*J. Korean Soc. Appl. Biol. Chem.* **53**(5), 598-604 (2010) **Article**

# Effects of Azadirachtin and Neem-based Formulations for the Control of Sweetpotato Whitefly and Root-knot Nematode

**Ohn Mar Lynn†, Woo-Geun Song, Jae-Kyoung Shim, Jang-Eok Kim, and Kyeong-Yeoll Lee***

*School of Applied Biosciences, Kyungpook National University, Daegu 702-701, Republic of Korea*

Received February 12, 2010; Accepted May 31, 2010

**Toxicity of the plant-derived natural pesticide azadirachtin and two types of commercial neem (*Azadirachta indica* A. Juss)-based formulations, Neema (liquid type) and Neema-plus (pellet type), were evaluated based on the mortality rate and developmental inhibition of the sweetpotato whitefly (*Bemisia tabaci*) and root-knot nematode (*Meloidogyne incognita*). In a laboratory assay, when *B. tabaci* adults were fed leaves containing 5 or 10 ppm of azadirachtin solutions, the rates of female oviposition, subsequent egg hatch, and adult eclosion were significantly reduced to 23.1, 53.2, and 26.6% of the control, respectively. At a tomato greenhouse, the rates of adult colonization, oviposition and egg hatch were reduced to 78.2, 47.0, and 71.2% by Neema foliar spray and 31.3, 34.1, and 66.8% by soil treatment with Neema-plus relative to the control, respectively. When isolated soil nematodes were exposed to various concentrations of azadirachtin, Neema, and Neema-plus, the immobility of juvenile nematodes showed no change at 2 h after treatment, whereas a reduction of 36.3% was observed at day 1 with 10 ppm of azadirachtin. Nevertheless, the effects of neem formulations were faster and much higher than those of azadirachtin. At a cucumber greenhouse, soil treatments with neem formulations significantly reduced the numbers of soil nematodes and plant root-knots; the reduction with Neema was 12.1 and 9.0%, and with Neema-plus 26.4 and 24.6% of the control, respectively. Furthermore, soil treatment with Neema-plus greatly improved the growth of cucumber plants in nematode-infested pots. These results showed that azadirachtin and neem-based formulations were highly effective on the developmental inhibition of both whiteflies and root-knot nematodes. Thus, soil application of the neem-based formulations would be applicable for the control of both leaf-sucking and soil pests.**

**Key words:** azadirachtin, *Bemisia tabaci*, neem, plant-derived pesticides, root-knot nematodes

The neem tree (*Azadirachta indica* A. Juss) is the most well known plant with powerful insecticidal properties since its discovery in 1966 [Mordue (Luntz) *et al*., 2005; Morgan, 2009]. Although various parts of the neem plant have been used for pest control, neem seeds have been the main source for the production of commercial neem formulations [Copping and Duke, 2007]. Neem extract contains various compounds that are toxic to insects, among which azadirachtin, a triterpenoid of the limonoid class, is the most active compound for the inhibition of insect growth and development [Schmutterer, 1990; Ascher, 1993; Mordue (Luntz) and Blackwell, 1993]. It is effective on many groups of arthropods, as well as nematodes and annelids, but has no toxic effect on vertebrates [Mordue (Luntz) *et al*., 2005].

The roles of azadirachtin have been identified in the physiological systems of digestion, endocrine, and reproduction of insects [Mordue (Luntz) *et al*., 2005]. Azadirachtin has a strong antifeedant activity on various phytophagous insects. In addition, when larval insects ingest this compound, their growth and development are inhibited due to the blocking of the biosynthesis of insect hormones, such as ecdysteroids. Furthermore, when insects ingest this compound, the development of reproductive organs, such as the ovary and testis, is significantly inhibited, and the fertility and fecundity of the adults are also reduced. Recently, there has been a resurgence of research devoted to improving the insect-controlling efficacy in practical applications for a specific pest in the field. In addition, recently developed molecular

†Present address: Department of Entomology, Yezin Agricultural University, Myanmar

*Corresponding author
Phone: +82-53-950-5759; Fax: +82-53-950-6758
E-mail: leeky@knu.ac.kr

doi:10.3839/jksabc.2010.092

techniques have been applied for the study of its mode of action and molecular interaction at both cellular and molecular levels [Salehzadeh *et al*., 2003; Robertson *et al*., 2007].

Various types of neem-based commercial products have been used for the control of several pests attacking crops in different ways [Copping and Duke, 2007]. Herein, we focused on two major greenhouse pests: the sweetpotato whitefly (*Bemisia tabaci*), which damages plants by leaf sucking, molding by honey dew production, and plant virus transmission, and the root-knot nematode (*Meloidogyne* spp.), which attacks the roots by making galls in the soil. Both pests cause great damages to most horticultural crops, including tomato, cucumber, sweet melon, paprika, and pepper crops. Recently, damage caused by *B. tabaci* has increased significantly, particularly in tomato greenhouses in Korea. Many tomato cultivars have been extensively damaged by tomato yellow leaf curl virus (TYLCV) disease, which is only transmitted by a single vector, *B. tabaci* [Czosnek, 2007]. Root-knot nematode problems are particularly serious in fields repeatedly cultivated with a single crop.

Due to pesticide resistance and the difficulties associated with their application, both *B. tabaci* and root-knot nematodes have not been successfully eliminated by the application of synthetic pesticides. Thus, from an environmental perspective, azadirachtin is an exceptionally good control agent as it is a plant-derived natural pesticide [Morgan, 2009]. As a rule, neem products have been applied as a foliar spray to plants. Recently, soil application has been known to be effective for the control of whiteflies and for its ease of application in the greenhouse [Kumar *et al*., 2005; Kumar and Poehling, 2006].

In the present study the insect-controlling efficacy of azadirachtin and two different types of neem-based formulations (liquid and pellet) were examined on both the sweetpotato whitefly and soil nematodes, especially the root-knot nematodes. The effects of soil application of neem-products for the control of both pest species in the greenhouse were also evaluated.

## Materials and Methods

**Azadirachtin and neem-based formulations.** Azadirachtin (>95% purity; Sigma-Aldrich, St Louis, MO) was dissolved in isopropanol and diluted with distilled water. All solutions were kept at −20°C until used. Two types of commercial neem-based formulations obtained from Bicosys (Kajo, Korea) were used: Neema, a liquid-type formulated with the extracted oil of neem seeds, and Neema-plus, a pellet-type formulated with seed remnants after oil extraction. Various dilutions of Neema were made with tap water. Neema-plus was ground with an electric blender before various dilutions were made with tap water.

**HPLC analysis of neem-based formulations.** A standard solution of azadirachtin was prepared by dissolving it in an HPLC-grade acetonitrile. Serial dilutions were made in the range of 40 to 2 μg/mL to plot the calibration curve. The sample (20 μL) was injected into an HPLC (Acme 9000 HPLC system; Younglin Instrument Co., Name of City, Korea). The separation of azadirachtin was achieved using an acetonitrile-water gradient at a flow rate of 1 mL/min, and the peaks were monitored at 215 nm.

Either Neema or Neema-plus were mixed with 20 mL water: acetonitrile (1:1). After adding 20 mL hexane, the tube was shaken for 10 min and filtered, and the upper layer was discarded. Sample aliquots were transferred to 250-mL separator funnels and mixed with 100 mL distilled water, 50 mL saturated NaCl, and 50 mL dichloromethane. The mixture was shaken for 10 min, and the aqueous phase containing azadirachtin was extracted two times with dichloromethane. The pooled layer was dried at 30°C using a rotary evaporator. The residue was dissolved in 10 mL of ethyl acetate and analyzed by HPLC.

**Collection of sweetpotato whiteflies and soil nematodes.** *B. tabaci* was reared on tomato and cucumber plants in insect-proof cages measuring 45×60×90 cm at 27°C and 70% relative humidity (RH). Experimental plants, including approximately 4-week-old cucumber plants and 6-week-old tomato plants, had at least four fully developed leaves. Soil heavily infested by root-knot nematodes was obtained from a cucumber greenhouse in Gunwi, Korea. Nematodes were extracted from soil samples using the Baermann funnel technique [Ronald and Thompson, 1983]. The standard Baermann apparatus consisted of a glass funnel with a mesh brass wire screen placed approximately 3 cm below the top of the funnel. A rubber tube was connected to the bottom of the funnel, and a clamp was attached to the tube approximately 10 cm below the funnel. Nematodes were forced out of the soil by filling the funnel with water, thereby driving the nematodes into a vessel. The nematodes extracted from the soil were then counted under a dissection microscope.

Species of root-knot nematodes were identified by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Genomic DNA was extracted from female nematodes isolated from root-knots in cucumber plants. Primers used were previously designed to amplify the region between COII and LrRNA