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Toxicity of three pesticides to the European red mite *Panonychus ulmi* and its predator, *Typhlodromus* (*T.*) *setubali* (Acari: Phytoseiidae, Tetranychidae).

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Abstract

The present study was conducted to assess the contact toxicity of the recommended doses of three pesticides, namely avermectin (abamectin), Teppeki (flonicamid) and Thiovit based on sulfur, against *Panonychus ulmi* and its predator *Typhlodromus* (*T.*) *setubali* adults. In the laboratory trials, bioassays were performed with populations of mites collected in two plots with different crop protection backgrounds. Abamectin caused 97.48 % mortality within 24-48 hours of *P. ulmi* originating from the plot with a low frequency of treatments and 68.76 % among those collected in plot frequently treated. For both predator populations, mortality for flonicamid and sulfur was significantly higher than abamectin, which showed a selective effect against *T.* (*T.*) *setubali*, the highest mortalities were observed among individuals collected in the plot with a low frequency of treatments (65.39 % and 53.47 %, respectively). Results showed that the susceptibility of mites collected in the plot where these pesticides had been frequently used, was significantly reduced. Based on these results, if these pesticides are to be considered for integrated pest control programs in plots where *T.* (*T.*) *setubali* is present, their use should be minimized in plots where they have rarely been applied.

Keywords: Biological Control; Chemical Control; Management; Pesticides; Phytoseiidae; Toxicity.

Introduction

Currently, there are two economically important phytophagous mites in Moroccan apple orchards, the European red mite, *Panonychus ulmi* (Koch) and the two-spotted spider mite, *Tetranychus urticae* (Koch) **[1, 2]**. The immature and adult stages of these phytophagous mites and other mite pests cause a decrease in photosynthesis resulting in a decrease in the size of the fruits, which encourages their premature fall and affects the fruiting in the following season **[3, 4]**.

The innumerable effects of the use of acaricides on microarthropod pests have been hierarchically explored within an ecotoxicology framework applied to integrated pest management. Also, comprehensive risk assessments have increasingly explored the effects on non-target species, contrasting with the majority of efforts focused on target species **[5].** Given this intensive use of chemical pesticides**,** mite pests develop resistance against many active ingredients only after a few applications **[6]**, with the disruption of natural equilibriums within the agro-ecosystem scale and noxious consequences on health and environment **[7]**.

Or, chemical pesticides are not often friendly with natural enemies and causing pest resurgence **[8, 9].** Among the economically and ecologically useful microarthropods, Phytoseiidae is the most important family of plant inhabiting predatory mites. These mites are extensively used as biological control agents for the management of mite and insect pests of crops grown in greenhouses and in open field.

The interest for searching on phytoseiids has steadily increased worldwide during the last 50 years, resulting in a large number of papers regarding their taxonomy, biology, ecology and practical use **[10, 11]**. In the absence of these useful mites, whatever population of mite pest is left, it has an open field to multiply and destroy the crop almost completely. So, there is a great need to understand the effect of commonly used pesticides on the predatory mite like *T.* (*T.*) *setubali* **(**Dosse**)**, which is used as biological control agent of mite pests in Arbor orchard, Oulmes, Morocco, since its introduction in 2015 (Ouassat et al. unpublished data)**.** Moreover, integrated pest management (IPM) of Tetranychid mites is carefully achieved via biological control, provided mainly by phytoseiid mites **[12-14].**

The pesticides used in experiments are an acaricide (abamectin), an insecticide (flonicamid) and a fungicide (sulfur). These specialties are marketed by Syngenta in Morocco. Avermectins represent a novel class of macrocyclic lactones that have demonstrated nematicidal, acaricidal, and insecticidal activity. They are a mixture of natural products produced by a soil actinomycete, *Streptomyces avermitilis* MA-4680 (NRRL 8165). The discovery of the avermectins from this organism in 1976 has greatly influenced the arsenal of chemicals available for control of household and agricultural arthropod pests as well as parasites of mammals **[15].**

Thiovit is a fungicide widely used for controlling powdery mildew, rust and mites in pome and stone fruit and some vegetables. This product has been registered and its active ingredient contains about 80 % of elemental sulfur. It is a unique micronized sulfur formulation, wettable with spherical particles that mix easily with water to form a spray with good spreading and sticking properties. It is classified as a fungicide/miticide and practically used in crop protection worldwide **[16].**

Teppeki (Flonicamid) has a systemic effect insecticide. This product belongs to the chemical family of pyridine-carboxamids, used for controlling the most common insects, such as aphids, whose some strains are resistant to many aphicides. Where the resistance of aphids to products containing flonicamid occurs, Teppeki is unlikely to give satisfactory control. Repeat treatments result in lower levels of control. This feature makes Teppeki a key player in preventing the emergence of resistance. Flonicamid has a translaminar action and an upward migration that ensure homogeneous protection of the plant **[17]**. It acts on aphids and biting insects by contact and ingestion; the stop of the feeding is immediate and thus makes it possible to stop the damage by punctures.

One of the main objectives of this study is to know if the regular exposure of *P. ulmi* and its predator *T.* (*T.*) *setubali* to the active substances used would reduce their toxicity. The toxicity of the active substances to the predatory mite is defined according to the toxicity criteria described, under laboratory conditions, by the IOBC (International Organization for Biological Control) **[18]**.

Material and methods

Origin of spider mite and phytoseiid populations

The two sampled orchards are located in the central plateau in Morocco and experimental exploitations had during three consecutive years, 2015, 2016 and 2017 chemical treatments of variable frequency. The study was conducted on two populations of the European red mite, *Panonychus ulmi* and two populations of the predatory mite, *T.* (*T.*) *setubali*. A population of each species has sampled in plots with a phytosanitary history concerning the three pesticides vertimec (abamectin), Teppeki (Flonicamid), and fungicides including Thiovit. The first plot from which, population 1 was taken received 4 to 6 treatments per year. A second population of each species was collected in apple plot where these same active substances were applied with a low frequency (1 to 3 treatments per year).

Rearing of mites

P. ulmi and of *T.* (*T.*) *setubali* populations were reared at 25 ± 1 °C, 65 ± 5 % RH, and a photoperiod of 16: 8 (L: D). Rearing of phytoseiids was carried out on planted green bean (*Phaseolus vulgaris*) containing colonies of *P. ulmi.*

Toxicological tests

Contact toxicity tests were realized according to the method described by Knight et al. (1990) **[19]**. Ten adult females of *P. ulmi* are placed on a bean leaf disc (4 cm in diameter), applied to moistened filter paper. The set is placed in a Petri dish (9 cm in diameter). The toxicological tests carried out on Phytoseiids will consist of place, in the same Petri dish, ten females of *T.* (*T.*) *setubali* and five adults of *P. ulmi*. The spider mites will be used as food for Phytoseiides tested.

Aqueous mixtures have been prepared. For each active ingredient, the concentration corresponds to the recommended dose in the field **(Table 1)**. The preparations obtained are pulverized on leaf discs containing mites by a hand-held sprayer **[20]**. The volume of mixture applied as previously measured by the same authors is 9.6 ± 0.83 $\mu l.cm^{-2}$.

Pesticides	Active ingredient	Recommended dose on apple
Vertimec 1, 0.8 % EC	Abamectin	200 $ml.hl^{-1}$
Thiovit ® Jet	Sulfur	1000 $mg.hl^{-1}$
Teppeki	Flonicamid	14 $g.hl^{-1}$

Table1: Pesticides employed in toxicity test on phytophageous mite *Panonychus ulmi* and its predator *T.* (*T.*) *setubali.*

The witnesses received a blank treatment: they are treated in the same way, but with distilled water only. The mortality rates presented are adjusted by the Henderson-Tilton formula **[21]** and the mortality limit acceptable for witnesses was fixed at 10% **[22]**.

$$Corrected\,(\%) = \left(1 - \frac{ninCobefortreatment * ninTaftertreatment}{ninCoaftertreatment * ninTbefortreatment}\right) * 100$$

After 24 hours, dead individuals are counted under the magnifying glass binocular. For each dose, each population and each species, five repetitions are performed.

To classify the secondary effects of pesticides, the criteria established by the International Organization of Biological Control (IOBC) for tests on natural enemies, in laboratory conditions were used. A pesticide that causes 0-30 % mortality among natural enemies is considered being relatively selective. A pesticide that generates 30-79 % of mortality is classified as being of moderate toxicity. Finally, a product is considered toxic when inducing more than 80 % mortality among predatory mites treated.

Statistical analysis

Mortality percentages are corrected by Henderson-Tilton formula. The statistical treatments are realized by using analysis of variance (ANOVA), followed by Tukey tests, allowing comparison of average two to two. The different statistical treatments are running on the R program (i386.3.4.3).

Results

Effectiveness of pesticides on *P. ulmi*

Our results show that the pesticides tested have differential efficacy on mite pest (F = 53.47; df = 3; *P* < 0.001). The recommended dose of abamectin shows higher effectiveness on both populations of *P. ulmi* in comparison with those of Flonicamid and sulfur. Abamectin, a specific acaricide belonging to the family avermectins has a deadly effect on red mites whatever the frequency of anterior treatments, this product kills 97.48 ± 7.43 % of *P. ulmi* collected in the plot having received treatments with a low frequency and reducing to 68.76 ± 3.68 % for individuals collected in heavily treated plot during the growing season. In light of this result, abamectin is more effective in the control of the European red mite.

In comparison with flonicamid, a systemic insecticide of the family pyridine-carboxamids, the mortality rates obtained are lower among individuals from both populations (*P* < 0.05). On average, flonicamid eliminated 17.43 ± 4.13 % and 10.82 ± 2.11 % of treated *P. ulmi*. Thiovit shows moderate toxicity in both populations of the red mite, resulting in mortality rates ranging from 34.35 ± 5.63 to 14.92 ± 4.71 %.

Generally, mite pests collected in the poorly treated plot are mostly sensitive to the tested products, those originating from parcels frequently treated were are less sensitive to the pesticides tested (*P* < 0.05). Indeed, the application of abamectin on spider mites originating from low-treated apple trees produce a mortality rate close to 100 % compared to 68.76 ± 3.68 % when they come from frequently treated apple trees, a reduction of 28.72 % in the mortality rate. The lowest reduction has been made with Teppeki, flonicamid eliminates 6.61 % of spider mite populations. In the same way, pre-field treatments with sulfur fungicide appear to be able to reduce 19.43 % of mite pests **(Figure 1)**.

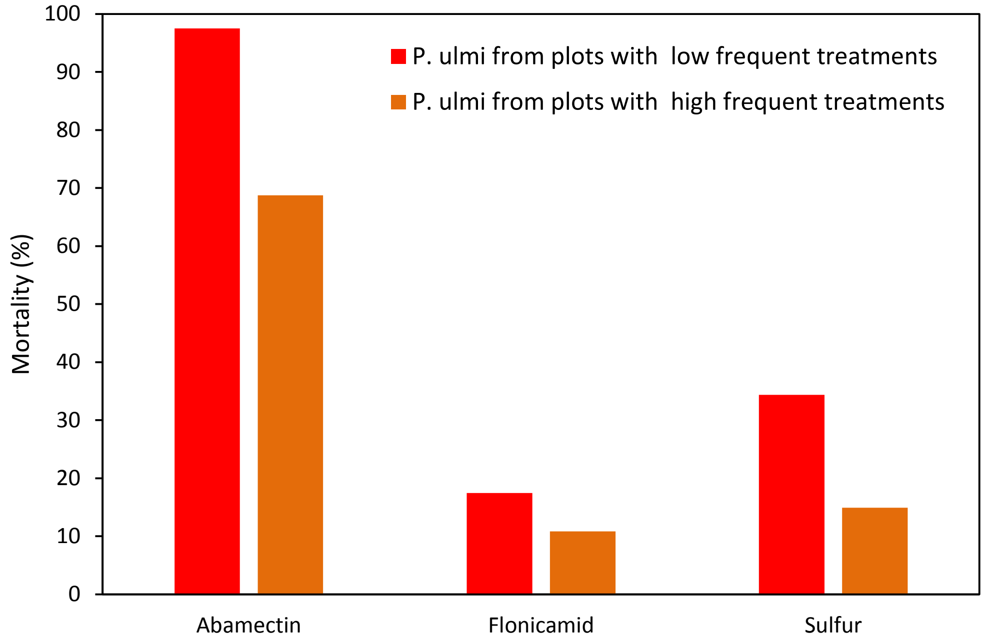

Figure 1: Percentages of mortality caused by the three pesticides sprayed on two populations of the red mite *P. ulmi*. Pesticides are applied at the recommended doses on apple trees in the laboratory study (n = 5 repetitions per treatment).

Effectiveness of pesticides on *Typhlodromus* (*Typhlodromus*) *setubali*

All pesticides tested have toxicity significantly different on *T.* (*T.*) *setubali* adults (F = 27.18, df = 3, $P < 0.001$). Abamectin appears to be the most selective active ingredient on the predatory mites *T.* (*T.*) *setubali* ($P < 0.05$). In average, this acaricide removes 12.68 ± 4.85 % of Phytoseiid mites. The results are graphically given in **Figure 2.**

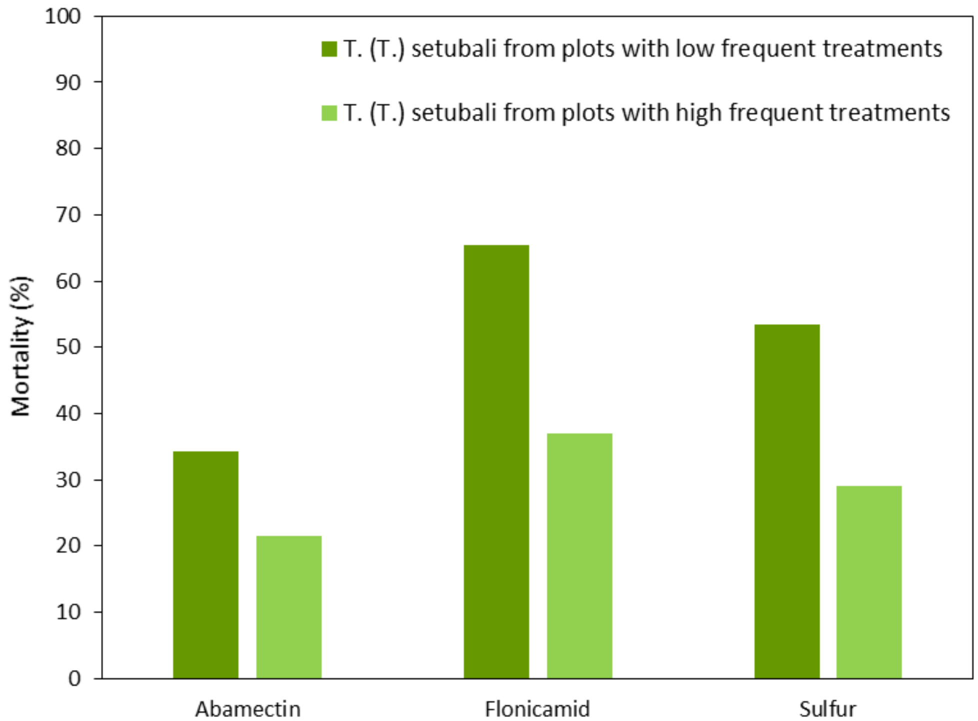

Figure 2: Percentages of mortality caused by the three pesticides sprayed on two populations of *T.* (*T.*) *setubali*. Pesticides are applied at the recommended doses on apple trees in the laboratory study (n = 5 repetitions per treatment).

Results reveal that Flonicamid and sulfur appear to be more harmful to *T.* (*T.*) *setubali* adults in comparison with abamectin. These active ingredients eliminate 28.47 ± 6.13 % and 24.44 ± 5.22 % of the predatory mite, respectively.

The impact of pesticides on *T.* (*T.*) *setubali* varies with the phytopharmaceutical history of the plots from which they were collected (F = 13.27, df = 2, $P < 0.05$). The predatory mites originating from the poorly treated plot are mostly sensitive; those originating from a plot that has received a frequent treatment are less sensitive to these chemical pesticides ($P < 0.05$).

Discussion

In Morocco, the production of the apple requires the use of a range of acaricides, insecticides, and fungicides to control major pests and diseases that can reduce yield. This study revealed that the three pesticides tested would be able to suppress mite pest populations even if they are used against other targets. However, the impact of these products on spider mites and the predatory mite *T.* (*T.*) *setubali* changes with history phytosanitary of the plots from which they are taken.

The study of pesticide effects on *P. ulmi* reveals a high efficacy of abamectin, which caused a mortality rate of 97.48 % among spider mites originating from the plot with low treatments. The same efficacy of this active substance on spider mites has also been described by Raudonis (2006) **[23]**, who has shown that abamectin (18 $g.l^{-1}$) at 18 $g.ha^{-1}$ and 27 $g.ha^{-1}$ is very effective on *P. ulmi* female adults and can causes a ranged mortality rate from 92.9 to 99 % after the first treatment and 95 % in *P. ulmi* larvae **[24].** The current application of abamectin for three years resulting in, reduction of its effectiveness about 28.72 %.

Our results are consistent with those obtained by Beers et al. (1998) **[25]**. Authors showed that after 7 to 8 years of wide spraying of abamectin, all populations of *Tetranychus urticae* studied showed a lower sensitivity level to abamectin. In the plots sampled, applied treatment programs included two nonmite-specific products, flonicamid sulfur. When these products are applied on *P. ulmi* from population 1, sulfur removes 34.35 %, whereas flonicamid removes only 17.43 %.

Unintended effects of applied phytopharmaceutical products were observed and assessed on the main species of the family Phytoseiidae in Moroccan orchards, *T.* (*T.*) *setubali*. The side effects of pesticides were evaluated according to IOBC instructions on several species of the family Phytoseiidae especially *Phytoseiulus persimilis* (Athias-Henriot) **[26-28]**. On the other hand, little work has so far been focused on the side effects of pesticides on Phytoseiids indigenous to Moroccan crops.

Among the products tested, abamectin appears to be the lowest offensive pesticide on the predatory mite, *T.* (*T.*) *setubali*. On average, this active ingredient causes approximately 27.92 % mortality and therefore returns among the products classified in class 1, according to the criteria of OILB. However, the toxicity of abamectin to phytoseiid decreases when *T.* (*T.*) *setubali* is originating from plot treated regularly with this active substance (12.68 % mortality). Flonicamid appears the most toxic product to typhlodrome whatever the frequency of treatments and given *T.* (*T.*) *setubali* population. Mortality rates of the predator from the first and second population, caused by flonicamid were 36.92 % and 65.39 %, respectively.

The present study reveals that sulfur has high to moderate toxicity to *T.* (*T.*) *setubali* adults. Collected from the frequently treated plot, sulfur caused mortality of 29.03 % (OILB, class 1). This rate is approximately 53.47 % when the predators collected from the plot weakly treated with sulfur and appear having moderate toxicity (OILB, class 2). For all pesticides, their toxicity depends on the plant protection history of the plots from which mites were collected.

Conclusion

The present findings might help to integrate biological and chemical control approaches to suppress *P. ulmi*. Before selecting any pesticide, one should look for its effectiveness against both pest and predatory mites. There should be a workable marriage between the target pest mite and predatory mite. This will help the proper suppression of pest mite and do not affect natural predatory mite. From the present study, it appears that abamectin might be a good choice because of its strong efficacy and the persistent of control against *P. ulmi* and its limited negative impact on natural enemies (*T.* (*T.*) *setubali*). Therefore, the use of abamectin may be suggested in integrated pest management programs (IPM) and augment successfully by releasing predatory mite to suppress *P. ulmi* population.

Flonicamid and sulfur have unintended effects on the red mite and the predatory mite *T.* (*T.*) *setubali*. The toxicity level of all products tested here in terms of risk on mites depends essentially on the phytosanitary history of treatments with these active ingredients. Preventive use of these pesticides should consider the presence of auxiliary fauna, which is able to keep the pest population below the economic threshold.

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