# Soil application of neem products in IPM: Controlling thrips (Thysanoptera: Thripidae) in vegetable crops

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**Summary**

The extract of the neem tree *Azadirachta indica* (Meliaceae) with its insecticidal and ecological properties has been classified as one of the most important bioactive compounds of plants for integrated pest management. Currently, neem products have been mainly applied as spray treatment on the crop canopy with varying levels of success in pest control. The potential of neem applications to the soil and the use of the systemic properties of the botanical ingredients in controlling thrips were investigated in this study.

Thrips such as *Frankliniella occidentalis* and *Ceratothripoides claratris* (Thysanoptera: Thripidae) are important pests causing high economic damage in a wide range of crops worldwide. Their characteristic life cycle with plant- and soil-dwelling stages, cryptic feeding behaviour, short generation time and high mobility led to a fast development of resistances against insecticides, which makes thrips control very difficult.

The presented studies were carried out in protected vegetable cultivation in temperate zone in Germany and in the tropics in Thailand to acquire that way a more complex analysis on the potential use of soil-applied neem ingredients in pest control.

To study the systemic effects of active neem ingredients the substrate of bean plants was treated with NeemAzal-U (NA-U) solutions (17% azadirachtin (AZA)). Afterwards the translocation and persistence of AZA, 3-tigloyl-azadirachtol, salanin and nimbin and the effects on *F. occidentalis* were studied. Residues of the active components from substrates with different contents of organic matter (pure culture substrate (CS), CS-sand mixture) and from various plant parts of *Phaseolus vulgaris* were quantified by HPLC-MS. The dissipation trend of AZA and 3-tigloyl-azadirachtol was similar within the same substrates. A slower decline of both active ingredients was measured with CS versus CS-sand mixture. The residue analyses from bean plants showed that only small proportions of the initial amount of the active ingredients applied to the substrate were measured in the plant (0.3% – 8.8%). Variable amounts of residues of the active components in relation to plant parts and time of analysis indicated a different translocation pattern of active ingredients. Mortality of *F. occidentalis* after NA-U soil applications reached up to 95% on CS-sand mixture compared to 86% in CS.

In the second part, the efficacy of soil treatments using AZA in combination with the two different predatory mite species *Amblyseius cucumeris* (Acari: Phytoseiidae) and *Hypoaspis aculeifer* (Acari: Laelapidae) to control *F. occidentalis* was tested. The study also looked at side effects on the antagonists and was conducted in the laboratory and greenhouses using French bean, *P. vulgaris*. The release of a single predatory mite species resulted in unreliable and varying reductions of thrips numbers. Antagonist combinations improved efficiencies ranging from 54% to 85%. NA-U caused mortalities from 70% to 98% after soil application. A combination of AZA with predatory mites enhanced not only consistency in thrips control but also resulted in efficacies up to 99%. No detrimental effects of NA-U on the survival of both predators were recorded. However, a significant reduction in population development of *H. aculeifer* was noted.

Moreover, the effects of soil-applied neem products on *C. claratris* were investigated on *Lycopersicon esculentum* cultivated under tropical conditions in netted greenhouses in Thailand. NA-U soil applications demonstrated systemic effects against *C. claratris:* More than 85% mortality on young tomato plants was achieved when high AZA concentrations were repeatedly applied. Different application schedules as well as organic matter content of typical growing substrates resulted in no significant influence on thrips control. However, plant age did have an influence on the outcome. The younger the plants the stronger were the effects after neem soil treatments. A delayed soil application with AZA resulted in increasing thrips populations. Of the different Neem products tested Thai Neem Oil 111 showed the lowest efficiency compared to NA-U and Thai Neem Pellet 222.

Overall, soil-applied neem products can be a promising approach for integrated thrips control. Although, thrips control efficacy after neem soil treatment in the tropics (*C. claratris*, tomato) was limited compared to strong effects against *F. occidentalis* (French bean) under temperate climate.

**Keywords**: neem, soil application, thrips

**Zusammenfassung**

Extrakte vom Neembaum *Azadirachta indica* (Meliaceae) gehören aufgrund ihrer insektiziden und gleichzeitig umweltverträglichen Eigenschaften zu den vielversprechendesten botanischen Pflanzenschutzmitteln im integrierten Pflanzenschutz. Bisher wurden Neempräparate hauptsächlich als Sprühapplikation auf oberirdische Pflanzenteile und mit unterschiedlichem Bekämpfungserfolg eingesetzt. Die Möglichkeiten zur Nutzung von Neem-Bodenapplikationen und der systemischen Wirkung der Neeminhaltstoffe am Beispiel der Kontrolle von Thripsen wurde in dieser Arbeit untersucht.

Thripse wie *Frankliniella occidentalis* and *Ceratothripoides claratris* (Thysanoptera: Thripidae) verursachen als bedeutende Schädlinge weltweit hohe ökonomische Schäden an vielen Kulturpflanzen. Der typische Lebenszyklus der Thripse mit Entwicklungsstadien auf der Pflanze und im Boden, die versteckte Lebensweise, eine kurze Entwicklungszeit und die hohe Mobilität bedingen eine schnelle Resistenzentwicklung gegenüber Insektiziden, was die Bekämpfung von Thripsen sehr schwierig macht.

Die Studien wurden in geschütztem Gemüseanbau in gemäßigtem Klima (Deutschland) und in den Tropen (Thailand) durchgeführt, um den Einsatz von Neem-Bodenapplikation zur Thripskontrolle umfassender untersuchen zu können.

Eine Zielsetzung dieser Arbeit war es, die systemische Wirkungsweise von Neemwirkstoffen nach Bodenbehandlungen näher zu erforschen. Die Verteilung und Persistenz der Wirkstoffe Azadirachtin (AZA), 3-Tigloyl-Azadirachtol, Salanin und Nimbin in Substrat und Pflanze (*Phaseolus vulgaris)* nach einer NeemAzal-U (NA-U) Bodenbehandlung (17% AZA) und die Wirkung auf *F. occidentalis* wurden untersucht. Mit Hilfe von HPLC-MS wurden Rückstandsanalysen der Wirkstoffe aus Substraten mit unterschiedlichem Anteil organischer Substanz (reines Kultursubstrat (KS), KS-Sand Mischung) und verschiedenen Pflanzenteilen durchgeführt. Das Abbauverhalten von AZA and 3-Tigloyl-Azadirachtol war vergleichbar im selben Substrat, wobei ein langsamerer Abbau beider Wirkstoffe im KS beobachtet wurde. In den Bohnenpflanzen konnten nur sehr geringe Anteile der zu Beginn auf das Substrat ausgebrachten Wirkstoffgehalte wiedergefunden werden (0,3%-8,8%). Unterschiedliche Rückstandsmengen je nach Pflanzenteil und Zeitpunkt der

Probenahme lassen eine unterschiedliche Verlagerung der Wirkstoffe erkennen. Es konnten Bekämpfungserfolge gegenüber *F. occidentalis* bis zu 95% bzw. 86% bei Verwendung der KS-Sand Mischung bzw. KS erzielt werden. Weiterhin wurde die Effizienz von Neem-Bodenapplikationen kombiniert mit dem Einsatz der Raubmilben *Amblyseius cucumeris* (Acari: Phytoseiidae) und *Hypoaspis aculeifer* (Acari: Laelapidae) zur Kontrolle von *F. occidentalis* getestet. Auch die Nebenwirkungen gegenüber den Antagonisten wurde untersucht. Die Studien wurden im Labor und Gewächshaus an *P. vulgaris* durchgeführt. Mit dem Einsatz einzelner Raubmilbenarten konnte nur ein schwankender und unzuverlässiger Bekämpfungserfolg erzielt werden. Der kombinierte Einsatz konnte die Wirksamkeit verbessern (54%-85%) und NA-U als Bodenapplikation resultierte in Mortalitäten von 70% bis 98%. Mit der Kombination von Neem und Raubmilben konnten die Wirkungsgrade bis auf 99% erhöht werden. Nebenwirkungen von NA-U auf das Überleben der beiden Räuber wurde nicht beobachtet. Allerdings wurde eine signifikante Verringerung der Populationsentwicklung von *H. aculeifer* verzeichnet.

Die Wirkung von Neem-Bodenapplikationen gegenüber *C. claratris* wurde an *Lycopersicon esculentum* in Netzgewächshäusern in Thailand untersucht. Durch Bodenbehandlungen mit NA-U wurden korrigierte Thripsmortalitäten von über 85% erzielt, wenn junge Tomatenpflanzen verwendet und hohe AZA Konzentrationen wiederholt appliziert wurden. Unterschiedliche Applikationsabstände und der Anteil organischer Substanz im Substrat haben keinen Einfluss auf den Bekämpfungserfolg gezeigt. Das Pflanzenalter hingegen hat die Bekämpfungseffizienz stark beeinflusst. Die Wirkung nach Neem-Bodenbehandlungen war umso stärker, je jünger die Tomatenpflanzen waren. Eine zeitverzögerte Behandlung hat zum Ansteigen der Thripspopulation geführt. Von verschiedenen Neemprodukten hat Thai Neem Oil die geringste Wirksamkeit im Vergleich zu NA-U und Thai Neem Pellet gezeigt.

Insgesamt konnte die Neem-Bodenbehandlung - trotz begrenzten Bekämpfungserfolgen in den Tropen (*C. claratris*, Tomate), jedoch sehr guten Kontrolleffizienzen in gemäßigtem Klima (*F. occidentalis*, Bohne) - einen vielversprechenden Ansatz für die integrierte Bekämpfung von Thripsen bieten.

**Schlagworte**: Neem, Bodenapplikation, Thripse

**Contents**

[1]Thoeming G, Draeger G and Poehling H-M. Soil application of azadirachtin and 3-tigloyl-azadirachtol to control the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae): translocation and persistence in bean plants. Submitted to Pest Management Science.

[2]Thoeming G and Poehling H-M. Controlling *Frankliniella occidentalis* (Thysanoptera: Thripidae) with azadirachtin as soil application and the predatory mites, *Amblyseius cucumeris* (Acari: Phytoseiidae) and *Hypoaspis aculeifer* (Acari: Laelapidae) in a combined treatment. Submitted to Environmental Entomology.

[3]Thoeming G and Poehling H-M. Soil application of different neem products to control *Ceratothripoides claratris* (Thysanoptera: Thripidae) on tomatoes grown under protected cultivation in the humid tropics (Thailand). Submitted to International Journal of Pest Management.

## Abbreviations

| | |
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| AZA | Azadirachtin |
| CM | Corrected mortality |
| CS | Culture substrate, Fruhstorfer Erde |
| CS-sand | Culture substrate-sand mixture, ratio 1:1 |
| HPLC-MS | High-performance liquid chromatography–mass spectrometry |
| L1 | First instar larva |
| L2 | Second instar larva |
| NA-U | NeemAzal-U |
| OM | Organic matter content |
| WFT | Western flower thrips |

# 1 General Introduction

The need for food is increasing permanently due to the constantly expanding population, with currently more than 6.3 billion people worldwide (FAO 2004). Based on Food and Agriculture Organisation (FAO) estimates, the world's population will increase to 8.5 billion by 2025 and around 83% of these people will live in developing countries. At present, 17% of the total population in developing countries is undernourished, and in some regions malnutrition rates of up to 40% have been recorded (FAO 2003a). In economically more developed countries a growing demand for quality food and a wide range of products is created as a result of economic growth and increasing incomes (FAO 2003a). An intensification of the food production on existing cultivated land can help satisfy these growing requirements for food.

Estimates indicate a global crop loss caused by pest organisms of 25% to 50%. Due to a lack of knowledge and the absence of alternative plant protection strategies, synthetic pesticides are often used prophylactically, too heavily or inconsiderately. The unavoidable consequences are the contamination of food and the environment with toxic pesticide residues resulting in detrimental effects on human and non-target organisms, the development of resistant pest populations, pest resurgence, and the outbreak of secondary pest infestations (Pingali and Roger 1995, Kacew et al. 1996, Tinker 1997, Eddleston et al. 2002, Horrigan et al. 2002). This momentous environmental impact by synthetic insecticides is one of the most important green issues today. Thus, the development of sustainable and non-polluting plant protection strategies is of global importance for population's food situation and for conservation of a functional environment.

Today, integrated pest management (IPM), as a combination of biological control with host plant resistance, appropriate farming practices and a minimal use of pesticides, is an important part of agriculture and horticulture worldwide and provides a basis for pest management in the future (Kogan 1998, Trumble 1998, Hillocks 2002, Phipps and Park 2002, Feder et al. 2004).

Neem products are components in IPM concepts as a result of the rediscovery and detailed research on the bioactive ingredients of the neem tree, *Azadirachta indica* A. Juss. (Meliaceae) over the last three decades (Schmutterer 1985, 1990, Prakash and Rao 1997, Hellpap and Dreyer 2002, Ermel et al. 2002). Parts of *A. indica* and other *Azadirachta* and *Melia* plants have been used in plant protection in the tropics for centuries, especially in south and southeast Asia where the neem tree originates from (Schmutterer 1985, Jacobson 1988, Dreyer and Hellpap 1991, Govindachari 1992, Hellpap and Dreyer 2002). However, these traditional methods have mostly been replaced with the introduction of synthetic pesticides over the last sixty years. Today, with the global focus on environmental problems, and extensive knowledge of insect biology available, as well as sophisticated technical equipment and experience in isolation, extraction and analysis of biological active ingredients, an improvement in the use of neem compounds in IPM is possible.

Azadirachtin (AZA), as the main active component of the neem tree, has demonstrated a remarkable impact in plant protection (Saxena 1989, Schmutterer 1990, 2005, Govindachari 1992, Ley et al. 1992, Prakash and Rao 1997, Mordue et al. 1998, Immaraju 1998, Kraus 2002). So far, more than one hundred ingredients have been isolated from *A. indica* but few components have been studied in detail for biological activity and structure related activity (Govindachari 1992, Kraus 2002). Most of the active ingredients belong to the group of tetranortripenoids and alongside AZA, 3-tigloyl-azadirachtol, salanin and nimbin are currently some of the best-investigated components, all with insecticidal properties (Nisbet et al. 1995, Stark and Walter 1995a, Jarvis et al. 1997, Mordue et al. 1998, Kraus 2002, Sharma et al. 2003, Barrek et al. 2004, Simmonds et al. 2004, Schmutterer 2005). For pest control, the most important mode of action of AZA is its effect on metamorphosis through the inhibition of the release of prothoracicotropic hormones, allatotropins and allotoinhibins (Banken and Stark 1997, Gonzales et al. 1999, Rembold 2002). Moreover, active neem ingredients impact on feeding behaviour, reproduction, growth, fitness and mobility as well as in repellent effects (Rembold 1989, Schmutterer 1985, 1990, Prakash and Rao 1997, Mordue et al. 1998).

The environmental compatibility of neem products due to short persistence of neem ingredients in environment is of particular importance for their use in IPM. A general statement on toxicology and ecotoxicology of 'neem' is difficult due to the mixture of components in neem extracts and variations in the concentration of active ingredients in neem preparations. These variations are caused by differences in the production process, storage conditions, harvest and origin, or contamination with mycotoxins such as aflatoxin (Johnson et al. 1996, Efuntoye 1999, Ermel et al. 2002, Jenkins et al. 2003). Registered neem formulations with defined contents of active ingredients like NeemAzal®-T/S (1% AZA, Trifolio-M GmbH, Lahnau, Germany) has been classified as safe for environment and non-target organisms, if applied at recommended dose rates (Spollen and Isman 1996, Sundaram 1996b, Ruch et al. 1997, Immaraju 1998, BVL 2005a, b, Schmutterer 1997, 2005). The LD50 / LC50 values (lethal dose / concentration 50% = dose administered that kills half the test population) of NeemAzal®-T/S with LD50 oral > 5000 mg/kg rat, LD50 dermal > 2000 mg/kg rat and LC50 inhalation > 5.4 mg/l/4h (rat) indicated a low health hazard of such neem products to humans (Niemann and Hilbig 2000, Niemann et al. 2002, Trifolio 2004). Ecotoxicology tests in the framework of registration indicated no hazard of NeemAzal®-T/S to honey bees (B4) and the beneficials *Typlodromus pyri* Scheuten (Acari: Phytoseiidae), *Poecilius cupreus* Linnaeus (Coleoptera: Carabidae), *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae) and *Aphidius rhopalosophi* De Stephani-Perez (Hymenpotera: Aphidiidae), but harmful effects on *Episyrphus balteatus* De Geer (Diptera: Syrphidae) were recorded (Trifolio 2004, BVL 2005a, b). Detrimental effects on earthworms (LD50 > 1000 mg/ kg) and aquatic organisms (EC50 > 50 mg/l (Daphnia), LC50 = 440 mg/l (after 24h, Trout)) were not detected (Ruch et al. 1997, Pussemeier 2000, Trifolio 2004, BVL 2005a, b). A rapid and complete degradation of neem ingredients reduces toxic residues on food, soil and water when applied to plants or soil (Ruch et al. 1997, Pussemeier 2000, Thompson et al. 2004).

To date, neem extracts have been mainly applied as spray treatment on the crop canopy with varying levels of success on a variety of pests (Schmutterer 1990, Immaraju 1998, Prabhaker et al. 1999, Fournier and Broduer 2000,

Pearsall and Hogue 2000). The use of such a treatment is limited by the sensitivity of the active ingredients to UV light and temperature, depending on the acidity of the medium (Ruch et al. 1997, Pussemeier 2000, Barrek et al. 2004). With the extraction of active components from leaves or seeds of *A. indica* the surrounding conditions of the ingredients are altered. In doing so, a faster degradation of bioactive neem ingredients with increasing light intensity and temperature dependent on media pH occurs. Due to this short persistence of bioactive neem ingredients, especially after spray treatments under field conditions with high UV irradiation, repeated spray applications are often required to assure adequate pest control (Saxena and Kidiavai 1997, Immaraju 1998). These repeated treatments might harm predators and parasitoids developing in above ground plant parts and restrict the potential combination of antagonists and neem components in IPM (Spollen and Isman 1996, Stark 1996, Immaraju 1998, Raguraman and Singh 1999, Tedeschi et al. 2001, Schmutterer 2002). In addition, pests living and feeding in enclosed microhabitats, like thrips, are difficult to control by topical treatments, even though the spray applications are frequently repeated (Schmutterer 1990, Saxena and Kidiavai 1997, Immaraju 1998, Pearsall and Hogue 2000). The application of neem extracts to the soil/substrate and the use of the systemic properties of the botanical ingredients may eliminate such limitations.

Currently, simple preparations like neem cakes, seed kernels or leaf powder are used as soil-applied fertiliser, nitrification inhibitor and pesticide on a small scale by those farmers mainly in growing regions of *A. indica* (Kareem et al. 1989, Dreyer and Hellpap 1991, Raguraman and Saxena 1994, Saxena et al. 2001, Musabyimana et al. 2000, Ketkar and Ketkar 2002, Uyovbisere and Elemo 2002). Oftentimes deficient pest control effectiveness after neem soil treatment is due to variations in the concentration of active ingredients in most of these preparations. This is reflected in the diverse opinion of neem soil treatments of Asian farmers today (pers. comm., farmer interviews). The development of commercial neem products for soil application with defined contents of active ingredients commenced recently. However, there is still a lack of knowledge on the systemic effects of neem ingredients especially after soil treatments. The studies of Gill and Lewis (1971) provided the first scientific evidence of systemic action of AZA against *Schistocerca gregaria* Forskål

(Orthoptera: Acrididae). Thereafter, systemic effects of neem extracts on different arthropod species of Coleoptera (Nauman et al. 1994), Lepidoptera (Meisner et al. 1978, 1985, 1990, Kubo and Klocke 1982, Osman and Port 1990, Rovesti and Deseo 1991, Koul and Shankar, 1995), Diptera (Lindquist et al. 1986, Meisner et al. 1986, Larew 1988, Parkman and Pienkowski 1990, Weintraub and Horowitz 1997), Homoptera (Meisner et al. 1992, West and Mordue 1992, Raguraman and Saxena 1994, Pavela et al. 2004), Thysanoptera (Thöming et al. 2003) and Acari (Sundaram et al. 1995) were investigated. Detailed studies on systemic action of neem ingredients on a bioanalytical and physiological level are still rare. The root uptake, systemic translocation, accumulation and dissipation of AZA in spruce trees and aspen plants have already been demonstrated and quantified by Sundaram et al. (1995, 1996a, 1997).

Valuable information on the environmental behaviour of AZA as a prerequisite for detailed investigations on soil treatments with neem ingredients has been gathered. Studies on the degradation and fate of AZA in soil and water have demonstrated the strong dependency of neem ingredients on environmental factors such as UV light, temperature, pH-value, microbial activity in soil and physical soil properties such as the organic matter content (Sundaram and Curry 1993a, Sundaram 1994, 1996b, Stark and Walter 1995a, Ruch et al. 1997, Pussemeier 2000, Barrek et al. 2004, Thompson et al. 2004). So far, all studies on systemic effects of neem ingredients have shown conflicting results regarding the efficiency in pest control. Moreover, facts on uptake, translocation, fate and persistence of bioactive neem components are still missing. Hence, a general statement on the actual efficiency of neem soil applications or an interpretation of the state of knowledge on systemic effects of neem components is difficult.

Studies on the systemic effects on *Frankliniella occidetalis* Pergande (Thysanoptera: Thripidae) after a soil application of NeemAzal®-T/S indicated strong efficiency on plant-sucking life stages due to systemic action (Thöming et al. 2003). These results motivated further extensive research on systemic effects in thrips control after neem soil application. Therefore, the first section of the presented study aims to clarify the mechanism of the systemic action of different bioactive neem ingredients and its impact for thrips control. Residue

analysis of AZA, 3-tigloyl-azadirachtol, salanin and nimbin in different growing substrates and plant parts were carried out, and biological tests on thrips control efficiency using *Phaseolus vulgaris* plants as a model system were arranged.

Due to the range of crops infested by thrips, the more than one hundred significant pests in the thysanoptheran family Thripidae, their small size and their ability to disperse widely, to multiply rapidly and to cause direct feeding damage and plant virus transmission, means thrips rank among the most important pests worldwide (Talekar 1991, Tommasini and Maini 1995, Lewis 1997a, Morse and Hoddle 2006). Thysanoptera occur worldwide with a predominance of tropical species, but with many temperate and a few artic ones (Ananthakrishnan and Gopichandran 1993, Mound 1997, Morse and Hoddle 2006). In the presented study two thrips species were examined: i) the Western Flower Thrips (WFT), *F. occidentalis*, which, since its unintended introduction in 1983, is the most important pest in European greenhouses (Tommasini and Maini 1995, Lewis 1997a), and ii) *Ceratothripoides claratris* Shumsher (Thysanoptera: Thripidae) as an example of a important tropical thrips species (Shumsher 1945, Okajima et al. 1992, Jangvitaya 1993, Mound and Kibby 1998, Murai et al. 2000, Rodmui 2002).

Thysanoptera cause direct plant damage by feeding on leaves, flowers and fruits, which can cause a range of results from deformations of single plant parts to the total loss of the plant (Tommasini and Maini 1995, Childers and Achor 1995, Lewis 1997a, Murai et al. 2000, Rodmui 2002). Thrips are able to feed in a piercing-sucking manner on epidermal, palisade and mesophyll cells using a strong suction mechanism (Chisholm and Lewis 1984, Ananthakrishnan and Gopichandran 1993, Tommasini and Maini 1995, Harrewijn et al. 1996a, b, Kirk 1997). A characteristic of thrips feeding behaviour is the saliva injection prior to sucking the cell content, which creates the ability of thrips to act as vectors of plant viruses (Chisholm and Lewis 1984, Harrewijn et al. 1996a, Kirk 1997). Transmission of plant pathogens, especially plant viruses, can induce indirect plant damage, which can pose more serious problems than the plant damage itself. WFT is known as a vector for viruses such as tomato spotted wilt virus, impatiens necrotic spot virus, groundnut ring spot virus and tomato chlorotic spot virus (Ullman et al. 1992, 1997, Parella 1995, Wijkamp et al.

1995, Moritz et al. 2004). *C. claratris* can transmit Capsicum chlorosis virus (CaCV, isolat of Asian Institute of Technology, Pathumthani, Thailand) (Premachandra et al. 2005a). In the life cycle of typical Thripidae the eggs are laid in above ground plant parts and the two active larval instars feed on leaves, buds, flowers and fruits. The late second instars finish feeding on the plant and move to soil or leaf litter for pupation. Whereas the adult and larval stages occupy similar protected microhabitats in aerial plant parts, the two relatively inactive non-feeding pupal instars are well sheltered in soil or leaf litter (Shumsher 1945, Tommasini and Maini 1995, Moritz 1997, Rodmui 2002, Berndt et al. 2004a). In tropical areas with mean temperatures ranging from 25 °C to 30 °C the total life cycle of *C. claratris* is 9 to 15 days (Rodmui 2002, Premachandra et al. 2004). At 20 °C in a temperate climate, WFT requires around 21 days to develop from egg to adult (Lewis 1997a, Tommasini and Maini 1995). This highlights the short generation time of the thrips.

The thrips' protected habitats on above ground plant parts or in soil and leaf litter makes their control with sprayed insecticides difficult, and requires repeated treatments. Despite recurrent spray applications, attempts in thrips control using synthetic pesticides are often ineffective, and cause environmental problems and interfere with IPM programmes (Riuvdavets 1995, Lewis 1997b, Morse and Hoddle 2006). Moreover, the repeated use of insecticides combined with the high fertility and short generation time of thrips has resulted in extensive pesticide resistances developing in many thrips populations and against various active ingredients (Immaraju et al. 1992, Brødsgaard 1994, Robb et al. 1995, Zhao et al. 1995, Lewis 1997b, Espinosa et al. 2002). At present, insecticides containing spinosad® applied as spray treatment are efficient in controlling Thripidae like *F. occidentalis* and *C. claratris* (Ishaaya et al. 2001, Jones et al. 2005, Premachandra et al. 2005b). Moreover, neonicotinoids like imidacloprid are used successfully in thrips control as foliar application, soil drench or seed treatment (Ester et al. 1997, Maienfisch et al. 2001, Riley and Pappu 2004, Tomizawa and Casida 2005). Nevertheless, the solely use of synthetic insecticides in thrips management runs the increased risk of resistance development. Recently, resistant strains to spinosad® were detected in WFT populations (Loughner et al. 2005). Thus, alternative thrips management measures such as biological and integrated control strategies

become increasingly important. Predatory mites or bugs like *Amblyseius* spp. (Acari: Phytoseiidae) and *Orius* spp. (Hemiptera: Anthocoridae) have demonstrated the highest potential in biological control of WFT so far (Ramakers 1995, Parella 1995, Brødsgaard et al. 1996, Castané et al. 1999, Jacobson et al. 2001a,b, Shipp and Wang 2003, Sengonca et al. 2004). Promising results in controlling soil-dwelling stages of WFT have occurred using entomopathogentic nematodes like *Steinernema* and *Heterorhabditis* spp. (Rhabditida: Steinernematidae, Heterorhabditidae) or ground-foraging *Hypoaspis* mites (Acari: Laelapidae) (Ehlers 2003, Ebssa et al. 2004, Berndt et al. 2004b). At present, for *C. claratris* only a mirid predator and the two parasitoids, *Ceranisus menes* Walker and *Goethena shakespearei* Girault (Hymenoptera: Eulophidae) have been identified as natural enemies (Murai et al. 2000, Rodmui 2002). Their efficacy in biological control of *C. claratris* however, just like bio-control in general, has yet to be investigated. To date, the use of natural enemies alone has seldom provided satisfactory thrips control (Brødsgaard 1995, Jacobson 1995, Parella 1995, Blaeser et al. 2004, Wiethoff et al. 2004). Currently, different IPM stategies such as natural enemies, ultraviolet light-refelective mulches, spinosad and/or different botanicals in combined treatments were tested for thrips control (Jacobson 1997, Reitz et al. 2003, Chiason et al. 2004, Jones et al. 2005, Morse and Hoddle 2006).

Thrips are one of the target pests of neem ingredients. But, the efficiency of foliar application is rarely satisfactory for practical thrips control as studies on different thrips species such as *F. occidentalis*, *Thrips tabaci* Lindeman, *Megalurothrips sjostedti* Trybom, *Sciothrips cardamomi* Ramakrishna, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) have indicated (Ivbijaro and Bolaji 1990, Malaipan et al. 1992, Santhosh 1994, Labanowski and Soika 1999, Pearsall and Hogue 2000, Schroer et al. 2001). Because of the relatively short persistence of the active ingredients and the high recolonization pressure from non-treated plant parts and soil, efficient thrips control can be achieved only with frequently repeated applications (Saxena and Kidiavai 1997, Immaraju 1998, Guiterrez 2000). As above mentioned, recurrent spraying of insecticides can pose a risk to non-target organisms living in the crop canopy, which limits the combined use of neem extracts and releases of beneficials in IPM (Immaraju 1998). Several studies indicated negative effects on antagonist after

neem spray treatments (Immaraju 1998, Schmutterer 1997, 2005). Mostly sublethal effects like reduction of fecundity or feeding intensity were recorded, e.g. on *Amblyseius cucumeris* Oudemans, *Cotesia plutella* Kurdjumov (Hymenoptera: Braconidae) and *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) (Spollen and Isman 1996, Perera et al. 2000, Tedeschi et al. 2001). Mortality of antagonist after neem treatments were dected more rare, e.g. on *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) *M. caliginosus* and *E. balteatus* (Spollen and Isman 1996, Tedeschi et al. 2001, Ahmed et al. 2003).

Soil applications of neem ingredients might overcome these disadvantages due to its systemic and contact effects on plant-feeding and soil-dwelling developmental stages of thrips respectively (Thöming et al. 2003). Therefore, IPM in this context was examined in the second part of the presented project. The combination of soil treatments containing neem ingredients with the two predatory mite species *A. cucumeris* and *Hypoaspis aculeifer* Canestrini to control WFT on French bean, *P. vulgaris* were examined in laboratory and greenhouse experiments. Additionally, possible detrimental impact on the antagonists was investigated, as a prerequisite for an implementation in IPM. These first studies were carried out to illustrate the potential of such combinations for integrated thrips control in European greenhouses.

To acquire a more complex analysis of the potential use of neem soil application in integrated thrips control, the studies of the presented project were carried out in two different horticultural systems under different climatic conditions: i) *F. occidentalis* on French bean in greenhouses in temperate climate (Hannover, Germany), and ii) *C. claratris* on tomato, *Lycopersicon esculentum,* in protected cultivation in the tropics in Pathumthani, Thailand.

In Germany, as in most European countries, the growing food demand has caused an intensification of agriculture since 1940. The excessive use of synthetic fertiliser and pesticides, intensive irrigation, large scale agriculture and monocropping has replaced traditional cropping systems. This increased use of pesticides has involved detrimental effects on human and non-target organisms, pest resurgence, the outbreak of secondary pest infestations and particularly

the development of resistant pest populations (Tinker 1997, Eddleston et al. 2002, Horrigan et al. 2002). Since 1970 the pesticide use has been dominated by the chemical classes organophosphates, carbamates and pyrethroids. However, resistant insects strains were soon selected and limit their effectiveness (Gunning and Moores 2001, Scott 2001, Siegfried and Scharf 2001, Hemingway et al. 2002, Weill et al. 2004). Over the last years new chemicals such as neonicotinoids with a high effectiveness, particularly via sytemic action against sucking insects, and in generally a low toxicity to vertebrates were introduced (Maienfisch et al. 2001, Tomizawa and Casida 2005). So far, neonicotinoids have proved to be relatively indestructible to resistance development, nevertheless resistant strains were recorded recently (Ishaaya et al. 2005, Nauen and Denholm 2005). A similar development has occurred in Thailand. Today the state is classified as a country with a consistent and strongly developing economy over the longer period, where agriculture is still the main source of employment, national income and foreign exchange. Over recent decades the country has increased the efficiency of its agriculture by using, for example, imported chemical fertilizer, pesticides and hybrid seeds. While, locally produced farm inputs such as compost, manure and botanical products have been replaced. However, the enhancement in food production has been primarily based on the expansion of cultivated land rather than intensifying agricultural productivity (Chaiwanakupt and Changprai 1991, FAO 1999, Thapinta and Hudak, 2000). This agricultural development has increasingly resulted in detrimental effects on the environment, such as deforestation, desertification, land degradation, increased salinity and all kinds of environmental pollution. Over the last years the environmental awareness and demand for food quality with pesticide-free products has increased also in Thailand. Thus, sustainability in agriculture with an intensification of food production on already existing cultivated land and reduced use of harmful pesticides has been proposed (Chaiwanakupt and Changprai 1991, Pookpakdi 1995, FAO 1999, Jitsanguan 2001).

Plant growth, as well as pest development and infestation, is enhanced in the tropics compared to the temperate zones. This results in a considerably stronger pest pressure on agricultural and horticultural crops cultivated in these regions, and highlights the need for effective plant protection. Based on FAO

estimates the consumption of synthetic pesticides in East-, Southeast Asia and China increased from 0.74 kg/ha in 1989-91 to 1.15 kg/ha in 1998-2000 (FAO 2003b). This alarming growth of pesticide use has caused an increase in human and environmental problems as well as pesticide resistance development. The detrimental impact of synthetic pesticides on the health of farmers, consumers and the environment in Thailand and the entire Southeast Asian region, following the growing demand for IPM is well established (Pingali and Roger 1995, Pookpakdi 1995, FAO 1999, Thapinta and Hudak 2000, Jitsanguan 2001, Jirachaiyabhas et al. 2004). Nevertheless, harmful pesticides are still overused, especially in vegetable and fruit crops (Bansiddihi and Poonchaisri 1991, Bodzian 1998, Thapinta and Hudak 2000, Jirachaiyabhas et al. 2004). In Thailand His Majesty King Bhumipol Adulyadej, the Department of Agriculture (DOA) and some private organizations initiated several projects funding the crop growing of chemical-free vegetables (FAO 1999, Thapinta and Hudak 2000). The use of neem-based insecticides is recommended in such programmes. The governmental and private promotion of neem products in pest control creates a good basis for the implementation of research results, and suggests Thailand as a practical region for the presented neem-research.

Over the last decades the value of vegetables has increased worldwide along with a considerable increase in the global vegetable supply, with a per annum growth rate of 4.4% (FAO 2004). In economically more developed countries with abundant food supply the low calorie content in vegetables is valued, whereas, in countries with a deficient food supply vegetables are important to satisfy the need for food. In Thailand the vegetable production is of particular importance due to its socio-economic role as an essential part of small scale farming, as well as its potential on the international market and in export (Manee and Pipob 1989, FAO 1999, 2004, AVRDC 2004). The royal project on crop substitution, a strategy designed to gradually replace opium production by growing other cash crops, encourages vegetable production in Thailand (FAO 1999). In recent years, the demand for vegetable crops in Thailand has grown constantly. Especially the tomato is one of the most economically important vegetables with a yearly increase in production of 7.9% (Manee and Pipob 1989, FAO 1999, 2004).

In the last section of the presented project the potential of neem soil treatments to control thrips in protected tomato production under tropical conditions in Thailand were evaluated. This aims to exemplify the potential practical use of such neem application in vegetable crops beyond the limits of European greenhouses. Thus, a completely different environment-plant-insect-system was examined in addition to the basic studies in Germany.

The main objective of this presented thesis is to clarify the ability of neem extracts as soil treatment to control sucking insects such as thrips. The study focused on three fields of activity:

(i) The biological basis of the systemic action of bioactive neem components after soil applications regarding the complete substrate-plant-insect-system, considering as example *F. occidentalis* on *P. vulgaris*

(ii) The practical use of soil-applied neem products for integrated thrips control in greenhouses in Germany, concerning efficiency of AZA soil treatments combined with predatory mites to control WFT, and the side effects on the antagonists, in laboratory and greenhouse on French bean

(iii) The implementation of neem soil treatments as IPM measure in the humid tropics, regarding the control of *C. claratris* on tomato in protected cultivation in Thailand

# 2 Soil Application of Bioactive Neem Ingredients to Control the Western Flower Thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae): Translocation and Persistence in Bean Plants[1]

## 2.1 Introduction

The interest in so called bio-pesticides such as botanicals is increasing. For instance extracts of the neem tree (*Azadirachta indica* A. Juss., Meliaceae) contain among other compounds, azadirachtin (= azadirachtin a, AZA), 3-tigloyl-azadirachtol (= azadirachtin b), salanin and nimbin as the most important active ingredients (Figure 2.1) and several structurally related tetranortripenoids, all possessing insecticidal properties (Jones et al. 1989, Govindachari 1992, Kraus 2002). Active ingredients can be extracted from all parts of the neem tree, but the highest concentration is found in the seeds.

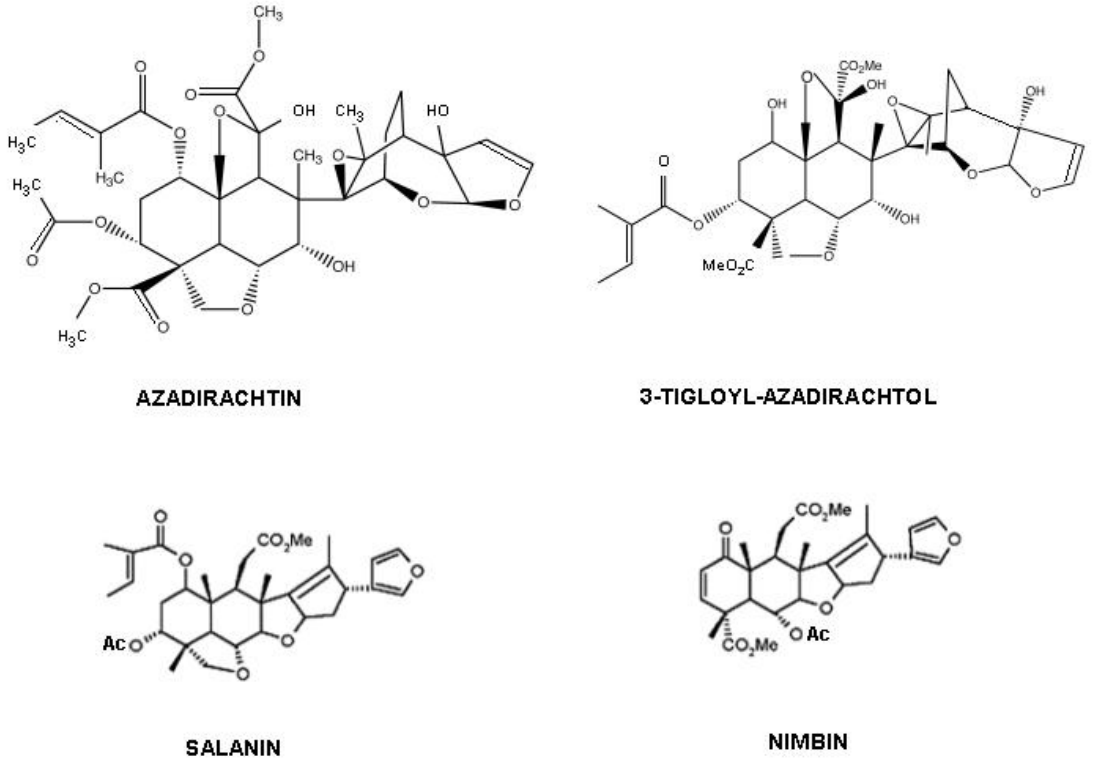


**Figure 2.1 Molecular structures of azadirachtin, 3-tigloyl-azadirachtol, salanin and nimbin**

[1]based on: Thoeming G, Draeger G and Poehling H-M. Soil application of azadirachtin and 3-tigloyl-azadirachtol to control the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae): translocation and persistence in bean plants. Submitted to Pest Management Science.

Neem extracts can exhibit antifeedant, repellent and growth regulating properties in insects, affecting many important pests of different crops (Schmutterer 1990, Mordue et al. 1998). Several commercially available neem formulations like NeemAzal®-T/S (1% AZA, Trifolio-M GmbH, Lahnau, Germany) are used in pest control worldwide. Advantages of neem products are the fast and complete degradation when applied to plants or soil (Sundaram 1996b, Ruch et al. 1997), the low risk to humans (Niemann and Hilbig 2000) and non-target organisms (Sundaram 1996b, Schmutterer 1997) and so far very low selection of resistant target organisms (Völlinger 1992, Feng and Isman 1995). An additional advantage of neem ingredients is their systemic activity (Gill and Lewis 1971, Sundaram et al. 1995, Weintraub and Horowitz 1997). Hence, protection of plants from pests can be achieved not only by direct treatments such as spraying on plant surfaces but also by selective application to lower plant parts or even to the roots by soil drenching. After that an uptake and acropetal translocation within the plant might occur. Although spraying of neem extracts can affect many major pests, the short persistence of the active ingredients, caused by its sensitivity to high temperatures and UV light, often requires repeated applications to assure adequate pest control (Saxena and Kidiavai 1997, Immaraju 1998, Barrek et al. 2004). Moreover, pest insects with a cryptic feeding behaviour such as thrips are often poorly controlled by foliar applications of neem extracts, even if treatments were frequently repeated. This additionally might harm beneficial predators and parasitoids occurring in the crop canopy (Raguraman and Singh 1999, Tedeschi et al. 2001), limiting the potential combination of neem extracts and beneficals in IPM. However, such constraints can be avoided when neem extracts are applied to the soil, exploiting the systemic properties of this botanical. Furthermore, neem soil treatments can control soil-borne pests such as nematodes or the soil-dwelling life stages of pest insects e.g. thrips (Agbenin 2004, Thöming et al. 2003).

The Western Flower Thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) is one of the most important pests of many horticultural crops (Tommasini and Maini 1995, Lewis 1997a). The economic significance of WFT is mainly caused by the difficulties in controlling the thrips. Due to its cryptic feeding behaviour, high mobility, short generation time, high fertility and soil-dwelling life stages, frequent applications of insecticides or the

use of persistent ingredients is usual practice to achieve sufficient control. This increases the hazards to non-target organisms and the selection of insecticide resistant pest populations (Immaraju et al. 1992, Espinosa et al. 2002). On the other hand biocontrol with predatory mites or bugs only, is often not efficient or reliable enough, so combinations of low risk pesticides and natural enemies seem to offer a new interesting opportunity for sustainable control.

In previous studies soil applications of NeemAzal®-T/S resulted in significant effects on WFT with corrected mortalities of up to 93%. This type of application affected the plant-feeding stages of thrips through systemic activity and in addition, the soil-dwelling life stages by direct contact (Thöming et al. 2003). However, little quantitative data on the uptake, translocation and persistence of the active components of neem in the substrate and the plants following a soil application of neem extracts are available (Sundaram et al. 1995, Stark and Walter 1995a). Therefore, the major objective of this study was to elucidate some aspects of the mechanism of the systemic distribution of neem's active ingredients i.e. their translocation and persistence in plants and substrate as well as their effects in thrips control.

## 2.2 Materials and Methods

### *Bean Plants, Substrates and Western Flower Thrips*

Bean seeds (*Phaseolus vulgaris* L., cv. Marona, Fabacea) were pre-germinated for three days before being planted in plastic seedling trays (50 × 30 × 6.5 cm, 50 seed/tray) containing a commercial substrate (CS) (Fruhstorfer Erde, Type P, Archut GmbH, Lauterbach-Wallenrod, Germany, 50% peat, 35% clay, 15% humus; pH 5.7-6.3; 124-185 mg N/l, 120-179 mg P2O5/l, 190-284 mg K2O/l, 0.8-1.4 g salt content/l). Then the seedlings were grown for six days under greenhouse conditions (21±2°C, 65-75% RH, 18:6 h [L:D] photoperiod). Thereafter, seedlings in the primary leaf stage were individually transplanted into plastic pots (11 × 7.5 × 8.5 cm) filled with CS (156 g substrate per pot) or with a substrate mixture of CS and sand in a 1:1 ratio (CS-sand) to reduce the content of organic matter (240 g substrate per pot) and grown under controlled conditions (23±2°C, 50-60% RH, 18:6 h [L:D] photoperiod). The physical properties of the substrates are shown in Table 2.1.

**Table 2.1 Physical properties (mean value ± SD) of Fruhstorfer Erde Typ P (CS) and a mixture of Fruhstorfer Erde and sand in a 1:1 ratio (CS-sand) analysed using the ISHS-method (DIN EN 13041, 2000).**

| | CS | CS–sand |
|---|---|---|
| Physical properties | Mean* ± SD | Mean* ± SD |
| Proportion organic matter (%) | 39.47 ± 1.35 | 8.46 ± 1.42 |
| Proportion mineral matter (%) | 60.53 ± 1.35 | 91.54 ± 1.42 |
| Pore volume (Vol. %) | 88.31 ± 0.20 | 64.61 ± 0.34 |
| Air capacity (Vol. %) | 8.65 ± 0.82 | -0.56 ± 0.84 |
| Container capacity (Vol. %) | 79.66 ± 0.70 | 65.17 ± 0.52 |
| Available water (Vol. %) | 34.03 ± 2.12 | 38.37 ± 0.49 |

***Average of four replicates**

To obtain uniformly aged life stages thrips were reared on pods of French beans (*Phaseolus vulgaris* L., cv. Marona) in glass jars in a climate chamber (23±2 °C, 50-60%RH and 18:6 h [L:D] photoperiod) according to the protocol described by Berndt et al. (2004a). First instar larvae (L1) were collected for experimental use.

### *Neem Product*

NeemAzal-U (17% AZA, Trifolio-M GmbH, Lahnau, Germany), a neem formulation intended for hydroponics and soil applications (registration pending) was used to conduct the experiments. It is a water-soluble powder with good root uptake properties. Analysis of the used NeemAzal-U batch showed an average content of 17% AZA, 2% 3-tigloyl-azadirachtol, 1.5% salanin and 0.35% nimbin. NeemAzal-U was selected instead of the already registered NeemAzal®-T/S since the latter formulation contains high amounts of oil (51%) and tenside (45%), which can accumulate in the substrate and thus cause adverse effects e.g., on roots, plant growth or substrate properties if used as soil application.

***Residue Analysis of Active Neem Ingredients in Different Substrates and Plant Parts***

Bean seedlings were drenched with 100 ml of a NeemAzal-U solution containing 0.59 g NeemAzal-U/l (100 mg AZA/l, 11.8 mg 3-tigloyl-azadirachtol/l, 8.9 mg salanin/l, 2.1 mg nimbin/l). This amounted to 10 mg AZA, 1.18 mg 3-tigloyl-azadirachtol, 0.89 mg salanin, 0.21 mg nimbin being applied per pot. Samples of the substrates and bean plants were collected one day before (1db), 1.5 hours after (0d), and 2, 4, 6, 10 and 14 days after (2da, 4da, 6da, 10da, 14da) the soil application of NeemAzal-U, respectively. At each sampling time 30 plants of each substrate type were randomly selected and uprooted. The bean plants were cut in sections and the upper leaves, lower leaves (primary leaves), stem and roots were separated. The roots were first carefully washed and stem samples were cut into pieces. Three replicate samples of 20 g each were collected from all test material (substrates and plant parts). All samples were put into plastic bags and stored at –20°C before being subjected to analyses. The samples were analysed using high-performance liquid chromatography – mass spectrometry (HPLC-MS) based on the protocol of Schaaf et al. (2000). All solvents used for extraction and analysis were HPLC grade. Analytical grade AZA, 3-tigloyl-azadirachtol, salanin and nimbin were provided by Trifolio-M GmbH (Lahnau, Germany) and used as the standard for the HPLC-MS analyses.

For extraction of the active neem ingredient residues from bean plants and planting substrates the frozen samples were cut into small pieces, i.e. leaves: 1 $cm^2$, stem and roots: 1 cm. These samples were immersed in 100 ml methanol each for 15 min and afterwards the solution was filtered using a fluted filter paper. The procedure was repeated three times. Then 150 ml water were added to the solution and afterwards transferred into a separating funnel and three times extracted with 50 ml dichloromethane. The methanol-water layer was discarded and the dichloromethane layers were collected, dried with magnesium sulphate and filtered (fluted filter paper). The extract was dried using a rotary evaporator (Typ RV05, IKA® Werke GmbH & Co KG, Germany; 400 mbar, room temperature), re-solubilised in 2 ml methanol and after an additional purification via syringe filters (13 mm, 0.2 µm nylon) analysed by HPLC-MS. Three replicates were done for each material at each sampling time.

Analyses were carried out with a Waters Alliance 2695 HPLC system (Waters Corp., Milford, MA, USA). A Luna 3u C18(2) HPLC column (50 x 2 mm, Phenomenex Ltd., Aschaffenburg, Germany) was used as stationary phase. At a flow rate of 0.2 mL $min^{-1}$ the mobile phase was 80% acetonitrile (containing 1% formic acid) and 20% acetonitrile (5 min isocratic). Over a period of ten minutes the percentage of acetonitrile was increased to 65% and kept for 5 min. To purge the column the amount of acetonitrile was raised to 100% within one minute. After 10 min purging the starting conditions were re-established within half a minute und and the column was reconditioned for 8.5 min. The HPLC system was connected to a Micromass LCT mass spectrometer (electrospray ionisation, time-of-flight mass determination) with a Lock Spray™ ion source (3000 V capillary voltage, 30 V sample cone voltage, 200°C desolvation temperature and 120°C source temperature; Micromass Ltd., Manchester, United Kingdom). The data were recorded and analysed with MassLynx™ 3.5 software (Micromass Ltd., Manchester, United Kindom).

***Biological Effects of NeemAzal-U on Western Flower Thrips***

The pots containing the two different substrates (CS, CS-sand) and one bean seedling each were covered individually with plexy glass cylinders (10 cm diameter x 30 cm high, AK Kunststoff Technik GmbH, Isernhagen, Germany). The cylinders were fixed to the pots using modelling clay to prevent the thrips from escaping. The top of the cylinders and six holes at the side for additional aeration were covered with nylon gauze (pore size ~ 64 µm, Heidland, Gütersloh, Germany). Two extra holes in the cylinder sides fitted with removable covers enabled the introduction of 20 uniformly-aged L1-larvae of *F. occidentalis* onto the leaves of each bean plant and the application of 100 ml NeemAzal-U solution. Two different concentrations with 0.30 and 0.59 g NeemAzal-U/l (50 and 100 mg AZA/l, 5.9 and 11.8 mg 3-tigloyl-azadirachtol/l, 4.45 and 8.9 mg salanin/l, 1.05 and 2.1 mg nimbin/l, respec.), and 0.59 g/l of the blank formulation as a control were applied to the substrates. In preliminary experiments no significant differences in thrips mortality were recorded between the blank formulation (0.59 g/l) and a tap water control. One hundred ml of each NeemAzal-U solution were applied one day before the introduction of the insects. Afterwards the bean plants were placed in a climate chamber (23±2°C,

50-60% RH, 18:6 h [L:D] photoperiod) for a period of eight days. Under these experimental conditions it takes eight days until almost all of the late L2-larvae migrate to the substrate for pupation (Berndt et al. 2004a). Therefore, after eight days the cylinders were removed and the bean plants were cut off. The pots were covered with photo eclectors, which consisted of inverted pots (11 × 7.5 × 8.5 cm) with a bases removed and four additional ventilation holes (diameter 2 cm) in the sides covered with nylon gauze (pore size ~ 64 µm). The removed bases of the pots were sealed with petri dish lids (diameter 8.5 cm) coated with insect glue (Temmen GmbH, Hattersheim, Germany) to trap WFT adults emerging from the soil. The two pots were sealed with modelling clay to prevent the insects escaping. The photo eclectors were kept for an additional 12 days in a climate chamber (see above). Trapped adults were counted every second day until no emerging adults were detected. Ten replicates per treatment were used, and the plexy glass cylinders and eclectors were arranged in a random pattern.

***Statistical Analyses***

The dissipation curves of the two substrates showing the residue data of active ingredients in two substrates were compared by means of analysis of covariance (ANCOVA) (Snedecor and Cochran 1989).

For the experiment on the biological effects of NeemAzal-U on WFT, the living thrips data was subjected to the Levene test to estimate variance homogeneity. The data were analysed by means of analysis of variance (ANOVA). Due to the factorial design of this experiment, the interaction effect was evaluated in addition to single factor effects. In case of significant F-values ($P<0.05$), treatment means were separated using Tukey test (Sokal and Rohlf 1995). All statistical analyses were conducted using SAS (SAS 1999).

## 2.3 Results

***Residues of Active Neem Ingredients in Substrates***

The average residues of AZA, 3-tigloyl-azadirachtol, salanin and nimbin (in mg/pot) in the CS and the CS-sand mixture over time are shown in Figure 2.2 and 2.3. The mean residue level found 1.5 hours (0d) after soil application in CS was 10.01 and 0.86 mg/pot for AZA and 3-tigloyl-azadirachtol, respectively and in the case of the CS-sand mixture 10.01 and 0.88 mg of AZA and 3-tigloyl-

azadirachtol/pot, respectively. In both substrate types, 99.9% of the applied dose of AZA, i.e. 10.02 mg/pot, was recovered 1.5 hours after the application of the treatment. For 3-tigloyl-azadirachtol in CS 73% and in CS-sand 75% of the initially applied quantity (1.18 mg/pot) were recovered (Figure 2.2). For salanin and nimbin the mean residue level found 1.5 hours after soil treatment using CS was 0.79 and 0.20 mg/pot, respectively, and on the substrate mixture 0.81 and 0.18 mg/pot, respectively. Related to the applied values of 0.89 mg salanin and 0.21 mg nimbin per pot, 88% and 93% were recovered in CS and 91% and 86% in CS-sand 1.5 hours after the treatment, respectively (Figure 2.3).

The neem ingredient residues found in the samples decreased exponentially according to the equation $Y = ae^{-bx}$, where *Y* is the amount of active ingredient present at time *x* (in days), *a* is the initial amount of ingredient at 0d and *b* is the dissipation constant (slope). With $R^2$-values ranging between 0.97 and 0.99 a good fit to the model of exponential decay was obtained. In case of AZA and 3-tigloyl-azadirachtol, the slope (b) of the dissipation curve was significantly greater in the case of the CS-sand mixture compared to CS (AZA: F=6.24; df=6, 29; P<0.0091; 3-tigloyl-azadirachtol: F=28.1; df=6, 29; P<0.0001). The DT50 (dissipation time 50%, i.e. the time required for one-half of the initial quantity of a pesticide to dissipate from a system according to the formula DT50 = LN(2)/b)) for CS was 12.16 days and times almost half of this were recorded in the CS-sand mixture with 6.58 and 5.05 days in the case of AZA and 3-tigloyl-azadirachtol, respectively (Figure 2.2). The slope of salanin's dissipation curve was significantly greater on CS compared to CS-sand (F=18.24; df=6, 29; P=0.0151) and for nimbin no significant differences in slope were recorded (F=15.00; df=6, 29; P=0.0770). For salanin a lower DT50 was recorded on CS (3.71 days) compared to CS-sand (5.11 days) and similar DT50 on both substrates for nimbin (CS: 5.68 days, CS-sand: 5.8 days) (Figure 2.3).

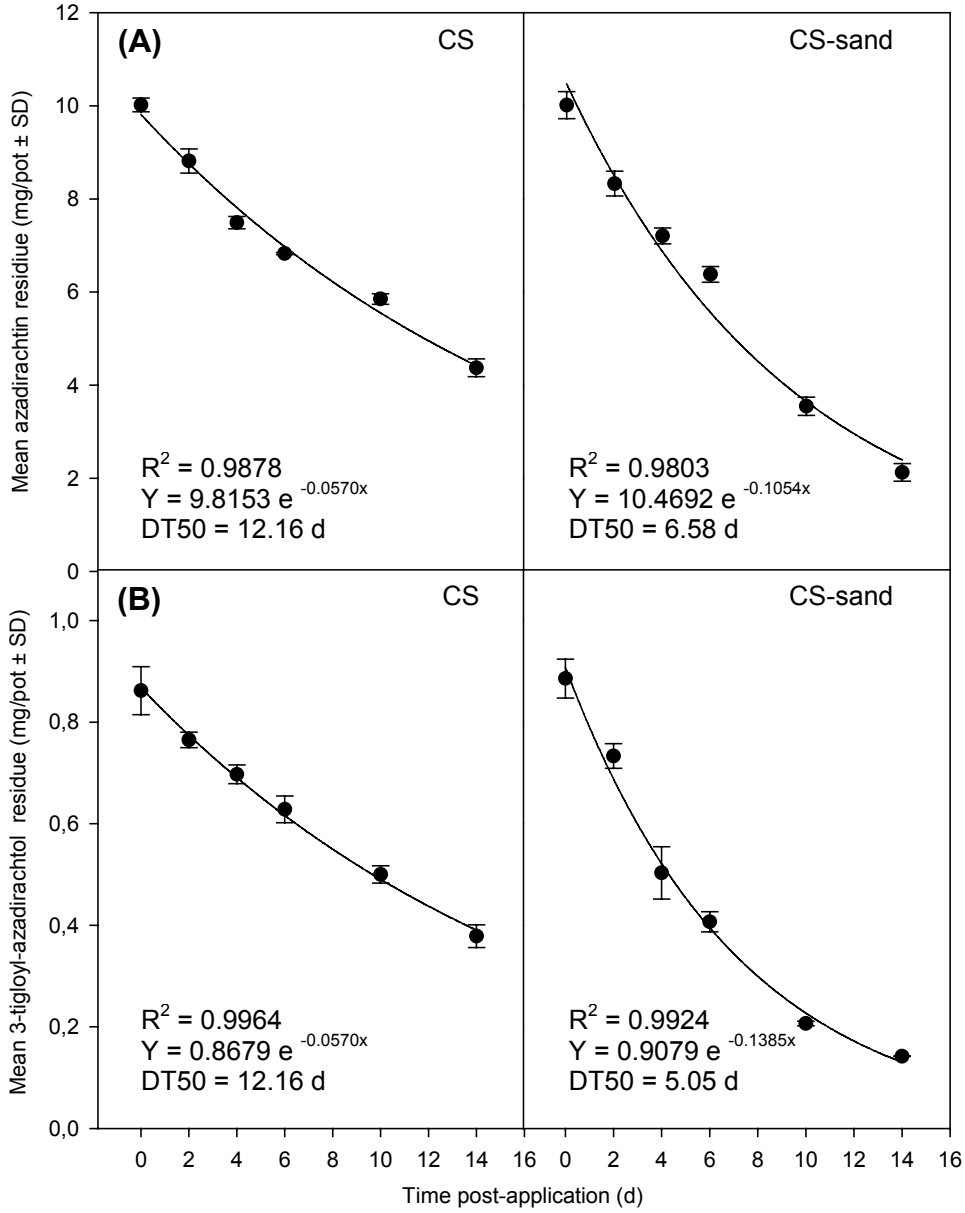


**Figure 2.2 Dissipation of AZA (A) and 3-tigloyl-azadirachtol (B) residues (mean ± SD) in the different substrates CS (156 g substrate/pot) and CS- sand mixture in ratio 1:1 (240 g substrate/pot) after soil application of NA–U (10 mg AZA and 1.2 mg 3-tigloyl-azadirachtol/pot) at different time intervals. The data were fitted to an exponential decay model, estimating regression factor ($R^2$) and dissipation time 50% (DT50), (n = 3).**

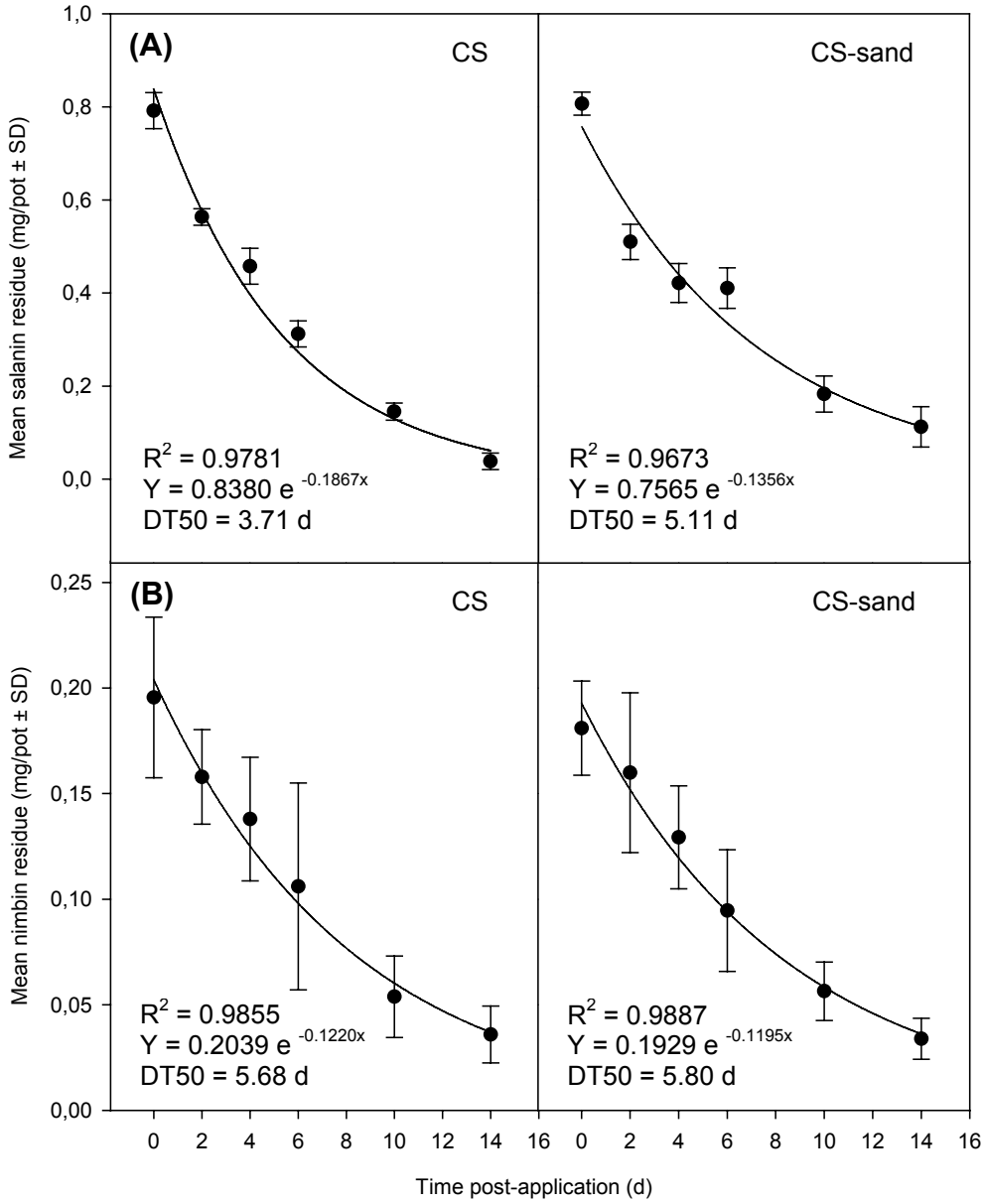


**Figure 2.3 Dissipation of salanin (A) and nimbin (B) residues (mean ± SD) in the different substrates CS (156 g substrate/pot) and CS- sand mixture in ratio 1:1 (240 g substrate/pot) after soil application of NA–U (0.89 mg salanin and 0.21 mg nimbin/pot) at different time intervals. The data were fitted to an exponential decay model, estimating regression factor ($R^2$) and dissipation time 50% (DT50), (n = 3).**

### *Residue of Active Neem Ingredients in Plants*

The mean residues of AZA and 3-tigloyl-azadirachtol (in µg/g fresh weight of sample) at different time intervals in roots, stem, upper and lower leaves after soil application of NeemAzal-U on two substrates are shown in Figure 2.4. The roots were able to incorporate only a very low percentage (1.1% – 6.5%) of the initial amount of active ingredients recovered in the substrate. Translocation within the bean plant was also low with a range between 0.3% and 8.1% from the initial amount of active components in the substrate (Table 2.2).

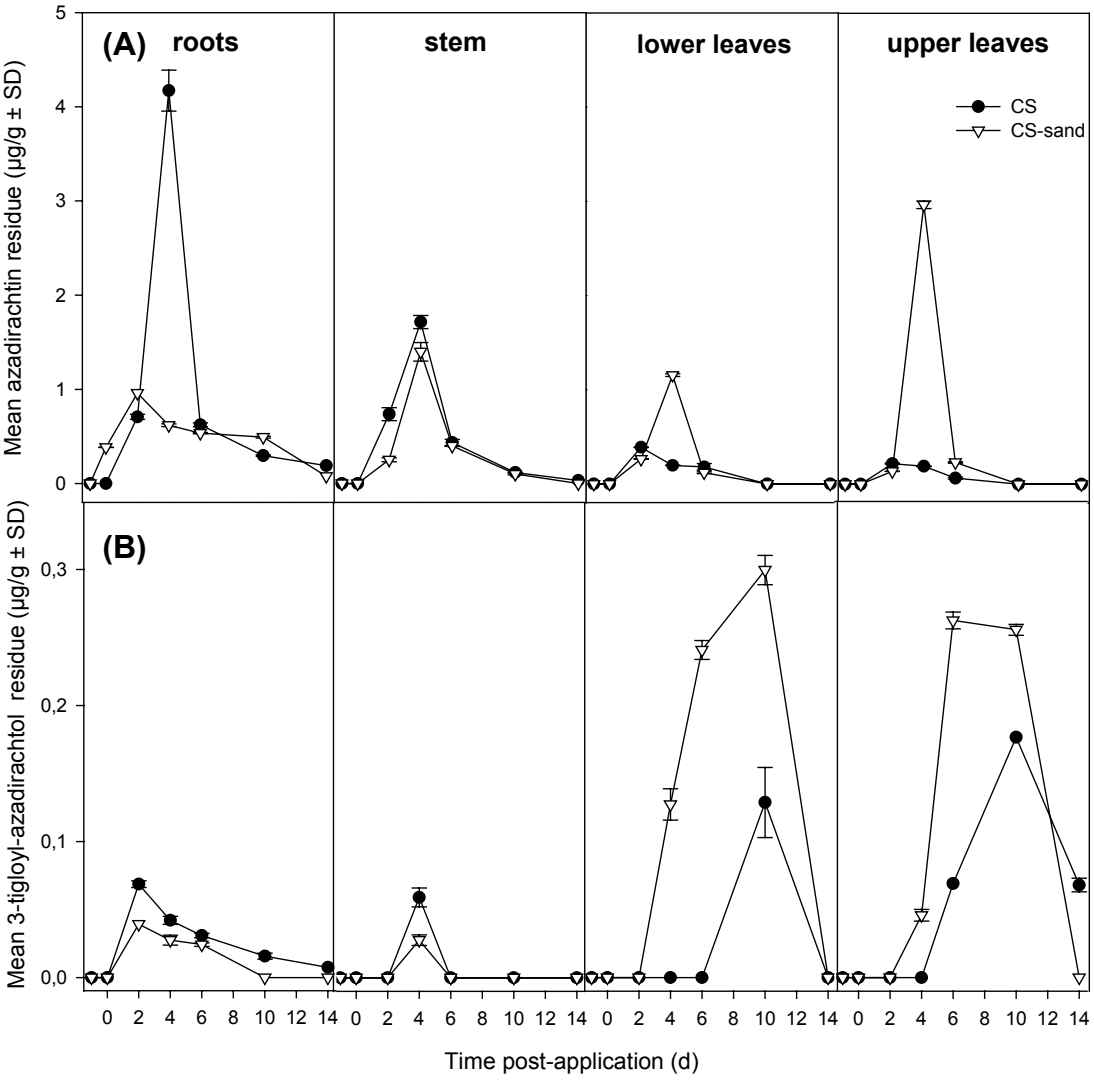


**Figure 2.4 Mean AZA (A) and 3-tigloyl-azadirachtol (B) residues in fresh weight of samples (µg/g ± SD) in successive plant parts of *Phaseolus vulgaris* plants at different time intervals on different substrates (CS, CS-sand) (n = 3).**

One and a half hours post treatment the average residue of AZA in roots of the CS-sand mixture was 0.38 µg/g. No residues were detected in any of the other plant parts in both substrates at this time. However, two and four days after application the maximum residue levels of AZA were detected in all plant parts from both substrates (Figure 2.4(A), Table 2.2). Peak residue levels of 3-tigloyl-azadirachtol from both substrates were recorded in roots on Day 2, in stems on Day 4 and in foliage at Day 6 or 10 (Figure 2.4(B), Table 2.2). After the peak a steady decline of the active component concentrations in all samples was observed. At Day 14 no active ingredients could be detected in all plant parts except for the roots (AZA: CS 0.191 µg/g, CS-sand 0.078 µg/g; 3-tigloyl-azadirachtol: CS 0.0075 µg/g) and 3-tigloyl-azadirachtol in the upper leaves from CS substrate (0.068 µg/g). The residues in roots and stems were usually higher in the CS than in the CS-sand system. In contrast, higher residues were detected in the foliage from the CS-sand mixture than from the CS substrate (Figure 2.4).

**Table 2.2 Maximum residue of AZA and 3-tigloyl-azadirachtol expressed in µg active ingredient per g fresh weight ± SD and in percentage of the initial amount of active ingredient in different substrates estimated at different time intervals.**

| | Mean* maximum residue | | | | | |
|---|---|---|---|---|---|---|
| | CS | | | CS-sand | | |
| **Azadirachtin** | (µg/g ± SD) | % | d | (µg/g ± SD) | % | d |
| Substrate | 64.21 ± 0.147 | 100 | 0 | 41.72 ± 0.291 | 100 | 0 |
| Roots | 4.17 ± 0.218 | 6.5 | 4 | 0.96 ± 0.001 | 2.3 | 2 |
| Stem | 1.71 ± 0.070 | 2.7 | 4 | 1.40 ± 0.010 | 3.4 | 4 |
| Lower leaves | 0.39 ± 0.001 | 0.6 | 2 | 1.16 ± 0.014 | 2.8 | 4 |
| Upper leaves | 0.21 ± 0.001 | 0.3 | 2 | 2.97 ± 0.042 | 7.1 | 4 |
| **3-Tigloyl-Azadirachtol** | | | | | | |
| Substrate | 5.53 ± 0.047 | 100 | 0 | 3.70 ± 0.038 | 100 | 0 |
| Roots | 0.07 ± 0.001 | 1.3 | 2 | 0.04 ± 0.001 | 1.1 | 2 |
| Stem | 0.06 ± 0.001 | 1.2 | 4 | 0.03 ± 0.001 | 0.8 | 4 |
| Lower leaves | 0.13 ± 0.033 | 2.4 | 10 | 0.30 ± 0.011 | 8.1 | 10 |
| Upper leaves | 0.17 ± 0.047 | 3.1 | 10 | 0.26 ± 0.001 | 7.1 | 6 |

***Average of three replicates**

No residues of nimbin were found in plant, and salanin residues were detected only in foliage. Salanin showed its maximum residues on CS two days after soil treatment in lower leaves with 0.014 µg/g, which is 1.7% from the initial amount of active ingredient in the substrate. Using CS-sand maximum residues were recorded four days after soil application in upper leaves with 0.071µg/g (8.8% from the initial amount of active component in CS-sand) (Table 2.3).

**Table 2.3 Mean salanin residues (µg/g fresh weight ± SD) in lower and upper leaves of *P. vulgaris* in different substrates estimated at different time intervals.**

| Days after treatment | Mean* salanin residue (µg/g ± SD) | | | |
|---|---|---|---|---|
| | CS | | CS-sand | |
| d | Upper leaves | Lower leaves | Upper leaves | Lower leaves |
| 2 | 0.007 ± 0.005 | 0.014 ± 0.002 | 0.006 ± 0.002 | 0.007 ± 0.001 |
| 4 | 0.006 ± 0.002 | 0.013 ± 0.003 | 0.071 ± 0.034 | 0.036 ± 0.048 |
| 6 | 0.003 ± 0.004 | 0.002 ± 0.001 | 0.021 ± 0.029 | 0.002 ± 0.001 |
| 10 | 0.002 ± 0.001 | 0.002 ± 0.001 | 0.001 ± 0.001 | 0.001 ± 0.002 |

***Average of three replicates**

### *Biological Effects of NeemAzal-U on Western Flower Thrips*

The numbers of thrips were significantly lower in the neem treatments than in the control. Significantly higher numbers of thrips were recorded for both substrate types after treatment with the low dose rate (50 mg AZA/l, 5.9 mg 3-tigloyl-azadirachtol/l, 4.45 mg salanin/l, 1.05 mg nimbin/l) compared to the high dose rate (100 mg AZA/l, 11.8 mg 3-tigloyl-azadirachtol/l, 8.9 mg salanin/l, 2.1 mg nimbin/l) ($F=147.97$; $df=5, 54$, $P<0.0001$). For both concentrations, soil treatment with NeemAzal-U resulted in significantly lower numbers of thrips in the CS-sand than in the CS system ($F=147.97$; $df=5, 54$, $P<0.0001$) (Figure 2.5). After application of low and high dose rates on the CS, corrected mortalities (Abbott 1925) of 57% and 86% were obtained, respectively. Yet in the CS-sand system 71% and 95% corrected mortality resulted from treatments with the same amounts of active ingredients, respectively.

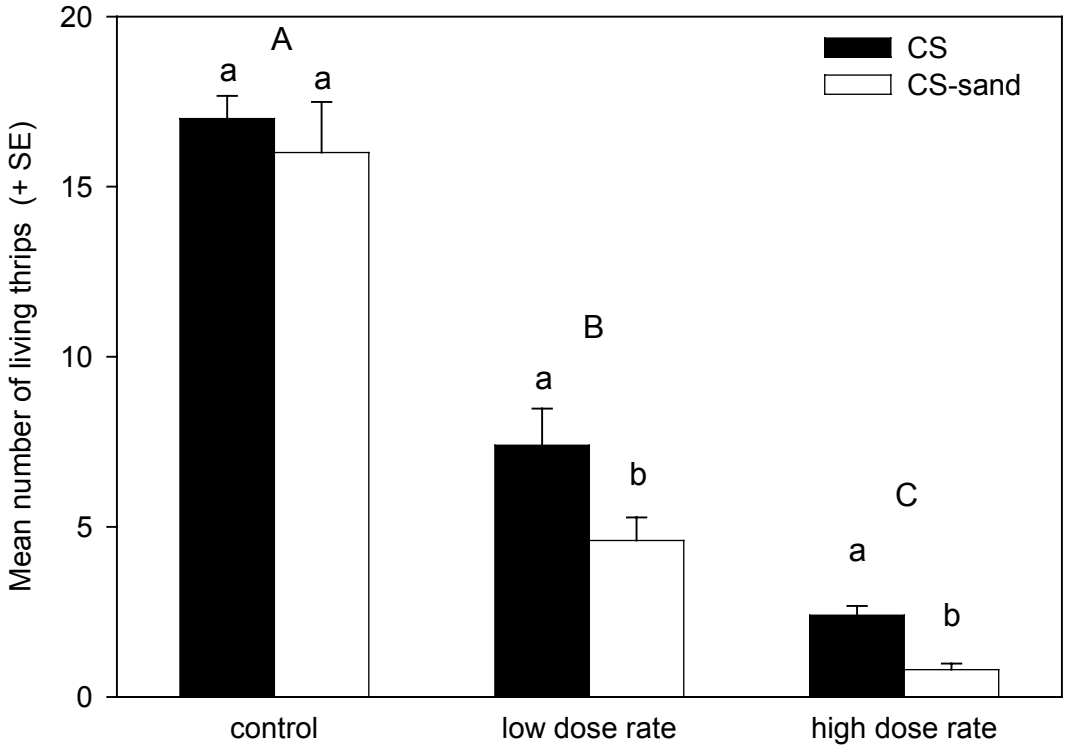


**Figure 2.5 Number of living thrips (mean + SE) after a soil treatment with two different NA-U dose rates (low: 50 AZA/l, 5.9 mg 3-tigloyl-azadirachtol/l, 4.45 mg salanin/l, 1.05 mg nimbin/l, high: 100 mg AZA/l, 11.8 mg 3-tigloyl-azadirachtol/l, 8.9 mg salanin/l, 2.1 mg nimbin/l) and a blank formulation as control on two substrates with different organic matter content (CS and CS-sand). Interactions between substrate type and dose rate were not significant. Consequently, dose rates were compared regardless of substrate type (upper case letter) and vice versa (lower case letter). Columns marked with the same letter are not statistically different, P>0.05.**

## 2.4 Discussion

### *Residues of AZA and 3-Tigloyl-Azadirachtol in Different Substrates*

Considering the DT50 and the rate of decrease of AZA and 3-tigloyl-azadirachtol in our test substrates, both tetranortripenoids degraded in a similar fashion. Stark and Walter (1995a) reported similar results on the persistence of AZA and 3-tigloyl-azadirachtol in soils under comparable experimental conditions. However, it cannot be excluded that under different environmental conditions other dissipation trends of AZA and 3-tigloyl-azadirachtol will occur

(Thompson et al. 2004). Moreover, we recorded differences in the decrease of the tested tetranortripenoids comparing the CS-sand mixture and the pure CS. The persistence of AZA applied to different soil types varied considerably in terms of DT50 within different studies (Sundaram et al. 1995, 1997, Pussemeier 2000). The degradation of AZA is influenced by several environmental parameters such as microbial activity, temperature, pH, light intensity and physical soil properties such as organic matter content (Stark and Walter 1995a, Pussemeier 2000, Barrek et al. 2004). This could explain the variation in persistence in different substrates. Obviously the content of organic matter in soil/substrate has a strong influence on the rate of degradation of azadirachtin components. We recorded a significantly slower decrease of AZA and 3-tigloyl-azadirachtol using the pure CS with higher organic content compared to a faster dissipation in the CS-sand mixture, which had a smaller amount of organic matter. Sundaram et al. (1995, 1997) reported similar results: a soil with higher organic matter content (72%) led to an increased DT50 (25.77days) compared to a soil with 3.5% organic matter where a DT50 of only 30.3 hours was recorded. Soils with low organic matter content result in higher rates of leaching and lower absorption of AZA (Sundaram 1996b, Pussemeier 2000), which could explain these findings.

***Residue of AZA and 3-Tigloyl-Azadirachtol in Different Plant Parts***

Compared to the high amounts of AZA and 3-tigloyl-azadirachtol found in both substrates, the residues in the different plant parts were very low. A maximum recovery of 8% of the initial quantity was measured in the substrate, indicating a low efficiency of uptake and translocation of the tetranortripenoids from soil into the plant. In aspen plants similar results with maximum recovery in plant of only 5% of the applied AZA amount were recorded after a neem soil treatment (Sundaram et al. 1995).

In contrast to identical dissipation trends of AZA and 3-tigloyl-azadirachtol in the growing substrate, the translocation and/or persistence of both components in bean plants *Phaseolus vulgaris* seem to differ depending on the plant part. AZA showed a relatively fast translocation into all plant parts with peaks only two to four days after soil application, whereas 3-tigloyl-azadirachtol peaked at the same time in roots and stems but later (Day 6 or 10)

in the leaves. As we know of no other studies explaining this different distribution pattern it can be concluded at this time that the different molecular structures of both substances may be responsible for that phenomenon. Studies on the distribution of AZA and 3-tigloyl-azadirachtol in insect tissue show a specific binding of each molecule to different membrane proteins according to the chemical structure. This was demonstrated in several studies, e.g. with the locust *Schistocerca gregaria* Forskål (Orthoptera: Acrididae) (Nasiruddin and Mordue 1994, Nisbet et al. 1995, Mordue et al. 1998, Sharma et al. 2003). The specific translocation of AZA and 3-tigloyl-azadirachtol into the plant after soil treatments resulted in a different temporal distribution of the peak concentrations of both ingredients. This might result in a synergistic effect and explain the relative long effects on feeding insects of at least 14 days.

Moreover, the substrate type influenced the translocation of the tested neem ingredients from the soil to the foliage. In roots and stems the highest residues of AZA and 3-tigloyl-azadirachtol were recorded using the pure CS. In foliage the maximum amounts of both substances were measured from plants grown in the CS-sand mixture. A convincing explanation for this phenomenon is difficult on the basis of our data. We suppose that the amount of uptake is mainly dependent on the available “free” neem ingredient in the rhizosphere and available water. Due to the property of organic matter in substrate/soil to absorb AZA the active ingredients may be more tightly bound and accumulated in CS (Sundaram 1996b, Pussemeier 2000). This could have facilitated a slow release from organic matter to rhizosphere and limited uptake by roots. The translocation on the other hand from roots to leaves should, as it is known from the behaviour of pesticides, travel in the xylem vessels and therefore depend on evaporation (Scheunert 1992). This is strongly related to the gradient of water from soil to the microclimate around foliage. With more available water in the CS–sand mixture, a faster transport to the leaves could have occurred which may explain the quicker removal from the roots and the higher accumulation in the leaves.

***Residue of Salanin and Nimbin in Substrate and Plant***

In our study NeemAzal-U concentrations were used, which should represent dose rates for practical use. These application rates contained only AZA and 3-

tigloyl-azadirachtol in adequate amount for analysable residue evaluation concerning the used analytic method. Thus, the residue analyses could result in exact residue data of active ingredient in different plant parts and substrate after soil treatments for AZA and 3-tigloyl-azadirachtol only. Nevertheless, salanin and nimbin should be noted as well. As expected, it was not possible to record evaluable data of salanin and nimbin residue in all plant parts and over the total period of 14 days. No nimbin residues were detected in plant, and salanin were recorded only in foliage. Similar to the results of AZA and 3-tigloyl-azadirachtol a trend of higher salanin residues in foliage was recorded using the substrate mixture compared to the pure substrate. Whereas, in substrate salanin and nimbin showed different dissipation behaviour compared to AZA and 3-tigloyl-azadirachtol. A strong influence of organic matter on the degradation of salanin and nimbin as recorded for the both other ingredients were not detected. Studies on photooxidation of different tetranortriterpenoids of the neem tree indicated that salanin and nimbin were much more unstable and degraded faster than azadirachtin (Jarvis et al. 1997). This is indicated in comparable low DT50 ranging from 3 to 5 days for salanin and nimbin in our studies. Results on structure related activity of salanin and nimbin in comparison to AZA using different pest insects are conflictive so far (e.g. Govindachari et al. 1996, Aerts and Mordue 1997, Jarvis et al. 1997). In general, only few data on these two bioactive neem ingredients, their bioanalytical and physiological properties, environmental behaviour and structure related activity are available. Further residue studies on soil-applied salanin and nimbin in adequate dose rates - irrespective of NeemAzal-U - are required for a proper interpretation of the existing facts. Nevertheless, the recorded salanin amounts in foliage from day two to ten after a NeemAzal-U soil application demonstrated a translocation of salanin in bean plants.

***Biological Effects of NeemAzal-U on Western Flower Thrips***

The efficacy of the substrate treatments against WFT was strongly influenced by the amount of organic matter in the growing substrate regardless of the active ingredient dose rate. Thrips mortality was higher using the CS-sand mixture compared to pure CS. This correlated with the higher residue amounts of AZA, 3-tigloyl-azadirachtol and salanin in foliage, the main feeding sides of

WFT on bean plants. Several studies on systemic effects of neem extracts against different insect species as Orthoptera, Homoptera and Thysanoptera indicated similar results with higher mortalities on substrate with lower organic matter (Gill and Lewis 1971, Oßiewatsch 2000, Thöming et al. 2003). As shown above the amounts of neem ingredients in substrate, roots and stem were lower using the CS-sand mixture compared to CS. However, the higher residues in foliage correspond well with the higher thrips mortality. Unfortunately, so far little is known about the metabolic pathways of the active neem components in plants making it difficult to properly interpret our findings.

Our studies demonstrated that substrate applied AZA and 3-tigloyl-azadirachtol are taken up by roots and translocated acropetally to upper plant parts. A good control efficacy against *F. occidentalis* after a soil treatment indicated systemic properties of neem ingredients. Even though only relatively low proportions of the applied amount of active components were translocated into the plant, it was sufficient to cause high thrips mortalities. These findings may lead to an improvement of the control strategies for plant-feeding pests in general and thrips in particular. However, a considerable lack of knowledge on the metabolic pathway of active neem ingredients and of the dynamics of their translocation in particular at the feeding sites still exists and warrants further research.

# 3 Controlling *Frankliniella occidentalis* with Azadirachtin as Soil Application and the Predatory Mites, *Amblyseius cucumeris* and *Hypoaspis aculeifer* in a Combined Treatment[2]

## 3.1 Introduction

The use of azadirachtin (AZA), the main active component of the neem tree *Azadirachta indica* A. Juss. (Melaiceae), for pest control in horticultural crops has mainly focused so far on spray applications on above ground plant parts. Several studies with a topical application of active neem ingredients have shown varying degrees of impact on many important pests (e.g. Schmutterer 1990, Pearsall and Hogue 2000). The basic mode of action seems to be the inhibition of release of prothoracicotropic hormones, allatotropins and allotoinhibins (Banken and Stark 1997, Gonzales et al. 1999). However, the relatively short persistence of AZA due to its sensitivity to temperature, UV light and pH-value (Barrek et al. 2004) make frequent spray applications necessary to achieve an efficient control. Especially for pests such as thrips, which can rapidly build up again from non-treated plant parts or re-colonize from refuges such as soil (Saxena and Kidiavai 1997, Immaraju 1998, Chiasson et al. 2004). Furthermore, spraying of neem products on above ground plant parts and/or the soil surface will contaminate the foraging patches of natural enemies dwelling in the crop canopy. This in turn affects their efficacy if released as a combined (integrated) treatment. Several studies reported detrimental effects of neem products on non-target organisms such as *Amblyseius cucumeris* Oudemans (Acari: Phytoseiidae), *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) and *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) if direct contact occurred (Spollen and Isman 1996, Tedeschi et al. 2001). A soil application of AZA may allow targeting the plant-feeding developmental stages of herbivorous pests in a more precise and selective fashion through a systemic effect. Soil-dwelling stages of pests are directly targeted while plant-dwelling non-target organisms will be unharmed. Systemic effects of AZA were verified in several studies (Gill and Lewis 1971, Weintraub and Horowitz 1997, Thöming

[2]based on: Thoeming G and Poehling H-M. Controlling *Frankliniella occidentalis* (Thysanoptera: Thripidae) with azadirachtin as soil application and the predatory mites, *Amblyseius cucumeris* (Acari: Phytoseiidae) and *Hypoaspis aculeifer* (Acari: Laelapidae) in a combined treatment. Submitted to Environmental Entomology.

et al. 2003). Therefore, making use of systemic translocation of active neem components in plants could not only be an option for the selective management of thrips but also for other organisms feeding cryptically on multiple plant parts thereby escaping control by natural enemies (Sundaram et al. 1995).

The Western Flower Thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) is a key pest in many crops by directly causing plant damage from sucking on flowers, leaves and fruits and indirectly through transmission of plant pathogenic virus such as Tomato Spotted Wilt Virus (van Rijn et al. 1995, Lewis 1997a, van de Wetering et al. 1999). WFT oviposits in almost all the above ground plant parts and first and early second instars feed on plant material in a cryptic manner. Late second instars stop feeding and the majority then migrates to the ground for pupation. Prepupae are slightly mobile, pupae are motionless and both pupal stages do not feed (Kirk 1997, Moritz 1997, Berndt et al. 2004a). Currently, insecticides containing imidacloprid or spinosad® can control WFT (Ishaaya et al. 2001, Riley and Pappu 2004, Jones et al. 2005). Nevertheless, efficient chemical thrips control is difficult because of their cryptic feeding behaviour, high mobility, soil-dwelling life stages and short generation time combined with high fertility. Particularly in vegetables, insecticide use limits their marketing (waiting periods), raises concerns in consumers because of residues and enhances the risk of selecting resistant biotypes (Zhao et al. 1995, Espinosa et al. 2002). Some promising but greatly varying results in biological control of WFT have been achieved using predatory mites or bugs such as *Amblyseius* spp. and *Orius* spp. (Hemiptera: Anthocoridae) (Castané et al. 1999, Shipp and Wang 2003). Furthermore, soil-dwelling thrips stages can be attacked by entomopathogentic nematodes such as *Steinernema* and *Heterorhabditis* spp. (Rhabditida: Steineranematidae, Heterorhabditidae) or ground-foraging predatory mites of the genus *Hypoaspis* (Acari: Laelapidae) (Ebssa et al. 2004, Berndt et al. 2004b). But at present, natural enemies of plant- and soil-dwelling life stages of WFT alone seldom provide sufficient and reliable thrips control (Parella 1995, Blaeser et al. 2004).

Preceding laboratory studies on systemic effects of AZA against WFT have shown, that a soil application of the commercial neem formulation NeemAzal®-T/S (1% AZA, Trifolio-M GmbH, Lahnau, Germany) affected plant-sucking life stages of *F. occidentalis* systemically, soil-dwelling pupal stages

directly and migrating late second instars in a repellent way (Thöming et al. 2003). Thus, soil applied neem products could become an additional building block for a more reliable integrated strategy with greater efficacy, especially in conjunction with antagonists such as *A. cucumeris* and/or *Hypoaspis aculeifer* Canestrini. These are two commercially available predatory mites that feed on plant- and soil-dwelling developmental stages of thrips. However, little is known about efficiency and tolerability of such mutual treatments. Thus, the objective of the study was to evaluate the potential of the combination of soil applied AZA with predatory mites for integrated WFT management. Special emphasis was laid on synergistic or antagonistic effects under laboratory and greenhouse conditions.

## 3.2 Material and Methods

### *Western Flower Thrips, Predatory Mites and Host Plants*

*F. occidentalis* were reared in glass jars in a climate chamber (23±2°C, 50-60% RH and 18:6 h [L:D] photoperiod) using pods of French beans (*Phaseolus vulgaris* L., cultivar Marona, Fabacea) as host plant. The rearing procedure was based on the protocol of Berndt et al. (2004a) to acquire uniformly aged life stages of *F. occidentalis* for experiments. As antagonists two commercially available predatory mites were selected: *A. cucumeris* (Katz Biotech AG, Baruth, Germany), a predator feeding on plant-dwelling young larval stages of WFT, and *H. aculeifer* (ÖRE Bio-Protect GmbH, Raisdorf, Germany), a ground-foraging predatory mite feeding on soil-dwelling pre-pupae and pupae.

Bean seeds were pre-germinated for three days and afterwards planted in plastic seedling trays (50 × 30 × 6.5 cm) filled with a commercial growing substrate (CS) commonly used in nurseries (Fruhstorfer Erde, Type P, Archut GmbH, Lauterbach-Wallenrod, Germany, 50% peat, 35% clay, 15% humus; organic matter (OM) 39.47%; pH 5.7-6.3). The trays were placed in a greenhouse (22±2°C, 65-75% RH, 18:6 h [L:D] photoperiod) for six days until the primary leaf stage.

### *Neem Preparation*

In the AZA treatment of the substrate, NeemAzal-U (NA-U, 17% AZA) (Trifolio-M GmbH, Lahnau, Germany, registration intended), a new water-soluble

powder especially developed for hydroponics and soil treatments, was used. NeemAzal-U is more convenient for root uptake and maintaining substrate quality as compared to NeemAzal®-T/S. This product contains high amounts of oil (51%) and tenside (45%), which can accumulate in the substrate and thereby result in negative effects on roots, plant growth or substrate properties if used as soil application.

***Microcosm Experiment 1***

Bean seedlings in their primary leaf stage were transplanted singly into plastic pots (11 × 7.5 × 8.5 cm) filled with the CS "Fruhstorfer Erde" as substrate or a mixture of the pure substrate with sand in ratio 1:1 (CS-sand) (OM 8.46%). Microcosms were constructed by covering the pots with plexy glass tubes (10 cm diameter x 30 cm high, AK Kunststoff Technik GmbH, Isernhagen, Germany), which were fixed to the pots using plasticine. For air ventilation the top and additional holes at the side of the cylinders were closed with nylon tissue (pore size ~ 64 µm, Heidland, Gütersloh, Germany). Two removable lids allowed the introduction of WFT, predatory mites as well as the application of 50 ml NeemAzal-U solution (100 mg AZA/l, i.e. 0.59 g NA-U/l) and the equal volume of 0.59 g/l blank formulation as control. NeemAzal-U (N) was applied one day before 25 uniformly aged first instars of WFT, together with either none, three and/or ten predatory mites (A3, A10 = 3, 10 *A. cucumeris*, H3, H10 = 3, 10 *H. aculeifer*), were placed in the microcosms. Thrips larvae and *A. cucumeris* were put on bean leaves and *H. aculeifer* on the surface of the substrate. All possible single (N, A3, A10, H3, H10) and combined treatments of antagonists and NeemAzal-U (A3H3, A3H10, A10H3, A10H10, A3N, H3N, A10N, H10N, A3H3N, A3H10N, A10H3N, A10H10N) were tested. The microcosms were kept for eight days in a climate chamber (23±2°C, 50-60% RH and 18:6 h [L:D] photoperiod). Previous studies have shown that under such conditions it takes eight days until nearly all late second instars (~ 98%) of WFT had left the plant for pupation in the substrate (Berndt et al. 2004a). Then the cylinders were removed, the bean plants cut off and the thrips and foliage-dwelling predatory mites on the above ground plant parts were counted under a binocular microscope. For extraction of the soil-dwelling arthropods from the substrate a combination of photo-eclector and modified Berlese apparatus (Wiethoff et al.

2004) was used within a period of 14 days extraction time in a climate chamber (23±2°C, 50-60% RH and 18:6 h [L:D] photoperiod). After 14 days the trapped WFT adults and predatory mites were counted under a binocular microscope. Eight replicates per treatment were set up in which the microcosms and eclectors were arranged in a completely randomized design.

***Microcosm Experiment 2***

In this trial the effects of AZA application time in conjunction with the release of predatory mites on WFT and antagonists were studied. 50 ml NeemAzal-U solution (see above) was applied to the substrate, i) at five and three days before (5db, 3db), ii) on the same day (0d) and iii) three and five days after (3da, 5da) placing 25 L1 of WFT and three *A. cucumeris*, three *H. aculeifer* or no predator in the microcosms. The control plants were treated with 0.59 g/l blank formulation together with the 0d-treatment. The remaining experimental protocol was identical to that described for Experiment 1.

***Microcosm Experiment 3***

In the following trial the impact of AZA-treated substrate on the reproduction of the soil-dwelling predatory mite *H. aculeifer* was studied. Microcosms were constructed as described in Trial 1. The pure substrate was drenched with 50 ml NeemAzal-U solution (see Experiment 1) three days before (3db), on the same day (0d) and three days after (3da) placing 25 first instars of WFT and five *H. aculeifer* (three females, two males) in the microcosm. The control plants were treated with 0.59 g/l blank formulation on the same day the arthropods were introduced (0d-treatment). The microcosms were kept 8, 16 or 24 days in a climate chamber (see above). Every fifth day, seven first instars of WFT were introduced to the microcosms to provide an adequate food source. After 8, 16 or 24 days, respectively, the cylinders were removed, the bean plants cut off and the substrate transferred into a combination of photo eclector and modified Berlese apparatus. After that it was cultivated for an additional 14 days in a climate chamber. In this way the predatory mites had a total reproduction time of 22, 30 or 38 days, respectively, after their release into the microcosms. The remaining experimental protocol was identical to that described for Microcosm Experiment 1.

***Greenhouse Experiments***

Two bean seedlings per pot were transplanted at the primary leaf stage into plastic pots (11 × 7.5 × 8.5 cm) filled with the CS or CS-sand mixture. The bean plants were cultivated for six weeks in plant cages (0.6 x 0.6 x 1.1 m, 5 pots per cage) in a greenhouse. The back and the bottom of the cage consisted of plywood sheets, the top of plexy glass and the sidewalls of nylon tissue (pore size ~ 64 µm). On the first experimental day (d 1) 20 L1 and five adults of WFT were placed in each pot. AZA treatments were split into three application schedules with changing application rates and times as presented in Table 3.1. Three *A. cucumeris* and/or *H. aculeifer* per pot were released on Day 4 and 17 (d 4, d 17) to the foliage or the substrate, respectively. All possible single (N, A, H) and combined treatments of predatory mites and AZA (AH, AN, HN, AHN) were tested. Leaf samples were taken once a week by removing one leaf per plant before an AZA application on Day 3, 9, 16, 23, 30, 37. WFT were then counted under a binocular microscope. The bean plants were watered every day and fertilized weekly (Wuxal Super, NPK 8/8/6, Spezialdünger GmbH & co. KG, Düsseldorf, Germany). Air temperature and humidity (Tinytag Plus, -30°C - +50°C; 0–100% RH) and substrate temperature (Tinytalk, -40°C - +75°C, Gemini Dataloggers (UK) Ltd, Chichester, West Sussex, England) were measured during the entire experiment (air: 26±6°C, 50-70% RH, substrate: 24±2°C). After six weeks the bean plants were cut off and the substrates were extracted with the photo-eclector-Berlese apparatus combination for 14 days as previously described. The above ground parts of the bean plants were destructively sampled and plant weight, pod number and pod weight were recorded.

**Table 3.1 Application schedules of the AZA treatments with different dose rates and application times for the three greenhouse trials.**

| | AZA dose rates | | | Application times | |
|---|---|---|---|---|---|
| | Per application | | Per week | Time interval | Exp. day |
| | (mg AZA/l) | (mg NA-U/l) | (mg AZA/kg substrate) | | (d) |
| Trial 1 | 100 | 590 | 64 | Weekly | 3, 9, 16, 23, 30 |
| Trial 2 | 50 | 295 | 32 | Weekly | 3, 9, 16, 23, 30 |
| Trial 3 | 100 | 590 | 32 | Biweekly | 3, 16, 30 |

***Statistical Analysis***

For the microcosm trials, percent WFT mortalities were computed per treatment and mortality data were corrected for mortality in the control treatment (Abbott 1925). Percent mortalities were subjected first to Levene test to evaluate for variance homogeneity and then transformed using arcsine-square root transformation if necessary. Differences among treatments were assessed by analysis of variance (ANOVA). For factorial experiments, the interaction effect was evaluated additionally to single factor effects. If significant F-values were obtained ($P<0.05$), treatment means were compared using Tukey's test (Sokal and Rohlf 1995).

In the greenhouse trials, the numbers of thrips per leaflet were subjected to Levene test to evaluate for variance homogeneity, and transformed using log-transformation if necessary. Differences among treatments over the entire experimental period were analysed by a repeated measurement analysis of variance (ANOVA) using AUC (Area Under the Curve) estimation and the interaction effect was evaluated additionally to single factor effects. In the case of significant F-values ($P<0.05$), treatment means were compared using Tukey's test (Sokal and Rohlf 1995). Additionally corrected efficacy was calculated (Henderson and Tilton 1955).

Differences among soil arthropod densities in microcosm and greenhouse experiments were analysed with Wilcoxon-Mann-Whitney test for pairwise comparison (Wilocoxon 1945, Mann and Whitney 1947) (Microcosm Experiment 1) or analysis of variance (ANOVA) followed by Tukey's test (Sokal and Rohlf 1995) (Microcosm Experiment 2, 3, Greenhouse Experiments).

Differences among the treatments considering the plant evaluation were assessed by multiple analysis of variance (MANOVA). Where significant F-values were obtained ($P<0.05$), treatment means were discriminated using Tukey' test (Sokal and Rohlf 1995). All statistical analyses were performed using the statistical package SAS (SAS 1999).

## 3.3 Results

***Microcosm Experiment 1***

All treatments caused significantly higher thrips mortality compared to the control, in which mortality did not exceed 15% in any of the microcosm

experiments (F=18.81; df=17, 110; P<0.0001). No differences in corrected mortality (CM) among the single predatory mite treatments (A3, A10, H3, H10) and between the different combinations of *A. cucumeris* and *H. aculeifer* (A3H3, A3H10, A10H3, A10H10) were recorded (Figure 3.1(A), 3.2(A)). Using CS the combination of ten *A. cucumeris* with three and ten *H. aculeifer* (A10H3, A10H10) showed significantly higher CM with up to 85% compared to the single predator treatments that resulted in CM between 23% - 41% (A3, H3, H10) (F=18.81; df=17, 110; A10H3: P=0.0004, P<0.0001, P=0.0133, A10H10: P=0.0123, P=0.0030, P=0.0300, respectively). An exception occurred for high densities of *A. cucumeris* reaching 51% CM (A10) (F=18.81; df=17, 110; A10H3: P=0.1904, A10H10: P=0.3209) (Figure 3.1(A)). Using the CS-sand mixture, all predator combinations (A3H3, A3H10, A10H3, A10H10) showed significant higher CM than single treatments in low densities (A3, H3) (F=18.65; df=17, 111; A3: P=0.0254, P<0.0001, P=0.0004, P<0.0001; H3: P=0.0101, P<0.0001, P=0.0001, P<0.0001, respectively) (Figure 3.2(A)). The substrate drenching with NeemAzal-U alone caused significantly higher CM compared to the release of three predatory mites per pot (A3, H3) (F=18.81; df=17, 110; A3: P=0.0253, H3: P=0.0099) in CS and to all treatments with one predatory mites species (A3, H3, A10, H10) using CS-sand mixture (F=18.65; df=17, 111; A3: P=0.0002, H3: P=0.0014, A10: P=0.0039, H10: P=0.0139). Moreover, AZA combined with the release of predatory mites (single or mixed) showed higher CM than treatments without AZA. High densities of single predatory mites or predator combinations together with NeemAzal-U (A10N, H10N, A3H3N, A3H10N, A10H3N, A10H10N) resulted in CM of 91%-100% (Figure 3.1(A), 3.2(A)). The single AZA treatment showed significant higher CM using the substrate mixture compared to the pure substrate (F=5.81; df=1, 14, P=0.0302). Otherwise, no significant differences were recorded between the two substrates. As for the survival of predatory mites, no significant differences in recapture rates of *A. cucumeris* and *H. aculeifer* between substrate drenching with NeemAzal-U and the control treatment were recorded (CS: A3: P=0.0901, A10: P= 0.0818, H3: P=0.2651, H10: P=0.5912; CS-sand: A3: P=0.0523, A10: P= 0.0578, H3: P=0.2668, H10: P=0.9220), regardless of the amount of organic matter in the substrate (Figure 3.1(B), 3.2(B)).

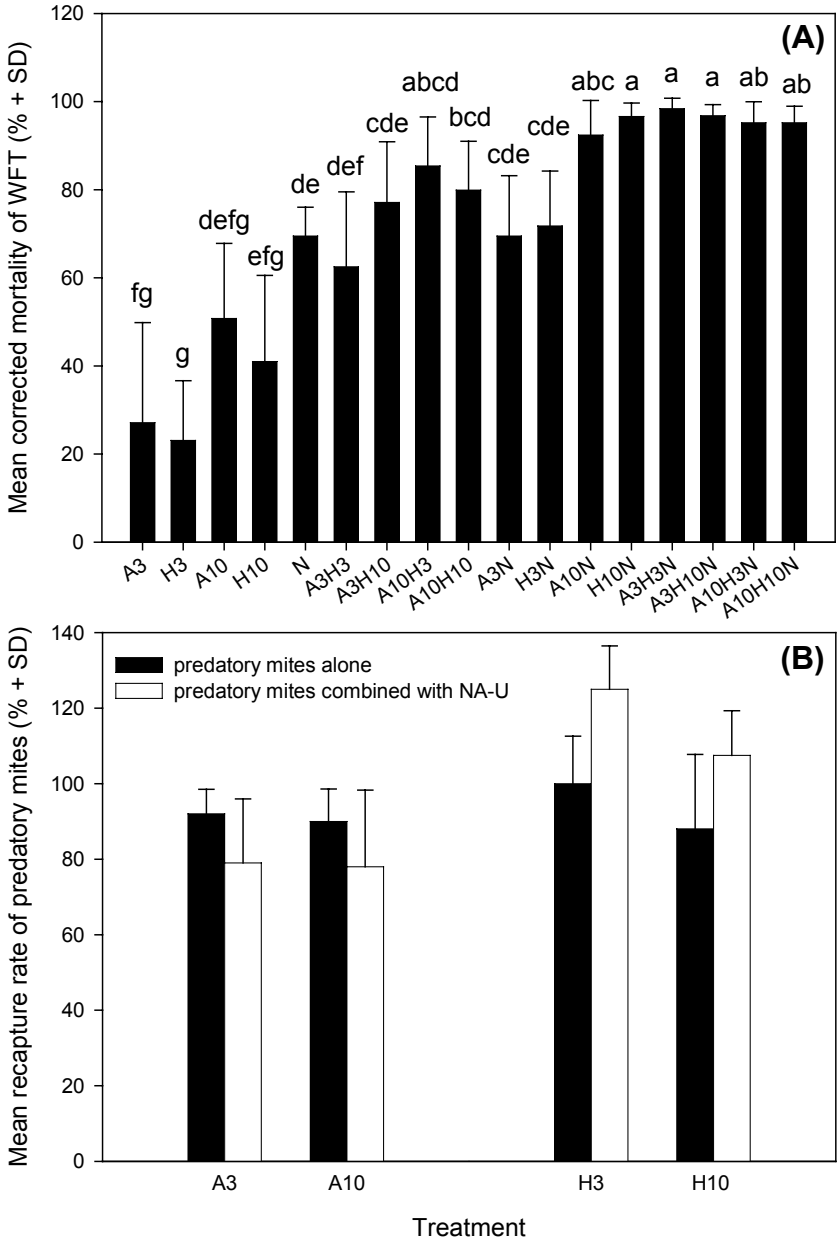


**Figure 3.1 Mean corrected mortality (% + SD) of WFT caused by single treatments of predatory mites in rates of 3 or 10 mites/pot (A3, A10, H3, H10), NA-U (N, 0.59 g NA-U/l) or 0.59 g/l blank formulation applied to CS with 39.47% OM (50 ml/pot). All possible combinations of antagonists and AZA follow single treatments (A). Mean recapture rate of predatory mites (% + SD) with and without neem substrate drenching (B). Bars marked with different letters are statistically different, P>0.05.**

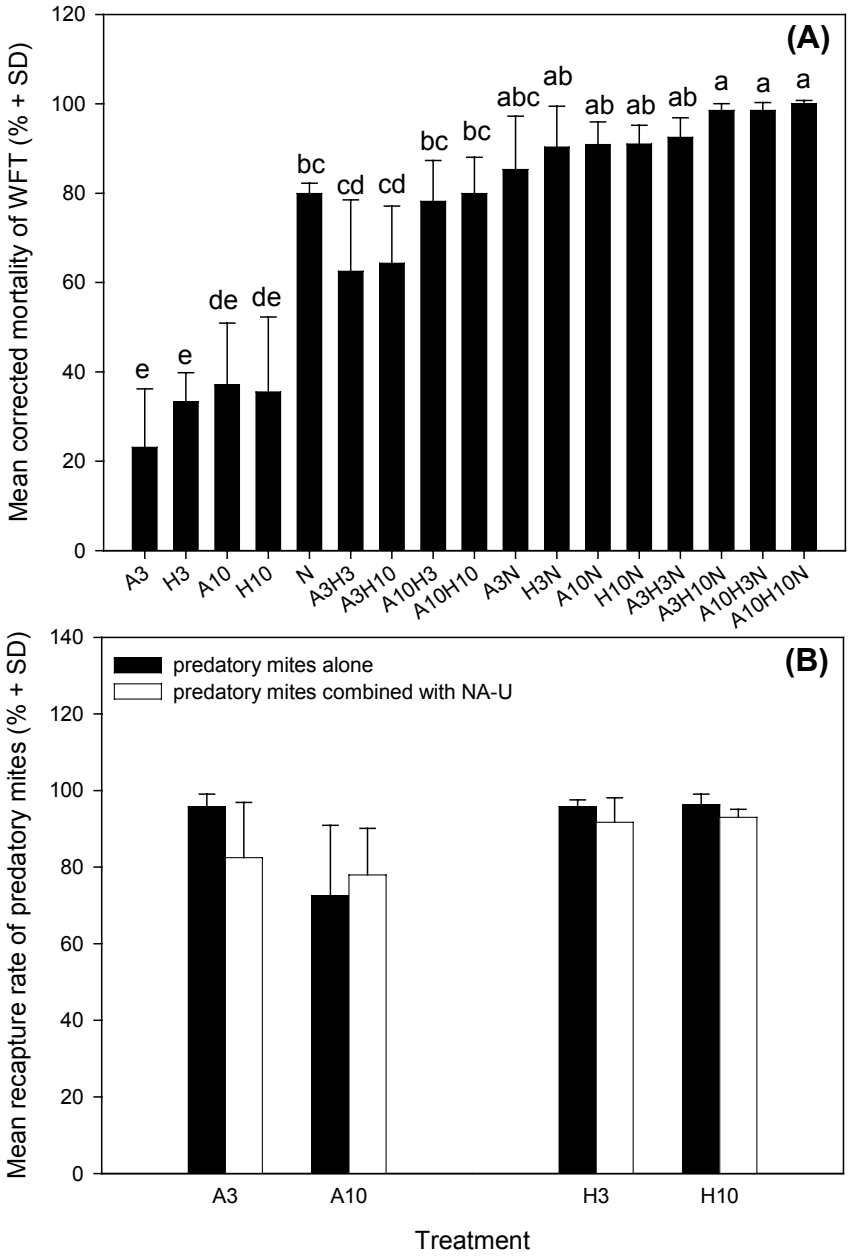


**Figure 3.2 Mean corrected mortality (% + SD) of WFT caused by single treatments of predatory mites in rates of 3 or 10 mites/pot (A3, A10, H3, H10), NA-U (N, 0.59 g NA-U/l) or 0.59 g/l blank formulation applied to CS-sand mixture with 8.46% OM (50 ml/pot). All possible combinations of antagonists and AZA follow single treatments (A). Mean recapture rate of predatory mites (% + SD) with and without neem substrate drenching (B). Bars marked with different letters are statistically different, P>0.05.**

***Microcosm Experiment 2***

Using CS the application of AZA resulted in higher thrips mortality if soil drenching occurred before the release of WFT and predatory mites (5db, 3db) compared to AZA treatments after thrips and predator introduction (3da, 5da). This was apparent regardless of predatory mite species or the application of AZA with or without predators. In the 5da-treatment, CM of only 30% (with *A. cucumeris*), 24% (with *H. aculeifer*) and 15% (without predatory mites) were recorded, whereas the 3db- or 5db-treatments resulted in CM up to 98% (with *A. cucumeris*), 84% (with *H. aculeifer*) and 73% (without predatory mites), respectively (Figure 3.3(A)). Lower time effects were recorded in the CS-sand mixture with CM ranging only from 68% in applications after thrips and predator introduction to 98% in soil treatments before releases of the arthropods (Figure 3.4(A)). On both substrates no significant differences were detected in recapture rates of *A. cucumeris* ($F=2.23$; $df=8, 71$; $P=0.0860$) and *H. aculeifer* ($F=1.28$; $df=8, 71$; $P=0.2665$) during the different treatments of this experiment (Figure 3.3(B), 3.4(B)).

***Microcosm Experiment 3***

Additional studies on the impact of NeemAzal-U substrate drenching on soil-dwelling predatory mites resulted in no significant differences in the population growth of *H. aculeifer.* That was after AZA treatments three days before (3db), at the same day (0d) and three days after (3da) release of the arthropods versus the control treatment, considering the population development 22 and 38 days after the antagonist introduction (22 d: $F=1.89$; $df=3, 28$; $P=0.1534$, 38 d: $F=0.92$; $df=3, 28$; $P=0.4452$). However, 30 days after the release of the mites a significantly lower number of predators was recorded in the AZA treatment (3da) compared to the control ($F=4.71$; $df=3, 28$; $P=0.0087$). Finally, after a period of 38 days the initial population of five *H. aculeifer* per microcosm increased to a mean number of 39 predatory mites in the control and 36 (3db), 39 (0d) and 43 (3da) in the AZA treatments (Figure 3.5).

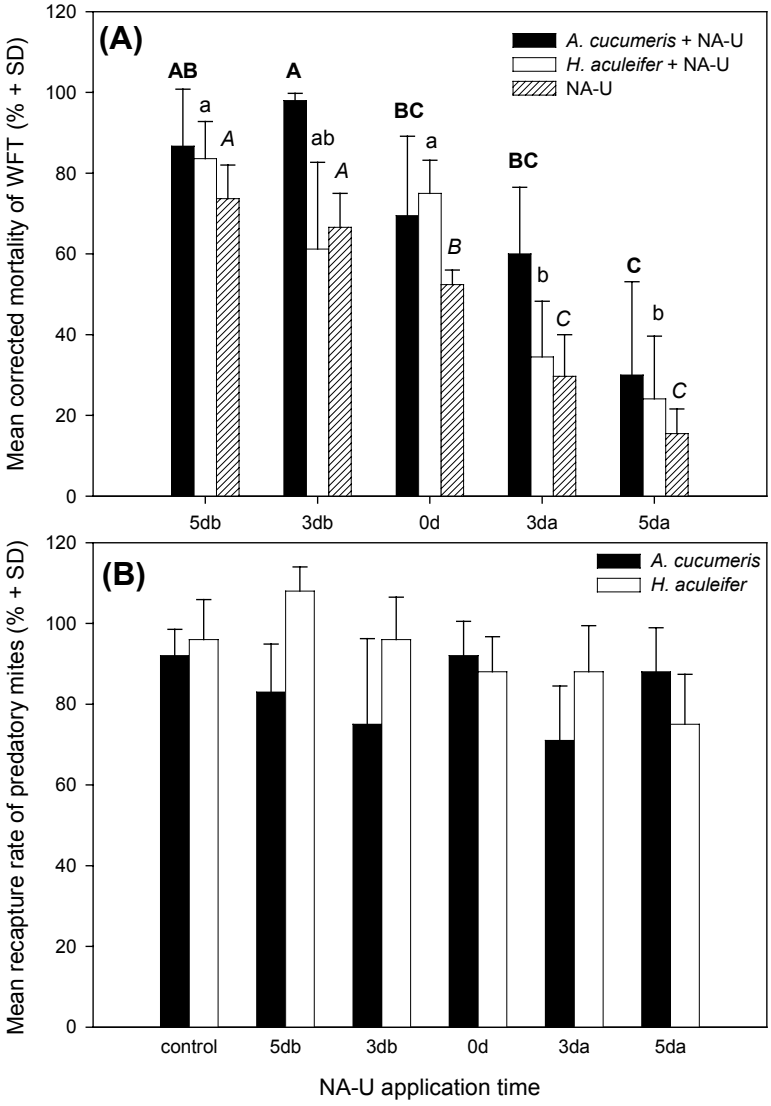


**Figure 3.3 Mean corrected mortality (% + SD) of WFT (A) and mean recapture rate of predatory mites (% + SD) (B) caused by NA-U (0.59 g NA-U/l) or 0.59 g/l blank formulation applied to CS with 39.47% OM (50 ml/pot) at different times before and after introduction of 25 L1 and 3 predatory mites/pot (3db, 5db, 0d, 3da, 5da). Bars marked with different letters are statistically different, comparisons among *A. cucumeris* treatments combined with AZA were marked with upper case letters, bold, *H. aculeifer* combined with AZA with lower case letters and single AZA treatment with upper case letters, italic, P>0.05.**

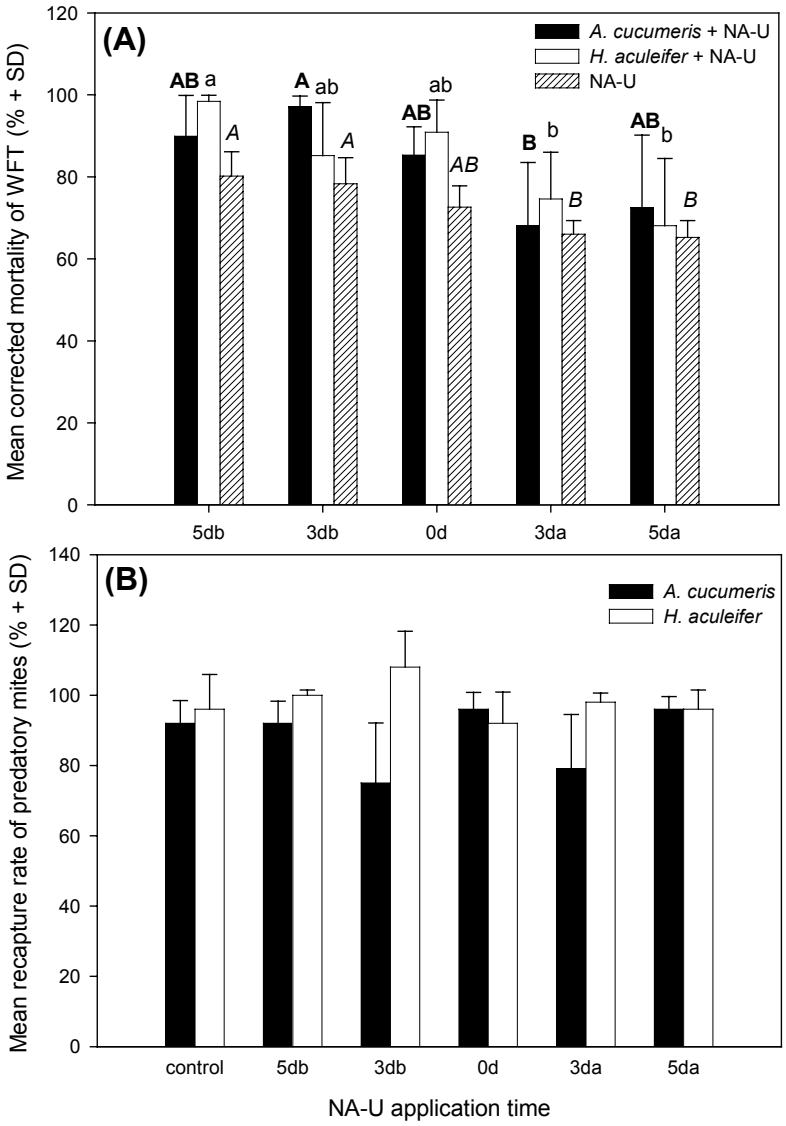


**Figure 3.4 Mean corrected mortality (% + SD) of WFT (A) and mean recapture rate of predatory mites (% + SD) (B) caused by NA-U (0.59 g NA-U/l) or 0.59 g/l blank formulation applied to CS-sand mixture with 8.46% OM (50 ml/pot) at different times before and after introduction of 25 L1 and 3 predatory mites/pot (3db, 5db, 0d, 3da, 5da). Bars marked with different letters are statistically different, comparisons among *A. cucumeris* treatments combined with AZA were marked with upper case letters, bold, *H. aculeifer* combined with AZA with lower case letters and single AZA treatment with upper case letters, italic, P>0.05.**

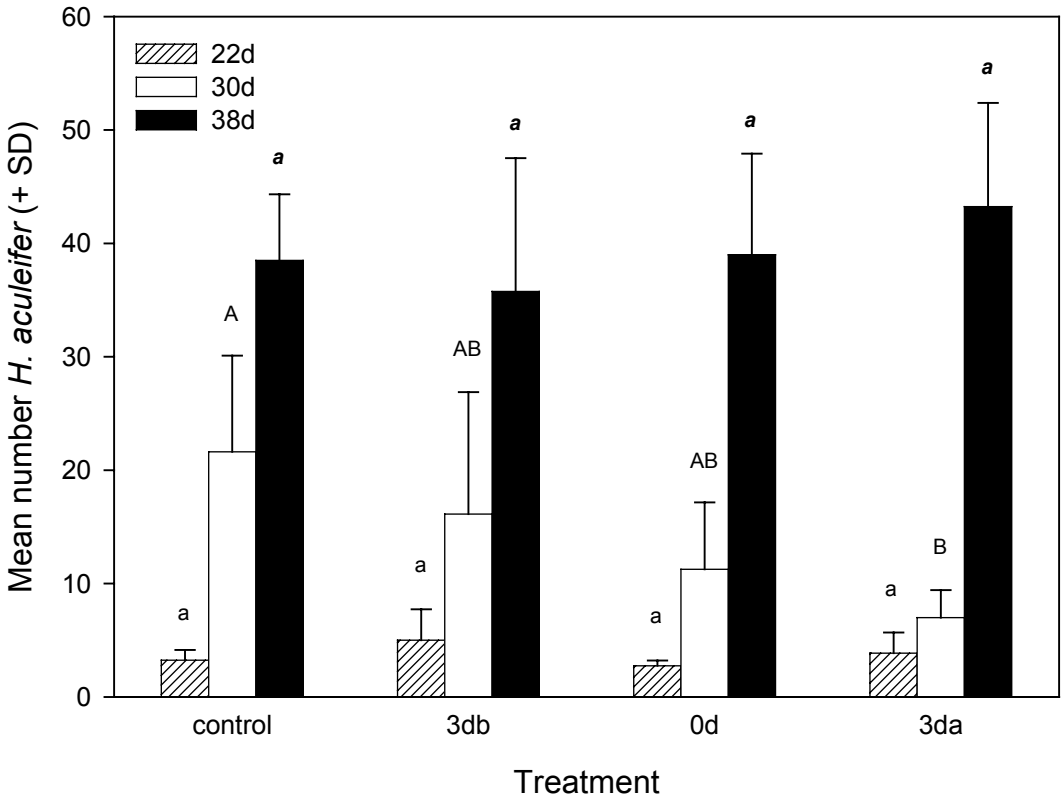


**Figure 3.5 Mean number of *H. aculeifer* (+ SD) 22, 30 and 38 days after predatory mite release after substrate drenching with 50 ml NA-U (0.59 g NA-U/l) three days before (3db), at the same day (0 d) and three days after (3da) placing 25 L1-larvae and five *H. aculeifer* in the microcosm. The control is an application of 0.59 g/l blank formulation on the same day the arthropods were introduced (0d-treatment). Bars marked with different letters are statistically different, comparisons for 22 days observation time represented with lower case letters, 30 days with upper case letters and 38 days with lower case letters, italic, bold, P>0.05.**

### *Greenhouse Experiments*

Considering the mean number of WFT over the entire experimental time of five weeks all treatments resulted in lower thrips numbers compared to the control treatment (Table 3.2 – 3.4). For the single *H. aculeifer* release (H) significantly higher WFT numbers were recorded compared to *A. cucumeris* alone and the combination of both antagonists (A, AH) (Table 3.2 – 3.4). The thrips densities in the substrate confirmed the results, except that no significant differences

among control (C) and single *H. aculeifer* treatment (H) in Trial 1 (CS: F=68.93; df=7, 72; A: P=0.1910; CS-sand: F=42.24; df=7, 72; A: P=0.1684) and in Trial 3 using the CS-sand mixture (F=38.90; df=7, 72; A: P=0.0.5669) were observed (Table 3.2 – 3.4). Studies with weekly soil application of NeemAzal-U solution with 100 mg AZA/l (64 mg AZA/kg substrate/week) showed no differences among all AZA treatments (N, HN, AN, AHN) (Table 3.2). While a reduced AZA dose rate with 32 mg AZA/kg substrate/week (weekly, 50 mg AZA/l and every second week, 100 mg AZA/L) caused mainly significant lower WFT numbers in the combination of NeemAzal-U with both predatory mites (AHN) versus the AZA soil application alone (N) (CS: Trial 2: F=346.54; df=7, 24; P<0.0001, Trial 3: F=452.54; df=7, 32; P=0.0034; CS-sand: Trial 2: F=261.90; df=7, 24; P=0.0002, Trial 3: F=258.08; df=7, 32; P<0.1191) (Table 3.3 + 3.4). Particularly in treatments with reduced AZA dose rates the single and combined AZA treatment resulted in efficiencies of more than 88% (Table 3.2 – 3.4). Considering the impact of AZA-treated substrate on the soil-dwelling antagonist, no significant differences in numbers of *H. aculeifer* among the treatments with and without neem components (H, AH, HN, AHN) were recorded in all greenhouse experiments. Except that in Trial 3 using the CS-sand mixture significant lower number of soil-dwelling predatory mites resulted in the combined use of AZA with both antagonists (AHN) (F=4.77; df=3, 16; H: P=0.0197, AH: P=0.0437) (Table 3.4). In general, no differences in efficacy of the different treatments between the two substrates were recorded (Table 3.2 – 3.4). In all greenhouse trials, plant weight, pod number and pod weight showed no significant differences between the different treatments using the same substrate. However, significant lower number of pods and lower plant and pod weight were recorded on the CS-sand mixture compared to CS (Table 3.5 – 3.7).

**Table 3.2 Mean number of WFT per leaf (± SD) recorded on different sampling days (3, 9, 16, 23, 30, 37), corrected mortality (CM) in % and mean arthropod number per pot (± SD) in different treatments (treat.) (C, H, A, N, AH, HN, AN, AHN) on two substrates (sub.): CS and CS-sand (mix). Neem as 100 mg AZA/l (0.59 g NA-U/l) was applied weekly.**

| Sub. | Treat. | WFT number/leaf (mean* ± SD)# | | | | | | | CM (%) | Arthropod number/pot (mean* ± SD)# | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | d3 | d9 | d16 | d23 | d30 | d37 | | | WFT | | *H. aculeifer*. | |
| CS | C | 0.4 ± 0.9 | 8.6 ± 4.4 | 5.0 ± 2.9 | 33.2 ± 15.4 | 81.2 ± 12.3 | 122.2 ± 32.7 | A | | 146.3 ± 17.2 | A | | |
| | H | 0.0 ± 0.0 | 3.8 ± 0.8 | 4.0 ± 1.6 | 10.0 ± 2.2 | 58.6 ± 15.9 | 87.8 ± 13.2 | B | 27.8 | 76.8 ± 5.5 | A | 19.0 ± 8.4 | A |
| | A | 0.4 ± 0.5 | 5.0 ± 0.7 | 2.6 ± 1.1 | 4.0 ± 1.6 | 36.0 ± 3.5 | 41.0 ± 2.9 | C | 66.4 | 30.6 ± 5.1 | B | | |
| | AH | 0.0 ± 0.0 | 4.2 ± 1.9 | 2.0 ± 1.7 | 10.2 ± 5.3 | 28.2 ± 3.8 | 27.2 ± 8.4 | C | 77.9 | 29.9 ± 5.0 | B | 16.9 ± 2.6 | A |
| | N | 0.4 ± 0.5 | 3.4 ± 1.1 | 0.6 ± 0.5 | 1.8 ± 0.4 | 0.6 ± 0.5 | 1.2 ± 0.8 | D | 98.4 | 4.6 ± 2.1 | C | | |
| | HN | 0.4 ± 0.5 | 2.2 ± 1.5 | 0.6 ± 0.5 | 0.2 ± 0.4 | 1.2 ± 0.8 | 0.8 ± 0.4 | D | 99.8 | 4.6 ± 3.1 | C | 13.0 ± 4.9 | A |
| | AN | 0.6 ± 0.5 | 1.8 ± 1.3 | 0.2 ± 0.4 | 0.2 ± 0.4 | 0.6 ± 0.5 | 0.4 ± 0.5 | D | 99.8 | 5.9 ± 3.5 | C | | |
| | AHN | 0.2 ± 0.4 | 0.0 ± 0.0 | 0.2 ± 0.4 | 0.8 ± 0.4 | 0.8 ± 0.4 | 0.2 ± 0.4 | D | 99.2 | 3.7 ± 3.1 | C | 13.6 ± 7.0 | A |
| Mix | C | 0.4 ± 0.5 | 6.8 ± 3.1 | 8.0 ± 3.4 | 19.4 ± 12.9 | 79.6 ± 38.5 | 117.8 ± 17.4 | A | | 167.2 ±20.9 | A | | |
| | H | 0.6 ± 0.5 | 5.4 ± 1.1 | 4.0 ± 1.6 | 22.0 ± 6.7 | 43.2 ± 10.4 | 57.6 ± 1.8 | B | 50.9 | 71.2 ± 12.2 | AB | 22.4 ±11.4 | A |
| | A | 0.4 ± 0.5 | 7.2 ±3.8 | 1.4 ± 0.9 | 12.4 ± 1.1 | 20.0 ± 5.7 | 34.6 ± 9.0 | C | 70.3 | 54.0 ± 28.8 | B | | |
| | AH | 0.2 ± 0.4 | 4.8 ± 4.6 | 3.4 ± 1.1 | 9.4 ± 2.4 | 15.4 ± 8.2 | 37.4 ± 9.7 | C | 68.6 | 23.0 ± 5.4 | B | 18.0 ± 8.7 | A |
| | N | 0.2 ± 0.4 | 3.4 ± 2.5 | 0.8 ± 0.8 | 0.2 ± 0.2 | 1.0 ± 1.2 | 0.8 ± 1.3 | D | 99.1 | 5.4 ± 4.2 | C | | |
| | HN | 0.4 ± 0.5 | 1.4 ± 1.1 | 0.2 ± 0.4 | 0.2 ± 0.4 | 0.8 ± 0.4 | 1.0 ± 0.7 | D | 99.0 | 4.2 ± 2.9 | C | 13.8 ± 6.3 | A |
| | AN | 0.2 ± 0.4 | 1.4 ± 1.1 | 0.4 ± 0.5 | 0.4 ± 0.5 | 0.4 ± 0.5 | 0.4 ± 0.5 | D | 99.7 | 1.4 ± 2.2 | C | | |
| | AHN | 0.8 ± 0.8 | 0.6 ± 0.5 | 0.2 ± 0.1 | 0.4 ± 0.5 | 0.2 ± 0.4 | 1.2 ± 0.8 | D | 98.9 | 1.6 ± 1.8 | C | 10.6 ± 4.3 | A |

***Average of five replicates, #Values followed by the same letter within columns are not significantly different, P>0.05**

**Table 3.3 Mean number of WFT per leaf (± SD) recorded on different sampling days (3, 9, 16, 23, 30, 37), corrected mortality (CM) in % and mean arthropod number per pot (± SD) in different treatments (treat.) (C, H, A, N, AH, HN, AN, AHN) on two substrates (sub.): CS and CS-sand (mix). Neem as 50 mg AZA/l (0.3 g NA-U/l) was applied weekly.**

| Sub. | Treat. | WFT number/leaf (mean* ± SD)# | | | | | | | CM (%) | Arthropod number/pot (mean* ± SD)# | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | d3 | d9 | d16 | d23 | d30 | d37 | | | WFT | | *H. aculeifer*. | |
| CS | C | 1.4 ± 0.5 | 12.4 ± 4.0 | 26.4 ± 4.4 | 29.4 ± 5.5 | 46.4 ± 2.7 | 130.2 ± 3.6 | A | | 87.4 ± 15.2 | A | | |
| | H | 0.4 ± 0.5 | 6.8 ± 1.5 | 14.6 ± 3.2 | 11.6 ± 4.4 | 29.0 ± 3.5 | 107.2 ± 12.6 | B | 17.7 | 40.0 ± 8.3 | B | 18.8 ± 8.0 | A |
| | A | 1.0 ± 0.7 | 1.8 ± 0.8 | 10.6 ± 2.5 | 7.0 ± 4.5 | 21.0 ± 4.8 | 76.8 ± 7.6 | C | 40.8 | 28.2 ± 4.1 | C | | |
| | AH | 1.2 ± 0.4 | 2.2 ± 0.8 | 9.6 ± 1.1 | 10.0 ± 3.2 | 23.2 ± 2.3 | 59.8 ± 12.3 | C | 53.9 | 26.2 ± 3.6 | CD | 23.8 ± 6.8 | A |
| | N | 0.6 ± 0.5 | 4.2 ± 1.3 | 6.4 ± 1.1 | 9.4 ± 1.7 | 15.2 ± 1.5 | 12.6 ± 4.2 | D | 90.0 | 14.4 ± 2.1 | D | | |
| | HN | 1.4 ± 0.5 | 2.8 ± 0.4 | 3.8 ± 0.8 | 5.4 ± 0.5 | 15.2 ± 0.8 | 14.6 ± 3.5 | DE | 88.5 | 6.6 ± 0.9 | E | 17.4 ± 6.3 | A |
| | AN | 0.8 ± 0.4 | 0.8 ± 0.4 | 2.6 ± 1.8 | 5.2 ± 1.3 | 8.2 ± 2.3 | 10.6 ± 1.9 | EF | 91.5 | 3.2 ± 0.8 | E | | |
| | AHN | 1.0 ± 0.7 | 3.2 ± 1.6 | 0.6 ± 0.5 | 0.4 ± 0.5 | 2.6 ± 5.3 | 2.2 ± 1.9 | F | 98.5 | 3.0 ± 1.4 | E | 19.4 ± 10.1 | A |
| Mix | C | 1.2 ± 1.1 | 14.2 ± 3.9 | 26.4 ± 2.3 | 28.4 ± 6.4 | 47.6 ± 2.8 | 122.6 ± 3.3 | A | | 145.6 ± 16.7 | A | | |
| | H | 1.6 ± 1.3 | 10.4 ± 4.6 | 15.8 ± 2.5 | 14.4 ± 1.1 | 33.0 ± 4.6 | 103.0 ± 8.8 | B | 47.7 | 93.8 ± 9.2 | B | 25.2 ± 8.9 | A |
| | A | 0.8 ± 0.8 | 2.2 ± 0.4 | 11.2 ± 1.8 | 18.0 ± 3.5 | 28.0 ± 2.4 | 61.2 ± 11.2 | C | 50.4 | 55.6 ± 10.9 | C | | |
| | AH | 1.0 ± 1.0 | 2.6 ± 0.5 | 8.4 ± 0.9 | 11.0 ± 2.8 | 26.6 ± 6.2 | 57.2 ± 6.0 | C | 53.7 | 61.2 ± 17.9 | C | 14.4 ± 6.8 | A |
| | N | 1.2 ± 0.4 | 3.2 ± 0.4 | 5.4 ± 0.9 | 8.2 ± 0.8 | 13.8 ± 3.0 | 9.8 ± 0.8 | D | 91.9 | 8.0 ± 4.2 | D | | |
| | HN | 0.8 ± 0.4 | 2.8 ± 0.8 | 4.6 ± 1.1 | 6.4 ± 1.5 | 11.4 ± 3.0 | 12.4 ± 3.4 | D | 90.2 | 12.6 ± 5.5 | DE | 24.6 ± 15.7 | A |
| | AN | 1.0 ± 1.0 | 0.2 ± 0.4 | 2.4 ± 1.8 | 4.0 ± 1.6 | 8.8 ± 1.3 | 8.6 ± 2.1 | DE | 92.7 | 9.2 ± 2.8 | DE | | |
| | AHN | 0.8 ± 0.8 | 1.6 ± 1.3 | 1.4 ± 1.1 | 0.2 ± 0.4 | 2.0 ± 1.9 | 2.6 ± 1.7 | E | 97.6 | 5.2 ± 4.1 | E | 17.0 ± 11.2 | A |

***Average of five replicates, #Values followed by the same letter within columns are not significantly different, P>0.05**

**Table 3.4 Mean number of WFT per leaf (± SD) recorded on different sampling days (3, 9, 16, 23, 30, 37), corrected mortality (CM) in % and mean arthropod number per pot (± SD) in different treatments (treat.) (C, H, A, N, AH, HN, AN, AHN) on two substrates (sub.): CS and CS-sand (mix). Neem as 100 mg AZA/l (0.59 g NA-U/l) was applied every second week.**

| Sub. | Treat. | WFT number/leaf (mean* ± SD)# | | | | | | | CM (%) | Arthropod number/pot (mean* ± SD)# | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | d3 | d9 | d16 | d23 | d30 | d37 | | | WFT | | *H. aculeifer* | |
| CS | C | 1.2 ± 0.4 | 33.0 ± 15.6 | 39.4 ± 11.6 | 47.8 ± 9.5 | 288.2 ± 25.7 | 465.0 ± 26.0 | A | | 330.6 ± 50.0 | A | | |
| | H | 1.0 ± 0.0 | 12.2 ± 6.1 | 19.2 ± 1.9 | 23.2 ± 2.9 | 72.6 ± 18.5 | 219.8 ± 23.0 | B | 52.9 | 216.2 ± 26.8 | B | 31.2 ± 12.8 | A |
| | A | 0.8 ± 0.4 | 15.8 ± 9.0 | 17.0 ± 6.0 | 13.4 ± 3.3 | 61.0 ± 22.9 | 136.4 ± 14.6 | C | 70.7 | 95.8 ± 18.9 | C | | |
| | AH | 0.8 ± 0.4 | 17.2 ± 6.0 | 20.8 ± 3.6 | 15.4 ± 2.5 | 62.8 ± 12.4 | 136.0 ± 19.7 | C | 70.7 | 90.0 ± 24.3 | C | 34.2 ± 6.9 | A |
| | N | 1.0 ± 0.7 | 5.4 ± 2.7 | 11.4 ± 5.1 | 5.6 ± 1.1 | 24.2 ± 3.8 | 39.8 ± 6.9 | D | 91.4 | 56.0 ± 7.2 | CD | | |
| | HN | 0.6 ± 0.5 | 4.0 ± 1.9 | 6.8 ± 4.1 | 2.4 ± 1.5 | 6.0 ± 2.5 | 13.4 ± 2.9 | DE | 97.1 | 18.6 ± 5.9 | DE | 20.2 ± 7.8 | A |
| | AN | 1.2 ± 0.4 | 7.4 ± 2.9 | 4.0 ± 2.5 | 0.4 ± 0.5 | 10.6 ± 3.6 | 7.8 ± 4.4 | DE | 98.3 | 17.8 ± 3.3 | DE | | |
| | AHN | 1.4 ± 0.5 | 2.2 ± 1.3 | 0.8 ± 0.4 | 0.8 ± 0.4 | 1.0 ± 0.7 | 3.8 ± 1.5 | E | 99.1 | 2.2 ± 0.8 | E | 19.2 ± 8.1 | A |
| Mix | C | 0.8 ± 0.8 | 19.4 ± 3.0 | 31.8 ± 18.4 | 47.8 ± 19.5 | 274.0 ± 33.6 | 449.2 ± 29.6 | A | | 314.6 ± 62.3 | A | | |
| | H | 1.0 ± 0.7 | 12.6 ± 4.4 | 17.0 ± 1.0 | 21.8 ± 7.0 | 75.0 ± 20.6 | 224.6 ± 15.5 | B | 49.9 | 90.6 ± 50.6 | AB | 33.8 ± 14.4 | A |
| | A | 1.0 ± 0.2 | 11.8 ± 7.6 | 18.8 ± 9.2 | 13.0 ± 2.2 | 67.2 ± 23.0 | 127.2 ± 25.4 | C | 71.7 | 128.0 ± 66.3 | B | | |
| | AH | 1.2 ± 0.4 | 14.0 ± 6.0 | 24.8 ± 1.3 | 16.8 ± 3.3 | 64.8 ± 23.9 | 129.0 ± 19.7 | C | 71.3 | 136.2 ± 69.6 | B | 31.4 ± 10.1 | A |
| | N | 0.8 ± 0.4 | 7.6 ± 3.0 | 9.2 ± 3.3 | 5.6 ± 1.1 | 15.0 ± 3.2 | 29.0 ± 7.0 | D | 93.5 | 20.6 ± 15.1 | C | | |
| | HN | 1.0 ± 0.2 | 4.4 ± 1.8 | 6.0 ± 2.5 | 2.8 ± 0.8 | 3.8 ± 1.3 | 12.0 ± 2.8 | D | 97.3 | 7.2 ± 4.4 | C | 21.4 ± 5.4 | A |
| | AN | 1.0 ± 1.0 | 7.0 ± 3.8 | 4.6 ± 4.0 | 1.0 ± 0.7 | 3.8 ± 1.5 | 5.8 ± 3.0 | D | 98.7 | 11.6 ± 9.4 | C | | |
| | AHN | 0.6 ± 0.5 | 3.6 ± 2.9 | 0.6 ± 0.5 | 0.4 ± 0.5 | 1.0 ± 1.0 | 1.8 ± 0.4 | D | 99.6 | 5.4 ± 4.0 | C | 14.0 ± 3.6 | B |

***Average of five replicates, #Values followed by the same letter within columns are not significantly different, P>0.05**

**Table 3.5 Mean pod number, pod weight (g) and total plant weight (g) per bean plant (± SD) measured five weeks after the first soil application in different treatments, and the statistical analysis for the substrate comparison. NA-U was applied weekly with 100 mg AZA/l (0.59 g NA-U/l) to CS and CS-sand.**

| Treatment | Pod number (mean* ± SD) | | Pod weight (g) (mean* ± SD) | | Total plant weight (g) (mean* ± SD) | |
|---|---|---|---|---|---|---|
| | CS | CS-sand | CS | CS-sand | CS | CS-sand |
| C | 9.6 ± 1.1 | 7.8 ± 1.3 | 29.8 ± 4.5 | 24.1 ± 3.0 | 18.5 ± 3.6 | 17.2 ± 2.0 |
| H | 9.0 ± 1.5 | 12.0 ± 0.7 | 24.3 ± 6.1 | 36.4 ± 3.5 | 22.6 ± 2.6 | 25.2 ± 1.5 |
| A | 11.5 ± 0.4 | 6.3 ± 1.3 | 37.6 ± 1.1 | 18.7 ± 5.8 | 27.9 ± 0.1 | 17.3 ± 4.2 |
| AH | 12.8 ± 4.3 | 11.2 ± 2.1 | 34.6 ± 8.2 | 38.0 ± 7.5 | 28.2 ± 5.1 | 26.5 ± 4.5 |
| N | 9.0 ± 1.2 | 7.0 ± 1.4 | 25.6 ± 4.5 | 15.6 ± 2.3 | 19.2 ± 3.5 | 12.9 ± 1.7 |
| HN | 11.4 ± 1.3 | 5.8 ± 0.8 | 30.2 ± 5.1 | 14.7 ± 3.0 | 22.0 ± 3.0 | 10.5 ± 1.7 |
| AN | 10.0 ± 1.4 | 4.2 ± 0.6 | 30.6 ± 7.0 | 9.4 ± 2.1 | 23.1 ± 4.5 | 9.3 ± 3.8 |
| AHN | 8.8 ± 1.0 | 6.0 ± 1.0 | 26.4 ± 2.3 | 15.5 ± 2.0 | 21.8 ± 2.2 | 12.3 ± 1.5 |
| Statistics | | | | | | |
| F-value | 16.02 | | 11.40 | | 14.25 | |
| df | 1, 68 | | 1, 68 | | 1, 68 | |
| P-value | 0.0002 | | 0.0012 | | 0.0003 | |

***Average of five replicates**

**Table 3.6 Mean pod number, pod weight (g) and total plant weight (g) per bean plant (± SD) measured five weeks after the first soil application in different treatments, and the statistical analysis for the substrate comparison. NA-U was applied weekly with 50 mg AZA/l (0.3 g NA-U/l) to CS and CS-sand.**

| Treatment | Pod number (mean* ± SD) | | Pod weight (g) (mean* ± SD) | | Total plant weight (g) (mean* ± SD) | |
|---|---|---|---|---|---|---|
| | CS | CS-sand | CS | CS-sand | CS | CS-sand |
| C | 11.0 ± 1.0 | 8.2 ± 0.8 | 34.9 ± 4.0 | 25.4 ± 4.4 | 24.1 ± 3.0 | 14.9 ± 3.3 |
| H | 14.0 ± 0.8 | 6.7 ± 0.5 | 36.8 ± 2.0 | 23.6 ± 2.3 | 28.9 ± 0.4 | 16.1 ± 3.6 |
| A | 10.3 ± 2.0 | 9.0 ± 1.6 | 36.5 ± 0.9 | 31.3 ± 2.2 | 26.1 ± 1.2 | 28.3 ± 1.4 |
| AH | 11.0 ± 1.7 | 10.2 ± 0.8 | 35.1 ± 2.2 | 36.2 ± 4.2 | 23.6 ± 3.6 | 28.6 ± 3.4 |
| N | 11.8 ± 1.7 | 9.4 ± 1.6 | 37.7 ± 4.5 | 29.9 ± 2.9 | 30.3 ± 2.5 | 23.2 ± 3.5 |
| HN | 12.2 ± 1.6 | 7.2 ± 0.6 | 38.3 ± 3.6 | 23.3 ± 4.6 | 31.8 ± 3.7 | 19.3 ± 3.3 |
| AN | 11.4 ± 0.8 | 9.0 ± 1.2 | 41.5 ± 4.1 | 25.7 ± 4.4 | 34.1 ± 3.0 | 21.7 ± 2.5 |
| AHN | 12.0 ± 0.9 | 8.2 ± 1.0 | 36.9 ± 2.0 | 21.6 ± 2.5 | 29.1 ± 4.5 | 18.2 ± 1.5 |
| Statistics | | | | | | |
| F-value | 29.18 | | 19.43 | | 17.04 | |
| df | 1, 68 | | 1, 68 | | 1, 68 | |
| P-value | <0.0001 | | <0.0001 | | <0.0001 | |

***Average of five replicates**

**Table 3.7 Mean pod number, pod weight (g) and total plant weight (g) per bean plant (± SD) measured five weeks after the first soil application in different treatments, and the statistical analysis for the substrate comparison. NA-U was applied every second week with 100 mg AZA/l (0.59 g NA-U/l) to CS and CS-sand.**

| Treatment | Pod number (mean* ± SD) | | Pod weight (g) (mean* ± SD) | | Total plant weight (g) (mean* ± SD) | |
|---|---|---|---|---|---|---|
| | CS | CS-sand | CS | CS-sand | CS | CS-sand |
| C | 11.4 ± 1.7 | 6.8 ± 1.0 | 34.0 ± 4.2 | 23.6 ± 5.0 | 23.4 ± 1.8 | 17.8 ± 2.0 |
| H | 11.0 ± 1.0 | 6.5 ± 2.4 | 31.9 ± 2.0 | 21.0 ± 7.0 | 21.4 ± 1.1 | 11.4 ± 2.5 |
| A | 10.0 ± 0.8 | 7.3 ± 1.0 | 28.5 ± 1.2 | 23.6 ± 3.7 | 14.4 ± 2.6 | 16.5 ± 3.5 |
| AH | 9.4 ± 0.4 | 9.8 ± 1.4 | 29.5 ± 2.6 | 27.7 ± 2.3 | 22.7 ± 1.6 | 17.9 ± 3.5 |
| N | 10.8 ± 0.4 | 6.8 ± 1.3 | 34.7 ± 0.6 | 25.7 ± 4.5 | 29.2 ± 1.8 | 24.7 ± 2.5 |
| HN | 11.0 ± 1.1 | 6.6 ± 1.5 | 36.2 ± 3.6 | 26.7 ± 6.5 | 30.5 ± 3.0 | 25.1 ± 3.3 |
| AN | 11.6 ± 0.8 | 7.6 ± 0.6 | 40.5 ± 3.1 | 27.3 ± 2.5 | 32.6 ± 1.5 | 23.5 ± 1.5 |
| AHN | 12.6 ± 1.0 | 9.8 ± 0.7 | 38.3 ± 3.7 | 31.5 ± 2.5 | 31.2 ± 1.6 | 26.3 ± 1.1 |
| Statistics | | | | | | |
| F-value | 32.60 | | 22.04 | | 14.65 | |
| df | 1, 68 | | 1, 68 | | 1, 68 | |
| P-value | <0.0001 | | <0.0001 | | 0.0003 | |

***Average of five replicates**

## 3.4 Discussion

### *Impact of Predatory Mites on Western Flower Thrips*

Even though foliage- and soil-dwelling predatory mites are established biological control agents for WFT management, the efficiency of the antagonists in thrips control showed variable results (Glockemann 1992, Brødsgaard et al. 1996, Jacobson et al. 2001). In our microcosm trials, single predatory mite releases with three mites/plant as approximately common application rate or ten predators/plant caused only mortalities lower than 51%. Berndt et al. (2004a) observed reduction rates of WFT of 58% and 81% at applications rates of five or twenty *H. aculeifer*/plant on French bean in comparable microcosm trials. Mortality in our greenhouse trials ranged for single releases of *H. aculeifer* from 18% to 53% and from 41% to 72% using *A. cucumeris* (three mites/plant). Wiethoff et al. (2004) found in greenhouse studies on cucumber with application rates of 528 *H. aculeifer*/$m^2$ (three plants per 2.16 $m^2$ plot) a reduction in WFT population of approximately 70% and thrips suppression rates ranging from 56% to 60% using *A. cucumeris* (50 mites/$m^2$). Similarly to our results, a high variability in efficiency after the release of the antagonists was observed. Biological thrips control using predatory mites is influenced in a complex way, which can result in a rather unreliable pest control. For example, the availability of alternative prey can reduce or enhance the establishment and the efficiency of predatory mites in the system (Brødsgaard et al. 1996, Jacobson et al. 2001). Moreover, the quality and therewith the efficacy of commercially available biological control agents can differ (O'Neil et al. 1998, Vasquez et al. 2004) and the efficiency of predatory mites is limited by the environmental conditions in the greenhouse (Rodriguez-Reina et al. 1994). Using combinations of different antagonists additional factors may accrue, e.g. interactions such as cannibalism, competition or intraguild predation among the organisms can have an effect on the success of pest control (Losey and Denno 1998, Schausberger and Walzer 2001, Berndt et al. 2003). No synergistic or additive effects were recorded in studies of Wiethoff et al. (2004) on combining *A. cucumeris* and *H. aculeifer* to optimize biological WFT control, therefore no such effects were expected in our studies. The combination of plant- and soil-dwelling predatory mites for thrips control in our trials showed with mortalities between 54% and 85% slightly higher control efficacies compared to single predatory mite

releases. Similar to our results, no differences among *A. cucumeris* alone and its combination with *H. aculeifer* (CM from 56% to 88%) but a significantly lower efficacy for the single release of *H. aculeifer* with 207 individuals/$m^2$ were recorded in greenhouse experiments of Wiethoff et al. (2004).

***Impact of AZA Soil Application Alone and Combined with Release of Predatory Mites on Western Flower Thrips***

The integration of active neem ingredients applied as soil treatment with releases of beneficials can improve the reliability and efficiency of thrips management. Overall, NeemAzal-U substrate drenching alone and in combination with antagonists showed in all trials the strongest effects on WFT with mortalities from 70% to 100%. These effects went along with a low variability in efficacy compared to the earlier mentioned variability after the use of predatory mites. Comparably, Thöming et al. (2003) reported high WFT mortalities after NeemAzal®-T/S substrate drenching using 100 mg AZA/l and 50 ml/pot. Substrate treatments with Azatin® XL (31.7 g AZA/l, Olympic Horticultureal Products, Bradenton, FL) for management of *F. occidentalis* and *Bradysia coprophila* Lintner (Diptera: Sciaridae) with a dose rate of 40 mg AZA/l (90 ml/pot, every sixth day) caused a reduction in WFT populations around 27% to 73% (Ludwig and Oetting 2001). In our microcosm trials the single AZA treatment (N) caused a similar effect as with the combination of predatory mites (A3H3, A3H10, A10H3, A10H10) with mortalities between 63% and 93%. Whereas under greenhouse conditions even significantly higher efficacies of up to 99% were recorded using AZA soil treatments alone (N) compared to predatory mites treatments (A, H, AH), indicating a high efficacy of single AZA soil treatments. Using soil treatments with 64 mg AZA/kg substrate/week no significant differences among the different NeemAzal-U drenchings (N, AN, HN, AHN) were recorded. The tested AZA dose rate probably had such a strong impact on WFT that it blotted out the effect of the antagonist. Thus, the dose rate used for AZA soil treatments if combined with predatory mites should be reduced compared to AZA concentrations if used by itself. The AZA dose rate reduction in the following experiments using 32 mg AZA/kg substrate/week confirmed this assumption, resulting in significant differences among single AZA treatments (N) and the combination of NeemAzal-U soil applications with

predatory mites (except Trial 3 using CS-sand mixture). This still yielded efficacies of 98% (AHN). Thus, substrate drenching using 32 mg AZA/kg substrate/week complemented with releases of predatory mites seems to be a more optimal combination of treatments. Combinations of AZA substrate drenching with *A. cucumeris* showed a similar efficacy to those with both predators and can be recommended as the most efficient combination considering efficacy in WFT control and monetary investment. The practicability of integrated thrips control with such combinations has also been suggested in other studies (Ludwig and Oetting 2001).

Varying time schedules of AZA soil application on pure CS in our microcosm trials showed higher mortality from NeemAzal-U treatments before the introduction of thrips and antagonist (5db, 3db) compared to treatments after the arthropod release (3da, 5da). This was regardless of a single AZA treatment or the combination of predatory mite species. Using the CS-sand mixture only reduced time effects were recorded. Similar effects in thrips control were recorded on French beans with soil drenching of NeemAzal®-T/S (100 mg AZA/l, 50 ml/pot) (Thöming et al. 2003). A relatively slow translocation of active neem ingredients from soil to the feeding sites of the pest on the plant can cause a delayed efficacy of AZA substrate drenching (Meisner et al. 1986, Sundaram et al. 1995). Thus, in case of the use of AZA soil treatments in IPM concepts this ’lag period’ should be considered.

### *Influence of Organic Matter Content on Efficiency of AZA Soil Application in Thrips Control*

The amount of organic matter in the growing substrate slightly influenced treatment efficacy in our studies. In the microcosm experiments after single AZA treatment higher corrected mortality was recorded using the CS-sand mixture with lower organic content compared to the pure CS with a higher amount of organic matter. Former studies indicated comparable results with higher efficacy in WFT control due to AZA soil drenching of a substrate-sand mixture (93% CM) compared to the pure substrate (76% CM) using French bean seedlings (Thöming et al. 2003). Gill and Lewis (1971) reported on lower systemic anti-feeding effects on *Schistocerca gregaria* Forskål (Orthoptera: Acrididae) after soil application of neem extracts due to high organic content of the substrate.

The available amount of AZA in soil/substrate can differ depending on parameters such as proportion of organic matter, soil type, microbial activity, temperature, light intensity and acidity (Stark and Walter 1995a, Pussemeier 2000, Barrek et al. 2004). A lower absorption of AZA by the organic matter in soil can enhance the efficacy of the active ingredient after soil treatments using substrates with lower organic content (Sundaram 1996b, Pussemeier 2000). Moreover, reduced time effects on CS-sand mixture were recorded compared to more explicit effects after different time treatments of AZA soil application using pure substrate (see above). This highlighted the influence of the amount of organic matter in growing substrate on control efficiency of neem soil treatments, in combined treatment with predatory mites as well. A longer persistence of AZA in WFT control was observed after soil application on a pure substrate compared to reduced persistence using a substrate-sand mixture with lower organic content (Thöming et al. 2003). However, in greenhouse trials no such explicit effects of organic content in substrate on thrips control efficacy were recorded. The repeated soil application seems to provide a continuous reservoir of AZA in the growing substrate, which makes the organic matter content as factor insignificant. Moreover, the evaluation of plant weight, pod number and pod weight in greenhouse trials showed no significant effects of the AZA soil treatment on plant development. Whereas, lower plant growth parameters were recorded using the CS-sand mixture compared to CS. Thus, negative effects on plant growth can occur although a substrate with lower content of organic matter may possibly enhance the control efficacy against thrips after neem soil treatments. This should be considered during the selection of substrate or growing medium in case of the use of soil-applied neem products in practice, according to the particular cultivation system.

***Side Effects of AZA Soil Application on Predatory Mites***

By the use of soil treatments with AZA compared to topical applications, a protection of plant-dwelling non-target organisms could be hypothesised. However, soil-dwelling organisms in general are much more under risk due to direct contact with AZA during and after substrate drenching. Thus, in case of soil dwelling predators such as *Hypoaspis* mites detrimental side effects are possible. Several studies reported on the harmful effects of AZA on non-target

organisms after more or less direct contact with active neem compounds (e.g. Stark 1996, Spollen and Isman 1996). Considering the survival of *A. cucumeris* and *H. aculeifer* after AZA substrate drenching, our experiments showed - as expected - no effects on the foliage dwelling mites but moreover, the soil-dwelling mites were conserved as well. Neither different application times before and after the introduction of the arthropods nor a weekly reapplied NeemAzal-U soil treatment for five weeks caused a strong detrimental impact on the antagonists. In spite of these observations, in greenhouse trials lower numbers of *H. aculeifer* were detected in AZA treatments using high dose rates of 100 mg AZA/l compared to control treatments. Thus, some impact could be suspected with higher AZA dosages. Detailed records under laboratory conditions on population development of the soil-dwelling predators confirmed this assumption. A decreased population growth of *H. aculeifer* was recorded 30 days after the release of the predator in the AZA treatment versus the control treatment, assumedly due to a reduced reproduction or delayed developmental period caused by direct contact with neem ingredients. Nevertheless, the detected effects can be neglected due to a fast recovery of the *H. aculeifer* population already eight days later (38 days after mite introduction). Studies on the effects of different pesticides containing fenpropimorph, primicarb and growth regulators methoprene (Apex® 5E) and diflubenzuron (Dimilin® 25wp) showed no negative impact on *Hypoaspis* species in soil (Krogh 1995, Ali et al. 1999). However, Krogh (1995) recorded adverse effects on the reproduction of *H. aculeifer* after applying dimethoate at about three times the recommended field dose rate, but a stimulating effect at the recommended dose rate. An explanation could be an increasing reproduction capacity of *H. aculeifer* under stressful conditions or effects on the behaviour of prey organisms at low dose rates making themselves an easier prey. These could result in higher prey capture rates, food consumption and reproduction of the predator. Thus, *H. aculeifer* seems to be capable of adapting to low level contaminated environments and is therefore suitable for IPM concepts. Overall, our results showed no significant differences in population development of *H. aculeifer* among treatments with and without AZA after a period of 38 days, although AZA was applied directly on the habitats of the mites. Therefore, minor effects of NeemAzal-U on *H. aculeifer* suggest that the use of AZA products combined

with foliage- and soil-dwelling predatory mites in IPM programs might not interfere with the biological control efficacy of the antagonists. In literature, several IPM concepts including antagonists and AZA products were recommended as more sustainable management systems in pest control than conventional control systems (Reddy and Guerrero 2000, Reddy 2001, Tang et al. 2002).

Overall, the combination of AZA substrate treatment with foliage- and/or soil-dwelling predatory mites improved the reliability and efficiency of WFT control with efficacies up to 99%. And even though minor side effects of NeemAzal-U on *H. aculeifer* were recorded, our results indicated that the release of predatory mites complemented by AZA soil applications could be a suitable combination for integrated WFT management.

# 4 Soil Application of Different Neem Products to Control *Ceratothripoides claratris* (Thysanoptera: Thripidae) on Tomatoes Grown under Protected Cultivation in the Humid Tropics (Thailand)[3]

## 4.1 Introduction

*Ceratothripoides claratris* Shumsher (Thysanoptera: Thripidae) is a tropical thrips species that causes severe damage to commercial tomato production in Thailand (Shumsher 1945, Okajima et al. 1992, Jangvitaya 1993, Mound and Kibby 1998, Murai et al. 2000, Rodmui 2002, Premachandra et al. 2005c). The general biology of *C. claratris* is very similar to that of other herbivorous Thripidae species such as *Franklinella occidentalis.* It lays its eggs in the aboveground plant parts and the first and second larval instar feed on the plant tissue. The late second instar stops feeding and moves to the soil or other off-plant refuges for pupation. Pre-pupae and pupae are more or less inactive and non-feeding (Rodmui 2002). In addition to tomatoes *C. claratris* can attack and damage other important vegetables, particularly through its feeding activity on leaves and fruits (Murai et al. 2000, Rodmui 2002). The transmission of plant viruses such as CaCV, Isolat AIT (Capsicum chlorosis virus, Isolat of Asian Institute of Technology, Pathumthani, Thailand) has also been attributed to this thrips species (Premachandra et al. 2005a). The control of *C. claratris* and other thrips species in the tropics is complicated for different reasons, especially in greenhouses. Due to their minute size thrips are easily distributed by wind and can pass through nets with mesh sizes, which usually provide efficient barriers for white flies or aphids. Thrips proof netting, often used in temperate regions, are not suitable in the tropics because they restrict air ventilation. The efficient adaptation of thrips' life cycle to high temperatures with high reproduction rates and a short generation time has led to extremely fast population increases after establishing in crops (Rodmui 2002, Premachandra et al. 2004, 2005c). This necessitates an efficient "task-force" control. Little is known so far about efficient chemical or biological strategies. Insecticides like Spinosad have been found to reduce *C. claratris* population growth (Premachandra et al. 2005b). However,

[3] based on: Thoeming G and Poehling H-M. Soil application of different neem products to control *Ceratothripoides claratris* (Thysanoptera: Thripidae) on tomatoes grown under protected cultiviation in the humid tropics (Thailand). Submitted to International Journal of Pest Management.

due to the cryptic feeding behaviour and the strong re-colonization pressure from life stages temporarily escaping to off-crop refuges (e.g. growing substrates), intensive spraying with complete wetting of above ground plant parts must be frequently repeated. This creates concerns due to the regular presence of farmers in a limited ventilation environment. There is also an increased risk of pests developing resistance.

Nowadays, seed or leaf extracts from the Neem tree (*Azadirachta indica* A. Juss., Meliaceae) containing azadirachtin (AZA) as the main active ingredient are becoming more and more important in pest management due to their insecticidal properties and environmental compatibility (Schmutterer 1990, Ruch et al. 1997). Neem products have several advantages compared to conventional synthetic pesticides, e.g. their fast degradation, low human and mammal toxicity (Ruch et al. 1997, Niemann and Hilbig 2000, Ullrichs et al. 2001) and so far, very low development of resistant pest biotypes (Völlinger 1992, Feng and Isman 1995). Topical AZA treatments can result in antifeedant, repellent and growth-regulating effects on many insects including thrips directly or via residues on plant surfaces (Schmutterer 1990, Mordue et al. 1998, Prabhaker et al. 1999, Pearsall and Hogue 2000). However, in the case of *C. claratris* the short persistence of AZA, particularly under tropical light and temperature conditions (Barrek et al. 2004), needs even higher frequencies of neem sprayings to achieve acceptable control levels (Saxena and Kidiavai 1997, Immaraju 1998). This reduces the comparative advantage to conventional pesticides. Neem soil applications might overcome these drawbacks if AZA is (i) directly applied to the soil-dwelling stages of the thrips and (ii) is delivered as a systemic treatment to target the plant-feeding stages. Recent studies in greenhouse and laboratory have shown the potential of soil applications with AZA to control the Western Flower Thrips, *F. occidentalis* (Ludwig and Oetting 2001, Thöming et al. 2003). *C. claratris* was shown to be sensitive to direct NeemAzal®-T/S and systemic application of NeemAzal-MD (5% AZA, Trifolio-M GmbH, Lahnau, Germany) but so far these studies have been carried out only under small scale laboratory conditions (Premachandra et al. 2005b).

Therefore, the overall objective of this study was to evaluate the potential of systemic effects of AZA to control *C. claratris* in netted greenhouses under practical, large scale growing conditions within a subtropical climate.

## 4.2 Material and Methods

The experiments were performed within the frame of the interdisciplinary research project 'Protected cultivation, an approach to sustainable vegetable production' on the campus of the Asian Institute of Technology (AIT) in Pathumthani, Thailand, in tropical greenhouses (200 $m^2$) with plastic roofs and netted side walls (0.18 mm diameter of mesh pores, Econet M, Ludvig Svensson, Hellevortsluis, The Netherlands). For all experiments tomato plants, *Lycopersicon esculentum* Mill., cv. King Kong II (Solanaceae), were used which were reared in a completely closed and pest free nursery. Three weeks after sowing, plants with four leaves were transferred to the experimental greenhouses. The plants were irrigated and fertilised on an average 7-9 times per day (2.5 l/d) via a drip irrigation system controlled by solar light integral (Freshco Intertrade Co., LTD, Bangkok, Thailand). The fertilisers used were: Hakaphos® (N-P-K, 2.5 kg/100 l, COMPO Austria, GmbH, Thailand) and Bai-plus® (Calcium, 1.8 kg/100 l, Bayer Ltd., Thailand). Climatic data, i.e. temperature and humidity, were recorded using a data logging system (ITG data logger, University Hannover, Germany). Mean air temperature, relative humidity and substrate temperature ranged from 27-35°C, RH 65-98% and 28-37°C, respectively. Preliminary experiments revealed that drip irrigation had no significant impact on the efficiency of neem soil application in thrips control compared to manual irrigation (Table 4.1). Thus, the drip irrigation system was used in all trials. A completely randomised design with ten plants per treatment was established.

### *Ceratothripoides claratris*

In all experiments tomato plants were naturally colonised by *C. claratris*. However, in cases of low infestation (i.e. less than 5 – 10 thrips per plant) or a patchy distribution of the thrips in the experimental greenhouse, adult *C. claratris* were added to the plants in a uniform pattern. They were taken from a stock culture kept on potted tomato plants of the same variety in a 36 $m^2$ greenhouse.

**Table 4.1 Corrected mortality (%) and thrips number per leaflet (mean ± SD) over the entire experimental term of 3 weeks after weekly soil application with NA-U blanc formulation (1.2 g/l) as control and NA-U (200 mg AZA/l) using different irrigation types (automatic drip irrigation (auto.), manual irrigation with 1.5, respect. 0.75 l/d).**

| Irrigation type | CM (%) | Number of thrips / leaflet (mean ± SD) d after 1st treatment | | | | |
|---|---|---|---|---|---|---|
| | | -1d* | 7d* | 14d* | 21d* | Total# |
| control, auto. | | 5.3 ± 2.4 a | 119.2 ± 48.6 a | 25.8 ± 11.4 a | 72.0 ± 37.6 a | A |
| control, manual 1.5 l/d | | 7.4 ± 2.9 a | 151.1 ± 49.8 a | 24.1 ± 9.3 a | 44.0 ± 12.5 a | A |
| control, manual 0,75 l/d | | 6.7 ± 1.5 a | 156.7 ± 39.0 a | 20.9 ± 5.9 a | 46.4 ± 14.7 a | A |
| Neem, auto. | 81.5 | 6.2 ± 3.0 a | 25.5 ± 13.1 b | 3.8 ± 1.6 b | 16.0 ± 7.8 b | B |
| Neem, manual 1.5 l/d | 77.27 | 6.7 ± 3.4 a | 41.4 ± 17.0 b | 2.9 ± 1.1 b | 9.8 ± 5.8 b | B |
| Neem, manual 0.75 l/d | 73.9 | 6.6 ± 2.2 a | 45.7 ± 25.1 b | 2.7 ± 0.9 b | 11.7 ± 9.6 b | B |

**Means followed by the same letter within columns are not significantly different (Tukey's test).**

**#Differences among treatments over the entire experimental term were marked with upper case letters**

***Differences among treatments for particular time interval were marked with lower case letters**

***Neem Products***

NeemAzal-U (NA-U) (17% AZA, Trifolio-M GmbH, Lahnau, Germany), a neem extract formulation intended for hydroponics and soil applications (registration pending) was used in the experiments. NeemAzal-U is a water-soluble powder and was used instead of the already registered NeemAzal®-T/S (1% AZA) since the latter formulation contains high amounts of oil (51%) and tenside (45%), which can have detrimental effects on root systems.

Additionally, the local neem products were used. Thai Neem Oil 111 (TNO) (1% AZA), recommended for spray applications on aerial plant parts, and Thai Neem Pellet 222 (TNP) (0.01% - 0.5% AZA) (both: Thai Neem Products Co., LTD, Suphanburi, Thailand). Although the pellet formulation has a variable AZA content, we have selected this product for our experiments since it is a low-priced neem product for soil treatments (insecticide and organic fertilizer) and widely used in Thailand. In analyses of active ingredient content the recorded amount of AZA in the used TNP batch was on average 52 mg AZA per 20g pellets (0.26%).

***Experiment 1. Effects of Concentration and Application Interval***

Three-week old tomato plants were planted into plastic pots (30 x 25 cm) filled with a common Thai culture substrate (11.4% organic matter, 38% sand, 20% silt, 42% clay, pH 5.4, Dinwondeekhankaset, Dinsontong company, Ayuthaya, Thailand). The plants were randomly arranged in six rows with 60 plants per row and 160 cm distance between the rows. The first soil treatments were applied as soon as a uniformly distributed thrips population (5 - 10 thrips per plant) was recorded. This occurred on average five to seven days after transplanting. AZA concentrations of 50, 200, 400 mg AZA/l were made up by taking 200 ml of three different concentrations of NeemAzal-U (0.3, 1.2, 2.4 g NA-U/l). These amounts and the blank formulation (1.2 g/l) of NeemAzal-U as control treatment were applied at three different application intervals, i.e. twice a week, weekly and every second week. In preliminary experiments, no significant differences in thrips numbers per leaflet among different concentrations of the blank formulation (0.3, 1.2, 2.4 g/l) and tap water were recorded. A weekly spray treatment of the aerial plant parts with the pyrethroid Cypermethrin (0.2%) was used as a positive standard and performed in a separate part of the

greenhouse to avoid contamination. The soil drenching was performed after the last irrigation cycle during the evening to prevent an immediate wash-out of the bio pesticide. Numbers of *C. claratris* were recorded following the procedure used by Premachandra et al. (2004, 2005 b, c): a sample of one leaflet per plant randomly selected from the middle stratum characterises infestation of the whole tomato plant. First samples were taken before the first application, and thereafter, weekly. On each leaflet the number of thrips was counted under a stereo microscope. Four weeks after the first treatment the experiment was terminated and the following parameters were assessed: plant-, root-, stem- and leaf weights, plant length, leaf area, number of leaves, florescences and fruits.

***Experiment 2. Effects of Substrate Type***

The efficacy of AZA applications on thrips populations using four substrates differing in their organic matter content was evaluated. Three-week old tomato plants were planted into plastic pots filled with: i) a high organic matter substrate used for nursery (21.6% organic matter, 68% sand, 4% silt, 28% clay, pH 5.4; Pindstrup Substrate No1, Imported from Pindstrup Mosebrug A/S, Pindstrup, Denmark), ii) a local Thai culture substrate (see Experiment 1), and iii) both substrates were mixed in a 2:1 ratio with sand to reduce the content of organic matter to 6.9% and 4%, respectively. The substrate analyses were carried out at the Department of Soil Science, Faculty of Agriculture, Kasetsart University of Bangkok, Thailand. Pots were arranged and treated as described in the previous experiment applying weekly 200 ml of NeemAzal-U solution (200 mg AZA/l). The control treatments were carried out in the Thai culture substrate. Experimental protocol and data assessment were the same as described for Experiment 1. Six weeks after the first treatment the experiment was terminated. Tomato plants were evaluated as in the first trial, four and six weeks after the first treatment.

***Experiment 3. Effects of Tomato Plant Age***

Two, three, four and five week old tomato plants were transplanted at the same time into plastic pots filled with the Thai culture substrate. Once the expected number and distribution of thrips per plant were recorded (see above), 200 ml of

NeemAzal-U solution (200 mg AZA/l) and the control treatments were applied weekly. Data recording was performed as described for Experiment 1 and 2.

***Experiment 4. Effects of Neem Extract Application Date***

Three-week old tomato plants in Thai culture substrate were infested with five adult thrips each, three days after transplanting. The same treatments as described in the previous experiment were applied two days before (2db), on the same day (0d) and two, five and seven days after (2, 5, 7da) thrips infestation. Thrips counting and evaluation of plants were carried out as described in Experiments 1 and 2.

***Experiment 5. Effects of Thai Neem Products***

This experiment was conducted to compare the efficacy of soil applications of the two previously mentioned Thai neem products on *C. claratris*. The age of tomato plants and the substrate type were identical as in Experiment 3 and 4. In the TNP treatment, 20 g pellets were added to the substrate under roots during transplanting. Once a sufficiently high and stable thrips infestation was reached (see above) 200 ml of NeemAzal-U and TNO solution, both with a dose rate of 200 mg AZA/l, and control treatments (see above) were applied. Data were recorded as described in Experiment 1 and 2.

***Statistical Analysis***

The corrected mortality was calculated as described by Henderson and Tilton (1955). The number of thrips per leaflet from each of the experiments was subjected to Levene test to evaluate for variance homogeneity, and normalized using log-transformation if necessary. In each experiment the data were analysed by means of repeated measures ANOVA (SAS 1999), considering dates of evaluation as a repeated parameter, using AUC (area under the curve) estimation (Zerbe 1979). If ANOVA showed significant differences among treatments, Tukey's test (Sokal & Rohlf 1995) was used for multiple comparisons. To identify particular time intervals in which treatment effects were different, individual ANOVAs were used. Plant growth data was analysed using MANOVA (SAS 1999) and treatment means were compared using Tukey's test. A significance level of $\alpha=0.05$ was selected in all analyses.

## 4.3 Results

### *Experiment 1*

The number of thrips per leaflet was significantly reduced after an application of AZA and Cypermethrin ($F=105.75$; $df=10, 99$; $P<0.0001$) in all experiments for all tested AZA treatments (Table 4.2 - 4.6). A significant dose-dependent efficiency in thrips control after AZA soil treatment using different dose rates was recorded (Table 4.2). All 400 mg AZA/l treatments as well as the soil application twice a week with 200 mg AZA/l resulted in similar effects to the chemical spray treatment with Cypermethrin (Table 4.2). Similarly, high dose rates of NeemAzal-U (400 mg AZA/l) and the Cypermethrin treatment resulted in greater leaf area (Figure 4.1(A)), leaf weight (Figure 4.1(B)) and plant weight (Figure 4.1(C)) compared to the control and the lower AZA concentrations (200 and 50 mg AZA/l). Overall, the different application intervals showed no influence on thrips control and plant growth parameter within each tested dose rate (Table 4.2, Figure 4.1).

### *Experiment 2*

The effect of NeemAzal-U on the number of thrips per leaflet was not significantly different after soil application on different types of growing substrate with one exception: significantly lower thrips numbers were found using the culture substrate-sand mixture compared to the other three substrate types two and three weeks after the start of the treatment (14d: $F=42.00$; $df=5, 64$, $P<0.0001$; 21d: $F=45.23$; $df=5, 64$; Culture substrate: $P<0.0059$; Nursery substrate: $P<0.0044$; Nursery-sand substrate: $P<0.0005$) (Table 4.3). No influence of the substrate type on tomato plant growth after NeemAzal-U soil treatment was detected (Figure 4.2). Moreover, no effects were recorded for tomato plant growth parameters six weeks after the first treatment in all experiments.

**Table 4.2 Corrected mortality (%) and thrips numbers per leaflet (mean ± SD) over the entire experimental term of 4 weeks after soil application with NA-U blank formulation (1.2 g/l) as control, Cypermethrin (0.2%) as positive standard and NA-U in different AZA-concentrations for different application rates.**

| Dose rate (mg AZA/l), application rate | CM (%) | Number of thrips / leaflet (mean ± SD) d after the first treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | -1d* | 7d* | 14d* | 21d* | 28d* | Total# |
| Control, twice a week | | 1.4 ± 1.1 a | 44.7 ± 11.3 a | 58.0 ± 15.4 a | 74.8 ± 18.0 a | 83.9 ± 13.1 a | A |
| 50, every second week | 58.9 | 1.7 ± 0.9 a | 15.6 ± 8.9 b | 27.5 ± 19.0 b | 28.1 ± 9.9 b | 41.8 ± 9.7 b | B |
| 50, weekly | 73.1 | 2.6 ± 1.6 a | 15.2 ± 6.7 b | 19.5 ± 7.4 b | 29.9 ± 8.0 b | 41.9 ± 10.4 b | B |
| 50, twice a week | 61.3 | 1.6 ± 1.3 a | 14.7 ± 8.8 b | 20.1 ± 8.0 b | 32.5 ± 19.1cb | 37.1 ± 6.9 b | B |
| 200, every second week | 71.4 | 1.4 ± 1.0 a | 11.7 ± 3.1 cd | 10.4 ± 1.5 c | 15.4 ± 9.8 cd | 24.0 ± 14.3 c | CD |
| 200, weekly | 81.9 | 1.8 ± 0.8 a | 5.8 ± 7.7 bc | 4.6 ± 5.7 c | 13.0 ± 7.8 d | 19.5 ± 3.8 dc | CD |
| 200, twice a week | 86.9 | 2.0 ± 1.3 a | 5.5 ± 2.9 cd | 4.9 ± 2.0 c | 11.0 ± 6.5 d | 15.7 ± 7.4 de | CDE |
| 400, every second week | 85.5 | 0.9 ± 0.3 a | 2.9 ± 1.5 de | 1.9 ± 1.6 d | 2.7 ± 1.9 e | 7.8 ± 3.5 e | DE |
| 400, weekly | 92.7 | 1.6 ± 0.8 a | 2.2 ± 2.0 def | 0.6 ± 0.7 de | 2.6 ± 2.6 e | 7.0 ± 3.5 e | DE |
| 400, twice a week | 95.8 | 0.8 ± 0.6 a | 1.8 ± 1.4 ef | 0.4 ± 0.5 de | 0.6 ± 0.7 e | 2.0 ± 1.6 f | E |
| Cypermethrin, weekly | 89.6 | 0.4 ± 0.6 a | 0.0 ± 0.0 f | 0.2 ± 0.4 e | 1.2 ± 1.5 e | 2.5 ± 1.1 f | E |

**Means followed by the same letter within columns are not significantly different (Tukey's test).**

**#Differences among treatments over the entire experimental term were marked with upper case letters**

***Differences among treatments for particular time interval were marked with lower case letters**

**Table 4.3 Corrected mortality (%) and thrips numbers per leaflet (mean ± SD) over the entire experimental term of 6 weeks after weekly soil application with NA-U blank formulation (1.2 g/l) as control, Cypermethrin (0.2%) as positive standard and NA-U (200 mg AZA/l) using different substrates.**

| Substrate | CM (%) | Number of thrips / leaflet (mean ± SD) d after the first treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | -1d* | 7d* | 14d* | 21d* | 28d* | 35d* | 42d* | Total# |
| Control | | 6.1 ± 2.7 a | 102.0 ± 30.3 a | 29.4 ± 11.4 a | 78.4 ± 30.6 a | 52.1 ± 16.9 a | 101.0 ± 28.7 a | 118.6 ± 31.2a | A |
| Nursery | 75.1 | 6.8 ± 3.1 a | 23.1 ± 9.0 b | 5.0 ± 2.4 b | 16.5 ± 8.8 b | 16.9 ± 6.5 b | 44.9 ± 18.4 b | 32.9 ± 9.3 b | B |
| Culture | 71.5 | 6.2 ± 3.0 a | 25.5 ± 9.8 b | 3.8 ± 1.1 b | 16.0 ± 6.2 b | 14.3 ± 5.7 b | 37.8 ± 13.1 b | 34.4 ± 13.4 b | B |
| Nursery-sand | 62.6 | 5.5 ± 2.8 a | 31.8 ± 11.3 b | 6.4 ± 2.6 b | 22.5 ± 9.6 b | 14.1 ± 5.0 b | 45.5 ± 18.6 b | 40.0 ± 10.0 b | B |
| Culture-sand | 58.7 | 5.3 ± 2.6 a | 31.3 ± 11.0 b | 1.1 ± 0.7 c | 12.7 ± 4.4 c | 19.1 ± 5.9 b | 40.3 ± 14.2 b | 42.6 ± 18.4 b | B |
| Cypermethrin | 96.6 | 6.2 ± 2.4 a | 0.1 ± 0.3 c | 0.3 ± 0.7 c | 1.1 ± 1.3 d | 2.4 ± 0.8 c | 4.0 ± 1.2 c | 4.1 ± 1.4 c | C |

**Means followed by the same letter within columns are not significantly different (Tukey's test).**

**#Differences among treatments over the entire experimental term were marked with upper case letters**

***Differences among treatments for particular time interval were marked with lower case letters**

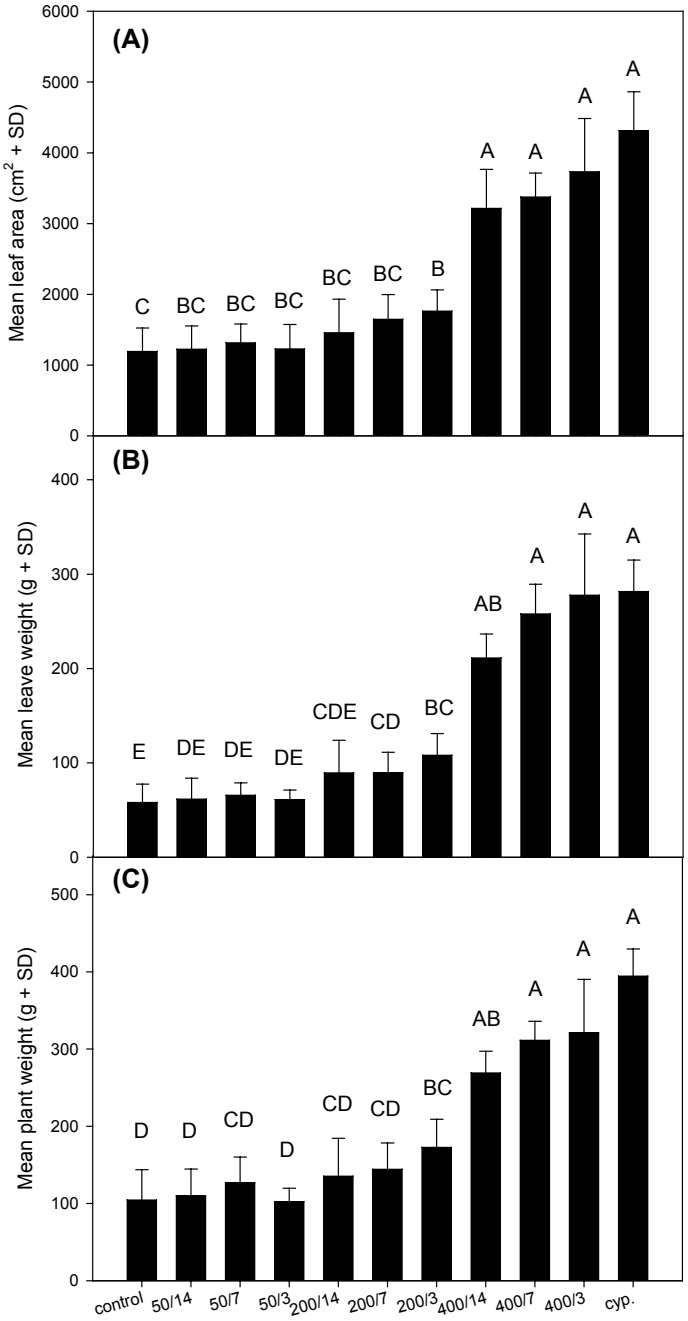


**Figure 4.1 Mean leaf area ($cm^2$ + SD) (A), mean leaf weight (g + SD) (B), and mean plant weight (g + SD) (C) of tomato plants after soil applications of NA-U using different AZA doses (50, 200, 400 mg AZA/l) and different application intervals (14, 7, 3) compared with NA-U blank formulation (1.2 g/l) (control) or Cypermethrin (0.2%) as positive standard (cyp.), evaluated 4 weeks after the first treatment. Columns marked with the same letter are not statistically different, P>0.05.**

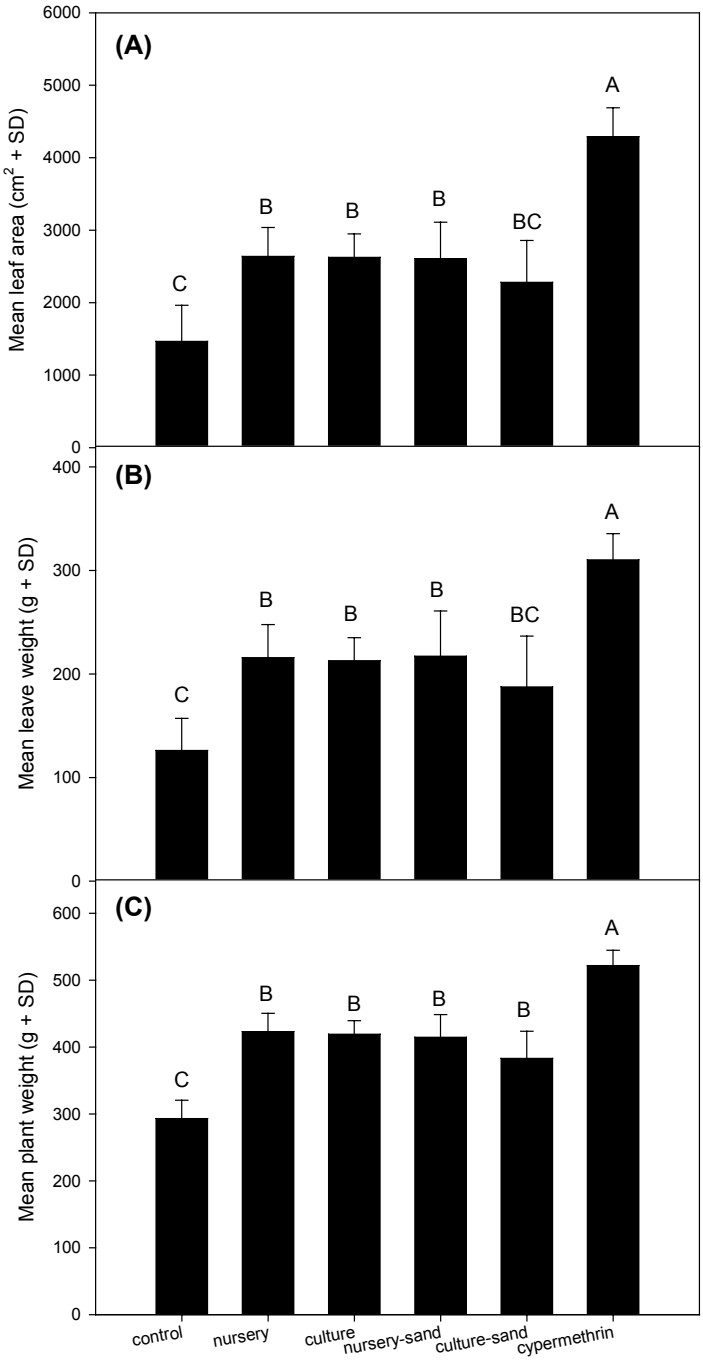


**Figure 4.2 Mean leaf area ($cm^2$ + SD) (A), mean leaf weight (g + SD) (B), and mean plant weight (g + SD) (C) of tomato plants after soil applications of NA-U soil application (200 mg AZA/l) and treatment witj NA-U blank formulation (1.2 g/l) (control) or Cypermethrin (0.2%) as positive standard using different substrates, evaluated 4 weeks after the first treatment. Columns marked with the same letter are not statistically different, P>0.05.**

***Experiment 3***

A strong influence of plant age on the efficiency of AZA soil application on the development of *C. claratris* was revealed with significantly lower thrips numbers per leaflet on young tomato plants (two and three week old) compared to older plants (four and five week old) ($F=239.56$; $df=5, 64$; $P<0.0001$) (Table 4.4). From the third week after the first treatment, significant differences were recorded also between two and three week old plants (Table 4.4). The estimation of plant growth parameters four weeks after the first neem soil application verified these results. Leaf area (Figure 4.3(A)), leaf (Figure 4.3(B)) and plant weight (Figure 4.3(C)) were significantly greater after AZA soil and Cypermethrin treatment in two and three week old plants compared to the control.

***Experiment 4***

Thrips numbers increased substantially in relation to greater time intervals between infestation and first treatment (Table 4.5). Treatments applied seven days after thrips infestation (7da) resulted in a significantly higher number of thrips compared to earlier treatments (2da, 0d, 2db) ($F=282.3$; $df=6, 39$; $P<0.0011$; $P<0.0216$; $P<0.0003$, respectively) (Table 4.5). Overall, no influence of application date of NeemAzal-U soil treatment on tomato plant growth was detected (Figure 4.4).

***Experiment 5***

The TNO soil application had little success in thrips control yielding a significantly higher number of *C. claratris* compared to NA-U and TNP ($F=437.01$; $df=5, 34$; $P<0.0001$) (Table 4.6). During the early phase of population build up (21d and 28d after the first infection) the NA-U soil application resulted in the highest control efficacy considering the different neem products (21d: $F=129.19$; $df=5, 34$;TNO: $P<0.0001$; TNP: $P<0.0012$; 28d: $F=209.06$; $df=5, 34$; TNO: $P<0.0001$; TNP: $P<0.0162$). Later on, soil treatment with TNP was significantly better than the NA-U soil application (35d: $F=486.18$; $df=5, 34$; $P<0.0001$; 42d: $F=496.25$; $df=5, 34$; $P<0.0001$) (Table 4.6). Within the three different AZA treatments no significant differences in the tomato plant growth parameters were recorded (Figure 4.5).

**Table 4.4 Corrected mortality (%) and thrips numbers per leaflet (mean ± SD) over the entire experimental term of 6 weeks after weekly soil applications with NA-U blank formulation (1.2 g/l) (control, 3 w.), Cypermethrin (0.2%) as positive standard (Cyp.) and NA-U (200 mg AZA/l) using different plant ages (plants were planted in green house 5, 4, 3, 2 weeks after sowing).**

| Treatment, plant age (week) | CM (%) | Number of thrips / leaflet (mean ± SD) d after the first treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | -1d* | 7d* | 14d* | 21d* | 28d* | 35d* | 42d* | Total# |
| Control | | 0.2 ± 0.4 a | 17.4 ± 9.8 a | 42.6 ± 13.3 a | 55.1 ± 15.9 a | 85.8 ± 19.7 a | 96.4 ± 9.6 a | 125.8 ± 8.5a | A |
| NA-U, 5 | 41.4 | 0.3 ± 0.5 a | 3.8 ± 2.7 b | 6.0 ± 4.7 b | 14.5 ± 8.4 b | 52.5 ± 9.9 b | 76.7 ± 9.8 b | 110.5 ± 16.3 b | B |
| NA-U, 4 | 44.1 | 0.3 ± 0.5 a | 3.3 ± 2.1 b | 5.9 ± 3.9 b | 21.5 ± 10.8 b | 48.2 ± 20.7 b | 72.7 ± 25.1 b | 105.5 ± 17.7 b | B |
| NA-U, 3 | 57.9 | 0.2 ± 0.4 a | 0.3 ± 0.5 c | 0.6 ± 0.8 c | 4.7 ± 3.1 c | 7.9 ± 3.6 c | 27.6 ± 6.4 c | 52.9 ± 3.6 c | C |
| NA-U, 2 | 87.3 | 0.4 ± 0.5 a | 0.3 ± 0.5 c | 1.3 ± 1.3 c | 1.1 ± 1.0 d | 2.6 ± 1.4 d | 15.7 ± 6.4 d | 32.0 ± 7.9 d | CD |
| Cyp. | 98.1 | 0.4 ± 0.7 a | 0.5 ± 0.8 c | 0.2 ± 0.4 c | 0.9 ± 1.1 d | 2.4 ± 0.8 d | 4.3 ± 1.1 e | 4.7 ± 1.4 e | D |

**Means followed by the same letter within columns are not significantly different (Tukey's test).**

**#Differences among treatments over the entire experimental term were marked with upper case letters**

***Differences among treatments for particular time interval were marked with lower case letters**

**Table 4.5 Corrected mortality (%) and thrips numbers per leaflet (mean ± SD) over the entire experimental term of 6 weeks after soil applications with NA-U blank formulation (1.2 g/l) on the day of thrips infestation as control (0d), Cypermethrin (0.2%) as positive standard and NA-U (200 mg AZA/l) employing different time intervals (AZA soil application 7d (7da,) 5d (5da), 2d after (2da), 0d, 2 d before (2db) thrips infestation).**

| Treatment, day of treatment | CM (%) | Number of thrips / leaflet (mean ± SD) d after thrips infection | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0d* | 7d* | 14d* | 21d* | 28d* | 35d* | 42d* | Total# |
| Control | | 0.1 ± 0.2 a | 2.8 ± 1.2 a | 8.3 ± 1.6 a | 22.2 ± 3.7 a | 39.7 ± 3.4 a | 57.0 ± 4.2 a | 77.5 ± 4.1 a | A |
| NA-U, 7d a | 58.5 | 0.2 ± 0.3 a | 2.2 ± 0.8 a | 4.7 ± 0.8 ab | 13.0 ± 2.9 b | 26.8 ± 3.2 b | 45.0 ± 4.9 bc | 64.3 ± 6.9 b | B |
| NA-U, 5d a | 71.7 | 0.3 ± 0.4 a | 0.9 ± 0.5 b | 3.3 ± 1.2 b | 10.7 ± 1.9 bc | 17.2 ± 1.5 b | 39.2 ± 3.1 c | 65.8 ± 2.4 b | BC |
| NA-U, 2d a | 60.6 | 0.2 ± 0.2 a | 0.0 ± 0.0 b | 0.0 ± 0.0 d | 7.3 ± 2.0 de | 15.8 ± 2.0 b | 38.8 ± 7.5 bc | 61.0 ± 5.4 b | D |
| NA-U, 0 d | 79.7 | 0.4 ± 0.6 a | 0.2 ± 0.1 b | 0.2 ± 0.4 cd | 8.0 ± 2.4 cd | 17.0 ± 1.3 b | 46.2 ± 4.3 bc | 63.2 ± 2.0 b | CD |
| NA-U, 2d b | 82.8 | 0.5 ± 0.5 a | 0.0 ± 0.0 b | 1.2 ± 1.6 cd | 5.0 ± 1.1 e | 16.5 ± 0.8 b | 43.5 ± 6.9 bc | 66.5 ± 3.4 b | CD |
| Cypermethrin | 96.9 | 0.2 ± 0.4 a | 0.0 ± 0.0 b | 0.0 ± 0.0 d | 1.0 ± 1.2 f | 2.3 ± 1.3 c | 4.0 ± 1.4 d | 4.8 ± 1.3 c | E |

**Means followed by the same letter within columns are not significantly different (Tukey's test).**

**#Differences among treatments over the entire experimental term were marked with upper case letters**

***Differences among treatments for particular time interval were marked with lower case letters**

**Table 4.6 Corrected mortality (%) and thrips numbers per leaflet (mean ± SD) over the entire experimental term of 6 weeks after soil applications with NA-U blank formulation (1.2 g/l) (control), TNO (200 mg AZA/l), NeemAzal-U (200 mg AZA/l) (NA-U), TNP (52 mg AZA/20g pellet) and spray treatment of Cypermethrin (0.2%) as extra standard.**

| Treatments | CM (%) | Number of thrips / leaflet (mean ± SD) d after the first infection | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0d* | 7d* | 14d* | 21d* | 28d* | 35d* | 42d* | Total# |
| Control | | 0.3 ± 0.5 a | 2.9 ± 1.6 a | 8.8 ± 1.9 a | 23.0 ± 3.9 a | 37.2 ± 3.2 a | 57.7 ± 5.2 a | 79.5 ± 4.7 a | A |
| TNO | 46.5 | 0.3 ± 0.5 a | 0.5 ± 0.5 b | 0.3 ± 0.8 b | 7.5 ± 0.8 b | 12.7 ± 6.3 a | 33.5 ± 1.0 b | 42.5 ± 2.1 b | B |
| NA-U | 60.0 | 0.3 ± 0.4 a | 0.2 ± 0.4 b | 0.8 ± 1.6 b | 1.3 ± 0.5 c | 4.5 ± 3.1 c | 24.0 ± 2.3 c | 31.8 ± 2.9 c | C |
| TNP | 71.2 | 0.3 ± 0.3 a | 0.3 ± 0.5 b | 0.7 ± 1.9 b | 4.3 ± 1.2 b | 7.9 ± 1.9 b | 11.1 ± 2.5 d | 22.9 ± 4.9 d | C |
| Cypermethrin | 90.9 | 0.2 ± 0.3 a | 0.0 ± 0.0 b | 0.0 ± 0.0 b | 1.0 ± 1.2 c | 2.3 ± 1.3 c | 4.0 ± 1.4 e | 4.8 ± 1.3 e | D |

**Means followed by the same letter within columns are not significantly different (Tukey's test).**

**#Differences among treatments over the entire experimental term were marked with upper case letters**

***Differences among treatments for particular time interval were marked with lower case letters**

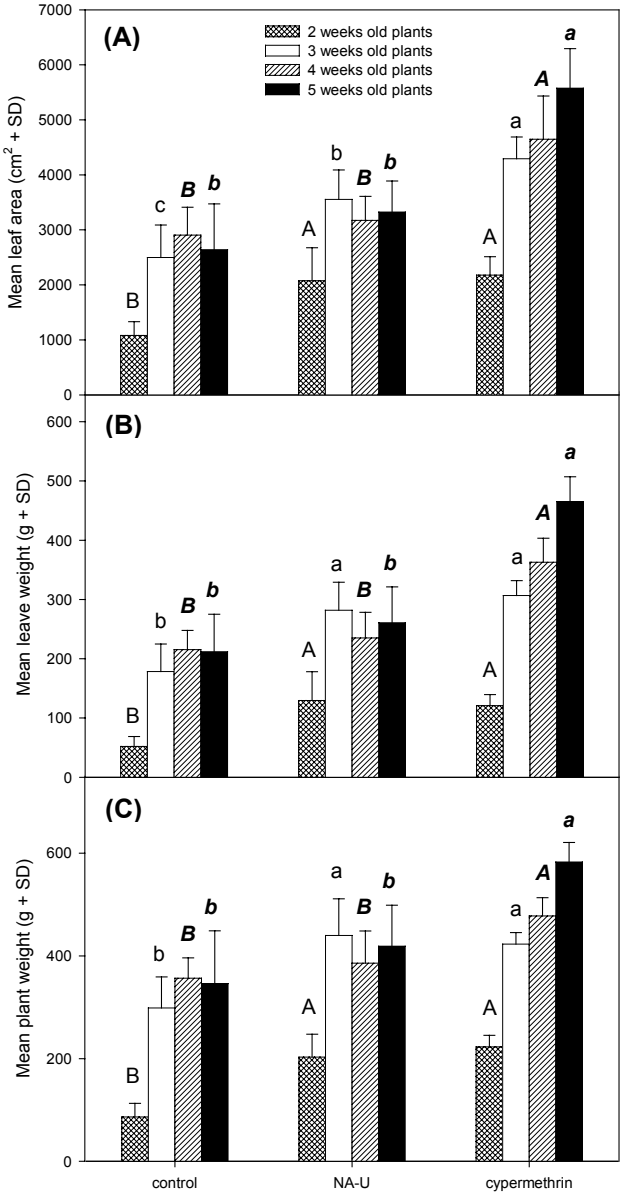


**Figure 4.3 Mean leaf area ($cm^2$ + SD) (A), mean leaf weight (g + SD) (B), and mean plant weight (g + SD) (C) of tomato plants after NA-U soil applications (200 mg AZA/l) and treatment with NA-U blank formulation (1.2 g/l) (control) or Cypermethrin (0.2%) as positive standard using different plant ages (2, 3, 4, 5 week old plants), evaluated 4 weeks after the first treatment. The different treatments were compared separately for every plant age; 2 weeks old plants marked with upper case letter, 3 weeks old plants with lower case letter, 4 weeks old plants upper case letter, bold, italic and 5 weeks old plants with lower case letter, bold, italic. Columns marked with the same letter are not statistically different, P>0.05.**

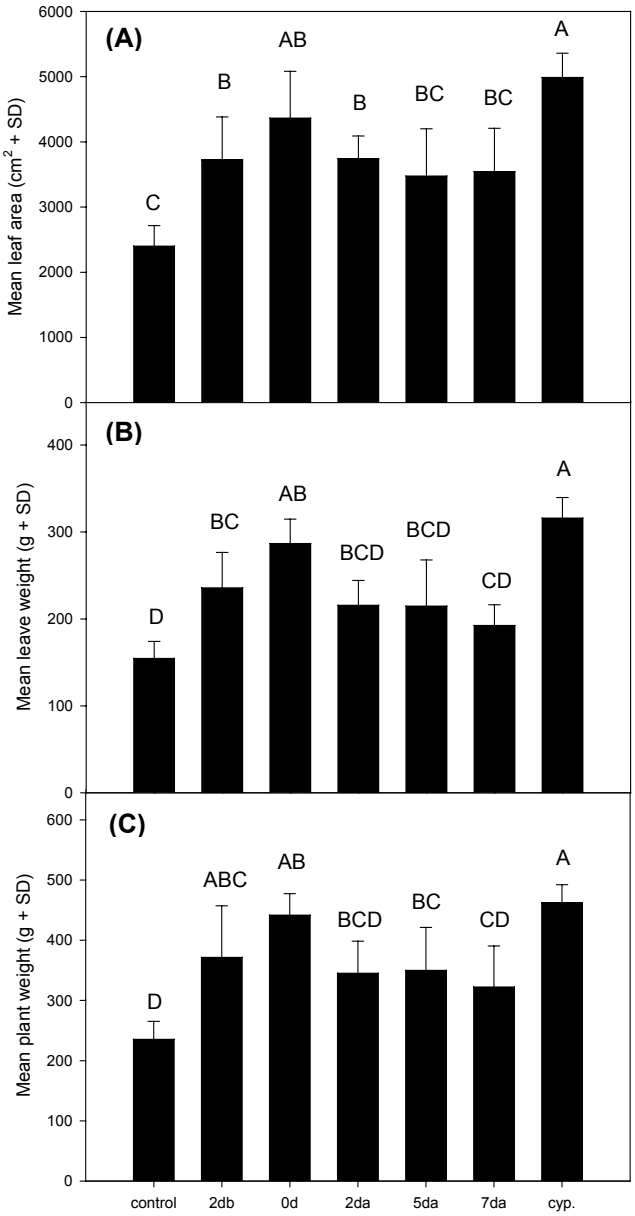


**Figure 4.4 Mean leaf area ($cm^2$ + SD) (A), mean leaf weight (g + SD) (B) and mean plant weight (g + SD) (C) of total tomato plants after NA-U soil application (200 mg AZA/l) using different time treatments (neem soil application seven days (7da,) five days (5da), two days after (2da), at the same day (0d), two days before (2db) thrips infestation) or NeemAzal-U blank formulation (1.2 g/l) at the day of thrips infestation as control, Cypermethrin (0.2%) as positiv standard (cyp.), evaluated 4 weeks after the first treatment. Columns marked with the same letter are not statistically different, P>0.05.**

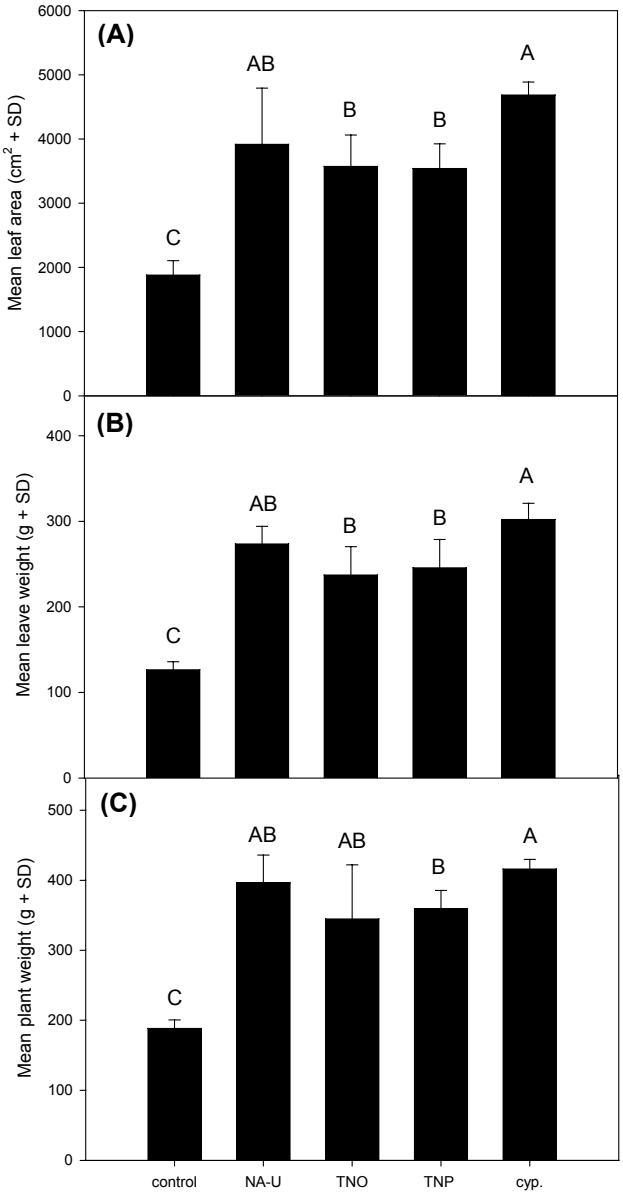


**Figure 4.5 Mean leave area ($cm^2$ + SD) (A), mean leaf weight (g + SD) (B) and mean plant weight (g + SD) (C) of total tomato plants after weekly soil application with NA-U blank formulation (1.2 g/l) as control, spray treatment of Cypermethrin (0.2%) as positiv standard (cyp.), soil treatment with NA-U (200 mg AZA/l), Thai Neem Oil 111 (200 mg AZA) (TNO) and Thai Neem Pellets 222 (52 mg AZA/20g pellet) (TNP), evaluated 4 weeks after the first treatment. Columns marked with the same letter are not statistically different, P>0.05.**

## 4.4 Discussion

***Effects of Concentration and Application Interval***

NeemAzal-U applied to the soil can reduce population growth of *Ceratothripoides claratris* on tomatoes under tropical greenhouse conditions over a period of four weeks as common synthetic insecticides (i.e. Cypermethrin) can do. However, high doses of AZA (400 mg AZA/l, i.e. 80 mg AZA/kg substrate) are needed to achieve acceptable levels of control with an efficacy of more than 85%. In contrast, laboratory studies with soil drenching using NeemAzal MD (5% AZA) had less impact even with high AZA dose rates (10, 12.5, 15 g NeemAzal MD/l) and only affected mainly the L1 *C. claratris* larvae (Premachandra et al. 2005b). However, it is difficult to compare both experiments on the basis of the available data because of the different experimental setup. For instance, due to the different microclimatic conditions in the laboratory experiments, where plants grow in closed cages in a very humid environment, the evaporation and water transport from roots to leaves and therefore the translocation of soil-applied AZA is reduced. Moreover, considering the optimal temperature for development of *C. claratris* at 32-33°C (Premachandra et al. 2004), the conditions for the thrips development were sub-optimal at laboratory with mean temperatures of 25°C. This reduces feeding intensity and AZA uptake of the insects. Thus, the measured systemic effects on *C. claratris* could be much lower in laboratory experiments compared to the more optimal conditions for plant growth, AZA uptake and distribution and thrips development in the greenhouse. Similar greenhouse trials in a temperate climate with *F. occidentalis* on *Phaseolus vulgaris* resulted in high mortalities after NeemAzal-U soil application even with lower AZA concentrations (100 mg AZA/l, i.e. 64 mg AZA/kg substrate) for a period of at least six weeks (Chapter 3). A possible reason for the need of high amounts of active ingredient in a tropical climate could be the higher substrate and surrounding environment temperatures. On the one hand high temperature facilitates plant growth (Papadopoulos et al. 1997, Jones 1999), and therefore, more AZA is required to provide all plant parts with sufficient levels of active ingredient to affect thrips. On the other hand, a faster degradation of AZA in soil due to high substrate temperatures could be expected in comparison to temperate regions (Sundaram 1994, Stark and Walter 1995a, Barrek et al 2004).

***Effects of Substrate Type***

A negative relationship between systemic efficacy and organic matter content of the substrate was shown in several laboratory studies (e.g. Gill and Lewis 1971, Oßiewatsch 2000, Thöming et al. 2003). Organic matter can absorb AZA, thus influencing the amount of available active components in the rhizosphere and the uptake into the roots (Sundaram 1994, Ruch et al. 1997, Pussemeier 2000). However, no explicit influence of organic matter content on AZA induced thrips mortality could be recorded in our greenhouse experiment. Possibly other factors such as the high and variable temperature and the high irrigation frequency outweighed the substrate effects found in laboratory experiments in a controlled environment.

***Effects of Tomato Plant Age***

Plant age showed a strong influence on the efficiency of soil-applied neem extract to control *C. claratris*. The systemic distribution of AZA seems to be more concentrated the younger the plants are. Sundaram (1996a) demonstrated that systemic effects of neem ingredients were most efficient using young spruce seedlings and nursery plants, similar to our results. The distribution of AZA after root uptake into various plant organs is not homogenous. It was shown that after AZA soil treatments the active ingredient is taken up by the root system, translocated via xylem vessels and concentrated in areas of new growth (Sundaram et al. 1995, Sundaram 1996a). Such a selective distribution pattern with “loading” of new leaf material has been recently quantified in one of our studies with slow growing bean plants in the greenhouse under temperate climate conditions (Chapter 2). Tropical climates, however, accelerate plant growth much more. Thus, in these trials the main part of active neem ingredients was probably rapidly transported to plant parts with maximum growth whereas older plant parts received less. Studies on distribution and population dynamics of *C. claratris* on tomatos in Thailand indicated that this thrips species infests first lower leaf strata and subsequently moves to the top of the plant (Premachandra et al. 2005c). Thus, *C. claratris* infests mainly older plant parts, containing lesser amounts of AZA. Furthermore, younger plants have a lower amount of biomass compared to older plants. For instance, the mean fresh weight of two weeks old plants in total was 144.4 g

versus the average weight of five weeks old plants of 419.1 g. Nevertheless, plants of both ages were provided with the same amount of AZA (80 mg AZA weekly). Thus, mean AZA concentrations were lower in older plants (e.g. 0.19 g AZA/g plant) compared to younger plants (e.g. 0.55 g AZA/g plant). These differences in concentrations could explain the more effective thrips control on younger plants.

***Effects of Neem Extract Application Date***

A trend of increased numbers of *C. claratris* per leaflet and greater plant damage were observed with a delayed AZA soil application. Similar effects due to different application dates of neem extracts were recorded with *F. occidentalis* on *Phaseolus vulgaris* after NeemAzal®-T/S soil application (100 mg AZA/l) in laboratory studies (Thöming et al. 2003). A slow transport of active ingredients after soil drenching with a neem extract can result in such delayed efficacy (Meisner et al. 1986). Thus, the start of such soil treatment as soon as possible after plant infestation could improve the efficacy of soil-applied neem extracts against thrips. The results so far indicate that weekly AZA soil applications from the beginning of transplanting of young tomato plants and the use of doses ranges of 200 to 400 mg AZA /l give the most effective control of *C. claratris* for at least four weeks.

***Effects of Thai Neem Products***

All tested neem products reduced the numbers of *C. claratris* on the tomato plants after soil application compared to untreated control plants. The weakest effects were observed with Thai Neem Oil. The oily neem extract formulation was developed for spray applications on aboveground plant parts, whereas NeemAzal-U and Thai Neem Pellet were prepared especially for soil treatments. Thus, the kind of formulation could have influenced the thrips control efficacy (Stark and Walter 1995b). During the first weeks of crop development NeemAzal-U seems to provide the most efficient control of *C. claratris*, whereas later Thai Neem Pellets were more successful. In contrast to NeemAzal-U as a water-based solution, neem pellets such as neem cakes or other ‘slow-dispenser’ formulations for soil application facilitate a continuous slow release of the active neem components. This can result in delayed but

strong long-term effects in pest management due to a longer AZA supply (Rajappan et al. 2000, Singh and Singh 2003). Thus, Thai Neem Pellet seems to be a promising alternative to applications with highly purified but more expensive soluble neem products. A simple comparison of product prices and recommended concentrations for one greenhouse unit (200 $m^2$, 360 tomato plants, weekly soil application) indicates that NeemAzal-U (currently estimated price per kg NeemAzal) at 8111.22 Baht/month (158.98 Euro/month) and Thai Neem Oil at 11580.00 Baht/month (226.97 Euro/month) are very high priced. Thai Neem Pellets are at 162.00 Baht/month (3.18 Euro/month) much cheaper but still more expensive when compared to a common Cypermethrin spray treatment with 11.84 Baht/month (0.23 Euro/month). Importantly, when compared to chemical pesticides neem products such as the Thai Neem Pellets are non-polluting and cause lower risks for farmers, consumers and minimises resistance development. These factors go along way in compensating for the higher price. The use of Spinosad (0.15%), which has been recently propagated for thrips control because of its high efficacy and low risk to humans, non-target organisms and environment (Thompson et al. 2000, Reitz et al. 2003), has at 150 Baht/month (2.94 Euro/month) a comparable price to Thai Neem Pellet. However, the risk of resistance development for Spinosad seems to be much higher compared to neem extracts. Several Studies have already recorded resistance development against Spinosad e.g. in strains of *Heliothis virescens* (Lepidoptera: Noctuidae), *Liriomyza trifolii* (Diptera: Agromyzidae), *Plutella xylostella* (Lepitoptera: Plutellidae) and *F. occidentalis* (Wyss et al. 2003, Ferguson 2004, Sayyed et al. 2004, Loughner et al. 2005).

In summary, soil-applied neem extracts has a systemic effect on *Ceratothripoides claratris* on potted tomato plants under conditions of protected cultivation in the humid tropics. However, control is only satisfactory if high AZA concentrations are used and if the first infestations with thrips on young plants are targeted early. Long-term protection with soil treatments seems to be difficult and needs additional measures of integrated pest management. The highest efficacy in young plants could be achieved with the particular formulation of NeemAzal as it is well studied and in relation to the content of active ingredient a very reliable product. However, even the inexpensive and

locally available Thai Neem Pellet product demonstrated sufficient efficacy against the thrips to avoid unacceptable damage during the first four weeks of crop development. Hence, AZA soil treatments especially with local neem products could become an efficient tool in integrated pest management of *C. claratris* on tomatoes in protected cultivation in the tropics and subtropics.

# 5 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.

A strong thrips control efficacy due to systemic action after neem soil application, as recorded in preliminary investigations (Thöming et al. 2003), could be confirmed in the presented study. In addition, the root uptake and acropetally translocation of different active neem components to upper plant parts of French bean were demonstrated in the results of Chapter 2. The studies on degradation kinetics of the tested active ingredients in different substrates and on translocation pattern of the components in the bean plant clarified the physiological and bioanalytical basis of the systemic activity. Considering the practical use of soil-applied neem products, the results in Chapter 3 and 4 demonstrated the ability of neem products as soil treatment for thrips control. The reliability and efficiency in controlling thrips (*Frankliniella occidentalis)* was improved with substrate-applied neem compounds and predatory mites in a combined treatment with only minor side effects on the soil-dwelling predator (Chapter 3). This promising efficacy of thrips control with soil application of neem products was achieved not only on French beans in closed greenhouse systems in a temperate zone (Germany), but also on tomatoes cultivated under tropical conditions in net houses in the humid tropics (Thailand). However, soil-applied neem ingredients could affect thrips (*Ceratothripoides claratris*) in the tropical system only if comparably high AZA dose rates were applied to the soil, and if treatments were scheduled to combat first thrips infestations on young plants (Chapter 4). Thus, the presented strong effects of neem extract soil treatment against WFT on French bean are not universal for thrips control. Under tropical climate conditions in protected tomato production the thrips control efficacy after neem soil application was limited. An adequate thrips management over a longer period, as it was possible under temperate climate conditions, could not be achieved. Nevertheless, soil-applied neem extracts can be a promising approach for integrated thrips control.

## 5.1 Systemic Action of Bioactive Neem Ingredients

Systemic effects of neem extracts after soil treatments include the root uptake and translocation of active ingredients in the plant as well as the resulting impact on the pest organism. These different steps can be influenced by a lot of factors, which makes the study of underlying mechanism challenging. Detailed studies on systemic action of active neem ingredients on a molecular and physiological level are still seldom. And so far only little work has been done considering structure related activity of neem compounds.

In Chapter 2 AZA, 3-tigloyl-azadirachtol, salanin and nimbin were selected for residue analysis to exemplify systemic effects of neem ingredients. These tetranortripenoids are the most investigated neem compounds regarding structure related activities and environmental behaviour (Nisbet et al. 1995, Stark and Walter 1995a, Jarvis et al. 1997, Sharma et al. 2003, Barrek et al. 2004, Simmonds et al. 2004). However, apart from this main substances neem extracts contain a complex blend of minor compounds, which are identified and investigated in the beginning of neem research activities (Kraus 2002). These components are not considered in our studies because of their extremely low concentrations and missing sensitivity of the analytical methods. Nevertheless, they could influence the uptake, distribution and mode of activity of the here selected compounds in an even complex manner. This makes the comparison with other published data so difficult, and may explain the variable results presented and discussed from others dealing with the same main compounds but different “background” compositions. To set an example, AZA was the most effective component in comparison with salanin and nimbin considering antifeedant, growth and molt disrupting effects in studies on different species of Lepidoptera, Orthoptera and Homoptera (Govindachari et al. 1996, Aerts and Mordue 1997, Jarvis et al. 1997). Godindachari et al. (1996) indicated that salanin caused growth-regulating activities against *Spodoptera litura* Fabricius, *Pericallia ricini* Fabricius and *Oxya fuscovittata* Marschall, which were comparable to that of AZA. In contrast, Jarvis et al. (1997) recorded no growth inhibition effects on *S. littoralis* Boisduval caused by salanin. Moreover, neem compounds can be affected by light but remain still active: The photooxidation products of nimbin and salanin were more effective than photooxidation products of AZA and showed similar antifeedant effects against *S. littoralis* like

AZA. Whereas, the photoisomerized product of AZA indicated only low activity against *Schistocerca gregaria* and no effects on *S. littoralis* and *Locusta migratoria* Linnaeus (Jarvis et al. 1997). These varying insecticidal properties of the photooxidation products of active neem ingredients (Jarvis et al 1997) as well as the conflictive results in studies on structure-activity relationship in one insect genus (Godindachari et al. 1996, Jarvis et al. 1997) highlight the complexity of the mode of action of neem ingredients. A similar complexity in systemic mode of action of the tested tetranortripenoids was suggested in the results presented in Chapter 2. For instance, identical dissipation trends of AZA and 3-tigloyl-azadirachtol were recorded in the substrate, whereas the translocation of both ingredients in bean plants seems to differ the temporal pattern depending of the plant part. AZA indicated a fast translocation in all plant parts with residue maxima two to four days after soil application. Whereas, 3-tigloyl-azadirachtol was transported slower into the leaves with peak residues in foliage not until Day six after treatment. With the present state of knowledge these observations are difficult to explain. Many questions are raised and warrant further investigation. In the presented study data on residue amounts of salanin and nimbin in the French bean and any of the active ingredients isomers, degradation products or related compounds in substrate and plant are missing because of improper analytical methods. These data are necessary to complete the basic knowledge on uptake and translocation of active neem ingredients by the plant after soil application in the tested model system. Moreover, the uptake and translocation of neem ingredients by plants differed with factors such as plant species (Sundaram et al. 1995, 1996a, 1997, Oßiewatsch 2000), composition of substrate (Sundaram and Curry 1993a, Sundaram 1994, Pussemeier 2000, Oßiewatsch 2000) and environmental conditions (Ruch et al. 1997, Barrek et al. 2004, Thompson et al 2004). This motivates to study the systemic activity of neem compounds in different model systems. To elucidate the systemic distribution of neem ingredients across trophic levels the residues must be analysed in thrips and if possible also in antagonists (predators, parasitoids) attacking the herbivors. The approach to analyse residue amounts in pest insect and predators in this study failed. Only insufficient analytical methods not sensitive enough to detect AZA in extremely small specimens such as thrips and predatory mites were available, although

pooling thousands of individuals (e.g. averaged 0.025 – 0.04 g fresh weight per thousands of thrips). Thus, the pathway of AZA and 3-tigloyl-azadirachtol after soil application could be observed from the substrate, via root uptake to the translocation into the plant, but not further into the pest and antagonist. Oßiewatsch (2000) recorded AZA residues in *Myzus persicae* Sulzer (Homoptera: Aphididae) after soil treatments with neem seed kernel extract analysing samples of 1.8 – 2 g fresh weight (thousands of aphids). Thus, systemic translocation from soil via plant into the insect is in principal detectable but needs too much insect material if very small species are considered. For the future, a further improvement of analytical methods might facilitate more sensitive analysis to detect neem residues in thrips as well, possibly even for a given individual. Over the last years some improvement in such technology could already be achieved, starting with several HPLC methods (e.g. Sundaram and Curry 1993b) and ranging from supercritical fluid chromatography (SFC) (Johnson and Morgan 1997), fractionation with Biotage flash chromatography followed by thin layer chromatography or SFC (Jarvis et al. 1999), HPLC-MS (Schaaf et al. 2000) and HPLC-MS-MS (Barrek et al. 2004) to enzyme-linked immunosorbent assays (Schütz et al. 1997). However, the present analytical equipment is not yet sensitive enough for residue analysis of such diminutive organisms as thrips. Residue analysis of thrips feeding on plants, which were treated with soil-applied neem extracts, and moreover, analysis of their antagonists would complete the demonstration of systemic action of neem compounds.

Therefore, this study could only make a first move to elucidate systemic action of neem ingredients. However, the overall results of this work demonstrate a good efficiency of soil-applied neem ingredients in thrips control, which encourages to expand the in-depth knowledge on systemic action of neem compounds with further studies.

## 5.2 Controlling Thrips by Systemic Effects of Soil-Applied Neem Compounds

In addition to the here presented results, systemic effects after soil treatments with neem extracts to control Thysanoptera are studied scarce. However, in preceding studies strong systemic effects on plant-sucking life stages of

*F. occidentalis* after a NeemAzal®-T/S soil treatment were recorded (Thöming et al. 2003). Ludwig and Oetting (2001) tested AZA and different other insect growth regulators as soil application to control *F. occidentalis* and *Bradysia coprophila* Lintner (Diptera: Sciaridae). They suggested such substrate treatments as potential tool in thrips control due to an evidently suppression of WFT population. Spray treatments of neem extracts often showed poor results in thrips control (Saxena and Kidiavai 1997, Immaraju 1998, Pearsall and Hogue 2000). Moreover, studies on systemic effects of neem ingredients have shown very conflicting results considering the control efficiencies testing different pest arthropods: Neem soil treatments resulted in very low systemic effects e.g. on *Liriomyza trifolii* Burgess (Diptera: Agromyzidae) and larvae of *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae) versus direct effects after neem spraying with mortalities up to 100%, using the same dose rates (Meisner et al. 1985, Larew 1988). In contrast, in studies on *Earias insulana* Boisduval (Lepitoptera: Noctuidae) and *Asymmetrasca decedens* Paoli (Homoptera: Cicadellidae) systemical treatments resulted in comparable effects like spray applications did (Meisner et al. 1990, 1992). Automatically the questions raise: i) why soil-applied neem extracts should result in such strong thrips control efficacies compared to sprayings of neem products, and ii) why systemic effects should cause particularly in thrips control such good efficacies.

In addition to neem extracts, soil applications and insecticides with systemical properties for crop protection against thrips were commonly used over the past years (Maienfisch et al. 2001, Riley and Pappu 2004, Coutts and Jones 2005, Tomizawa and Casida 2005). Systemically effective insecticides such as the neonicotinoid imidacloprid led to good control efficacies in thrips control (Ester et al. 1997, Riley and Pappu 2004). For instance, soil applications of imidacloprid caused mortality rates of *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae) up to 80% on avocado seedlings. Moreover, the soil-applied imidacloprid was quantified within the leaves of the avocado plants, which correlated with the thrips mortality data (Byrne et al. 2005). Neonicotinoids are used primarily as plant systemics by application to soil/substrate, seeds or foliage. The active ingredients of neonicotinoids are translocated to plant parts of maximum growth and afford plant protection against sucking insects over a long period (Maienfisch et al. 2001, Tomizawa

and Casida 2005). This outcome for soil-applied imidacloprid in thrips control is comparable to our results (Chapter 2), indicating both strong effects in thrips control by systemic activity.

Advantages of soil-applied active ingredients compared to insecticide sprayings in thrips control were discussed above (Chapter 1). The protected habitats of Thripidae on above ground plant parts, in soil/substrate and leaf litter makes their control with spray treatments so difficult (Riuvdavets 1995, Lewis 1997b, Morse and Hoddle 2006). The untreated plant parts and soil enable a rapid reestablishment of the thrips population after insecticide sprayings, and requires repeated spray applications. Whereas, after soil applications the insecticide can affect i) feeding thrips stages via systemic effects also at their cryptic habitats on the plant, and ii) soil-dwelling thrips stages via direct contact effects in soil/substrate or leaf litter. Moreover, plant systemics applied to the soil afford a long-term protection against sucking pests, and therefore low application rates are required (Sundaram et al. 1995, Maienfisch et al. 2001, Tomizawa and Casida 2005). Thus, frequently repeated treatments as necessary for insecticide sprayings can be avoided using soil applications. This limits the risk of resistance development and reduces detrimental effects on non-target organisms and environment. The efficacy of neem spray treatments is additionally limited by the fast degradation of the active ingredients under high temperature and UV light (Ruch et al. 1997, Barrek et al. 2004). Applying neem ingredients to the soil/substrate the UV exposure can be reduced. This decelerates the disappearance kinetic of bioactive neem components and affords a long-term plant protection compared to neem spray treatments. These special features of neem soil application could cause the higher control efficacies in thrips management after soil treatments versus sprayings of neem based insecticides.

Additionally, the characteristic feeding behaviour of the Thysanoptera could result in the presented strong effects in thrips control after systemic treatments. A strong suction intensity with an average ingestion of 10% - 20% of their body weight per hour was described for *Thrips tabaci* Lindeman and *Limothrips cerealium* Haliday (Chisholm and Lewis 1984, Harrewijn et al. 1996a, b, Kirk 1997). In comparison to the ingestion rate of phloem feeding aphids, which are taking up the liquid via passive ingestion from the phloem

vessels, Thysanoptera have a eight times higher ingestion rate per body weight (Harrewijn et al. 1996a). Thus, thrips probably ingest the active ingredients of insecticides, which are translocated systemically to feeding places of the pests, with a similar eight fold ingestion rate. This might result in a much higher efficacy of such systemic treatments in case of Thysantoptera compared to other pest such as aphids. Moreover, this could explain the strong systemic effects of neem extracts in thrips control in our studies, although only very low amounts of active compounds were recovered in plant material (Chapter 2). Therefore, the use of systemic insecticides in general seems to be very feasible for thrips control.

On the other hand, the ability of thrips to transmit plant viruses seems to conflict with the use of systemic effects in thrips control. As above mentioned, thrips' feeding behaviour, ontogenesis and biology in general plays a significant part in the ability of thrips to act as plant virus vector (Harrewijn et al. 1996a, Kirk 1997, Moritz et al. 2004) (Chapter 1). Large epidemics in many agricultural and horticultural crops are caused by virus transmission of thrips, especially in mild climate and protected cultivation with continuous cropping and permanent presence of thrips populations. Already small numbers of thrips are sufficient to cause high dispersion of viruses and therewith virus-related epidemics. Moreover, the transmission of viruses by thrips occurs very fast after short feeding or even probing duration (Ullman et al. 1992, 1997, Parella 1995, Wijkamp et al. 1995, Kumar et al. 1995, Premachandra et al. 2005a). Thus, the use of systemic insecticidal ingredients in thrips control, which allows and needs first feeding activity on the crop, seems to implicate simultaneously risk of virus transmission. This contradiction was discussed for other systemic insecticides like imidacloprid in several studies with the result that the assumption was rebutted (Riley and Pappu 2004, Coutts and Jones 2005, McPherson et al. 2005). For instance, Coutts and Jones (2005) recorded after an imidacloprid substrate drenching of young tomato plants a suppression of Tomato Spotted Wild Virus up to 80%. It is concluded that such soil treatments with systemic insecticides kill very fast particularly the most sensitive young larval stages of thrips and therefore prevent virus acquisition, which is possible only in that stages. This principle could apply accordingly for soil treatments with neem extracts. In Chapter 4 the impact of tomato plant age on the control efficacy on

*C. claratris* after neem soil treatments were indicated with stronger systemic effects using younger plants, similar to the results of Coutts and Jones (2005). However, a possible suppressing of virus spread due to soil application with neem based insecticides using young plants must be tested in further studies.

Overall, soil-applied neem extracts could fill a gap in thrips control due to their good control efficacy, and their more ecological properties and so far only few resistant strains against AZA compared to other systemically active insecticides (Zhao 1995, Völlinger and Schmutterer 2002, Nauen and Denholm 2005).

### 5.3 Soil Treatments with Neem Products in Practical IPM

The surrounding conditions and therewith the initial situation of using neem in IPM is completely different in Germany and Thailand. In the presented study, only Thripidae as serious key pest, neem extracts as potential tool for thrips control and protected cultivation of vegetable crops were issues of shared concern. Thus, the presented work with its studies in greenhouses in Germany and Thailand was an attempt to elucidate the potential of neem soil treatments in integrated thrips control in a more extensive way. Only a tentative draft for the implementation of neem extracts in practical IPM could be proposed.

In economic more developed countries like Germany spray treatments with commercial neem formulation are commonly used in IPM, especially in protected cultivation of vegetables and ornamental plants (Prakash and Rao 1997, Isman 1997, Immaraju 1998, Hummel and Kleeberg 2001, Stadler and Staucke 2002). Soil treatments of neem products are not used commonly in practice at present. However, the limited effectiveness of synthetic insecticides due to increasing resistance development, particularly in thrips control, and the growing demand of the consumers for more “ecological products” furthers the use of neem products. At present, the main drawbacks using neem products in IMP are: i) the fast decomposition of active compounds, which often results in labile pest control efficacies, ii) the standardization of active ingredient concentrations in neem products and quality control, and iii) registration barriers (Isman 1997, Immaraju 1998, Ermel et al. 2002, Stadler and Staucke 2002). A further development of analytical methods for estimation of bioactive components in neem products (Johnson and Morgan 1997, Schütz et al. 1997,

Jarvis et al. 1999, Schaaf et al. 2000, Barrek et al. 2004) tries to overcome these obstacles. Moreover, new formulations should stabilize active ingredients in neem products (e.g. Kumar and Parmar 1999, Johnson et al. 2003) or open up novel access paths for pest control. One example is NeemAzal-U as new formulations for soil application and hydroponics. The here presented findings demonstrate neem based soil treatment as feasible tool for thrips control. However, the results have to be verified in more practical situation on different crops and against several pests before neem soil applications can be assessed as good and reliable part for IPM concept. First steps with promising results were done recently: The release of *Amblyseius* and *Hypoaspis* predatory mites combined with NeemAzal®-T/S foliar and soil treatments to control *F. occidentalis* on bean, tomato, egg-plant, cucumber, castor-oil, tobacco and okra plants plants resulted in the eradication of pest population in greenhouse (Schmid and Guyer 2004). Moreover, effects on other pest species like *Aphis fabae* Scopoli (Homoptera: Aphididae) after soil application with different neem products in greenhouse and field trials using *Vicia fabae* were studied in detail. These studies demonstrated larvae mortalities up to 75% after soil application with NeemAzal-U. In a combined treatment with the predator *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) soil application of NeemAzal-U caused reduced side effects compared to neem spray treatments. A longer development time and a decrease in weight but no mortality of *C. carnea* larvae were recorded after such soil treatments (Islam 2005). Side effects of such neem soil treatments on soil-dwelling non-target organismns are of particularly importance. In studies on the impact of NeemAzal-U soil applications on entomopathogenic and phytopathogenic nematodes a much higher mortality on the phytopathogenic nematode *Meloidogyne incognita* (Tylenchida: Meloidogyninae) were recorded compared to the entomopathogenic one *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae). The mortality of *H. bacteriophora* was lower than 15% using AZA concentration (500 and 1000 mg AZA/l) much higher than the recommend dose rate of 50 to 100 mg AZA/l. However, a reduced parasitation rate indicated negative effects on the efficacy of the entomopathogenic nematode after a combined use with NeemAzal-U soil application (Meyer 2004). So far only low side effects of neem soil treatments on soil-dwelling antagonist of pest insects such as predatory mites and

entomopathogenic nematodes were recorded (Stark 1996, Meyer 2004). However, more data are needed due to detailed studies on possible negative effects on non-target organisms living in soil/substrate are a precondition for the use of neem soil treatments in IPM. Nevertheless, with further development of standardized neem products for soil treatments and additional studies indicating reliable pest control under practical conditions soil-applied neem extracts might become important for IPM in greenhouses in the temperate zone of Europe.

In tropical countries like Thailand vegetables are among the crops which were attacked most badly by arthropod pests, with thrips of particular importance (Bansiddihi and Poonchaisri 1991, Okajima et al. 1992, Hiroshi et al. 1996). This extreme pest pressure has caused an overuse of synthetic pesticides, which resulted in high hazards for environment and health as well as resistance problems (Thapinta and Hudak 2000, Jirachaiyabhas et al. 2004). In this regard, neem products as alternative methods of pest control are particularly suitable for tropical IPM concepts on the basis of: i) the established traditional use of *A. indica* preparations for pest control especially in south and southeast Asia, ii) the distribution and growing area of *A. indica* in the tropics, which simplifies the production and use of neem products and its acceptance by farmers, and iii) homemade neem products as low priced alternatives to synthetic insecticides, which are often not affordable for small scale farmers in developing countries. In addition to homemade preparations many commercial neem products are available in tropical regions (Parmar and Sinha 2002). Based on farmer interviews in North and Central Thailand, small scale farmers commonly use soil treatments of neem cakes and similar homemade neem compounds (pers. comm., farmer interviews in Chiang Mai, Chiang Rai, Suphanburi, Chainat, Ratchaburi, 2004). This might facilitate the acceptance by farmers and implementation of the overall results for practical IPM. However, the prevailing opinion and experience of Thai farmers concerning neem products for pest control as spray and soil treatments is rather conflictive (pers. comm., farmer interviews). On the one hand neem treatments often cause more or less unreliable efficacies in pest control and result always in lower and temporally delayed effects compared to synthetic insecticides. Otherwise, royal and governmental projects support farmers in producing particularly vegetables with the use of neem based insecticides instead of chemical input, and farmer

field schools teach about the importance and potential efficacy of different IPM concepts (FAO 1999, Thapinta and Hudak, 2000, Elsey and Sirichoti 2003). Actually, neem products are still used limited and particularly neem based soil treatments were applied rather as fertiliser than for pest control (Tran and Perry 2003, pers. comm., farmer interviews). The here presented results along with studies on *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) (Hossain et al. 2005) and *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) (Kumar et al. 2005) confirmed at least the capability of neem soil treatments in controlling thrips, leafminers and white fly in protected tomato cultivation under tropical climate conditions. A long-term protection and pest control beyond conditions under protected cultivation using such soil treatments seems to be hardly feasible and need additional measures of IPM. In general, for a successful implementation of IPM in Thailand the tradition must be balanced with modern methods in farming practice. Nevertheless, many farmers were open-minded about the use of neem soil application for pest control after positive personal experience (pers. comm., farmer interviews). Thus, neem soil treatments as traditional oriented methods in crop protection could be one way to enhance further adoption of IPM in Thailand.

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