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## Use of homeophathic preparations for red spider mite, *Tetranychus urticae* (Acari: Tetranychidae) control in strawberry plants

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The *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae) mite is one of the most important quarantine pests globally. In strawberries (*Fragaria x ananassa* Duch.) (Rosales:Rosaceae), one of the most exported fruits in Mexico, *T. urticae* causes large losses in crop production, causing chlorotic spots on the upper surface of leaves, vigor loss in plants and leaf deformations thereby affecting productivity, size, yield and fruit quality (Valerio-Salgado *et al.* 2019).

The conventional control of T. urticae is made through chemical acaricides, which promote mites' resistance (Villegas et al. 2010). Among the alternatives to control the mite, the use of natural enemies (Soto et al. 2013) and the application of plant extracts [e.g. Cymbopogon winterianus Jowitt ex Bor (Poales: Poaceae)] (Vicentini et al. 2015), could be mentioned. Another less explored pest control alternative could be the use of homeopathics, like in the case of the homeopathic of adults of Acanthoscelides obtectus (Say, 1831) (Coleoptera: Chrysomelidae) weevils, where their offspring was controlled from 80 to 100% in bean plants (Deboni et al. 2017). Wyss et al. (2010) reported that the number of juvenile descendants of the rosy apple aphid (Dysaphis plantaginea Pass.) (Hemiptera: Aphididae) in apple plant seedbeds, decreased by 17% with the Lycopodium clavatum L. (Lycopodiales: Lycopodiaceae) homeopathic at 15 CH (it refers to Centesimal Hahnemannian dilution, the amount of initial active material is diluted with a successive dilution number, the CH number) and by 14% with a homeopathic based on leaves damaged by D. plantaginea (nosode) at 6 CH. It is worth mentioning that no reports were found on the use of homeopathics to control T. urticae in strawberry, let alone the use of homeopathics based on the same mite-infested plant. Due to the foregoing, the objective of this work was the evaluation of the effect of commercial homeopathic preparations of Árnica [Arnica montana L. (Asterales: Asteraceae)] (Árni), Belladonna [Atropa belladonna) (Solanales: Solanaceae) (Bellad), Ferrum sulphuricum (it is a mineral) (FS), Bovista (Bovista sp.) (Agaricales: Agaricaceae) (Bov) and a homeopathic based on leaves infested with T.

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*urticae* (*Agroh*) at 6 and 30 CH, on the incidence and severity control of the damage caused by *T*. *urticae* in strawberry plants.

The work was performed in the greenhouses of the Universidad de Costa Rica located in San Pedro, Montes de Oca (latitude 9° 56′ 09″ N and longitude 84° 03′ 07″ W, 1200 m a.s.l.). The breeding of mites was performed on Camino Real strawberry variety 22 days after transplanting (DAT) in pots with inceptisol soil. Ten mites (adult females) per plant were inoculated and were left to grow at 23  $\pm$  1 °C and a relative humidity of 59  $\pm$  1%. The mites were previously collected from the foliage of strawberry plants and identified by Dr. Hugo Aguilar Piedra, in accordance with the keys described by Ochoa *et al.* (1991). The plants were placed in a metal cage wrapped with organza fabric, fertilized every three days (0.5 g of NPK 10:30:10) and watered every two days as indicated by González *et al.* (2015).

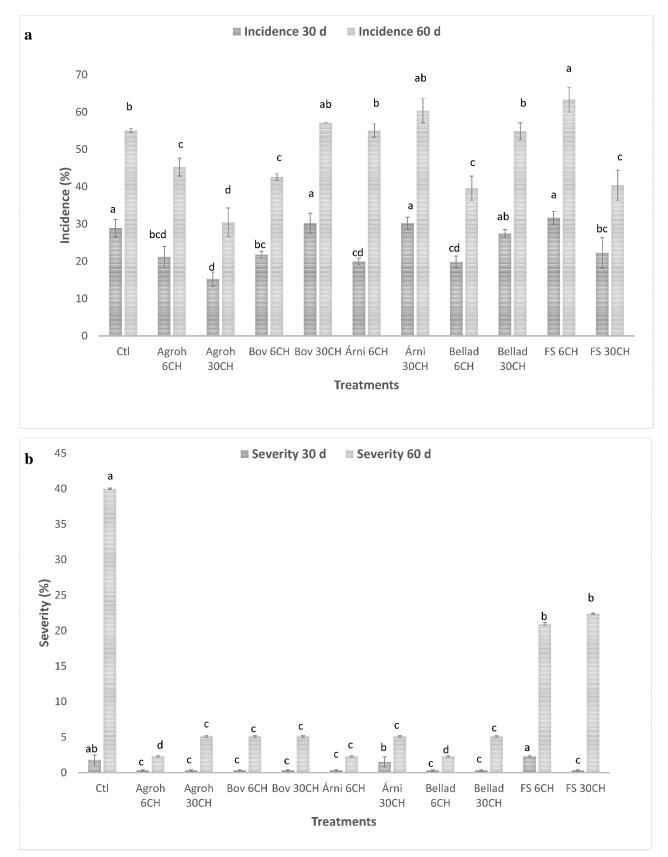
For the preparation of 1 CH and 2 CH of the homeopathic of mite-infested strawberry leaf, the technique reported by Rodríguez *et al.* (2014) was used, with modifications in the amount of sample used (1 g). The 3 CH was made in the liquid phase, for which one gram of the material of 2 CH was added to an amber bottle of 30 mL, 10 mL of 87% ethanol and 10 mL of distilled water were added, and followed by a 3 minutes of succussion were carried out. The 4 CH was prepared with 1 mL of 3 CH, 10 mL of 87% ethanol and 10 mL of distilled water; the mixture was added to an amber bottle of 30 mL followed by 3 minutes of succussion. The foregoing procedure was performed consecutively until the 6 CH and 30 CH were obtained.

The homeopathics *Bovista*, *Belladonna*, *Árnica* and *Ferrum sulphuricum* were bought from Scholler laboratory and were evaluated for mite control as indicated in the Handbook of Agrohomeopathy (Viecelli *et al.* 2010). They were acquired at a 6 CH concentration, from which the 30 CH was prepared following the same procedure used for liquid preparations.

Plants of Camino Real strawberry variety 22 DAT were used. The plants were placed in one litter pots with one kg of sterile inceptisol soil. Acaricide TALSTAR 10 EC (0.5 g per 500 mL of water) was applied four days before starting the experiment. The treatments were the following: **a**) plants treated with the 6 and 30 CH agrohomeopathic of strawberry leaves infested with *T. urticae* mites (*Agroh*); **b**) plants treated with the 6 and 30 CH *Bovista* (*Bov*); **c**) *Belladonna* (*Bellad*); **d**) *Arnica* (*Árni*); **e**) *Ferrum sulphuricum* (*FS*); and **f**) distilled water (*Ctl*). Eight plants per treatment were used. The homeopathics were prepared by placing 0.5 mL per every 500 mL of distilled water and were applied by spraying them on both the front and back of the leaves, applying five mL per plant, as indicated by Viecelli *et al.* (2010). The plants were sprinkled with the different treatments two times a day in five consecutive days and ten female adults were placed per plant immediately thereafter (Soto *et al.* 2013).

The plants were kept in greenhouse at  $23 \pm 1$  °C and relative humidity of  $59 \pm 1\%$ , they were fertilized and watered as described above. Thirty and 60 days afterwards the mite infestation and severity were determined by using the seven levels scale reported by Cardoso and Meirelles (2012), where the last level implies the loss of the plant; while the incidence was determined by counting the number of mite-infested leaves regarding the total number of plant leaves. Percentages of severity and incidence were determined using the formulas described by Jiménez *et al.* (2015). An analysis of variance (ANOVA) was applied to data, and the comparison of means was performed with the Tukey test (p < 0.05) using the SAS software for Windows V9.

Regarding the incidence of *T. urticae* mites in strawberry plants, after 30 days, the treatments: 30 CH *Agroh* (15.238 %), 6 CH *Árni* (19.940 %) and 6 CH *Bellad* (19.805 %), promoted a lower incidence, 0.5 to 0.7 times less than that retrieved from the control treatment (Fig. 1a), highlighting the treatment with 6 CH *FS* that surpassed 1.14 times the incidence of the control. The incidence after 60 days in plants treated with 30 CH *Agroh* was 30.4 %, which was the lowest incidence observed in all of the treatments and 1.8 lower than that retrieved from the control. The plants with 6 CH *FS* treatment had the highiest percentage of incidence (63.3 %) of all the treatments, surpassing the control treatment (55.05%) 0.86 times (Fig. 1a).



**Figure 1.** Degree of **a**) Incidence, and **b**) severity caused by *T. urticae* in strawberry plants 30 and 60 days after applying the treatments: Distilled water (*Ctl*); 6 and 30 CH Strawberry leaves homeopathic (*Agroh*); 6 and 30 CH *Bovista* (*Bov*); 6 and 30 CH *Belladonna* (*Bellad*); 6 and 30 CH *Arnica* (*Árni*) and 6 and 30 CH *Ferrum sulphuricum* (*FS*). Treatments with the same letter are not significantly different between them (p < 0.05) (n = 8).

USE OF HOMEOPHATIC PREPARATIONS FOR RED SPIDER MITE CONTROL IN STRAWBERRY

The results observed in this work concur with those obtained by Rolim *et al.* (2010), who reported the use of homeopathics to control *T. urticae* mites in tomato plants. The results revealed a lower mite incidence (2.43 %) in plants treated with 30 CH *Staphysagria* (Ranunculales: Ranunculaceae) and 200 CH *Thuja occidentalis* (Pinales: Cupressaceae) (2.35 %), being significantly different from the control (3.67 %).

Furthermore, the difference observed in the 6 CH FS treatment incidence is interesting; this was higher than the 30 CH dilution of the same treatment both after 30 and 60 days. This reveals the importance of determining the adequate dosage to attain the control effect even when the same homeopathic is used. The foregoing was also observed in the treatment reported by Rauber *et al.* (2007), who used the homeopathics: 60 CH *Camomilla (Matricaria chamomilla)* (Asterales: Asteraceae), 60 CH *Thuya (Thuja* sp.) (Pinales: Cupressaceae) and water, on the incidence of insects such as *Diabrotica speciose* (Coleoptera: Chrysomelidae), *Epitrix* sp. (Coleoptera: Chrysomelidae) and natural enemies in potato plants of different potato germplasms. Among the results, it is reported that only the plants treated with the 60 CH *Thuya* homeopathics had a lower incidence of pest insects, higher yield and higher number of natural enemy species; however, the 60 CH *Camomilla* preparation had a higher number of insects and incidence of causative agents of disease.

On the other hand, the severity of strawberry plants showed a different degree of damage caused by *T. urticae* mites. After 30 days, the plants with 6 CH *Agroh*, *Bov*, *Árni* and *Bellad* and 30 CH *FS* had a degree of damage of 0.3% compared with the control (2.3%) (Fig. 1b).

After 60 days, the plants with 6 and 30 CH *Agroh*, *Bov*, *Árni* and *Bellad* had the lowest severity of damage caused by mites with percentages ranging from 2.5 to 5.1%, which were 16 and 7.8 lower than those retrieved from Ctl (40%). The plants treated with 6 and 30 CH *FS* showed greater percentage of damage severity with 20.9 and 22.4 % respectively, this showed an attack severity between 7.6, and 5.6 times greater than that obtained by the rest of the homeopathics evaluated (Fig. 1b).

In this work, it was observed that most of the homeopathics controlled the severity of the damage caused by *T. urticae* in the strawberry plant; however, 30 CH Strawberry leaves homeopathic (*Agroh*) was the one that presented the best result in all analyses. These results partially coincide with those reported by Giesel *et al.* (2012), who mentions that the acaricide effect of the 30 CH *Belladona* homeopathics allowed the decrease of the *Acromyrmex laticeps* (Hymenoptera: Formicidae) and *A. heyeri* (Hymenoptera: Formicidae) ants' activity by spraying the homeopathics on the primary feeding trails of each of the ant nests. In this work, the 6 and 30 CH *Belladona* homeopathics controlled the severity of the damage caused by the mites after 30 days and this control was kept up to 60 days.

Most of the homeopathics used herein accomplished *T. urticae* control in strawberry plants, except for *FS*, whose 6 CH treatment, after 30 days, allowed a damage degree even greater than the control, whose severity was surpassed. However, this same treatment at 30 CH allowed a reduction of the damage severity in the plants after 30 days. These results coincide with those reported in the work of Cavalca *et al.* (2010), which evaluated the larvicidal effect against *Aedes aegypti* (Diptera: Culicidae) of the *Eucalyptus cinereal* (Myrtales: Myrtaceae) essential oil at 3, 6, 9, 12, and 30 CH dilutions and one control treatment. The 6, 9 and 12 CH dilutions decreased the number of larvae 11, 5 and 7.5% in comparison with the 3 and 30 CH dilutions and the control treatment. However, in the 30 CH dilution a decrease in the number of production cycles of the adult female mosquito was observed.

A hypothesis that could explain the homeopathics' mode of action in the reduction of severity of the damage caused by the mites focuses on the possibility that they may have a stimulating effect of the release, or inductive effect of the production, of metabolites related to the defense of plants. This hypothesis is supported on the results reported by Toledo *et al.* (2015), who evaluated the 6, 12, 30 and 60 CH *Propolis, Sulfur* and *Ferrum sulphuricum* homeopathics to control the fungus *Alternaria solani* (Pleosporales: Pleosporaceae); it was observed that the 12 and 30 CH Sulfur, 6, 12 and 30 CH Propolis and *Ferrum sulphuricum* decreased the severity of the disease by 17–49%, but it was also

mentioned that the 60 CH *Sulfur* solution produced a systemic effect on the induction of resistance. This also coincides with the report by Mioranza *et al.* (2017), who controlled the nematode *Meloidogyne incognita* (Kofoid & White, 1919) (Tylenchida: Heteroderidae) in *Solanum lycopersicum* L. (Solanales: Solanaceae) tomato plants with homeopathic solutions of *Thuya occidentalis* (Pinales: Cupressaceae) 6, 12, 24, 50, 100, 200 and 400 CH. The 100 CH dilution produced the decrease of juvenile stage 2 nematodes and the dilutions 24, 50, 200 and 400 CH developed the enzymatic activity of peroxidase, polyphenol oxidase at 200 CH, and phenylalanine ammonia lyase at 24 and 50 CH dilutions.

In addition to the activation of defense mechanisms, the use of homeopathics in plants has the advantage of not leaving residues on them due to the diluted form in which they are applied, in comparison with the use of other extracts. For future research works, it would be important to design strategies so as to determine the mechanisms applied to attain control. The results obtained lead to conclude that the treatment with *Agroh, Bov*, and *Bellad*, both at 6 CH and 30 CH, allowed the decrease of severity of the mite infestation of strawberry plants after 30 and 60 days of sampling.

These results show the potential use of the homeopathics evaluated and in particular those prepared from *T. urticae* mites to control them in strawberry plants.

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