibition of the release of prothoracicotropic hormones (Rembold 1989 and 2002; Gonzales et al. 1999) which favours most detrimental effects during larval-pupal development. Younger larvae showed a higher sensitivity to AZA compared to older larval and pupal stages. Due to their high relation of surface vs body mass, younger larvae receive highest amounts of active ingredient per body mass if in contact with NeemAzal®-U loaded tissue in mines; in addition its the most intensively feeding stage relative to body mass and consequently small larvae take up more systemically delivered substances as compared to older ones or non feeding pupae. Furthermore, younger larval stages are more affected by minute alterations in hormonal titers upsetting

developmental regulation. The differences in larval susceptibility may be of interest for the timing of applications. The highest efficacy for managing the population development could be expected if Neem had already been distributed within the plant before L1 started to feed on the leaf tissue.

Adult emergence

Most larvae surviving on NeemAzal®-U treated plants were killed during the pupal stage. No adults emerged from any of the pupae from plants treated with the high concentrations of 2.25 and 3.0 g/lw NeemAzal®-U. Those results agree with the findings of other authors. The above already mentioned studies of Weintraub and Horowitz (1997) and Larew et al. (1985) reported no adults emerging from any of the pupae of *L. huidobrensis* developing on bean plants or *L. trifolii* on chrysanthemums drenched with Neem extracts. Moreover, Parkman and Pienkowski (1990) found a 65.4% and 77.3% reduction of adult emergence of *L. trifolii* with pot grown chrysanthemum drenched with 250 ml of 1 ppm and 2 ppm Azadirachtin, respectively.

Overall, the literature offers a body of results all agreeing that *L. sativae* is extremely susceptible to Neem during pupal development. The mechanism or mode of action is still ambiguous. In some cases we observed that pupae fully developed but failed to ecdyse to adult stage but most treated cages containing pupae showed no developed adults which indicates an interference with the development from prepupae to pupae. It could be concluded that the hormonal antagonism activity of Azadirachtin plays the major role during this process.

Persistence effects

NeemAzal®-U has been found to be effective for at least 7 days after soil drenching in both laboratory and greenhouse bioassays. Larval mortality ranged from 9.38 % to 100% with an increasing concentration of NeemAzal®-U (0.75 to 3.0 g/lw). Mortality of the larvae declined steadily with the time span between application and infestation but still after 7 days significant mortality could be recorded. Larew et al. (1985) reported that insecticidal activity against *L. trifolii* lasted for up to 3 weeks and Thoeming et al. (2003) found that soil drenching with NeemAzal-T/S® (1% AZA, Trifolio-M GmbH, Lahnau, Germany) resulted in comparable persistent effects on *F. occidentalis*. In another study, Otto (1996) fed *Leptinotarsa decemlineata* larvae with potato leaves cut from plants which had received a soil application of NeemAzal (100 ppm Azadirachtin). Leaves

that were cut 15 d after Neem treatment resulted in the same level of control as leaves that were cut 3 d after the treatment, indicating a constant uptake of Neem by the plant rhizosphere from a reservoir in the soil or a long persistence of the active compound within the plants. In contrast, Ascher et al. (2000) found that residues of 0.1% Neemix-45 applied topically on cotton against *F. occidentalis* were only active for 5 and 3-4 days in the greenhouse and outside, respectively. Thus, the persistence of systemic effects after soil drenching seems to be more pronounced compared to topical application. The short persistence of Azadirachtin after topical treatments is due to the rapid degradation under high temperature and UV light (Johnson et al. 2003; Barrek et al. 2004). In contrast, after a soil drenching AZA is protected in the substrate from the detrimental UV-radiation, which maintains its persistence.

Effects on soil-inhabiting life stages

The literature bears no information so far on the direct influence of Azadirachtin on the soil-inhabiting life stages of *L. sativae*. Our findings reveal that NeemAzal®-U if applied to the soil about 36 h before late L3 or prepupae started to drop to the soil for pupation induced a significant effect as compared to the control treatment but with only low mortality even with the deployment of the highest NeemAzal®-U concentrations. But when NeemAzal®-U was applied immediately before or during migration of the late L3 larvae or prepupae to the soil, a considerable higher mortality could be recorded. Most treated specimens or insects died inside the cocoon. Under the described experimental conditions, most of the prepupae would have already dropped to soil prior to the soil treatment. Therefore, the effects could only be a result of external contact with the active ingredient. A similar result of 89% pupal mortality was obtained by the mentioned studies of Larew et al. (1985) with *L. trifolii*. Hormonal imbalances should be particularly detrimental in the early phase of pupal development where the initial differentiation of adult tissue from imaginal buds takes place. On the other hand, the high sensitivity of young pupae compared to older ones may be simply caused by the facilitated NeemAzal®-U penetration through the incompletely or partly sclerotisized cuticula.

Results from soil-dwelling life stages (prepupae, pupa) of *Frankliniella occidentalis* exposed by Thoeming et al. (2003) to NeemAzal-T/S in the soil corroborate our findings: No significant effects were recorded when Neem was

applied 48 hrs before late L2 left the bean plants for pupation into soil but applications when soil-dwelling life stages were already present in the substrate, resulted mortalities up to more than 70%, due to direct contact effects. The direct effects on pupae could be exploited for practical purposes. If leafminer pupate not only in the pot soil but in dense crops stands on the soil surface (e.g. of greenhouses), contact sprays with Neem could reduce this reservoir of re-infestation of the crop.

Conclusion

The results of our study display the high systemic efficiency of NeemAzal[®]-U against larvae of *L. sativae* in the tropics where the high temperature and high load of UV-radiation prevail. Those results were promising both under controlled laboratory experiments with controlled temperature conditions and low radiation as well as in typical net houses. Although high temperature leads to an early impediment of a sustainable activity, the persistence is rather long compared to spray applications to aerial parts of the crop. This could be related to the protection thus slower degradation of the Neem ingredients in the soil or the plant. The drawback of the rapid degradability of Neem after foliar applications was addressed recently by Pavela et al. (2004). In addition, the contact of the young pupae with fresh Neem solution could additively promote the management efficiency of the leafminer population. In summary soil treatments with Neem could be a key component of IPM strategies in protected cultivation even in the humid tropics. Further studies will focus on possible side effects of Neem soil application on parasitoids of *L. sativae* under laboratory and greenhouse conditions and try to work out a strategy of combining parasitoids with Neem treatments.

3 Effects of topical application of Azadirachtin (commercial product: NeemAzal®-T/S) on different immature life stages of the leafminer *Liriomyza sativae* (Dip.: Agromyzidae) on tomatoes in the humid tropics[2]

3.1 Introduction

The genus *Liriomyza,* first documented in 1894, contains more than 300 species, with 23 species of economical importance. Its larvae can cause severe damage to agricultural and ornamental plants by feeding in mesophyll tissues of leaves and forming serpentine mines (Spencer, 1973). Among the several species of economic importance, the two major species of *Liriomyza* that cause major economic damage of concern particularly in tomatoes are the vegetable leafminers, *Liriomyza sativae* Blanchard and *L. trifolii* (Burgress) (Zoebisch and Schuster, 1987). In tomatoes *L. sativae* can cause losses of up to 70% (Waterhouse and Norris, 1987). In addition, the highly polyphagous *L. sativae* attacks a wide variety of crops (Parrella, 1987; Spencer, 1990).

In previous years leafminers such as *L. sativae* were primarily controlled by the use of synthetic insecticides, like Permethrin and Fenvalerate (Mason et al., 1987). However, frequent applications resulted in the selection of pesticide resistant strains, increased toxicological risks to farmers and consumers by persistent residuals on the produce and adverse effects on non-target organisms, namely beneficial parasitoids and predators. (Raguraman and Singh, 1999). Moreover, the classical "secondary pest" problem locally can increase the leaf miner problem. Long term use of broad spectrum pesticides i.e. Methomyl to control primary vegetable pests such as *Heliothis zea* (Boddie), *Keiferia lycopersicella* (Walsingham) and S*podoptera exigua* (Hubner) resulted in outbreaks of *L. sativae* on fresh market tomatoes by destruction of the agromyzid's effective natural enemies (Oatman and Kennedy, 1976; Johnson et al., 1980).

The described drawbacks of synthetic pesticides increased consumers and grower's interest in natural insecticides originating from plants (Tedeschi et al., 2001) and their usage increased in recent years (Weathersbee and Tang,

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[2]Hossain MB and Porhling H-M. Effects of topical application of Azadirachtin (commercial product: NeemAzal®-T/S) on different immature life stages of the leafminer *Liriomyza sativae* (Dip.: Agromyzidae) on tomatoes in the humid tropics. Submitted to Phytoparasitica.

2002). Advantages of "bio-pesticides" such as Rotenone, Pyrethrins or Neem include their fast degradability in the environment, low human toxicity, lower risk of selection of resistant pest biotypes and selective properties concerning some beneficial organisms. Moreover, they can be used under the specific regulations of organic farming. One of the most interesting biopesticides is Neem products with the active ingredient Azadirachtin (AZA). Numerous studies have shown that topical applications of Azadirachtin can affect many important pests of agricultural and horticultural crops (Schmutterer, 1990; Mordue, 1998) but often application revealed moderate or even low toxicity to non-target organisms (Lowery and Isman, 1995; Naumann and Isman, 1996). Residues on plants, plant products or the soil degrade rapidly, particularly if exposed to UV-radiation (Isman, 1999).

Neem compounds mainly act as feeding inhibitor or as insect growth regulator or both. Antifeedant properties have been reported for several species of Diptera (Warthen, 1979). Furthermore, effects on growth were shown with numerous species of phytophagous insects (Warthen, 1979; Sieber and Rembold, 1983; Schmutterer, 1988; Isman, 1990; Walter 1999). The insect growth regulatory effects of Azadirachtin in contrast to antifeedant effects are remarkably similar among target species (Mordue and Blackwell, 1993). Typical symptoms of growth regulating activities are inhibition of embryonic and postembryonic development, malformation during molting processes, larval and adult mortality and a reduction in reproductive success (Ascher, 1993). Neem products particularly affect early larval instars of different target organisms (Hassan, 1998) due to the sensitive reactions of such stages to hormonal imbalances (Schmutterer, 1988).

Leafminers are found to be sensitive to Neem treatments as shown in studies with *L. trifolii* (Burgress) and *L. sativae* Blanchard (Webb et al., 1983; Fagoonee and Toory, 1984; Parkman and Pienkowski, 1990; Babu et al., 2002; Azam et al., 2003). In addition to larval mortality sub-lethal effects expressed in both lower fecundity and longevity of the adults are reported. However, the use of Neem products against leafminers has not been tested in detail under the most critical climate conditions, in protected cultivation in the humid tropics with its high temperatures and intensive UV radiation which may severely inhibit efficiency. Therefore, the objective of this study was to evaluate the effects of

NeemAzal®-T/S, a commercially available Neem product, on *L. sativae* in tomatoes under greenhouse conditions in the humid tropics compared to climate controlled environments and to assess in detail the most effective rates and timing of NeemAzal®-TS application.

3.3 Materials and Methods

General frame

The experiments were carried out in air conditioned laboratory rooms and greenhouses of 6 m x 6 m each (plastic roof, side walls covered with 40-mesh net) established at the Asian Institute of Technology (AIT) in the peri-urban area of Bangkok, Thailand. All experiments were repeated twice except Exp. 1, which was repeated three times.

Insects and plant sources

The *L. sativae* strain used in the experiments was selected in July 2002 from tomato plants (v. King Kong II) grown in the greenhouse complex at AIT. Thereafter, a stock culture of *L. sativae* was continuously reared from the same tomato variety in cages placed in air conditioned rooms at 29±1 ^{o}C, 60-65% RH and 16:8 [L:D]. Synchronized adults were obtained by placing two day old adults (males and females) on young potted tomato plants for 6 hours. After oviposition, the adults were removed. This short oviposition time ensured a uniformly age of the eggs and subsequent larvae, pupae and thereafter emerging adults.

Experiments in air conditioned environment

The laboratory experiments were established in air conditioned rooms at 29±1 ^{o}C, 60-65% RH and 16:8 h [L:D]. Acrylic cages (65 cm x 61 cm x 61 cm) with top side and two holes in each side wall (25 cm Ø) covered with 78-mesh net for ventilation served as experimental arenas. Thirty-five day old tomato plants containing approximately 5-6 fully expanded true leaves grown in pots (8 cm high and 10 cm Ø) containing 180 g of a clay loam substrate composed of silt, sand and clay (39.2, 29.9 and 30.9%, respectively) and 27.9% organic matter were used in all experiments. The pots were watered twice a day with 50 ml tap water, which satisfies the substrate saturation capacity.

Greenhouse experiments

Greenhouse experiments were carried out in two research greenhouses each with a total area of 36-m^2 (see also above). Forty-five day old tomato plants containing 7-8 fully expanded true leaves grown in pots of 15 cm high x 20 cm Ø and the same substrate as described above were used. The day-night temperature ranged from 26.2-34.9 ^{o}C with relative humidity from 65 - 70%, respectively. Mean daily temperature and RH were maintained throughout the experimental period. Plants were watered three times per day with drip irrigation water mixed with fertilizers at local recommended dosages.

Neem

The commercially available Neem product NeemAzal$^®$-T/S (Trifolio–M GmbH, Germany) containing 1% Azadirachtin was used for all experiments. Application solutions of with dosage rates of 1, 3, 5, 7 and 10 ml/lw were produced by mixing the respective amount of NeemAzal$^®$-T/S in tap water and stirring for 10 minutes at room temperature. As a control, tap water was used in all experiments. All concentrations were prepared immediately prior to use.

Experiments

Exp. 1. Direct toxicity: Sensitivity of different immature developmental stages of L. sativae under laboratory conditions (climate rooms)

To obtain different immature stages of leafminer, plants were infested as described above. Thereafter, infested plants containing leafminer eggs were removed from the cages and arranged into 4 sets for different developmental stages (eggs, L1, L2 and L3) with 30 plants each (6 treatments x 5 replications). Each set of plants was placed in different insect proof cages (ten replications). The group of egg containing plants was treated immediately after egg counting. Therefore, the aerial parts of the tomato plants were sprayed with a hand held sprayer equipped with a fine-mist nozzle (Apollo International Spray, Thailand) with above mentioned NeemAzal$^®$-T/S dilutions until run-off. Pot soil was covered with polyethylene paper during spraying to avoid possible dropping of NeemAzal$^®$-T/S solutions to the pot soil to prevent uptake by roots and systemic translocation. Similarly, 2, 4 and 5 days after infestation the plants carrying L1, L2 and L3 stages were sprayed, respectively. The initial number (before treatment) of each stage per plant was counted before spraying. Different larval instars were determined by stereo-microscope with a micrometer scale

following the procedure by Petitt (1990) who distinguished different larval instars of *L. sativae* by measuring the length of cephalopharyngeal skeleton, L1 (0.058-0.111 mm), L2 (0.123-0.173 mm) and L3 (0.196-0.249 mm). In each treated stage, mortality was recorded 1 day after spraying. After treatment of egg stage mortality of eggs but also of hatched L1 after escaping from the egg shell was monitored. Dead larvae were marked by placing a small black dot on leaflets on every sampling day. In the case of living larvae, leaflets were checked daily until prepupae dropped for pupation or died on the foliage. Surviving prepupae were collected and reared to pupae; afterwards adult emergence was noted.

Exp. 2. Dosage dependent residual efficacy of NeemAzal®-T/S on oviposition and development under laboratory conditions

The aerial parts of tomato plants (both upper and lower surface of leaves) were sprayed as described in Exp. 1. Treatments were performed 7, 5, 3 and 1d, and 6 h (fresh) prior to introducing the leafminers. Plants were arranged in a completely randomized design and at hour 0 one-day-old uniformed aged adult leafminers of both sexes (150/cage) from the stock culture were released for a period of 24 h. Different treatments were randomly arranged in the same cages to allow the leafminers a free choice for oviposition on treated and untreated plants. Ten replications were run but spaced over two time periods so that a total of 30 plants per treatment per date were evaluated. Thereafter, all adult leafminers were removed from the cages using an aspirator. After adult removal from the cages, the eggs were counted using a stereo-microscope with substage lighting. After 48 h, the eggs were checked again to record the number of hatched individuals. From 72 h onwards, the plants were inspected daily under a microscope to determine larval mortality until the larvae dropped for pupation. Pupae were further observed as described above.

Exp. 3. Dosage dependent residual efficacy of NeemAzal®-T/S on oviposition and development under greenhouse conditions

Potted tomato plants were placed in the above described greenhouses and treated 7, 5, 3 and 1 d prior to the introduction of the leafminers with five NeemAzal®-T/S dosages as described above. Water treatment served as control and five replications per treatment were arranged in a completely randomized design. At day 0, approximately 7000 one-day-old adult leafminers of the same age and of both sexes were released. Thus, the females were

given a choice between treated and non-treated plants for oviposition. After forty eight hours plants were transferred to another identical but leafminer free greenhouse. As the middle leaves of tomato plants are relatively more preferred by the leafminer for oviposition (Issa and Marcano, 1993; Zehnder and Trumble 1984), an infested middle leaf of each plant was tagged. Five days later the tagged leaves were excised and checked in the laboratory for dead larvae. Surviving late third instars/prepupae were trapped in plastic bags and kept for pupation. Prepupae were reared to pupae and until adult eclosion as in Exp. 1. The initial number of larvae was calculated from the sum of prepupae, pupae and dead larvae.

Statistical procedures

Data with numbers (count values) and percentages were subjected to HOVTEST = LEVENE option of SAS to account for homogeneity of variance and normality. In case of non-homogeneity, percent values were transformed using arcsine–square-root (arcsine$\sqrt{}$) transformation and insect count values were transformed by square-root ($\sqrt{}$) transformation before running an ANOVA. Whenever, significant interaction was observed between factors, the level of one factor was compared to each level of the other factor by all pair wise multiple comparisons. Where significant F values were obtained ($P < 0.05$), treatments means were separated using Tukey's test. All statistical analysis was performed using the GLM procedure in SAS (2002).

3.4   Results

Exp. 1. Direct toxicity: Sensitivity of different immature developmental stages of L. sativae

Egg mortality measured by the proportion of eggs not developing to first instar larvae was not affected by NeemAzal®-T/S treatments ($F = 0.06$; df = 5, 89; $P = 0.99$) compared to the untreated control but significant differences were detected in larval mortality ($F = 1162.79$; df = 5. 89; $P < 0.0001$) (Table 3.1). Larval mortality increased with increasing NeemAzal®-T/S concentrations (1 - 10 ml/lw) and significant differences were detected between the individual developmental stages, L1 ($F = 1153.08$; df = 5, 89; $P < 0.0001$), L2 ($F = 859.13$; df = 5, 89; $P < 0.0001$) and L3 ($F = 419.30$; df = 5, 89; $P < 0.0001$) at 1, 3, 5, 7 and 10 ml/lw NeemAzal®-T/S concentrations. Mortalities ranged from 30.35% to

100% for L1, 10.13% to 100% for L2 and 5.27% to 84.35% for L3 when evaluating low (1 ml/lw) to high (10 ml/lw) NeemAzal®-T/S concentrations, respectively, (Table 3.1). Furthermore, adult eclosion was significantly reduced if eggs (F = 380.13; df = 5, 89; P < 0.0001), L1 (F = 373.86; df = 5, 89; P < 0.0001), L2 (F = 240.18; df = 5, 89; P < 0.0001) and L3 (F = 298.74; df = 5, 89; P < 0.0001) were treated (Table 3.2).

Table 3.1 Effects of NeemAzal®-T/S on different immature stages of *L. sativae* under laboratory conditions

NeemAzal®-TS (ml/lw)	Mean (±SE) % mortality				
	Eggs	L1 (hatched from treated eggs)	L1	L2	L3
Control = 0	1.68±0.77a	1.76±0.88a	1.75±0.70a	1.37±0.64a	1.58±0.78a
1	1.91±0.78a	33.62±0.99b	30.35±0.78b	10.13±1.27b	5.27±1.11b
3	1.97±0.69a	68.13±0.93c	67.92±0.69c	46.97±0.53c	30.61±0.82c
5	2.04±0.83a	98.54±0.74d	98.14±0.88d	91.87±0.74d	74.88±0.95d
7	2.07±0.98a	100±0d	100±0d	98.51±0.69e	82.88±1.49d
10	2.22±0.89a	100±0d	100±0d	100±0e	84.35±0.78d

Mean (±SE) numbers within a column followed by same letter are not significantly different (P > 0.05, ANOVA, Tukey's HSD test).

Table 3.2 Effects of NeemAzal®-T/S on adult eclosion from different immature stages of *L. sativae* under laboratory conditions

NeemAzal®-TS (ml/lw)	Mean (± SE) % adult eclosion			
	Eggs	L1	L2	L3
Control = 0	78.25±1.09a	74.90±0.84a	74.54±0.90a	75.11±0.84a
1	5.57±1.73b	8.83±2.04b	13.66±1.87b	29.77±0.91b
3	0.74±0.74c	0±0c	5.38±1.81c	13.72±0.76c
5	0±0c	0±0c	0±0d	5.90±2.28d
7	0±0c	0±0c	0±0d	0±0e
10	0±0c	0±0c	0±0d	0±0e

Mean (±SE) numbers in a column followed by the same letter are not significantly different (P > 0.05, ANOVA, Tukey's HSD test).

Exp. 2. Dosage dependent residual efficacy of NeemAzal®-T/S on oviposition and development under laboratory conditions

Data for oviposition and egg hatch showed no significant interactions (days*treatments, $F = 0.30$; df = 20, 299; $P = 1.00$ and $F = 0.38$; df = 20, 299; $P = 0.99$). Hence, data were pooled. Density of egg laying seemed to be slightly lower on NeemAzal®-T/S treated compared to untreated plants but all reductions by the NeemAzal®-T/S treatments were not significant ($F = 0.71$; df = 5, 299; $P > 0.62$) (Table 3.3). Furthermore, nearly all eggs hatched irrespectively of treatment. Regarding larval mortality interactions between days and treatments were significant ($F = 40.37$; df = 20, 299; $P < 0.0001$). All five concentrations of NeemAzal®-T/S resulted in higher larval mortality than the untreated control and significant differences on larval mortality were recorded among different NeemAzal®-T/S concentrations ($F = 4261.73$; df = 5, 299; $P < 0.0001$) (Table 3.4). Mortality ranged from 8.79% (NeemAzal®-T/S applied 7 days before release, 1 ml/lw) to 100% (NeemAzal®-T/S applied 1 day before release, 5, 7 and 10 ml/lw). Mortality declined steadily with increasing ages of residues. Adult eclosion was significantly affected by all tested NeemAzal®-T/S concentrations in all residual age groups ($F = 1444.47$; df = 5, 299; $P < 0.0001$). No adults eclosed from any of the pupae which developed on plants treated with 10 ml/lw NeemAzal®-T/S and only very few adults finally hatched on plants treated with 3 ml/lw (5 and 7 days residual age groups), 5 ml/lw (7 day residual age group) and 7 ml/lw (7 day residual age group) (Table 3.5).

Table 3.3 Effect of NeemAzal®-T/S on oviposition of *L. sativae* under laboratory conditions

NeemAzal®-TS (ml/lw)	Eggs/leaf
Control = 0	26.12±1.17a
1	24.74±1.07a
3	24.76±0.97a
5	24.12±0.88a
7	23.56±0.93a
10	23.82±0.88a

Mean (±SE) numbers of eggs per leaf in column followed by the same letter are not significantly different ($P > 0.05$, ANOVA, Tukey's HSD test).

Table 3.4 Effects of NeemAzal®-T/S on larval mortality with differently timed topical spray (residual effect) under laboratory conditions

NeemAzal®-TS (ml/lw)	Mean (±SE) % mortality (Age of residues, days)				
	Fresh	1	3	5	7
Control = 0	1.93±0.89aA	1.37±0.59aA	1.70±0.87aA	2.07±0.89aA	2.14±1.11aA
1	33.50±0.54bA	32.81±0.60bA	24.06±0.66bB	15.00±0.71bC	8.79±0.41bD
3	71.10±0.79cA	70.98±0.74cA	58.75±0.85cB	45.31±0.76cC	39.66±0.61cD
5	100±0dA	99.35±0.65dA	89.20±0.58dB	72.76±0.95dC	61.57±0.78dD
7	100±0dA	100±0dA	92.58±0.46dB	80.65±0.71eC	70.79±0.38eD
10	100±0dA	100±0dA	98.83±0.48eA	87.21±0.95fB	74.37±0.80eC

Mean (±SE) numbers followed by same letter are not significantly different: upper case letters within a row, lower case letters within a column ($P > 0.05$, ANOVA, Tukey's HSD test).

Table 3.5 Effects of NeemAzal®-T/S on adult eclosion with differently timed topical spray (residual effect) under laboratory conditions

NeemAzal®-TS (ml/lw)	Mean (±SE) % adult eclosion (Age of residues, days)				
	Fresh	1	3	5	7
Control = 0	74.39±1.07aA	75.36±1.05aA	75.65±0.75aA	74.54±1.16aA	76.52±0.44aA
1	9.43±1.51bA	9.64±0.77bA	14.62±0.91bB	24.09±0.75bC	30.29±0.36bC
3	0±0cA	0±0cA	0±0cA	7.45±2.56bcB	12.24±2.20cC
5	0±0cA	0±0cA	0±0cA	0±0dA	6.19±2.24dB
7	0±0cA	0±0cA	0±0cA	0±0dA	4.86±2.73dA
10	0±0cA	0±0cA	0±0cA	0±0dA	0±0eA

Mean (±SE) numbers followed by same letter are not significantly different: upper case letters within a row, lower case letters within a column ($P > 0.05$, ANOVA, Tukey's HSD test).

Exp. 3. Dosage dependent efficacy of NeemAzal®-T/S on L. sativae under greenhouses conditions

Larval densities did not differ between NeemAzal®-T/S treated and untreated control plants (F = 1.12; df = 5, 239; P > 0.35) indicating similar oviposition intensity (Table 3.6). However, all concentrations of NeemAzal®-T/S resulted in significantly higher larval mortality compared to control treatment (F = 1002.19; df = 5, 239; P < 0.0001) and significant differences of larval mortality were found among different NeemAzal®-T/S concentrations within all time treatments (Table 3.7). The highest larval mortality was recorded in 10 ml/lw NeemAzal®-T/S treated plants. Efficiency of NeemAzal®-T/S was significantly reduced with an increasing time span between treatment and infestation (F = 794.32; df = 3, 239; P < 0.0001). A pre-spraying time span of seven days resulted in low mortalities between 2.13% to 28.09% in 1 and 10 ml/lw NeemAzal®-T/S treatments, respectively, suggesting a comparatively fast degradation of NeemAzal®-T/S under the greenhouse conditions. Significant differences were apparent in adult eclosion with respect to time (F = 966.39; df = 3, 239; P < 0.0001). All NeemAzal®-T/S concentrations significantly affected adult emergence (Table 3.8) while success of adult development increased in relation to the age of residues.

Table 3.6 Effect of NeemAzal®-T/S on larval density of *L. sativae* per leaf under greenhouse conditions

NeemAzal®-TS (ml/lw)	Mean (±SE) larval density per leaf
Control = 0	31.22±1.91a
1	27.63±1.96a
3	26.93±2.01a
5	26.65±1.36a
7	26.38±1.94a
10	25.83±1.47a

Mean (±SE) numbers followed by same letter are not significantly different (P > 0.05, ANOVA, Tukey's HSD test).

Table 3.7 Effect of NeemAzal®-T/S on larval mortality with differently timed topical spray (residual effect) under greenhouse conditions

NeemAzal®-TS (ml/lw)	Mean (±SE) % mortality (Age of residues, days)			
	1	3	5	7
Control = 0	2.33±1.04aA	1.95±1.00aA	1.30±0.72aA	1.46±0.77aA
1	25.89±0.42bA	11.84±1.19bB	1.85±0.80aC	2.13±0.98aC
3	56.99±1.01cA	37.11±0.90cB	13.95±1.63bC	2.96±0.89aD
5	72.28±0.68dA	56.17±0.89dB	33.05±2.69cC	12.98±1.07bD
7	100±0eA	82.15±1.08eB	48.33±3.18dC	21.94±1.42cD
10	100±0eA	86.69±1.03eB	62.87±0.70eC	28.09±0.68cD

Mean (±SE) numbers followed by same letter are not significantly different: upper case letters within a row, lower case letters within a column (P > 0.05, ANOVA, Tukey's HSD test).

Table 3.8 Effect of NeemAzal®-T/S on adult eclosion with differently timed topical spray (residual effect) under greenhouse conditions

NeemAzal®-TS (ml/lw)	Mean (±SE) % adult eclosion (Age of residues, days)			
	1	3	5	7
Control = 0	76.41±1.10aA	75.92±1.06aA	76.37±0.97aA	76.56±0.76aA
1	14.46±0.53bA	41.86±2.39bB	74.08±0.94aC	74.02±1.07aC
3	9.50±1.84bA	33.34±2.44cB	62.89±1.38bC	74.04±0.88aD
5	0±0cA	14.12±1.27dB	43.61±1.27cC	64.46±0.81bD
7	0±0cA	8.98±3.07dB	19.56±1.57dC	32.42±1.00cD
10	0±0cA	0±0eA	10.33±2.95eB	24.69±1.07dC

Mean (±SE) numbers followed by same letter are not significantly different: upper case letters within a row, lower case letters within a column (P > 0.05, ANOVA, Tukey's HSD test).

3.4   Discussion

The results obtained from the different treatments either under laboratory or greenhouse conditions revealed a high susceptibility of *L. sativae* to NeemAzal®-T/S if sprayed on the aerial plant parts.

Oviposition

Oviposition repellency has been reported for Neem products against many pests, i.e. melon fly *Bactrocera cucurbitae* and oriental fruit fly *Bactrocera dorsalis* (Singh and Singh, 1998). However, NeemAzal®-T/S spraying did not influence oviposition by *L. sativae* suggesting that neither preference nor avoidance of treated plants was obvious which is in agreement with other studies. In a choice experiment, Webb et al., (1983) found no effects on oviposition of *L. sativae* when lima bean plants were sprayed with Neem seed kernel extracts. Weintraub and Horowitz (1997) reported that spraying of 15 ppm Neemix-45 (4.5% Azadirachtin) on bean plants *Phaseolus vulgaris* had no effect on oviposition of *L. huidobrensis* and from a greenhouse experiment Larew (1986) described that foliar spray with 0.5% Neem seed kernel extract in 0.25% Tween-20 did not inhibit oviposition of *L. trifolii* in bean plants.

Egg hatch

Both direct and residual toxicities in laboratory showed no deleterious effects of NeemAzal®-T/S on egg hatch. No supportive studies with leafminers were found to corroborate our results, but similar observations are reported with other species. Azadirachtin treatments (50, 100 mg AZA/l) did not affect the egg hatch of *Chrysoperla carnea* (Stephens) (Medina et al., 2004) or the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Hom.: Aleyrodidae) (von Elling et al., 2002). In a similar study Coudriet et al. (1985) found that about 99% of sweet potato white fly *Bemisia tabaci* Gennadius (Hom.: Aleyrodidae) eggs hatched after spraying with Neem seed extracts. An explanation for this phenomenon could be that the active ingredient (AZA) of Neem or other Neem products could not penetrate into eggs due to impermeable egg membrane or chorion of leafminer eggs.

Larval mortality

Both laboratory and greenhouse results indicated that NeemAzal®-T/S is highly toxic to leafminer larvae with all tested dosage rates of 3, 5, 7 and 10 ml/lw. This indicates a fast translaminar penetration into tomato leaves of the active

compound Azadirachtin to *L. sativae* feeding sites within the mines. Mortality is age specific with young larval stages being more susceptible than older ones. Supporting results can be found in other studies. Webb et al. (1983) recorded 100%, 100% and 99% mortalities for L1, L2 and L3, respectively of *L. sativae* larvae in bean plants sprayed with 0.1% Neem (Vikwood Ltd., Sheboygan, Wis. USA). Weintraub and Horowitz (1997) obtained 63.1% and 71% larval mortality of *L. huidobrensis* (Blanchard) when Neemix-45 (4.5% Azadirachtin) was sprayed on bean plants at 1 and 15 ppm Azadirachtin, respectively. Larew (1986) found that both sides of bean leaves painted with 1% aqueous Neem extract killed 98.1% of *L. trifolii* larvae. Moreover, Meisner et al. (1986) reported a 98.5% reduction in pupal numbers when bean plants were sprayed with 1% Neem extract (methanolic extracts) before exposure to *L. trifolii*. All these studies show the intensive translaminar penetration of Azadirachtin and the high susceptibility of insect larvae feeding within mines.

It is generally accepted that the tetranortriterpenoid Azadirachtin is responsible for the majority of biological effects observed in insects exposed to Neem compounds (Isman et al., 1990; Mordue and Blackwell, 1993; Verkerk and Wright, 1993) and that one key function of Azadirachtin is its impact on the insect hormonal system. AZA inhibits the release of prothoracicotropic hormones, allatotropins and allotoinhibins (Rembold, 1989 and 2002; Gonzalez et al., 1999) and this is of special relevance for immature development. Thus, the high susceptibility of larval and pupal stages is an expected consequence. The reason for the especially high vulnerability of the younger larval stages may be its higher feeding intensity when compared to older stages (relation of food uptake to body mass). Furthermore, younger larval stages are more affected by minute alterations in hormonal titers upsetting developmental regulation.

Adult eclosion

Most larvae surviving on NeemAzal®-T/S treated plants were killed during the pupal stage. Adult eclosion was greatly affected by all NeemAzal®-T/S concentrations in both direct and residual toxicities in laboratory. In direct toxicity study, even the pupae developed from treated L3 larvae (1 to 10 ml/lw NeemAzal®-T/S concentrations) were strongly reduced for adult emergence. In residual studies, the adult eclosion decreased from 30.29% to 0% when the concentration of NeemAzal®-T/S increased from 1 to 10 ml/lw. Still for pupa that

developed on plants treated 7 days before infestation and even under greenhouse conditions no adults at all emerged from any of the pupae from the plants treated with higher concentrations (5, 7 and 10 ml/lw) 1 and 3 days before infestation.

Our findings support results of many other researchers: Larew (1986) reported that nearly no adults of *L. trifolii* emerged from pupa derived from plants treated with Neem seed kernel extracts or 1.6% Margosan-0 (containing 3000 ppm Azadirachtin). Weintraub and Horowitz (1997) found that only 0.8% and 1.8% of adults emerged from pupae of *L. huidobrensis* (Blanchard) on bean plants sprayed with 15 ppm Azadirachtin (from Neemix-45 with 4.5% Azadirachtin) immediately after egg laying and at first instar larvae, respectively. In another study, Venzon et al. (2004) reported completely inhibited pupal development of the coffee leafminer (*Leucoptera coffeea*) with solutions derived from NeemAzal[TM] T/S.

Residual performance

The results of our studies imply that bioactivity of NeemAzal[®]-T/S degraded in a different way under greenhouse than under laboratory conditions. The effects of NeemAzal[®]-T/S on immature stages in laboratory declined steadily but slowly, however much faster in the greenhouse. Under the protected UV free and temperature controlled conditions of the laboratory, in relation to increasing dosage rates larval mortality ranged from 33.50 to 100% in leaves treated 1 day before infestation while same dosage rates gave 8.79 to 74.37% mortality in plants treated 7 days before infestation. On the contrary, same treatments (dosage rates) in the greenhouse with high temperature and only slightly reduced UV radiation compared to open field conditions resulted in similar high efficacy (25.9% to 100%) on plants with 1 day old residues. However, here a more rapid decrease of efficacy (2.13 to 28.09%) could be observed in plants with 7 day old residues indicating a progressive destruction of the active ingredient. This relatively fast reduction of active compounds in Neem products by environmental effects is described from other studies. Stokes and Redfern (1982) stated that Azadirachtin (1 µg/1µl acetone) could be reduced by approximately 50% after seven days of exposure to sunlight. Johnson et al. (2003) reported that the half–life time of Azadirachtin-A exposed as thin film on leaf surfaces, was only effective for 2.47 days under sunlight.

Conclusion

In conclusion, the results of our study demonstrate a high efficiency of NeemAzal®-T/S as foliar application against *L. sativae*. From the practical point of view, our results have elucidated a complete collapse of *L. sativae* population for successive generations under both conditions controlled environment and tropical net greenhouse. However, in the netted house under high temperatures and high load of UV-radiation, higher concentrations of Neem and more frequent (weekly) applications may be required over time to ensure a sufficient efficacy. The big advantage of using Neem is not only its broad availability in the tropics but its different mechanism of action. It can help to reduce selection pressure of permanent cycles of conventional insecticides and improve resistance management strategies. Furthermore, its low toxicity reduces hazards to farmers, consumers (residues) and the environment. Thus, Neem products could fit well as one tool in Integrated Pest Management systems. Further studies will focus on possible side effects of Neem on parasitoids of *L. sativae* under laboratory and greenhouse conditions and try to work out a strategy of combining parasitoids with Neem treatments.

4

A comparative study of the residual effects of Azadirachtin, Spinosad and Avermectin on *Liriomyza sativae* (Dip.: Agromyzidae) on tomatoes[3]

4.1   Introduction

L. sativae is a highly polyphagous herbivore and a serious pest that attacks a wide array of vegetable and ornamental crops (Parrella, 1987; Spencer, 1990; Zhao, 2002). In tomatoes, *L. sativae* can cause losses of up to 70% (Waterhouse and Norris, 1987). The leafminer species, particularly *L. sativae*, *Liriomyza trifolii* and *Liriomyza huidobrensis* are primarily controlled with chemical insecticides such as Permethrin, Fenvalerate, Methamidophos, Chlorpyrifos and Cyromazine (Webb, et al. 1979; Tryon and Poe, 1979; Johnson et al., 1980; Mason et al. 1987; Leibee, 1988; Weintraub, 2001) and the development of resistant strains has already been described (e.g. Spencer, 1990). Moreover, Weintraub (2001) reported the adverse effects of pesticides on non-target organisms, namely *Diglyphus isaea* a parasitoid of *L. huidobrensis.*

As alternatives to synthetic products those from natural sources like plants or microorganisms have been discussed. Such "biopesticides" like Azadirachtin from the Neem tree *Azadirachta indica* A. Juss (Tedeschi et al., 2001), Spinosyns (Spinosad) (Jones et al., 2005) or Avermectin (Abamectin) (Weintraub, 2001) from soil microorganisms are expected to degrade completely and quickly on produce and in the environment to leave no toxic residues but still be able to efficiently control the target pest organism without severe side effects to non-target invertebrates, especially "beneficials".

The biological activities of Neem extract (or its most active constituent, Azadirachtin) are known for more than 400 insect pests (Schmutterer and Singh, 1995). As a foliar spray, Neem seed extracts with Azadirachtin as the main active ingredient act as either a feeding inhibitor or as an insect growth regulator or both against a wide variety of insects (Warthen 1979; Schmutterer 1990; Mordue 1998). However, equally important, Neem extracts, if properly

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[3]based on Hossain MB and Poehling H–M. A comparative study of the residual effects of Azadirachtin, Spinosad and Avermectin on *Liriomyza sativae* (Dip.: Agromyzidae) on tomatoes. To be submitted to Pest Management Science.

used, can have low toxicity to non-target organisms such as parasitoids, predators, and pollinators (Lowery and Isman 1995; Naumann and Isman, 1996) and can degrade rapidly in the environment (Isman, 1999).

Spinosad is a newly developed microbial-derived insecticide with active ingredients isolated from the soil bacterium *Saccharopolyspora spinosa* (Actinomycetales) (Boek et al., 1994; Sparks et al., 2001) and the commercial formulations are a mixture of spinosyns A and D to control insects pests (Sparks et al., 2001). Spinosad has a novel and unique mode of action initially causing involuntary muscle contractions and tremors by exciting neurons in the central nervous system. Spinosad exhibits wide margins of safety to the environment (Sparks et al., 2001), beneficial insects and related organisms (Schoonover and Larson, 1995). Jones et al. (2005) found Spinosad to be harmless to *Amblyseius cucumeris*, but of moderate toxicity for *Orius insidiosus*, the biological control agents of western flower thrips *Frankliniella occidentalis*.

Abamectin is a fermented natural product derived from a soil actinomycete, *Streptomyces avermitilis* Burg (Fisher and Mrozik, 1989)*,* consisting of a mixture of 80% Avermectin B_{1a} and 20% Avermectin B_{1b} (Leibee, 1988) and is commercially available for killing insects, mites and nematodes (Putter et al., 1981). Exposure of insects to Avermectin results in increased mortality (Wolfenbarger et al., 1985; Bull, 1986) and reduces feeding (Pienkowski and Mehring, 1983), disrupts development (Wright, 1984; Robertson, 1985), damages ovaries (Glancey et al., 1982) and reduces fecundity (Bariola, 1984; Beach and Todd 1985; Reed et al., 1985; Cochran, 1985). Abamectin rapidly degrades on plant surfaces (Bull et al., 1984). Abamectin is far less toxic to non-target arthropods compared to synthetically produced pesticides from the classes of organophosphates or pyrethroids but has been shown to be harmful to very sensitive organism like parasitoids (Prijono et al., 2004). However, with proper application strategies, it can be used in an environmentally friendly manner for integrated pest management programs (Dybas, 1989).

Leafminers, *Liriomyza* are found to be sensitive to Neem (Webb et al., 1983*;* Weintraub and Horowitz, 1997) and Abamectin (Schuster and Everett, 1983). Although Spinosad currently is labeled only to control of Lepidoptera and certain Thysanoptera, spinosyns generally have some broad spectrum activities including many species from different insect orders, including Coleoptera,

Diptera, Homoptera, Hymenoptera, Isoptera, Orthoptera and Siphonaptera, as well as mites (Salgado et al., 1997).

The aim of our present study was to evaluate and compare the potential of these three biopesticides in controlling *L. sativae* on tomatoes under the humid tropical climatic conditions in which we are developing sustainable vegetable production systems with protected cultivation considered as one tool.

4.2   Materials and Methods

Leafminer culture and plant sources

The experiments were conducted on tomato plants *Lycopersicon esculentum* Mill (v. King Kong II). The *L. sativae* strain used in the experiments was initially collected in July 2002 from tomato plants of the same variety grown outdoors at the greenhouses complex at the Asian Institute of Technology, Thailand. Subsequently, a stock culture of *L. sativae* was continuously reared on the same tomato variety in cages placed in air-conditioned rooms at 29±1 ^{o}C, 60-65% RH and a photoperiod of 16:8 [L:D] h. Synchronized adults were obtained by placing two day old adults (males and females) on young potted tomato plants for 6 hours. After oviposition, the adults were removed. This short oviposition period ensured a uniformly age of eggs and subsequent larvae, pupae and emerging adults. The L1, L2 and L3 instars used in the experiment were distinguished by stereo-microscope with micrometer scale based on the method of Petitt (1990) who distinguished different larval instars of *L. sativae* by measuring the length of the cephalopharyngeal skeleton: L1 (0.058-0.111 mm), L2 (0.123-0.173 mm) and L3 (0.196-0.249 mm).

Locations and conditions

The experiments were conducted in air conditioned laboratory rooms and greenhouses measuring 6 m x 6 m each (plastic roof, side walls covered with 40-mesh net) at the Asian Institute of Technology (AIT), Bangkok, Thailand. All experiments were repeated two times. The first round of experiments were established in air conditioned rooms at 29±1 ^{o}C, 60-65% RH and 16:8 h [L:D]. Acrylic cages (65 cm x 61 cm x 61 cm) with the upper side and two perforated side holes (25 cm Ø) covered by 78-mesh net to allow ventilation were used. Four week old tomato plants grown in pots (7.5 cm high and 6.5 cm Ø) containing a clay loam substrate composed of silt, sand and clay (39.2, 29.9

and 30.9%, respectively) and organic mater (27.9%) were used. The pots were watered manually with tap water. The second round of experiment was run in the greenhouses. In this trial, 35 day old potted (15 cm high x 20 cm Ø) tomato plants were used. The day-night temperature ranged from 21.5-36.9 ^{o}C with relative humidity from 65 - 75%. Mean daily temperature and RH were maintained throughout the experimental period. Plants were watered three times a day with drip irrigation water mixed with fertilizers at the local recommended dosages.

Pesticides (NeemAzal®-T/S, Success® and Abamectin)

Aqueous solution of NeemAzal®-T/S (Trifolio–M GmbH, Germany) containing 1% of Azadirachtin, Success® [Spinosad, 12% wt:vol) SC, Dow Agrosciences, Indianapolis, IN] and Abamectin [1.8% EC Avermectin, wt:vol, Exphoreflex, Industrial, Thailand; Imported by: Inter Crop Co., Ltd., Thailand] were used in all experiments. As Success® and Abamectin are not registered for controlling *L. sativae* in Thailand, the concentrations of Success® and Abamectin were prepared based on the labelled recommended dosage of 20-40 ml/20 l water (4 days interval) for other pests, i.e. *Plutella xylostella*, *Helicoverpa armigera* and *Spodoptera* spp. on Brassicaceous crops in the field conditions. Consequently, we used dilutions of NeemAzal®-T/S (5 ml and 10 ml/lw), Success® (2 ml and 4 ml/lw) and Abamectin (2 ml and 4 ml/lw) dissolved in tap water, and tap water was used as the control. Tomato leaflets were sprayed with tests solutions on both sides until run off using a fine-mist hand-held sprayer (Apollo International Spray, Thailand).

Experiments

Exp. 1. Sensitivity of different immature developmental stages of L. sativae (no choice test)

This experiment was performed with no choice option to investigate the susceptibility of the different immature stages of *L. sativae* to NeemAzal®-T/S, Success® and Abamectin. To acquire different immature life stages of leafminers, plants were placed in leafminer cages for 6 hours to allow oviposition. Thereafter, plants were removed from the cages and arranged into 4 sets for different developmental stages (eggs, L1, L2 and L3). Each set of plants was placed in different insect proof cages (ten replications). The group of egg containing plants was sprayed immediately after counting of the eggs with

the different NeemAzal®-T/S, Success® and Abamectin concentrations, as described above. Similarly, 2, 4 and 5 days after infestation the plants carrying L1, L2 and L3 stages were sprayed, respectively. The initial number of each stage per plant was counted before spraying. In each treated larval stage, mortality was recorded 1 day after spraying and checked daily until prepupae dropped for pupation or died on the foliage.

Exp. 2. Residual and dosage-dependent efficacy on L. sativae oviposition and development under laboratory conditions (choice test)

After spraying aerial parts of tomato plants with the above described pesticide solutions 1, 3, 5, 7, 10 and 14 days before infestations (day 0) plants were arranged in a completely randomized design. At day 0, one-day-old uniformly aged adult leafminers of both sexes (200/cage) from the stock culture were released for a period of 24 h. Different treatments were randomly arranged in the same cages to allow leafminer adults a free choice for oviposition on treated and untreated plants. Ten replications were run, but were split over two time periods. Thereafter, all adult leafminers were removed from the cages using an aspirator. Upon removal of adults from the cages, eggs were counted using a stereo-microscope with substage lighting. After 48 h, eggs were checked again to record the number of hatched ones. From 72 h onwards, the plants were inspected daily under the microscope to determine larval mortality until the larvae dropped for pupation. Larvae that dropped from the foliage were collected in plastic bags. Then pupae were transferred to petri dishes (9 cm Ø) and retained until adult emergence.

Exp. 3. Residual and dosage-dependent efficacy under greenhouse conditions (choice test)

Potted tomato plants were placed in the above described greenhouses, and 1, 3, 5, 7, 10 and 14 d old residues were established prior to leafminers' introduction (see Exp. 2). Five replications per treatment per date were arranged in a completely randomized design. At day 0, approximately 10,000 one-day-old evenly aged adult leafminers of both sexes were released. Thus, the females were given a choice between treated and non-treated plants for oviposition. After forty-eight hours, adults were removed and plants transferred to another identical but leafminer free greenhouse. An infested middle leaf of each plant was tagged. Five days later, the tagged leaves were excised and

checked in the laboratory for dead larvae. Surviving late third instar/prepupae were trapped in plastic bags and kept for pupation. Prepupae were reared to pupa and until adult eclosion, as in Exp. 2. The initial number of larvae was calculated from the sum of prepupae, pupae and dead larvae.

Statistical procedures

Data with numbers (count values) and percentages were subjected to HOVTEST = LEVENE option of SAS to account for homogeneity of variance and normality. In case of non-homogeneity, percent values were transformed using arcsine–square-root (arcsine√) transformation and insect count values were transformed by square-root (√) transformation before running an ANOVA. Whenever significant interaction was observed between factors, the level of one factor was compared to each level of the other factor by all pair-wise multiple comparisons. Where significant F values were obtained ($P < 0.05$), treatments means were separated using Tukey's test. All statistical analysis was performed using the GLM procedure in SAS (2002).

4.3   Results

Exp. 1. Sensitivity of different immature developmental stages of L. sativae (no choice test)

Egg mortality calculated by the proportion of eggs that did not develop to first instar larvae was strongly affected by Abamectin ($F = 2535.01$; df = 6, 69; $P < 0.0001$) compared to NeemAzal®-T/S, Success® and the untreated control. Moreover, all pesticides caused absolute mortality of young larvae during or immediately after hatching from treated eggs ($F = 4760.5$; df = 6, 69; $P < 0.0001$) (Table 4.1). Significant differences in larval mortality were detected between the individual developmental stages, L1 ($F = 4193.61$; df = 6, 69; $P < 0.0001$), L2 ($F = 1992.06$; df = 6, 69; $P < 0.0001$) and L3 ($F = 327.97$; df = 6, 69; $P < 0.0001$). In most cases, mortalities achieved 100% for L1 and L2 with all pesticides treatments and decreased for L3 to 76.65% and 90.14% for NeemAzal®-T/S (5 ml and 10 ml/lw), and 93.68% and 97.73% for Success® (2 ml and 4 ml/lw) treated foliage. In contrast, 100% larval mortalities were recorded when evaluating Abamectin (2 ml/lw to 4 ml/lw).

Table 4.1 Effects of NeemAzal®-T/S, Success® and Abamectin on different immature stages of *L. sativae* under laboratory conditions

Treatments (ml/lw)	Mean (±SE) % mortality				
	Eggs	Larval mortality hatched from treated eggs	L1	L2	L3
Control = 0	0.86±0.65a	0.55±0.37a	0.62±0.42a	1.43±0.76a	1.90±0.89a
NeemAzal®-T/S 5 ml	1.15±0.82a	100±0b	100±0b	100±0b	76.65±0.99b
NeemAzal®-T/S 10 ml	1.55±1.03a	100±0b	100±0b	100±0b	90.14±0.87c
Success® 2 ml	0.53±0.55a	100±0b	100±0b	100±0b	93.68±0.57c
Success® 4 ml	1.39±0.74a	100±0b	100±0b	100±0b	97.73±1.30d
Abamectin 2 ml	85.8 ±1.09b	100±0b	100±0b	100±0b	100±0d
Abamectin 4 ml	92.80±0.95c	100±0b	100±0b	100±0b	100±0d

Mean (±SE) numbers within a column followed by same letter are not significantly different (P > 0.05, ANOVA, Tukey's HSD test).

Exp. 2. Residual and dosage dependent efficacy on oviposition and development under laboratory conditions (choice test)

Significant interactions between (days*treatments) in oviposition (F = 9.26; df = 30, 419; P < 0.0001) (Table 4.2) were determined. Intensity of egg depositions was significantly lower in Abamectin treated foliage. NeemAzal®-T/S and Success® treated plants showed no significant differences on oviposition compared to the untreated control. Thus neither preference nor avoidance effects were obvious. Data for egg hatch showed no significant interactions (days*treatments) (F = 0.64; df = 30, 419; P = 0.93). Hence, data were pooled for presentation. Significant differences of egg hatch were found among treatments (F =1575.90; df = 6, 419; P < 0.0001) (Table 4.3). NeemAzal®-T/S and Success® did not influence egg hatch compared to the untreated plants but both concentrations of Abamectin strongly reduced egg hatch. Regarding larval mortality, interactions between days and treatments were significant (F = 74.58; df = 30, 419; P < 0.0001). All concentrations of NeemAzal®-T/S, Success® and Abamectin resulted in higher larval mortality than the untreated control and significant differences of larval mortality were recorded among different NeemAzal®-TS, Success® and, Abamectin concentrations (F = 5959.42; df = 6, 419; P < 0.0001) (Table 4.4). NeemAzal®-T/S induced larval mortality ranged from 42.64% (NeemAzal®-T/S applied 14 days before release, 5ml/lw) to 100% (NeemAzal®-T/S applied 1 day before release, 5ml and 10 ml/lw) and mortality declined steadily with the increased age of residues. Success® caused a slight decrease in mortality on plants treated 10 and 14 d before exposure to leafminers. In contrast, irrespective of concentrations, Abamectin caused 100% mortalities in all residual ages. Adult eclosion was significantly affected by all tested NeemAzal®-T/S, Success® and Abamectin concentrations in all residual age groups (F = 4301.41; df = 6, 419; P < 0.0001). Only very few adults eclosed from the pupae which developed on plants treated with 5 and 10 ml NeemAzal®-TS /lw from 10 and 14 d old residues. In the case of Success® and Abamectin no adults were finally developed on plants with 1 to 14 days old residues.

Table 4.2 Residual effects of NeemAzal®-T/S, Success® and Abamectin on oviposition under laboratory conditions

Treatments (ml/lw)	Eggs/leaf (1…14 days old residues before leafminer infestation)					
	1	3	5	7	10	14
Control = 0	28.2±1.44aA	29.3±2.62aA	28.3±2.43aA	28.9±2.80aA	27.3±1.71aA	29.4±1.89aA
NeemAzal®-T/S 5 ml	27.1±2.08aA	27.9±1.98aA	28±1.74aA	26.2±2.61aA	28.6±2.62aA	29.1±1.76aA
NeemAzal®-T/S 10 ml	26.3±2.42aA	26.61±1.86aA	27.7±1.65aA	28.6±1.97aA	29.7±2.84aA	27±2.53aA
Success® 2 ml	27.8±1.94aA	28.5±2.49aA	26±2.44aA	24±2.55aA	27.5±3.02aA	26.5±1.8aA
Success® 4 ml	26.9±1.42aA	28.2±2.16aA	27.1±1.96aA	29.2±2.02aA	28.5±2.77aA	27.4±2.37aA
Abamectin 2 ml	5.5±0.34bA	6.4±0.31bA	8.5±0.70bA	15±0.71bB	25.7±0.84aC	28.8±0.66aC
Abamectin 4 ml	5.4±0.30bA	5.8±0.33bA	8.2±0.39bAB	12.7±0.97bB	18±1.15bBC	24.6±1.78aC

Mean (±SE) numbers followed by same letter are not significantly different: upper case letters within a row, lower case letters within a column ($P > 0.05$, ANOVA, Tukey's HSD test).

Table 4.3 Effects of NeemAzal®-T/S, Spinosad and Abamectin on egg hatch under laboratory conditions

Treatments (ml/lw)	% Egg hatch
Control = 0	98.81±0.26a
NeemAzal®-T/S 5 ml	98.76±0.31a
NeemAzal®-T/S 10 ml	98.34±0.35a
Spinosad 2 ml	98.45±0.32a
Spinosad 4 ml	98.65±0.31a
Abamectin 2 ml	27.9±0.65b
Abamectin 4 ml	23.44±0.63c

Mean (±SE) numbers followed by same letter are not significantly different ($P > 0.05$, ANOVA, Tukey's HSD test).

Table 4.4 Residual effects of NeemAzal®-T/S, Success® and Abamectin on larval mortality under laboratory conditions

Treatments (ml/lw)	Mean (±SE) % mortality (1…14 days old residues before leafminer infestation)					
	1	3	5	7	10	14
Control = 0	1.39±0.73aA	1.87±0.85aA	2.07±0.89aA	1.64±0.90aA	1.67±0.90aA	1.77±0.76aA
NeemAzal®-T/S 5 ml	100±0bA	91.91±0.93bB	75.04±0.61bC	62.63±1.37bD	53.21±0.81bE	42.64±0.47bF
NeemAzal®-T/S 10 ml	100±0bA	100±0bcA	95.28±0.35cB	76.37±0.47cC	69.75±0.58cD	62.72±0.78cE
Success® 2 ml	100±0bA	100±0cA	100±0dA	100±0dA	98.27±0.79dA	91.37±1.18dB
Success® 4 ml	100±0bA	100±0cA	100±0dA	100±0dA	100±0dA	98.89±0.74eA
Abamectin 2 ml	100±0bA	100±0cA	100±0dA	100±0dA	100±0dA	100±0eA
Abamectin 4 ml	100±0bA	100±0cA	100±0dA	100±0dA	100±0dA	100±0eA

Mean (±SE) numbers followed by same letter are not significantly different: upper case letters within a row, lower case letters within a column ($P > 0.05$, ANOVA, Tukey's HSD test).

Exp. 3. Residual and dosage-dependent efficacy under greenhouse conditions (choice test)

The interactions for initial larval densities between days and treatment were significantly different (F = 5.14; df = 30, 419; P < 0.0001). Larval densities were significantly reduced only on Abamectin treated plants compared to NeemAzal®-T/S, Success® and untreated control plants (F = 139.76; df = 6, 419; P < 0.0001) (Table 4.5). However, all concentrations of NeemAzal®-T/S, Success® and Abamectin yielded significantly higher larval mortality compared to control treatments, and significant differences of larval mortality were found among different NeemAzal®-T/S, Success® and Abamectin concentrations within all time treatments (Table 4.6). The highest larval mortalities (100%) were recorded in NeemAzal®-T/S (10 ml/lw), Success® (2 ml to 4 ml/lw) and Abamectin (2 to 4 ml/lw) treated plants. Efficiency of NeemAzal®-T/S was significantly reduced with the increasing time span between treatment and infestation (F = 16.84; df = 5, 419; P < 0.0001) followed by Success®, and Abamectin treatments, respectively. This suggests a comparatively fast degradation of NeemAzal®-T/S under the greenhouse conditions. Significant differences were apparent in adult eclosion with respect to time (F = 271.05; df = 5, 419; P < 0.0001). All pesticides used greatly affected adult emergence (Table 4.7). The success of adult development increased in relation to the age of residues of NeemAzal®-T/S. Irrespective of concentrations, only few adults eclosed from pupae of Success® treated plants from 10 and 14 d age of residues and no adults were emerged from any of the pupae of Abamectin treated plants.

Table 4.5 Residual effects of NeemAzal®-T/S, Success® and Abamectin on larval densities under greenhouse conditions

Treatments (ml/lw)	Mean (±SE) larvae/leaf (1…14 days old residues before leafminer infestation)					
	1	3	5	7	10	14
Control = 0	34.3±1.65aA	30.4±1.63aA	30.7±1.59aA	32.1±1.53aA	29.1±2.12aA	31.4±2.27aA
NeemAzal®-T/S 5 ml	30.2±2.30aA	29±2.66aA	31.6±2.45aA	30.5±2.49aA	34.2±2.30aA	32.4±2.40aA
NeemAzal®-T/S 10 ml	26.4±2.96aA	29.7±2.26aA	30±2.42aA	32±2.44aA	28.2±2.12aA	31.2±2.41aA
Success® 2 ml	28.7±1.99aA	29.5±2.40aA	30.5±1.88aA	35±2.45aA	28.1±2.51aA	33.9±2.27aA
Success® 4 ml	29.5±2.77aA	32.8±2.58aA	28.4±2.36aA	31.8±2.13aA	29.4±2.58aA	32±2.66aA
Abamectin 2 ml	7.4±0.54bA	7.7±0.70bA	13.6±0.92bB	16.6±1.67bB	19.3±1.27bBC	24.6±1.48abC
Abamectin 4 ml	5.3±0.54bA	6.4±0.64bAB	9.8±0.74bB	15.4±0.90bC	18.7±1.17bC	20.2±2.00bcC

Mean (±SE) numbers followed by same letter are not significantly different: upper case letters within a row, lower case letters within a column ($P > 0.05$, ANOVA, Tukey's HSD test).

Table 4.6 Residual effects of NeemAzal®-T/S, Success® and Abamectin on larval mortality under greenhouse conditions

Treatments (ml/lw)	Mean (±SE) % mortality (1…14 days old residues before leafminer infestation)					
	1	3	5	7	10	14
Control = 0	1.58±0.69aA	0.73±0.73aA	1.10±0.79aA	1.25±0.84aA	2.25±1.17aA	1.07±0.71aA
NeemAzal®-T/S 5 ml	75.87±1.53bA	58.14±0.82bB	36.35±1.01bC	17.36±0.58bD	7.95±1.40bE	5.68±0.84bE
NeemAzal®-T/S 10 ml	100±0cA	88.72±0.43cB	67.18±0.53cC	32.13±0.52cD	19.30±1.50cE	11.69±0.47cF
Success® 2 ml	100±0cA	100±0dA	100±0dA	98.30±0.87dA	74.33±0.87dB	53.90±0.90dC
Success® 4 ml	100±0cA	100±0dA	100±0dA	100±0dA	83.27±1.12eB	62.63±0.67eC
Abamectin 2 ml	100±0cA	100±0dA	100±0dA	100±0dA	95.27±1.15fB	77.08±0.79fC
Abamectin 4 ml	100±0cA	100±0dA	100±0dA	100±0dA	100±0gA	85.68±0.86gB

Mean (±SE) numbers followed by same letter are not significantly different: upper case letters within a row, lower case letters within a column (P > 0.05, ANOVA, Tukey's HSD test).

Table 4.7 Residual effects of NeemAzal®-T/S, Success® and Abamectin on adult eclosion under greenhouse conditions

Treatments (ml/lw)	Mean (±SE) % adult eclosion (1…14 days old residues before leafminer infestation)					
	1	3	5	7	10	14
Control = 0	74.78±0.83aA	73.61±0.93aA	78.33±0.92aA	76.03±0.55aA	77.02±0.81aA	74.60±1.02aA
NeemAzal®-T/S 5 ml	0±0bA	9.73±2.32bB	35.84±0.83bC	57.64±2.40bD	65.10±0.76bD	71.39±1.46aE
NeemAzal®-T/S 10 ml	0±0bA	0±0cA	5.56±2.52cB	18.70 ±1.29cC	29.19±1.45cD	38.36±0.99bE
Success® 2 ml	0±0bA	0±0cA	0±0dA	0±0dA	6.94±3.54dB	20.71±1.33cC
Success® 4 ml	0±0bA	0±0cA	0±0dA	0±0dA	0±0eA	12.74±1.57dB
Abamectin 2 ml	0±0b	0±0c	0±0d	0±0d	0±0e	0±0e
Abamectin 4 ml	0±0b	0±0c	0±0d	0±0d	0 ±0e	0±0e

Mean (±SE) numbers followed by same letter are not significantly different: upper case letters within a row, lower case letters within a column ($P > 0.05$, ANOVA, Tukey's HSD test).

4.4   Discussion

Strong toxicities of NeemAzal®-T/S, Success® and Abamectin against *L sativae* were found in our laboratory and greenhouse studies.

Oviposition

NeemAzal®-T/S and Success® spraying did not influence oviposition by *L. sativae*. Oviposition repellency had previously been reported for Neem products against many pests, i.e. *Spodoptera litura* (F.) (Joshi and Sitaramaiah, 1979), melon fly *Bactrocera cucurbitae* (Dimetry et al., 1995), oriental fruit fly *Bactrocera dorsalis* (Singh and Singh, 1998), Diamondback moth *Plutella xylostella* (Loke et al., 1992) and *Heliothis armigera* Hubner (Lepidotera: Noctuidae) (Jeyakumar and Gupta, 1999). However, this seems to be typical for leaf miners since in a choice experiment, Webb et al., (1983) found no effects on oviposition of *L. sativae* when lima bean plants were sprayed with neem seed kernel extracts and similar observations were obtained by Weintraub and Horowitz (1997) who reported that spraying of 15 ppm Neemix-45 (4.5% Azadirachtin) on bean plants *Phaseolus vulgaris* had no effect on oviposition of *L. huidobrensis*. So far no reports have been found in the literature for detailed studies of the effects of Spinosad on oviposition of leafminers, but for other insects no ovicidal effects have been described: Premachandar et al. (2005) observed no ovicidal effect of Spinosad against thrips *Ceratothripoides claratris* when applied on tomatoes. In contrast, Abamectin induced high oviposition deterrent effects in all concentrations tested in our experiments. Schuster and Taylor (1987) found fewer eggs of *L. trifolii* on treated tomato plants compared to untreated control plants when applying Abamectin (MK 936, 0.15 emulsifiable concentrate, [4.54 g AI/378.5 liters] at 748 liters/ha) and later they corroborated this findings (Schuster and Taylor, 1988) when they exposed females of *L. trifolii* for 24 h in laboratory experiments to the same product (at dosage rate 1.2 g [AI]/100 liters) treated leaflets.

Egg hatch

In our study, we did not find any detrimental effect of NeemAzal®-T/S and Success® on embryonic development of *L. sativae.* Eggs hatched to L1 which emerged at a rate of 98-100% on treated tomato foliage. No supportive studies with leafminers were found to corroborate our results, but similar observations have been reported with other species. Azadirachtin treatments (50, 100 mg

AI/l) did not affect the egg hatch of *Chrysoperla carnea* (Stephens) (Medina et al., 2004) or the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Hom.: Aleyrodidae) (von Elling et al. 2002). Adan et al. (1999) reported a lack of toxicity of Spinosad on the egg development of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). In contrast, both concentrations of Abamectin strongly inhibited final hatch of developed L1 from the egg. In nearly every instance, the sickle-shaped mandibles of the larvae were already visible through the eggs chorion of treated eggs but L1 died before complete eclosion. Schuster and Everett (1983) found that 93.3% eggs did not hatch when tomato foliage was treated with Abamectin (MK 936, 0.03SL emulsifiable concentrate, 1.2 g [AI/100 liters]) against *L. trifolii.* Mujica et al. (2000) reported 79.6% embryo mortality when Abamectin was applied at 0.15% against *L. huidobrensis*. Our results agree with these studies.

Larval mortality

In our no choice study under laboratory conditions, highly toxic effects of NeemAzal®-T/S, Success® and Abamectin were found against all tested larval stages (e. g. L1, L2 and L3). The particular sensitivity of NeemAzal®-T/S, Success® and Abamectin against *L. sativae* larval stages is in agreement with previous laboratory and greenhouse studies by other researchers. Webb et al. (1983) found 100% mortality of L1, L2 and L3 of *L. sativae* when exposed to dosage rates of 0.1% Neem seed extract on bean plants. We did not find any research studies on Spinosad against *Liriomyza* spp. Supportive studies can be retrieved from other researchers on other pests i.e thrips. Premachandra et al. (2005) recorded a successful reduction of *Ceratothripoides claratris* with 100% larval mortality when treated with Spinosad at dosage rates of 5, 10 and 20 ml/20 lw on tomatoes.

In a greenhouse study, Mujica et al. (2000) reported that mortality of L1, L2 and L3 were 66.0%, 81.3% and 57.2%, respectively when Abamectin applied at 0.15% against *L. huidobrensis* on bean plants and Schuster and Everett (1983) recorded 100% L1 mortality when Abamectin (MK 9836 0.03SL) was applied at dosage rates of 1.2 and 0.6 g-AI/100 l water on tomato foliage. Hurni (1992) reported that in a laboratory test Abamectin at 0.05% caused 100% larval mortality of *L. huidobrensis.*

In the choice test studies, reduction of activities of NeemAzal®-T/S, Success® and Abamectin were assessed both in laboratory and greenhouse with increasing age of residues and we detected different loss of activity between laboratory and greenhouse experiments. The mortality decreased only slowly under laboratory conditions with all pesticides but in the greenhouse a much faster loss of activity was observed particularly with NeemAzal®-T/S. Immature mortalities reached 100% with all pesticides and concentrations tested if plants with 1 day old residues were infested both in the laboratory and the greenhouse with the exception of NeemAzal®-T/S 5 ml/lw in greenhouse test. In the laboratory, the mean percentage mortalities for NeemAzal®-T/S, Success® and Abamectin were 100% at each concentration of all three pesticide if foliage was treated 1 d before exposure to leafminer and declined to 42.64% and 62.72%, and 91.37% and 98.89% for NeemAzal®-TS (5 ml and 10 ml/lw) and Success® (2 ml and 4 ml/lw), respectively. In contrast, 100% mortality from Abamectin treated foliage at 1 d before exposure to leafminer did not influence any reduction at 14 d old residues in laboratory. In greenhouse, percentage mortalities of 75.87% to 5.68% and 100% to 11.69% for NeemAzal®-T/S (5 and 10 ml/lw, respectively), of 100% to 53.90% and 100 to 62.63% for Success® (2 ml and 4 ml/lw, respectively) and of 100% to 77.08% and 100% to 85.68% for Abamectin (2 ml and 4 ml/lw, respectively) in foliage treated 1 to 14 d before exposure to leafminer suggest that NeemAzal®-T/S degrade much faster in greenhouse than in laboratory. These results confirm the stronger and longer persistency of Success® and Abamectin in greenhouse conditions than of NeemAzal®-T/S. Studies from other researchers corroborate these findings:
Ascher et al. (2000) reported that residues of 0.1% Neemix-45 applied topically on cotton against *F. occidentalis* were only active for 5 days in a greenhouse and 3-4 days outside. Saunders and Bret (1997) reported that half-lives for spinosyn A were 1.6 to 16 days depending on the amount of sunlight received. Spinosad applied to field crops generally loses activity after a week (Brunner and Doerr, 1996; Liu et al., 1999). Jones et al. (2005) reported 96% larval mortality of western flower thrips *Frankliniella occidentalis* on cucumber treated with Spinosad (Conserve® SC 60 mg/lw) 28 days before exposure to thrips under greenhouse conditions.

Schuster and Everett (1983) found 100% and 98.1% larval mortality when tomato foliage was exposed to *L. trifolii* after 1 and 3 days of treatments with Abamectin (MK 936, 0.03SL emulsifiable concentrate, 1.2 g [AI/100 liters]). In a field study, Schuster and Taylor (1987) found 7 day residual effects on oviposition, and mortality when Abamectin (MK 936, 0.15 emulsifiable concentrate, 4.54 gm [AI/378.5 liters]) was applied on tomato against *L. trifolii.* Weintraub (2001) reported that a single application of Abamectin, 10-14 day before peak of adult *L. huidobrensis* infestation killed the larvae. Dybas (1989) reported that residual activity depends on pests feeding on foliage that has absorbed the toxicant.

As the leafminer larval stages continue their development inside the leaf, the experiments clearly show the ability of the ingredients to penetrate the leaf translaminar. The observed reduced mortalities in our greenhouse study compared to the laboratory could be attributed to UV- and thermodegradation of the active ingredients of the NeemAzal®-T/S, Success® and Abamectin. Stokes and Redfern (1982) stated that Azadirachtin (1 µg/1µl acetone) could be reduced by approximately 50% after seven days of exposure to sunlight. Spinosad residues are subject to degradation in sunlight with half-lives of 3 to 7 d in the field condition on soybean (Boyd and Boethel, 1998). In a study Reis et al. (2004) reported a reduction efficacy of Abamectin from 100% to 19% on coffee plants after 5 days of application. Wislocki et al. (1989) reported that in the sunlight the half-life of Abamectin as a thin film on vegetation was 4 to 6 hrs.

Adult eclosion

All three pesticides significantly reduced the *L. sativae* adult eclosion both in the laboratory and the greenhouse. In most cases, larvae surviving from NeemAzal®-T/S, Success® and Abamectin were found to be aberrant puparial forms and died during pupal development. In the case of NeemAzal®-TS, adult eclosion increased over the period of residual age both in laboratory and greenhouse. In laboratory experiment, no adults were eclosed from any of the pupae from 5 ml and 10 ml/lw NeemAzal®-T/S treated foliage of 1 to 7 d old residues and only a few adults (6.57% and 10.89% for 5 ml/lw and 3.24% and 5.44% for 10 ml/lw) were eclosed from 10 and 14 d old residues, respectively. However, no adults developed from tomato foliage sprayed with Success® and Abamectin in any residual age in laboratory. Both concentrations of Success®

significantly reduced pupal development and exclusively few adults emerged from the surviving pupae from 10 and 14 d old residues in greenhouse study. The larvae that survived from Abamectin treated plants of 10 and 14 old residues in the greenhouse experiment were completely killed during pupal development. The results of many other researchers corroborate our findings. In a greenhouse experiment, Larew (1986) reported that nearly no adults of *L. trifolii* emerged from pupa derived from plants treated with Neem seed kernel extracts or 1.6% Margosan-0 (containing 3000 ppm Azadirachtin). Weintraub and Horowitz (1997) found only 0.8% and 1.8% of adults emerged from pupae of *L. huidobrensis* (Blanchard) on bean plants when these plants were sprayed with 15 ppm Azadirachtin (from Neemix-45 with 4.5% Azadirachtin) immediately after egg laying. As in the case of NeemAzal®-T/S, we found no study to support our findings for Success®. Leibee (1988) reported that cowpeas in the primary leaf stage treated with 0.65 ppm Abamectin against *L. trifolii* influenced adult emergence only 0.5%. Schuster and Everett (1983) recorded very few adult eclosion when Abamectin (MK 936 0.03 SL) was applied at dosage rate 0.2 g -AI/100 l water.

Conclusion

In conclusion, our results show that all three pesticides tested can have significant impact on *L. sativae* preventing it multiplying in successive generations. The high efficacy of Spinosad and Abamectin against larvae coupled with longer persistence can help even to completely eliminate *L. sativae* populations. The effects of the tested biopesticides are strong under both controlled environment and tropical net greenhouse. However, in the net house under high temperatures and high load of UV-radiation higher concentrations of NeemAzal®-T/S and more frequent applications may be required over time to ensure a sufficient efficacy. Spinosad and Abamectin have been considered outstanding chemicals for controlling leafminer. For a more complete evaluation whether these products are convenient for IPM with a strong biocontrol background, further studies should consider the impact of all the three biopesticides on parasitoids of *L. sativae*.

5 **Side effects of Azadirachtin, Spinosad and Avermectin on *Opius* (*Opiothorax*) *chromatomyiae* (Hymenoptera: Braconidae) and *Neochrysocharis formosa* (Hymenoptera: Eulophidae), two endo-larval parasitoids of *Liriomyza sativae* (Diptera: Agromyzidae) under laboratory conditions[4]**

5.1   Introduction

Leafminers in the family Agromyzidae are among the world's most economically important pests of vegetables and floricultural crops. Various species of leafminers cause extensive economic damage to a broad range of host plants under both field and greenhouse conditions (Belokobylskij et al. (2004). Among the economic important *Liriomyza* spp., *Liriomyza sativae* cause substantial damage to tomatoes for instance infestation strongly reduces the photosynthetic activities resulting in high yield losses (Parrella et al., 1985; Waterhouse and Norris, 1987).

Several species of parasitoids attack *Liriomyza* spp. (Diptera: Agromyzidae) and under pesticide free conditions parasitoids can regulate leafminers populations (Murphy and LaSalle, 1999). Chen et al. (2003) reported that *Opius caricivorae*, a parasitoid of *L. sativae* caused 84.4% parasitization of the host larvae. In Europe, Westerman and Minkenberg (1986) reported that inundative release of *Diglyphus isaea* and *Chrysocharis parksi* in the greenhouses against *Liriomyza bryoniae* caused 99% and 97% mortality due to parasitism, respectively. In Thailand, six species of parasitoids belonging to the families of Braconidae and Eulophidae were described from *Liriomyza* larvae, i.e. *Asecodes* sp. nr. *notandus* (Silvestri) (Hymenoptera: Eulophidae), *Hemiptarsenus variconis* (Girault) (Hymenoptera: Eulophidae); *Cirrospilus ambiguous* Hansson & LaSalle (Hymenoptera: Eulophidae); *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae); *Quadrastichus* sp. nr *Liriomyzae* (Hymenoptera: Eulophidae); and *Opius dissitus* (Muesebeck (Hymenoptera: Braconidae) (Petcharat et al., 2002). In our studies for

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developing IPM strategies under protected vegetable production we found two endo-parasitoids *Opius* (*Opiothorax*) *chromatomyiae* Belokobylskij & Wharton and *Neochrysocharis formosa* Westwood (larval-pupal and larval parasitoids, respectively) in the study area (AIT campus, Bangkok), where *O. chromatomyiae* had not been reported in the past. Both species occurred regularly and attacked *L. sativae* larvae with high rates of parasitization and were selected as promising candidates for parasitoid based biocontrol of leafminers under protected cultivation conditions.

Under conventional production conditions leafminers such as *L. sativae* were primarily controlled by the use of synthetic insecticides like Permethrin and Fenvalerate (Mason et al., 1987). However, frequent applications bear a high risk for selection of pesticide resistant strains and adverse effects on non-target organisms, namely parasitoids and predators (Raguraman and Singh, 1999). More IPM compatible pesticides originating from natural sources like plants or microorganisms have been discussed as alternatives and have begun to replace synthetic products. The so called biopesticides such as Azadirachtin from the Neem tree *Azadirachta indica* A. Juss (Tedeschi et al., 2001), Spinosyns (Spinosad) or Abamectin (Avermectins) from soil microorganisms (Jones et al., 2005; Weintraub, 2001) are described as efficiently controlling different important pests but with shorter persistency in the environment, lower human toxicity (residues) and lower impact on non-target organisms particularly beneficials than most conventionally used synthetic insecticides.

Numerous studies have shown that Azadirachtin, the active constituent of Neem can affect many important pests of agricultural and horticultural crops (Schmutterer 1990; Mordue 1998) and often application revealed moderate or even low toxicity to non-target organisms (Lowery and Isman 1995; Naumann and Isman, 1996). Residues on plants, plant products or even in the soil degrade rapidly, particularly when exposed to UV-radiation (Isman, 1999).

Spinosad is a newly developed microbial-derived insecticide with active ingredients isolated from the soil bacterium *Saccharopolyspora spinosa* (Actinomycetales) (Boek et al., 1994; Sparks et al., 2001) and can control many target pests (Kristensen and Jespersen, 2004). Spinosad exhibits wide margins of safety to beneficial insects and related organisms (Schoonover and Larson, 1995). Jones et al. (2005) found harmless effects from Spinosad on *Amblyseius*

cucumeris, moderate toxicity to *Orius insidiosus* and high toxicity *to Encarsia formosa* the parasitoids of thrips *Frankliniella occidentalis*.

Abamectin is a fermented natural product derived from soil Actinomycete, *Streptomyces avermitilis* Burg (Fisher and Mrozik, 1989). In recent years, Abamectin has been considered an outstanding chemical against leafminer flies on tomatoes (Schuster and Everett 1983). Abamectin rapidly degrades on plants surfaces (Bull et al., 1984). Although Abamectin has been shown to be harmful to many beneficial parasitoids e. g. *Gronotoma micromorpha* it is still far less toxic than the very often used Chlorpyriphos (Prijono et al., 2004) and can be also characterized as a biorational pesticide (Dybas, 1989).

So far, little information is available regarding the effect of insecticides on leafminers parasitoids. The objective of the present study was to investigate the sensitivity of two endoparasitoids of *L. sativae*, *O. chromatomyiae* and *N. formosa* to Neem based pesticides (NeemAzal®-U and NeemAzal®-T/S) and the two biorational pesticides, Success® (Spinosad) and Abamectin (Avermectin). Both parasitoid species were selected for the reasons mentioned above. However, since the tomato crop we studied was under protected cultivation and apart from leafminers particularly thrips frequently occur as severe pests (Premachandra et al., 2005), temporal use of the above mentioned biopesticides is necessary and detailed knowledge about the compatibility may help to develop efficient IPM strategies. However, under the typical growing conditions of vegetables and particularly tomatoes in Thailand pesticides are the major means of pests control.

5.2   Materials and Methods

Plant sources

The experiments were conducted on 4 week old tomato plants *Lycopersicon esculentum* Mill (v. King Kong II) grown in pots (7.5 cm high and 6.5 cm Ø) containing a clay loam substrate composed of silt, sand and clay (39.2, 29.9 and 30.9%, respectively) and organic mater 27.9%. Pots were watered manually with tap water.

Leafminer and Parasitoid cultures

The *L. sativae* strain and its two parasitoids, *O. chromatomyiae* and *N. formosa* were initially collected in July 2002, November 2002 and December 2004,

respectively from tomato plants grown outdoors at the greenhouses complex at the Asian Institute of Technology, Bangkok, Thailand. The two parasitoid species were identified by Dr. John LaSalle, CSIRO Entomology, Australia. Subsequently, stock cultures of *L. sativae, O. chromatomyiae* and *N. formosa* were continuously reared on the same tomato variety in cages placed in air conditioned rooms at 29±1 ^{o}C, 60-65% RH and a photoperiod of 16:8 [L:D] h. Synchronized adult leafminers were obtained by placing two day old adults (males and females) on young potted tomato plants for 6 hours. After oviposition, the plants were transferred to other insect free cages. This short oviposition period ensured a uniformly age of eggs and subsequently larvae, pupae and emerging adults. The L2 and L3 instars of *L. sativae* used in the experiments were distinguished by stereo-microscope with micrometer scale based on the method of Petitt (1990) who distinguished different larval instars of *L. sativae* by measuring the length of the cephalopharyngeal skeleton: L1 (0.058-0.111 mm), L2 (0.123-0.173 mm) and L3 (0.196-0.249 mm). *O. chromatomyiae* and *N. formosa* were reared on *L. sativae* L3 and L2 larvae, respectively, and uniformly aged parasitoids were maintained with similar methods of *L. sativae* rearing.

Locations and conditions

The experiments were conducted in air-conditioned laboratory rooms at the Asian Institute of Technology (AIT), Bangkok, Thailand. All experiments were replicated 15 times but split within 3 time periods. The experiments were carried out in acrylic cages (45 cm x 40 cm x 40 cm) with upper side and two perforated side holes (25 cm Ø) covered by 78-mesh net to allow sufficient ventilation.

Pesticides (NeemAzal$^{®}$-U, NeemAzal$^{®}$-TS, Success$^{®}$ and Abamectin)

NeemAzal$^{®}$-U, NeemAzal$^{®}$-T/S, Success$^{®}$ and Abamectin were tested in different dilutions of the stock product in tap water (Table 5.1). NeemAzal$^{®}$-U and NeemAzal$^{®}$-T/S solutions were prepared based on the labelled dosage rates and previous studies (M. B. Hossain, unpublished data). NeemAzal$^{®}$-U is specifically developed for soil drenching whereas NeemAzal$^{®}$-TS is registered for spray applications. As Success$^{®}$ and Abamectin are not registered for controlling *L. sativae* in Thailand, the concentrations of Success$^{®}$ and Abamectin were prepared according to the recommended field dosage rates for other pests, i.e. *Plutella xylostella*, *Helicoverpa armigera* and *Spodoptera* spp.

on Brassicaceous crops in the field conditions. Tap water was used as control. Tomato leaflets were sprayed with tests solutions from both sides until run off using a fine-mist hand-held sprayer (Apollo International Spray, Thailand).

Table 5.1 Pesticides tested against *Opius* (*Opiothorax*) *chromatomyiae* and *Neochrysocharis formosa*

Pesticides	Active ingredients	Concentrations used	Manufacturers
NeemAzal®-U	17% Azadirachtin	0.75, 1.5, 2.25 and 3.0 g/l water	Trifolio–M GmbH, Germany
NeemAzal®-T/S	1% Azadirachtin	5 ml and 10 ml/l water	Trifolio–M GmbH, Germany)
Success®	Spinosad 12% wt:vol, SC	2 ml/l water	Dow Agrosciences, Indianapolis, IN
Abamectin	Avermectin 1.8% EC wt:vol	2 ml/l water	Exphoreflex, Industrial, Thailand; Imported by: Inter Crop Co., Ltd.,

Experiments

Exp. 1. Effects of NeemAzal®-U on soil-inhabiting life stages of Opius (Opiothorax) chromatomyiae and longevity

To evaluate the impact of Azadirachtin on *O. chromatomyiae* within the soil-dwelling life stages of *L. sativae*, NeemAzal®-U was used. Tomato plants were first disposed to two-day-old uniformly aged adult leafminers of both sexes (50 pair/cage) for 6 h to allow oviposition. Thereafter, plants were transferred to new cages. After five days when *L. sativae* larvae just had reached the early L3, two-day-old *O. chromatomyiae* (50 pairs/cage) were released for 6 hrs for oviposition in the host larvae. Thereafter, the larvae (larvae that had exposed to parasitoid) per plant were counted using a stereo-microscope with substage lighting. Then the plants were excised at soil level and the lower stems of plants were immersed individually in glass vials (9.5 cm high and 2 cm Ø) filled with tap water. The vials were tightly sealed with parafilm, which prevents prepupae/pupae from falling into the water filled vials. Subsequently, the vials were placed on the top of pot soil and covered with fitted and ventilated plexi glass cylinders (30 cm high and 10 cm Ø) so that all emerging adults (leafminer and parasitoid) could be trapped and counted. Afterwards, when the

larvae/prepupae (larvae that had exposed to parasitoid) started dropping into soil for pupation, the soil was drenched with the different NeemAzal®-U concentrations and tap water. Vials with plants were removed from the plexi glass cylinders after all prepupae left the foliage. Plants were rechecked after removal from the plexi glass cylinders to observe whether there were any pupae or dead larvae. The pots with soil covered by the plexi glass cylinders were then maintained until adult emergence. Adult emergences were monitored for five consecutive days to ensure trapping of all hatching adults. Percentage of adult emergence was calculated based on the initial number of larvae that drop from the foliage. The same whole procedure was run without parasitoids for comparison of emergence of parasitized vs. unparasitized leafminers under the insecticide regime.

For longevity evaluation, a single pair (male and female) of *O. chromatomyiae* from the newly emerged adults of each treatment (total of 15 pairs) was gently isolated using aspirator from the plexi glass cylinder and reared in a plexi glass cylinder to observe longevity. Diluted honey droplets were provided daily in the plexi glasses for parasitoids feeding.

Exp. 2. Effects of NeemAzal®-T/S, Success® and Abamectin on the development of Opius (Opiothorax) chromatomyiae in L. sativae larvae

This study evaluated the side effects of topical application of NeemAzal®-T/S, Success® and Abamectin on *O. chromatomyiae* developing within leafminers larvae/pupae. Rearing of *L. sativae* L3 and parasitization process were identical to Exp. 1. Immediately after parasitization of *L. sativae* L3 larvae, plants were sprayed with NeemAzal®-T/S, Success® and Abamectin with above-mentioned concentrations until run off. Single treated plants were taken as a replication. Thereafter, the larvae/prepupae that escaped from treated foliage and dropped for pupation were collected in polyethylene bags and reared for adult emergence in plastic containers (10.5 cm high and 6.5 Ø) both sides of which were ventilated with net (pore size ≈ 64 μm) for adult emergence. The emerging adult leafminers and parasitoids from the emergence containers were recorded. The same whole procedure was run without parasitoids for comparison of emergence of parasitized vs. unparasitized leafminers under the insecticide regime.

Exp. 3. Effects of NeemAzal®-T/S, Success® and Abamectin on the development of Neochrysocharis formosa in L. sativae larvae

This experiment was performed to investigate the susceptibility of the different immature stages of *N. formosa* to NeemAzal®-T/S, Success® and Abamectin. To acquire different immature life stages of *N. formosa*, plants were initially placed in leafminer cages for 6 hours to allow oviposition. Thereafter, plants were removed from the cages and relocated in other insect free cages for rearing of *L. sativae* L2 larvae as L2 of *L. sativae* was the normal stage for *N. formosa* oviposition. Hence, after 4 d when early *L. sativae* L2 larvae were observed *N. formosa* (100 pair/cage) were introduced to the cages 12 h for oviposition. The plants bearing *L. sativae* L2 with parasitoid eggs were sprayed immediately after counting the parasitized leafminer larvae with the different NeemAzal®-T/S, Success® and Abamectin concentrations as described above. Similarly, larvae of *L. sativae* were sprayed when *N. formosa* had reached the larval (2 days) or pupal (5 days) stage. The initial number (before treatment) of each stage per plant was counted before spraying. Afterwards, plants were covered with fitted plexi glass cylinders and sealed with artificial clay to trap the emerging adult parasitoids and beginning from approximately 12, 10 and 7 d, respectively later emergence of adult parasitoids was noted daily. After finish of hatching period plants were removed from the plexi glass cylinders and rechecked for dead larvae or pupae. A parallel experiment with plants bearing *L. sativae* larvae without parasitization was run to compare the pesticidal effects on parasitized and non-parasitized leafminers.

Furthermore, to evaluate the longevity of *N. formosa*, single pairs (male and female) from the newly emerged adults of each treatment was gently isolated using an aspirator from the plexi glass cylinder and reared in a plastic containers (10.5 cm high and 6.5 cm Ø) with two vertical openings for ventilation covered with nylon tissue (pore size ≈ 64 μm) to observe longevity. The replications were 15 times but split over 3 time periods. Diluted honey droplets were placed daily in the containers through a side lid to feed the parasitoids.

Statistical procedures

The data collected on numbers and percentages were subjected to transformations into square-root values and arcsine values, respectively and

analyzed statistically using analysis of variance (ANOVA) (Linear Model). The treatment means were compared using the Tukey's procedure for honestly significant difference (HSD) (SAS, 2002).

5.3   Results

Exp. 1. Effects of NeemAzal®-U on soil-inhabiting life stages of Opius (*Opiothorax*) *chromatomyiae and longevity*

Leafminer emergence was strongly affected by the NeemAzal®-U treatment in a concentration related manner (see emergence without parasitoids Table 5.2). *L. sativae* larvae that had not been exposed for parasitization were nearly completely killed during pupal development by contact toxicity of NeemAzal®-U. Very few adult leafminers emerged from treated soil compared to untreated soil ($F = 324.17$; df = 4, 74; $P < 0.0001$). The parasitoid alone (control treatment with parasitoid) reduced survival of *L. sativae* by about 65% percent. However, the parasitoid developing inside the host pupa suffered only marginally from the NeemAzal®-U treatments. Significant differences were observed on the emergence of adult *O. chromatomyiae* from NeemAzal®-U treated soil compared to untreated soil ($F = 11.70$; df = 4, 74; $P < 0.0001$). Moreover, differences occurred among the different NeemAzal®-U treatments (Table 5.2). Even with the strongest NeemAzal®-U treatment (3.0 g/lw) from more than 50% of the *L. sativae* pupae parasitoid emerged compared to about 65% in the untreated control. Only few adult leafminers emerged from treated soil ($F = 33.21$; df = 4, 74; $P < 0.0001$).

Parasitoid longevity was not different between adult parasitoids isolated from newly emerged adult parasitoids from different treatments ($F = 1.05$; df = 4, 74; $P > 0.39$ and $F = 0.72$; df = 4, 74; $P = 0.86$) for both male and female (Table 5.2).

Table 5.2 Effects of NeemAzal[®]-U on the adult emergence and longevity of _Opius chromatomyiae_

NeemAzal[®]-U (g/lw)	Mean (±SE) % adult emergence		Longevity (Mean days)	
	Leafminer	Parasitoid	Male	Female
With parasitoids				
Control = 0	18.02±2.04a	64.96±1.63a	20.8±1.34a	23.67±1.41a
0.75	8.28±1.62b	59.05±1.67b	19.93±1.14a	23.46±1.38a
1.5	2.17±0.81c	55.96±1.30bc	19.53±0.79a	22.6±1.12a
2.25	1.25±0.72c	54.85±1.17bc	18.87±0.74a	22.13±1.28a
3.0	0.70±0.40c	52.85±1.07c	17.8±0.93a	20.87±1.31a
Without parasitoids				
Control= 0	76.84±1.11a	-		-
0.75	36.20±1.35b	-		-
1.50	14.96±0.95c	-		-
2.25	9.11±0.78d	-		-
3.0	5.34±1.05e	-		-

Within columns, mean (±SE) percentages of adult emergence and longevity followed by the same letter are not significantly different (P > 0.05, ANOVA, Tukey's HSD test).

Exp. 2. Effects of NeemAzal[®]-T/S, Success[®] and Abamectin on Opius chromatomyiae in L. sativae larvae

In the case of adult leafminer emergence from the _L. sativae_ larvae alone (without parasitization), significant differences occured among treatments. Very few adult leafminers emerged from the pupae on plants treated with NeemAzal[®]-T/S (5ml/lw) and Success[®] (2 ml/lw) (F = 686.63; df = 4, 74; P < 0.0001) (Table 5.3) whereas the plants bearing _L. sativae_ L3 larvae without parasitization treated with NeemAzal[®]-T/S (10 ml/lw) and Abamectin (2 ml/lw) concentrations showed completely kill off of _L. sativae_ L3. In contrast, about 29% leafminer and 61% parasitoid emerged from the control treatment (parasitoid alone) However, significantly lower numbers of _O. chromatomyiae_ developed to adulthood from the parasitized _L. sativae_ larvae compared to the untreated control when the plants bearing parasitized larvae were treated with NeemAzal[®]-T/S, Success[®] and Abamectin (F = 187.22; df = 4, 74; P < 0.0001)

and adult leafminers mortality was as extremely high as in the unparasitized treatments ($F = 174.04$; df = 4, 74; $P < 0.0001$).

Table 5.3 Effects of NeemAzal®-T/S, Success® and Abamectin on the adult emergence of *Opius chromatomyiae*

Treatments (ml/lw)	Mean (±SE) % adult emergence		
	Without parasitoid	With parasitoid	
	Leafminer	Leafminer	Parasitoid
Control = 0	77.42±1.26a	29.11±2.13a	61.01±2.95a
NeemAzal®-T/S 5 ml	6.33±0.92b	2.52±0.87b	8.73±0.77b
NeemAzal®-T/S 10 ml	0±0c	0±0c	4.02±0.53c
Success® 2ml	0.61±0.34c	0±0c	3.15±0.57cd
Abamectin 2 ml	0±0c	0±0c	1.36±0.55d

Within columns, mean (±SE) percentages of adult emergence followed by the same letter are not significantly different (P > 0.05, ANOVA, Tukey's HSD test).

Exp. 3. Effects of NeemAzal®-T/S, Success® and Abamectin on the development of Neochrysocharis formosa in L. sativae larvae

L. sativae larvae alone (without parasitization) were completely killed by NeemAzal®-T/S, Success® and Abamectin concentrations before pupation. ($F = 9338.49$; df = 4, 74; $P < 0.0001$) (Figure 5.1i). However, there were no significant differences on the emergence of adult *N. formosa* from the eggs, larvae (with the exception of NeemAzal®-T/S 10 ml/lw) and pupae stages of *N. formosa* treated with NeemAzal®-T/S compared to untreated plants. In every instance, parasitoid emergences were highly affected by Success® and Abamectin. Significantly lower numbers of parasitoids emerged from the egg ($F = 386.85$; df = 4, 74; $P < 0.0001$), larvae ($F = 536.35$; df = 4, 74; $P < 0.0001$), and pupae ($F = 593.46$; df = 4, 74; $P < 0.0001$) stages of *N. formosa* in the host larvae in the plants treated with Success® and Abamectin (Figures 5.1ii, 5.1iii and 5.1iv). Virtually no differences were found in longevity of newly emerged adult *N. formosa* isolated from different NeemAzal®-T/S and control treatments, but the longevity of newly emerged adults from Success® and Abamectin treated host larvae was strongly reduced in males [(egg, $F = 33.97$; df = 4, 74; $P < 0.0001$) (larvae, $F = 77.16$; df = 4, 74; $P < 0.0001$) and (pupae, $F = 50.27$; df = 4, 74; $P < 0.0001$)] and females [(egg $F = 53.83$; df = 4, 74; $P < 0.0001$)

(larvae F = 105.22; df = 4, 74; P < 0.0001) (pupae F = 129.81; df = 4, 74; P < 0.0001)] (Table 5.4) from each individual stage.
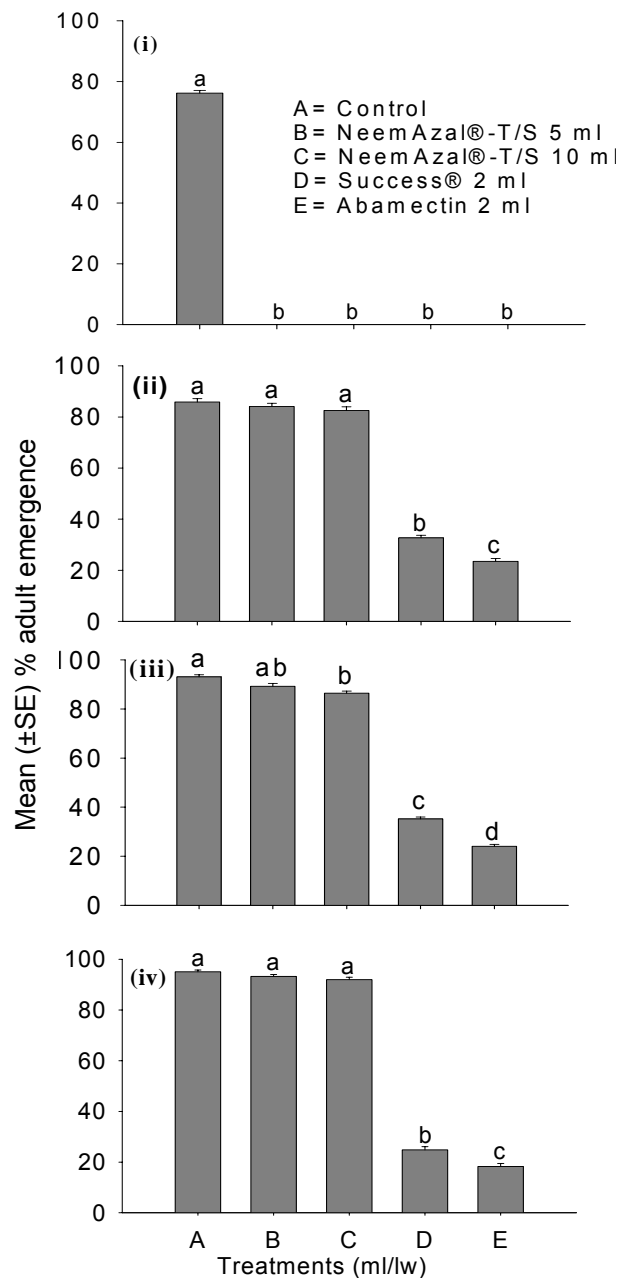

Figure 5.1 Mean (±SE) percentage adult emergence of leafminer and *Neochrysocharis formosa* from different treatments applied on (i) leafminer larvae without parasitoid and immatures stages of *Neochrysocharis formosa*, (ii) eggs, (iii) larvae and (iv) pupae. Columns marked with the same letter are not statistically different (P > 0.05, ANOVA, Tukey's HSD multiple mean comparisons).

Table 5.4. Effects of NeemAzal®-T/S, Success® and Abamectin on the longevity of newly emerged adult of *Neochrysocharis formosa*

Treatments (ml/lw)	Mean (±SE) longevity (days)					
	(Adults from treated eggs, larvae and pupae)					
	Egg		Larvae		Pupae	
	Male	Female	Male	Female	Male	Female
Control = 0	13.73±1.11a	15.8±1.0a	13.8±0.99a	16.33±1.05a	14.4±1.26a	16.13±0.96a
NeemAzal®-T/S 5 ml	13.27±0.94a	15.53±0.93a	12.87±0.95a	15.66±0.96a	13.86±1.37a	16.06±0.99a
NeemAzal®-T/S 10 ml	12.07±1.17a	14.93±0.89a	12.73±1.01a	15.46±1.06a	13.26±1.32a	15.67±0.83a
Success® 2 ml	5.13±0.53b	6.6±0.50b	2.86±0.31b	3.73±0.40b	2.8±0.33b	3.17±0.34b
Abamectin 2 ml	4.07±0.33b	4.4±0.51b	2.66±0.33b	2.93±0.36b	2.53±0.36ab	2.6±0.36b

Within columns, mean (±SE) longevity followed by the same letter is not significantly different (P > 0.05, ANOVA, Tukey's HSD test).

5.4   Discussion

There have been as yet no reports of studies dealing with the impact of Neem, Spinosad and Abamectin on *O. chromatomyiae* and *N. formosa.*

In our study, all NeemAzal®-U concentrations caused low mortality to the life stage of *O. chromatomyiae* which developed in *L. sativae* pupal sheets in the soil. The emergence of *O. chromatomyiae* ranged between 52.85% to 59.05%, and 64.96% when the soil containing parasitized prepupae was drenched directly with high to low (3.0 - 0.75 g/lw NeemAzal®-U) concentrations and tap water for control, respectively. We assume that this strong insensitivity can be only explained by a very low "uptake" of active ingredient through the pupal tissue of *L. sativae* which was protecting the parasitoid from encounter of a toxic amount of NeemAzal®-U. The not feeding host stage in soil cannot actively enrich Neem. Moreover, the parasitoid is protected by its own cuticle. In contrast, only very few leafminer adults survived from the pupae of NeemAzal®-U treated soil that had not been parasitized indicating that the contact toxicity of NeemAzal®-U is just strong enough to kill the soft bodied *L. sativae* prepupae. Moreover, the longevity of *O. chromatomyiae* was unaffected by the NeemAzal®-U treatments indicating no severe sublethal effects. The high sensitivity of leafminer prepupae to direct contacts with Neem is supported by results from Larew et al. (1985). They found 95% pupal mortality when pot soil was drenched with 0.4% Neem against leafminers prepupae that had dropped to the soil for pupation and subsequent adult emergence. On the other hand also indications for low sensitivity of parasitoids eggs treated with Neem are reported. When eggs of *Clavigralla scutellaris* (Hemiptera: Heteroptera: Coreidae) parasitized by the egg parasitoid *Gryon fulviventre* were exposed to 5% Neem suspension, parasitoids developed well and longevity of males and females *of Gryon fulviventre*, was unaffected (Mitchell et al., 2004).

NeemAzal®-T/S, Success® and Abamectin had strong toxic effects on *O. chromatomyiae* when sprayed on leaves bearing parasitized larvae of *L. sativae*. Larvae of *L. sativae* continue with feeding until they drop down for pupation even though they were being parasitized by *O. chromatomyiae*. Thus, the pesticides could reach the parasitoid via diffusion through the soft host cuticle and by ingestion of the host during feeding. Consequently, *O. chromatomyiae* feeding inside the contaminated *L. sativae* larvae upon

hatching was receiving relative high dosages of the pesticides. Even though the L3 of *L. sativae* larvae feeds in leaves only for a relatively short time, consumption is high due to high increase in body mass during that stage. In our investigation, we found high toxicities of all three pesticides including all concentrations against *O. chromatomyiae* and the success of adult emergence of parasitoids were only 8.73% and 4.02%, 3.15%, and 1.36% when plants bearing parasitized larvae had been treated by NeemAzal®-T/S, Success® and Abamectin concentrations, respectively. We did not find any study on the impacts of pesticides on *O. chromatomyiae* to support our findings but support could be retrieved from other more or less comparable studies with other parasitoids species:

In a study, Srivastava et al. (1997) reported that 0.5% w/w emulsions of Neem seed kernels when applied on parasitoid eggs in the host larvae caused 100% mortality of the *Bracon brevicornis* Wesm. (Hym: Braconidae), a larval parasitoid of *Corcyra cephalonica* (Lep.: Pyralidae).

Penagos et al. (2005) reported 100% and 70% reduction of the reproduction of *Chelonus insularies* Cresson (Hymenoptera: Braconidae), the parasitoid of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), when Spinosad at dosage rates of 200 ppm and 20 ppm, respectively, was applied to eggs of the parasitoid. Newman et al. (2004) found that Spinosad (Success®) at the field rate (96 g ai/ha) caused 100% corrected mortality of the leafroller parasitoid *Dolichogenidea tasmanica* (Hym.: Braconidae).

Iqbal and Wright (1996) found significant reduction in adult emergence of *Diadegma semiclausum* Hellen (Hymenopter: Ichneumonidae) (12% and 26% between Abamectin and control, respectively) when Abamectin was applied at a dosage rate of 0.014 (μg ai ml^{-1}/)^2 on second instar of *Plutella xylostella* Linnaeus (Lepidoptera: Yponomeutidae). Weintraub (2001) recorded higher toxicity of Abamectin on *Diglyphus isaea* (Hymenoptera: Braconidae) in Abamectin treated potatoes than for Cyromazine treated potatoes up to 20 days of application.

It is evident that Success® and Abamectin strongly affect the success of adult emergence of *N. formosa*. The effects of Success® and Abamectin on eggs, larvae and pupae of *N. formosa* could be addressed to direct toxic effects as they penetrate the leaflet surface or could have been a result of toxic effects as

emerging adults chewed the treated leaflets to gain openings for their way out. In most cases, the parasitoid was affected during adult emergence and normal development of immature stages was continued to pupae formation. The data from the studies demonstrate that pupae of *N. formosa* were more affected compared to eggs and larvae during adult emergence when treated with Success[®] and Abamectin; the cause of this discrepancy might be that the times from treatment to times of adult emergence were shorter (Schuster, 1994).

Surprisingly we did not find any detrimental effects of NeemAzal[®]-T/S on adult emergence of *N. formosa*. We recorded 84.07% and 82.52%, 89.26% and 86.48%, and 93.21% and 91.99% adult emergence from eggs, larvae and pupae, respectively, when treated with 5 ml and 10 ml NeemAzal[®]-T/S, a similar trend for emerged adults of *N. formosa* was obtained from untreated foliage. The possible explanation could be as the *L. sativae* larvae become paralyzed immediately after being parasitized and stopped feeding on the leaves, thus the toxicity may not be harmful for *N. formosa* adult emergence as eggs, larvae and pupae without ingestion of the contaminated *L. sativae* larvae. The results of emergence success suggest that foliar application of NeemAzal[®]-T/S may not have contact effects on *N. formosa* if *L. sativae* larvae stop feeding immediately after being parasitized. In contrast, we found 100% mortality of *L. sativae* L2 larvae when NeemAzal[®]-T/S was applied on tomato foliage that contained *L. sativae* L2 larvae without parasitization at dosage rates of 5 ml and 10 ml/lw. Moreover, the longevity of newly emerged *N. formosa* was not affected by NeemAzal[®]-T/S, whereas longevity was strongly affected when treated with Success[®] and Abamectin at each individual immature stage.

Mitchell et al. (2004) reported that exposure of parasitized eggs of *Clavigralla scutellaris* (Hemiptera: Heteroptera: Coreidae) by *Gryon fulviventre* (Hymenoptera: Scelionidae) were not affected for adult emergence treated with 5% Neem suspension. In another study, Raguraman and Singh (1999) reported that pretreatment of host eggs (*Corcyra cephalonica* Stainton) with 0.6% and 0.3% Neem seed oil did not affect adult emergence of an internal parasitoid *Trichogramma chilonis* compared to control. In his study, Schuster (1994) found that Abamectin 0.15EC at dosage rate of 0.01 g (ai)/lw caused 73% larval and 38% pupal mortality of *Diglyphus intermedius* from the host larvae *Liriomyza trifolii*.

Conclusion

The above observations provide a clear idea of the impacts of biopesticides on *L. sativae* parasitoids *O. chromatomyiae* and *N. formosa.* Our laboratory results demonstrate that Neem is effective against *L. sativae* larvae but it does not reduce the parasitoid emergence and longevity by pure contact toxicity when the parasitoid develops within a protected host stage which cannot actively increase the Neem concentration inside. Therefore, the results here demonstrate slight or no contact toxicities of Neem formulations on *O. chromatomyiae* and *N. formosa* when applied directly for contact toxicities and/or if parasitized larvae/prepupae stopped feeding after being parasitized. The longevity of newly emerged adults of these parasitoids was unaffected. This may be due to the lack of contact toxicities. However, when NeemAzal[®]-T/S, Success[®] and Abamectin were applied on parasitized *L. sativae* larvae, and thereafter larvae continue their feeding on treated spheres until larval drop down for pupation, high toxicities to adult development of *O. chromatomyiae* was strongly demonstrated. Moreover, Success[®] and Abamectin showed highly detrimental effects on adult emergence and longevity of *N. formosa* with even the larvae stopping feeding on treated spheres after being parasitized. From the findings of this research, recommendations could be deduced for selection of biopesticides, application schemes and dosages if parasitoids should be incorporated as biocontrol agents.

6 Final Discussion

Main details of our studies are discussed in the chapters above, here we will give a final short and comprehensive review and valuation of the achieved results and their broader importance in addition.

Several studies demonstrated that *L. sativae* among the Agromyzid leafminers is a serious pest of vegetables and floricultural plants (Chen et al., 2003) particularly tomatoes and chrysanthemum (Oatman and Kennedy, 1976; Zoebisch and Schuster, 1987). In addition, *L. sativae* has been listed as an A1 quarantine pest by EPPO (OEPP/EPPO, 1984). The control of *L. sativae* using synthetic pesticides is still not satisfactory and not reliable enough. The typical natures of *Liriomyza* spp. (i.e. *L. sativae*) with the hidden egg deposition, larval feeding under the epidermis of the leaves and pupae sheltered in soil makes it difficult to control hit all life stages which impedes the effectiveness of pesticides, thus frequent spraying of pesticides is necessary. However, the multiple sprayings bear a high risk to select pesticides resistance biotypes of the leafminers (Parrella, et al., 1984). Resistance against pesticides is wide spread and especially cross-resistance has already been described in *Liriomyza* spp. (Ferguson, 2004). To overcome these difficulties, uses of biopesticides, biorational pesticides and biological control strategies have been of increased importance in recent years. From a management perspective of view, the findings of our studies could be a benefit for the tomato growers of the Southeast Asia and could help to reduce the use of synthetic pesticides. Hence, apart from economic terms, ecological and toxicological (residues) improvements could be achieved. The results indicate that Neem based biopesticides (NeemAzal®-U and NeemAzal®-T/S) and novel biorational pesticides such as Success® and Abamectin are effective to combat *L. sativae* and can help to optimize control measures in the greenhouse tomato production in the humid tropics. In the presented studies, it was possible to complete diminish populations of *L. sativae*. Moreover, the study on the impacts of these pesticides on parasitoids (chapter 5) may give open further venues to combine soft pesticides with beneficials in an IPM programs. Our findings are discussed in detail in the different chapters; the following may give a final overview and compilation.

The results from chapter 2 demonstrate that Neem (NeemAzal®-U) strongly affects the foliar-inhabiting and soil-inhabiting life stages of *L. sativae* through ingestion and contact effects. NeemAzal®-U caused a significant mortality of *L. sativae* immatures when soil was drenched with different concentrations and the mortality of *L. sativae* immatures reached nearly 100% in most treatments. Hence, a complete elimination of this notorious pest from the crop is possible if Neem products are applied with proper dosages and with an optimized application time schedule. The study also demonstrates the high systemic properties and a relatively long persistence of NeemAzal®-U (17% Azadirachtin) against *L. sativae.* As *L. sativae* prefers to pupate in the soil, NeemAzal®-U and soil would be the method of choice since by the direct contamination also this reservoir of reinfestation could be eliminated. Furthermore, the systemic application of NeemAzal®-U can avoid rapid degradation due to high UV and temperature in the greenhouse environments. Our studies clearly demonstrate that NeemAzal®-U applied to the root system is rapidly translocated via stem and leaf petioles to the epidermal and/or mesophyll tissues, the typical feeding site of the here studied pest which corroborates findings like those of Larew (1985) who reported strong effects of soil drenching with 0.1% Neem seed kernel extract in 0.05% Tween-20 on adult emergence of *L. trifolii* or that of a similar study with the western flower thrips *Frankliniella occidentalis* (Pergande). Here Thoeming et al. (2003) recorded strong systemic effects on the leaf feeding stages as well as contact effects of Neem on the soil-dwelling life stages of the thrips. Since Azadirachtin is in principle very efficient as a systemic insecticide with soil drenching, we suggest further studies to optimal integrate its application in the greenhouse drip irrigation schemes.

In addition, topical application of NeemAzal® T/S (Chapter 3) could induce very high mortality of foliar dwelling life stages of *L. sativae*. We recorded strong larval mortality shortly after application of NeemAzal®-T/S which decreased only slowly with the age of residues in the laboratory. But mortality induced by NeemAzal®-T/S dropped sharply under greenhouse conditions with aging of the residues. This demonstrates that surface residues of NeemAzal®-T/S on tomato plants may be rapidly dissipate if exposed to high temperature and particularly high UV load in greenhouse or even open field conditions (Schmutterer, 1988; Premachandra et al., 2005). Therefore, for an enhanced control of *L. sativae* by

the usage of NeemAzal®-T/S, frequent (weekly) spraying on the aerial parts of tomato plants would be necessary and a more prophylactic application would be desirable to greatly reduce the risk of the initial invasion of *L. sativae* by accumulating efficient residues of NeemAzal®-T/S on tomato plants.

The merely reliance on single pesticide application intensely boosts the risk of resistance development in the pest (Williams and Dennehy, 1996), and resistance development of insects also against so called biopesticides has already been reported as in the case of Spinosad (Ahmad et al., 2002; Young et al., 2002). Thus the comparative study with NeemAzal® T/S, Success® and Abamectin (Chapter 4) was of crucial importance for developing a long term strategy based on biopesticides to control *L. sativae.* All the three pesticides showed strong effects on foliar inhabiting life stages of *L. sativae* and in terms of efficiency can be used as alternatives. The results indicate that NeemAzal®-T/S degrades faster than Success® and Abamectin in contrast, Success® and Abamectin showed longer persistency on tomato plants and its slow and steady degradation in greenhouse conditions suggest that bi-weekly spraying is enough to achieve a high level of control for *L. sativae* in the greenhouses. Similar findings were found by other researchers on *L. sativae* (Webb et al., 1983) and *L. trifolii* (Leibee 1988) with Neem and Abamectin, respectively but for the control of *Liriomyza* spp with Spinosad these data are new. Furthermore, it should be noticed that we used recommended dosages of Success® and Abamectin for other pests. From the strong effects found against *L. sativae* it can be hypothesized that lower dosages could be efficient as well. Reducing the dosage rate could help to improve selectivity concerning non-target organisms and could reduce the residual load of the tomato fruits at harvest. In conclusion the data presented here could help to find an optimal combination of these natural pesticides based on judging (weighting) between efficacy, risk of resistance selection but also regarding possible residues on the produce in such a sensible crop with continuous harvest and marketing.

The findings described in the last chapter (Chapter 5) revealed an apparent scenario of the impacts of Neem, Spinosad and Abamectin on the two important parasitoids, *Opius* (*Opiothorax*) *chromatomyiae* and *Neochrysocharis formosa* of *L. sativae.* NeemAzal®-U when used as soil drench against prepupae is highly efficient against the target but surprisingly safe for the internal life stage

of *Opius* (*Opiothorax*) *chromatomyiae*. We did not find any such study to support our findings. In contrast, the results from foliar applications of NeemAzal®-T/S, Success® and Abamectin showed harmful effects on *Opius* (*Opiothorax*) *chromatomyiae* when these pesticides were sprayed on leaves carrying newly parasitized *L. sativae* larvae. We assumed that strong differences in sensitivity of the parasitoid in these different situations are a result of the amount of active ingredient reaching the parasitoid developmental stages. In case of soil dwelling stages Neem can reach sensitive parasitoid tissue only through diffusion and has to pass two cuticles. However, leaf dwelling host larvae continue with feeding even when parasitized and accumulate much more Neem which is taken up by the parasitoid when feeding from the contaminated host tissue.

The interesting selectivity of Neem is further demonstrated by the studies with *N. Formosa.* Each immature stage (egg, larvae and pupae) of *N. formosa* within leaves (and the host *L. sativae*) treated with NeemAzal®-T/S continued development until adult emergence with similar success rates as in to the untreated control. However, Success® and Abamectin were found to be very harmful to *N. formosa* compared to Neem treatment and untreated control using the same experimental approach. Corroborative results can be found in similar studies with other species of parasitoids, *Corcyra cephlonica* (Raguraman and Singh, 1999), *Bracon brevicornis* (Srivastava et al., 1997) and *Chelonus insularies* (Penagos et al., 2005). We assume that this low detrimental effect of Neem is mainly a consequence of the early interruption of the feeding activity by the parasitoid which reduces the uptake of Neem into the host tissue. In conclusions, the results presented in these studies show that the immature stages of *L. sativae* are highly vulnerable to the biopesticides - NeemAzal®-U, NeemAzal-T/S, Success® (Spinosad) and Abamectin (Avermectin). The achieved data can provide a strong baseline for developing an efficient but environment and consumer safe integrated control strategy for *L. sativae* under greenhouse conditions in the humid tropics. The study indicates especially the strong advantage of using NeemAzal®-U as soil drench concerning not only stability but particularly reduced contamination of the environment as well as higher selectivity to leaf miner parasitoids. On the other hand the results suggest that Abamectin use is incompatible with the indigenous natural

enemies but can be very efficient to completely eliminate pest populations and thus could be a preferred pesticide if the risk of resistance selection by multiple treatments with neem is of major concern.

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Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, dass die vorliegende Dissertationnicht schon
als MSc-Arbeit oder eine ähnliche Prüfungsarbeit verwendet worden ist.

....
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