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NoVil: Hunt for weevil control: Update!

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Abstract: The virulence of *Metarhizium robertsii* isolate CPD6 has been reported against pepper weevil, *Anthonomus eugenii* and cranberry weevil, *Anthonomus musculus*, and the isolate is being developed as mycoinsecticide under the trade name of NoVil. In addition to these two insects, NoVil has been found to be pathogenic against other arthropods that are pests of greenhouse pepper (*Capsicum* spp.). In bioassays, NoVil caused mortality of 97.0 ± 3.0% of adult tarnished plant bug, *Lygus lineoralis*, 4 days post-inoculation at the concentration of 10^8 conidia/ml. Spray applications of NoVil in the greenhouse resulted in reduction over the control of 87.6% of green peach aphid, *Myzus persicae*, on pepper plants and 80.8% of two-spotted spider mite, *Tetranychus urticae*, on cucumber plants. Preliminary results seem also to indicate that NoVil is compatible with other biocontrol agents and could play an important role as a component of integrated pest management (IPM) of pepper crop.

Key words: arthropod pests, pepper plant, entomopathogenic fungus, *Metarhizium*, aphid, mite, lygus, control

Introduction

Greenhouse pepper (*Capsicum* spp.) is host to many arthropod pests including green peach aphid (GPA), *Myzus persicae*, Western flower thrips, *Frankliniella occidentalis*, two-spotted spider mite (TSSM), *Tetranychus urticae*, whitefly, *Trialeurodes vaporariorum*, pepper weevil, *Anthonomus eugenii*, and tarnished plant bug (TPB), *Lygus lineolaris* (https://phytopath.ca/wp-content/uploads/2015/03/DPVCC-Chapter-24-greenhouse-pepper.pdf). Pepper weevil (PAW) is a quarantine pest from Mexico origin and has spread throughout most of Central America and the Caribbean, and to the southern part of the United States and Canada (Costello and Gillespie, 1993; Clark and Burke, 1996). It was recently reported in Europe (Gaag et al., 2013; Speranza et al., 2014). The control of PAW includes the use of synthetic chemical insecticides and sanitation. Yellow sticky cards can also be used to monitor populations (Riley and Schuster, 1994).

Entomopathogenic fungi are being considered as alternatives to synthetic chemical insecticides for the control of adult PAW. A few fungal isolates belonging to the genera *Metarhizium, Beauveria* and *Isaria* were screened against pepper weevil in the laboratory (Maniania et al., unpublished) and among them, *Metarhizium robertsii* isolate CPD6 outperformed the other isolates and was selected as a potential mycoinsecticide candidate. In greenhouse, application of CPD6 against pepper weevil-infested pepper plants resulted in 70% reduction of weevil populations over the control, comparable to the synthetic chemical insecticide Flagship 25WG (Thiamethoxam) (Figure 1) (Maniania et al., unpublished). Furthermore, application of CPD6 reduced feeding, number of oviposition marks and adult

emergence as compared to the control. The fungal isolate has been given the tentative trade name of NoVil. Further tests showed that NoVil was virulent against cranberry weevil, *Anthonomus musculus,* in the laboratory (Maniania et al., unpublished). The objective of this report is to give an update on NoVil, specifically on its virulence against three other arthropod pests associated with greenhouse pepper crop, namely, GPA, TPB and TSSM.

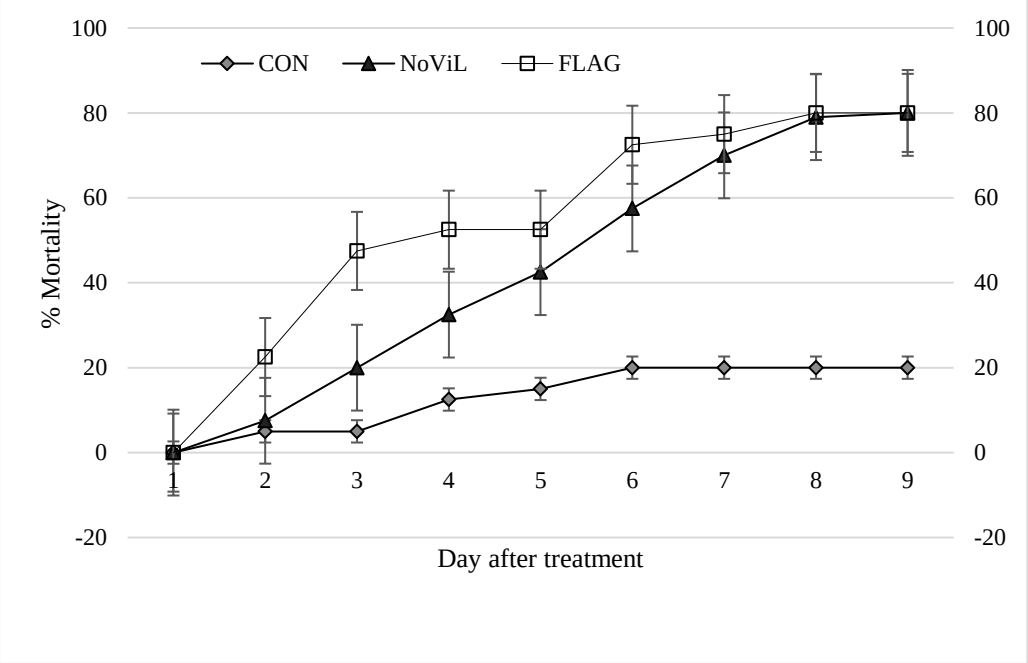

Figure 1. Efficacy of *Metarhizium robertsii* isolate CPD6 and insecticide Flagship against adult pepper weevil on pepper crop in greenhouse peppers.

Material and methods

Insects/Mite

Tarnished plant bugs were obtained from Dr. Maribel Portilla, USDA/ARS, Southern Insect Management Research Unit, Stoneville, MS. They were maintained on non-autoclaved semi-solid diet at room conditions (23-27 °C, 60-80% rh). The adult stage was used in the study. The GPA originated from naturally-infested four-month old pepper plants in the greenhouse. Since TSSM could not establish on pepper plants, two-month old cucumber plants were used instead. Plants were transferred individually in 1/2-gallon plastic nursery plastic pots in bugdorm (W 60 × D 60 × H 180 cm). Feritlizer solution was provided daily. Plants were treated when the pest population was high

Fungi

Three isolates of *Metarhizium robertsii* (CPD6, CPD30 and CPD31), one isolate of *M. brunneum* (CPD37), one isolate of *Beauveria bassiana* (CPD28) and one isolate of *Isaria fumosoroseus* were used for screening against adult TPB. They were obtained from Crop Defenders' Fungus Culture Collection and maintained on Sabouraud dextrose agar supplemented with 0.5% yeast extract. A conidial concentration of 1.0×10^8 conidia/ml was

prepared and used for bioassays against adult TPB. For greenhouse trials, conidia of CPD6 were produced on rice as substrate in SAC O_2 bags and harvested using a MycoHarvester (MH6). They were formulated in organic canola oil (0.5%) and Silwet 408 (0.05%), and corresponded to the rate of 1.0×10^{12} conidia/ha.

Treatments

Adult TPB were chilled for approx. 2 min to immobilize them. They were then dipped in the fungal suspension for 2-3 sec. and transferred to 9.5 cm-aerated plates whose bottom was lined with moist filter paper. 3.5 cm-pouches containing non-autoclavable diet was introduced in each plate and served as food. Insects in the control were dipped in 0.05% Silwet 408. Twenty insects were used per treatment and replicated four times. Mortality was recorded at day 4 and day 5 post-inoculation.

Pepper or cucumber-infested plants were removed from bugdorms and brought outside where they were sprayed using a Dramm BP-4 backpack sprayer. Control plants were treated with 0.5% organic canola oil and 0.05% Silwet 408. Plants were then returned to the bugdorms. For evaluation of the effectiveness of the treatments, population density was recorded before treatments and 7 days after treatments (7 DAT) by randomly collecting leaves from bottom, middle and top of the plant. Each plant in the bugdorm represented a replicate. Four cucumber plants were used for TSSM while 8 pepper plants for GPA.

Results and discussion

Virulence of NoVil against adult tarnished plant bug

Mortality in the control was 12.6 ± 2.3 and 31.1 ± 4.9% 4 and 5 days post-treatment, respectively. In the fungus treatments, mortality varied between 20.0 ± 9.6 and 97.0 ± 3.0% 4 days after treatment, with CPD6 causing the highest mortality, followed by CPD30 (Table 1). Mortality remained the same with these two fungal isolates after 5 days post-inoculation. However, a substantial increase in mortality was recorded with CPD28, reaching 95% (Table 1).

Table 1. Mortality of adult tarnished plant bug *L. lineolaris* following exposure to *M. robertsii, M. brunneum, B. bassiana* and *I. fumosorosea* isolates at the concentration of 10^8 conidia/ml.

Treatment	% Mortality (X ± SE)	
	4 DAT	5 DAT
Control	12.6 ± 2.3	31.1 ± 4.9
M. robertsii CPD6	97.0 ± 3.0	97.0 ± 3.1
M. robertsii CPD30	88.3 ± 1.5	88.3 ± 1.5
M. robertsii CPD31	40.5 ± 16.6	52.3 ± 17.6
M. brunneum CPD37	33.3 ± 17.6	60.0 2 ± 20.8
B. bassiana CPD28	36.0 ± 3.8	95.0 ± 1.0
I. fumosorosea (accession number not available)	20.0 ± 9.6	24.3 ± 9.4

Performance of NoVil against green peach aphid and two-spotted spider mite in greenhouse
GPA populations were siginificantly different between control and NoVil before and after application of treatments ($F_{1,27}$ = 26.6; P < 0.001) (Table 2). Significantly more aphids/plant were recorded in the control compared to the NoVil treatment 7 days post-treatment (Table 2).

TSSM population/plant was significantly higher in NoVil than in the control treatments ($F_{1,15}$ = 12.5; P < 0.001) before application of treatments (Table 2) but decreased significanlty ($F_{1,15}$ = 12.5; P < 0.001) in NoVil treatment 7 days post-treatment; while it increased considerably in the control treatment (Table 2).

Table 2. Performance of NoVil (*M. robertsii* isolate CPD6) against green peach aphid *M. persicae* and two-spotted spider mite *T. urticae* in greenhouse

Host/Treatment	Density per plant (X ± SE)		% decrease over the control
	Before treatment	7 days after treatment	
Green peach aphid			
Control	14.5 ± 4.2a	1477.4 ± 257.8a	-
NoVil	27.0 ± 9.1b	183.3 ± 65.0b	87.6
Two-spotted spider mite			
Control	171.3 ± 55.5a	560.3 ± 332.5a	-
NoVil	298.3 ± 49.3b	107.8 ± 13.0b	80.8

Within column and host pest, means (± SE) followed by the same letter are not significanntly different by the Tukey HSD test.

All the fungal isolates tested were pathogenic to adult TPB. However, mortality varied according to fungal species and fungal isolates. For example, *I. fumosorosea* was the least pathogenic compared to the other species, causing 24.3% mortality after 5 days. Among the isolates of *M. robertsii*, isolates CPD6 and CPD30 were virulent while CPD31 was moderately pathogenic against TPB. Interespecific and intraspecific variations among entomopathogenic fungi have been reported on many groups of arthropods (Bugeme et al., 2009; Migiro et al., 2010; Bayissa et al., 2016 a; Mweke et al., 2018). Screening for strain selection is therefore an important step in the development of entomopathogenic fungi as mycoinsecticides (Zimmerman, 1986).

Spray applications of *M. robertsii* isolate CPD6 (NoVil) against GPA and TSSM resulted in significant reduction of both pests 7 days post-treatment, 87.6 and 80.8% reduction in GPA and TSSM, respectively (Table 2). The efficacy of *Metarhizium* isolates in controlling aphids and spider mites in greenhouse and field studies have already been reported elsewhere. For instance, application of *M. anisopliae* ICIPE78 resulted in significant reduction of TSSM densities in greenhouse and field on common bean crop and yields increased on treated plants (Bugeme et al., 2015). Furthermore, Bayisa et al. (2016 a) reported a decline in the number of three species of aphids (*Brevicoryne brassicae*, *Lipaphis pseudobrassicae*) following application of *M. anisopliae* ICIPE62 on okra and crucifers in addition to negative effects on population growth of the aphids compared to the controls. Similarly, a good control of *Aphis craccivora* was achieved on cowpea crop with the same isolate of *M. anisopliae* (Mweke et al., 2019).

One of the advantages of mycoinsecticides, is their compatibility with other components of IPM, especially predators and parasitoids (Bayissa et al., 2016 b; Maniania et al., 2016; Saito and Brownbridge, 2018; Mweke et al., 2019). No negative effect of NoVil was observed on the predatory mite *Phytoseiulus persimilis* (Maniania, pers. observation).

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