The Potential of *Beauveria brongniartii* and Botanical Insecticides Based on Neem to Control *Otiorhynchus sulcatus* Larvae in Containerised Plants – Short Communication

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Abstract

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Otiorhynchus sulcatus is considered as an important pest of strawberry fields and on potted ornamental plants. The efficacy to control this pest by new Polish products containing azadirachtin and the entomopathogenic fungus *Beauveria brongniartii* were tested. The aim of the laboratory investigation was to assess the influence of different forms of azadirachtin (A and B) and the entomopathogen on mortality and physiological development of the insect. Mortality after treatment ranged between 86–93%. There were significant differences in the mean number of surviving stages of the insect between *Beauveria* and neem treatments, but the final total mortality was not statistically different. Sensitivity of all developmental stages to the fungus was observed. In the neem treatments the physiological development of larvae was completely stopped. The level of control achieved by *Beauveria* and neem for use in outdoor containers is acceptable for practical application.

Keywords: *Otiorhynchus sulcatus; Beauveria brongniartii*; azadirachtin; control

Both adults and larvae of the black vine weevil, *Otiorhynchus sulcatus* Fabricius (Coleoptera: Curculionidae) are serious pests of small fruits, horticultural crops and ornamental containerised plants in nurseries. The species is parthenogenetic, long-lived and highly fecund (Moorhouse *et al.* 1992). Insecticide treatments of plant foliage can reduce the population of adults but, unfortunately, they are not selectively toxic and have negative ecological consequences. Larvae are extremely difficult to control with pesticides. Yet they are the most dangerous because one larva can consume almost all the roots of a seedling, leading to economical losses. Many control strategies have been developed, including application of insectivorous nematodes (Vainio & Hokkanen 1993; Sampson 1994), entomopathogenic fungi (Stenzel 1992; Tkaczuk *et al.* 2005) and botanical insecticide (Cowles 2004; Gaffney *et al.* 2005). Numerous studies have shown that entomopathogenic nematodes (EPNs) are the most effective against later instars of the weevil larvae in greenhouse containers. They can be applied through the irrigation system that must be minimised due to the low volumes of the containers and the desired long retention of EPNs in the containers. Test trials have also shown that entomopathogenic nematodes are ineffective against the pupae and adults of the black vine weevil. Thus, different pest management strategies must only target susceptible life stages.

One promising natural insecticide is a botanical insecticide derived from the neem tree (*Azadirachta indica* A. Juss), is effective against many insect groups, and is biodegradable (Barrek *et al.* 2004). Azadirachtin ($C_{35}H_{44}O_{16}$) is a compound belonging to the limonoids. The seed extract contains a mixture of several structurally related tetranotriterpenoids. The high insecticidal activity is due to the major tetranotriterpenoid isomer azadirachtin A (AZ-A) (Schmutterer 1990). The primary active ingredient of most neem-based pesticides has shown an excellent insecticidal activity against lepidopteran larvae (Koul *et al.* 2004) and insect pests of stored grain (Koul 2004). Antifeedant and growth inhibitory activity of azadirachtin was studied earlier (Koul *et al.* 1990; Szczepanik *et al.* 2000). Over 400 species of insects are susceptible to azadirachtin (Sampson 1994; Schmutterer & Sing 1995). Azadirachtin can be applied in combination with *Bacillus thuriengiensis* (Sing 2007). A high concentration of azadirachtin can act as a repellent to insects, potentially it can also disrupt reproduction. Current research shows that azadirachtin applied on the plant surface affects aphids (Stark 1992). Neem extracts have minimal toxicity to non-target organisms such as parasitoids, predators and pollinators (Naumann 1996) and degrade rapidly in the environment (Barrek *et al.* 2004). Nevertheless, the rapid degradability is a disadvantage for foliar applications. For this reason, optimal timing of an application with higher concentrations of azadirachtin is necessary (Pavela & Holy 2003). Further, new types of applications, such as tree injection (Naumann 1994) and soil applications (Soundaram *et al.* 1995) resulting in uptake by the root system are desirable. The roots of plants take up dissolved azadirachtin and incorporate it into their tissue as a natural metabolite (Zounos 1999). The effects on insect pests by azadirachtin taken up by the root system is not clear. Different formulations of products obtained from the neem tree were used in the protection of field crops (Pavela & Holy 2003), greenhouse crops (Surviliene & Raudonis 2003; Premachandra *et al.* 2005) and in the forest (Malinowski 2002). Plant protection products containing azadirachtin are recommended to control Curculionidae insects in the field. Azadirachtin is registered in many countries as a relatively non-toxic, general pesticide. Two forms of azadirachtin exist: A and B. However, azadirachtin A is most frequently used to produce this botanical insecticide.

The objective of this study was: (*i*) to evaluate different forms of azadirachtin and *Beauveria brongniartii* (Saccardo) Petch in their control of black vine weevil larvae, (*ii*) to compare the effectiveness of Polish plant protection products that are under development with a commercially available product, and (*iii*) to assess the possibility of using these products as a preventive method to protect containerised plants.

MATERIAL AND METHODS

Insect. The larvae of *O. sulcatus* were collected from infected plants of *Heuchera* spp. growing in containers in the nursery.

Biological control agent. *Beauveria brongniartii* is a pathogen which occurs naturally in soils. It is commonly isolated from larvae and adults of *Otiorhynchus* spp. The product named Bovecol (Rol-Eko, Poland) containing a Polish isolate of *B. brongniartii* and propagated on seeds of wheat was used. It will be registered in the near future.

Chemicals. A water-based solution of NeemAzal-T/S (1% of Azadirachtin A, Trifolio, Germany) was used at the concentration 0.5%. Treex-Bio (3% Azadirachtin A plus B, Pestinova, Poland) was applied at the same concentration.

Bioassays. Laboratory trials were performed in PVC pots (diameter 6 cm) without plants, filled with loamy soil mixed with peat. Three larvae of *O. sulcatus* were placed into each pot. Each of the four variants consisted of 20 replicates of 3 larvae per pot. The test were done under laboratory conditions at room temperature of 21°C, with 40% RH and 8:16 h as an L:D. The first variant used Bovecol; three kernels of wheat covered with *B. brongniartii* were applied to the soil of each pot, at deep 2 cm (equivalent to a rate of 120 kg/ha). In the second and third variants with azadirachtin, 2 ml of a water solution of NeemAzal-T/S and Treex-Bio, resp., were applied to the surface of the soil in the pots The control variant was 2 ml water applied to the surface of the soil. The efficacy of treatments was evaluated in October/November during 30 days by counting dead larvae, pupae and adults, every second day. After 30 days the total mortality of insects in each variant was assessed.

Statistical analysis. The data obtained were normalised through Freeman-Tukey's transformation and subjected to ANOVA. The significance of differences was examined using Tukey's multiple range test at $P < 0.05$.

Table 1. Effect and influence of the tested insecticides on physiological development and mortality of *Otiorhynchus sulcatus* larvae in containers under laboratory conditions, 30 days after treatment

Treatment	Rate	Efficacy of control (%)		FOI	Mean of surviving per pot		Total
		larvae	pupae	DAT	larvae	imagines	mortality/pot
Bovecol	120 kg/ha	26.6	60	12	2.10 ± 0.55 b	0.75	2.60 ± 0.5 a
NeemAzal-T/S	0.5%	92.8	0	6	0.60 ± 0.68 c	0	2.75 ± 0.44 a
Treex-Bio	0.5%	92.8	0	4	0.90 ± 0.55 c	0	2.75 ± 0.44 a
Control (untreated)	water	0	0		3.00 ± 0.0 a	2.95	0.00 ± 0.0 b

Significantly different from control at $P < 0.05$, $n = 3$/pot; FOI – first dead insects; DAT – day after treatment

RESULTS AND DISCUSSION

The level of mortality of *O. sulcatus* depended on the treatment (Table 1). All treatments gave a very good control effect. In the variant treated with *B. brongniartii,* the larvae, pupae, and imagoes of the insect were infected by the fungus. Treatment with azadirachtin completely stopped the physiological development of larvae. This observation finds support from other studies, which showed the growth-disruptive properties of neem formulations (Gaffney *et al.* 2005). Any insects that recovered from one of the neem treatments were in the larval stage. Differences between effects caused by products containing different forms of azadirachtin (A, A + B) were observed only at the onset of insect mortality. The first dead insects (FOI) were found 4 days after application of Treex-Bio and 6 days after NeemAzal-T/S. In the *Beauveria* treatment the best results of control were observed after 12 days; thus, the time to kill the insect with the fungus was longer than that with the neem treatments and some of larvae began pupation and only than were infected by fungus. The results reported by other researchers support that all stages of *O. sulcatus* are susceptible to the different entomopathogenic fungi used in field trials (Zimmerman 1996; Tkaczuk *et al.* 2005). There were significant differences between the mean numbers of surviving insects at different life stages after *Beauveria* and neem treatments, but the total mortality after different treatments was not statistically different. The efficacy of control of all treatments were satisfactory for practical use. This evaluation shows that the formulations of *Beauveria* and neem are acceptable for use in the control of black vine weevil larvae in outdoor containers. The two tested Polish products under development are promising for use in practice in the future and could be used as preventive methods to protect potted plants, thus avoiding economical losses by growers. Poland urgently needs registration of new bio-insecticides, which could also be used for the protection of organic crops. At present only seven insecticides are qualified for use on organic crops in Poland, against mites, and codling moth in orchards.

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