# Combining the Botanical Insecticides NSK Extract, NeemAzal T 5%, Neemix 4.5% and the Entomopathogenic Nematode *Steinernema feltiae* Cross N 33 to Control the Peach Fruit Fly, *Bactrocera zonata* (Saunders)

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**Abstract**

Mahmoud M. F. (2007): **Combining the botanical insecticides NSK extract, NeemAzal T 5%, Neemix 4.5% and the entomopathogenic nematode *Steinernema feltiae* Cross N 33 to control the peach fruit fly, *Bactrocera zonata* (Saunders)**. Plant Protect. Sci., **43**: 19–25.

Botanical insecticides based on azadirachtin and the entomopathogenic nematode *Steinernema feltiae* were evaluated for their control of the peach fruit fly, *Bactrocera zonata*. Laboratory bioassays determined the potential of combinations between the biological control agent *S. feltiae* and the botanical insecticides NSK, NeemAzal T 5%, Neemix 4.5% against $3^{rd}$ instar larvae of *B. zonata.* Of 25 treatment combinations between azadirachtin from NSK extract and *S. feltiae,* 18 gave synergistic responses, 4 were additive, none antagonistic and 3 without any response. The same number of combinations with NeemAzal T 5% showed 19 synergistic responses, 1 additive, none antagonistic and 5 without any response. Combinations of Neemix 4.5% and *S. feltiae* showed 11 synergistic responses, 5 additive, 3 antagonistic and 6 without any response. The combined use of botanical insecticides based on azadirachtin, especially NSK extract and NeemAzal T 5%, with the entomopathogenic nematode *S. feltiae* may offer an integrated approach to increase the efficacy of control of the peach fruit fly, *B. zonata,* by entomopathogenic nematodes.

**Keywords**: entomopathogenic nematode; *Steinernema feltiae*; the peach fruit fly; *Bactrocera zonata*

The peach fruit fly, *Bactrocera zonata* (Saunders), is recognised as one of the most important and serious pests attacking fruit crops. It is established in South and South-East Asia (White & Elson-Harris 1994; Allwood *et al.* 1999). In Egypt, it has been established since the late 1990s, owing to the suitability of climate and the extension in planting favourable host fruits such as peach, guava, mango and apricot (CABI 1996).

*Bactrocera zonata* is a strong flier capable of dispersing more than 24 km in its search for host plants. It is active throughout the year when temperatures exceed 10°C. Adults appear in early spring, feeding on nectar, plant sap, and decaying fruit. The preoviposition period (including sexual maturation of 8 to 16 days) is 10 to 23 days. The female lays an average of 137 eggs in batches of two to nine under the rind of the host fruit. A female can lay up to 93 eggs/day, and as many as 564 in its lifetime. Under favorable conditions, the eggs hatch into larvae within 2 days. The larvae feed in the fruit for 4 to 21 days, depending upon temperature. They burrow 2.5 to 12.5 cm in the ground to pupate. The pupal period varies from 4 days in summer to over 6 weeks in winter. It can apparently survive winters in temperate climates.

There are several generations a year if conditions are favorable (Christenson & Foote 1960; Fletcher 1987; Qureshi *et al.* 1993).

Current control methods of this pest rely heavily on the aerial application of malathion, bait sprays, or ground cover sprays of potent organophosphorus pesticides (Roessler 1989). These methods have a negative impact on the environment, and specifically on the Phytoparasitica populations of beneficial organisms. Thus, environmentally friendly methods of control are much in need (Roessler 1989).

In recent years, the Neem tree, *Azadirachta indica* A. Juss. (Meliaceae) has been used as a botanical insecticide (Koul *et al.* 1990; Ascher 1993). The first commercial Neem insecticide, Margosan-O, was registered by the EPA in the United States in July, 1985 (Jacobson 1989). Since that time, the EPA has exempted Margosan-O from food crop tolerances and several other commercial Neem insecticides have been developed worldwide (Ascher 1993; Schmutterer 1990).

The entomopathogenic nematode, *Steinernema feltiae,* is highly infective on a wide range of insect hosts (Poinar 1990). Lindegren and Vail (1986) demonstrated that late 3rd instar larvae of the fruit flies *Ceratitis capitata*, *Dacus cucurbitae* and *Dacus dorsalis* were susceptible to *Steinernema* (= *Neoaplectana*) *carpocapsae* and ceased feeding soon after infection, but pupae were not susceptible. Mahmoud and Osman (2006) found that 3rd instar larvae and 1 day old pupae of *B. zonata* were significantly more susceptible to nematode infection than 2nd instar larvae and 4, 6 days old pupae at all concentrations of *S. feltiae* tested.

Recently, the combination of entomopathogenic nematodes and botanical insecticides based on azadirachtin has opened up new possibilities of promising control methods against the peach fruit fly, *B. zonata*. The subject of the present study was to evaluate the combined effect of the botanical insecticides NSK extract, NeemAzal T 5%, Neemix 4.5% and the entomopathogenic nematode *S. feltiae* for possible successful use against *B. zonata*.

## MATERIALS AND METHODS

(1) NSK extract – an aqueous suspension of neem was prepared by stirring 20 g of powdered NSK in 100 ml of distilled water for 3–4 h, followed by filtering through a fine muslin cloth. The remains on the cloth were squeezed into the beaker. The freshly prepared suspension was taken as a 20% stock solution, which was diluted to lower concentrations with distilled water to 0.6, 1.25, 2.5, 5.0 and 10.0%.

(2) NeemAzal T 5% – a commercial formulation and was tested as suspension in distilled water at concentrations of 0.15, 0.3, 0.6, 1.25 and 2.5%.

(3) Neemix 4.5% – a commercial formulation and was tested as suspension in distilled water at concentrations of 0.1, 0.2, 0.5, 1.0 and 2.0%.

The peach fruit fly, *B. zonata,* used in the present study was taken from a population initially obtained from infested peaches in Sinai orchards and reared in the laboratory at 23 ± 2°C and 65–75% RH under a 12L:12D photoperiod on the artificial diet developed by Mahmoud (1997) for *Ceratitis capitata* (Wied.). *Steinernema feltiae* on the other hand, was obtained from Prof. Dr. Sergei E. Spiridonov, Institute of Parasitology, Moscow, Russia, and was cultured *in vitro* by using a foam substrate.

Infective nematodes were extracted by the Baermann funnel technique, collected and stored in sterilised distilled water at 5°C for 7–21 days before use (Woodring & Kaya 1988).

The appropriate number of nematodes was counted under a microscope and added to the filter paper in 1.0 ml distilled water in a Petri dish to obtain concentrations of 50, 100, 200, 400 and 800 infective juveniles/ml.

The effect of combinations with azadirachtin was tested using a filter paper assay. Five 3rd instar larvae of *B. zonata* were placed in a Petri dish of 9 cm diameter, lined with a filter paper disk. Different concentrations of the nematodes *S. feltiae* and of NSK, NeemAzal T 5% and Nemix 4.5%, respectively, were applied to the filter paper. In this bioassay, pest larvae were exposed to the combinations of insecticides and nematodes for 72 h, at which larval mortality was recorded.

Analyses for additive, antagonistic, or synergistic interactions was based on a binomial test and comparison of the expected and observed mortality rates as adapted from Robertson and Preiser (1992).

Expected mortality at a set concentration of insecticides based on azadirachtin and the entomopathogenic nematode was based on formula $P_E = P_0 + (1-P_0)/(P_1) + (1-P_0)(1-P_1)/(P_2)$, where $P_E$ is the expected mortality of a combination of nematodes and insecticides, $P_0$ is the natural mortality, $P_1$ is the mortality after treatment with insecticide alone, and $P_2$ is the mortality after treatment with the nematode alone.

$X^2 = (L_0 - L_E)^2/L_E + (D_0 - D_E)^2/D_E$ , with $L_0$ the number of living larvae observed, $L_E$ the number of living larvae expected, $D_0$ the number of dead larvae observed, and $D_E$ the number of dead larvae expected. Additivity was indicated if $X^2 < 3.84$. Antagonism was indicated if $X^2 > 3.84$ and $P_C < P_E$,

Table 1. Effect of interaction between NSK extract (azadirachtin) and the entomopathogenic nematode *S. feltiae* on mortality rate of 3rd instar larvae of *B. zonata*

| Concentration of Neem (azadirachtin) | Infective juvenile nematodes | Mortality (%) | | $X^2$ | Response |
|---|---|---|---|---|---|
| | | observed | expected | | |
| 0 | 0 | 0 | – | – | |
| 0.6 | 0 | 13.3 | – | – | |
| 1.25 | 0 | 20.0 | – | – | |
| 2.5 | 0 | 26.6 | – | – | |
| 5.0 | 0 | 66.0 | – | – | |
| 10.0 | 0 | 80.0 | – | – | |
| 0 | 50 | 32.0 | – | – | |
| | 100 | 52.0 | – | – | |
| | 200 | 68.0 | – | – | |
| | 400 | 80.0 | – | – | |
| | 800 | 88.0 | – | – | |
| 0.6 | 50 | 40.0 | 15.0 | 48.9 | ++ |
| | 100 | 44.0 | 10.2 | 124.7 | ++ |
| | 200 | 52.0 | 9.2 | 219.2 | ++ |
| | 400 | 56.0 | 8.4 | 294.4 | ++ |
| | 800 | 76.0 | 10.5 | 456.4 | ++ |
| 1.25 | 50 | 44.0 | 25.5 | 17.9 | ++ |
| | 100 | 60.0 | 21.5 | 87.7 | ++ |
| | 200 | 72.0 | 19.8 | 171.5 | ++ |
| | 400 | 84.0 | 21.5 | 231.3 | ++ |
| | 800 | 88.0 | 19.7 | 294.8 | ++ |
| 2.5 | 50 | 72.0 | 56.8 | 9.4 | ++ |
| | 100 | 80.0 | 38.9 | 71.02 | ++ |
| | 200 | 92.0 | 36.4 | 133.5 | ++ |
| | 400 | 96.0 | 30.4 | 213.7 | ++ |
| | 800 | 100 | 28.8 | 247.2 | ++ |
| 5.0 | 50 | 92.0 | 100 | 0.6 | + |
| | 100 | 96.0 | 100 | 0.16 | + |
| | 200 | 100 | 94.6 | 5.7 | ++ |
| | 400 | 100 | 80.4 | 24.3 | ++ |
| | 800 | 100 | 73.1 | 36.7 | ++ |
| 10.0 | 50 | 100 | 100 | – | ± |
| | 100 | 100 | 100 | – | ± |
| | 200 | 100 | 100 | – | ± |
| | 400 | 100 | 97.7 | 2.35 | + |
| | 800 | 100 | 88.9 | 1.12 | + |

+ additive, – antagonism, ++ synergism, ± without response

where $P_C$ is the observed mortality from an insecticide and nematode combination, and $P_E$ is the expected mortality by the combination. Synergism was indicated if $X^2 > 3.84$ and $P_C > P_E$.

## RESULTS

The mortality responses to combinations of botanical insecticides based on azadirachtin

Table 2. Effect of interaction between NeemAzal T 5% and the entomopathogenic nematode *S. feltiae* on mortality rate of 3rd instar larvae of *B. zonata*

| Concentration of NeemAzal T 5% | Infective juvenile nematodes | Mortality (%) | | $\chi^2$ | Response |
|---|---|---|---|---|---|
| | | observed | expected | | |
| 0 | 0 | 0 | – | – | |
| 0.15 | 0 | 12.0 | – | – | |
| 0.3 | 0 | 16.0 | – | – | |
| 0.6 | 0 | 44.0 | – | – | |
| 1.25 | 0 | 76.0 | – | – | |
| 2.5 | 0 | 92.0 | – | – | |
| 0 | 50 | 32.0 | – | – | |
| | 100 | 52.0 | – | – | |
| | 200 | 68.0 | – | – | |
| | 400 | 80.0 | – | – | |
| | 800 | 88.0 | – | – | |
| 0.15 | 50 | 48.0 | 16.2 | 33.12 | ++ |
| | 100 | 64.0 | 18.2 | 137.4 | ++ |
| | 200 | 72.0 | 44.9 | 29.6 | ++ |
| | 400 | 88.0 | 81.5 | 2.7 | + |
| | 800 | 100 | 100 | – | ± |
| 0.3 | 50 | 60.0 | 21.1 | 90.8 | ++ |
| | 100 | 72.0 | 21.1 | 155.5 | ++ |
| | 200 | 96.0 | 60.09 | 53.7 | ++ |
| | 400 | 100 | 92.8 | 7.7 | ++ |
| | 800 | 100 | 100 | – | ± |
| 0.6 | 50 | 68.0 | 23.8 | 107.7 | ++ |
| | 100 | 84.0 | 24.0 | 197.3 | ++ |
| | 200 | 100 | 62.0 | 61.2 | ++ |
| | 400 | 100 | 92.8 | 7.7 | ++ |
| | 800 | 100 | 100 | – | ± |
| 1.25 | 50 | 100 | 34.0 | 194.1 | ++ |
| | 100 | 100 | 28.6 | 249.5 | ++ |
| | 200 | 100 | 62.7 | 59.2 | ++ |
| | 400 | 100 | 93.0 | 7.5 | ++ |
| | 800 | 100 | 100 | – | ± |
| 2.5 | 50 | 100 | 34.0 | 194.1 | ++ |
| | 100 | 100 | 28.6 | 249.5 | ++ |
| | 200 | 100 | 62.6 | 59.4 | ++ |
| | 400 | 100 | 92.9 | 7.6 | ++ |
| | 800 | 100 | 100 | – | ± |

+ additive, – antagonism, ++ synergism, ± without response

(NSK, NeemAzal T 5% and Neemix 4.5%) with the entomopathogenic nematode *S. feltiae* were primarily synergistic (Tables 1–3), namely 18 out of 25 combinations with NSK (Table 1), 19 out of 25 with NeemAzal T 5% (Table 2) and 11 out of 25 with Neemix 4.5% (Table 3). The responses were synergistic in 76.0% of the combinations with NeemAzal T 5% at concentrations of 0.15, 0.3, 0.6,

Table 3. Effect of interaction between Neemix 4.5% and the entomopathogenic nematode *S. feltiae* on mortality rate of 3rd instar larvae of *B. zonata*

| Concentration of Neemix 4.5% | Infective juvenile nematodes | Mortality (%) | | $\chi^2$ | Response |
|---|---|---|---|---|---|
| | | observed | expected | | |
| 0 | 0 | 0 | – | – | |
| 0.1 | 0 | 28.0 | – | – | |
| 0.2 | 0 | 44.0 | – | – | |
| 0.5 | 0 | 60.0 | – | – | |
| 1.0 | 0 | 84.0 | – | – | |
| 2.0 | 0 | 88.0 | – | – | |
| 0 | 50 | 32.0 | – | – | |
| | 100 | 52.0 | – | – | |
| | 200 | 68.0 | – | – | |
| | 400 | 80.0 | – | – | |
| | 800 | 88.0 | – | – | |
| 0.1 | 50 | 28.0 | 22.8 | 1.53 | + |
| | 100 | 44.0 | 35.5 | 3.15 | + |
| | 200 | 52.0 | 20.2 | 62.7 | ++ |
| | 400 | 80.0 | 26.6 | 146.0 | ++ |
| | 800 | 84.0 | 25.5 | 180.1 | ++ |
| 0.2 | 50 | 36.0 | 47.06 | 4.9 | – |
| | 100 | 52.0 | 42.2 | 3.93 | ++ |
| | 200 | 68.0 | 42.3 | 21.1 | ++ |
| | 400 | 88.0 | 46.7 | 68.5 | ++ |
| | 800 | 92.0 | 44.4 | 91.7 | ++ |
| 0.5 | 50 | 50.0 | 90.3 | 185.4 | – |
| | 100 | 76.0 | 86.2 | 8.7 | – |
| | 200 | 88.0 | 75.5 | 8.4 | ++ |
| | 400 | 96.0 | 70.09 | 31.9 | ++ |
| | 800 | 96.0 | 63.7 | 45.1 | ++ |
| 1.0 | 50 | 92.0 | 100 | 0.6 | + |
| | 100 | 92.0 | 100 | 0.6 | + |
| | 200 | 100 | 100 | – | ± |
| | 400 | 100 | 100 | – | ± |
| | 800 | 100 | 93.4 | 7.06 | ++ |
| 2.0 | 50 | 100 | 100 | – | ± |
| | 100 | 100 | 100 | – | ± |
| | 200 | 100 | 100 | – | ± |
| | 400 | 100 | 100 | – | ± |
| | 800 | 100 | 97.9 | 2.1 | + |

+ additive, – antagonism, ++ synergism, ± without response

1.25, 2.5 and with all nematode concentrations; in 72% of combinations with NSK at concentrations of 0.6, 1.25, 2.5, 5.0; but only in 44% of the combinations with Neemix 4.5% at concentrations of 0.1, 0.2 and 0.5. The percentage of combinations between botanical insecticides and the entomopathogenic nematode that elicited no response was 12% for NSK, 20% for NeemAzal T 5% and 24% for Neemix 4.5%. An additive response was observed in 16% of the combinations with NSK, and in 4% with NeemAzal T 5%. There was no antagonistic response in any of the combinations with NSK and NeemAzal T 5%, whereas 12% with Neemix 4.5% indicated an antagonistic response (Tables 1–3).

The combined effects of botanical insecticides based on azadirachtin and entomopathogenic nematode differed from applications of the insecticides alone or nematodes alone. Most combinations of the nematode with NSK, NeemAzal T 5% or Neemix 4.5% significantly increased host mortality.

## DISCUSSION

Combinations of entomopathogenic nematodes and chemical insecticides have been shown to be synergistic in insect suppression (Koppenhofer & Kaya 1998; Nishimatsu & Jackson 1998). Increased efforts in recent years have been focused on biological control using entomopathogenic nematodes of the families Steinernematidae or Heterorhabditidae combined with insecticides and insect growth regulators (Mannion *et al.* 2000).

Entomopathogenic nematodes are tolerant to many herbicides and fungicides but sensitive to certain insecticides and nematicides (Grewal 2000). The entomopathogenic nematode *Steinernema feltiae* has been used successfully to suppress a wide range of insect pests including a variety of caterpillars, cutworms, corn borers, grubs, corn root worm, thrips, fungi gnat, and beetles (Kaya & Koppenhofer 1999).

Botanical insecticides based on azadirachtin have favorable toxicological properties, rapid degradation, low residues and are safe for the consumer. The rapid degradation makes it necessary to choose the time of application carefully to obtain a high bioefficacy in the field (Kleeberg 2001). In several crops, researchers have proposed different mechanisms as reasons for the increased efficacy such as increased activity and changes in nematode behavior. However, the most common suggestion is that the insecticide weakens the host insect, making it more susceptible to nematode attack.

The interaction between botanical insecticides based on azadirachtin and nematodes may allow for reduced chemical application rates. Additionally, the nematodes may become established and begin to offer a long term reduction in the larval populations (Klein & Georgis 1992). In the present work, most combinations resulted in synergistic responses, especially in NeemAzal T 5% and NSK. This study gives additional support to the importance of combinations between botanical insecticides and entomopathogenic nematodes for the control of insect pests.

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