Effects of Neem (*Azadirachta indica* A. Juss) on *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae), a Parasitoid of *Nezara viridula* (L.) (Hemiptera: Pentatomidae)[1]

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Abstract The effects of neem (*Azadirachtin indica* A. Juss) on *Trissolcus basalis* (Wollaston), an egg parasitoid of *Nezara viridula* (L.), were assessed in the laboratory and in cowpea (*Vigna unguiculata* [L.] Walpers). Treatment of *N. viridula* eggs with 0.5% (225 ppm azadirachtin) aqueous solution of neem had no effect on parasitization by *T. basalis,* using both choice and no-choice tests. Parasitoid development and emergence from host eggs treated before or after parasitization also were not affected by neem compared with controls. Additionally, neem did not affect longevity of adult parasitoids from treated eggs or the reproductive activity of females compared with controls. In a field choice test using treated and control eggs, parasitism levels were similar. Also, parasitism of eggs was similar in treated and control cowpea plots. These results suggest that neem may be safe to parasitoids of *N. viridula* and could be an important component of an integrated pest management program in cowpea.

Key Words *Trissolcus basalis, Nezara viridula,* parasitoids, parasitism, neem, Neemix, azadirachtin, cowpea

Nezara viridula (L.) (Hemiptera: Pentatomidae) is a highly polyphagous pest species with cosmopolitan distribution on agricultural and horticultural crops. It feeds on fruits, pods and seeds and reduces crop yield and quality (Todd and Herzog 1980, Todd 1989) and attacks more than 30 families of cultivated plants (Jones 1918, Drake 1920, Hoffman 1935, Todd and Herzog 1980, Todd 1989, Panizzi 1997). *Nezara viridula* is an important pest of cowpea in the U.S. and in several other countries (Singh 1980, Nilakhe et al. 1981a,b, Jackai and Daoust 1986, Abudulai and Shepard 2001). Adults and nymphs feed on pods resulting in pod abscission, abortion, shriveling or discoloration of seeds (Nilakhe et al. 1981a, Schalk and Fery 1982, Abudulai and Shepard 2001).

The egg parasitoid, *Trissolcus basalis* (Wollaston), is a major biological control agent of *N. viridula* (L.) (Shepard et al. 1994, Jones et al. 1996). Both indigenous populations and field releases of *T. basalis* have been used to control *N. viridula* in Hawaii, Australia, New Zealand, Brazil and in other countries (Caltagirone 1981, Shepard et al. 1994, Corrêa-Ferreira and Moscardi 1995, Follett et al. 2000). Addi-

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tionally, several predators such as coccinellids, formicids and orthopterans attack eggs and nymphs of *N. viridula* in the field causing significant mortality (Stam et al. 1987, Follett et al. 2000). However, synthetic chemical insecticides used in control programs for *N. viridula* (Chalfant 1976, Hoffmann et al. 1987) negatively impact non-target beneficial organisms (Croft and Brown 1975, Croft 1990, Smilanick et al. 1996, Abudulai et al. 2001) and, as a result, often are not compatible with a sustainable integrated pest management (IPM) approach. For example, Justo (1994) reported that season-long parasitism of *N. viridula* eggs by *T. basalis* was significantly reduced by esfenvalerate treatments in tomatoes in Charleston, SC. Also, Sales (1978) reported that applications of carbaryl, methomyl and parathion in soybean significantly reduced emergence of *T. basalis* from host eggs. Thus, interest in alternatives to synthetic insecticides for IPM programs has increased, particularly if these materials are less harmful than commercial chemical insecticides to biological control organisms (Ascher 1993, Schmutterer 1997).

Extracts from the neem tree, *Azadirachta indica* A. Juss, have received considerable attention (Isman et al. 1991, Mordue and Blackwell 1993, Lowery and Isman 1995, Schmutterer 1997, Tedeschi et al. 2001). The major active ingredient in neem, azadirachtin, has low mammalian toxicity and yet it is effective against over 200 insect pests (Schmutterer 1990, Ascher 1993, Mordue and Blackwell 1993, Singh and Saxena 1999). Neem compounds act as repellents or antifeedants on phytophagous insects (Schmutterer 1990, Mordue and Blackwell 1993) and also affect reproduction and development of pest insects by inhibiting oviposition and by interfering with larval molts (Schmutterer 1990). Except for a few reports, effects of neem on insect natural enemies have not been adequately investigated (Lowery and Isman 1995, Schmutterer 1997).

Schmutterer (1997) reported that neem products are safe to spiders, numerous beneficial insects and eggs of predators such as coccinellids, with only slight side-effects observed under semi-field and field conditions. No adverse effects were observed in studies of the effects of neem extracts on the egg parasitoid, *Telenomus remus* Nixon (Joshi et al. 1982), and the larval parasitoid, *Bracon hebetor* Say (Raguraman and Singh 1998). Parasitization of eggs of *Helicoverpa zea* Boddie by *Trichogramma pretiosum* Riley following neem treatments in a melon field also was not affected (Schmutterer 1997). However, Raguraman and Singh (1999) reported that neem oil treatment of eggs of the rice moth, *Corcyra cephalonica* (Stainton), resulted in oviposition deterrence and mild toxicity to its parasitoid, *Trichogramma chilonis* Ishii. Treatment of host eggs, however, had no adverse effects on the development of the parasitoid. Srivastava et al. (1997) studied the effects of neem ecotypes found in India on the braconid larval parasitoid, *Bracon brevicornis* Wesmael. They reported that alcohol and hexane extracts of 17 neem ecotypes were toxic to the egg, larval and pupal stages of the parasitoid. Tedeschi et al. (2001) reported that neem was toxic to the mirid predator, *Macrolophus caliginosus* Wagner, but they found no significant differences on mortality and fecundity of surviving females compared with controls 5 d after treatment.

The present studies were conducted to evaluate the effects of the commercial neem formulation Neemix 4.5 EC (Certis USA, Columbia, MD) on *T. basalis,* a major parasitoid of *N. viridula,* under laboratory and field conditions. These data may be useful in the development of a pest management approach that integrates biological control with neem for control of *N. viridula* in cowpea.

Materials and Methods

Insect colonies. Eggs from colony-reared *N. viridula* were used as hosts for *T. basalis.* Colonies of *N. viridula* and *T. basalis* were maintained in the laboratory at 24 ± 0.5°C room temperature, 55 to 65% RH, and a photoperiod of 14:10 (L:D) h. *Nezara viridula* colonies were established from bugs collected from collard (*Brassica oleracea* L.) and green bean (*Phaseolus vulgaris* [L.]) fields in Charleston, SC. Bugs were reared on green beans in metal screen cages (36 × 36 × 36 cm). Egg masses were harvested daily from paper towel strips used as oviposition sites in the cages and were either used for colony maintenance or kept frozen at –20°C until ready for use (Powell and Shepard 1982). Colonies of *T. basalis* were established by placing *N. viridula* egg masses in cowpea fields for parasitization. Parasitized eggs were kept in test tubes in the laboratory until *T. basalis* emergence. Parasitoid colonies were maintained using *N. viridula* eggs as hosts. Honey solution (80%) was provided as food.

Neem compound. Neemix® (Certis, Columbia, MD), a commercial neem formulation containing 4.5% azadirachtin as the active ingredient, was used in this study. The formulation was diluted with distilled water to form a solution of 0.5% (225 ppm azadirachtin) for the tests. This concentration was the rate used for *N. viridula* control in the field (Abudulai et al. 2002).

Laboratory choice and no-choice tests for *T. basalis* oviposition. Oviposition preference for *T. basalis* in neem-treated and distilled water-treated control host eggs was examined in choice and no-choice tests in the laboratory. Egg masses were treated singly using a pipette to deposit a 1-ml droplet of neem solution or distilled water. The droplet was spread over the entire egg surface using a fine camel-hair brush. After 2 h of drying at room temperature, egg masses were glued (Elmer's Glue-All, Borden Inc., Columbus, OH) to strips of labeled index cards (2 × 4 cm). In the choice test, a neem-treated egg mass and one treated with distilled water were paired on a strip. A neem-treated or a water-treated egg mass on a strip was used in the no-choice test. One mated 2 to 3-d-old female *T. basalis* was transferred into a Petri dish (10 × 1.5 cm) containing a single egg strip using a fine camel-hair brush and allowed to oviposit for 24 h. A cotton ball moistened with 80% honey solution served as food for parasitoids in the Petri dishes. There were 10 replicates for each treatment in both tests, and the experiments were repeated two times. The behavior of parasitoids on neem-treated and control eggs was observed for about 30 min after parasitoid introduction into the Petri dishes. Individual parasitized egg masses were held in Petri dishes under laboratory conditions previously described for *T. basalis* rearing.

After emergence, parasitoid sex ratio was determined and longevity was recorded by daily counts of dead adults. To evaluate the reproductive activity of females, ten 2 to 3-d-old mated females each from treated and control groups were each presented an egg mass and allowed to oviposit for 24 h. At the end of the tests, each egg that failed to hatch was dissected to determine whether a parasitoid was present. Percent parasitism, emergence, sex ratio, longevity, and reproductive activity of females were recorded.

Effect of neem on developing *T. basalis.* This laboratory test examined the effect of neem on *T. basalis* developing within host eggs. The experiment was conducted as described for the no-choice test except that egg masses on index card strips were treated with neem or distilled water control 8 d after exposure to female parasitoids. Parasitoids were in the pupal stage within host eggs at the time of treat-

ment application (Orr et al. 1989, Smilanick et al. 1996). Treated egg masses were allowed to air dry and then held in Petri dishes for adult *T. basalis* emergence, which occurred 2 to 4 d after treatment. There were 10 replications per treatment, and the experiment was repeated two times. After emergence, data were taken on percent emergence, sex ratio, longevity, and reproductive activity of females.

Parasitism and predation on neem-treated and water-treated egg masses in cowpea. A neem-treated egg mass and one treated with water, were placed on opposite sides of individual cowpea plants by stapling index cards (2 × 4 cm) containing *N. viridula* egg masses to the under surface of leaves. Twenty egg masses (10 pairs) were randomly placed in each of four plots measuring 20 × 30 m. Eggs were placed in the field when plants were in the late podding stage on 12 August 2000. The experiment was repeated beginning on 16 August 2001. Eggs remaining after 7 d were collected, transported to the laboratory and held in test tubes for 1 month for parasitoid emergence. After this time, numbers of parasitized eggs per egg mass were determined by counting the individual parasitoids that emerged and by dissecting eggs that showed symptoms of the presence of parasitoids. Percent parasitism and emergence and percent were recorded.

Response of *T. basalis* to field applications of neem. Augmentative releases of *T. basalis* were made in cowpea on 15 August 2000 and again on 21 August 2001, to determine if neem-treated fields would affect parasitism of *N. viridula* egg masses. Twenty egg masses, each glued to an index card strip (2 × 4 cm), were fastened under cowpea leaf surfaces in a field plot using paper clips. Egg placement and parasitoid releases were done one day after neem application. Treated plots were sprayed with 0.5% aqueous neem solution (934.9 L/ha) using a tractor-mounted boom (5.5 m swath width). Untreated plots served as controls. Plot sizes used in both years were 30 × 15 m with a distance of 3.6 m between plots and 10 m spacing between blocks. Plots were arranged in a randomized complete block design with four replications.

Parasitoid releases were carried out by placing parasitized *N. viridula* eggs with *T. basalis* expected to emerge within 24 h midway between adjacent treated and untreated plots. Eggs were glued to the inside of a plastic container (15 × 6 cm) that was fixed bottom up on top of a wooden stake (1 m long). Parasitized eggs were placed in the field in the evening around 1800 h (EDT). Food was provided to emerging *T. basalis* adults by placing cotton balls moistened with 80% honey solution near the inner rim of the container. The base of the wooden stake was coated with Tangle-Trap® (The Tanglefoot Co., Grand Rapids, MI) to prevent crawling predators from attacking the eggs. After 7 d, egg masses were collected and numbers of *T. basalis* released in the field were determined by counting the exit holes made by emerging adult *T. basalis*. About 1,800 and 3,280 *T. basalis* adults were released per ha of cowpea in 2000 and 2001, respectively.

After 7 d, eggs were collected, brought to the laboratory, and held in test tubes at 24 ± 0.5°C, 55 to 65% humidity and a photoperiod of 14:10 (L:D) h for 1 month for parasitoid emergence. Unhatched eggs were dissected to determine if parasitoids were present. Percent egg parasitism was calculated based on parasitoids that emerged and those counted by dissecting the eggs.

Data analyses. Data were analyzed by using *t*-tests to compare treated and controls and means were separated at $P < 0.05$ (SAS Institute 1996). Data from the two repetitions in the laboratory tests were pooled for analysis.

Voucher specimens. Voucher specimens of *T. basalis* have been deposited in

the Department of Entomology Arthropod Museum at Clemson University, Clemson, SC. Identification of parasitoids, *Anastatus* sp. (Hymenoptera: Eupelmidae) and *Ooencyrtis* sp. (Hymenoptera: Encyrtidae) were confirmed Dr. Walker Jones of USDA (Weslaco, TX).

Results

Laboratory choice and no-choice tests for *T. basalis* oviposition. During the 30-min observation period, parasitoids settled on egg masses without observed differences between neem-treated and water-treated control eggs in both tests. In the choice test, parasitoids settled on 85% of the neem-treated eggs (n = 20) and on 80% of the water-treated controls (n = 20). Parasitoids settled on 90% of treated eggs (n = 20) and 100% of control eggs (n = 20) in the no-choice test. There was no indication that neem treatment repelled *T. basalis* or inhibited them from oviposition (Fig. 1). No differences were detected in *T. basalis* development and emergence ($P > 0.05$) between treated and control eggs (Fig. 1). Female-to-male ratio was 15:1 (n = 167) in controls and 16:1 (n = 217) in neem treatments in the choice test. The ratio was 14:1 (n = 512) and 12:1 (n = 339) in control and neem-treated eggs in the no-choice test, respectively. Longevity of parasitoids that emerged from neem-treated eggs was not significantly ($P > 0.05$) different from controls in both tests (Fig. 1). Also, reproductive activity (percent parasitism) of *T. basalis* from treated eggs was not significantly ($P > 0.05$) different from controls.

Effect of neem on developing *T. basalis*. Treatment of *N. viridula* eggs when *T. basalis* were pupae did not significantly ($P > 0.05$) affect adult parasitoid emergence. Female to male ratio was 15:1 (n = 249) in controls and 16:1 (n = 228) in treated eggs. Although emergence of adult parasitoids from neem-treated egg masses was lower (74.4 ± 4.21%) compared with the controls (80.1 ± 3.0%), this difference was not significant ($P > 0.05$). Longevity of parasitoids from treated eggs was not significantly different from controls (9.1 ± 0.03 and 8.4 ± 0.2 d, respectively) and per cent parasitism between neem-treated egg masses (35.5 ± 5.3) and controls (49.3 ± 4.4) was not significantly different in these tests.

Parasitism on neem-treated and water-treated egg masses in cowpea. In 2000, only *T. basalis* were recovered from the field-exposed *N. viridula* egg masses. Eighty-nine percent of available neem-treated egg masses (n = 18) and 65% of water-treated controls (n = 17) were parasitized by *T. basalis.*

In 2001, in addition to *T. basalis,* an encyrtid, *Ooencyrtus* sp. and an eupelmid, *Anastatus* sp., were recovered from the egg masses. A single parasitoid emerged from each egg. Only one egg mass was parasitized by *Anastatus* sp. and three egg masses by *Ooencyrtus* sp. out of a total of 55 egg masses from which parasitoids were recovered. *Trissolcus basalis* were recovered from 51 egg masses composing approximately 93% of total egg mass parasitism. Forty-seven percent of neem-treated egg masses and 53% of water-treated controls were parasitized.

Percent egg parasitism was not significantly ($P > 0.05$) different between neem-treated eggs and water-treated controls in 2000 and 2001 (Fig. 2). Emergence of *T. basalis* from eggs also was not significantly ($P > 0.05$) different between the treatments (Fig. 2).

Response of *T. basalis* to field applications of neem. In 2000, *Ooencyrtus* sp. was recovered from 7.5% and *T. basalis* from 43% of available egg masses (n = 93)

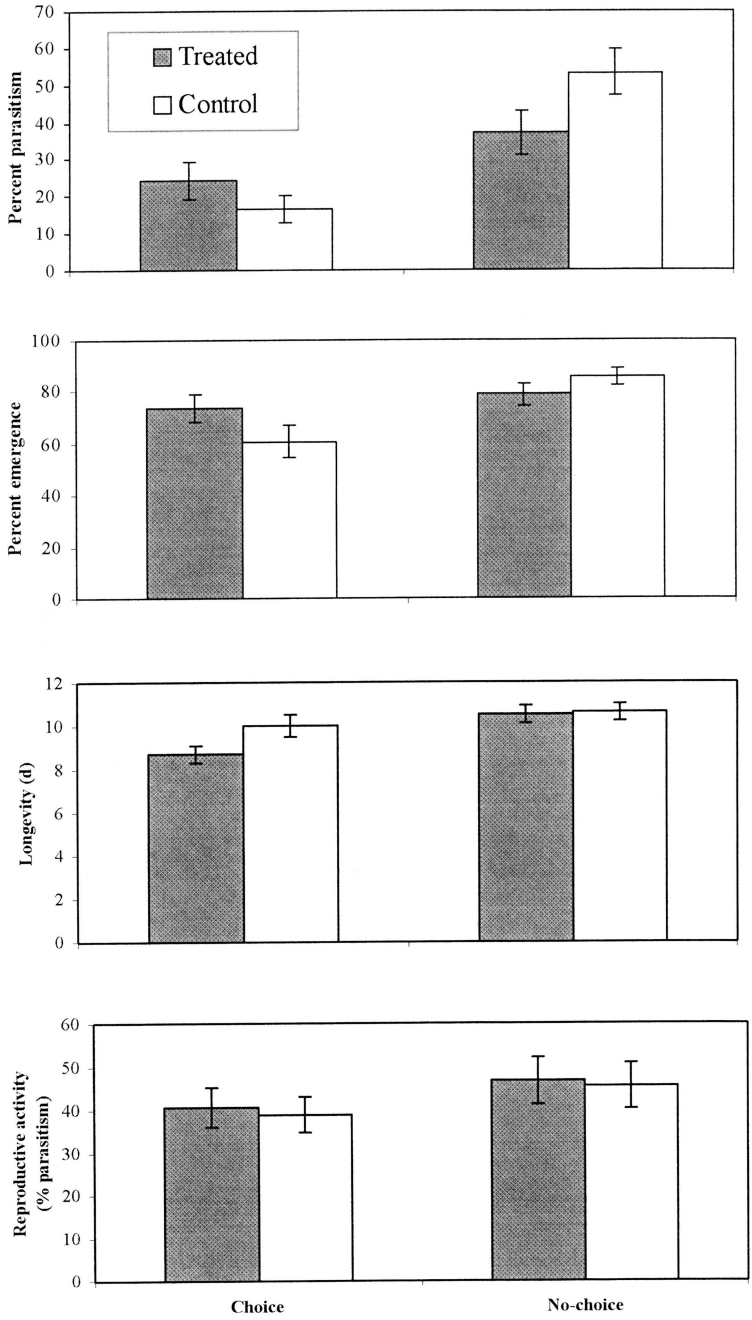

Fig. 1. Mean (±SE) percent parasitism, emergence, longevity and reproductive activity of *Trissolcus basalis* (Wollaston) from *Nezara viridula* (L.) eggs treated with 0.5% neem solution or distilled water control in laboratory choice and no-choice tests. No significant differences were detected between the treatments (*t*-test, $P > 0.05$).

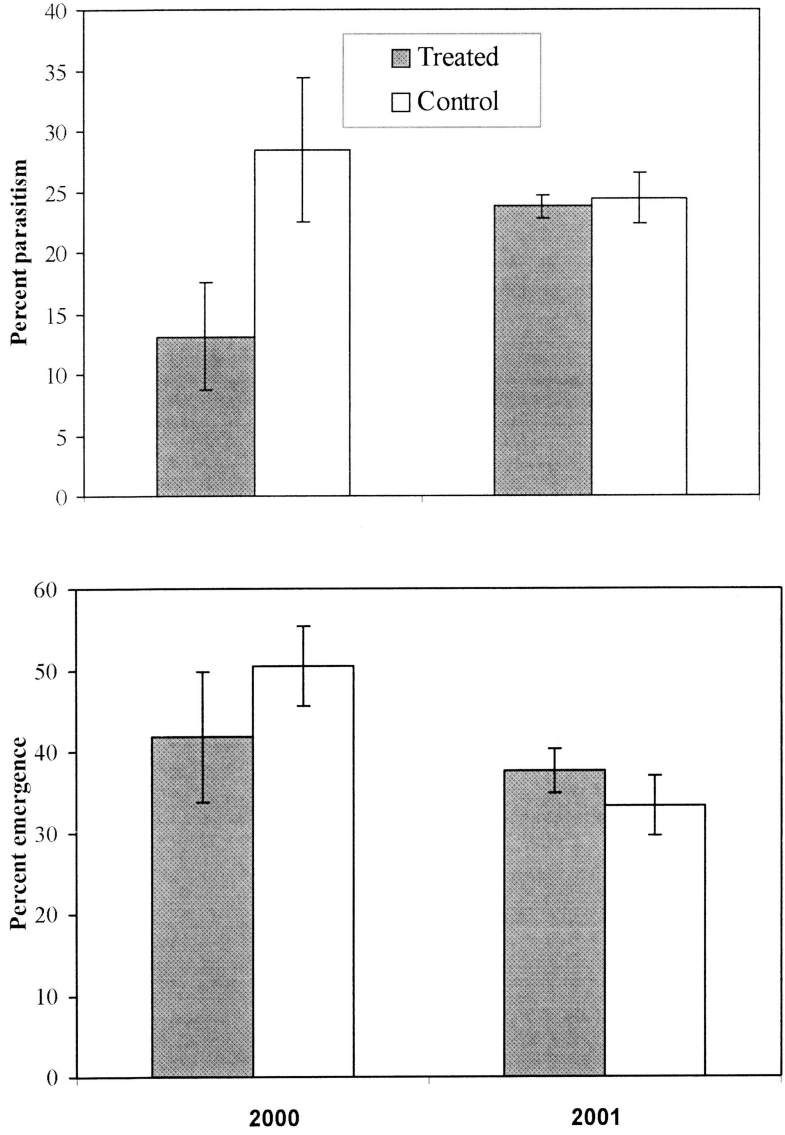

Fig. 2. Mean (±SE) percent parasitism and emergence of *Trissolcus basalis* (Wollaston), from *Nezara viridula* eggs treated with neem or distilled water control in field choice tests in 2000 and 2001. No significant differences were detected between the treatments (*t*-test, $P > 0.05$).

in the field. Of those parasitized, 55% of the egg masses were from treated plots and 45% were from untreated controls. In 2001, 43% of available egg masses (n = 131) were parasitized by *T. basalis*. Forty-five percent of the parasitized egg masses were from treated plots and 55% were from untreated controls. Twenty percent of egg

masses (n = 80) from treated plots and 16% of egg masses (n = 80) from untreated controls were lost to predators.

Percent parasitism of eggs in a mass was similar ($P > 0.05$) in treated and untreated plots in 2000 and 2001 (Fig. 3). Also, parasitoid emergence was not significantly ($P > 0.05$) different between the treatments during both years (Fig. 3).

Discussion

Although a considerable amount of literature is available on the effects of neem on phytophagous insects, there is little information about the effects of neem on natural enemies. Jacobson (1986) stated that neem is selective against insect pest species. Neem-based insecticides have been reported to have little effect on beneficial organisms such as honeybees, predators and parasitoids (Lowery and Isman 1995, Schmutterer 1997). It is generally believed that this is due to lack of contact toxicity in most cases or because most natural enemies do not feed on plants, thus lessening the possibility of ingestion of neem (Ascher 1993).

Results from the present study indicate that Neemix, applied at 0.5% (225 ppm azadirachtin) aqueous concentration to *N. viridula* eggs, did not deter its parasitoid, *T. basalis,* from ovipositioning in treated eggs using choice and no-choice tests in the laboratory or in the field. Likewise, parasitization of *H. zea* eggs by *Trichogramma pretiosum* Riley in Nicaragua was not reduced in melon plots treated with the neem seed kernel extract, NIM-20, compared with untreated controls when parasitoids were released in the field after treatment (Schmutterer 1997). However, treatment of eggs of *Plutella xylostella* (L.) (Klemm and Schmutterer 1993) and *C. cephalonica* (Raguraman and Singh 1999) with neem oil reduced parasitization by *Trichogramma* spp. in the laboratory and in the field. Schmutterer (1997) noted that due to some physical properties of neem oil formulations, they have a higher degree of toxicity to very small beneficial species, such as parasitoids, compared with oil-free neem formulations. Neem oil also has large quantities of salannin, which apart from being toxic, deters insects from feeding (Schwinger et al. 1984).

The relative susceptibility of *T. basalis* to pesticides depends on its stage of development at the time of treatment application (Orr et al. 1989). In the present study, parasitoid development and emergence from eggs treated before parasitization or during the pupal stage developing within host eggs was not affected by neem. This suggests that egg chorions provided a barrier to neem penetration (Orr et al. 1989). Longevity of parasitoids that emerged from treated eggs also was not affected in the present study. Joshi et al. (1982) found that neem treatment of *S. litura* eggs before or after parasitization did not affect development and emergence of adult *T. remus* parasitoids. They observed that adult parasitoid longevity was reduced when eggs were treated before parasitization; however, longevity was increased when eggs were treated after parasitization. Raguraman and Singh (1999) reported that treatment of host eggs before or after parasitization with neem seed oil did not influence the developmental stages and adult emergence in the egg parasitoid, *Trichogramma chilonis* Ishii. Also, they found that longevity of adult parasitoids was unaffected by neem as in the present study.

Neem has been reported to cause sterility and reduced fecundity in phytophagous insects (Koul 1984, Schmutterer 1990). In this study, parasitism was similar for parasitoids from neem-treated and water-treated control eggs. Neem extracts had no

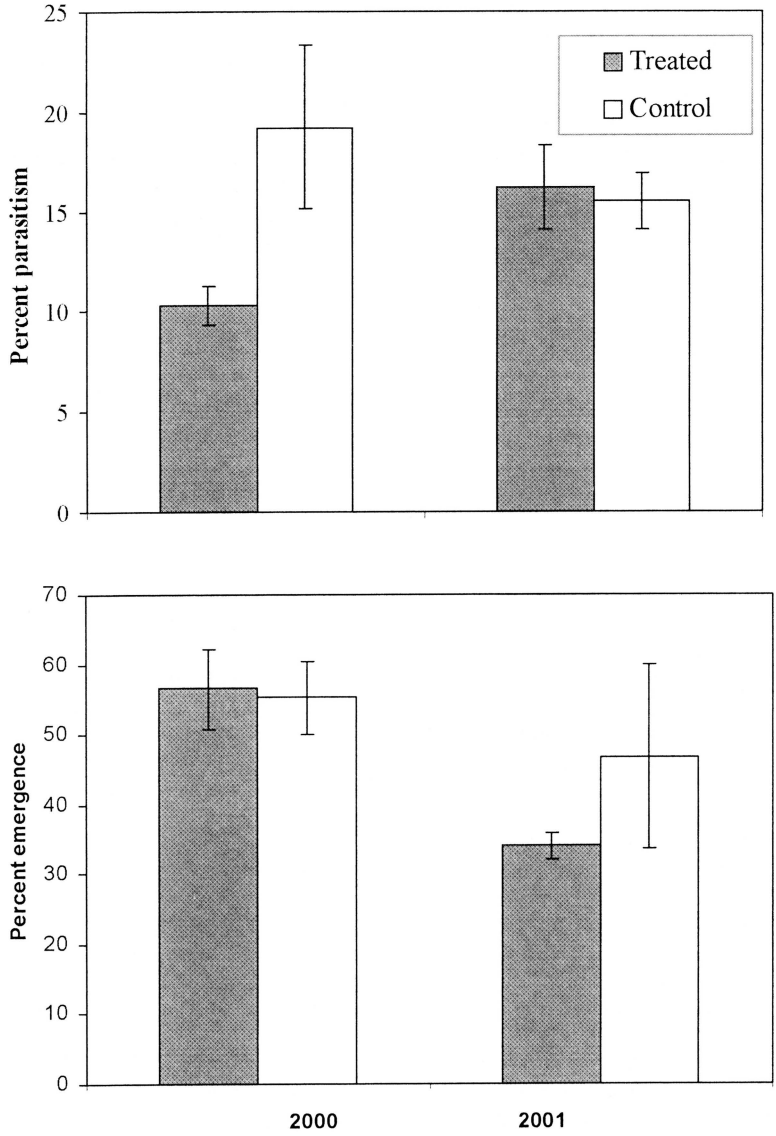

Fig. 3. Mean (±SE) percent *Trissolcus basalis* (Wollaston) parasitism and emergence, from *Nezara viridula* (L.) eggs in neem-treated and untreated cowpea field plots in 2000 and 2001. No significant differences were detected between the treatments (*t*-test, $P > 0.05$).

effect on fecundity of progeny of *B. hebetor* that emerged from treated hosts (Raguraman and Singh 1998) and reproduction of the braconid parasitoids, *Diachasmimorpha tryoni* (Cameron) and *D. longicaudata* (Ashmead) that developed in fruit flies larvae exposed to azadirachtin was unaffected (Stark et al. 1992).

In our study, the sex ratio in *T. basalis* progeny was not altered by neem. *Trissolcus basalis* progeny emerging from treated and control host eggs were female-biased, which corroborate findings of Buschman and Whitcomb (1980) and Powell and Shepard (1982) that *T. basalis* produce female-biased progenies.

In summary, results from the present study suggest that 0.5% Neemix (225 ppm azadirachtin) did not have a negative impact on parasitism by *T. basalis,* the major biocontrol agent of *N. viridula,* as indicated by laboratory and field studies. In addition, *N. viridula* populations were reduced after neem application (Abudulai et al. 2002). Thus, neem may have promise for use in IPM programs for *N. viridula.*

Acknowledgments

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