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Effects of a commercial formulation of a plant resistance elicitor on non-target organisms

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Abstract: The toxicity of Fyto11, a plant resistance elicitor commercial formulation used to increase the natural resistance of Cucurbitaceae (cucumber, zucchini and melon) and Solanaceae (tomato and sweet pepper) against powdery mildew under greenhouse conditions, has been assessed on several beneficial arthropods: the honeybee *Apis mellifera*, the predatory mite *Typhlodromus pyri*, the aphid parasitic wasp *Aphidius rhopalosiphi* and the aphid predator *Episyrphus balteatus*.

Tested at 0.5% and 1.0% spray dilution on plants, Fyto11 did not lead to significant corrected mortalities for *E. balteatus* larvae and adult wasps of *A. rhopalosiphi*, with a maximum of 5.4% mortality. No effects on reproduction capacity of the parasitic wasps were observed.

The exposure of *T. pyri* protonymphs to Fyto11 on glass plates lead to corrected mortalities of 1.1% and 32.6% at 0.5% and 1.0% and to a reduction of female fertility of 9.0% and 25.4%, compared to the control performance. When assessed on the honeybee by contact and oral exposure, Fyto11 did not lead to significant effects, with LD50 estimated as > 10 µg a.i./bee (oral) and 12.5 µg a.i./bee (contact), leading to HQ ratio < 50 and classifying the product at no or low risk for honeybees.

These results showed that the product was harmless for all beneficials at the proposed commercial rate, except for *T. pyri* at 1.0% on glass plates, where the product was rated as slightly harmful. Even if all the beneficial species' diversity has not been assessed with this product, negative effects on non-target organisms are not expected in field conditions and Fyto11 can be considered as compatible with IPM programs.

Introduction

Fyto11 is a plant resistance elicitor commercial formulation used to increase the natural resistance of Cucurbitaceae (cucumber, zucchini and melon) and Solanaceae (tomato and sweet pepper) against powdery mildew under greenhouse conditions. This product is intended to be used in IPM and reduced pesticide systems context and be in contact with several beneficial arthropods used for pest control and pollination. Therefore, the compatibility of this product with beneficial was assessed on four different species: the honeybee *Apis mellifera* L. (Hym.; Apidae), the predatory mite *Typhlodromus pyri* Scheuten (Acari; Phytoseidae), the aphid parasitic wasp *Aphidius rhopalosiphi* (Destefani-Perez) (Hym., Aphidiidae) and the aphid predator *Episyrphus balteatus* (Degeer) (Dipt.; Syrphidae). The two first studies strictly followed the guidelines for the registration process and were performed in this context. The two last studies were performed to assess the compatibility of the product with IPM and the methods used followed IOBC standards for extended laboratory tests on natural substrates.

Material and methods

Test product

Fyto 11 is a commercial formulation containing 12.5 g/l Oligosaccharidic complex (COS-OGA). The product is used as foliar spray at a commercial rate of 0.5%, with repeated applications at one week interval in case of high diseases infection pressure. In this study, the product was tested at 0.5%, corresponding to the commercial field rate for a single application and at 1.0%, to simulate repeated application on the three beneficial arthropods species. The spray volume was 200 l/ha for glass plates tests and 400 l/ha (run-off) for extended lab test.

For honeybees, the products were topically applied on the bees (contact test) or offered on a sugar solution (oral toxicity test), on a range of different doses in order to determine the $LD_{50.}$ Water was used as control for all tests.

Test species and methods

For *A. rhopalosiphi* tests, the products were applied on barley seedlings infested with cereal aphids (*S. avenae*). Preliminary trials have shown that the products had no impact on aphid population development. Soon after treatments, adult wasps were confined on the plants. After 48h of exposure, the units were opened and the mortality was assessed. The plants and the aphids were further kept 10 days to let the aphid parasitized during the exposure to develop. The number of aphid mummies found per unit was then counted and the mean calculated. For each object, there were 10 replicates of 10 wasps (5 males, 5 females).

With *E. balteatus*, the products were applied till run-off at 0.5 and 1.0% on potted sweet pepper infested with *Myzus persicae*. Preliminary tests have shown that repeated applications (up to 4) of Fyto11 at 0.5% had no impact on *M. persicae* populations in these conditions. Soon after product application, 2-3 day old larvae were released on the plants. The larvae were left to develop on the plants, feeding on treated aphids, till pupation and adult emergence. Adults were then harvested and counted and the mortality recorded. There were 5 plants with 10 larvae for each object.

The test performed with *T. pyri* were similar than those used for registration studies on glass plates (open cell method, Blümel *et al*., 2001). The product was applied on glass plates, 2-3 day old protonymphs were confined on these plates during 7 days. They were then counted for mortality record, sexed and transferred into similar units previously treated for fertility assessment. At day 14, the number of eggs produced per female was counted and used to detect any sublethal effect. The mites were fed with *Pinus* trees pollen during all the experiments.

The tests performed with honeybees followed the OECD guidelines 213 and 214 for acute oral and contact toxicity (Anonymous, 1998a, b). For contact toxicity, the products were diluted in water + triton X and 1 µl droplets were topically applied on the thorax of bees. For oral toxicity test, the products were diluted in a water/sugar solution and offered to the bees for maximum 6 hours. The amount of product ingested was calculated by weighing the sucrose solution just before and after being offered to the bees. For each test, there were 4 replicates of 20 bees per dose and for the control. The mortality was recorded after 48h. The test product was assessed at 5 different doses in a serial dilution in order to estimate the LD_{50}.

Test conditions

Aphidius and *Episyrphus* tests were performed in a climatic room at 20 °C ± 2 °C, 60-90% RH, with a 16/8 L/D regime provided by sodium lamps (8,000-12,000 lux). *T. pyri* test was performed at 25°C ± 2 °C, 60-90% RH, with a 16/8 L/D regime provided by sodium lamps (about 1,200 lux). The honey bee test was performed in an incubator, in the dark at 25 ± 2 °C with humidity 50-70% RH.

Results

The results of the test performed on the three beneficial species are summarised in Table 1. With *A. rhopalosiphi*, the observed mortalities were lower than in the control (7.0% and 1.0% at 0.5% and 1.0% compared to 8.0% in the control). The aphid mummy production was also higher with Fyto11 than with the control, leading to an increase of the beneficial capacity. Fyto11 was classified as harmless at both tested rate.

With *E. balteatus*, the observed mortalities reached 26.0%, 30.0% and 24.0% in the control and with Fyto11 at 0.5% and 1.0%, respectively. Fyto11 was classified as harmless at both tested rates.

T. pyri was the only tested species slightly affected by the product at 1.0%, with a reduction in beneficial capacity of 50.3%, with a combination of effects on mortality and on fertility. No effects were observed at 0.5%. Fyto11 was classified as harmless at 0.5% and slightly harmful at 1.0%. However, it must be stressed that the test was performed on glass plates, known to be a very worst case scenario. According to this and to the absence of effects at 0.5%, no adverse effects of this product are expected in field conditions.

Table 1. Effects of Fyto11 applied at 0.5% and 1.0% on three beneficial arthropods species. Type of test, corrected mortalities, reduction in fertility performance, reduction in beneficial capacity (E) and IOBC classification

	Tier testing	Tested rates	Corrected mortality	Reduction of fertility performance	E	IOBC class
Aphidius rhopalosiphi	Extended laboratory test	0.5% 1.0%	-1.1% -7.6%	-48.0% -44.0%	-49.6%[1] -54.9%[1]	Harmless Harmless
Episyrphus balteatus	Extended laboratory test	0.5% 1.0%	5.4% -2.7%	-	5.4% -2.7%[1]	Harmless Harmless
Typhldromus pyri	Initial toxicity	0.5% 1.0%	1.1% 32.6%	9.0% 25.4%	10.0% 50.3%	Harmless Slightly harmful

[1] a negative value is indicating an increase in beneficial capacity (performance higher than in the control)

The results obtained with the honeybees are listed in Table 2. No tested doses, during the oral or the contact toxicity test lead to a corrected mortality higher than 50%. Therefore, the acute oral and contact LD50 of Fyto11 for workers of *A. mellifera* were estimated as higher than 10.0 µg a.i. and 12.5 µg a.i./bee, the highest tested rates. On basis of a field application of 12.5g a.i./ha, the calculation of HQ ratio rated Fyto11 as at no risk for honeybees, as the value were much lower than the threshold value of 50.

Table 2. Acute toxicity of Fyto11 for honeybee workers. 48h- oral and contact toxicity tests according to OECD guidelines and HQ ratio on basis of an application of 12.5g a.i./ha.

Tier testing	Tested rates	Effects	HQ ratio
Initial contact toxicity (OECD 214)	0.80-12.5 µg a.i./bee	No mortality > 50.0% LD50 > 12.5 µg a.i./bee	1
Initial oral toxicity (OECD 213)	0.78-10.0 µg a.i./bee	No mortality > 50.0% LD50 > 10 µg a.i./bee	1.25

Conclusions

The tests performed indicated that Fyto11 applied at 0.5% in the laboratory were not toxic for the aphid parasitic wasp *A. rhopalosiphi*, the hoverfly larvae *E. balteatus* and the predatory mite *T. pyri*. The only effect recorded was a slight increase of mortality and a slight decrease of fertility of the predatory mite on glass plates at 1.0%. No acute effects on honeybees were recorded. These results suggest that the use of Fyto11 will be compatible with IPM programs and that unlikely adverse effects are not expected.

References

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