# Management of Sweetpotato Whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) on tomato using biorational pesticides (Neem, Abamectin and Spinosad) and UV-absorbing nets and films as greenhouse cover in the humid tropics

Von der Naturwissenschaftlichen Fakultät
der Universität Hannover
zur Erlangung des Grades

**Doktor der Gartenbauwissenschaften**
**- Dr. rer. hort. -**

genehmigte Dissertation
von

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geboren am 27. December 1970 in Muzaffarpur, India

2005

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**Tag der Promotion:** 08.02.2006

***---- Dedicated to my late grandparents ----***

***Sri. Ramnandan Mishra ji***

***&***

***Smt. Ahilaya Devi***

**Summary**

The sweetpotato (**W**hite**f**ly, WF) *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) originates from tropical and subtropical regions, now having a worldwide distribution as a serious pest of open field vegetable production (Tropics, Sub-tropics and Mediterranean regions) and crops grown under protected cultivation. The short and multiple life cycles with high reproduction rates under tropical conditions, fast selection of resistant biotypes to different classes of insecticides including organophosphates, pyrethroids, cyclodiens and even first, second generation neurotoxin nicotinoids, and even growth regulators are major control constraints. In addition, the waxy shelters protecting the immobile larval and pupal WF stages, high immigration and generation time, wide range of hosts (over 600 plant species) are characteristics that make its control extremely difficult.

Subject of the present studies were exploring the potential of the botanical pesticides, neem using its various application methods and concentrations to control WF and evaluating its persistency compared to so-called bio-rational natural pesticides like spinosad and abamectin. In addition, physical control strategy by using a combination of UV-blocking nets and plastics were explored to learn their potential to manipulate the immigration behavior (entry) of WF and other small sucking insect-pest of tomatoes like thrips and aphids taking into consideration also the thrips related spread of a tospovirus.

In first series of experiments, neem was tested using three different treatment methods (seed, soil and foliar) and two different commercial neem products (NeemAzal® T/S 1% Azadirachtin and NeemAzal® U 17% Azadirachtin) against WF on tomato plants. Studies were conducted in cages in air conditioned cultivation rooms. All three methods of neem treatments resulted in reduced colonization and oviposition by WF. Overall oviposition intensity was significantly reduced by the treatment of tomato seeds (261 eggs in control compared to 147 eggs at a dose-rate of 3.0g/l of NeemAzal® U) but an even higher reduction was achieved through soil drenching (345 egg in control compared to 90 eggs at 3.0g/l of NeemAzal® U) and foliar spraying (286 eggs in control compared to 53 eggs at 10 ml/l of NeemAzal®) TS. In contrast, in soil and foliar treatment fecundity per female increased at highest tested concentrations (from 19 eggs/female in blank treatments to 28 eggs per female

at 3.0 g/l NeemAzal® U and from 15 eggs/female to 22 at NeemAzal® TS at 10 ml/l in foliar treatment). Reduced egg hatch could be observed only at high neem concentrations; 62 and 51% of deposited eggs hatched at highest dose-rates of NeemAzal®U at 3.0 g/l in case of seed and soil drenching treatments respectively; whereas only 43% of deposited eggs hatched in case of foliar treatments at highest dose-rates of 10 ml/l using NeemAzal® T/S. Seed (35%), foliar (93%) and soil treatments (91%) caused a significantly higher mortality of immatures and reduced number of hatching adults compared to control plants treated with a blank formulation or water. The mortality amongst immatures increased in relation to azadirachtin concentrations. Concerning susceptibility of different developmental stages, young larvae showed the most sensitive reaction. The most efficient treatment was foliar treatment, which achieved 100 % mortality for all three larval stages at high concentrations (10.0 ml/l of NeemAzal® T/S) compared to 78-87% mortality with soil treatment (at 3.0g/l of NeemAzal® U).

To further explore the possibilities of developing synergy with locally available parasitoids of WF, persistence of foliar and systemic application of azadirachtin was tested for 7 days (1,3,5 and 7) in air conditioned rearing rooms and tropical netted greenhouses using the same two products described for the first experiments. Foliar application induced under closed room conditions at dose-rates of 7 and 10 ml NeemAzalTS/l immature mortality of 32 and 44 % respectively 7-days post application, where as under greenhouse conditions these rates declined to 5 and 7 % during the same period indicating rapid dissipation of active ingredient. However, systemic application resulted in more stable effects under both laboratory and greenhouse conditions. After soil drenching with solutions of 3.0 g NeemAzalU/l until 7-d, immature mortality declined from 88% for the first day to almost half (45%) on 7-d. However in case of laboratory, it was 90% on first day and declined to 64% on 7-d post application. Similar trends of responses of the *B. tabaci* were obtained for other parameters like adult colonization, egg deposition and egg hatch. The loss of efficiency of the neem products was clearly related to the dose-rate, methods of application and environment (temperature and UV). Soil application is therefore a convenient approach to achieve high efficiency and persistence with neem products under the critical conditions in tropical greenhouse environments.

In third experiments, direct and residual toxicity of NeemAzal TS (azadirachtin), spinosad (Spinosyne) and abamectin (Avamectin) were tested against different life stages of WF under laboratory conditions and in a tropical net greenhouse. NeemAzal TS and abamectin deterred the settling of adults on the plant and consequently reduced egg deposition. No such effect was detected for spinosad. All three pesticides influenced egg hatch. Effects of NeemAzal TS were significantly altered if applied to different aged eggs (1, 3, and 5-d old). In contrast, abamectin treated eggs failed to hatch at any given age-class. Moreover, spinosad and NeemAzal TS influenced egg hatch in a concentration dependent manner. All three products caused heavy mortality of all three larval stages of *B. tabaci*, where the first instar larvae was found to be most susceptible compared to other two larval stages. Larval mortalities of 100% were achieved with NeemAzal TS at twice the recommend dose-rate (10ml/l) and at all tested concentrations of abamectin and spinosad. The daily mortality rates were highest for abamectin, all treated larvae at every larval stage died within 24 h post application. In contrast, 100% larval mortality in case of NeemAzalTS and spinosad was reached 6-9 days post application. The daily mortality rates were clearly concentration dependent. Abamectin caused 100% immature mortality at all residue ages (1, 5, 10 and 15-d) in the laboratory and greenhouse as well. Persistence of spinosad was comparable high in the laboratory but in the greenhouse a faster decline of activity was evident by increased egg deposition, egg hatch and reduced rates of immature mortality. Toxicity of NeemAzalTS however strongly declined under greenhouse conditions with time (5-d) post application.

The last series of experiments explored the possibility of integrating UV-blocking nets and plastics to develop appropriate physical control strategies for WF. The studies were conducted to investigate the effect of ultraviolet blocked greenhouses made from combination of net and plastics on the immigration of three important pest of tomatoes; WF (*Bemisia tabaci*), thrips (*Ceratothripoides claratris*), and aphid (*Aphis gossypii*) and occurrences of viruses e.g. tospovirus. Fewer WF*,* aphids and thrips immigrated and consequently were trapped either, when gates kept open whole day (complete ventilation) or partially open from 6.00 – 10.00 (partial ventilation) in greenhouses made from the combination of UV-blocking nets and plastics compared to non UV-blocking nets and plastic

greenhouse. Similarly, significantly less number of alate aphids and adult *B. tabaci*/leaf were counted within greenhouses with low intensity of the UV over those with more UV light intensity. Thrips were the most occurring pests, that too were recorded significantly less under GH with lower UV-intensity and consequently significantly lower levels of leaf damage were recorded under these greenhouses. During, open gates experiments (complete ventilation), a 96-100% virus infestation was recorded under non UV-blocking greenhouses compared to 6-10% under UV-blocking greenhouses, having majority of the plants tested positive for the tospovirus, CaCV (isolate AIT). The virus spreads were remarkably delayed for several days under greenhouses with lower UV light. These results suggests that greenhouses made from the combination of the UV-blocking nets and plastics have a significant influence on the both the immigration and virus spread vectored by some of these insects. The results are discussed in context of improved management of sucking insect-pests of tomatoes in the humid tropics under protected cultivation.

**Keywords:** *Bemisia tabaci*, Biorationals, UV-blocked greenhosues

**Zusammenfassung**

Die Weiße Fliege (WF) *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) ursprünglich aus den Tropen und Subtropen stammend ist heute weltweit verbreitet und ein bedeutender Schädling im Feldgemüsebau wärmerer Klimaregionen aber auch vieler Gewächshauskulturen der gemäßigten Zonen. In den Tropen führen der kurzen Entwicklungszyklus mit multiplen Generationen im Jahr zusammen mit hohen Reproduktionsraten zu schnellen und dauerhaften Massenvermehrungen. Die intensive Anwendung von Insektiziden führt unter diesen Bedingungen zu einer schnellen Selektion insektizidresistenter Biotypen. Resistenz ist heute gegenüber verschiedenen Wirkstoffgruppen belegt, so organischen Phosphorsäureestern, Pyrethroiden, Cyclodienen und jüngst sogar den erst seit wenigen Jahren eingesetzten Nicotinoiden und Wachstumsregulatoren. Zudem werden die immobilen Larvenstadien und das Entwicklungsstadium im Puparium durch Wachsüberzüge vor Kontaminierung mit Kontaktinsektiziden geschützt. Aufgrund der großen Polyphagie (bis zu 600 Pflanzenarten sind als Wirtspflanzen bekannt) besteht in der Regel ein hoher Immigrationsdruck in neu etablierte Kulturen. Diese Faktoren insgesamt machen eine effektive Kontrolle allein mit herkömmlichen Insektiziden außerordentlich schwierig, zudem sind dabei aufgrund der Toxizität und Persistenz vieler Wirkstoffe erhebliche Risiken für Farmer und Konsumenten gegeben.

Ziel der hier vorgestellten Studien ist die Analyse des Potentials des botanischen Insektizids Neem unter Berücksichtigung verschiedener Applikationstechniken und Aufwandmengen zur Kontrolle von *B. tabaci* und eine Bewertung der Persistenz im Vergleich zu den sogenannten „Biopestiziden“ Spinosad und Abamectin, die Produkte natürlicher Bodenorganismen sind. Zusätzlich sollten Möglichkeiten der Manipulation des Einwanderungsverhaltens von WF mittels UV-sorbierender Netze und Folien untersucht werden, wobei auch andere mobile saugende Schädlinge der Tomate wie Thripse und Blattläuse einbezogen wurden und der Übertragung von Tospoviren durch Thripse ein besonderes Augenwerk geschenkt wurde.

In einer ersten Serie von Experimenten wurde die Wirkung von zwei kommerziellen Neem-Präparaten (NeemAzal® T/S (1% azadirachtin) and NeemAzal U® (17% azadirachtin)) auf *B. tabaci* bei verschiedenen

Applikationsmethoden (Saatgutbehandlung, Boden- und Blattapplikation) untersucht. Die Untersuchungen erfolgten in Käfigen in klimatisierten Zuchträumen. Alle drei Anwendungsverfahren führten zu einer verringerten Besiedlung der Tomatenpflanzen und zu reduzierter Eiablage. Insgesamt war die Intensität der Eiablage durch die Behandlung der Samen signifikant vermindert (261 Eier in der Kontrolle im Vergleich zu 147 Eier bei einer Aufwandmenge von 3,0g/l of NeemAzal® U). Eine intensivere Reduktion wurde durch die Bodenbehandlung (345 Eier in der Kontrolle im Vergleich zu 90 Eiern bei 3,0g/l of NeemAzal® U) und durch eine Sprühbehandlung der Blätter (286 Eier in der Kontrolle verglichen mit 53 Eiern bei 10 ml/l of NeemAzal TS®)) erreicht. Im Gegensatz dazu wurde bei Boden- und Blattbehandlungen eine höhere Fekundität pro Weibchen bei den höchsten geprüften Konzentrationen beobachtet (von 19 Eiern/Weibchen in Kontrollen bis zu 28 Eiern pro Weibchen bei 3,0 g/l NeemAzal U® und von 15 Eiern/Weibchen bis zu 22 mit NeemAzal TS® bei einer Aufwandmenge von 10 ml/l).

Ein reduzierter Schlupf der Eilarven konnte nach Anwendung hoher Neem Konzentrationen beobachtet werden; 62% und 51% der abgelegten Eier schlüpften bei der höchsten Dosierung von NeemAzal® U (3,0 g/l) bei Samen- und Bodenbehandlungen während nur 43% der Eier im Fall von Blattapplikationen mit hohen Aufwandmengen von 10 ml/l NeemAzal® T/S schlüpften. Samen- (35%), Blatt- (93%) und Bodenbehandlungen (91%) führten zu signifikant höheren Mortalitätsraten der Larvenstadien und verringerten die Anzahl schlüpfender Adulter verglichen mit Kontrollbehandlungen. Dabei nahm die Mortalität mit zunehmender Konzentration an azadirachtin zu. Die höchste Empfindlichkeit zeigten junge Entwicklungsstadien. Die effizienteste Applikationsform stellte die Blattbehandlung dar, mit der eine 100 %ige Mortalität aller drei Larvenstadien bei hohen Dosierungen (10,0 ml/l NeemAzal® T/S) erreicht werden konnten, verglichen mit 78-87% Mortalität bei Bodenbehandlungen (3,0g/l NeemAzal®U).

Weiterhin wurde die Persistenz der Wirkung von Blatt- und Bodenapplikation von Azadirachtin überprüft, indem die Behandlungen in einem maximalen Zeitraum von 7 Tagen (1, 3, 5 und 7 Tage) vor der Besiedlung durch B. tabaci durchgeführt wurden. Die Behandlungen wurden vergleichend in klimatisierten und vor UV-Licht geschützten Räumen sowie in Netzhäusern mit freier

Sonneneinstrahlung angelegt. Blattbehandlungen induzierten unter den Bedingungen der klimatisierten Zuchträume bei Dosierungen von 7 and 10 ml NeemAzalTS/l eine Larvalmortalität von 32% und 44 % auf sieben Tage vor Besiedlung behandelten Pflanzen wohingegen unter Gewächshausbedingungen diese Raten auf 5% und 7 % abnahmen und damit den schnelleren Abbau der aktiven Substanzen im Gewächshaus dokumentierten. Die systemischen Behandlungen resultierten in stabileren Effekten unter beiden äußeren Bedingungen. Nach Bodenbehandlung mit 3,0 g NeemAzalU/l nahm die Larvenmortalität von 88% auf 45% innerhalb von Tag eins bis sieben im Gewächshaus, im Labor nur von 90% auf 64% ab. Ähnliche Trends in der Reaktion von *B. tabaci* wurden auch bei anderen Parametern beobachtet wie dem Schlupf von Adulten, der Eiablage und dem Eischlupf. Abnehmende Effizienz war jeweils verknüpft mit abnehmender Dosierungsrate, der Behandlungsmethode und den Umweltfaktoren (Temperatur, UV). Bodenbehandlungen mit Neem bieten somit einen geeigneten Ansatz eine hohe Effizienz zusammen mit einer hohen Persistenz zu erreichen selbst unter den kritischen Bedingungen tropischer Gewächshäuser.

In einem dritten Experiment wurden direkte und residuale Effekte von NeemAzal TS (azadirachtin), Spinosad (Spinosyne) and Abamectin (Avamectin) auf verschiedene Entwicklungsstadien der Weißen Fliege unter Laborbedingungen und in tropischen Gewächshäusern vergleichend untersucht. NeemAzal TS and Abamectin übten einen Deterrent-Effekt auf die Ansiedlung der Adulten auf den Pflanzen aus mit der Konsequenz einer Reduktion der Eiablage. Entsprechendes konnte für Spinosad nicht beobachtet werden. Alle drei Insektizide beeinflussten zudem den Eischlupf. Die Effekte von NeemAzal TS prägten sich significant unterschiedlich aus, wenn unterschiedlich alte Eistadien (1, 3, und 5 Tage alt) behandelt wurden. Im Gegensatz dazu wurde der Eischlupf durch Abamectin vollständig bei allen Alterklassen der Eier unterbunden. Zudem beeinflussten Spinosad und NeemAzal TS den Eischlupf konzentrationsabhängig. Alle drei Produkte führten zu hoher Mortalität der Larvenstadien von *B. tabaci.* Das erste Stadium erwies sich als besonders empfindlich. Larvalmortalitäten von 100% wurden mit NeemAzal TS bei einer Aufwandmenge von 10ml/l und allen Dosierungen von Abamectin und Spinosad erreicht. Die täglichen Mortalitätsraten waren am

höchsten für Abamectin, alle behandelten Larven und alle Larvalstadien starben innerhalb von 24 Stunden nach Behandlung. Im Gegensatz dazu wurde eine 100% Larvalmortalität im Fall von NeemAzalTS und Spinosad 6-9 Tagen nach Behandlung errreicht. Die täglichen Mortalitätsraten waren klar konzentrationsabhängig. Abamectin führte zu einer 100% igen Abtötung der Larven bei allen Altersgruppen der Spritzbeläge (1, 5, 10 und 15 Tage) im Labor wie auch im Gewächshaus. Die Persistenz von Spinosad war im Labor vergleichbar hoch, nahm jedoch im Gewächshaus schneller ab, erkennbar an zunehmender Eiablage, erhöhtem Eischlupf und einer reduzierten Larvalmortalität. Die Wirkung von NeemAzal TS hingegen nahm unter Gewächshausbedingungen mit der Zeit besonders stark ab.

Die letzte Serie von Experimenten analysierte die Möglichkeit UV-sorbierende Netze und Folien als physikalische Kontrolle von WF zu nutzen. Die Untersuchungen wurden durchgeführt, um den Einfluß UV blockierender Gewächhausmaterialien als Kombination von Netzen und Folien auf die Einwanderung von drei bedeutenden Schädlingen der Tomate, der Weißen Fliege *Bemisia tabaci*, dem Thrips *Ceratothripoides claratris*, und der Aphide *Aphis gossypii* einschließlich des Auftretens von Virosen (Tospoviren) zu erfassen. Weniger Weiße Fliegen, Aphiden und Thripse immigrierten in die Gewächshäuser, die mit einer Kombination UV sorbierender Netze und Folien bespannt waren, obwohl die Tore ganztägig oder teilsweise (6.00 – 10.00) zur Ventilation offen gehalten wurden. Gleichermassen wurden weniger geflügelte Aphiden und Adulte *B. tabaci* pro Blatt in Gewächshäusern mit einer geringen Intensität an UV verglichen mit Häusern, die höhere UV Intensität innen aufwiesen, gezählt. Thripse waren besonders abundant und wurden ebenfalls signifikant weniger in GH´s mit niedriger UV Intensität gefangen. Konsequenterweise ergaben sich signifikant geringere Schadsymptome an den Blättern. Mit offen Türen und normalen nicht UV blockierenden Gewächhausmaterialien wurden Virussymptome an 96 bis 100% der Pflanzen festgestellt, während nur 6 bis 10% der Pflanzen in UV sorbierenden Häusern infiziert wurden. Die Mehrzahl der Pflanzen mit visuelle erkennbaren Symptomen wurde positiv auf das Tospovirus CaCV (Isolat AIT) getestet. Die Virusausbreitung war deutlich verzögert unter geringen UV Intensitäten. Diese Ergebnisse deuten an, daß Gewächshäuser aus den erwähnten Materialien

signifikant zur Reduzierung der Immigration saugender Schädlinge und Virusausbreitung beitragen können. Die Ergebnisse werden im Hinblick auf ein verbessertes integriertes Management saugender Insekten an Tomaten in den humiden Tropen unter Bedingungen des geschützten Anbaus diskutiert.

**Stichworte:** *Bemisia tabaci*, Biopestiziden, UV-sorbierende Netze und Folien

**Contents**

[1] Part of this chapter was published as: Kumar, P., H.-M. Poehling and C. Borgemeister. 2005. Effects of different application methods of azadirachtin against sweetpotato whitefly *Bemisia tabaci* Gennadius (Hom., Aleyrodidae) on tomato plants. J. Appl. Entomol. 129 (9/10), 489–497.

[2] Kumar, P., and H-M. Poehling. Persistence of soil and foliar azadirachtin treatments to control Sweetpotato Whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) on tomatoes under controlled (laboratory) and field (netted greenhouse) conditions in the humid tropics. Submitted to Journal of Pest Sciences.

[3] Kumar, P., and H-M. Poehling. Effects of Azadirachtin, Avamectin and Spinosad on Sweetpotato Whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) on tomato plants under laboratory and greenhouse conditions in the humid tropics. Submitted to Journal of Economic Entomology.

[4] Kumar, P., and H-M. Poehling. Impact of UV-blocking plastic covers and netting on the pest status of *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae), *Ceratothripoides claratris* Shumsher (Thysanoptera: Thripidae) and *Aphis gossypii* Glover (Homoptera: Aphididae) on tomatoes in the humid tropics. Submitted to Environmental Entomology.

**Abbreviations**

| | |
|---|---|
| AIT | Asian Institute of Technology |
| ANOVA | Analysis of variance |
| arcsin√ | Arcsine–square-root |
| Ca | Calcium |
| CaCV | Capsicum chlorosis virus |
| d | day |
| d.f. | Degree of freedom |
| DAS-ELISA | Double antibody sandwich enzyme-linked immunosorbent assay |
| DoA | Department of Agriculture, Royal Govt. of Thailand |
| F | Statistical F-value |
| g/l | Grams per liter |
| GBNV | Groundnut bud necrosis virus |
| GH | Greenhouse |
| GHs | Greenhouses |
| GHWF | Greenhouse whitefly (*Trialeurodes vaporaiorum*) |
| h | Hours |
| ha | Hectare |
| IPM | Integrated pest management |
| K | Potassium |
| L : D | Relation of light to darkness |
| Lab | Laboratory |
| L1 | First instar larva |
| L2 | Second instar larva |
| L3 | Third Instar lava |
| LSD | Least significant difference |

| | |
|---|---|
| ml/l | Milliliters per liter |
| Mt | Million tons |
| N | Nitrogen |
| *P* | Statistical probability value |
| P | Potassium |
| rH | Relative humidity |
| SAS | Statistical analysis system |
| SE | Standard error |
| t | Statistical t-value |
| UV-B | Ultraviolet blocking |
| UV-NB | Ultraviolet Non-blocking |
| WF | Whitefly (*Bemisia tabaci*) |

# 1 General Introduction

Tomato, *Lycopersicon esculentum* (Mill) (Solanaceae) originated from the South America in the Peru and Ecuador region, is now widely cultivated throughout the world in tropical, sub-tropical and temperate climatic zones (Tindall 1983, Taylor 1986). Tomato was brought to the Asian continent by the Spanish colonists, first to the Philippines, from where, it moved to Southeast Asia and then to the entire Asian continent (Anonymous 2005a, see fig. 1.1).

Tomato is very good source of Vitamin A, B and excellent source of Vitamin C (Madhavi and Salunkhe 1998). The area under tomato production in Asia has doubled in last decade from 1,440,744 to 2,585,292 ha and production increased from 33,232,543 to 59,662,771 Mt in 2004 (FAOSTAT 2005). In Thailand, the tomato area increased from 9,760 ha in 1994 to 10,200 ha in 2005 with a total production of 248,000 Mt (FAOSTAT 2005) and it is widely grown in all regions but concentrated in the central and north-eastern part of Thailand (Anonymous 2005b).

The realization of optimal yields of vegetable crops including cultivated tomatoes, particularly in the warm humid lowlands of the tropics, is often constrained by a number of serious arthropod pests and viral diseases vectored by them (Deang 1969, Gomaa et al. 1978, Lange and Bronson 1981, Berlinger et al. 1988, Kakar et al. 1990, Berlinger 1992, Jinping 1994, Ketelaar and Kumar 2002). Tomato production in Thailand is constrained by WF (*Bemisia tabaci*), Thrips, Leafminers, Fruit worm (*Helicoverpa sp*.), etc. and among them Bemisia vectored TYLCV is major production constraints (Attathom et al. 1990, Sawangjit et al. 2005). About 1300 whitefly species in over 120 genera have been described (Anonymous 2001, Mound and Halsey 1978) and the genus Bemisia contains at least 37 species (Mound and Halsey 1978). The genus is thought to have originated in Asia with *Bemisia tabaci* being of possible Indian origin (Fishpool and Burban 1994). The first *B. tabaci* in the New World were collected in 1897 in the United States on sweetpotato. It was originally described as *Aleyrodes inconspicua* Quaintance and given the common name of sweetpotato whitefly (Quaintance, 1900).

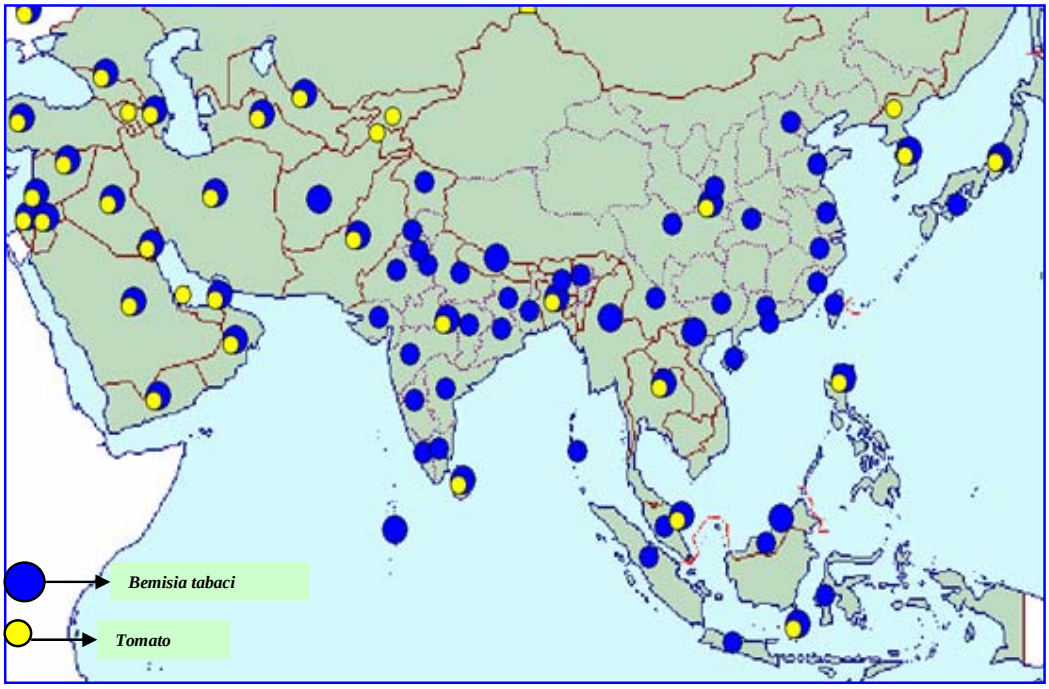



**Fig. 1.1.** Distribution of tomato cultivation area and *B. tabaci* presence in Asia[1]

In 1928, it was found in Brazil described as *B. costalimai* Bondar (Mound and Halsey 1978) and in 1933, in Taiwan and described as *B. hibisci* (Mound and Halsey 1978). Further, *B. tabaci* spread to other geographical range from subtropical and tropical agriculture systems has occurred to include temperate climate areas; the species is now globally distributed and found on all continents except Antarctica (Martin 1999, Martin et al. 2000). It is widely present in most of the countries in Asia (see figure 1.1). *B. tabaci* was first described as a pest of tobacco in Greece in 1899 (Cock 1986). In warmer regions (Tropics, Mediterranean), it is a serious pest in open field vegetable production but crops grown under protected cultivation (film tunnels, net houses) are equally suffering from heavy infestation with WF and severe damage is frequently reported. In addition, it has recently become a significant pest of protected horticulture in temperate regions (Butler and Heneberry 1986, Denholm et al. 1996). WF has been recorded in over 600 different plant species (Mound & Halsey 1978, Greathead 1986, Cock 1986, Secker et al. 1998) and can easily adapt to a new environment. It feeds on a wide variety of dicotyledonous horticultural crops such as tomato, pepper, beans, eggplant and cucumber.

[1] Source: Crop Protection Compendium, CAB International 2002 ed.

The polyphagous nature of *Bemisia tabaci* has been documented worldwide (Bird 1957, Costa and Russell 1975, Bird and Marmorosch 1978, Butler et al. 1986, Costa and Brown 1990 & 1991, Costa et al. 1991, Burban et al. 1992). Large numbers of cultivated crops, weeds, non-cultivated annual and perennial plant species are reported in several studies as acceptable feeding and/or reproductive hosts (Butler and Henneberry 1986, Bedford et al. 1992, 1994, Brown et al. 1992 &1995). Of the total host-plant species listed by Mound and Halsey (1978), almost half belong to five families: Fabaceae, Asteraceae, Malvaceae, Solanaceae and Euphorbiaceae. Tomato is one of the major vegetable hosts of the Bemisia in Thailand beside a root /starch crop Cassava.

*B. tabaci* adult and nymphs damages the tomato crops directly through sap feeding, produces massive quantities of honeydew that encourages the growth of sooty mould on leaves inhibiting photosynthesis and causing cosmetic damage (De Barro 1995). It causes uneven ripening of tomato (see fig. 1.2 (B); Maynard and Cantliffe 1989, Bharathan et al. 1990, Yokomi et al. 1990, Schuster et al. 1990, Matsui 1992), and on vegetables, melons, and ornamentals, honeydew and sooty mould reduce quality and marketability (Riley and Palumbo 1995).

An indirect effect of feeding by some whiteflies is the transmission of plant viruses, many of which are of economic importance. Whitefly instar nymphs and adults feed by inserting their proboscises into the leaf, penetrating the phloem and withdrawing sap. It is during this feeding process that plant viruses are acquired. Adult whiteflies may disperse and transmit the virus to new plants while feeding (Jones 2003). *B. tabaci* has been of increasing importance as a pest and vector of virus diseases of food, fiber and ornamental plants since the early 1980s. This has been due to the emergence of the B biotype and its rapid expansion in geographic distribution and host range. The whiteflies, and the viruses it transmits, are now responsible for significant crop losses in many regions with tropical, subtropical, arid and Mediterranean climates. Cassava, cotton, cowpea, cucurbits, crucifers, tobacco, tomato, potato, soybean, sweet potato, okra, lettuce, pea, bean, pepper, poinsettia and chrysanthemum are some of those crops that are vulnerable (De Barro 1995).

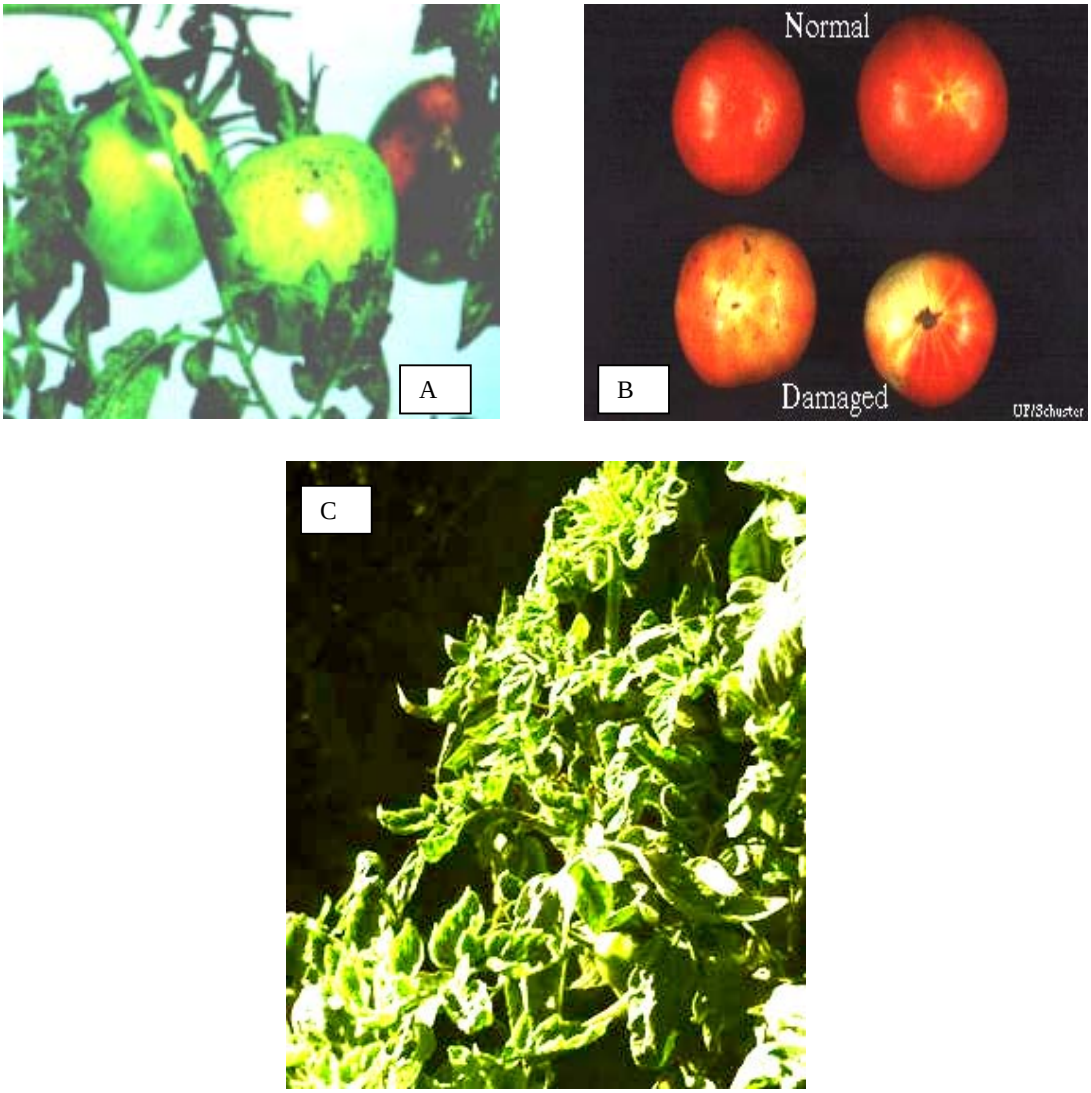



**Fig. 1.2.** Sooty mould growth on tomato (A)[2]; uneven ripening in tomato (B)[3]; Tomato Yellow Leaf Curl Virus infected tomato plant (C).

*B. tabaci* is a vector of 111 plant viruses recognized as species in the genera *Begomovirus* (*Geminiviridae*), *Crinivirus* (*Closteroviridae*), *Carlavirus* or *Ipomovirus* (*Potyviridae*) (Jones 2003). Begomoviruses are the most numerous of the *B. tabaci*-transmitted viruses and cause crop yield losses of between 20% and 100% (Brown and Bird 1992, Rapisarda and Garzia 2002) and its symptoms includes yellow mosaics, yellow veining, leaf curling, stunting and vein thickening (Anonymous 2001).

[2] http://www.crop.cri.nz/home/products-services/publications/broadsheets/91.pdf. (Assessed on 16.09.2005)

[3] http://whiteflies.ifas.ufl.edu/wfly0013.htm. (Assessed on 15.09.2005)

In conclusion, the high degree of polyphagy, ingestion of large amounts of phloem sap by feeding and transmission of plant viruses between hosts all contributes to the pest status of this species (Duffus 1987, Byrne et al. 1990).

The *Bemisia* life cycle consists of egg, 3 nymphal (larval) instars, pupal and adult stage. The eggs are about 0.2 mm long and pear shaped. They are laid on the under surface of young leaves. After hatching, individuals during their immature stages also stay on the under surface of leaves. The first instar nymphs (crawlers) move a very short distance after hatching over the leaf surface until they find a suitable site for feeding. Once settled, they remain sessile until they reach the adult stage, except for brief periods during moults. The fourth instar (the so called “pupa”) is about 0.7 mm long. Its red eye spots, which become eyes at the adult stage, are characteristic of this instar (Hill and Walker 1991, Kumar P. 2005 *unpublished data*). In a study on life cycle of *B. tabaci* from Thailand, Charungphan (2002) reported that pre-oviposition period of female WF was 1.38±0.49 days (1-2 day) and the oviposition period was 5.03±1.17-d. The number of eggs/female averaged 73.97±14.01 and incubation period was 6.60±0.84 –d. The nymphs underwent three instars of development and duration of each successive three instars was 2.84±0.75 days; 3.34-d; 2.59±0.61-d. respectively. The total nymphal period was 8-10 days; pupal duration was 5-7 days (see fig. 1.3 A-E; Charungphan 2002, Kumar P. 2005 *unpublished data*).

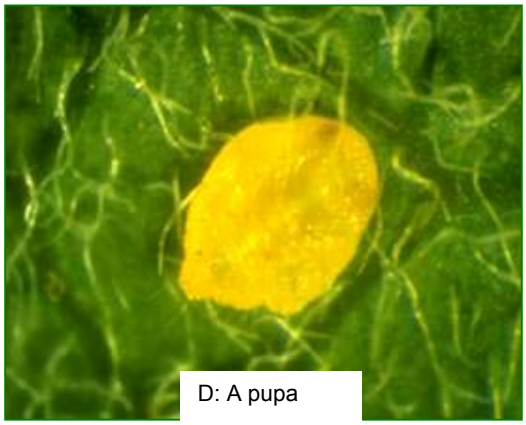
The direct damage of *B. tabaci* adult and nymphs along with its virus transmission abilities lead to high losses in tomato production in Thailand. Therefore, suitable management strategies against *B. tabaci* are urgently needed to reduce the overall loss of yield and quality of tomato production.

Chemical based pest management strategies are common feature of Asian vegetable production and tomato production in Thailand is not an exception in this regard. Thailand is a major market for pesticides with an annual growth rate since1982-92 of 8.8%, with some slowing down since then.

A: Eggs

B: Immatures of *B. tabaci*

C: empty pupal case



D: A pupa

E: Freshly emerged adult

F: An emerging adult from puparia

**Fig.1 3. (A-E).** Some important development stages of the of *Bemisia tabaci*[4]

[4] 1.3 (B) Source: http://www.entocare.nl/nl/foto's/images/Bemisia_tabaci_larve.jpg). Accessed on 15.09.2005

1.3 (F) Source: http://www.whitefly.org/UnderConst.asp). Assessed on 15.09.2005

Thailand is one of the biggest users of pesticides in the Southeast Asian region with an annual sales amounted in 1994 was US$247 million. Lot of pesticides are imported and, of imported pesticides, 73% fall into the WHO hazard categories Ia, extremely hazardous, and Ib, highly hazardous, and a further 33% are category II, moderately hazardous category (Jungbluth 1996).

Between 1980 and 1999 the quantity of pesticides imported to Thailand has increased from 9,855 to 33,969 tons, at an annual growth rate of 6.7% (Anonymous, 2002). In Thailand, misuse and over-use of pesticides results into 39,600 pesticide poisoning cases a year, with total annual health costs of about 13 million Baht[5] (Jungbluth 1996). Unlike some other SE Asian counties like Indonesia, the overall pesticide market in Thailand still remain largely unaffected by national and international IPM efforts (Oudejans 1999).

Several work have been reported so far against insecticidal management of the *Bemisia tabaci* in tomato crop e.g. pyrethroids or combination of conventional pesticides (Schuster 1994 &1995a, b, Stansly and Cawley 1994a, Stansly and Conner 1995). Despite the fact that the larval stages of the WF are susceptible to these active ingredients (Prabhaker et al. 1989), control of immature populations on plants with conventional treatments is inherently difficult to achieve, because the sessile nymphs reside on the abaxial surface of leaves and are difficult to contact with sprays (Palumbo and Coates 1996). Similarly, lot of work were reported against *B. tabaci* on tomato using novel first generation neurotoxic nicotinoids like imidacloprid either as foliar spray or pre or post planting drench with some but variable success for *B. tabaci* management in tomato (Schuster (1993a, 1993b, 1995, 1996, 1997a&b, 1998, 2000a and 2000b); Schuster and Polston, (1997a & b, 1998). Moreover, imidacloprid failed to prevent the transmission of the TYLCV in a recently reported study (Rubinstein et al. 1999). A more successful use is reported for the second generation nicotinoids like Thiamethoxam, Acetamiprid, Thiamethoxam either as foliar sprays or drench (Schuster and Polston 1998, Stansly and Conner 1998, Stansly et al. 1999, Schuster 2000 a & b, Stansly and Conner 2000).

[5] 1 US $ = 41 Thai Baht (approximately) as of Nov. 2005

However this compound also failed to provide an effective and reliable management of the TYLCV spread and gave so far only inconsistent results (Schuster 2000a, Stansly and Conner 2000).

Insect growth regulators are yet another group of novel chemistry, a being successfully integrated for management of *B. tabaci* in vegetable cropping ecosystem with good success (Palumbo et al. 2001). The major limitations in using these very effective growth regulators are their restrictive effects on only certain life stages of *B. tabaci* and rapid development of resistance (Horowitz et al. 1999a & b, Denholm et al. 1998, Ellsworth et al. 1996, Dennehy et al. 1996).

Rapid development of resistance against insecticides has been well document in *B. tabaci* for several conventional insecticides, alone or in combination, (Dittrich et al. 1990a, Cahill et al. 1995, Horowitz and Ishaaya 1996, Denholm et al. 1996). The high potential of *B. tabaci* to develop resistance is documented by the recent development against the chloronictinyls as well. Resistance of *B. tabaci* against Imidacloprid as first leading compound of this group is more and more often reported (Prabhaker et al. 1997, Denholm et al. 1998, Cahill and Denholm 1999, Elbert and Nauen 2000) and even the repeated application of second generation nicotinoids like acetamiprid resulted in 5-10 fold decrease in susceptibility of *B. tabaci* to the compound (Horowitz et al. 1999a). Furthermore IGR`s with a unique mode of action have proven select resistant populations of *B. tabaci (*Horowitz and Ishaaya 1994, Cahill et al. 1996, Elbert and Nauen 2000).

To avoid selection of resistant biotypes (Talekar and Shelton 1993, Williams and Dennehy 1996), a careful management with frequent changes of active ingredients (change of targets) is necessary. Control with insecticides is not only difficult because of resistance but also to its deleterious effect on natural enemies, contamination of water sources, and direct health hazards to both farmers and consumers (Saha 1993). Pronounced systemic properties of the pesticides are needed because WF feeding sites are on the abaxial surface of leaves and by production of their wax shelters they are difficult to target by contact poisons (James 2003). Short time after immigration, typically all developmental stages of WF are continuously present on the plants (Prabhaker et al. 1989); any control strategies not targeting all development stages of the

WF would be insufficient. This is particular important for the partly feeding pupal stages. Furthermore, according to the philosophy of Integrated Pest Management (IPM) effective pesticides but with low mammalian toxicity, low persistence in the environment and high degree of selectivity are desired. Along with so called “bio-pesticides” several others environmentally sound management techniques are recommended like use of resistant varieties (de Jager and Butot 1993, Shelton et al. 1998) and/or habitat management (Suzuki and Miyara 1984, Riddell-Swan 1988). Biological control using aphelinid parasitoids like *Encarisa* sp. and *Eretmocerus* sp. has played an important role in the control of the whitefly in greenhouses and in field world wide (van Lenteren et al. 1980, van Lenteren 1983, Hoddle et al. 1998) but till date no candidate has been widely used and adopted in the humid tropics.

To overcome most of the mentioned problems related to chemical pesticides so called biopesticides like neem and two recent novel pesticides of microbial origin spinosad and abamectin along with physical control options like Ultra-violet blocking plastics and nets are discussed as promising candidates but have to be critically tested under the dynamic and extreme conditions of the humid tropics before they could become a good and accepted option for both protected crops as well as field crops.

Azadirachtin (neem), a steroid-like tetranortriterpenoid derived from neem trees (*Azadirachta indica* Juss.), is a strong anti-feedent, repellent and growth regulating compound for a wide variety of phytophagous insects, including WF. It delays and prevents moulting, reduces growth, development and oviposition; and can cause significant mortality particularly in immatures (Coudriet et al. 1985, Flint and Sparks 1989, Prabhaker et al. 1989, Schmutterer 1990, Liu and Stansly 1995, Mitchell et al. 2004). Neem preparations are commercially available in most countries in the humid tropics for control of plant sucking insects including WF; however the efficacy seems to be highly variable particularly under field conditions (Puri et al. 1994, Leskovar and Boales 1996, Akey and Henneberry 1999). The major drawback of neem and neem based triterpenoids is their rapid dissipation and degradation in presence of light, which can reduce its bio-efficacy considerably (Stokes and Redfern 1982, Barnaby et al. 1989, Johnson et al. 2003, Barrek et al. 2004).

Spinosad (Spinosyn A, 85%: Spinosyn D, 15%) is a bio-rational pesticide derived from aerobic fermentation of the actinomycete soil bacterium *Saccharopolyspora spinosa* with a world wide use on over 200 crops against insect-pests of several orders like Lepidoptera, Diptera, Thysanoptera, Siphonaptera, Coleoptera and Hymenoptera etc. and with high selectivity concerning mammals or wildlife. It is classified as a reduced-risk pesticide by the US Environment Protection Agency (Cleveland et al. 2001). However, it is relatively less active against mites and sucking insect-pests (Boek et al. 1994, Dow 1997, Bret et al. 1997, Thompson et al. 2000). Spinosad acts through ingestion and contact and kills the insects through action on their nervous system (Salgado 1997 and 1998, Thompson et al. 2000, Cowles et al. 2000, Tjosvold and Chaney 2001). For non-target insects and beneficial its toxicity is quite specific. Whereas, selectivity is described for mammals or wildlife fresh residues are described to affect pollinators like Honey Bees or Bumble Bees (Miles et al. 2002, Mayes et al. 2003, Morandin et al. 2005). It is moderately toxic to commonly used biological control agents like *Amblyseius cucumeris* Oudeman (Acarina; Phytoseiidae) and *Orius indidiosus* Say (Hemiptera:Anthocoridae) (Pietrantonio and Benedict 1999, Ludwig and Oetting 2001). However, it was found highly toxic to the commonly used whitefly parasiotid, *Encarsia formosa* (Hym: Aphelinidae) even after 28-day post application (Jones et al. 2005) or the egg parasiotid *Anaphes iole* (Hymenoptera: Mymaridae) (Williams III et al. 2003) to give only two striking examples. The persistency of spinosad is limited to few days in presence of sunlight (Saunders and Brett 1997), thus devoid of any long term persistent effects to the natural enemies.

Abamectin is also derived from a soil bacterium *Streptomyces avermitilis* (avermectins: 80% avermectin B1a and 20% avermectin B1b) and it acts by affecting the nervous system of insects. It is highly toxic to a broad spectrum of insects if they are contaminated by fresh spraying solutions or residues and mammals can be affected if ingesting too high dosages since the LD 50 value is in the toxic range (Ray 1991). Similar to spinosad, it is highly toxic to the honey bees and other pollinators and to water organism but it is subject to rapid degradation when present as a thin film, as on treated leaf surfaces. Under

laboratory conditions and in the presence of light, its half-life is short, regardless of surface or foliage type (Wislocki et al. 1989).

Abamectin does not persist or accumulate in the environment. Its instability as well as its low water solubility and tight binding to soil, limit abamectin's bioavailability for non-target organisms and, furthermore, prevent it from leaching into groundwater or entering the aquatic environment (Lasota & Dybas 1990).

Some species of insects like whitefly, thrips and aphids have been shown to be dependent on UV light to orient themselves during flight and may use UV-light reflectance patterns as cues in recognizing host plants and flower species (Kring 1972, Rossel and Wehner 1984, Scherer and Kolb, 1987, Greenhough et al. 1990, Kring and Schuster 1992, Gold Smith 1993, Costa and Robb 1999). Furthermore this idea was supported by previous findings that *Bemisia argentifolii* and *Frankliniella occidentalis* are attracted to the UV light (Mound 1962, Matteson and Terry 1992, Antignus et al. 1996, Antignus 2000) and incidence of aphids and aphid-borne virus diseases were delayed and reduced by use of UV-blocking plastic mulches in squash and other crops (Brown et al. 1993, Summers and Stapleton 1998, Stapleton and Summers 2002). Field studies from Israel reported the significant reduction in incidences of whitefly (*Bemisia tabaci*), aphids and thrips, in protected crops by UV-blocking plastics or nets when compared with UV- non blocking materials (Antignus et al. 1996 & 1998 & 2001, Antignus 2000).

Regarding the aspects discussed this thesis is divided in 4 more chapters. After introduction (chapter 1), the major objectives of the chapter 2 were to study the effects of various neem application methods (seed, foliar and soil drenching) at various dose-rates on the colonization behavior, overall and individual fecundity, immatures mortality and adult emergence of *B. tabaci*. In addition the efficacies of each application method at various dose-rates were compared in relation to potential use of neem in the humid tropics.

In chapter 3, the residual toxicity of the soil and foliar application of neem under laboratory and greenhouse conditions were compared using the colonization behavior, overall and individual fecundity, immatures mortality and adult

emergence of *B. tabaci* as dependent variables. Furthermore, residual toxicity of application methods were compared in relation to their potential use in protected cultivation.

A comparative study of neem with the two novel pesticides of microbial origin, spinosad and abamectin is presented in the chapter 4. Studies were conducted both in air conditioned, UV protected environments and under more open conditions in net greenhouses to check for influences of the exposure conditions on intensity and duration of residual activity. In addition, in no-choice studies, toxicity of these novel pesticides were determined against various life stages of the *B. tabaci* at different dose-rates and results were discussed in context of their potential use in the humid tropics.

In the last chapter, chapter 5, immigration of three important sucking insect-pests of tomatoes in lower Bangkok plains and related virus spread inside greenhouses using different combinations of UV-blocking nets and plastics as greenhouse cover were compared. Conditions of partial (partial ventilation) or open access (complete ventilation) to the structures regulated by the doors were tested to simulate different ventilation conditions. In addition, the attractions of WF and thrips to the walls of the GH were also determined and attempts were made to separate the thrips transmitted tospovirus and other viruses in the experiments. All experiments were carried out in laboratories (Entomological Laboratory 2; Whitefly Laboratory) and separately built greenhouses constructed under the framework of the DFG Research group FOR 431 entitled “Protected cultivation - an approach to sustainable vegetable production in the humid tropics” at AIT campus during 2002-2005. They are part of a larger study which aims to establish sustainable and environmentally friendly vegetable production systems under protected cultivation in the humid tropics.

# 2 Use of seed, foliar and soil treatments of Azadirachtin to control Sweetpotato Whitefly *Bemisia tabaci* (Hom.: Aleyrodidae) on tomato plants[6]

## 2.1 Introduction

The WF, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) originates from tropical and subtropical regions with a worldwide distribution as a serious pest of open field vegetable production (Tropics, Sub-tropics and Mediterranean regions) and crops grown under protected cultivation (Butler and Heneberry 1986, Denholm et al. 1996). WF has been recorded from over 600 different plant species (Mound & Halsey 1978, Greathead 1986, Cock 1986, Secker et al. 1998) and it causes damage to the tomatoes in many ways such as direct sap feeding, virus transmission (Tomato Yellow Leaf Curl), sooty mould (reduced cosmetic value of fruits and photosynthetic area of plant) and uneven ripening of the fruits (Maynard and Cantliffe 1989, De Barro 1995, Rapisarda and Garzia 2002).

Chemical control is the primary method to manage WF. However control with pesticides is difficult for several reasons. Penetration of active ingredients after topical treatments can be inhibited by the waxy shelters protecting the immobile larval and pupal stages (James 2003) and all feeding stages colonize the abaxial surface of leaves and spraying from the top of the canopy results in incomplete coverage. Furthermore, shortly after immigration, typically all developmental stages of WF are present on the plants (Prabhaker et al. 1989). Thus, any control strategies not targeting all stages would be inefficient. This is particularly relevant for the largely non-feeding pupal stages. Moreover, the short and multiple life cycles with high reproduction rates, particularly under tropical conditions, favors fast selection of resistant biotypes to different classes of insecticides especially organophosphates, pyrethroids and cyclodiens. Even for the relatively young group of chloro-nicotinyl insecticides (leading substance: imidacloprid) resistant biotypes are already described (Prabhaker et al. 1989, Cahill et al. 1995, Dittrich et al. 1990a & b, Byrne et al. 2003).

[6] Part of this chapter was published as Effects of different application methods of azadirachtin against sweetpotato whitefly *Bemisia tabaci* Gennadius (Hom., Aleyrodidae) on tomato plants P. Kumar, H.-M. Poehling and C. Borgemeister. J. Appl. Entomol. 129 (9/10), 489–497.

Additionally, natural enemies, which can play an important role in the integrated control of pest complexes particularly in protected environments, can be seriously affected by pesticide treatments (e.g. Gonzalez-Zamora et. al. 2004). Even neem products (see below), often claimed to be selective, can significantly affect natural enemies such as *E. formosa* (Feldhege and Schmutterer 1993).

Azadirachtin, a steroid-like tetranortriterpenoid derived from neem trees (*Azadirachta indica* Juss.), is a strong anti-feedent, repellent and growth regulator for a wide variety of phytophagous insects, including WF (Coudriet et al. 1985, Flint and Sparks 1989, Prabhaker et al. 1989, Schmutterer 1990, Liu and Stansly 1995, Mitchell et al. 2004). The efficiency of neem against WF has been tested in numerous experiments in field and greenhouse studies but with variable success (Puri et al. 1994, Leskovar and Boales 1996, Akey and Henneberry 1999). Main advantages of using so-called bio-pesticides like neem are reduced human toxicity, fast and complete degradation in the environment, low risk for resistance and sometimes selective properties concerning non-target organism (Feng and Isman 1995, Immaraju 1998, Walter, 1999). Most control strategies and related studies, however, focus on foliar applications of neem products. The results are often unsatisfactory for several reasons such as: side effects on natural enemies (Feldhege and Schmutterer 1993), rapid photo-degradation and insufficient distribution within the crop canopy (Stokes and Redfern 1982, Larew 1988, Barnby et al. 1989). Systemic distribution of neem as recently described for thrips control (Thoeming et al. 2003) could help to overcome these shortcomings, to improve the efficiency, and to enable growers to achieve a higher level of reliability and sustainability in WF management with neem. Moreover, it could be hypothesized that soil application would strongly reduce the contamination of plant foraging parasitoids or predators and would open the door for synergistic use of the bio-pesticide (“fast task force”) and parasitoids or predators (“long term sustainable control”).

A detailed comparison of application methods (topical vs. systemic) regarding possible alterations in sensitivity of different developmental stages has not been conducted to date. In order to test the assumptions listed above we undertook a series of experiments under controlled conditions to measure the effects of three different methods of neem treatment on the colonization, oviposition, as

well as egg hatch and mortality of immature stages of *B. tabaci* on tomato plants. The experiments are part of a project aimed at developing a WF management strategy for tomato production under protected cultivation in the humid tropics.

## 2.2 Materials and Methods

### *Location, host plant and rearing of whiteflies*

The study was part of an interdisciplinary research project funded by the German Research Foundation (FOR 431) entitled “Protected cultivation - an approach to sustainable vegetable production in the humid tropics”. Experiments were conducted on tomato plants (*Lycopersicon esculentum* Mill (Solanaceae), cv. King Kong II) at the greenhouse and laboratory complex provided for the AIT-Hanover Project, Asian Institute of Technology, Bangkok, Thailand. The initial whitefly culture was obtained from the DoA (Department of Agriculture) Virology section, *Chatuchak*, Bangkok. This culture was maintained on eggplant and cotton seedlings for the past 2 years without any pesticides. Thereafter, the culture was mass reared in air conditioned rooms using the above mentioned tomato variety. The plants were kept in insect-proof cages (1.20 x 65 x 65 cm) at 24± 2°C and 60-70% relative humidity (rH). WF of the same age, i.e. L1, L2 and adults, were obtained by allowing female, *B. tabaci* (approximately 400 with a 1:1 male and female ratio) to lay eggs for 24 h on caged tomato plants. Thereafter, adults were removed from the cages using an aspirator. Plants with eggs were then stored in insect-proof cages for further synchronized development of *B. tabaci*. Plants with L1, L2, L3 or pupae were used for the neem experiments (see below) or kept until adult emergence in order to obtain adults of similar age.

### *Neem Formulations*

Two types of neem products, NeemAzal-U® (17% Azadirachtin A) and NeemAzal®-TS (1% Azadirachtin A) (Trifolio M GmbH, Lahnau, Germany) were used either in choice or no-choice tests. NeemAzal-U® was used for seed soaking and soil drenching-experiments, whereas NeemAzal-TS® only for foliar applications. Different concentrations of drenching solution were prepared by dissolving 0.75 (Azadirachtin = 0.1275 g), 1.50 (Azadirachtin = 0.255 g), 2.25 (Azadirachtin = 0.3825 g) and 3.0 g (Azadirachtin = 0.51 g) NeemAzal-U® in 1

liter tap water, which was then shaken for 30 minutes on a mechanical shaker before use. For foliar applications, 1 (0.01 g AZA), 3 (0.03 g AZA), 5 (0.05 g AZA) 7 (0.07 g AZA) and 10 ml (0.1 g AZA)/NeemAzal T/S were dissolved in 1 l tap water, and then shaken vigorously for approximately 10 min.

Before spraying, solutions were shaken again to ensure proper distribution of the oil-based formulation in water. For spraying, a local hand-held water sprayer of 1 l capacity was used. Control treatments were performed with a blank formulation of 3.0g/l NeemAzal® -U or with tap water in the case of NeemAzal® -T/S. Seeds were soaked in 50 ml of each dilution of NeemAzal®-U and pot substrates were drenched with 50 ml of the NeemAzal®-U solutions. For foliar spraying approximately 50 ml of NeemAzal® -T/S solutions were applied per plant.

***Treatments***

All experiments described below were conducted on tomato plants cv. King Kong II grown and/or planted in 10 cm diameter plastic pots with 180 g of local substrate (pH-5.3; organic matter - 28%; sand - 30%; silt - 39%; clay - 31%; total N - 0.4% ; K - 0.65%; P - 0.18%; Ca - 0.08%). Plants were kept in an air-conditioned laboratory at 24± 2°C, 60-70% rH with a photoperiod of 16:8 h (light: dark). Tomato plants were treated with the respective neem formulations as described below with ten replications per treatment and trial and with three repetitions over time.

***Experiments***

***Seed Soaking***

Tomato seeds were gently shaken in a Petri dish for 36 hours in 50 ml of 0.75, 1.5, 2.25 and 3.0 g NeemAzal®-U/l and 3.0 g blank /l formulation to ensure a uniform soaking of neem. In a preliminary test, no negative effects of seed soaking on germination were observed. Treated seeds were planted in pots and kept for two weeks in a climate controlled environment. A total of 50 plants were used in the experiment. Afterwards, plants were randomly placed in a transparent acrylic box (1.2 m height, 75 cm width with 30 meshes net fixed at the top and at two sides for proper ventilations and air circulations) for exposure to WF. Approximately 400 same-aged adult WF (2-d old) were released into the cages for 72 hours to allow adult WF sufficient time for choice of plants and oviposition. Starting one day after the release for three consecutive days, all

adult WF per plant were counted to record the colonizing preference of WF. Thereafter, adults were removed from the boxes and WF eggs on each leaflet counted using a microscope. Plants were maintained in WF-free cages and after 30 days when the majority of surviving WF had completed their development plants were removed from the cages. Then the number of living and dead immatures and empty pupal cases were counted to record adult emergence and immature mortality. Immatures were considered dead when they lost their normal yellow-green color, turgidity and smooth cuticle structure.

**Soil treatment**

***Choice Experiment***

Soil treatments were carried out with the same blank and four neem solutions as described for the seed soaking experiment. The substrate of two-week old tomato plants was treated with 50 ml of the neem solutions. After a 48 hour waiting period for uptake and translocation of neem ingredients plants were exposed to WF. Further experimental details were similar to the seed-soaking experiment.

***No-choice experiments, stage specific sensitivity***

Plants with different synchronized developmental stages of *B. tabaci* were produced as described above. Once the WF reached the desired development stage, numbers of larval instars and pupae were reduced to 50/plant with the help of an entomological pin directed under a microscope. Only in the case of eggs no adjustment was made and the number of eggs on each leaflet was counted before treating the tomato plants. Each of the 50 individuals left was marked for the purpose of easy counting and identification. Afterwards plants were treated with 50 ml NeemAzalU solution /pot and 10 replications were run for each treatment. Treated plants with eggs were stored until emergence of L1. Six days later the numbers of hatched eggs were counted to record the proportion of hatched individuals. In case of immatures, plant substrates were treated 7 (L1), 10 (L2), 14 (L3) and 17 days (pupae) after egg laying. The growth and development of WF development was monitored until adult emergence. By counting the empty pupal cases, live and dead larva, mortality and the proportion of hatched pupae could be calculated.

***Foliar treatments***

***Choice experiments***

Potted tomato plants were sprayed with 1, 3, 5, 7 and 10 ml/l NeemAzalTS on adaxial and abaxial leaf surfaces until runoff. Plants sprayed with tap water alone served as controls. Afterwards, plants were exposed to WF and subsequent maintenance was similar to that described for soil treatments. After 30 days of neem application (until emergence of all adults), dead immature and empty pupal cases were counted to determine immature mortality.

***No-choice experiments, stage specific sensitivity***

Different developmental stages of *B. tabaci* on tomato plants were established as described above. NeemAzalTS was applied as foliar spray directly on the adaxial and abaxial surfaces of leaves carrying desired stages of WF. Growth and development were monitored until adult emergence followed by counting of the proportion of empty pupal cases, dead and alive larvae to calculate mortality rates.

***Statistical analyses***

Data with percentage egg hatching, percentage immature mortality and percentage adult emergence were subjected to HOVTEST = LEVENE option of SAS to account for homogeneity of variance and normality. In the case of non-homogeneity, percent values were transformed using arcsine–square-root (arcsine$\sqrt{}$) transformation. Insect count values were transformed by square-root ($\sqrt{}$) transformation before running an ANOVA. (Steel and Torrie 1980, Gomez and Gomez 1984). Data were analyzed using the PROC GLM procedure in SAS (SAS, 1999). In case the ANOVA yielded significant F-values, means were compared using Tukey's HSD procedure unless mentioned otherwise. A significance level of $\propto$ = 0.05 was used in all analyses.

## 2.3 Results

***Seed-Soaking experiments***

The mean number of adults per plant, the number of laid eggs, the percentage of hatched eggs and the mortality of immature WF on plants grown from neem treated seeds are summarized in the table 2.1. Neem seed treatments with 2.25 and 3.0 g NeemAzal U /lw resulted in a significant and dose dependent reduction in the number of adults that colonized the plants (F = 18.92; df = 4,

145; *P*<0.0001) and in the number of deposited eggs as well (F= 33.34; df = 4, 145; *P*<0.0001). However, no significant difference in individual fecundity (eggs deposited per female) were observed (F = 2.06; df = 4, 145; *P* = 0.0885). WF did not discriminate between plants grown from seeds treated with blank formulation, 0.75 and 1.50 g NeemAzalU /l for egg deposition. With respect to egg hatch a significant reduction by neem treatments could be observed (F = 119.90; df = 4, 145; *P*<0.0001) resulting in fewer immatures on the plants treated with increasing neem concentrations (F = 373.53; df = 4, 145; *P*<0.0001). Moreover, the mortality of immatures increased in relation to the dosage of neem almost 3-fold, if plants from blank treated seeds were compared with those treated with 3.0 g NeemAzalU/l.

***Soil treatment***

***Choice Experiment***

The mean number of adult WF and eggs per plant, percentage eggs hatched and percent mortality on plants treated by soil application using different concentrations of NeemAzal®U solutions are summarized in table 2.2. NeemAzal®U significantly reduced plant colonization by adult WF (F = 500.33; df = 4, 145; *P* < 0.0001) as well as the number of deposited eggs compared to the blank treatment (F=334.64; df = 4, 145; *P* <0.0001). In contrast, the females deposited more eggs on tomato plants treated with highest concentrations (2.25 and 3.0 g/l) of azadirachtin (F = 34.78; df = 4, 145; *P* <0.0001). Moreover, neem significantly affected the percentage of hatched WF eggs (F = 1862.49; df = 4, 145; *P*<0.0001) and induced increasing immature mortality (F = 4946.55; df = 4, 145; *P* <0.0001) in dose dependent manner with significant differences between treatments.

**Table 2.1. Mean (±SE) number of adult whiteflies, total number of eggs deposited, % hatched eggs, % mortality of immature stages of *Bemisia tabaci* on tomato plants with seeds treated with NeemAzal U or a blank solution (control).**

| Neem concentrations | No. adult | No. eggs | % eggs hatched | % Mortality |
|---|---|---|---|---|
| Blank | 30.97± 0.60a | 261.40± 6.27a | 83.75± 0.32a | 13.01±0.35a |
| 0.75 g/l | 30.20± 0.74a | 260.23± 11.13a | 78.18± 0.72b | 23.16±0.31b |
| 1.50 g/l | 28.23± 1.07ab | 246.20± 7.86a | 75.73± 1.23bc | 23.94±0.31c |
| 2.25 g/l | 25.93± 0.95b | 206.53± 10.09b | 75.03±0.29c | 28.10±0.34c |
| 3.0 g/l | 21.20± 1.17c | 147.77± 5.90c | 62.93±0.34d | 35.67±0.76d |

Values in columns followed by same letters are not significantly different (Tukey's HSD test; $P<0.05$)

**Table 2.2. Mean (±SE) number of adult whiteflies, total number of eggs deposited, % hatched eggs, % mortality of immature stages on tomato plants after treatment of substrate with NeemAzal U or a blank solution (control).**

| Neem concentrations | No. adult | No. eggs | % Eggs hatched | % Mortality |
|---|---|---|---|---|
| Blank | 35.50± 0.79a | 345.83±13.52a | 93.26 ± 0.27a | 10.09 ± 0.10a |
| 0.75 g/l | 24.73± 0.47b | 169.46 ± 4.68b | 72.11± 0.18b | 52.15 ± 0.38b |
| 1.50 g/l | 17.71± 0.57c | 144.13 ± 4.12c | 62.20±0.22c | 61.57 ± 0.29c |
| 2.25 g/l | 9.93± 0.38d | 106.00±1.26d | 54.74 ± 0.73d | 73.69 ± 0.30d |
| 3.0 g/l | 6.86± 0.29e | 90.36± 1.16e | 51.40 ± 0.40e | 91.59 ±0.49e |

Values in columns followed by same letters are not significantly different (Tukey's HSD test; $P<0.05$)

***No-choice experiments, stage specific sensitivity***

Significant differences between all treatments ($F = 1066.56$; df = 4, 145; $P<0.0001$) could be observed for the percentage of eggs hatched ($F = 1066.56$; df = 4, 145; $P<0.0001$) and for the mortality of L1 ($F = 1223.93$; df = 4, 145; $P < 0.0001$), L2 ($F = 1888.34$; df = 4, 145; $P < 0.0001$), L3 ($F = 3932.93$; df = 4, 145; $P<0.0001$) and the pupal stage ($F = 3932.93$; df = 4, 145; $P <0.05$) (table2.3).

Again, regarding all stages, efficacy of neem increased with the concentration of the applied solution. When comparing the reaction of the immature stages, L1 was obviously the most sensitive one.

***Foliar treatments.***

***Choice experiments***

Colonization behavior of adults was strongly affected by foliar treatments with NeemAzal TS ($F = 346.69$; $df = 5, 174$; $P<0.0001$) (see table 2.4). Moreover, significant differences were detected in the number of eggs deposited ($F = 557.80$; $df = 5, 174$; $P<0.0001$). The foliar treatment resulted in significantly less eggs developing finally to the larval stage compared to the tap water treated plants ($F = 3590.31$; $df = 5, 174$; $P<0.0001$). Similar to the soil application fecundity per female WF increased at highest (7 & 10 ml/l) concentration of NeemAzal TS tested in the experiment ($F= 11.92$; $df= 5, 174$; $P<0.0001$). It could be observed that most developing L1 larvae (crawlers) died within the eggshell immediately before or during hatching (7 –d after egg laying). Mortality of immatures from neem treated plants was significantly different compared to control treatments ($F = 2053.47$; $df = 5, 174$; $P<0.0001$), which resulted in a fewer number of adults developing on these plants. The dose relation was similar to the experiments described above.

**Table 2.3. Mean (±SE) % hatched eggs, % mortality of larval stages (L1 – L3) and pupa on tomato plants with substrate treated with NeemAzalU after infestation with different synchronized developmental stages of *B. tabaci.***

| Neem concentrations | % Eggs hatched | ← Mortality (%) → | | | |
|---|---|---|---|---|---|
| | | L1 | L2 | L3 | Pupae |
| Blank | 92.56±0.35a | 13.46±0.96a | 14.26±0.72a | 9.66± 0.40a | 9.60±0.49a |
| 0.75 g/l | 73.87±0.28b | 35.80±0.77b | 32.26±0.85b | 31.93± 0.20b | 31.40±0.82b |
| 1.50 g/l | 65.21±0.84c | 52.33±0.91c | 47.80±0.37c | 45.00±0.35c | 40.33±0.28c |
| 2.25 g/l | 57.25±0.43d | 71.66±0.68d | 68.40±0.36d | 65.86±0.49d | 52.73±0.35d |
| 3.0 g/l | 54.25±0.32e | 87.00±0.32e | 83.93±0.72e | 78.93±0.40e | 73.73±0.70e |

Values in columns followed by same letters are not significantly different (Tukey's HSD test; $P<0.05$)

**Table 2.4. Mean (±SE) number of adult whiteflies, total number of eggs deposited, % eggs hatched, % mortality of larvae and % emerged adults on tomato plants treated with foliar application of NeemAzal TS and water (control).**

| Neem concentrations | No. adult | No. eggs | % eggs hatched | % Mortality |
|---|---|---|---|---|
| Water | 39.40 ± 1.20a | 286.53±9.01a | 94.24 ± 0.21a | 5.71 ± 0.17a |
| 1 ml/l | 26.90 ± 1.32b | 247.86 ± 6.42b | 71.90 ± 0.22b | 63.54 ± 0.22b |
| 3 ml/l | 18.73± 0.51c | 158.03 ± 3.48c | 60.70 ± 0.29c | 68.61 ± 0.46c |
| 5 ml/l | 11.86 ± 0.52d | 102.03±1.65d | 55.26 ± 0.27d | 73.31 ± 0.90d |
| 7 ml/l | 7.73 ± 0.22e | 83.76 ± 1.12e | 49.35 ± 0.34e | 86.06 ±0.52e |
| 10 ml/l | 5.23 ±0. 24f | 53.63 ± 1.22f | 43.26 ± 0.49f | 93.47 ± 0.52f |

Values in columns followed by same letters are not significantly different (Tukey's HSD test; $P<0.05$).

***No-choice experiments, stage specific sensitivity***

The results of these experiments are summarized in the table 2.5. The foliar treatment during the early egg stage of WF resulted in a significant lower amount of eggs completing development to L1 ($F = 4874.36$; df = 5, 174; $P<0.0001$) compared to the untreated control. Moreover, significant differences in percent mortality were observed between control and foliar neem treatments regarding L1 ($F = 4288.40$; df = 5, 174; $P < 0.0001$), L2 ($F = 6471.62$; df = 5, 174; $P<0.0001$) L3 ($F = 10156.5$; df = 5, 174; $P<0.0001$) and the pupal stage ($F = 5441.06$; df = 5, 174; $P<0.0001$). The pupal stage was less susceptible compared to all three larval stages of WF. The mortality of L1 and pupae steadily increased with the neem concentration applied.

**Table 2.5. Mean (±SE) % eggs hatched, % mortality of larval stages (L1 – L3) and pupa on tomato plants treated after infestation with different synchronized developmental stages of B*. tabaci* with foliar spraying of NeemAzal TS.**

| Neem concentrations | Egg hatch | ← Mortality (%) → | | | Pupa |
|---|---|---|---|---|---|
| | | L1 | L2 | L3 | |
| Water | 93.97±0.36a | 9.80±0.51a | 7.93±0.52a | 7.400±0.43a | 7.73±0.28a |
| 1 ml | 68.72±0.51b | 72.53±0.52b | 70.33±0.54b | 69.53±0.33b | 29.53±0.20b |
| 3 ml | 64.86±0.39c | 88.93±0.81c | 81.20±0.34c | 81.36±0.37c | 51.00±0.35c |
| 5 ml | 44.49±0.56d | 97.06±0.18d | 95.60±0.26d | 95.46±0.23d | 67.20±0.41d |
| 7 ml | 17.57±0.49e | 100.0±0.00e | 100.00±0.00e | 100.00±0.00e | 69.73±0.29e |
| 10 ml | 11.26±0.50f | 100.0±0.00e | 100.00±0.00e | 100.00±0.00e | 81.73±0.28f |

Values in columns followed by same letters are not significantly different (Tukey's HSD test; $P<0.05$)

## 2.4 Discussion

***Plant choice and oviposition***

The choice experiments either with seed, soil or foliar application of NeemAzal, demonstrated deterrent effects resulting in fewer adults settling on the treated tomato plants compared to untreated controls. Moreover, this effect was clearly dose dependent and particularly pronounced when a foliar application was used. The observation of repellent effects of neem on adult WF corroborates reports of Coudriet et al. (1985), Hilje et al. (2003) & Nardo et al. (1997) working with *Bemisia tabaci*, and of Prabhaker et al. (1999) with *B. argentifolii*. Similar results are also described for other pests attacking tomatoes such as the leafminers, *Liriomyza trifolii* (Burgess) and *L. sativae* Blanchard (Webb et al.1983), *Spodoptera litura* F. (Joshi and Sitaramaiah 1979) or even the locust (*Schistocerca gregaria*) (Schmutterer 1985 & 1988). In addition to the deterrence of adults we could observe lower deposition rates of eggs on all treated plants independent of application method. The numbers of eggs laid were especially low after treatments with higher neem concentrations. Reduced oviposition is a normal consequence if adults try to avoid settling on a host plant. In contrast, in soil and foliar treatment experiments, individual fecundity per female was higher compared to the respective controls like in case of soil application 19 eggs were deposited at control (blank) against 22 and 28 eggs per female at dose-rates of 2.25 and 3.0 g/l NeemAzal U. Similarly individual fecundity per female increased from 15 (control) to 22 at highest dose-rate tested (10 ml/l of NeemAzal TS). Moreover, these differences were not so apparent at other dose-rates tested in both experiments. No such effect was detected in the case of seed-treatment experiment. The reason for the increased fecundity is still unclear. It is possible that the lesser crowding on these treatments reduces intra-specific competition; on the contrary, similar effects attributed to sub-lethal insecticide stress effects are reported in Bemisia by Dittrich et al. (1990 a&b). Furthermore, although not measured in our experiments, a reduced uptake of phloem sap by adults avoiding feeding or changing the feeding site may more frequently have resulted in reduced numbers of ripened eggs ready for deposition. Our results are in agreement with findings of other authors who have studied neem compounds or related substances from *Melia azadirach* on *B. tabaci* (Coudriet et al. 1985, Nardo et al.

1997, Abou-Fakhr Hammad et al. 2001). The difference in the magnitude of host preference alteration between the three application methods may be related to the presence of different amounts of neem residues in or on the leaves: foliar treatment should result in much higher amounts of active ingredient on the leaf surface being directly encountered by the plant dwelling adults compared to seed and soil treatments, where neem compounds are translocated internally to the leaves. However, we could not differentiate in our observations between adults reacting immediately after plant contact or after first feeding (probing). In total, the clear feeding deterrent effects measured indicate a very sensitive reaction of adults to select non-treated plants for feeding.

***Egg hatch***

All three treatment-methods influenced the maturing and hatching of larvae from eggs deposited on the treated plants. The reduction was lowest in seed treatments compared to the soil and foliar applications, which corroborates earlier findings of Prabhaker et al. (1999) with *B. argentifolii*. Observation of the process of hatching revealed that the apparent reduction in successful egg hatch was due to neem on crawlers after eclosion from viable eggs when they came into contact with neem residues on the plant leaves and on the egg chorion. Hence, the reduction was not due to a disruption or inhibition of embryogenesis. We suspect that residual activity of neem on the egg chorion was toxic to the emerging crawlers as they were trying to come out from their eggs shell. We observed several of such half-emerged dead crawlers (under the microscope). These observations are similar to ones reported by von Elling et al. (2002).

***Mortality of immatures***

All three methods of neem treatment resulted in strong lethal effects on the immatures. Consequently, on the treated plants, much lower numbers of WF completed development to the adult stage. Direct effects after topical treatments on a large number of insects and WF (see e.g. von Elling et al. 2002) are reported and should not stay in focus here. More interesting are the strong effects shown without direct application to the targets. The results indicate that neem is efficiently absorbed through seeds or roots, transported via stems to the leaves or absorbed by the leaves and distributed translaminar. It could be

also concluded, regarding the feeding habits of whiteflies, which active compounds occur in the phloem vessels, the primary feeding site of WF. Systemic activity of neem has been reported in several studies in different herbivore-plant systems like in Tenthredinidae larvae (Keelberg 1992), Colorado potato beetle *Leptinotarsa decemlineata* Say. (Col.: Chrysomelidae), (Otto 1994) and larvae of *Liriomyza huidobrensis* Blanchard (Dipt. Agromyzidae) (Weintraub and Horowitz 1997).

Only a few earlier studies have used the active uptake by non-manipulated seeds or roots, rather than the artificial loading of plants by immersion of cut stems or leaves in neem solution. Our results are in agreement with earlier findings of Prabhaker et al. (1999) with *B. Argentifolii*, Thoeming et al. (2003) and Ossiewatsch (2000) with western flower thrips, *Frankliniella occidentalis* and Larew's (1986 and 1988) studies with aphids. All these studies showed systemic translocation of neem after treatment of bottom parts of intact plants resulting in strong effects on these sucking insects. Furthermore, with insect-pests having different feeding habits, such as the leafminer *Liriomyza trifollii,* seed treatments with neem showed similar systemic properties in ornamental plants (Larew et al.1985).

***Effects of foliar application and stage specific mortality***

Our results indicate that all three larval stages of *B. tabaci* are highly susceptible to the foliar treatment with neem. The L1 was most susceptible compared to L2 and L3. The pupal stage was least susceptible compared to all three larval stages. This could be due to the fact that the pupal stage is a largely non-feeding stage, where feeding occurs only in the first part of the development (Gill 1990). Additionally, due to the presence of thick cuticular layers it avoids any chance of contact toxicity. These results agree with earlier findings of Coudriet et al. (1985), Lindquist and Casey (1990), Price and Schuster (1991).

The different intensity of WF reaction to foliar sprays compared to seed and soil treatments supported findings of Liu and Stansly (1995), who found similar differences in nymphal mortality of *B. tabaci* comparing a spray and leaf-dip method for treatments with the neem product Margosan-O (Grace Grace-Sierra Horticultural Products Company, Fogelsville, PA).

***Conclusion***

Neem as a natural botanical pesticide with a low risk of toxicity to humans and animals could be one important plant protection agent in IPM programs. The results presented here show that neem is systemically translocated in tomato plants, and that this feature is of paramount importance for the control of plant sucking insects including WF. In particular, immatures of *B. tabaci* are highly susceptible to neem if the compound is allowed to be translocated systemically. The use of neem as a systemic pesticide has advantages in protected cultivation, i.e. where plants can be grown in pots or on artificial substrates; and where the infection pressure can be reduced by the use of mechanical barriers such as nets.

Making use of the systemic properties of neem can help to overcome two major drawbacks of neem if used for canopy spraying: fast degradation because of strong ultra-violet light (Johnson et al. 2003) and deleterious side effects on beneficial non-target organisms. However, concerning the latter point, more detailed studies in tropical greenhouses are needed to determine the possible side effects of neem on the indigenous or released natural enemy communities of *Bemisia tabaci*. These largely comprise Aphelenidae parasitoids and some general predators. Further studies by our group will focus on using these findings on the systemic properties of neem to improve complex pest – beneficial communities for better management of Bemisia in humid tropics.

# 3 Persistence of soil and foliar azadirachtin treatments to control Sweetpotato Whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) on tomatoes under controlled (laboratory) and field (netted greenhouse) conditions in the humid tropics[7]

## 3.1 Introduction

The WF, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) is a polyphagous pest feeding on over 600 plant species worldwide (Mound & Halsey 1978, Greathead 1986, Cock 1986, Secker et al. 1998). Tomatoes grown both in temperate and tropical regions, under protected cultivation, are highly vulnerable to whitefly damage (Butler and Heneberry 1986, Denholm et al. 1996). The pest status of this species is due to a number of factors: high degree of polyphagy, ingestion of phloem sap, massive honey dew secretion that reduces both the cosmetic value of the tomato and the available leaf area for photosynthetic activities, uneven ripening in tomatoes and transmission of plant viruses like TYLCV (Duffus 1987, Maynard and Cantliffe 1989, Byrne et al. 1990, De Barro 1995, Rapisarda and Garzia, 2002).

Chemical control is the primary method for managing WF. However, the use of chemicals has been inadequate principally because of the rapid emergence of resistance to different classes of insecticides, especially organophosphates, pyrethroids and cyclodienes. Even for the relatively new group of chloro-nicotinyl insecticides (leading substance imidacloprid) resistant biotypes have been described (Prabhaker et al. 1989, Dittrich et al. 1990a, Cahill et al. 1995, Byrne et al. 2003).

Alternatively, certain chemicals, derived either from plants or from certain micro-organisms, which we term here as biopesticides have been promoted in recent years. These include especially the azadirachtins, as well as avermectins and spinosyns. Azadirachtin, a steroid-like tetranortriterpenoid derived from the neem tree (*Azadirachta indica* Juss.), acts as a strong anti-feedent, repellent and growth regulator for a wide variety of phytophagous insects, including WF (Coudriet et al. 1985, Schmutterer 1990). It delays and prevents moulting,

[7]To be published as Kumar, P., and H-M. Poehling. Persistence of soil and foliar azadirachtin treatments to control Sweetpotato Whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) on tomatoes under controlled (laboratory) and field (netted greenhouse) conditions in the humid tropics. Submitted to Journal of Pest Sciences.

reduces growth, development and oviposition; and can cause high mortality, particularly in immatures, as documented for a wide group of phytophagous insects including WF (Coudriet et al. 1985, Flint and Sparks 1989, Prabhaker et al. 1989, Schmutterer 1990, Liu and Stansly 1995, Mitchell et al. 2004, Kumar et al. 2005). Neem products have been developed to address many pest problems, and are registered in many countries. Local production in most countries in the humid tropics makes them economic and readily available for smallholders.

The major problem with neem products based on triterpenoids as the active ingredient is the rapid photo-degradation by UV radiation when applied to the crop canopy as a foliar application (Pradhan and Jotwani 1968, Stokes and Redfern 1982, Saxena et al. 1982, Meisner et al. 1982, Hellap 1984, Barnaby et al. 1989, Caboni et al. 2002, Johnson et al. 2003, Barrek et al. 2004). Soil treatments making use of the systemic properties of azadirachtin (Thoeming et al. 2003, Kumar et al. 2005) may lessen instability and prolong persistency of the products.

A detailed comparison of persistency under different application methods (systemic, and topical) would help in choosing the optimal method and application frequencies to improve the overall neem use efficiency, and enable the growers to achieve a higher level of reliability and sustainability in WF management. Additionally, neem used for soil drenching would largely reduce direct toxicity to plant-foraging natural enemies such as parasitoids, thereby allowing its effective use as a component in IPM strategies.

This paper describes experiments to evaluate the persistence of different application methods, optimal product concentrations and timing of application for two commercial neem products in two environmental situations: climate-controlled rearing rooms (air conditioned and artificially illuminated, i.e., with intermediate temperature and low UV) and netted tropical greenhouses (high temperature and high UV). Impacts on WF investigated included: colonization preference, oviposition, eggs hatch and immature mortality.

## 3.2. Materials and Methods

### *Location, host plant and rearing of whiteflies*

The study was part of an interdisciplinary research project funded by the German Research Foundation (FOR 431) entitled “Protected cultivation - an approach to sustainable vegetable production in the humid tropics”. Experiments were conducted on tomato plants (*Lycopersicon esculentum* Mill (Solanaceae), cv. King Kong II) at the greenhouse and laboratory complex of the AIT-Hanover Project, Asian Institute of Technology, Bangkok, Thailand. The initial WF culture was obtained from the Department of Agriculture Virology section, *Chatuchak*, Bangkok and mass reared using insect-proof cages (1.20 x 65 x 65 cm) in air conditioned rooms (at 24± 2°C and 60-70% relative humidity (rH)) on the above mentioned tomato variety. WF of the same age were obtained by allowing female *B. tabaci* (approximately 400 with a 1:1 male and female ratio) to oviposit for 24 h on caged tomato plants. Thereafter, adults were removed and plants with eggs stored in insect-proof cages for further synchronized development.

The laboratory experiments were carried out in an air-conditioned laboratory (24- 25ºC; rH 65-75%, photoperiod 16: 8 [light: dark], whereas the greenhouse experiments were performed in two identical greenhouses (6x3x3 meters:72 mesh size, Econet®; Ludvig Swensoon, Sweden) at temperature range of 29-39ºC; rH 55-75% and natural photoperiod. During the experimental period, daily UV-A and temperature were measured with a Radiometer UV-Sensor (Dr. Grobel UV-Elektronik GmbH, Germany) and thermometer respectively inside greenhouse and in the laboratory. The measured mean UV-A for GH was in the range of 15-16.0 w/m$^2$, whereas it was 0.6-1.0 w/m$^2$ in the laboratory during the period of the experiments.

### *Neem Formulations*

Two types of neem, NeemAzal-U® (17% Azadirachtin A) and NeemAzal-TS® (1% Azadirachtin A) (Trifolio M GmbH, Lahnau, Germany) were used in bioassays as choice tests. NeemAzal®-U formulated as powder for water-based solutions was used for soil drenching experiments, whereas the NeemAzal®-TS, formulated as liquid product with a high content of oil, was used for foliar applications.

Different concentrations of drenching solution were prepared by dissolving 0.75 (Azadirachtin = 0.1275 g), 1.5 (Azadirachtin = 0.255 g), 2.25 (Azadirachtin = 0.3825 g) and 3.0 g (Azadirachtin = 0.51 g) NeemAzal-U® in 1 liter tap water, which was then shaken for 30 minutes on a mechanical shaker (*Orbit shaker, Edmund Buhler Co., Dreieich, Germany)* before use. For foliar applications 1, 3, 5, 7 and 10.0 ml NeemAzalT/S® /liter water were dissolved in tap water, followed by a vigorous shaking for approximately 10 minutes. Before spraying, solutions were shaken again to ensure proper distribution of the oil-based formulation in water. A local hand-held sprayer of 1 l capacity was used. In the case of NeemAzal-U®, 3.0g/l blank formulation of NeemAzal®-U (Trifolio-M, GmbH, Lahnau, Germany) was used as a control, while in the case of NeemAzal®-T/S tap water was used as a control. Pot substrates were drenched with 50 ml of the NeemAzal®-U solutions. For foliar spray, approximately 50 ml of suspension were sprayed until run off.

***Treatments***

All choice experiments were conducted on tomato plants cv. King Kong II grown and/or planted in 10 cm diameter plastic pots with 180 gram of local substrate (pH-5.3; Organic matter - 28%; Sand - 30%; Silt - 39%; Clay - 31%; Total N - 0.4%; K - 0.65%; P - 0.18%; Ca - 0.08%). Plants were either kept in an air conditioned laboratory or under GH conditions as discussed above. Tomato plants were treated with the respective neem formulations as described below with eight replications per treatment per trial, and three replication trials over time.

***Experiments***

***1. Persistency of soil treatment with NeemAzalU***

***A. Greenhouse (GH)***

Soil treatments were carried out with 0.75, 1.5, 2.25 and 3.0 g Neem-Azal®-U/lw and tap water as control. Each potted tomato plant was drenched with 50 ml neem at 7, 5, 3, and 1 day prior to introducing WF. Afterwards, plants were arranged for a choice test in 8 replications in eight separate well ventilated acrylic boxes (1.2 m height, 75 cm width; top and sides 72 mesh nets) containing one plant of each treatment in a randomized block design and exposed to WF under prevailing greenhouse (GH) conditions. Approximately 400 same-aged (1:1 male and female approximately) adult WF (2-d old) were

aspirated and released into the cages for 72 h to give adult WF sufficient time for plant choice and oviposition. Starting one day after the release for three consecutive days, all adult WF per plant were counted, and then returned, to record the colonizing preference of WF. Thereafter, WF adults were removed from the boxes and WF eggs on each leaflet counted using a microscope. Plants carrying WF eggs were marked and placed inside a WF-free GH to allow juveniles to develop. After 30 days, plants were removed from the GH and the number of living and dead immatures and empty pupal cases were counted to record adult emergence and mortality amongst immatures. Immatures were considered dead if they had lost their normal yellow-green color, turgidity and smooth cuticle structure. Three times per day water losses from the soil were replenished, but without any drainage from the pots, to maintain optimum moisture during the period of experiments.

***B. Air conditioned laboratory***

Similar experiment as in A. was conducted but with treated plants kept under laboratory conditions, as described above.

***2. Persistency of foliar treatments of NeemAzalTS***

***A. Greenhouse***

Potted tomato plants were sprayed with 1, 3, 5, 7 and 10 ml/l Neem-Azal T/S® on adaxial and abaxial leaf surface until runoff at 7, 5, 3, and 1- day prior to introducing WF. Plants sprayed with tap-water alone served as controls. Thereafter, the arrangements of plants, exposure to the WF, maintenance of tomato plants carrying WF eggs and data evaluation were carried out similar to the above described soil drenching experiment (experiment 1).

***B. Air conditioned laboratory***

Similar experiment as in A was conducted with treated plants kept under laboratory conditions described above.

***Statistical Analyses***

Data with percentage egg hatching, immature mortality were subjected to HOVTEST = LEVENE option of SAS to account for homogeneity of variance and normality. In the case of non-homogeneity, percent values were transformed using arcsine–square-root (arcsine√) transformation. Insect and eggs count values were transformed by square-root (√) transformation before running an ANOVA (Steel and Torrie 1980, Gomez and Gomez 1984). Data

were analyzed using the PROC GLM procedure in SAS to determine single or interaction effects of factors (SAS 1999). Whenever significant interaction was observed between factors, the level of one factor was compared to each level of the other factor by all pair-wise multiple comparison procedures (Tukey's test) unless mentioned otherwise. All data are presented as Mean± SE. A significant level of $\propto$ = 0.05 was used for all analyses.

### 3.3. Results

***1. Persistency of soil treatment with NeemAzalU***

***A. Greenhouse (GH)***

The interaction of the factors i.e. dose-rate* day was found significant for all variables studied in the experiment i.e., adult colonization (F = 19.051; df = 12, 479; *P*<0.0001); egg deposition (F = 12.367; df = 12, 479; *P*<0.0001); egg hatch (F = 17.52; df = 12, 479; *P*<0.0001); eggs laid per female (F = 3.805; df = 12, 479; *P*<0.0001) and immatures mortality (F = 62.39; df = 12, 479; *P*<0.0001). The dose-rate was found to have significant effect on all variables, i.e., adult colonization (F = 285.556; df = 4, 479; *P*<0.0001); egg deposition (F = 257.662; df = 4, 479; *P*<0.0001); eggs laid per female (F = 5.427; df = 4, 479; *P*<0.0001);egg hatch (F = 235.588; df = 4, 479; *P*<0.0001) and immatures mortality (F = 2191.559; df = 4, 479; *P*<0.0001). The reduced persistency of NeemAzalU with time was apparent with all parameters measured i.e. adult colonization i.e., adult colonization (F = 158.607; 3, 479; *P*<0.0001); egg deposition (F = 89.207; df = 3, 479; *P*<0.0001); eggs laid per female (F = 10.788; 3, 479; *P*<0.0001); egg hatch (F = 180.451; 3, 479; *P*<0.0001) and immatures mortality (F = 941.200; 3, 479; *P*<0.0001).

The mean number of adult colonization, total eggs deposited, and eggs deposited per female on the plants as well as the number of eggs hatched and the immature mortality across the dose-rates and days are summarized in tables 3.1, 3.2, 3.3, 3.4 and 3.5 respectively. The soil treatment reduced colonization, egg deposition and egg hatch rate and caused mortality amongst immatures. Moreover, higher individual fecundity was recorded which gradually reduced over time. For instance, 25 eggs/female on 7-d reduced to 20 eggs/level (level of control) on day 5. However, at low dose-rates (0.75 and 1.5 g/l) persistence of effects rapidly decreased compared to the dose rate of 2.25

and 3.0 g/l, which remain highly effective until the 7-d post application. For instance, the mortality of immatures with 3.0 g/l was reduced to almost half (88 % on 1-d and 45 on 7-d), whereas with 0.75 g/l already control levels were reached at day 7.

**Table 3.1. Mean (±SE) numbers of WF adult on tomato plant untreated and treated with neem applied to the soil across the different residue level and dose-rates of NeemAzal®-U under laboratory and in greenhouse conditions**

| NeemAzal® -U (g/l) | Mean (±SE) total number of adult | | | |
|---|---|---|---|---|
| | Residue age, days | | | |
| | 1-d | 3-d | 5-d | 7-d |
| *Laboratory* | | | | |
| Blank | 24.46±0.55aA | 25.50±0.61aA | 24.00±0.37aA | 25.96±0.84aA |
| 0.75 | 19.04±0.42bA | 22.58±0.47bB | 24.88±0.64aB | 24.04±0.53aB |
| 1.50 | 20.00±0.43bA | 22.88±0.58bB | 24.50±0.58aB | 24.25±0.35aB |
| 2.25 | 11.38±0.42cA | 12.33±0.59cA | 17.17±0.64bB | 19.63±1.20bB |
| 3.0 | 8.71±0.20dA | 10.58±0.44cB | 13.75±0.47cC | 17.13±0.56bD |
| *Greenhouse* | | | | |
| Blank = 0 | 26.29±0.73aA | 25.17±0.41aA | 25.75±0.41aA | 25.50±0.77aA |
| 0.75 | 19.88±0.66bA | 22.96±0.42bB | 25.79±0.82aC | 25.79±0.83aC |
| 1.50 | 20.17±0.42bA | 23.67±0.34bB | 25.46±0.79aBC | 26.75±0.79aC |
| 2.25 | 14.13±0.19cA | 15.08±0.33cA | 19.63±0.50bB | 22.42±0.39bC |
| 3.0 | 10.96±0.39dA | 12.67±0.34dB | 18.96±0.40bC | 21.13±0.31bD |

Means followed by the same case small letters within column and upper case letters within the row are not significantly different ($P$ = 0.05, Tukey's multiple comparison test [SAS Institute 1999]. Data were subjected to square root transformation before the analysis; non-transformed data on mean number of adult colonized tomato plants are presented in the table.

**Table 3.2. Mean (±SE) numbers of deposited eggs on tomato plant untreated and treated with neem applied to the soil across the different residue level and dose-rates of NeemAzal®-U under laboratory and in greenhouse conditions.**

| NeemAzal® -U (g/l) | Mean (±SE) total numbers of egg deposition | | | |
|---|---|---|---|---|
| | Residue age, days | | | |
| | 1-d | 3-d | 5-d | 7-d |
| *Laboratory* | | | | |
| Blank = 0 | 250.92±3.56aA | 249.38±2.96aA | 248.38±4.07aA | 253.63±4.76aA |
| 0.75 | 197.92±3.28bA | 224.71±2.59aA | 245.33±2.13aB | 240.46±3.64aB |
| 1.50 | 196.88±3.38bA | 222.58±2.55aB | 240.79±4.61aB | 241.00±3.32aB |
| 2.25 | 141.79±3.50cA | 127.83±6.79bB | 168.21±3.99bC | 179.08±9.69bC |
| 3.0 | 116.33±3.76dA | 125.71±4.93bA | 141.17±4.41cB | 166.63±3.70bC |
| *Greenhouse* | | | | |
| Blank = 0 | 271.17±4.31aA | 263.29±5.53aA | 266.42±5.23aA | 263.00±3.52aA |
| 0.75 | 200.17±1.86bA | 236.92±3.46bB | 263.25±4.10aC | 262.88±6.46aC |
| 1.50 | 200.50±3.29bA | 235.96±3.64bB | 251.71±2.86aB | 252.42±10.79aB |
| 2.25 | 168.71±8.53cA | 164.04±3.03cA | 191.29±2.73bB | 222.13±3.61bC |
| 3.0 | 141.17±5.58dA | 139.67±4.10dA | 188.08±3.56cB | 206.25±2.85bC |

Means followed by the same case small letters within column and upper case letters within the row are not significantly different ($P$ = 0.05, Tukey's multiple comparison test [SAS Institute 1999]. Data were subjected to square root transformation before the analysis; non-transformed data on mean number of total deposited eggs are presented in the table.

**Table 3.3. Mean (±SE) numbers of deposited eggs per female on tomato plant untreated and treated with neem applied to the soil across the different residue level and dose-rates of NeemAzal®-U under laboratory and in greenhouse conditions.**

| NeemAzal® -U (g/l) | Mean (±SE) number of eggs/female (Residue age, days | | | |
|---|---|---|---|---|
| | 1-d | 3-d | 5-d | 7-d |
| *Laboratory* | | | | |
| Blank | 20.70±0.45aA | 19.74±0.39aA | 20.80±0.45aA | 19.95±0.68aA |
| 0.75 | 20.96±0.46aA | 20.12±0.53aA | 20.00±0.50aA | 20.01±0.46aA |
| 1.50 | 19.92±0.57aA | 19.71±0.50aA | 19.78±0.36aA | 19.97±0.39aA |
| 2.25 | 25.54±0.94bA | 20.96±0.98aB | 20.12±0.76aB | 19.70±1.50aB |
| 3.0 | 26.96±1.00bA | 24.02±0.79bB | 20.89±0.78aC | 19.95±0.84aC |
| *Greenhouse* | | | | |
| Blank = 0 | 20.83±0.39aA | 21.00±0.49aA | 20.84±0.57aA | 20.93±0.50aA |
| 0.75 | 20.60±0.64aA | 20.85±0.60aA | 20.83±0.65aA | 20.82±0.82aA |
| 1.50 | 20.02±0.40aA | 20.09±0.33aA | 20.34±0.91aA | 19.51±0.93aA |
| 2.25 | 23.74±0.98bA | 21.94±0.57aA | 19.85±0.66aB | 19.91±0.38aB |
| 3.0 | 25.78±0.54bA | 22.26±0.69aB | 20.00±0.48aC | 19.60±0.33aC |

Means followed by the same case small letters within column and upper case letters within the row are not significantly different ($P$ = 0.05, Tukey's multiple comparison test [SAS Institute 1999]. Data were subjected to square root transformation before the analysis; non-transformed data on mean number deposited eggs per female are presented in the table.

**Table 3.4. Mean (±SE) percentage eggs hatching on tomato plant untreated and treated with neem applied to the soil across the different residue level and dose-rates of NeemAzal®-U under laboratory and in greenhouse conditions.**

| NeemAzal® -U (g/l) | Mean (±SE) % egg hatching, Residue age, days | | | |
|---|---|---|---|---|
| | 1-d | 3-d | 5-d | 7-d |
| *Laboratory* | | | | |
| Blank = 0 | 95.74±0.64aA | 95.07±1.97aA | 95.57±1.20aA | 96.44±1.15aA |
| 0.75 | 72.52±1.79bA | 77.30±1.09bA | 85.81±0.79bB | 94.17±1.93aC |
| 1.50 | 63.75±0.81cA | 69.13±0.66cA | 78.09±0.85cb | 89.14±1.80bC |
| 2.25 | 56.51±0.85cdA | 63.27±0.81cdA | 74.15±0.74cdB | 79.71±1.40cC |
| 3.0 | 52.80±1.29dA | 59.72±0.61dA | 67.77±0.89dB | 72.85±1.33cB |
| *Greenhouse* | | | | |
| Blank = 0 | 95.99±0.85aA | 95.49±1.81aA | 95.94±0.99aA | 95.46±1.09aA |
| 0.75 | 71.88±2.14bA | 79.64±0.93bB | 95.87±1.49aC | 95.42±1.21aC |
| 1.50 | 62.15±2.71cA | 70.15±1.92cB | 82.74±0.72bC | 93.79±1.97aD |
| 2.25 | 55.90±1.61cdA | 69.84±1.11cB | 79.53±1.11bcC | 86.33±0.74bD |
| 3.0 | 50.49±1.22dA | 63.62±0.50cB | 72.49±1.66cC | 81.89±1.07bD |

Means followed by the same case small letters within column and upper case letters within the row are not significantly different ($P$ = 0.05, Tukey's multiple comparison test [SAS Institute 1999]). Data were subjected to arcsine–square-root (arcsine√) transformation before the analysis; non-transformed data on mean percentage eggs hatching are presented in the table.

### *B. Laboratory*

The interaction of the factors i.e. dose-rate*day was found significant for all variables studied in the experiment i.e. adult colonization (F = 10.253; df = 12, 479; $P$<0.0001); egg deposition (F = 7.480; df = 12, 479; $P$<0.0001); eggs laid per female (F = 5.057; df = 12, 479; $P$<0.0001 egg hatch (F = 9.464; df = 12, 479; $P$<0.0001;) and immatures mortality (F = 42.217; df = 12, 479; $P$<0.0001). The dose-rate of neem had significant effect on all variables, i.e., adult colonization (F = 349.383; df = 4, 479; $P$<0.0001); egg deposition (F = 439.417; df = 4, 479; $P$<0.0001); eggs laid per female (F =12.310 ; df = df = 4, 479; $P$<0.0001 ); egg hatch (F = 375.683; df = 4, 479; $P$<0.0001) and immatures mortality (F = 1792.576; df = 4, 479; $P$<0.0001). Whereas the persistency of neem reduced over time i.e. adult colonization (F =86.418; df = 3,479; $P$<0.0001); egg deposition (F = 59.041; df = 3,479; $P$<0.0001); eggs laid per

female (F = 17.237; df = df = 3,479; *P*<0.0001); egg hatch (F = 151.995; df = 3,479; *P*<0.0001) and mortality amongst immatures (F = 489.698; df = 3,479; *P*<0.0001).

**Table 3.5. Mean (±SE) percentage immatures mortality on tomato plant untreated and treated with neem applied to the soil across the different residue level and dose-rates of NeemAzal®-U under laboratory and in greenhouse conditions.**

| NeemAzal® -U (g/l) | Mean (±SE) % immatures mortality | | | |
|---|---|---|---|---|
| | Residue age, days | | | |
| | 1-d | 3-d | 5-d | 7-d |
| *Laboratory* | | | | |
| Blank = 0 | 5.42±0.57aA | 5.15±1.02aA | 5.99±0.83aA | 5.64±1.10aA |
| 0.75 | 48.52±1.60bA | 40.55±0.59bB | 23.92±0.33bC | 4.65±1.08aA |
| 1.50 | 59.95±1.49cA | 52.70±1.06cB | 34.93±1.36cC | 20.85±1.51bD |
| 2.25 | 71.31±0.88dA | 64.11±0.98dB | 46.01±1.94dC | 37.17±0.71cD |
| 3.0 | 90.16±0.73eA | 83.27±0.69eB | 67.04±1.60eC | 64.39±1.96dC |
| *Greenhouse* | | | | |
| Blank = 0 | 5.44±0.92aA | 5.40±0.51aA | 5.14±0.18aA | 5.14±0.19aA |
| 0.75 | 45.02±0.91bA | 39.20±0.71bB | 18.49±0.86bC | 5.92±0.88aD |
| 1.50 | 57.13±1.25cA | 49.59±1.50cB | 31.62±0.69cC | 13.75±0.43bD |
| 2.25 | 71.81±0.75dA | 67.68±1.10dA | 39.05±1.70dB | 27.93±0.52cC |
| 3.0 | 88.18±0.97eA | 84.39±1.26eB | 69.36±1.24eC | 45.22±1.86dD |

Means followed by the same case small letters within column and upper case letters within the row are not significantly different (*P* = 0.05, Tukey's multiple comparison test [SAS Institute 1999]). Data were subjected to arcsine–square-root (arcsine√) transformation before the analysis; non-transformed data on mean percentages immatures mortalities are presented in the table.

The mean number of plant colonization by adults, total, egg deposition, eggs deposition per female, percentages of eggs hatch and immatures mortality across the dose-rates and days are summarized in tables 3.1, 3.2 , 3.3, 3.4 and 3.5 respectively. The results indicate the stronger persistence of neem, when applied as a soil drench under laboratory conditions compared with GH conditions. This effect is expressed through: reduced colonization (from 8 WF to 17 WF at 1 and 7-d post application) and egg deposition (116 to 166 eggs at 1 and 7-day post application respectively). A higher individual fecundity from 1- until 3-d post application at 3.0g/l (22 and 24 eggs/female under GH and laboratory conditions respectively) were recorded, which reduced to the level of

controls 5-d post-application. Similarly, the dose rate of 2.25 and 3.0 g/l remained effective until the 7-d post–application, e.g. the immatures mortality was reduced from 90 to 64%; indicating a slower dissipation rate of applied neem under laboratory conditions.

***2. Persistency of foliar treatments of NeemAzalTS***

***A. Greenhouse***

The interaction of the factors i.e. dose-rate* day was found significant for all variables studied in the experiment i.e., adult colonization (F = 72.051; df = 15, 575; *P*<0.0001); egg deposition (F = 50.026; df = 15, 575; *P*<0.0001); eggs laid per female (F = 6.326; df = 15, 575; *P*<0.0001) egg hatch (F = 117.309; df = 15, 575; *P*<0.0001) and immatures mortality (F = 237.687; df = 15, 575; *P*<0.0001). The effect of dose-rate significantly affected all variables compared to their respective controls, i.e., adult colonization (F = 374.534; df = 5, 575; *P*<0.0001); egg deposition (F = 255.732; df = 5, 575; *P*<0.0001); eggs laid per female (F = 17.321; df = df = 5, 575; *P*<0.0001); egg hatch (F = 699.199; df = 5, 575; *P*<0.0001) and immatures mortality (F = 896.699; df = 5, 575; *P*<0.0001). Whereas the persistency of neem reduced over the time and affected all studied variables in the experiment i.e., adult colonization (F = 958.780; df = 3, 575; *P*<0.0001); egg deposition (F = 730.210; df = df = 3, 575; *P*<0.0001); eggs laid per female (F = 20.437; df = df = 3, 575; *P*<0.0001 );egg hatch (F = 1814.920; df = 3, 575; *P*<0.0001) and immatures mortality (F = 4176.632; df = 3, 575; *P*<0.0001). The mean number of adult colonization, total egg deposition, eggs deposition per female, eggs hatch and immatures mortality across the dose-rates and day are summarized in tables 3.6, 3.7, 3.8, 3.9 and 3.10 respectively.

**Table 3.6. Mean (±SE) numbers of adults colonization on tomato plant untreated and treated with foliar application of neem across the different residue levels and dose-rates of NeemAzal®-T/S under laboratory and in greenhouse conditions**

| NeemAzal®-T/S (ml/l) | Mean (±SE) number of adult | | | |
|---|---|---|---|---|
| | Residue age, days | | | |
| | 1-d | 3-d | 5-d | 7-d |
| *Laboratory* | | | | |
| Control = 0 | 36.88±1.23aA | 36.86±0.56aA | 35.74±0.41aA | 36.21±0.59aA |
| 1 | 22.52±1.63bA | 31.71±0.59bB | 33.42±0.45aB | 35.79±0.45aC |
| 3 | 17.97±0.43cA | 21.80±0.41cB | 29.13±0.75bC | 35.21±0.48aD |
| 5 | 12.72±0.75dA | 21.58±0.50cB | 21.75±0.55cB | 33.79±0.75aC |
| 7 | 7.08±0.17eA | 12.04±0.19dB | 17.10±0.70dC | 24.33±0.44bD |
| 10 | 6.09±0.30eA | 10.71±0.20dB | 13.13±0.54eC | 20.75±0.56cD |
| *Greenhouse* | | | | |
| Control = 0 | 35.67±0.76aA | 36.08±0.88aA | 36.09±1.18aA | 35.38±0.59aA |
| 1 | 24.07±0.69bA | 29.50±0.71bB | 35.96±1.16aC | 35.33±0.74aC |
| 3 | 16.32±0.36cA | 28.45±1.32bB | 35.54±0.90aC | 35.25±0.78aC |
| 5 | 11.22±0.35dA | 20.13±0.62cB | 34.13±0.82aC | 35.25±0.92aC |
| 7 | 7.19±0.29eA | 14.21±0.42dB | 25.04±0.73bC | 35.46±0.54aD |
| 10 | 6.31±0.27eA | 13.71±0.61dB | 22.33±0.52bC | 35.42±0.95aD |

Means followed by the same case small letters within column and upper case letters within the row are not significantly different ($P$ = 0.05, Tukey's multiple comparison test [SAS Institute 1999]. Data were subjected to square root transformation before the analysis; non-transformed data on mean number of adults colonized tomato plants are presented in the table.

**Table 3.7. Mean (±SE) numbers of deposited eggs on tomato plant untreated and treated with foliar application of neem across the different residue levels and dose-rates of NeemAzal®-T/S under laboratory and in greenhouse conditions**

| NeemAzal®-T/S (ml/l) | Mean (±SE) number of total deposited eggs | | | |
|---|---|---|---|---|
| | Residue age, days | | | |
| | 1-d | 3-d | 5-d | 7-d |
| *Laboratory* | | | | |
| Control = 0 | 312.75±9.50aA | 316.67±4.00aA | 315.46±4.30aA | 312.58±5.17aA |
| 1 | 182.67±6.70bA | 271.08±4.27bB | 284.88±4.81bB | 313.58±6.32aC |
| 3 | 152.63±3.59cA | 191.17±3.29cB | 247.08±6.18cC | 311.50±5.94aD |
| 5 | 139.42±7.32cA | 183.50±4.24cB | 185.25±3.79dB | 285.88±5.08bC |
| 7 | 83.38±2.12dA | 133.88±2.14dB | 191.29±7.34dC | 209.33±4.34cD |
| 10 | 81.92±3.41dA | 128.92±3.39dB | 153.67±.44eC | 177.00±5.54dD |
| *Greenhouse* | | | | |
| Control = 0 | 303.00±6.76aA | 308.92±6.59aA | 307.00±8.08aA | 301.79±5.37aA |
| 1 | 207.42±7.61bA | 255.04±12.51bB | 305.00±8.81aC | 303.75±6.02aC |
| 3 | 139.58±5.64cA | 235.79±9.57bB | 302.67±7.36aC | 304.92±4.98aC |
| 5 | 106.67±2.08dA | 166.75±6.36cB | 281.46±4.52aC | 309.58±8.54aC |
| 7 | 76.46±3.60eA | 138.50±3.86dB | 217.33±6.50bC | 305.67±5.42aD |
| 10 | 75.38±4.04eA | 134.54±3.80dB | 195.71±4.41bC | 304.04±7.67aD |

Means followed by the same case small letters within column and upper case letters within the row are not significantly different ($P$ = 0.05, Tukey's multiple comparison test [SAS Institute 1999]. Data were subjected to square root transformation before the analysis; non-transformed data on mean number of deposited eggs are presented in the table.

Neem applied through foliar application exhibited the persistency effect for several days under GH conditions only at the higher rates of 7.0 and 10.0 ml/l. Neem applied at other dose-rates at 3-d post application largely became ineffective e.g. 6-7 adults WF colonized plants 1-d post application and after 5-d there was no sig. difference observed in any tested dose-rates. Similarly, more eggs were laid with lapse of time, for instance 304 eggs were deposited on 7-d post application against 75 eggs on 1-d post application at 10.0 ml/l. Similar to the soil application, the female WF deposited more eggs on plants with fresh residue, which quickly came down to the level of the control e.g. 27 eggs at 10.0ml/l on 1-d post application against 17 eggs (similar sig. level of control) on 5-d post application. The result clearly indicates faster dissipation of the applied neem through foliar application over soil drenching.

**Table 3.8. Mean (±SE) numbers of deposited eggs per female on tomato plant untreated and treated with foliar application of neem across the different residue levels and dose-rates of NeemAzal®-T/S under laboratory and in greenhouse conditions**

| NeemAzal®-T/S (ml/l) | Mean (±SE) number eggs/female | | | |
|---|---|---|---|---|
| | Residue age, days | | | |
| | 1-d | 3-d | 5-d | 7-d |
| *Laboratory* | | | | |
| Control = 0 | 17.04±0.24aA | 17.24±0.19aA | 17.67±0.23aA | 17.28±0.19aA |
| 1 | 17.79±1.11aA | 17.19±0.33aA | 17.06±0.21aA | 17.58±0.41aA |
| 3 | 16.92±0.10aA | 17.74±0.54aA | 17.04±0.30aA | 17.69±0.23aA |
| 5 | 22.15±0.32bA | 17.05±0.26aB | 17.12±0.26aB | 17.01±0.25aB |
| 7 | 23.64±0.51bA | 22.32±0.42bB | 22.55±0.50bB | 17.24±0.26aC |
| 10 | 27.41±0.70cA | 24.17±0.61bB | 23.82±0.62bB | 17.10±0.35aC |
| *Greenhouse* | | | | |
| Control = 0 | 17.02±0.23aA | 17.27±0.45aA | 17.20±0.43aA | 17.07±0.14aA |
| 1 | 17.44±0.72aA | 17.27±0.68aA | 17.04±0.22aA | 17.24±0.27aA |
| 3 | 17.16±0.67aA | 16.76±0.29aA | 17.12±0.33aA | 17.39±0.29aA |
| 5 | 19.53±0.83bA | 16.61±0.41aB | 16.65±0.38aB | 17.58±0.21aB |
| 7 | 21.58±0.87cA | 19.60±0.38bA | 17.38±0.19aB | 17.25±0.19aB |
| 10 | 24.22±1.33dA | 20.21±0.77bB | 17.55±0.18aC | 17.20±0.15aC |

Means followed by the same case small letters within column and upper case letters within the row are not significantly different ($P$ = 0.05, Tukey's multiple comparison test [SAS Institute 1999]. Data were subjected to square root transformation before the analysis; non-transformed data on mean number deposited eggs per female are presented in the table.

**Table 3.9. Mean (±SE) percentage eggs hatching on tomato plant untreated and treated with foliar application of neem across the different residue levels and dose-rates of NeemAzal®-T/S under laboratory and in greenhouse conditions.**

| NeemAzal®-T/S (ml/l) | Mean (±SE) % egg hatching (residue age, days) | | | |
|---|---|---|---|---|
| | 1-d | 3-d | 5-d | 7-d |
| *Laboratory* | | | | |
| Control = 0 | 95.25±0.33aA | 95.86±0.43aA | 95.47±0.36aA | 95.11±0.20aA |
| 1 | 60.41±0.52bA | 84.43±0.19bB | 95.31±0.22aC | 95.10±0.46aC |
| 3 | 55.55±0.64cA | 74.45±0.18cB | 92.86±0.32bC | 94.15±1.31aD |
| 5 | 45.27±0.49dA | 52.32±0.26dB | 87.86±0.25cC | 95.09±0.49aD |
| 7 | 30.53±0.59eA | 43.86±0.21eB | 68.22±0.14dC | 84.20±0.51bD |
| 10 | 23.58±0.75fA | 39.51±0.27fB | 63.36±0.24eC | 80.58±0.47cD |
| *Greenhouse* | | | | |
| Control = 0 | 95.40±0.45aA | 95.21±0.74aA | 96.89±1.44aA | 95.73±0.50aA |
| 1 | 57.31±0.32bA | 95.50±0.41bB | 94.66±0.74aB | 94.73±0.56aB |
| 3 | 53.69±0.29bA | 82.52±0.50bB | 95.12±0.67aC | 95.49±0.51aC |
| 5 | 43.79±0.30cA | 67.31±0.49cB | 94.83±1.02aC | 95.56±0.53aC |
| 7 | 27.02±0.75dA | 47.76±0.25dB | 81.04±0.55bC | 95.50±0.83aD |
| 10 | 22.30±0.45dA | 43.18±0.84dB | 71.56±0.28cC | 87.87±0.39D |

Means followed by the same case small letters within column and upper case letters within the row are not significantly different ($P$ = 0.05, Tukey's multiple comparison test [SAS Institute 1999]. Data were subjected to arcsine–square-root (arcsine√) transformation before the analysis; non-transformed data on mean percentages eggs hatching are presented in the table.

### *B. Laboratory*

The interaction of the factors i.e. dose-rate* day was found significant for all studied variables i.e., adult colonization (F = 34.503; df = 15, 575; $P$<0.0001); egg deposition (F = 31.232; df =15,575; $P$<0.0001); eggs deposited/female (F = 21.957; df =15,575; $P$<0.0001);egg hatch (F = 220.380; df =15,575; $P$<0.0001) and immatures mortality (F = 329.330; df =15,575; $P$<0.0001). The effect of dose-rate significantly affected all variables compare to their respective controls, i.e., adult colonization (F = 849.330; df = 5, 575; $P$<0.0001); egg deposition (F = 682.430; df = 5, 575; $P$<0.0001); eggs deposited/female (F =126.711 ; df = 5, 575; $P$<0.0001); egg hatch (F = 2768.251; df = 5, 575; $P$<0.0001) and immatures mortality (F = 6532..024; df = 5, 575; $P$<0.0001).

**Table 3.10. Mean (±SE) ) percentage immatures mortality of *B. tabaci* on tomato plant untreated and treated with foliar application of neem across the different residue levels and dose-rates of NeemAzal®-T/S under laboratory and in greenhouse conditions.**

| NeemAzal®-T/S (ml/l) | Mean (±SE) % immatures mortality (residue age, days) | | | |
|---|---|---|---|---|
| | 1-d | 3-d | 5-d | 7-d |
| *Laboratory* | | | | |
| Control = 0 | 5.14±0.33aA | 5.51±0.35aA | 5.52±0.43aA | 5.17±0.26aA |
| 1 | 66.32±0.65bA | 20.27±0.23bB | 5.73±0.75aC | 5.17±0.27aC |
| 3 | 70.30±0.58cA | 40.07±0.52cB | 12.71±0.38bC | 5.50±0.32aD |
| 5 | 76.73±0.40dA | 45.81±0.69dB | 19.06±0.32cC | 6.88±0.53aD |
| 7 | 100.00±0.00eA | 90.57±0.85eB | 62.57±0.53dC | 32.87±0.81bD |
| 10 | 100.00±0.00eA | 91.29±1.80eB | 65.44±0.97dC | 44.97±0.32cD |
| *Greenhouse* | | | | |
| Control = 0 | 5.47±0.42aA | 5.99±0.52aA | 5.29±1.12aA | 5.90±0.84aA |
| 1 | 64.10±0.88bA | 8.12±0.40abB | 5.26±1.01aC | 5.70±0.72aC |
| 3 | 69.80±0.90cA | 11.12±0.18bB | 5.33±0.92aC | 5.85±1.19aC |
| 5 | 75.93±1.05dA | 17.14±0.31cB | 5.78±0.86aC | 5.16±0.55aC |
| 7 | 100.00±0.00eA | 50.77±1.27dB | 11.88±0.48bC | 5.60±0.37aD |
| 10 | 100.00±0.00eA | 61.06±2.14eB | 18.83±0.82cC | 7.81±0.44bD |

Means followed by the same case small letters within column and upper case letters within the row are not significantly different ($P$ = 0.05, Tukey's multiple comparison test [SAS Institute 1999]. Data were subjected to arcsine–square-root (arcsine√) transformation before the analysis; non-transformed data on mean percentages of immatures mortalities are presented in the table.

Whereas the persistency of neem reduced over time i.e., adult colonization (F = 577.638; df = 3,575; $P$<0.0001); egg deposition (F = 541.758; df = 3,575; $P$<0.0001); eggs deposited/female (F = 60.349; df = 3,575; $P$<0.0001); egg hatch (4145.183; df = 3,575; $P$<0.0001) and immatures mortality (F = 7003.502; df = 3,575; $P$<0.0001). The mean number of adult, egg deposition, eggs deposited per female, eggs hatch and immatures mortality across the dose-rates and day are summarized in tables 3.6, 3.7, 3.8, 3.9 and 3.10 respectively. Foliar applied neem in the laboratory exhibited longer persistency compared to GH conditions; for instance 6 adults WF colonized tomato plants at 10.0 ml/l on 1-d post application which increased to 20 adults (35 adults under GH conditions) 7-d post application. Reduced colonization by WF resulted in deposition of fewer eggs. However, an increased individual fecundity for longer time period (5-d over 3-d post application in GH) was recorded, clearly indicating persistency for several days. Similar to the soil application, where the

highest concentration persisted longest, the high foliar application of 10.0 ml/l persisted longest. The dose-rate of 5.0ml/w and less become largely ineffective in as soon as 4-d post applications and consequently there was little differences in hatching, egg laying and immatures mortality compared to the control. However, the mortality of immatures which was 100% for 7.0 and 10.0 ml/l on 1-d after foliar application reduced to the extent 44% and 32% respectively on 7-d post application.

## 3.4 Discussion

These studies investigate the importance of the persistence of azadirachtin after soil treatments and foliar applications for control of *Bemisia tabaci* in a typical climatic region of the humid tropics. We first discuss the observed effects on a set of chosen variables (plant choice by adults, total egg deposition, individual fecundity, eggs hatch and mortality of immature) comparing these two application methods. We then comment on dose relationships and the fundamental problem of neem degradation by environmental factors by comparison of laboratory (protected environment) and GH (close to open field) conditions.

***Persistency, adult colonization, egg deposition***

Both methods of NeemAzal application, i.e. foliar application and soil drenching, in the laboratory and in the GH resulted in reduced colonization by adults of the treated tomato plants compared to their respective controls. The difference in colonization preference between the two applications methods may be related to the presence of different amounts of neem residues on the leave surface after foliar treatment compared to soil application. With spraying, neem compounds were deposited directly on the plant surface, the first contact region for adults searching for feeding or egg deposition sites. After soil application azadirachtin must be translocated from the roots to the leaves. This difference is evident through different responses of WF in terms of adult colonization and subsequent egg deposition behavior. Moreover, the degradation of neem was dose dependent which has been shown by other authors who reported a decline of efficacy with dosage (Schmutterer1985 & 1988, Barnby et al. 1989). The deterrent effects of neem and compounds of related plant species (*Melia azedarach;* Meliaceae) against *Bemisia tabaci* have been reported (Nardo et al.

1997, Abou-Fakhr Hammad et al. 2000 & 2001). However, when numbers of eggs/female were calculated, it was found that, in all cases, (foliar and soil application) either in the laboratory or in GH, freshly applied neem at high dose-rates (7.0 and 10.0 ml/l or 2.25 or 3.0 g/l) did not reduce egg deposition; indeed females even deposited more eggs. Thus, negative effects on egg development can be ruled out which is in agreement with reported negative effects of azadirachtin on reduction of ovary weight, ovary proteins and vitellogenin synthesis (Ludlum and Sieber 1988, Rao et al. 1996), yolk synthesis, (Handler and Postlethwait 1978); and even on the inhibition of oogenesis and ovarian ecdysteroid synthesis (Sieber and Rembold 1983, Schulz and Schluter 1984, Rembold 1988).

Moreover, the overall reduction in eggs deposition seems mainly related to the anti-feedent and deterrence effect of neem. Anti-feedent actions of neem and similar plant species resulting into decreased egg deposition behaviour of WF have been reported in several earlier studies (Nardo et el. 1977, Coudriet et al. 1985, Abou-Fakhr Hammad et al. 2001, Hilje et al. 2003). This could be explained by the fact that oviposition by *Bemisia tabaci* occurs normally while the insect is feeding on the plant (Gammel 1974). Over time, more WF was feeding, resulting into higher number of eggs deposited. This is consistent with degradation of active azadirachtin on or within the leaves. The neem applied through foliar method was deposited on the leaf surface, and was therefore exposed to external factors, particularly light. It would therefore be expected to degrade faster than the internally translocated azadirachtin (see also Larew 1988).

Our findings are in line with the other reported results, where feeding and oviposition deterrence of applied neem products decreased over the time. Showler et al. (2004) showed that neem products [Agroneem (Ajay Bio-Tech, Pune, India), Ecozin (AmVaC, Los Angeles, CA), and Neemix 4.5 (Certis, Columbia, MD)], was effective against Gravid Boll Weevil on cotton bolls for only for 24-h. After 72 hrs the neem had degraded to the point that no feeding and oviposition deterrence was observed. Moreover, this reduction in effectiveness of applied neem was dose-rate and UV-dependent as discussed below.

***Persistency and eggs hatching***

In all experiments either in the GH and the lab, hatching of WF eggs was reduced after neem application either by topical spray or by soil drenching. However, the percentage of hatched eggs increased over the time and development was faster under GH compared to laboratory conditions. This again can be related to the progressive decrease of active azadirachtin. The hatch rate increased from about 50% up to 81% when eggs were deposited 1 or 7 day after drenching of tomato plants with 3.0 g/l NeemAzalU in the GH. Whereas, at same dose-rate, hatch rate reached only 72% under laboratory conditions 7-d post application, clearly indicating gradual dissipation of applied neem. In foliar treated plants, only 23% eggs hatched on 1-d old residues, a rate which increased to 87% 7-d post-application in GH compared to 80% under laboratory conditions.

The reduction in eggs hatch with soil and foliar application of neem corroborates earlier findings of Prabhaker et al. (1999) in a study with *B. argentifolii* and with the GH WF (*Trialeurodes vaporariorum*) by von Elling et al. (2002). Observation of the process of eclosion revealed that apparent reduction in egg hatch was due to the effects of neem on crawlers after hatching from viable eggs, when they come in contact with neem residues on the plant leaves and on egg chorion and not by disruption or inhibition of embryogenesis.

***Persistency and mortality of immatures***

The immature mortality was highest with fresh neem residue in foliar treatments (10.0 ml/l) reaching 100%. This reduced to 7% on 7-d treatments under GH conditions and 44% in the laboratory. Similarly, the mortality was 88% (GH) and 90% (laboratory) in soil applications, which decreased to 45% and 64% in the 7-d treatments under GH and laboratory conditions respectively. It is obvious from the results that degradation of applied neem was faster following foliar application compared to soil application. Foliar treatment provided excellent control of WF for the first few days, but rapidly degraded over time. Soil application caused over 90% mortality but degradation was much slower and overall effect against WF was more stable over the time The strong effect of topical neem spray on WF immatures corroborates findings by von Elling et al. (2002) against GHWF (*Trialeurodes vaporariorum* Westwood) using NeemAzal T/S® at 0.05% and Prabhaker et al. (1999) on *B. argentifolii,* using Azatin E (3%

[AI] of azadirachtin; Agridyne, Salt Lake City, UT). Similarly, our result on systemic translocation of neem agrees with the earlier reported work of Prabhaker et al. (1999) against *B. argentifolii.*

Systemically induced mortality of azadirachtin has been reported in several studies in different herbivore-plant systems. Keelberg (1992) achieved 100% mortality in Tenthredinidae larvae by inserting a birch twig in NeemAzal solutions (100 ppm azadirachtin). Similarly, 100% mortality in Colorado potato beetle *Leptinotarsa decemlineata* Say. (Col.: Chrysomelidae) and subsequent reduced fertility in F1 adults was reported after feeding on cut leaf stems of potato plant places in glasses with NeemAzal solutions (100 ppm azadirachtin) (Otto 1994). Also, systemic effect of neem against larvae of *Liriomyza huidobrensis* Blanchard (Dipt. Agromyzidae) after inserting bean leaves in a neem based insecticide (Neemix – 45, 4.5% azadirachtin; W. R. Grace & Co., Conn., Columbia, MD) was reported by Weintraub and Horowtiz (1997). Similar results were obtained against *western* flower thrips, *Frankliniella occidentalis* Thoeming et al. (2003) as well as aphids, Ossiewatsch (2000), Larew et al. (1985).

The decrease of activity with neem-based pesticides was demonstrated in several previous studies; a reduction in efficacy of foliar applied neem was shown with *F. occidentalis* larvae, where residues of 0.1% Neemix-45 (4.5% azadirachtin, produced by W.R. Grace & Co. - Conn., Columbia, MD, USA) on cotton seedling were in the laboratory highly active for 10-11 days compared to only 5 and 3-4 d in the GH and outside, respectively (Ascher et al. 2000). In a similar study with three aphid species, Ossiewatsch (2000) recorded 100% larval mortality after 5 d of neem application. Similarly a short residual life of only 24 h under tropical conditions was reported by Isman et al. (1991) and that of 6.85 days for Margosan-O, reported by (Sundaram 1996).

The progressive loss of activity of azadirachtin treatments especially under GH conditions clearly indicated the role of abiotic factors like UV and temperature responsible for the degradation of the active ingredient of NeemAzal. From our results it is difficult to separate temperature and UV radiation as the driving forces of degradation. Temperature was more or less stable under laboratory (24-25°C) conditions, whereas in the GH a fluctuating and higher temperature (29-39ºC) was recorded. On the other hand, the average mean UV intensity per

day recorded during the experiments under GH condition was in range of 15-16 w/m$^2$ compared to a constant value of <1 w/m$^2$ under laboratory conditions. We assume that this large difference in UV radiation might have been the main degradation factor resulting in different decrease rates of NeemAzal activity under these two growing conditions. The rapid environment driven neem degradation corroborates the earlier reported work of Barnaby et al. (1989), Stokes and Redfern (1982) as well as Johnson et al. (2003). Sundram (1996) reported fast degradation of neem if exposed to ultraviolet light or other environmental factors. Under tropical conditions a shorter lifetime of azadirachtin was reported by Scott and Kaushik (2000). Consequently, the rapid UV-induced degradation of the neem products, as happened under our GH conditions, would explain the need for frequent applications by growers in the humid tropics.

***Conclusion***

In summary, our study indicates that *B. tabaci* is highly susceptible to NeemAzal, if application and infestation are relatively closely synchronized in time. With more or less “fresh” azadirachtin residues in or on plants strong effects on egg deposition, egg hatches, but particularly larval survival, are obvious. In particular, soil drenching can lead to reliable and high efficiency. The active ingredient dissipates over the time but with a variable rate in relation to application method and the environmental conditions. The faster degradation under sunlight in the GH and the longer persistency with soil treatments when azadirachtin is protected from UV radiation within the soil or plant is best explained by a high sensitivity of azadirachtin to the UV radiation. These assumptions are corroborated by results of earlier reports such as those of Koul et al. (1990), Schmutterer, (1990) and Showler et al. (2004).

The area under protected cultivation in the tropics is steadily increasing especially in the last decade owing to higher consumer demands for safe, fresh and clean fruits and vegetables in peri-urban areas. Tomatoes that are cultivated under protected cultivation conditions, where they are UV exposed on one hand and grown in pots on another giving the opportunity for a very localized and concentrated application of neem products to the growing substrate Thus, we foresee that substrate treatments with neem can be a valuable tool to improve WF control on a sufficient level.

# 4 Effects of Azadirachtin, Avamectin and Spinosad on Sweetpotato Whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) on tomato plants under laboratory and greenhouse conditions in the humid tropics[8]

## 4.1 Introduction

The WF, *Bemisia tabaci* Gennadius (Hom.: Aleyrodidae) is typically adapted to the warm climate of tropical and subtropical regions but today enjoys a worldwide distribution. In warmer regions (tropics, mediterranean), it is a serious pest in open field vegetable production but crops grown under emerging protected cultivation (film tunnels, net houses) are equally suffering under heavy WF burden. In addition, it has recently become a significant pest of protected horticulture in temperate regions (Butler and Heneberry 1986, Denholm et al. 1996). WF has been recorded from over 600 different plant species (Mound & Halsey 1978, Greathead 1986, Cock 1986, Secker et al. 1998) and it feeds on a wide variety of dicotyledonous horticultural crops like tomato, pepper, beans, eggplant and cucumber. WF damages the crops through direct sap feeding and producing massive quantities of honeydew. This encourages the growth of sooty mould on leaves inhibiting photosynthesis, and causes cosmetic damage (De Barro 1995). It is a vector of important viruses, e.g. Tomato Yellow Leaf Curl Virus (TYLCV) (Rapisarda and Garzia 2002) and responsible for plant disorders like uneven ripening (Maynard and Cantliffe 1989) in tomatoes. In conclusion, the high degree of polyphagy, ingestion of phloem sap during feeding and transmission of plant viruses between hosts, all contribute to the serious pest status of this species (Duffus 1987, Byrne et al. 1990).

Chemical control is the primary method to manage WF, but it has two serious drawbacks: rapid development of insecticide resistance and negative effects on natural enemies (Gonzalez-Zamora et. al. 2004). Resistant biotypes of WF have been described for different classes of insecticides especially organophosphates, pyrethroids and cyclodiens, but even for the relatively new group of chloro-nicotinyl insecticides (leading substance imidacloprid)

[8] To be published as Kumar, P., and H-M. Poehling. Effects of Azadirachtin, Avamectin and Spinosad on Sweetpotato Whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) on tomato plants under laboratory and greenhouse conditions in the humid tropics. Submitted to Journal of Economic Entomology.

(Prabhaker et al. 1989, Dittrich et al. 1990a, Cahill et al. 1995, Byrne et. al. 2003). To avoid selection of resistant biotypes a careful management with frequent changes of active ingredients is desirable. Furthermore, conventional insecticides bear a high risk for farmers and consumers because of toxicity and residues on the produces after harvest, particularly if decreasing efficacy (resistance) is counteracted by increased dosage or application frequency. The philosophy of integrated plant management recommends effective pesticides that have low mammalian toxicity, low persistence in the environment and high degree of selectivity. To minimize the above problems this study investigates biopesticides or botanicals of natural origin under the special conditions of the humid tropics.

Azadirachtin (product: NeemAzal®TS), a steroid-like tetranortriterpenoid derived from Neem trees (*Azadirachta indica* Juss.), is a strong anti-feedent, repellent and growth regulating compound for a wide variety of phytophagous insects, including WF (Schmutterer 1990, Coudriet et al. 1985). It delays or prevents moulting, reduces growth, development and oviposition; and can cause significant mortality particularly in immatures (Coudriet et al. 1985, Flint and Sparks 1989, Prabhaker et al. 1989, Schmutterer 1990, Liu and Stansly 1995, Mitchell et al. 2004). Neem preparations are commercially available worldwide, but especially in most countries in the humid tropics. However, the efficacy seems to be highly variable (Puri et al. 1994, Leskovar and Boales 1996, Akey and Henneberry 1999). This is partly caused by variable contents of the active ingredient of different products. The NeemAzal used in this study is of a very reliable and consistent quality. A major drawback of neem active ingredients is their sensitivity to UV-radiation and temperature and fast degradation under open field conditions (Stokes and Redfern 1982, Barnaby et al. 1989, Johnson et al. 2003, Barrek et al. 2004).

Spinosad consisting of 85 % Spinosyn A and 15% Spinosyn D (product: Success®) is a bio-rational pesticide derived from aerobic fermentation of the soil microorganism *Saccharopolyspora spinosa* with a world wide use on over 200 crops against insect-pest of several orders including Lepidoptera, Diptera, Thysanoptera, Siphonaptera, Coleoptera and Hymenoptera. It is classified as a reduced-risk pesticide by the US Environment Protection Agency (Cleveland et al. 2001). It is reported to be relatively less active against mites and sucking

insect-pests (Boek et al. 1994, Dow 1997, Bret et al. 1997, Thompson et al. 2000). Spinosad acts through ingestion and contact and kills the insects through targeting the nervous system (Salgado 1997 and 1998, Thompson et al. 2000, Cowles et al. 2000, Tjosvold and Chaney 2001). Concerning its selectivity no general rule can be given. It is of low toxicity for mammals but for non-target insects a broader spectrum of activity is reported. Fresh residues are described to affect pollinators like honey or bumblebees (Miles et al. 2002, Mayes et al. 2003, Morandin et al. 2005). It is moderately toxic to commonly used biological control agents like *Amblyseius cucumeris* Oudeman (Acarina; Phytoseiidae) and *Orius insidiosus* Say (Hemiptera:Anthocoridae) (Pietrantonio and Benedict 1999, Ludwig and Oetting 2001). However, it is highly toxic to the commonly used whitefly parasitoid, *Encarsia formosa* (Hym: Aphelenidae) even after 28-day post application (Jones et al. 2005). It is also toxic to the egg parasitoid *Anaphes iole* (Hymenoptera: Mymaridae) (Williams et al. 2003). The persistency of spinosad is limited to a few days in presence of direct sunlight (Saunders and Brett 1997), thus devoid of any long term effects for natural enemies.

Abamectin (product: Avermectin) is derived from a soil microorganism *Streptomyces avermitilis*. It consists of 80% avermectin B1a and 20% avermectin B1b as active ingredients. It acts by affecting the nervous system of insects and is highly toxic to a broad spectrum of insects, if they are contaminated by fresh spraying solutions or residues. Mammals can be affected only by ingesting high dosages (Ray 1991). Similar to spinosad, it is toxic to honey bees and other pollinators and to water organisms. It could be rapidly degraded, when present as a thin film on treated leaf surfaces. In the presence of light, its half-life as a thin film was measured as 4- 6 h regardless of surface or foliage type (Wislocki et al.1998). However, other studies reported much longer persistence (Reis et al. 2004). Abamectin does not persist or accumulate in the environment. Its instability, as well as its low water solubility and tight binding to soil, limits its bioavailability for non-target organisms and prevents it from leaching into groundwater or entering the aquatic environment (Lasota & Dybas 1990).

Apart from our earlier studies on impact of Azadirachtin on *Bemisia tabaci* (Kumar et al. 2005) little is known about the efficacy of these natural pesticides

against WF in Thailand and elsewhere in the SE Asia. Efficacy of abamectin against WGWF, *T. Vaporariorum,* was reported by Wang et al. (2003) and a similar effect of spinosad in northwestern Europe against this species and against *Bemisia tabaci* in Israel was described by Schoonejans and Van der Staaij (2001) and Ishaaya et al. (2001) respectively.

We assume that these botanical pesticides could improve the management of *B. tabaci* particularly in terms of safety for growers and consumers in the humid tropics in general and in protected cultivation systems in particular. Hence, we conducted a series of experiments under controlled (air conditioned laboratory) conditions and in tropical net greenhouses to evaluate the direct contact toxicity and residual persistence of these botanicals at different concentrations on the colonization preference of WF adults, oviposition pattern, egg hatch and immature mortality.

## 4.2. Materials and Methods

### *Location, host plant and rearing of whiteflies*

The study was part of an interdisciplinary research project funded by the German Research Foundation (FOR 431) entitled “Protected cultivation - an approach to sustainable vegetable production in the humid tropics”. Experiments were conducted with tomato plants (*Lycopersicon esculentum* Mill (Solanaceae), cv. King Kong II) at the greenhouse and laboratory complex at the Asian Institute of Technology, Bangkok, Thailand. The initial WF culture was obtained from the DoA (Department of Agriculture) Virology section, *Chatuchak*, Bangkok, which was maintained there without any pesticide exposure for two years. For the experiments mass rearing was established on tomatoes grown in air conditioned rooms. WF was kept in insect-proof cages (1.20 x 65 x 65 cm) at 24± 2°C and 60-70% relative humidity (rH). WF stages of same age, i.e. L1, L2 and adults, were obtained by allowing female *B. tabaci* to lay eggs for 24 h on caged tomato plants. Thereafter, adults were removed from the cages using an aspirator. Plants with eggs were further cultivated for synchronized development of *B. tabaci*. Plants with L1, L2, L3 or pupae were used for the experiments (see below) or kept until adult emergence in order to obtain adults of similar age. The laboratory and greenhouse experiments presented below were carried out from September 2004 until February 2005.

***Pesticides***

Pesticides used were: NeemAzal®-TS (1% Azadirachtin A = AZA) (Trifolio M GmbH, Lahnau, Germany), Success® (Spinosad 12% (wt: vol) Sc, Dow Agrosciences, Indianapolis, IN], and Abamectin [1.8% Avermectin (wt: vol.) EC, produced by: Exphoreflex Industrial, Thailand; Imported by: Inter Crop Co. Ltd., Thailand]. No recommend dose-rates for abamectin and spinosad against WF were available in Thailand. Dose rates chosen were 2-6ml/l and were based on recommended dose-rates of 1-4 ml of both commercial products/liter water for *Plutella xylostella*, *Helicoverpa armigera* (Hubner) and *Spodoptera* spp. on Brassicaceous crops and experience from preliminary experiments with WF. Neem was applied at the recommend dose-rate of 5 ml (0.05 g AZA) NeemAzal® TS/l and, to study dose-relation further, with 10 (0.1 g AZA) and 15 (0.15 g AZA) ml/l. All three products were diluted to spraying solutions with tap water which was also used for the untreated control. Approximately 50 ml of the product solutions were applied per plant using a small (500 ml capacity) hand held sprayer.

***Treatments***

All experiments were conducted on tomato plants cv. King Kong II grown in 10 cm diameter plastic pots with 180 gram of local substrate (pH-5.3; organic matter - 28%; sand - 30%; silt - 39%; clay - 31%; total N - 0.4%; K - 0.65%; P - 0.18%; Ca - 0.08%). Plants were kept in an air-conditioned laboratory at 24± 2°C, 60-70% rH and a photoperiod of 16:8 h (Light: Dark).

***Experiment 1: Direct Toxicity***

The direct toxicity of NeemAzalTS (5, 10 and 15 ml/l), abamectin (2, 4 and 6 ml/l) and spinosad (2, 4 and 6 ml/l) was tested against eggs, larvae (L1, L2 & L3), and pupal stage of *B. tabaci*. All experiments were carried out with 6 replications of each treatment and the experiments were repeated thrice over time.

To measure ovicidal effects three different age group, i.e. 1, 3 and 5-d old eggs were selected from synchronized eggs batches with 50 eggs of each group/per plant (rest removed by means of an entomological pin under microscope). Afterwards, plants were treated with the compounds at the stated dose rates. Treated plants were stored until emergence of the L1 and, thereafter, the proportion of hatched individuals calculated.

Similarly, 50 synchronized immature stages per plant were marked for easy individual counting and identification. Afterwards, batches of plants were treated 7 (L1), 10 (L2) and 14 (L3) days after egg laying. Dead larvae/pupae on the leaflets were counted daily. Immatures (larvae/pupae) were considered dead when they lost their normal yellow-green color, turgidity and smooth cuticle structure.

The effects of all three products on *B. tabaci* pupae were checked again with three different age groups, i.e. 1, 3 and 5-d old pupae. Emerging adults were counted daily and the proportion of dead individuals calculated by comparison with the non-hatched numbers of pupa.

***Experiment 2. Residual toxicity***

***General procedure and plant treatments***

Potted 15-day old tomato plants were sprayed with 5 and 10 ml/l NeemAzalTS and 4 and 6 ml/l abamectin and spinosad on the adaxial and abaxial leaf surfaces until run-off at 15, 10, 5 and 1- day prior to introducing WF. Plants sprayed with tap-water served as controls. Plants were arranged in a randomized design in a transparent acrylic box (1.2 m height, 75 cm width) and at day 0 approximately 400 same-aged un-sexed adult WF (2-d old) were released into the cages for 72 h. to give adult WF sufficient time for plant choice and oviposition.

***Laboratory conditions***

Plants were cultivated in an air conditioned laboratory. Starting one -day after the release, all adult WF per plant were counted for three consecutive days to record the colonizing preference of WF. Thereafter, WF adults were removed from the boxes and WF eggs on each leaflet counted using a microscope. Plants were further maintained in WF-free cages to allow juveniles to develop. After 30 days, plants were removed from the boxes and the number of living and dead immatures and empty pupal cases were counted to record adult emergence and immature mortality.

***Greenhouse conditions***

After treatment, plants were arranged in acrylic boxes for exposure to WF as mentioned above. Boxes were established in a net greenhouse (6x3x3 meter: net 78 mesh, Econet®; Ludvig Swensoon, Sweden) and exposed to WF. Adults were counted for three days. Afterwards, plants carrying WF eggs were

removed from the boxes, eggs counted, marked and plants arranged inside a similar WF-free net house to allow juveniles to develop under greenhouse condition. Data for egg hatch and immature mortality were calculated as in the above-mentioned experiments. Both experiments were carried out with 6-replication for each treatment and 2 repetitions over time.

***Statistical Analyses***

Data for percentage egg hatch, immature mortality and adult emergence were subjected to HOVTEST = LEVENE option of SAS to account for homogeneity of variance and normality. In the case of non-homogeneity, percent values were transformed using arcsine–square-root (arcsine$\sqrt{}$) transformation. Insect count values were transformed by square-root ($\sqrt{}$) transformation before running an ANOVA (Steel and Torrie 1980, Gomez and Gomez 1984). The data was analyzed using the PROC GLM procedure in SAS to determine single or interaction effects of factors (SAS 1999). Whenever significant interaction was observed between factors, the level of one factor was compared to each level of the other factor by all pair wise multiple comparison procedures (Tukey's test) unless mentioned otherwise. All data are presented as mean± SE. A significant level of $\propto$ = 0.05 was used for all analyses.

## 4.3. Results

***Experiment1: Direct Toxicity***

Egg hatch was significantly affected by the interaction of the age of treated eggs (age class) and the concentration of NeemAzalTS (concentrations*age class: F=44.05; df =6,143; P< 0.0001). Hence percentage of the larval emergence of each age class was compared at each concentration level of NeemAzalTS (see table 4.1).

**Table 4.1. Mean (±SE) % of *B. tabaci* larvae hatching from eggs treated at different ages on tomato plants by foliar spraying with different concentrations of NeemAzalTS.**

| Concentration NeemAzalTS | Egg age-classes (d) treated | | |
|---|---|---|---|
| | 1-d | 3-d | 5-d |
| 0 ml/l (control) | 99.33±0.28aA | 98.33±0.17aA | 98.59± 0.17aA |
| 5 ml/l | 54.00±1.74bA | 45.33±0.99bB | 21.17±0.76bC |
| 10 ml/l | 7.33±0.62cA | 2.33±0.33cB | 0.00±0.00cC |
| 15 ml/l | 2.17±0.63dA | 0.00±0.00dB | 0.00±0.00dB |

Means followed by the same lower case letters within column and uppercase letters within the rows are not significantly different (*P*: 0.05; Tukey's multiple comparison test; SAS Institute 1999). Data on % egg hatching was subjected to (arcsine√) transformation before analysis; non transformed percentages of eggs hatching are presented in the table.

Hatch success was least from eggs treated on day-5 with all concentrations compared to 3-day and 1-day old WF eggs. In contrast, no significant interaction was found in larval emergence between the egg age-class and concentrations of either spinosad (F= 0.55; df =6,143; *P* = 0.767) or abamectin (F = 0.26; df = 6,143; *P* = 0.953). Thus, concentrations were compared irrespective of the levels of the age classes and vice versa (see table 4.2). spinosad significantly reduced larval emergence in relation to the water control (F = 3061.97; df = 3,143; *P* < 0.0001) in a dose dependent manner (see table 4.2). Abamectin treatment, however, completely inhibited larval development within the eggs.

In all NeemAzalTS treatments, cumulative larval mortalities increased rapidly with time reaching, in all larval stages, 100% mortality latest after 4 days with concentrations of 10 and 15 ml/l. Only with the lowest dosage of 5 ml/l a reduced initial efficacy could be observed (Fig. 4.1 A-C).

**Table 4.2. Mean (±SE) % of *B. tabaci* larvae hatching from eggs treated at different ages on tomato plants by foliar spraying with three concentrations of Abamectin and Spinosad**

| Bio-pesticides | Concentration of pesticides | | | |
|---|---|---|---|---|
| | 0 ml/l | 2 ml/l | 4 ml/l | 6 ml/l |
| Abamectin | 99.78±0.11a | 0±0b | 0±0b | 0±0b |
| Spinosad | 99.61± 0.13a | 68.00± 0.60b | 43.50± 0.73c | 22.22±0.78d |

Values in rows followed by same letters are not significantly different (Tukey's HSD test; $P<0.05$)

The cumulative mortalities of treatments compared to the control were significantly different at all three stages, L1 ($F = 2671.04$; df=3, 71; $P < 0.0001$), L2 ($F = 5950.98$; 3, 71; $P < 0.0001$), L3 ($F = 4845.60$; 3, 71; $P < 0.0001$) but within treatments above all the lowest concentration of 5 ml/l separated clearly from the 10 and 15ml/l dose-rates. Similarly, with spinosad, all concentrations resulted in 100% mortalities in all three larval stages latest at day 8 after treatment with no significant differences among concentrations (see fig 4.2 A-C). The final accumulated mortalities differed significantly from the control at all three larval stages, L1 ($F = 5997.45$; df =3, 71; $P < 0.0001$), L2 ($F = 9317.38$; df=3, 71; $P < 0.0001$), L3 ($F = 17573.4$; df=3, 71; $P < 0.0001$). In contrast, abamectin caused 100% mortalities in all concentrations and all three larval stages within 24 hrs of treatment, which was highly significant compared to the control. Hence daily cumulative mortalities were not calculated L1 ($F = 5120.59$; df=3, 71; $P < 0.0001$), L2 ($F = 38302.8$; df=3, 71; $P < 0.0001$, L3 ($F = 9317.38$; df=3, 71; $P < 0.0001$).

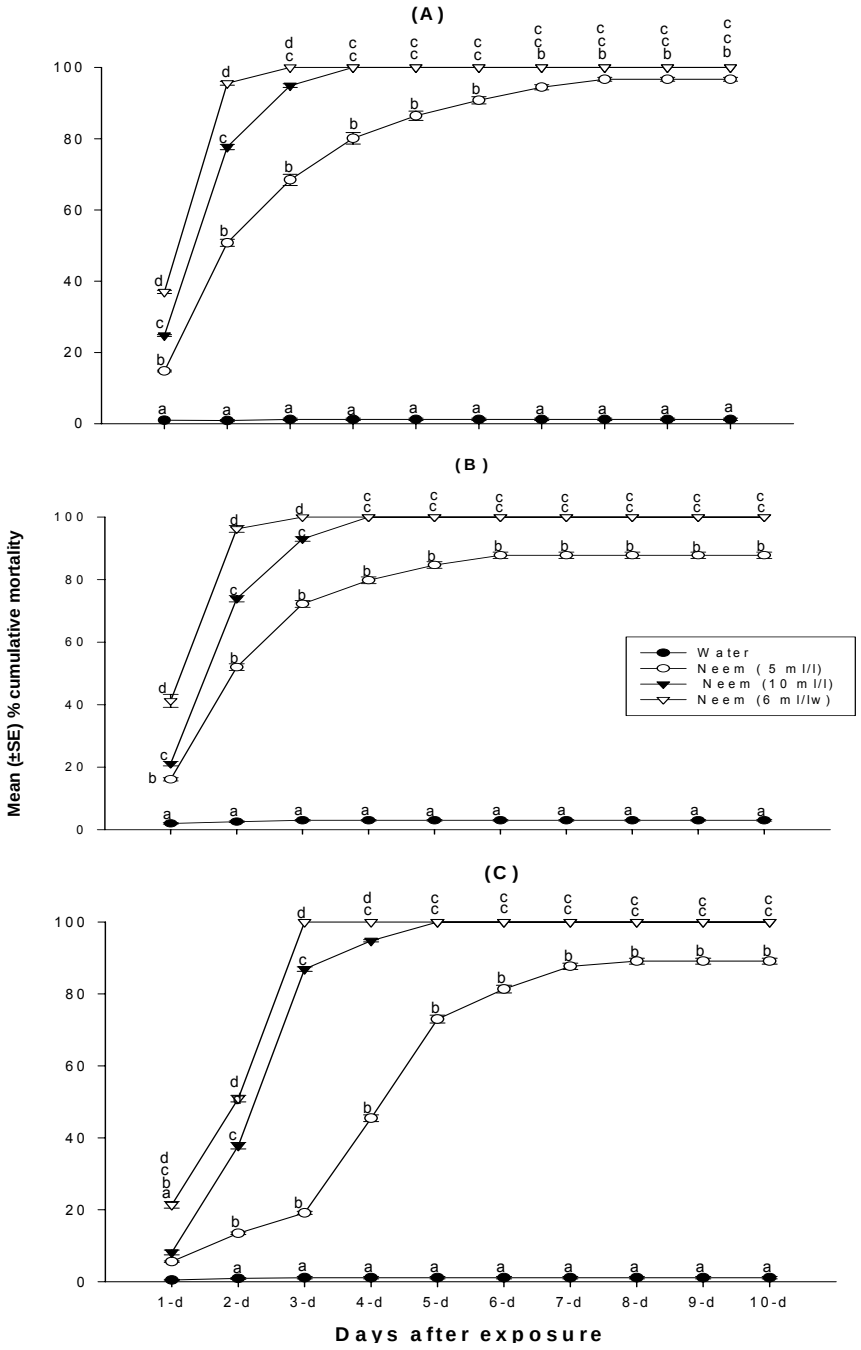



**Fig.4.1. Mean (±SE) percentage of cumulative mortality in the first larval stage (A), second stage larvae (B) and third stage larvae (C) of the *B. tabaci* to the three concentrations (5, 10 and 15 ml/l) of NeemAzalTS during 10 consecutive days. Values sharing a common letter(s) (within individual days after exposure) are not significantly different at $P < 0.05$, Tukey's HSD test).**

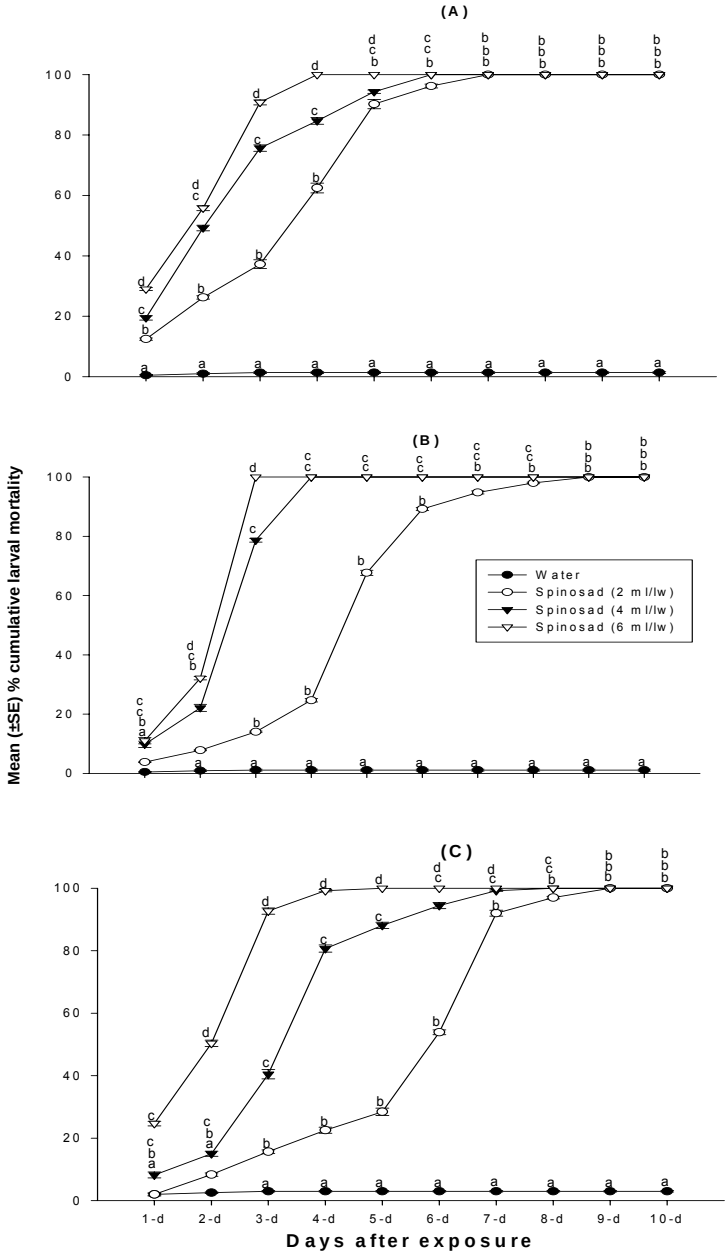



**Fig.4.2. Mean (±SE) percentage of cumulative mortality in the first larval stage (A), second stage larvae (B) and third stage larvae (C) of the *B. tabaci* to the three concentrations (2, 4 and 6 ml/l) of Spinosad during 10 consecutive days. Values sharing a common letter(s) (within individual days after exposure) are not significantly different at $P < 0.05$, Tukey's HSD test).**

Pupal mortality expressed by the proportion of empty pupal cases was affected significantly by the interaction of the pupal age when treated and NeemAzalTS concentrations (concentrations*age class); concentrations (F=7330.79; df =3,143; *P*< 0.0001); pupal age-class (F=6.60; df =2,143; *P* = 0.001).

Hence, the mortality of each age class was compared at each level of the tested NeemAzalTS concentrations (see table 4.3). Mortality did not differ for 1 and 3-d old pupa but increased significantly if pupae were already 5-d old at treatment with NeemAzalTS concentrations of 5 and 10 ml/l. In contrast, no significant interaction was found in pupal mortality between the pupal age-class and the tested concentrations of spinosad (F= 1.64; df=6,143; *P* = 0.141). Significant differences were observed for concentrations (F= 36242.6; df=3,143; *P*< 0.0001), but not for pupal age-class (F= 1.63; df=2,143; *P* = 0.1993). Similarly, no interaction in tested concentrations and age-class occurred for abamectin (F = 1.64; df = 6,143; *P* = 0.144). Thus, concentrations of spinosad and abamectin were compared irrespective of the levels of the age classes and vice versa.

**Table 4.3. % mortality (±SE) of B*. tabaci* pupae treated at different ages on tomato plants by foliar spraying with different concentrations of NeemAzalTS under laboratory conditions.**

| Concentration NeemAzalTS | Pupal age-class | | |
|---|---|---|---|
| | 1-d old | 3-d old | 5-d old |
| 0 ml/l(control) | 0.33±0.22aA* | 0.17±0.17aA | 0.33±0.22aA |
| 5 ml/l | 57.50±2.19bA | 58.00±2.26bA | 61.33±0.67bB |
| 10 ml/l | 79.83±0.76cA | 80.00±0.74cA | 85.50±1.02cB |
| 15 ml/l | 100±0dA | 100.±0dA | 100.00±0.00dA |

Means followed by the same lower case letters within column and upper case letters within the rows are not significantly different (*P*: 0.05; Tukey's multiple comparison test; SAS Institute 1999). Data on % pupal mortality was subjected to (arcsine√) transformation before analysis; non transformed percentages of eggs hatching are presented in the table.

### *Experiment 2. Residual Toxicity*

#### *Laboratory conditions*

Interaction of concentrations of all three biopesticides * days were significant for all variables (plant choice, egg deposition and hatch and mortality) studied: plant choice (F=25.70; df = 18, 335; *P*<0.0001); egg deposition (F = 39.42; df = 18,335; *P*<0.0001); egg hatch (F = 89.93; df = 18,335; *P*<0.0001) and immature mortality (F = 1428.07; df = 18,335; *P*<0.0001). The mean number of adult WF colonizing the plants, numbers of deposited eggs, percentage eggs hatched and mortality rates of immatures across the concentrations and days are summarized in the tables 4.4, 4.5, 4.6, & 4.7 respectively. The results showed that activity of abamectin residues persisted longest compared to spinosad and NeemAzalTS. Neem degraded faster than spinosad in all laboratory tests and its degradation was clearly concentration-dependent. In contrast, degradation of abamectin was less related to the applied concentrations; and spinosad was much less so.

**Table 4.4. Mean (±SE) numbers of adult whiteflies settling on tomato plants with different aged foliar residues of NeemAzalTS, Spinosad and Abamectin under laboratory conditions.**

| Treatments | Residue age, days | | | |
|---|---|---|---|---|
| | 1-d | 5-d | 10-d | 15-d |
| Water | 27.92±1.28aA | 29.92±1.28aA | 32.92±1.58aA | 28.50±1.93aA |
| Neem (5ml/l) | 11.93±0.39bA | 26.75±1.18aB | 30.50±1.46aB | 29.17±1.60aB |
| Neem (10 ml/l) | 7.13±0.37cA | 15.75±0.73bB | 30.00±1.42aC | 31.25±1.42aC |
| Abamectin (2 ml/l) | 5.67±0.36cA | 1.67±0.36cA | 01.58±0.29bA | 2.08±0.08bB |
| Abamectin (4 ml/l) | 0.50±0.19dA | 0.42±0.15dB | 0.67±0.14bBC | 1.25±0.13bC |
| Spinosad (2 ml/l) | 29.17±1.39aA | 28.08±1.21aA | 28.83±1.58aA | 29.08±1.48aA |
| Spinosad (4 ml/) | 26.08±1.48aA | 29.67±1.77aA | 30.00±1.44aA | 28.33±1.16aA |

Means followed by the same lower case letters within column and upper case letters within the rows are not significantly different (*P*: 0.05; Tukey's multiple comparison test; SAS Institute 1999). Data on number of adult WF was subjected to square-root transformation before analysis; non transformed numbers of adult WF are presented in the table.

**Table 4.5. Mean (±SE) numbers of egg deposition on tomato plants untreated and treated with foliar application of NeemAzalTS, Spinosad and Abamectin across the different residue levels and concentrations under laboratory conditions.**

| Bio pesticides Concentrations | Residue level | | | |
|---|---|---|---|---|
| | 1-d old | 5-d old | 10-d old | 15-d old |
| Water | 321.92±9.19aA | 324.00±13.25aA | 325.42±11.82aA | 323.75±13.60aA |
| Neem (5ml/l) | 116.25±4.16bA | 265.92±6.68bB | 327.00±6.51aC | 321.33±14.50aC |
| Neem (10ml/l) | 65.75±3.72cA | 185.75±7.78cB | 270.50±13.09bC | 323.17±13.78aD |
| Abamectin (2ml/l) | 25.08±1.34dA | 27.17±1.48dA | 26.33±1.40cA | 22.58±1.02bA |
| Abamectin (4ml/l) | 12.92±1.05eA | 15.67±0.87dA | 15.00±0.83dA | 14.17±0.88bA |
| Spinosad (2ml/l) | 307.83±9.35aA | 309.67±9.13abA | 312.17±11.67abA | 324.42±19.87aA |
| Spinosad (4ml/) | 302.75±7.38aA | 284.50±12.40aA | 319.50±13.19abA | 323.33±19.50aA |

Means followed by the same lower case letters within column and upper case letters within the rows are not significantly different (*P*: 0.05; Tukey's multiple comparison test; SAS Institute 1999). Data on number of eggs deposition was subjected to square-root transformation before analysis; non transformed number eggs depositions by adult WF are presented in the table.

**Table 4. 6. Mean (±SE) percentage of eggs hatching on tomato plants untreated and treated with foliar application of NeemAzalTS, Spinosad and Abamectin across the different residue levels and concentrations under laboratory conditions.**

| Bio pesticides & Concentrations | Residue level | | | |
|---|---|---|---|---|
| | 1-d old | 5-d old | 10-d old | 15-d old |
| Water | 97.75± 0.52aA | 96.61±0.60aA | 99.60±0.76aA | 98.98±0.22aA |
| Neem (5ml/l) | 45.37± 1.82bA | 88.18±0.51bB | 91.96±0.55bC | 98.58±0.35aD |
| Neem (10ml/l) | 24.32±0.60cA | 65.36±0.93cB | 85.61±0.77cC | 97.30±0.44aD |
| Abamectin (2ml/l) | 19.23±1.48cA | 18.95±1.70dA | 17.67±0.86dA | 20.48±0.79bA |
| Abamectin (4ml/l) | 6.34±0.97dA | 7.01±0.87eB | 7.55±1.29eC | 9.20±1.05cC |
| Spinosad (2ml/l) | 68.38±0.41eA | 67.98±0.97cA | 69.90±0.79fA | 77.99±0.37dB |
| Spinosad (4ml/) | 44.74±0.87bA | 44.89±0.96fA | 48.94±0.12gA | 56.23±0.43eB |

Means followed by the same lower case letters within column and upper case letters within the rows are not significantly different (*P*: 0.05; Tukey's multiple comparison test; SAS Institute 1999). Data on percentage eggs hatch was subjected to arcsine square-root transformation before analysis; non transformed percentage eggs hatch data are presented in the table.

**Table 4.7. Mean (±SE) percentage of immatures mortality on tomato plants untreated and treated with foliar application of NeemAzalTS, Spinosad and Abamectin across the different residue levels and concentrations under laboratory conditions.**

| Bio pesticides Concentrations | Residue level | | | |
|---|---|---|---|---|
| | 1-d old | 5-d old | 10-d old | 15-d old |
| Water | 2.76±0.24aA | 3.22±0.26aA | 3.11±0.14aA | 2.84±0.25aA |
| Neem (5ml/l | 76.31±0.76bA | 20.73±1.25bB | 3.91±0.26aC | 3.37±0.43aC |
| Neem (10ml/l) | 100.00±0.00cA | 65.11±1.16cB | 25.16±0.13bC | 7.71±0.43bD |
| Abamectin (2ml/l) | 100.00±0.00cA | 100.00±0.00dA | 100.00±0.00cA | 100.00±0.00cA |
| Abamectin (4ml/l) | 100.00±0.00cA | 100.00±0.00dA | 100.00±0.00cA | 100.00±0.00cA |
| Spinosad (2ml/l) | 95.77±0.17dA | 94.16±0.32eA | 93.22±0.48dB | 91.80±0.28dC |
| Spinosad (4ml/) | 100.00±0.00cA | 100.00±0.00dA | 100.00±0.00eA | 100.00±0.00eA |

Means followed by the same lower case letters within column and upper case letters within the rows are not significantly different (*P*: 0.05; Tukey's multiple comparison test; SAS Institute 1999). Data on percentage immatures mortality was subjected to arcsine square-root transformation before analysis; non transformed percentage immatures mortality data are presented in the table.

***Greenhouse conditions***

Similar to the laboratory, in greenhouse, residue bioassay for the interaction of concentration of all three pesticides*day were significant for all studied variables, plant choice (F = 28.81; df = 18, 335; *P*<0.0001); egg deposition (F = 31.47; df = 18,335; *P*<0.0001); egg hatch (F = 135.40; df = 18,335; *P*<0.0001) and immature mortality (F = 646.80; df = 18,335; *P*<0.0001). Comparable to the laboratory tests, the relevant data are listed in the tables 4.8, 4.9, 4.10, and 4.11. Like in the laboratory conditions, NeemAzalTS lost its activity faster than spinosad and abamectin as expressed through colonization, egg deposition, egg hatch and immature mortality of WF. Mortality for immatures decreased to control level even after 5 days and therefore much faster then in the laboratory. Abamectin showed longest persistency in the greenhouse, where its residue remained active for15-days post-application. Apparently, abamectin has low effect on hatch of eggs but it functioned as a strong oviposition deterrent and caused 100% mortality at all residue levels tested. spinosad residues remained effective for long time, particularly concerning immature mortality. But it neither

deters the WF to settle onto the tomato plants nor was it a strong oviposition deterrent, and had only a moderate effect on egg hatch.

**Table 4.8. Mean (±SE) numbers of adult whiteflies, on tomato plants untreated and treated with foliar application of NeemAzalTS, Spinosad and Abamectin across the different residue levels and concentrations under greenhouse conditions.**

| Bio pesticides Concentrations | Residue level | | | |
|---|---|---|---|---|
| | 1-d old | 5-d old | 10-d old | 15-d old |
| Water | 29.68±1.28aA | 31.08±1.52aA | 28.83±1.35aA | 30.92±1.45aA |
| Neem (5ml/l) | 8.33±0.53bA | 26.67±0.92aAB | 29.50±1.50aB | 31.33±1.73aB |
| Neem (10ml/l) | 6.30±0.52bA | 15.75±0.64bB | 29.75±1.59aC | 30.00±1.42aC |
| Abamectin (2ml/l) | 2.00±0.28cA | 1.67±0.36cA | 1.92±0.29bA | 2.25±0.13bA |
| Abamectin (4ml/l) | 1.08±0.08cA | 0.42±0.15cB | 1.08±0.08bC | 1.42±0.15bC |
| Spinosad (2ml/l) | 28.62±1.20aA | 31.92±1.56aA | 30.00±1.48aA | 31.25±1.30aA |
| Spinosad (4ml/) | 29.92±1.30aA | 30.42±1.60aA | 30.08±1.33aA | 29.33±1.39aA |

Means followed by the same lower case letters within column and upper case letters within the rows are not significantly different (*P*: 0.05; Tukey's multiple comparison test; SAS Institute 1999). Data on number of adult WF was subjected to square-root transformation before analysis; non transformed numbers of adult WF are presented in the table.

**Table 4.9. Mean (±SE) numbers of egg deposition on tomato plants untreated and treated with foliar application of NeemAzalTS, Spinosad and Abamectin across the different residue levels and concentrations under greenhouse conditions.**

| Bio pesticides Concentrations | Residue level | | | |
|---|---|---|---|---|
| | 1-d old | 5-d old | 10-d old | 15-d old |
| Water | 324.83±2.44aA | 330.58±11.51aA | 314.92±13.91aA | 321.00±17.00aA |
| Neem (5ml/l) | 114.42±9.47bA | 283.00±11.96aB | 311.00±8.57aB | 318.67±15.90aB |
| Neem (10ml/l) | 59.83±3.78cA | 202.92±12.98bB | 300.42±15.55aC | 320.25±12.98aC |
| Abamectin (2ml/l) | 24.33±1.37dA | 24.75±1.42cA | 32.33±2.57bA | 34.08±2.70bA |
| Abamectin (4ml/l) | 12.83±1.01eA | 14.08±0.83cAB | 18.33±1.74bBC | 27.50±2.32bC |
| Spinosad (2ml/l) | 311.83±11.89aA | 309.17±16.94aA | 316.67±9.89aA | 316.50±14.42aA |
| Spinosad (4ml/) | 314.00±17.07aA | 311.67±11.28aA | 318.67±13.13aA | 317.83±13.74aA |

Means followed by the same lower case letters within column and upper case letters within the rows are not significantly different (*P*: 0.05; Tukey's multiple comparison test; SAS Institute 1999). Data on number of eggs deposition was subjected to square-root transformation before analysis; non transformed number eggs depositions by adult WF are presented in the table.

**Table 4.10. Mean (±SE) percentage of eggs hatching on tomato plants untreated and treated with foliar application of NeemAzalTS, Spinosad and Abamectin across the different residue levels and concentrations under greenhouse conditions.**

| Bio pesticides Concentrations | Residue level | | | |
|---|---|---|---|---|
| | 1-d old | 5-d old | 10-d old | 15-d old |
| Water | 99.61±0.46aA | 98.63±0.48aA | 97.85±0.62aA | 99.73±0.29aA |
| Neem (5ml/l) | 45.12±0.87bA | 91.89±1.11bB | 97.15±0.36aC | 97.67±0.37aC |
| Neem (10ml/l) | 23.79±1.29cA | 72.19±0.91cB | 97.67±0.21aC | 97.46±0.59aC |
| Abamectin (2ml/l) | 18.90±1.12cA | 17.55±1.04dA | 17.81±1.76bA | 20.51±1.56bA |
| Abamectin (4ml/l) | 8.32±0.65dA | 7.35±0.40eA | 7.75±0.98cA | 8.94±1.69cA |
| Spinosad (2ml/l) | 67.34±0.93eA | 69.62±0.49cA | 74.95±0.27dB | 81.78±0.81dC |
| Spinosad (4ml/) | 45.78±0.45bA | 46.87±0.35fA | 49.76±0.72eBC | 54.60±1.50eC |

Means followed by the same lower case letters within column and upper case letters within the rows are not significantly different (*P*: 0.05; Tukey's multiple comparison test; SAS Institute 1999). Data on percentage eggs hatch was subjected to arcsine square-root transformation before analysis; non transformed percentage eggs hatch data are presented in the table.

**Table 4.11. Mean (±SE) percentage of immatures mortality on tomato plants untreated and treated with foliar application of NeemAzalTS, Spinosad and Abamectin across the different residue levels and concentrations under greenhouse conditions.**

| Bio pesticides Concentrations | Residue level | | | |
|---|---|---|---|---|
| | 1-d old | 5-d old | 10-d old | 15-d old |
| Water | 2.36±0.18aA | 1.90±0.17aA | 2.19±0.16aA | 2.87±1.75aA |
| Neem (5ml/l) | 74.39±0.96bA | 3.05±0.19aB | 2.86±0.13aB | 3.64±0.32aB |
| Neem (10ml/l) | 100.00±0.00cA | 19.64±0.31bB | 12.03±1.11bC | 4.26±0.29aD |
| Abamectin (2ml/l) | 100.00±0.00cA | 100.00±0.00cA | 100.00±0.00cA | 100.00±0.00bA |
| Abamectin (4ml/l) | 100.00±0.00cA | 100.00±0.00cA | 100.00±0.00cA | 100.00±0.00bA |
| Spinosad (2ml/l) | 97.64±0.24dA | 96.78±0.38dB | 89.65±0.57dC | 87.23±0.64cD |
| Spinosad (4ml/) | 100.00±0.00cA | 100.00±0.00cA | 97.10±0.46eB | 89.31±1.46dC |

Means followed by the same lower case letters within column and upper case letters within the rows are not significantly different (*P*: 0.05; Tukey's multiple comparison test; SAS Institute 1999). Data on percentage immatures mortality was subjected to arcsine square-root transformation before analysis; non transformed percentage immatures mortality data are presented in the table.

## 4.4. Discussion

### *Direct Contact Toxicity*

The results show that the sensitivity of *B. tabaci* eggs for azadirachtin changes with progressing development. This corroborates earlier findings of Prabhaker et al. (1999) with a similar species, *B. argentifolii.* However, no such age specific effects were observed in the case of abamectin and spinosad-treated eggs. These are in contrast to an earlier study of Wang et al. (2003) with *T. vaporariorum* treated with abamectin. The different results may be explained by the different concentrations of abamectin used. Our concentrations selected were in the saturation part of the dose-response curve. Inductions of embryonic disruptions by abamectin are reported from other abamectin-herbivore systems like *Liriomyza huidobrensis* (Schuster and Everett 1983, Ochoa and Carballo 1993, Buxton and McDonald 1994). In contrast, the missing concentration response of spinosad is in line with earlier reports on GHWF (*T. vaporariorum)*, where no effect of concentration was found on various egg stages and where an overall efficacy of over 98% was reported for all tested age-classes (Schoonejans and Van der Staaij 2001). Examination of the process of embryonic development revealed that abamectin-treated eggs changed color from dark brown to black presumably indicating the death of developing embryo. In neem and spinosad-treated eggs, no such color change took place and apparently more the influence on a successful egg hatch was the key mechanism resulting in killing the emerging crawlers immediately after eclosion from viable eggs, when they came into contact with neem and spinosad residues on the plant leaves and on the egg chorion (Schoonejans and Van der Staaij 2001 & Ishaaya et al. 2001). Byrne et al. (1990) demonstrated that WF eggs are closely connected to the leaf tissue, e.g. extracted water from plant tissue accounts for 50% of the egg mass. Consequently, also translaminar translocated ingredients can be expected to penetrate in small quantities via plant into the embedded eggs. With its high toxicity even small amounts of abamectin might have caused such deleterious effects and the penetration into the maturing egg may be more intensive then with younger stages (see Wang et al. 2003).

Moreover, abamectin was very toxic for the larval stages, since all died within 24 hours after treatment. In contrast, mortality induced by neem and spinosad

decreased gradually with aging of larvae; the first larval stage was found more susceptible than the other two older stages for both ingredients. In the case of neem, this findings agree with earlier studies of Coudriet et al. (1985), Lindquist and Casey (1990), Price and Schuster (1991) and the results corroborate with findings of Schoonejans and Van der Staaij (2001), who tested spinosad against *T. vaporariorum.* Comparing abamectin and spinosad, a striking difference was found in the speed of action: high mortality rates in abamectin were reached within 24 hrs whereas with spinosad it takes 6-9 d before the final mortality values were reached. The lower daily mortality from spinosad could be due to its slow penetration rates and slow metabolism once inside the insect body (Sparks et al. 1998, Sparks et al. 2001), which results in such a delayed but steady increasing activity.

Similar to the egg stage the intensity of reaction of *B. tabaci* pupae to NeemAzalTS depends on the pupal age at treatment. The least number of adult WF emerged from the 5-d old neem-treated pupae compared to 1 and 3-d old. Similar to its effect on egg stage it could be due to the presence of residues, killing the emerging WF coming out of the puparia. Our results corroborate earlier work with *T. vaporarium* where a concentration of 0.5% NeemAzal T/S significantly reduced the proportion of emerging adults (von Elling et. al. 2002). In contrast, all tested concentration of spinosad and abamectin killed the adults within the pupal stage by 100%. Similar results are reported with abamectin against pupae of *T. vaporarium* by Wang et al. 2003. However, our results do not agree with findings of Schoonejans and Van der Staaij (2001), who did not find any effect of spinosad on pupae of *T. vaporariorum.*

***Residual toxicity***

Abamectin most efficiently deterred both in laboratory and in greenhouse, the settling of WF adults on the tomato plants; followed by weaker but pronounced effects of neem. In contrast, spinosad showed no inhibition of adult colonization either as fresh or 15-d old residues. The dissimilar colonization behavior of adult WF resulted in unequal egg deposition. Anti-feedent actions of neem resulting in decreased egg deposition behavior of WF are reported in several studies (Nardo et el. 1977, Coudriet et al. 1985, Abou-Fakhr Hammad et al. 2001, Hilje et al. 2003). The intensity of oviposition by *B. tabaci* is normally in relation to its feeding activity (Gammel 1974) and deterrent effects often reduce not only

settling but also phagostimulation. Oviposition suppressant effects of neem products have also been documented for different other insect orders i.e. Orthoptera, Heteroptera, Homoptera, Hymenoptera, Lepidoptera, and Diptera (Saxena, 1989, Singh 1993, Schmutterer 1995, Isman 1996). The results with abamectin are consistent with studies of Horowitz et al. (1997), where abamectin considerably reduced oviposition of *B. tabaci* in a concentration-dependent manner. Such deterrent effects decreased in the case of NeemAzalTS with residual age and were less severe in the greenhouse compared to the laboratory environment.

Hatching of WF eggs was reduced by all three products both under lab and in theGH. With increasing age of residues, hatch rates increased. This is probably the result of decreasing activity of neem, abamectin and spinosad residues on the plants. However, the intensity of reduction varied in all three cases. It progressed rapidly in case of NeemAzalU, but was slower with spinosad and lowest with abamectin. The results are in agreement with studies reported by Premachandra et al. (2005) dealing with the thrips, *Ceratothripoides clarathris*, a major pest on tomatoes in Thailand.

All three products caused heavy residual mortality of the immature stages of the *B. tabaci.* Abamectin had the strongest performance and consequently caused 100% immature mortality at all residue levels followed by spinosad and NeemAzalTS. The higher persistency of spinosad and abamectin was reported also by Horowitz et al. (1997) and Premachandra et al. (2005). Whereas, abamectin showed nearly no loss of activity with time under the greenhouse conditions, toxicity of spinosad to immatures slightly decreased from 95% of fresh residues to 91% 15-d post application and same aged residues caused 87% mortality under greenhouse conditions. Concentrations of 5, 10 and 20 ml/ 20 l water for spinosad caused 100% mortality at larval instars and adult of the *Cetraothripoides claratis* until 7-days post application under greenhouse condition indicating the very strong persistency (Premachandra et al. 2005). Similarly, in greenhouse experiments with cucumber and tomatoes, Narocka (2002) recorded 100% mortality in western flower thrips, *F. occidentalis* at two spinosad concentrations. In addition, persistent toxicity of spinosad was reported from other economically important insect-pests, e.g. diamond back moth (Hill and Foster 2000), Cabbage looper *Trichoplusia ni* (Hubner)

(Lepidoptera: Noctuidae) (Liu et al. 1999) and Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae) (King and Hennessey 1996) and the eggplant flea beetle (Coleoptera: Chrysomelidae) on eggplants under field conditions (McLeod et al. 2002). In contrast, neem's toxicity decreased rapidly already 5-d post application in the greenhouse and mortality rates dropped to the level of control. This finding with neem is in line with work of Ascher et al (2000). In a similar neem bioassay against *F. occidentalis*, under laboratory conditions, residues of 0.1% Neemix-45 on cotton seedling were highly active for 10-11 days but only 5 and 3-4 d in the greenhouse and outside, respectively. The consistent progressive loss of activity with time more in the greenhouse compared to the laboratory could be explained by the more rapid degradation of the neem on exposure to sunlight, high temperatures and UV (Barnaby et al. 1989, Stokes and Redfern 1982, Johnson et al. 2003).

***Conclusion***

In summary, our studies indicate that *B. tabaci* is highly susceptible to NeemAzalTS spinosad and abamectin. However, the susceptibility varies with WF growth stage and time span between application and infestation as well as the presence and absence of sunlight. Spinosad affects adult WF but failed to reduce egg deposition. However, it affects egg hatching, causing high immature mortality and inhibiting adult emergence. Abamectin affects colonization, egg deposition, egg hatch and induces high mortality amongst immatures. Neem affects settling, egg deposition and egg hatch, as well as larval and pupal mortality; but the chemical shows the strongest sensitivity and loss of activity over time if exposed to adverse conditions (high temperature and intensive UV radiation).

The use of neem products can help to control the serious pest *B. tabaci* in a more safe and sustainable manner; particularly if only short term effects are necessary since remigration of the pest, e.g. in GH, is low. However it easily becomes ineffective in the presence of high temperature and strong ultra-violet light (Johnson et al. 2003). Thus, we foresee that WF management in tropically adapted greenhouses, if necessary for longer periods under heavy infestation pressure, cannot be achieved with this botanical alone. It requires a combination of neem and other safe products like spinosad or even abamectin, if there is a need for product rotation to avoid resistance selection. The highly

efficient spinosad seems to be at risk of rapid selection of resistant biotypes if it is used frequently (Zhao et al. 2002). Moreover, the possible combination of bio-pesticides, with release of natural enemies, should be studied in more detail. That requires reliable data about possible side effects under practical growing conditions. Data so far available does not give a clear picture. Jones et al. (2005) found spinosad to be highly toxic for *Encarsia* spp; but in another study Zchori-Fein et al. (1994) combined abamectin and *Encarsia* for integrated management of the WF. Therefore, in ongoing studies, we will elucidate possible side-effects of these chemicals on the indigenous parasitoids of *B. tabaci* in the humid tropics.

# 5 Impact of UV-blocking plastic covers and netting on the pest status of *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae), *Ceratothripoides claratris* Shumsher (Thysanoptera: Thripidae) and *Aphis gossypii* Glover (Homoptera: Aphididae) on tomatoes in the humid tropics[9]

## 5.1. Introduction

Tomato production under protected cultivation in the humid tropics is extremely vulnerable to abiotic stresses (temperature, humidity, air flow etc.) (Ajwang et al. 2002), and to biotic stresses represented by insects (whitefly, thrips, aphids) and, less directly, plant virus diseases vectored by these insects (Thongrit et al.1986, Attathom et al. 1990, Premachandra et al. 2005). The damage that whitefly (WF) inflicts on the host plant results from sap sucking, the heavy deposition of honeydew, plant disorders like uneven ripening (Schuster et al. 1990) and spread of diseases caused by 50-60 different kinds of geminiviruses (Markham et al. 1994, Brown et al. 1995). Similarly, thrips (*Ceratothripoides claratris* Shumsher; Thysanoptera: Thripidae) is a serious pest species attacking field- and greenhouse-grown tomatoes in Thailand (Premachandra et al. 2005). Major damage is caused directly by mechanical damage through feeding and oviposition and indirectly by transmitting tospoviruses (Murai et al. 2000, McMichael et al. 2002, Premachandra et al. 2005). Aphids, *Aphis gossypii* (Homoptera: Aphididae) is another pest of tomato in Thailand causing direct damage by sucking plant sap and reducing the overall quality and productivity. Often plants are attacked by a complex of these pests which can potentate direct damage and lead to detrimental infections by more then one type of virus (Summers et al. 2004).

Chemical control is the primary method to manage WF, thrips and aphids however management using pesticides has not been effective, provides only partial control (Denholm et al. 1996, Horowitz and Ishaaya 1996) or fails mainly

[9] To be published as Kumar, P., and H-M. Poehling. Impact of UV-blocking plastic covers and netting on the pest status of *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae), *Ceratothripoides claratris* Shumsher (Thysanoptera: Thripidae) and *Aphis gossypii* Glover (Homoptera: Aphididae) on tomatoes in the humid tropics. Submitteted to Enviornmental Entomology.

because of rapid selection of resistant pest biotypes of WF (Denholm et al. 1996, Prabhaker et al. 1998, Cahill et al. 1995, Elbert and Nauen 2000), thrips (Kontsedalov et al. 1998, Espinosa et al. 2002), or aphids (Foster et al. 2000). Botanicals like neem can be efficient with lower risk of resistance selection (e.g. Thoeming et al 2003, Kumar et al. 2005) but suffer from rapid dissipation and degradation in presence of UV light under tropical conditions, which reduces persistency (Barnby et al. 1989, Johnson et al. 2003, Barrek et al. 2004).

Some species of insects like WF, thrips and aphids have been shown to be dependent on UV light (mainly UV A from 320 – 400 nm) to orient themselves during flight. These species may use UV-light reflectance patterns as cues for recognizing host plants and flower species (Kring 1972, Rossel and Wehner 1984, Scherer and Kolb 1987, Greenough et al. 1990, Kring and Schuster 1992, Goldsmith 1993, Costa and Robb 1999). Furthermore, previous findings show that *Bemisia argentifolii* and *Frankliniella occidentalis* are attracted to UV light (Mound 1962, Matteson and Terry 1992, Antignus et al. 1996, Antignus 2000). Similarly reduced aphid movement and delayed spread of aphid-borne virus diseases were achieved by using UV-blocking plastic mulches for squash and other crops (Brown et al. 1993, Summers and Stapleton 1998, Stapleton and Summers 2002). Field studies from Israel demonstrated a significant reduction in crop infestation by *B. tabaci*, aphids and thrips when UV- blocking plastics were used as greenhouse covers (Antignus et al. 1996, 1998, 2001, Antignus 2000). These materials are also reported to reduce the incidence of WF transmitted geminiviruses.

The area under protected cultivation in the tropics is on the rise. This trend is complemented by the constant change and improvement in existing covering materials and other production technologies in the last decades, and consumer demand for safe food has encouraged growers in the tropics to shift towards protected cultivation (Giacomelli and Roberts 1993, Ashekanzi 1996). The aim of protected cultivation is not only to allow production under otherwise adverse climatic conditions (e.g. heavy rainfalls) but to reduce dependency on frequent pesticide use with all its drawbacks (e.g. residues, operator health, increased production costs and resistance. However, the use of screens as a physical means of control has limitations, particularly with small insects since very small mesh size in nets, or complete cover with plastics, reduces the efficiency of

natural ventilation. Good ventilation is a prerequisite for greenhouses without expensive cooling devices (Michelle and Baker 2000, Ajwang et al. 2002). Materials hindering insect invasion but permitting adequate ventilation are desired. UV-blocking materials may be a further advance in greenhouse development. All past studies like those of Antignus (1998, 2000) and others mentioned-above reported the use of UV-blocking nets/screen or plastics alone, and their efficiency in reducing immigration, dispersal and virus infection. However, none of these studies were performed under the conditions of the humid tropics, where a combination of rain blocking plastic roof materials and well ventilated side wall covers is necessary to allow year round production of sensitive vegetable such as tomatoes. Therefore, we undertook this study with different combined UV-blocking and UV-transmissible roof and wall materials in small experimental greenhouses to study the movement pattern of the more serious small plant sucking insects (WF, thrips and aphids) of tomatoes, and the incidence of viruses transmitted by these vectors in the humid tropics.

## 5.2 Materials and Methods

### *Location*

The study was part of an interdisciplinary research project funded by the German Research Foundation (FOR 431) entitled “Protected cultivation - an approach to sustainable vegetable production in the humid tropics”. Experiments were conducted on tomato plants (*Lycopersicon esculentum* Mill (Solanaceae), cv. King Kong II) at the greenhouse complex provided for the AIT-Hanover Project, Asian Institute of Technology, Bangkok, Thailand. The experiments were conducted during the later part of the spring (March) until end of rainy season (October) 2005.

***Nets & Plastics***

Two nets; UV-blocking, Bionet® and non UV-blocking (= UV transmitting), Anti Insect® nets (50 mesh: Polysack Plastic Industries, Israel) along with two plastics, UV-blocking (Sun Selector Diffused Antivirus®, Ginegar Plastic Product Ltd**,** Kibbutz, Israel) and UV-transmitting (= non blocking) plastic film, PE-1A (RKW AG, Germany) were used in the experiments. The spectral transmission properties of these films were analyzed using a PerkinElmer Lambda 900 UV/ VIS/NIR spectrophotometer (PerkinElmer Life and Analytical Sciences, Boston, MA) (see fig. 5.1).

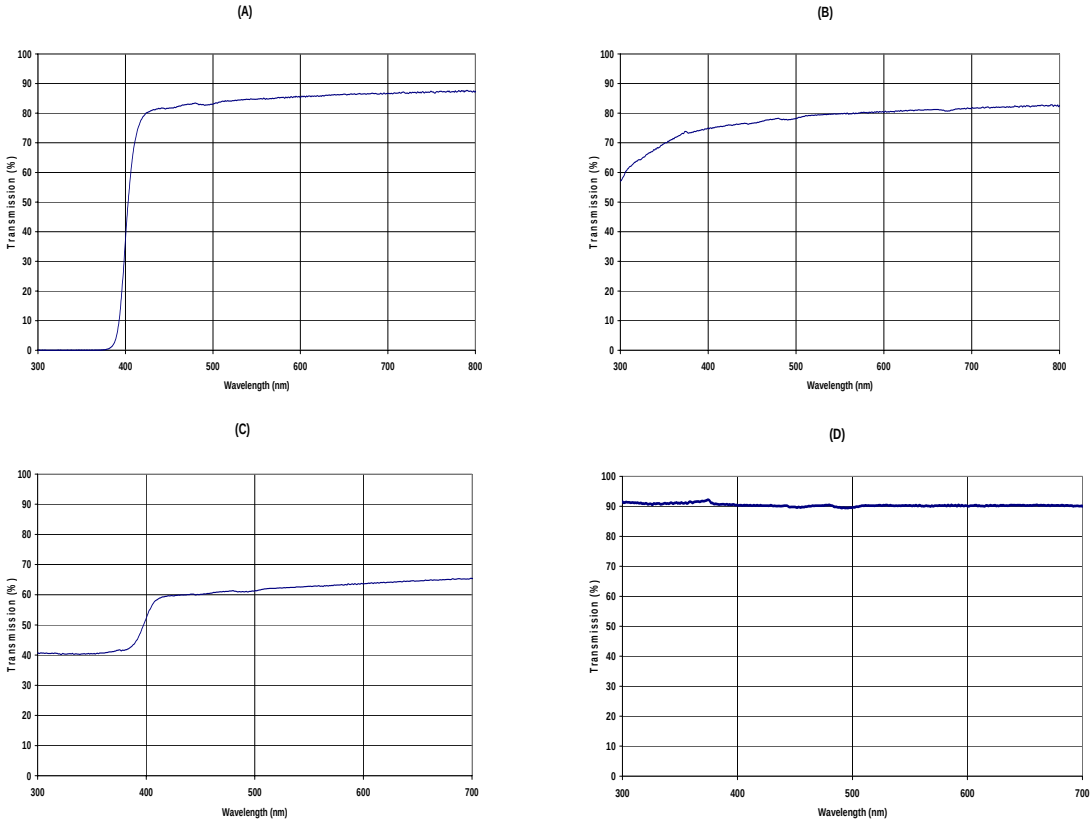



**Fig 5.1. Spectral transmissivity of UV-blocking plastic film (A, Sun Selector Diffused Anti Virus®, Ginegar Plastic Products Ltd., Israel), UV-transmitting plastic, PE-1A (B, RKW AG, Germany),UV blocking net (C, Bionet®,Polysack, Israel) and UV-transmitting nets (D, Anti-Insect®, Polysack, Israel) films measured with a PerkinElmer Lambda 900 UV/VIS/NIR spectrophotometer.**

***Treatments and greenhouses***

These two nets (UV-blocking and non UV-blocking; henceforth will be referred as UVB-N and NUVB-N, respectively) and plastics (UV-blocking and non-blocking; henceforth will be referred as UVB-P and NUVB-P, respectively) were permutated in 4 different combinations: UV blocking nets + UV blocking Plastics [henceforth referred as B (N+P)]; Non UV-blocking net + UV blocking plastic [NB-(N+BP)]; UV blocking nets + UV non-blocking Plastics [ BN+N-BP)]; UV Non-blocking nets + UV non-blocking plastic [NB (N+P)]. A total of eight greenhouses (GH) (7.5 m x 2 m x 2 m) were constructed with four GH each placed in identical orientations (either east/west or north/south direction) to avoid any effect of orientation. Furthermore, each greenhouse was provided with two identical doors at the length side. The front and rear end of the door walls were covered with identical nets used for the sidewalls of each greenhouse. The sidewalls of the greenhouses were always covered with either of the nets and the roofs with either of the plastics. Between GH, 1.5 meter space reduced shading from each other. The area around the GH complex was cleaned and all weed plants were removed prior to each series of experiments. Two replications of each treatment were arranged in a complete randomized block design. Between each series, greenhouses were thoroughly washed and cleaned approximately one week prior to new experiments. A total of 2 experimental series each of 6 weeks duration were carried out and each experiment was repeated once over the time. Data collection started one week after transplanting for 5 more weeks. A total of 30 potted (25 cm high and 27 cm Ø) tomato plants (2 weeks old) were transplanted in a commercial local media composed of clay, sand, and silt in proportions of 31, 30 and 39%, respectively, and 29% of organic matter. Tomato seedlings were grown in an insect free evapo-cooled nursery. Radiation triggered and scheduled drip irrigation combined with dosatron fertigation was provided to ensure the mineral balance and optimal growth and development of the tomatoes. Each GH was provided with a temperature, humidity and UV-A using Radiometer UV-Sensor (Dr. Grobel UV-Elektronik GmbH, Germany).

***CaCV detection by DAS-ELISA***

Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) was conducted for the confirmation of CaCV-AIT infection of tomato plants in addition to symptom diagnostics. Polyclonal and monoclonal antibodies raised against N-protein of Watermelon Silver Mottle Virus (WSMV) and Groundnut Bud Necrosis Virus (GBNV) (Agdia, Inc., Elkhart, ID, USA) were used. Plant leaves were homogenized at a ratio of 1: 5 in PBS-T (2.5 mM KCl, 1 mM KH2PO4, 8 mM Na2HPO4, 0.14 M NaCl and 0.6 ml/l Tween 20) containing 0.45 polyvinylpyrollidone (PVP). Leaves from healthy plants were used for the control treatment. Absorbance values were read with a microplate reader (BIO-Tek Instruments, Inc, Vermont, USA) at 405 nm, with PBS-T as a blank. The absorbance values were corrected by subtracting the average of three wells of the blank from samples means. Samples having absorbance means three times that of the control was considered as positive. For other viruses e.g. Tomato Yellow Leaf Curl Virus (TYLCV) visual counts were made on the basis of symptoms only.

***Experiment 1 and 2. Effect of UV blocking nets and plastics on the immigration of whitefly, thrips and aphids and occurrence of tospoviruses and TYLCV (reduced ventilation by partly open doors)***

Two rounds of experiment, were conducted using above-mentioned set ups of the 8 GH. The two parallel doors of the GH were simultaneously opened every morning from 6.00-10.00 am (partial ventilation), coinciding with the peak insect's activities time (Cohen and Melamed-Madjar 1978). The immigrating WF population were measured by yellow sticky traps (YST) (25 x 15 cm) positioned half at the plant canopy and half above canopy. The YST were made from yellow PVC sheets coated with insect-glue (Kosfix®, Kosmix Polymer, Bangkok, Thailand) on both sides. A total of 6 YST were placed for each GH, changed once a week and number of WF trapped at both side of the traps were counted. Each trap was considered as one replication and this way a total of 5 weekly readings were collected on the WF entering inside each of 8 GH during each experiment. Similarly, the numbers of adult WF per plants were counted by selecting one young fully developed leave per plant, gently turning it over and visually counting the number of adults present on the lower surface. The

counting was carried out in the early morning (7.00 am and before) from 3 randomly selected plants from each greenhouse.

Similarly, once a week, number of thrips entering in each GH was counted using Blue Sticky Traps (BST) of same dimension simultaneously with YST (12 replications). Additionally, once a week number of thrips infested leaves were counted from 3 pre-marked plants until the fifth week to asses cumulative weakly leaf damage. Once a week, number of virus infected tomato plants were counted and marked and towards the end of the experiments at 35 days after transplanting (DAT), DAS-ELISA tests were carried out to distinguish between the tospovirus and other viruses e.g. TYLCV. Since the tospovirus was the most commonly occurring one, the plants failed to test positive for the CaCV-AIT infection but showing virus symptoms were assumed to be infected with the TYCLV.

The number of immigrating winged aphids was monitored using same YST placed for the WF monitoring in similar manner as explained above. The immatures and wingless adults (henceforth will be refereed as immatures) were counted by selecting one young, fully expanded leaf per plant, gently turning it over and visually counting their numbers present on the lower surface.

***Experiment 3 and 4. Effect of UV blocking nets and plastics on the immigration and attraction of whitefly, thrips and aphids and occurrence of tospoviruses and TYLCV (full ventilation with complete open doors)***

Two rounds of experiments (June – July; August - September) were carried out in a similar GH set-up as discussed above with a single exception of timing of GH door opening. Two GH doors were kept open during the entire period of experiment (full ventilation). The numbers of WF and thrips were counted on the YST and BST as per the procedure explained above (weekly until 35 DAT). Similarly, number of thrips infested leaves and virus infected plants were counted, marked and plant viruses were monitored. Simultaneously with these 2 rounds of experiments, ability of WF and thrips to reach to the experimental GH were studied by attaching two YST and BST each at the outer walls (centrally placed). Traps were changed weekly followed by counting of thrips and WF. The position and orientation of the traps on all 4 GH types were similar.

***Data Analyses***

Adult whiteflies, thrips and aphids on traps, alate aphids & whiteflies on leaves, number of thrips infested leaves, percentage of virus infected plants were subjected to HOVTEST = LEVENE option of SAS to account for homogeneity of variance and normality. In case of non-homogeneity, percent values were transformed using arcsine–square-root (arcsin√) transformation. Insects on traps and plants and number of infested leaves count values were transformed by square-root (√) transformation before running an ANOVA followed by mean separation using Fisher's LSD test (Steel and Torrie 1980, Gomez and Gomez 1984). Data were then back transformed for presentation as Mean±SE. A significance level of $\propto$ = 0.05 was used in for all analysis.

## 5.3 Results

***Light Transmission and Temperature.*** No significant differences in temperatures and humidity inside the four tunnels were found during all 4 experiments. However, the UV light intensity varies under each GH type either during sunny and cloudy days during each four experiments (see figure 5.2). The UV levels drop to almost half during cloudy days. During experiments 1 and 2, approximately 20% of the 5 weeks long experiments were cloudy whereas it was approximately 40% during experiments 3 and 4.

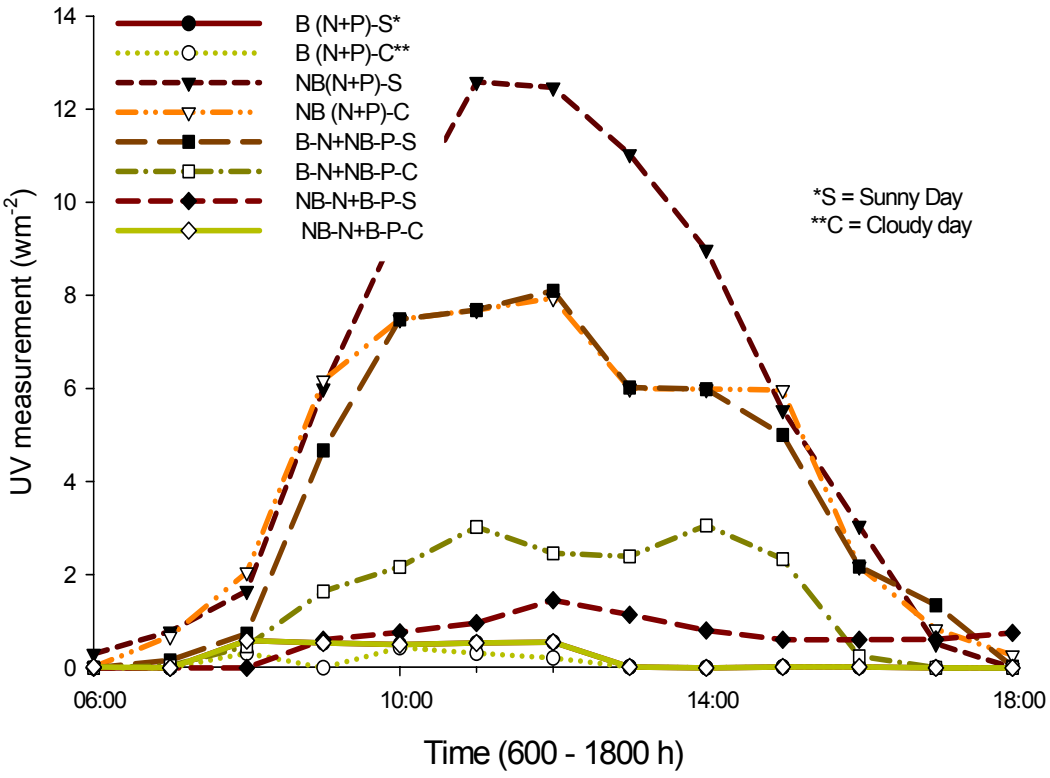



**Fig. 5.2. UV-A measurement ($wm^{-2}$) under each four greenhouses, UV-blocking net sidewalls with UV-blocking plastic film as roof [B (N+P)]; UV non-blocking nets as sidewalls and UV non blocking plastic films as roof [NB (N+P)]; UV-blocking nets as side walls and UV non blocking plastic films as roof [B-N+NB-P]; and, UV non blocking nets as side wall and UV-blocking plastics films as roof [NB-N+B-P] using Radiometer UV-Sensor (Dr. Grobel UV-Elektronik GmbH, Germany).**

***Experiment 1 and 2. Partial Ventilation***

***Whitefly.*** Significantly fewer whiteflies entered into the B (N+P) GH type compared to the other tested combinations during all sampling days (or periods). WF always preferred to enter the NB (N+P) GH type, irrespective of either initial low population (exp. 1) or at relatively higher population (exp. 2) (table 1). Comparing the other combinations, WF preferred to enter GHs with roofs made from the non-blocking plastics. In contrast, GHs with UV blocking plastic roofs had significantly lower number of WF on YST inside. Moreover, colonization was clearly related to the sidewall net properties (see table 5.1). Similarly, significantly fewer adult WF were recorded on leaves in the B (N+P) GH compared to the other tested GH types. Highest numbers of WF per leaf were recorded from the NB (N+P) type GH (see table 5.1). During the second round of experiments settling of WF followed the same trends (see table 5.2).

**Table 5.1. Weekly mean (±SE) number of *Bemisia tabaci* adults per leaf and on yellow sticky traps trapped inside GH during experiment 1.**

| Days | Treatments | | | |
|---|---|---|---|---|
| After Transplanting | B (N+P) | NB-N+ B-P | B-N+ NB-P | NB (N+P) |
| *WF per leaf* | | | | |
| 7 | 0.00±0.00a | 0.50±0.22b | 2.50±0.34c | 5.00±0.86d |
| 14 | 0.00±0.00a | 0.83±0.40ab | 2.00±0.52b | 10.33±1.65c |
| 21 | 0.17±0.17a | 1.50±0.22b | 5.67±0.71c | 15.50±2.28d |
| 28 | 0.50±0.34a | 2.00±0.45a | 7.67±1.86b | 22.17±3.12c |
| 35 | 1.50±0.43a | 2.83±0.54a | 10.00±2.14b | 22.67±2.54c |
| *WF per YST* | | | | |
| 7 | 0.00±0.00a | 0.42±0.15b | 1.83±0.37c | 8.92±1.04d |
| 14 | 0.17±0.11a | 1.00±0.28a | 6.75±0.45b | 24.83±4.31c |
| 21 | 0.75±0.41a | 2.58±0.56b | 10.58±0.69c | 25.17±1.97d |
| 28 | 0.92±0.26a | 1.42±0.38a | 11.58±0.68b | 32.58±3.59c |
| 35 | 0.08±0.08a | 1.92±0.47b | 7.50±1.14c | 28.58±3.84d |

ANOVA for each DAT was performed followed by mean separation using Fisher's LSD test. Means within DAT followed by the same letter (s) are not significantly different at $P = 0.05$.

**Table 5.2. Weekly mean (±SE) number of Bemisia *tabaci* adults per leaf and on yellow sticky traps trapped inside GH during experiment 2.**

| Days After Transplanting | Treatments | | | |
|---|---|---|---|---|
| | B (N+P) | NB-N+B-P | B-N+NB-P | NB (N+P) |
| *WF per leaf* | | | | |
| 7 | 0.00±0.00a | 0.67±0.21b | 2.83±0.40c | 6.50±1.52d |
| 14 | 0.00±0.00a | 0.83±0.40a | 4.33±0.21b | 19.33±3.63c |
| 21 | 0.17±0.17a | 2.33±0.33b | 7.50±1.06c | 30.67±7.79d |
| 28 | 0.83±0.48a | 3.50±0.76b | 9.67±1.31c | 29.00±4.43d |
| 35 | 1.50±0.34a | 2.33±0.42a | 11.17±2.14b | 34.67±6.29c |
| *WF per YST* | | | | |
| 7 | 0.00±0.00a | 0.42±0.19a | 1.58±0.56b | 10.75±1.04c |
| 14 | 0.17±0.11a | 2.58±0.66b | 10.67±1.36c | 33.33±1.97d |
| 21 | 0.33±0.22a | 1.17±0.39a | 6.75±0.86b | 47.25±4.26c |
| 28 | 0.42±0.26a | 1.92±0.61b | 9.75±1.58c | 71.92±5.09d |
| 35 | 0.08±0.08a | 5.25±0.87b | 20.00±1.56c | 93.17±5.68d |

ANOVA for each DAT was performed followed by mean separation using Fisher's LSD test. Means within DAT followed by the same letter (s) are not significantly different at $P = 0.05$.

***Aphids.*** Winged aphids followed the same entry trends as WF and significantly less aphids were trapped inside the B (N+P) GH compared to other tested treatments (see table 5.3 and 5.4). On 35 DAT both during exp. 1 and 2, highest counts were recorded on the YST. Moreover, for most sampling dates no significant differences were recorded inside B (N+P) and NB-N+B-P type GH. Significantly higher numbers of aphids per leaf were counted within the GH with more UV light intensity during both experimental periods (see table 5.3 and 5.4). It is obvious from the results that winged aphids preferred to immigrate into more UV receiving GH compared to the ones with less UV and that denser immatures and wingless adult populations developed on the leaves. Thus the GH made from the B (N+P) provided the best protection against the winged as well as the immature aphids.

**Table 5.3. Weekly mean (±SE) number of wingless adults and immatures aphids per leaf and winged aphid adults trapped on yellow sticky traps inside during experiment 1.**

| Days After Transplanting | Treatments | | | |
|---|---|---|---|---|
| | B (N+P) | NB-N+ B-P | B-N+NB-P | NB (N+P) |
| *Immatures and wingless adults per leaf* | | | | |
| 7 | 0.17±0.17a | 0.17±0.17a | 0.50±0.22a | 4.00±0.63b |
| 14 | 0.00±0.00a | 1.33±0.33b | 3.50±0.81c | 12.00±2.54d |
| 21 | 0.00±0.00a | 0.50±0.22a | 4.50±0.56b | 15.17±3.72c |
| 28 | 0.00±0.00a | 0.17±0.17a | 1.33±0.33b | 6.67±0.99c |
| 35 | 0.00±0.00a | 0.50±0.22a | 3.17±0.79b | 7.83±0.17c |
| *Winged adults per YST* | | | | |
| 7 | 0.00±0.00a | 0.00±0.00a | 0.75±0.18b | 6.08±1.87c |
| 14 | 0.50±0.19a | 1.00±0.35a | 3.47±0.42b | 10.83±1.09c |
| 21 | 0.17±0.11a | 1.42±0.42b | 3.92±0.81c | 12.83±1.64d |
| 28 | 0.42±0.19a | 0.67±0.22a | 4.75±0.79b | 14.50±2.18c |
| 35 | 0.25±0.13a | 0.75±0.18b | 3.92±0.71c | 15.92±0.90d |

ANOVA for each DAT was performed followed by mean separation using Fisher's LSD test. Means within DAT followed by the same letter (s) are not significantly different at $P = 0.05$.

**Table 5. 4. Weekly mean (±SE) number of wingless adults and immatures aphids per leaf and winged aphid adults trapped on yellow sticky traps inside GH during experiment 2.**

| Days After Transplanting | Treatments | | | |
|---|---|---|---|---|
| | B (N+P) | NB-N+ B-P | B-N+NB-P | NB(N+P) |
| *Immatures and wingless adults per leaf* | | | | |
| 7 | 0.00±0.00a | 0.00±0.00a | 1.50±0.50b | 4.17±0.70c |
| 14 | 0.17±0.17a | 1.00±0.37ab | 2.67±0.95b | 6.50±0.56c |
| 21 | 0.00±0.00a | 0.83±0.40b | 2.33±0.80c | 7.17±0.54d |
| 28 | 0.17±0.17a | 0.83±0.40a | 2.83±0.60b | 8.17±0.60c |
| 35 | 0.00±0.00a | 0.67±0.33b | 2.50±0.43c | 9.33±0.61d |
| *Winged adults per YST* | | | | |
| 7 | 0.00±0.00a | 0.00±0.00a | 1.08±0.73b | 5.08±1.37c |
| 14 | 0.25±0.13a | 0.67±0.22a | 4.17±0.95b | 11.58±2.76c |
| 21 | 0.00±0.00a | 1.75±0.98b | 5.08±1.47c | 15.00±2.92d |
| 28 | 0.67±0.22a | 1.58±0.73a | 6.42±2.26b | 21.33±3.92c |
| 35 | 0.33±0.22a | 2.33±0.92a | 9.67±1.71b | 25.92±4.29c |

ANOVA for each DAT was performed followed by mean separation using Fisher's LSD test. Means within DAT followed by the same letter (s) are not significantly different at $P = 0.05$.

***Thrips and leaf damage.*** Thrips was the most recorded pest and immigration followed similar trends that of WF and aphids. NB (N+P) GH attracted significantly the highest number of thrips compared to all other GH types (see table 5.5). During second round of experiments, more thrips per BST and more thrips damaged leaves were recorded. At 35 DAT, 162 and 176 thrips per BST were recorded under the NB (N+P) material during experiment 1 and 2 respectively against 0 and 3.75 thrips during same period inside B (N+P) GH types. For over 3 weeks significant differences in numbers of thrips were recorded inside B (N+P) and NB-N+B-P type GH during experiment 1 and 2. The higher number of immigrating thrips inside the NB (N+P) caused significantly higher cumulative number of thrips infested leaves (leaf damage) at 35 DAT compared to the other greenhouses (see table 5.5 and 5.6).

**Table 5.5. Weekly mean (±SE) number of adult thrips per BST trapped inside GH and cumulative leaf damage during experiment1.**

| Days | Treatments | | | |
|---|---|---|---|---|
| After Transplanting | B (N+P) | NB- N+ B-P | B-N+NB-P | NB (N+P) |
| *Adult per BST* | | | | |
| 7 | 0.00±0.00a | 0.00±0.00a | 0.25±0.13a | 9.75±0.75b |
| 14 | 0.25±0.13a | 0.67±0.22a | 6.75±1.08b | 17.42±1.99c |
| 21 | 0.17±0.11a | 1.00±0.35b | 11.50±0.89c | 33.42±1.59d |
| 28 | 0.42±0.19a | 1.58±0.31b | 20.00±0.83c | 72.83±4.52d |
| 35 | 0.00±0.00a | 1.75±0.48b | 24.08±0.54c | 162.67±2.25d |
| *Cumulative no thrips infested leaves/plant* | | | | |
| 7 | 0.00±0.00a | 0.67±0.21b | 1.00±0.45b | 2.00±0.26c |
| 14 | 0.33±0.21a | 1.17±0.40ab | 2.17±0.70b | 5.17±0.31c |
| 21 | 0.83±0.31a | 1.50±0.50a | 3.50±0.72b | 9.50±0.34c |
| 28 | 1.33±0.61a | 2.17±0.40ab | 4.83±1.17b | 12.67±0.33c |
| 35 | 1.67±0.71a | 2.83±0.60a | 7.00±1.34b | 13.33±0.49c |

ANOVA for each DAT was performed followed by mean separation using Fisher's LSD test. Means within DAT followed by the same letter (s) are not significantly different at $P = 0.05$.

**Table 5.6. Weekly mean (±SE) number of adult thrips per BST trapped inside GH and cumulative leaf damage during experiment 2.**

| Days After Transplanting | Treatments | | | |
|---|---|---|---|---|
| | B (N+P) | NB-N+B-P | B-N+NB-P | NB (N+P) |
| *Adult per BST* | | | | |
| 7 | 0.25±0.13a | 0.58±0.23a | 3.75±0.64b | 18.00±1.35c |
| 14 | 0.17±0.11a | 0.75±0.33a | 7.67±0.54b | 20.08±1.79c |
| 21 | 0.33±0.14a | 1.92±0.42b | 17.25±0.99c | 53.33±1.45d |
| 28 | 0.92±0.26a | 5.08±0.77b | 23.08±1.53c | 114.33±4.65d |
| 35 | 3.75±0.37a | 10.92±1.60b | 33.50±1.51c | 176.75±6.05d |
| *Cumulative no thrips infested leaves/plant* | | | | |
| 7 | 0.00±0.00a | 0.50±0.22ab | 1.17±0.54b | 3.33±0.76c |
| 14 | 0.33±0.21a | 1.17±0.48ab | 3.00±0.89b | 8.33±0.67c |
| 21 | 0.83±0.40a | 1.33±0.56a | 4.17±0.79b | 10.17±0.60c |
| 28 | 1.67±0.61a | 2.50±0.62b | 5.00±1.00c | 13.83±0.48d |
| 35 | 1.83±0.54a | 3.00±0.73b | 5.67±1.23c | 14.50±0.34d |

ANOVA for each DAT was performed followed by mean separation using Fisher's LSD test. Means within DAT followed by the same letter (s) are not significantly different at $P = 0.05$.

***Virus spread.*** Cumulative percent virus incidence at 35 DAT was significantly lower with 5.0% recorded inside B (N+P) GH compared to 45 % under NB (N+P) GH types ($F = 29.80$; df= 3, 7; $P = 0.0034$) (see fig. 5.3 A). Tospovirus constituted the major proportion and reached 88% and 66% respectively in B (N+P) and NB (N+P) greenhouse types (see fig 5.4 A) . Inside the NB (N+P) GH, first virus infected plants were recorded earlier and virus spread at faster rates, compared to the B (N+P) GH. During the second round of experiments, more plants showed virus symptoms but similar to the first experiment virus spread was significantly higher under NB (N+P) GH ($F = 243.73$; df= 3, 7; $P = 0.0001$) (see fig.5.3 B) compared to B (N+P) type GH. However no significant differences were found in B (N+P) and NB-N+B-P types GH. Out of these a total of 83.33 % plants were tested positive for the tospovirus (see fig 5.4 B). Percent cumulative infestation with tospovirus was significantly higher under the NB (N+P) type GH ($F = 24.30$; df= 3, 7; $P = 0.005$). Similar to the experiment 1, virus incidence started earlier at 14 DAT under the NB (N+P) GH types compared to 28 DAT under B (N+P) GH types. During both experiment 1 and 2 under the UV blocking plastic GH roof, most of the virus affected plants were found near to the doors, whereas in GH with UV non-blocking roof, infected plant were dispersed all over the GH. The results clearly indicate that the B (N+P) GH type provided the best protection against the virus infection.

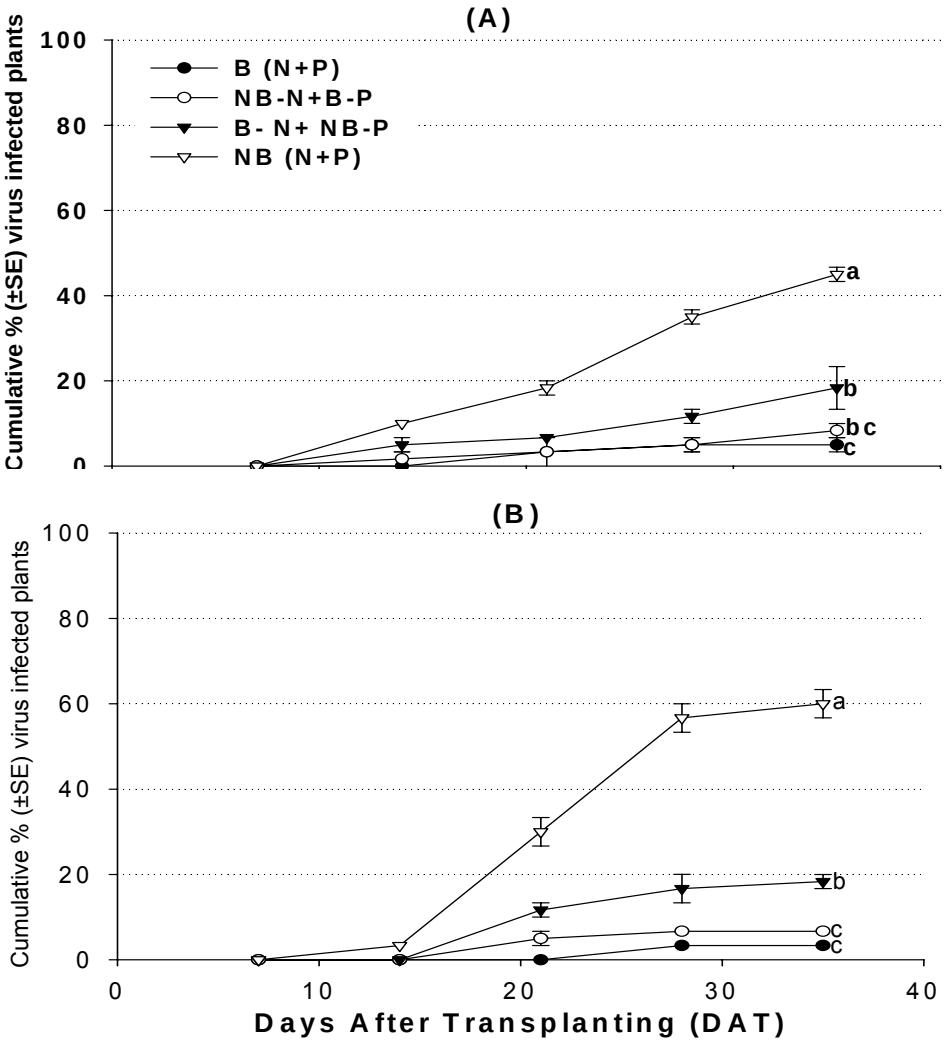



**Fig. 5.3. Percent cumulative virus infected tomato plants under greenhouses, UV-blocking net sidewalls with UV-blocking plastic film as roof [B (N+P)]; UV non-blocking nets as sidewalls and UV non blocking plastic films as roof [NB (N+P)]; UV-blocking nets as side walls and UV non blocking plastic films as roof [B-N+NB-P]; and, UV non blocking nets as side wall and UV-blocking plastics films as roof [NB-N+B-P], (A) during experiment 1 and (B) experiment 2, when greenhouse door was open for 6.00-10.00h. Cumulative percent at 35 days after transplanting sharing a common letter are not significantly different at *P* <0.05, Fisher's LSD.**

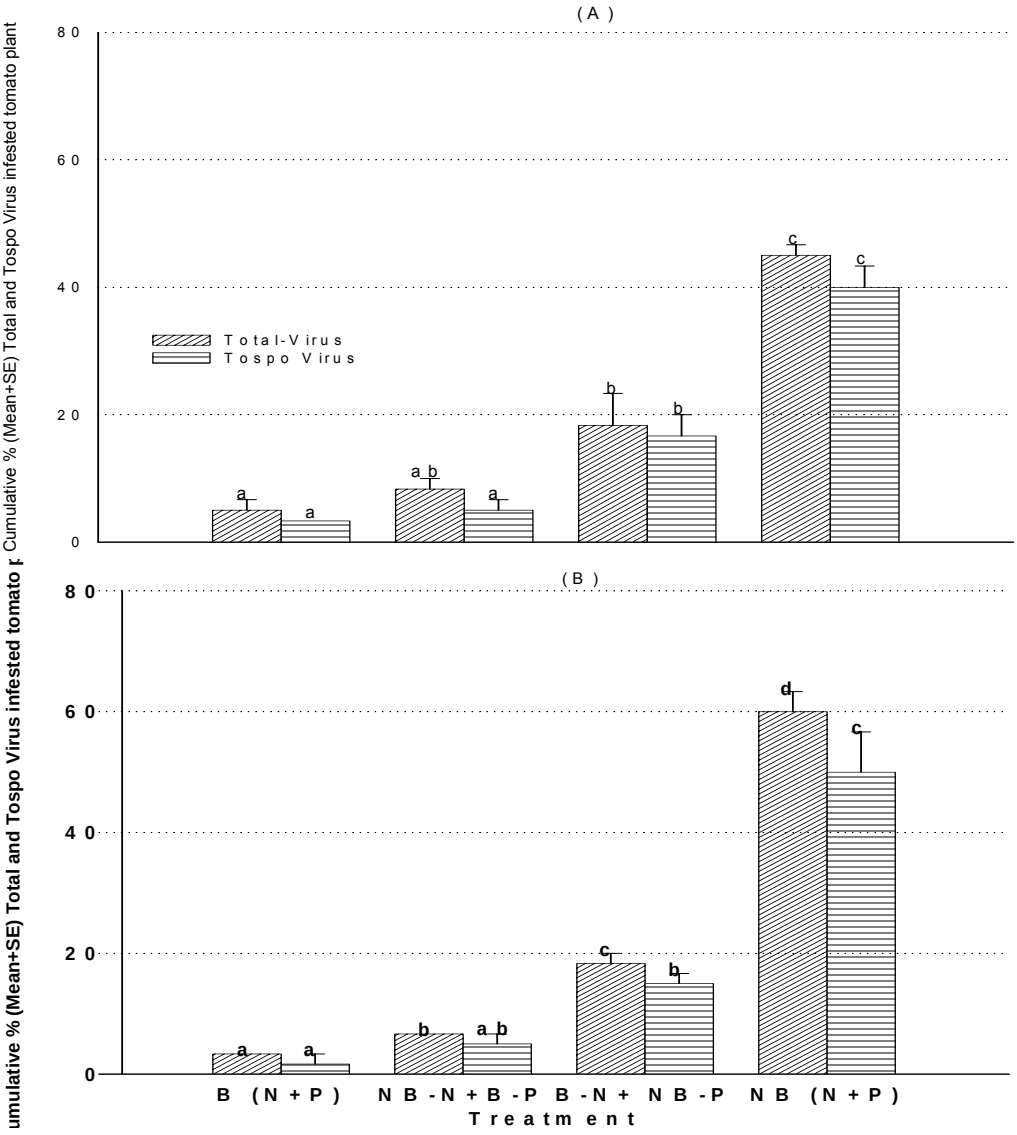



**Fig. 5.4. Proportion of tospovirus in comparison of total virus infected tomato plants under different greenhouses, UV-blocking net sidewalls with UV-blocking plastic film as roof [ B (N+P)]; UV non-blocking nets as sidewalls and UV non blocking plastic films as roof [NB (N+P)]; UV-blocking nets as side walls and UV non blocking plastic films as roof [B-N+NB-P]; and, UV non blocking nets as side wall and UV-blocking plastics films as roof [NB-N+B-P] during experiment 1 (A) and experiment 2 (B), when greenhouse doors open for 600-1000 h (partial ventilation). Bars sharing a common letter are not significantly different at *P* < 0.05, Fisher's LSD.**

***Experiment 3 and 4. Complete Ventilation***

***Whitefly.*** In total, a higher WF population was observed when gates were kept open to achieve complete ventilation. Similar to the entry trends under partial ventilation, significantly fewer number of WF entered inside the B (N+P) GH compared to other tested combinations during all sampling periods. Similar to the lower number trapped on YST, significantly fewer WF were found on leaves under B (N+P) GH over the sampling period (see table 5.7). These results yet again indicated the preference of WF to immigrate into to UV rich environment irrespective of the ventilation status under NB (N+P) type GH. During the second round of experiments entry and settling of WF followed the same trends (see table 8). The load of WF measured at outside walls of the NB (N+P) were significantly higher in either rounds of the experiments 3 and 4 (see table 5.7 and 5.8 respectively) compared to B (N+P) GH types.

**Table 5. 7. Weekly mean (±SE) number of *Bemisia tabaci* adult per leaf, on yellow sticky traps trapped inside GH and trapped on the yellow sticky traps on the outer walls of the GH during experiment 3.**

| Days After Transplanting | Treatments | | | |
|---|---|---|---|---|
| | B (N+P) | NB-N+ B-P | B-N+NB-P | NB (N+P) |
| *WF per leaf* | | | | |
| 7 | 0.17±0.17a | 0.50±0.22a | 4.33±1.65b | 15.17±2.21c |
| 14 | 2.17±0.48a | 3.00±0.37a | 9.50±1.63b | 37.50±3.80c |
| 21 | 2.33±0.21a | 3.50±0.62a | 18.67±1.78b | 53.67±9.04c |
| 28 | 2.67±0.56a | 4.00±0.37a | 20.17±1.72b | 60.00±9.15c |
| 35 | 3.83±0.54a | 5.17±1.01a | 15.17±1.76b | 36.00±2.18c |
| *WF per YST Inside* | | | | |
| 7 | 1.00±0.33a | 2.25±0.39ab | 4.92±0.34b | 15.00±4.49c |
| 14 | 0.83±0.24a | 3.58±0.98b | 19.25±2.75c | 43.17±7.64d |
| 21 | 1.42±0.29a | 2.58±0.31a | 19.58±2.27b | 109.83±6.64c |
| 28 | 1.58±0.38a | 2.50±0.80a | 23.33±2.42b | 131.25±17.32c |
| 35 | 1.25±0.28a | 2.67±0.61a | 25.67±1.32b | 133.92±11.42c |
| *WF per YST trapped on outer wall of GH* | | | | |
| 7 | 1.00±0.42a | 1.63±0.60a | 4.00±0.68b | 22.63±2.90c |
| 14 | 1.10±0.38a | 2.13±0.40b | 13.75±1.70c | 34.00±2.15d |
| 21 | 1.88±0.35a | 2.63±0.65a | 21.25±1.15b | 46.88±2.22c |
| 28 | 3.23±0.53a | 3.88±0.79a | 21.88±1.61b | 52.50±4.23c |
| 35 | 3.00±0.57a | 4.13±0.58a | 23.63±1.38b | 56.25±3.67c |

ANOVA for each DAT was performed followed by mean separation using Fisher's LSD test. Means within DAT followed by the same letter (s) are not significantly different at $P = 0.05$.

**Table 5.8. Weekly mean (±SE) number of *Bemisia tabaci* adult per leaf, on yellow sticky traps trapped inside GH and trapped on the yellow sticky traps on the outer walls of the GH during experiment 4.**

| Days after Transplanting | Treatments | | | |
|---|---|---|---|---|
| | B (N+P) | NB-N+B-P | B- N+ NB-P | NB (N+P) |
| *WF per leaf* | | | | |
| 7 | 0.17±0.17a | 0.67±0.33a | 5.00±1.39b | 17.17±2.69c |
| 14 | 2.17±0.60a | 3.33±0.67a | 10.33±0.92b | 38.50±5.85c |
| 21 | 1.67±0.61a | 3.50±0.76ab | 5.83±0.60b | 24.17±4.61c |
| 28 | 2.33±0.33a | 2.17±0.60a | 6.67±1.12b | 22.17±3.67c |
| 35 | 2.17±0.31a | 3.67±0.56a | 6.33±0.84b | 18.50±2.78c |
| *WF per YST tapped inside GH* | | | | |
| 7 | 2.75±0.48a | 4.49±0.50a | 10.17±0.27b | 21.33±3.02c |
| 14 | 5.33±0.99a | 7.67±0.45a | 17.42±0.56b | 50.00±6.28c |
| 21 | 5.93±0.84a | 7.33±0.83a | 20.50±1.02b | 52.58±4.09c |
| 28 | 4.58±1.33a | 11.75±0.62b | 36.50±1.80c | 98.75±11.99d |
| 35 | 6.83±0.81a | 10.75±0.68b | 31.50±1.34c | 90.92±7.69d |
| *WF per YST trapped on outer walls of GH* | | | | |
| 7 | 2.00±0.46a | 4.13±0.64a | 6.00±1.02b | 17.50±2.27c |
| 14 | 3.88±0.81a | 5.63±0.53a | 13.25±0.67b | 35.00±3.26c |
| 21 | 3.63±0.91a | 5.38±0.60a | 12.63±2.02b | 30.88±1.54c |
| 28 | 3.38±0.56a | 5.88±0.52b | 11.13±0.97c | 43.63±2.56d |
| 35 | 3.13±0.58a | 6.50±0.19b | 14.50±0.80c | 35.38±1.25d |

ANOVA for each DAT was performed followed by mean separation using Fisher's LSD test. Means within DAT followed by the same letter (s) are not significantly different at $P = 0.05$.

***Thrips and leaf damage.*** Again thrips was recorded as the most abundant pest and similar to the previously observed trends, significantly higher number of thrips entered and were trapped inside the NB (N+P) GH compared to other GH combinations tested in both rounds of experiments (see table 9 and 10). Moreover significantly higher cumulative leaf damage was observed under NB (N+P) type GH (table 9 and 10). Thrips followed the same trends of entry and attraction towards UV-rich environment and a higher number of thrips focused on sidewalls of NB (N+P) type compared to B (N+P) type GH in either of the two rounds of experiment (table 5.9 and 5.10).

**Table 5.9. Weekly mean (±SE) number of thrips on blue sticky traps inside GH and trapped on the outer walls of the GH and cumulative leaf damage during experiment 3.**

| Days After Transplanting | Treatments | | | |
|---|---|---|---|---|
| | B (N+P) | NB-N+B-P | B- N+ NB-P | NB (N+P) |
| *Thrips per BST trapped inside GH* | | | | |
| 7 | 3.33±0.66a | 4.25±0.93a | 16.83±1.70b | 60.50±11.13c |
| 14 | 4.17±1.17a | 5.92±1.55a | 101.83±20.36b | 270.42±37.35c |
| 21 | 11.42±2.52a | 17.08±2.53a | 86.67±7.86b | 327.92±35.40c |
| 28 | 17.33±3.32a | 27.33±3.76a | 102.00±22.99b | 442.17±25.95c |
| 35 | 11.75±2.56a | 24.75±3.93a | 130.17±19.77b | 578.83±32.88c |
| *Cumulative no thrips infested leaves/plant* | | | | |
| 7 | 0.33±0.21a | 0.67±0.21a | 1.50±0.22b | 2.67±0.21b |
| 14 | 1.33±0.33a | 2.00±0.00b | 4.17±0.31c | 8.67±0.42d |
| 21 | 1.67±0.42a | 2.67±0.21b | 5.83±0.40c | 11.17±0.48d |
| 28 | 1.83±0.48a | 3.67±0.21b | 8.17±0.40c | 14.00±0.63d |
| 35 | 2.33±0.33a | 5.00±0.37b | 11.33±0.33c | 21.00±0.68d |
| *Thrips per BST trapped on outer walls of GH* | | | | |
| 7 | 2.13±0.55a | 2.50±0.33a | 6.75±0.53b | 19.88±1.41c |
| 14 | 2.25±0.37a | 3.63±0.38a | 19.63±1.92b | 57.63±3.19c |
| 21 | 3.13±0.30a | 4.00±0.38a | 33.00±1.34b | 120.88±7.84c |
| 28 | 4.69±0.45a | 5.88±0.35a | 39.25±3.19b | 135.38±9.14c |
| 35 | 4.75±0.70a | 6.50±0.60a | 34.25±1.39b | 145.88±4.40c |

ANOVA for each DAT was performed followed by mean separation using Fisher's LSD test. Means within DAT followed by the same letter (s) are not significantly different at $P = 0.05$.

**Table 5.10. Weekly mean (±SE) number of thrips on blue sticky traps inside GH and trapped on the outer walls of the GH and cumulative leaf damage during experiment 4.**

| Days After Transplanting | Treatments | | | |
|---|---|---|---|---|
| | B (N+P) | NB-N+ B-P | B-N+NB-P | NB(N+P) |
| *Thrips per BST trapped inside GH* | | | | |
| 7 | 5.83±0.86a | 11.25±1.32b | 22.75±2.05c | 61.42±7.58d |
| 14 | 5.25±1.41a | 15.50±1.28b | 44.67±2.70c | 145.25±12.12d |
| 21 | 11.33±2.29a | 25.42±2.72b | 60.17±3.36c | 190.92±21.30d |
| 28 | 12.92±1.89a | 24.42±2.67b | 76.50±5.75c | 296.67±21.09d |
| 35 | 14.92±2.45a | 23.58±3.75a | 69.75±6.97b | 376.33±23.77c |
| *Cumulative no thrips infested leaves/plant* | | | | |
| 7 | 0.50±0.22a | 0.83±0.31a | 1.67±0.21b | 2.33±0.33b |
| 14 | 1.17±0.31a | 2.33±0.21b | 3.83±0.48ab | 5.00±0.58c |
| 21 | 1.33±0.33a | 3.17±0.31b | 5.67±0.61c | 8.00±0.68d |
| 28 | 2.00±0.52a | 3.83±0.48b | 8.50±0.67c | 12.33±0.67d |
| 35 | 2.83±0.48a | 4.33±0.49b | 10.67±0.92c | 18.33±0.88d |
| *Thrips per BST trapped on outer walls of GH* | | | | |
| 7 | 2.38±0.60a | 5.25±1.44ab | 11.63±2.06b | 34.75±11.92c |
| 14 | 4.50±0.91a | 14.00±1.64b | 28.63±2.21c | 47.13±4.84d |
| 21 | 5.00±0.60a | 9.63±1.40a | 29.75±0.96b | 68.38±8.96c |
| 28 | 6.00±1.86a | 9.13±1.16a | 21.50±2.04b | 71.25±6.82c |
| 35 | 2.75±0.73a | 9.13±1.30b | 17.13±1.42c | 73.00±2.43d |

ANOVA for each DAT was performed followed by mean separation using Fisher's LSD test. Means within DAT followed by the same letter (s) are not significantly different at $P = 0.05$.

**Virus spread.** Cumulative percent virus incidence at 35 DAT during exp. 3 was 8 % inside B (N+P) GH compared to 100% under NB (N+P) GH type ($F = 1588.25$; df= 3, 7; $P = 0.0001$) (see fig 5.5 A). Tospovirus constituted the major proportion and reached over 75% infection level under B (N+P) GH type ($F = 96.38$; df= 3, 7; $P = 0.0003$) (see fig 5.6 A ). Similar to the trends reported with the partial ventilation experiments, inside the NB (N+P) GH types, virus symptoms appeared early and spread at a faster rate compared to B (N+P) GH types. During second round of experiments, overall slightly less cumulative virus incidence was recorded at 96% under NB (N+P) GH type with similar trends as reported for the previous rounds ($F = 196.94$; df= 3, 7; $P = 0.0001$) (see fig. 5.5 B). Toppoviruses appeared in similar manner as of the experiment 3 (see fig 5.6 B) . Similarly the virus symptoms appeared earlier and then spread at faster rates under NB (N+P) GH type over B (N+P) GH types.

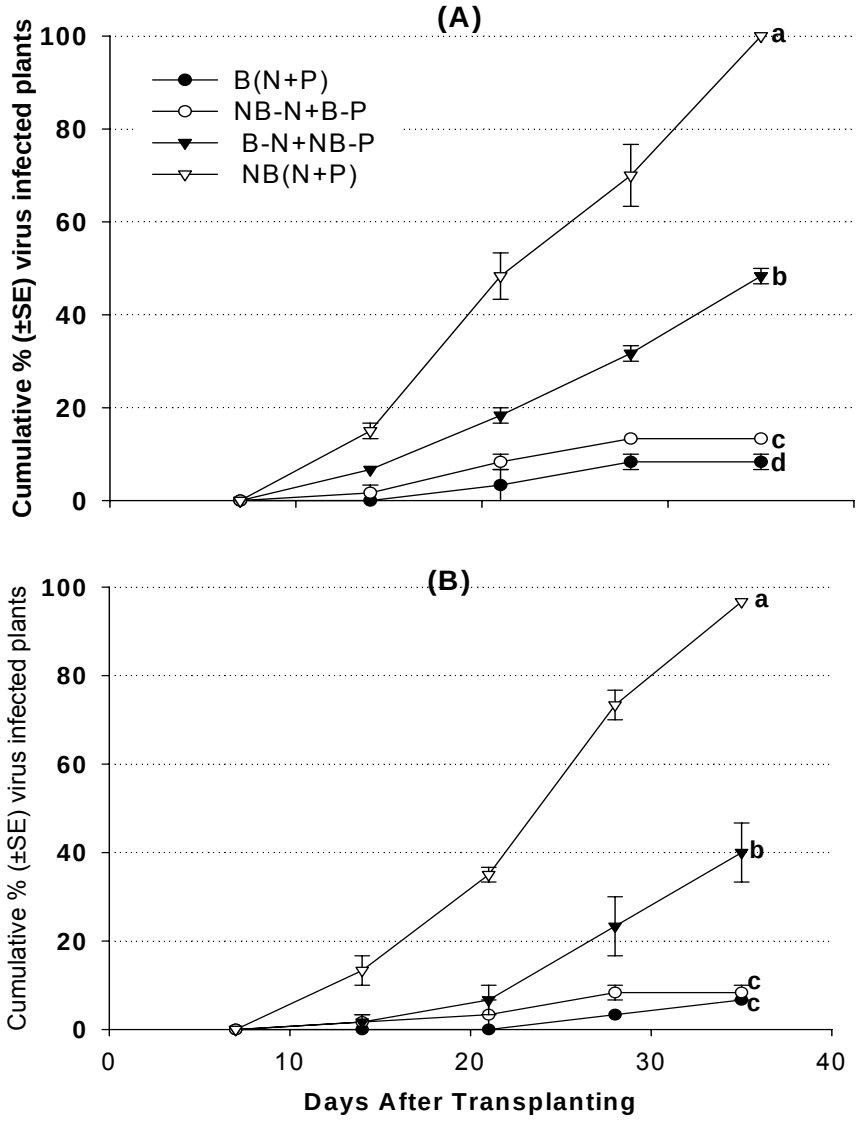



**Fig. 5.5. Percent cumulative virus infected tomato plants under greenhouses (treatments), UV-blocking net sidewalls with UV-blocking plastic film as roof [B (N+P)]; UV non-blocking nets as sidewalls and UV non blocking plastic films as roof [NB (N+P)]; UV-blocking nets as side walls and UV non blocking plastic films as roof [B-N+NB-P]; and, UV non blocking nets as side wall and UV-blocking plastics films as roof [NB-N+B-P], (A) during exp. 3 and (B), exp. 4, when greenhouse doors kept open (complete ventilation). Cumulative percent at 35 days after transplanting sharing a common letter are not significantly different at *P* <0.05, Fisher's LSD.**

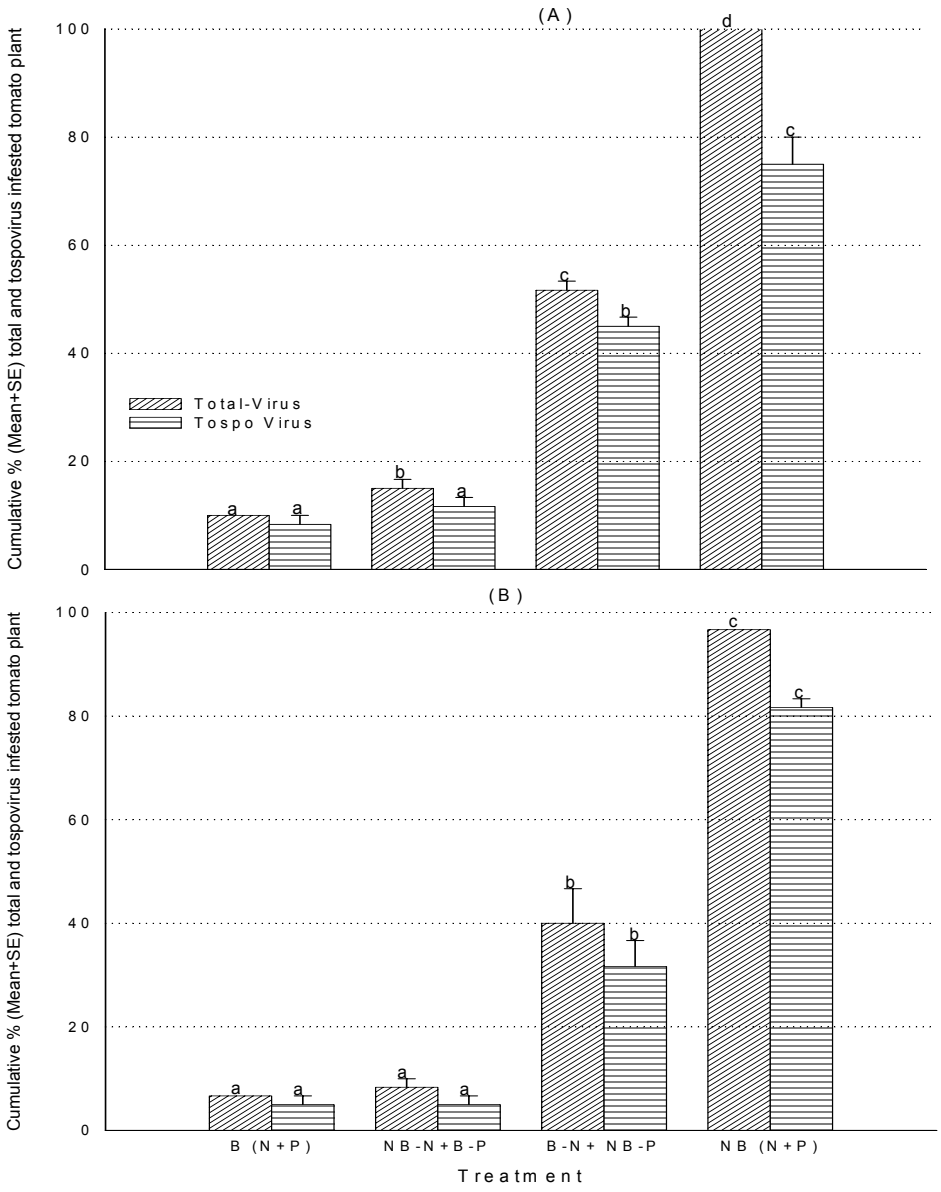



**Fig. 5.6. Proportion of tospovirus in comparison of total virus infected tomato plants under different greenhouses (treatments), UV-blocking net sidewalls with UV-blocking plastic film as roof [ B (N+P)]; UV non-blocking nets as sidewalls and UV non blocking plastic films as roof [NB (N+P)]; UV-blocking nets as side walls and UV non blocking plastic films as roof [B-N+NB-P]; and, UV non blocking nets as side wall and UV-blocking plastics films as roof [NB-N+B-P] during experiment 3 (A) and exp. 4 (B), when greenhouse doors kept open (complete ventilation). Bars sharing a common letter are not significantly different at *P* <0.05, Fisher's LSD.**

## 5.4. Discussion

These studies are probably the first of its kind from protected cultivation in SE Asia, investigating the entry of three plants sucking insect pest, WF, thrips and aphids and related virus spread in tropical greenhouses covered with UV–blocking material compared to those with non-blocking properties.

***Whitefly Immigration.*** The UV deficient environment in all three experiments reduced entry and attraction of WF towards or inside the greenhouses. Strongest differences were observed between greenhouses completely covered by UV blocking material (B (N+P) type GH) compared to those made from UV transmitting plastics and nets (NB (N+P) type GH). This entry trend was true irrespective of the length of the time GH gates were opened for ventilation but fewer WF immigrated and were trapped (YST) under the B (N+P) GH type, when gates were opened for 4-5 hrs per day only in the morning compared to experiments with parallel gates kept open long time for full ventilation. When the attraction of WF towards the structures was monitored by outside on the walls positioned traps much lower numbers were trapped around the UV blocking houses compared to the non-blocking ones. The results clearly indicate very sensitive reaction of WF adults to the presence of the total amount of UV inside a GH irrespective of the individual blocking properties of either nets or plastic used in the experiment.

The reduced immigration and attraction of WF inside UV deficient GH or towards sidewalls of UV-blocking material are in agreement with previously reported studies of Antignus et al. (1996, 1998, 2001) and Costa and Robb (1999). Similarly in recent studies Gonzalez (2004) working with *B. tabaci* and Mutwiwa et al. (2005) working with *T. vaporariorum* reported significantly lower numbers of WF trapped under UV low GH over GH with high UV. Most of these investigations showed a highly significant reduction in WF flight intensity and immigration into UV-poor tunnels/net house/greenhouse. Most of these studies used UV-blocking plastics, whereas Antignus et al. (1998, 2001) covered tunnels completely with UV-blocking nets and achieved a long-term protection of plants inside from *B. argentifolii.* Moreover, when we measured the incoming radiation inside these structures (see fig. 2), we found that plastic roofs of our small greenhouses blocked more efficiently the UV- radiation than nets at the sidewalls. Wherever we used the UV-blocking plastic roofs, internal UV-

radiation was lowest. The immigrating WF showed an UV-intensity dependent behavior. For instance, during experiment 1, on a typical sunny day at 12.00 h, inside GH types NB (N+P) we recorded UV intensity of 12.47 $wm^{-2}$ followed by 8.10 $wm^{-2}$ in the B-N+NB-P, 1.45 $wm^{-2}$ under NB-N+B-P and 0.55 $wm^{-2}$ under B (N+P) type GH (see figure 5.2). These levels of UV radiation decreased to half in respective GH types during cloudy days but the differences in attraction of WF persisted further on between the GH types. This indicates that not the absolute UV amount available triggers WF selection behavior but the relative difference between two light environments. Similar findings on reduced movement, dispersal and colonization under UV deficient conditions of another WF species in greenhouses, *T. vaporariorum* are recently reported by Doukas (2002) and Mutwiwa et al. (2005).

Similar to the trends of trapping with YST, significantly higher number of WF per leaf was recorded under the NB (N+P) GH either with short opening (4-5 hrs) or when gates kept open permanently. This indicates that YST trapping is giving a clear picture of WF settling and population development on the plants. Reduced population built up of WF under UV deficient environment is in line with previously published reports (e.g. Antignus et al. 1996, 1998, Summers et al. 2004). Our results seem to be only in disagreement with those of Costa et al. (2002), who found insignificant differences in WF numbers on plants in greenhouses made of UV-absorbing compared to UV-transmitting plastics. These contradictions could be due to the fact that in our experiment, only the gates were opened but not the sidewalls. However, we also found more WF, thrips and aphids on the tomato plants near the gates under B (N+P) GH compared to the centre of the GH. Even the virus infected plants in this type of GH are always recorded near the opening gates. Similar observations were made by Mutwiwa et al. (2005).

Clearly, the UV reduced GH environment achieved through the combination of the UV-blocking plastics and nets were able to dramatically reduce the number of WF movement to the wall of greenhouses, entering inside and numbers settling on plants. The exact mechanism of this effect is still unknown, but it is presumed that reduced immigration and dispersal levels result from interference with visual cues which trigger the selection of environment for flight activity and orientation to and selection of plants for settlement (Antignus 1996, Antignus et

al. 1998, 2000, Mutwiwa et al. 2005). That WF might be able to react to UV is shown by Mellor et al. (1997), who described UV sensitive photoreceptors for the greenhouse WF, *T. vaporariorum.* No such detailed information is available for *B. tabaci*.

***Aphid Immigration.*** Winged aphids followed similar trends considering the different GH types as previously discussed for WF independent whether they were trapped with YST or accounted on the plants These results are in line with earlier published reports by Antignus et al. (1996, 1998) or Chyzik et al. (2003) who reported trapping 50 times more alate aphids under normal condition over UV-blocked conditions. Recent studies (see Kirchner et al. 2005) show that aphids have photoreceptors in their compound eyes sensitive to light in the UVA range of the light spectrum; however detailed studies about the importance of light reception in the UV range for aphid behavior are still missing. The increased number of aphid nymphs inside the NB (N+P) GH could well be due to its increased propagation time over B (N+P) GH types. Propagation time of aphid (*Myzus persicae*) was reported to 1.5 – 2 times longer under regular film compared to UV-absorbing films and UV exposed aphids give more birth to new progeny (Chyzik et al. 2003).

***Thrips immigration and leaf damage.*** The thrips, *Ceratothripoides claratris* gave a very sensitive response to the changes in UV-environment and irrespective of ventilation period (partial or complete), preferred to enter inside UV-rich environment in a concentration-dependent manner. Thrips followed the same trend as WF and aphids in their attraction towards the various greenhouses. Higher numbers of thrips immigrating into NB (N+P) type GH resulted in higher number of damaged leaves per plant. Since no previous investigations with *C. claratris* are reported, results were compared with other thrips species. Our results are consistent with findings on WFT*, F. occidentalis* (Pergrande) from Israel, where significant reduction of the thrips were found under UV-absorbing plastic tunnels (Antignus et al. 1996). Similarly, in a choice study Costa et al. (1999) captured 90-98% of released *F. occidentalis* (Pergrande) under tunnels rich in UV over tunnels covered with UV-absorbing plastics. On the other hand Antignus et al. (1998), could not significantly reduce the immigration of *F. occidentalis* with tunnels made of 50-mesh UV –blocking Bionets®. The discrepancy to our results could be explained by the different set-

ups since we used a combination of UV-blocking plastics and nets with much higher UV-blocking capacity compared to Bionet only. Similar to aphids the ability of thrips to receive light in the UV range spectrum is well documented (Matteson et al. 1992) even a differentiation between UV-A and UB-B. Mazza et al. (1996, 2002) showed that the thrips *Caliothrips phaseoli* avoids UV-B but is attracted by UV-A and Vernon and Gillespie (1990) reported that high UV reflectance environment repels thrips. The selective sensitivity of thrips to different UV ranges becomes obvious when we compare our results with reports on the use of UV-reflective mulches against thrips. Some reports are available for tomato and capsicum crops, where use of UV-reflective mulch caused significant reduction in WFT, *F. occidentalis* (Pergrande) population (Scott et al. 1989, Greenough et al. 1990, Brown and Brown 1992, Kring and Schuster 1992, Vos et al. 1995, Costa et al. 2002, Stavisky et al. 2002, Gonzalez 2004). Similarly, other species of thrips were repelled using plastic reflective mulches in outdoor ornamentals and vegetable crops (Csizinski et al. 1995, Terry 1997). It could be speculated that the specific reflection pattern of UV is important in determining whether thrips is attracted to a host or repelled and that relative high amounts of reflected UV-B can overrule the attractive properties of UV-A. This interesting relation should be studied more in detail.

***Plant Virus.*** Thrips, *C. claratris* is recently reported to be a serious pest of protected cultivation of tomato in the greater Bangkok area and vector of tospovirus, CaCV (isolate AIT) (Premachandra et al. 2005). Number of plants showing virus symptoms, which was later confirmed through ELISA test, followed the trends of the immigrating thrips and WF, which was recorded least under the B (N+P) type GH over NB (N+P) type GH. B (N+P) GH reduced and delayed the virus infection in all experiments. Majority of recorded virus was the tospovirus as evident through the thrips as most occurring species. However, no further attempts were made to isolate other viruses but it could be speculated that Tomato Yellow Leaf Curl Virus (TYLCV) virus was one more virus, since symptoms were fitting. Furthermore it is transmitted by WF, and it is very frequently observed in field crops in the study area. In Israel, the spread of TYLCV were significantly reduced using UV-absorbing nets (Antignus et al., 1996, 1998, Gonzalez 2004) and the incidence of *cucurbit yellow stunting disorder virus* in melons were reported to be 70% less under UV-absorbing films

and the same film appeared to be effective against aphid-borne *Zucchini yellow mosaic virus* (Antignus 2000). Same way as discussed above is should be mentioned that UV-reflective mulches can significantly reduce the incidence of thrips vectored viruses as shown with *Tomato Spotted Wilt Virus*, which was vectored by *Frankliniella* spp (Stavisky et al. 2002). Moreover the use of aluminum or silver plastics mulches delayed the infection and spread of TYLCV in Jordan (Suwwan et al. 1988) and effectively protected tomato against *tomato mottle virus* in Florida (Csizinski et al. 1995).

***Conclusions***

In conclusion, our result show that the greenhouses made from a combination of the UV-blocking nets as side walls and roof from UV- blocking plastics are able to significantly limit immigration of WF, aphids and thrips into such structure and consequently tomato plants grown under such GH had fewer pest populations resulting into fewer leaf damage as well as reduced virus infections. Being in the tropics, the major amount of light filters though the roof, hence UV-blocking plastic on roof can efficiently reduce the incoming UV. Nets on sidewalls however are a prerequisite for low cost non-cooled greenhouses to achieve sufficient ventilation. UV-blocking nets although not so efficient as films in the blocking abilities can ideally supplement the UV blocking film roof material. Reducing immigration of the pests in greenhouse leads to a lower initial pest population density, which is a key factor for successful and effective control in general (Xu et al. 1984). Other potential benefits from the reduced UV-environment achieved through the use of UV-blocking net and plastics may include improved performance of entomopathogenic fungi (Costa et al. 2001), and baculoviruses (Goulson et al. 2003), improved management of some fungal pathogens (Reuveni and Raviv 1992, Elad 1997), reduced UV related degradation of botanicals like neem (Barnaby et al. 1989, Stokes and Redfern 1982, Johnson et al. 2003, Barrek et al. 2004), and overall improvements in the microclimate, but that has to be confirmed in further studies.

# 6 Final Discussion

Main details of our studies are discussed in the chapters above; here we will give a final short and comprehensive review and valuation of the achieved results and their broader importance for integrated pest management (IPM) of WF under protected cultivation in the humid tropics.

Tomato production in Thailand is seriously constrained by WF (*Bemisia tabaci*) and other insect-pests like thrips, leafminers, fruit worm (*Helicoverpa sp*.), etc. and among them *Bemisia* vectored TYLCV is major production constraint causing up to 100% losses (Attathom et al. 1990, Sawangjit et al. 2005). Over 600 different plant species have been recorded as host of WF (Mound & Halsey 1978, Greathead 1986, Cock 1986, Secker et al. 1998) and it can easily adapt to a new host and environment. It feeds on a wide variety of vegetable crops such as tomato, pepper, beans, eggplant and cucumber both under field and protected cultivation environment. The present focus on chemical management is seriously limited. Furthermore, faster resistance development leads to ineffective management of WF either with old conventional insecticides, or with first or second generation of nicotinoids [(Schuster (2000a and 2000b), Schuster and Polston (1997a, 1997b, 1998) Palumbo and Coates 1996)] or even with growth regulators (Horowitz et al. 1999 a & b, Denholm et al. 1998, Ellsworth et al. 1996, Dennehy et al. 1996).

Therefore, alternative control strategies for WF focusing on botanicals like neem are needed. A detail comparison of application methods (topical vs. systemic) at different dose-rates and learning the sensitivity of different WF developmental stages are of crucial importance (chapter 2) for sustainable tomato production under dynamic climatic condition of the humid tropics. Any attempt to combine successful bio-control agents like *Eretmocerus* and *Encarisa* with a botanical like neem would need information on the persistency (chapter 3) to develop the integrated control strategies. Similarly, so called novel bio-pesticides of microbial origin like abamectin and spinosad were compared in laboratory and in GH (chapter 4) with neem to provide detailed comparison and persistency to further dwell on the idea of the developing integrated control for WF. Moreover, reducing the infection pressure of WF by retarding the immigration into the GH environment by mechanical and optical barriers could contribute to sustainable

management. Consequently combinations of UV-blocking nets and plastics (Chapter 5) were tested.

Our findings related to neem and its various application methods (seed soaking, foliar and systemic) revealed that neem could provide excellent control of Bemisia in a concentration dependent manner (chapter 2). It first acts to repel the settling of adults on the treated plants resulting into reduction of the overall egg load on the plant; moreover, it caused reduction in egg hatching and high immature mortality. Similarly, we found that with different application methods, a different load of tomato leaves with active neem ingredients was achieved, where major feeding, egg laying and immatures development takes place. Foliar application was found a very efficient way to apply neem to the leaves, where it causes almost 100% immatures mortality followed by the systemic application and seed soaking. Most striking was the high efficacy of the systemic use of neem opening new venues to affect a leaf sucking herbivore pest without contaminating the crop canopy and wider environment. Therefore, an integrated strategy of using tomato seedlings grown out with neem seed soaking followed by a combination of foliar and soil application of neem is suggested as a first convenient tool to achieve an efficient and sustainable control of *B. tabaci* on tomatoes grown under tropical net houses.

When we studied the persistency of the neem applied by soil drenching or foliar spraying under GH and lab conditions (chapter 3) variable rates of degradation were evident measured by dynamic changes in adult colonization, and subsequent egg deposition, egg hatching and immature mortality. The neem ingredients applied to the plant roots were translocated into the plant vessel system and are there protected from abiotic degradation factors and less vulnerable to degradation compared to the neem applied on the foliage.

The reduction in the bio-efficacy of leaf sprayed neem was clearly related to the UV and temperature as dissipation rate was rapid under GH compared to lab conditions. Fresh foliar residues provided excellent control of Bemisia for first few days but quickly degraded to a point where no bio-efficacy was noted. In contrast, the systemically translocated neem steadily provided excellent control over a longer period of time. Thus, making the soil application a safer way to preserve the bio- efficacy of applied neem compared to the foliar applied neem. However, soil drenching requires higher quantity of neem compared to the foliar

application to achieve similar level of WF control, thus, making it economically costlier option for the growers. In addition, use of neem as a systemic pesticide for crops grown under protected cultivation, has advantages, i.e. where plants can be grown in pots or on artificial substrates; and where the infection pressure can be reduced by the use of mechanical barriers such as nets. Moreover, soil drenching of neem would least interfere with the foliage dwelling parasitoids because of lack of any direct contact, thus, would open the door for synergistic use of the biopesticide ("fast task force") and parasitoids or predators ("long term sustainable control").

Neem has already gained public acceptance in developed countries for use on food crops (Isman1994) because of reduced human toxicity, fast and complete degradation in the environment, low risk for resistance and sometimes selective properties concerning non-target organism (Feng and Isman 1995, Immaraju 1998, Walter 1999). A possible drawback of using neem is the cost of $1,500 US per ton of neem oil (Stone 1992) and the further cost of formulation. In contrast, neem being a native of India and part of Asia (developing world) is widely grown and a range of neem derived pesticides products (neem oil, kernel powder, oil cake, dried leaves etc.) are traditionally used and are available. Similarly being the producing countries of neem, costs are relatively very small e.g. in India cost of neem oil as low as Rs.20/kg[10] (Mruthyunjaya and Jha 1996). Thus, more then the pricing of neem products, quality and consistency of the marketed neem products would determine its wider use and adoptability by growers for vegetable production including tomatoes.

Our work with neem, spinosad and abamectin (chapter 4) revealed that *B. tabaci* are highly susceptible to neem, spinosad and abamectin. However, the susceptibility varies with WF growth stage and time span between application and infestation as well as the presence and absence of sunlight. The adult colonization was deterred by the neem and abamectin and consequently reduced egg deposition was observed. However, no such deterrency of adult and consequent reduced rate of oviposition was observed for spinosad. Abamectin treatment seriously affected the hatching of the WF eggs but only a concentration dependent response was observed for the neem and spinosad. Neem, spinosad and abamectin caused heavy mortality of all three larval stages

[10] 45 Indian Rupees (Rs.) = 1 US$ (2005 exchange rate).

of *B. tabaci*, where the first instar larvae was found to be most susceptible compared to other two larval stages. The abamectin treated larvae died faster (24 h) compared to 6-9 days in case of neem and spinosad. In terms of persistency, abamectin gave most persistent activity either under lab or in GH condition, whereas, there was considerable loss of efficacy of spinosad and neem was observed under GH condition, which was better under lab condition. However, neem products can help to control the serious pest *B. tabaci* in a more safe and sustainable manner particularly if only short term effects are necessary since remigration of the pest, e.g. in GH, is low. Thus, we foresee that WF management in tropically adapted GHs, if necessary for longer periods under heavy infestation pressure can not be achieved with this botanical alone. It requires a combination of neem and other safe products like spinosad or even abamectin, if necessity of product rotation to avoid resistant selection is considered. Particularly the highly efficient spinosad seems to be under risk of fast selection of resistant biotypes if used frequently (Zhao et al. 2002).

Regarding combined IPM strategies with a combination of pesticides and natural enemies, our results provide a promising future basis for integrating the WF parasitoid, *Eretmocerus nr. warrae*[11] (Hymenoptera: Aphelenidae) commonly present in and around the GH complex of AIT, Bangkok with a botanical pesticide like neem. However, several follow up studies would be important to increase our present understanding of such a combined strategy, like fate of applied neem inside plants; effect of neem application methods at different dose-rates on the overall fitness, development stages, behavior of the parasitoids, its effect on the second generation parasitoids. On another front, the knowledge on effects of brake-downs and analogs of azadirachtin on the Bemisia etc. would also be needed for successful and sustainable management of WF. Moreover the possible combination of biopesticides with release of natural enemies should be studied more in detail. That requires reliable data about possible side effects under practical growing conditions.

Vegetable crops like tomatoes grown under protected cultivation (net house, tunnels etc.) in humid tropics are vulnerable to abiotic stress (temperature, humidity, air flow etc.) (Ajwang et al. 2002) and biotic stresses represented by

[11]Identified by: Dr. Stefan Schmidt, Hymenoptera Section, Zoologische Staatssammlung Muenchen, Muenchhausenstr. 21, 81247 Munich, Germany

insects (WF, thrips, aphid) and plant virus diseases vectored by these insects like Tomato Yellow Leaf Curl (TYLCV) and tospovirus ((Tanapas et al. 1983, Thongrit et al.1986, Attathom et al. 1990, Ketelaar and Kumar, 2002, Premachandra 2004). As a novel attempt, it was planned to combine UV-blocking plastics as roof and UV-blocking nets as side walls to improve microclimate and reduce immigration of insects. A lower initial pest population density is a key factor for successful and effective control in general (Xu et al. 1984). The results (Chapter 5) revealed that GH made from a combination of UV-blocking nets as side walls and roofs with UV- blocking plastics are able to deter the immigrating WF, aphids and thrips. Consequently tomato plants grown under such GH`s had fewer leaf damage and we expect furthermore reduced virus infection including those of tosposvirus. Other potential benefits from the reduced UV-environment achieved through the use of UV-blocking net and plastics may include improved performance of entomopathogenic fungi (Costa et al. 2001), and baculoviruses (Goulsom et al. 2003), improved management of some fungal pathogens (Reuveni and Raviv 1992, 1997, Elad 1997), reduced UV related degradation of botanicals like neem (Barnaby et al. 1989, Stokes and Redfern 1982, Johnson et al. 2003, Barrek et al. 2004), and overall improvements in the microclimate leading to healthier production of crops like tomatoes. Thus, such GHs present itself as a viable option over all plastic made GHs in the humid tropics. However, additional questions like insects entry though the nets and their dispersal rates, effect of reduced UV-lights on the reproduction behavior of the WF and thrips etc. needs to be analyzed and will be subject of the further investigations.

In conclusion, the results presented in this work show that WF could be efficiently managed by the botanicals like neem and other so called bio-rational like spinosad and abamectin, if used properly. Moreover, under high UV environment of the humid tropics, selection of right concentration is essential to achieve sustainable level of management. Furthermore, under protected cultivation, physical control by using UV-blocking plastic and nets hold lot of promise, where several other non-chemical management options could be integrated to further reduce the WF damage levels. Data presented here can provide sound baseline information for the development of the IPM of the WF using alternatives to chemicals.

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**Acknowledgements**

My sincere thanks and gratitude to Professors, Dr. Hans-Michael Poehling (Supervisor) and Dr. Christian Borgemeister for giving me the opportunity to perform this research work in the framework of the FOR 431 project “Protected cultivation - an approach to sustainable vegetable production in the humid tropics”. I am greatly indebted to Professor Dr. Hans-Michael Poehling for his supervision especially during the period of preparation of manuscripts and thesis assembling, and particularly for the tedious editing work in preparing the manuscripts in this dissertation. In addition, my sincere thanks to Professor Dr. Christian Borgemeister for his invaluable comments and suggestions during the first 2 years of my work with the project. I am also grateful to Prof. Dr. Hans-Jürgen Tantau, Director, Institute of Horticultural and Agricultural Engineering (ITG), Hannover University, for providing support and advice on the UV part of the work and also for accepting the co-supervision of this dissertation.

Gratitude to Dr. Maxwell J. Whitten for constant encouragement and support that provided opportunity to learn about vegetable smallholders in South and SE Asia and elsewhere. Thanks to all vegetable farmers in India, Bangladesh, Thailand, Laos, Vietnam, China, PR and elsewhere for providing constant inspiration to find better ways to mange crops and pests. Thanks once again to Max for reading and commenting on all manuscripts of this dissertation.

Sincere thanks goes to Mr. Jan Willem Ketelaar, Chief Technical Advisor/IPM Expert of the Regional Vegetable IPM Programs and a good friend for his invaluable support and encouragements.

I am very much grateful to Dr. Paul Debarro, CSIRO, Australia for providing information on the species details of the Bemisia specimen and to Dr. Kurapan Kittipakorn (Department of Agriculture, Bangkok, Thailand) for providing the initial culture of whitefly. Also, sincere thanks go to Dr. Stefan Schmidt, Hymenoptera Section, Zoologische Staatssammlung Muenchen, Muenchhausenstr. 21, 81247 Munich, Germany for kindly identifying the parasitoids species more then once.

I am also grateful to Dr. E. Hummel of Trifolio-M GmbH (Lahnau, Germany) for providing the two neem products and their blank formulations. My special thanks go to Professor Dr.-Ing. Burkhard von Elsner, Institut fuer Technik in Gartenbau und Landwirtschaft, Universitaet Hannover, for kind assistance in measuring the spectral transmissivity of lots of plastic and net samples over the years and for timely advices throughout the UV trials.

Sincere thanks and gratitude to Prof. V. M. Salokhe, Coordinator, ASE Program (AIT, Bangkok) for providing constant encouragement and support towards making available brand new laboratory (ENTOMOLOGY LABORAOTRY II; WHITEFLY LAB) for research work, where I carried out most of my research and write-ups and once again his support in procuring the greenhouse structures for the UV-related experiments.

I am very much thankful to previous and present project coordinators; Dr. Thomas Achilles and Dr. Johannes Max respectively, for their great logistic support during the entire periods of my research. My special thanks to Mr. Lakchai Meenakanit (senior project consultant, AIT) and Ms. Patcharee Meenakanit (Ex Department of the Agriculture Extension, Bangkok, Thailand) for their great logistic support extended to me during the research work. I highly appreciate and I am thankful for the technical assistance provided to me by Ms. Janjira, Ms. Sopana, Mr. Swat, Mr. Songtham, Mr. Ganesh (AIT master student) and to all daily wage workers at AIT-Hanover Project. Moreover, I would like to thank all my friends in the FOR 431 project for their support and encouragement throughout the research.

I am highly grateful to the German Research Council (DFG) and the IPP of Hanover University for financial support of this research.

Last but not least, I would like to express my deepest gratitude to my wife, Abha Mishra for her immense support on every matter related to research and managing family. Without her support it could have been impossible to complete this research and dissertation. My love goes to my daughters' duo; Vagisha and Tanvi for making life colorful and happy amidst extremely hectic times during

research and later write-ups. My sincere regards goes to my father, Professor Dr. Sundareshwar Mishra and mother, Smt. Usha Mishra and my siblings Prashant, and two sisters Pratibha and Prabha and their families for their unconditional love and support to reach to this point in life.

I would also like to thank my all teachers in institutions from where I acquired basic knowledge eg. Tirhut College of Agriculture, Dholi, Muzaffarpur (Rajendra Agriculture University, Pusa, Samastipur, India) during undergraduate studies; Asian Institute of Technology, Bangkok, Thailand during M. Sc. studies. Especially to Drs. Steffen Johnsen (presently Advisor, CDC, Cambodia) and Prof. Dr. Richard L. Tinsley (Dick Tinsley) for their support during M.Sc. studies at AIT, Thailand.

Finally, prayers to the almighty God (the Cosmic Consciousness); who constantly provided inspiration to remain steadfast to the goals to serve humanity in all form and manifestation.

ॐ पूर्णमदः पूर्णमिदं पूर्णात् पूर्णमुदच्यते ।
पूर्णस्य पूर्णमादाय पूर्णमेवावशिष्यते ॥
ॐ शांतिः शांतिः शांतिः ॥

Aum. That unmanifested Brahman is perfect, and This manifested Brahman is also perfect. Fullness proceeds from fullness. Taking fullness from fullness, all that remains is fullness.

विद्यां चाविद्यां च यस्तद्वेदोभयं॰ सह
अविद्यया मृत्युं तीर्त्वा विद्ययाऽमृतमश्नुते

Knowledge and ignorance, he who knows the two together crosses death through ignorance and attains life eternal through knowledge.

***(Isha Upanishad; Verse 11)***

*!!!Aum Peace! Peace! Peace!!!*

## Curriculum vitae

**Personal Data**

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| Name | Prabhat Kumar |
| Date of Birth | 27.12.1970 |
| Place of Birth | Muzaffarpur, India |
| Family status | Married with 2 (two) children |

**Education Background**

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| 1989 – 1993 | B.Sc. (Agriculture & Hons.), Tirhut Collage of Agriculture, Rajendra Agriculture University, Pusa, India |
| 1995– 1996 | M. Sc. (Agricultural Systems), Asian Institute of Technology, Thailand. |
| 2002 – 2005 | PhD. Institute of Plant Protection and Plant Diseases, University of Hannover, Germany |

**Scholarship, Awards and Honours**

- Ranked ***first*** (1/80) in the class of B. Sc. (Ag.).
- Awarded ***the Keidanren Foundation Fellowship, Japan*** for Master's study at Asian Institute of Technology, Bangkok (January 1995- August 1996).
- Awarded ***MERIT*** Scholarship for the outstanding academic performance in B. Sc. (Ag).
- Awarded ***Thesis Research Grant*** from DANIDA for conducting master's thesis research

**Work Experience**

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| **1997 – 2001** | *Resident Vegetable IPM Consultant*, Food & Agril. Organization of the United Nations (FAO), Bangladesh, Thailand, Lao PDR, Cambodia and Vietnam. |
| **2001 – 2002** | *Senior Farming System Specialist*, AME (An Indo-Dutch bilateral Project), Bangalore, India. |
| **2002 – 2005** | *Project Researcher*, AIT-Hannover project for Sustainable Vegetable Production under the Protected Cultivation in the Humid Tropics, Thailand & *PTD IPM Expert* for Vegsys (EU-China-Vietnam) Project. |

**Publications**

- Tinsely, R. L., P. Kumar, and D.T.T. Huyen. 1998. Chemical usages on Vegetables in Asia. Workshop on Sustainability of Horticulture Systems in Southeast Asia, AIT, Thailand, 1-3 April, 1998.
- Kumar, P., S. Johnsen and R. L. Tinsley. 2000. Life Cycle Studies on Fruit and Shoot borer (*Leucinodes orbonalis*; Pyralidae; Lepidopetra) and natural enemies of eggplant (*Solanum melongena*) insect-pests, *Journal of Applied Biology*, Vol. 10 (2), 2000.
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- Ketalaar, J. W. K., and P. Kumar. 2002. Vegetable Integrated Production and Pest Management: the case for *farmers* as IPM Expert. International Conference on Vegetables held at the ITC Hotel Windsor Sheraton and Towers, Bangalore, India, 11-14 November 2002.
- Kumar, P., H.- M. Poehling, and C. Borgemeister. 2005. Effects of Different Application Methods of Neem against Sweetpotato Whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) on Tomato plants. Journal of Applied Entomology. 129:489–497.

**Contribution in Book**

- Tinsley, R. L. 2004. Developing Smallholder Agriculture: A Global Approach, AgBe Publishing, Brussels, Belgium. Contributor: Chapter 6 Sustainability of Smallholder Agriculture.

## Lebenslauf

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**Ausbildung**

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| 1989 – 1993 | B.Sc. Landwirtschft, Tirhut Collage of Agriculture, Rajendra Agriculture University, Pusa, Indien. |
| 1995– 1996 | M. Sc. (Agricultural Systems), Asian Institute of Technology, Thailand. |
| 2002 – 2005 | Doktorand, „Institut für Pflanzenschutz und Pflanzenkrankheiten“, Universität Hannover, Deutschland, Versuchsdurchführung am AIT, Thailand. |

**Stipendien und Auszeichnungen**

- Jahrgangsbester B. Sc.-Abschluß (1/80)
- Stipendium der ***Keidanren Foundation Fellowship, Japan*** für das Master-Studium am Asian Institute of Technology, Bangkok (Januar 1995 - August 1996).
- ***MERIT***-Stipendium für ausgezeichnete akademische Leistungen im B. Sc.–Studium.
- Forschungsstipendium der DANIDA-Stiftung für die Durchführung der Masterarbeit

**Arbeitserfahrung**

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| **1997 – 2001** | *IPM-Berater für Gemüsebau*, Food & Agril. Organisation of the United Nations (FAO), Bangladesh, Thailand, Laos, Kambodscha und Vietnam. |
| **2001 – 2002** | *Landwirtschaftsberater*, AME (bilaterales Projekt, Niederlande/Indien), Bangalore, Indien. |
| **2002 – 2005** | *Doktorand*, “Protected Cultivation – an approach for sustainable vegetable production in the humid tropics”, Thailand & *PTD IPM-Berater des* “Vegsys-Projektes (EU-China-Vietnam). |

**Mitgliedschaften**

Mitglied auf Lebenszeit der Indian Society of vegetable sciences

**Wissenschaftliche Veröffentlichungen**

- Tinsely, R. L., P. Kumar, and D.T.T. Huyen. 1998. Chemical usages on Vegetables in Asia. Workshop on Sustainability of Horticulture Systems in Southeast Asia, AIT, Thailand, 1-3 April, 1998.
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- Kumar, P., H.- M. Poehling, and C. Borgemeister. 2005. Effects of Different Application Methods of Neem against Sweetpotato Whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) on Tomato plants. Journal of Applied Entomology. 129:489–497.

Beiträge in Büchern

- Tinsley, R. L. 2004. Developing Smallholder Agriculture: A Global Approach, AgBe Publishing, Brussels, Belgium. Contributor: Chapter 6 Sustainability of Smallholder Agriculture.

**Eidesstattliche Erklärung**

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

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.....
(Prabhat Kumar)
9 December 2005
Bangkok, Thailand