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Efficacy of an Insect Immobilizer on The Two-Spotted Mite (*Tetranychus Urticae*) and Effect on i'ts Predators *Phytoseuilus Persimilis* and *Neoseiulus Californicus* Under Laboratory Conditions

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Abstract

Tetranychus urticae has been classified as one of the most significant pests globally. Integrated pest management strategies have incorporated tools such as acaricide application, which leads to resistance development in populations and contamination in the agroecosystem. The objective of this study was to evaluate the efficacy of an immobilizing insect mesh on *T. urticae* adults and nymphs, as well as its effect on their predators, *Phytoseiulus persimilis* and *Neoseiulus californicus*, under controlled laboratory conditions. Mortality rates exceeding 90% were observed with direct contact application on *T. urticae*, whereas predator mortality did not surpass 50%. On the other hand, surface-treated applications did not yield significant differences compared to the control, indicating that the most effective mode of entry for the polymer mesh is through direct contact.

Key words: Mite, integrated pest management, physical control, compatibility

Introduction

Pests generate economic losses in production systems due to their different feeding, reproduction and survival strategies (Jimenez, 2009). In terms of economic damage, it is found that insects and arthropods are capable of causing a decrease in productive yields, a clear example of this problem is observed with the species *Tetranychus urticae.*

This species is of great economic importance and is recognized for its broad spectrum, it feeds on more than 1,100 species of plants belonging to 140 families (North American Plant Protection Organization, 2014), its success lies in the survival mechanisms used in the feeding process, in which they are able to modulate the chemical defenses of the plant through 90 salivary proteins (Jonckheere et al., 2016), in addition, as indicated by Sparks and Nauen (2015), it is the pest most resistant to pesticides and this is due to the ability they have to eliminate the metabolites that enter the body with the help of ATP-dependent transporter genes or better known as ABC genes, which results in the detoxification of the individual (Dermauw et al., 2013).

A wide variety of strategies are implemented in the control of *T. urticae*. Among them is biological control in which they have used the release of predators such as *Phytoseiulus persimilis* and *Neoseiulus californicus*, Pérez (2017), reports in carnation cultivation a decrease of more than 20% in infestations of this pest with the release of 30 individuals per square meter, in addition Poliane (2012), reports the compatibility of the active ingredient imidacloprid with the predatory mite *Phytoseiulus persimilis*, which generates the integration of various tools in the control of this pest.

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Materials and Methods

The research was carried out in the research laboratory of the Entoma Institute, which is located in the municipality of Chía, Cundinamarca, under controlled conditions at an average temperature of 16°C and 70% relative humidity.

The biological material used consisted of bean plants (*Phaseolus vulgaris*) obtained by means of peat germination in a seedbed under average temperature conditions of 20 °C and 80% RH, which are transplanted 10 days after germination and subsequently used 14 days after transplanting, when the cotyledonal leaf has an approximate measure of 12 cm long by x 8 cm wide. The individuals of *Tetranychus urticae* were obtained in a bean crop located in the municipality of Tabio, the collection was made with the cutting of leaves infested with the different states of this pest, which were later placed in an airtight bag, inside a 20cm x 15cm x 10cm icopor refrigerator for transport. Following collection, the rearing of the individuals began under controlled laboratory conditions inside a 2m^3 entomological cage, inside which bean plants four weeks after transplantation were placed for the establishment of the individuals (see figure 1).

(A). Tetranychus urticae; (B). Phytoseiulus persimilis; (C). Neoseiulus californicus

Figure 1-. Biological species evaluated. To. *Tetranychus urticae*. B. *Phytoseiulus persimilis*. C. *Neoseiulus*

The obtaining of *Phytoseiulus persimilis* and *Neoseiulus californicus* was carried out through the company Bichopolis which is located in Tabio (Cundinamarca), the individuals are collected in containers of 4 cm diameter by 8 cm high, inside there is vermiculite substrate for the mobility of the mites inside. After acquisition, the individuals were immediately mounted to avoid stressful situations or cannibalistic behaviors.

The experimental unit consisted of a plastic and transparent tissue culture box of 5 cm in diameter by 3 cm high, inside a circumference of a cotyledonal bean leaf of the same diameter was located and with the help of a brush 10 individuals of each of the species were placed on top according to the treatment. In the case of predators, the experimental unit consisted of the aforementioned mechanism and 70 prey individuals were added for feeding (Forero et al., 2008). In the sealing of the tissue culture boxes, 1 cm wide masking tape was placed at the junction of both parts to prevent leakage.

The test substances used were the mechanical control (water) with the following characteristics: colorless, odorless, pH of 7.5 and the product PESTICK® (polymer mesh with a three-dimensional structure) applied at a concentration of 1 cc/L. The tests were carried out by treated surface and direct contact to the individuals, for the first case the microspray equipment was used, in which 95% coverage is guaranteed in the area and for the direct contact test the application is made with a micropipette, with which 1 microliter of product is applied directly on each individual in the case of adults. For the application of nymphs, 0.8 microliters/individual are applied. To observe the quality of the microspray, an application was made with a fluorescent product on the targets to be applied as the case may be (bean leaves or individuals applied) and then ultraviolet light was passed to observe the microdroplets on the substrate or the arthropods, this is because this product is an optical colorant, this ensures that the product is in contact with the biological targets (see Figure 2).

Figure 2 - Application quality methodology on bean leaf (Phaseolus vulgaris) seen with ultraviolet light. A. Water application. B. Product Application.

The research was proposed with a completely randomized design (DCA), which consisted of two treatments for each of the three species and two modes of entry for each, that is, a total of 24 treatments, each treatment had 5 repetitions with 10 individuals each for a total of 50 individuals per treatment. The evaluation was carried out 24 hours after application, because this product acts immediately. The response variable is efficacy, which was evaluated with the percentage of mortality due to treatment, data was collected in the corresponding log, and any individual who was dead or affected and did not show the possibility of recovery was taken as mortality.

The respective statistical analyses were performed through a Beta regression analysis using the betareg function, then a comparison test was performed to analyze the differences between treatments and for the calculation of efficacy a non-statistical Abbott analysis was performed, because the number of individuals per repetition was homogeneous. Analyses were performed using the freely distributed RStudio statistical software.

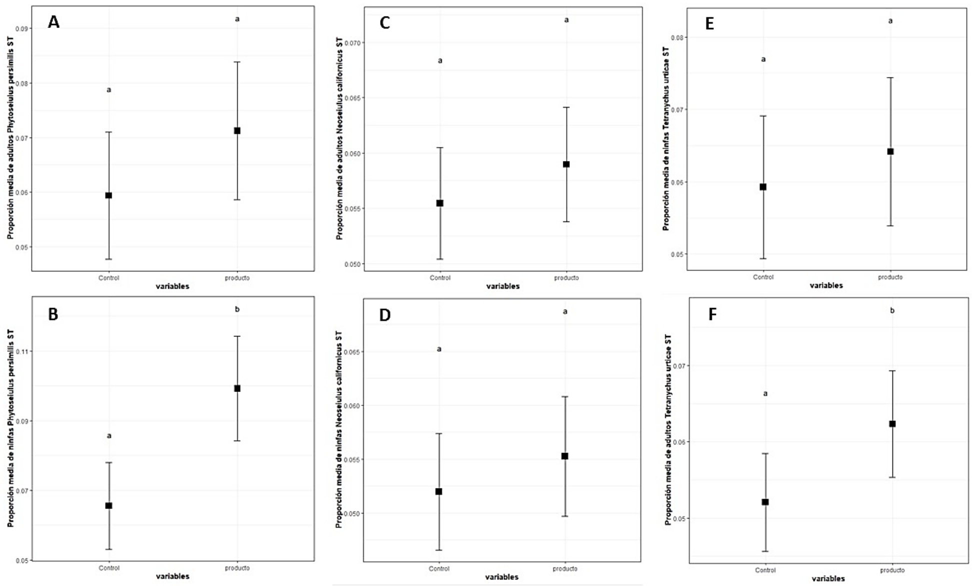
% Corrected Mortality

$$= \frac{\%mortality\ sample\ -\ \%witness\ mortality}{100\ -\ \%\ witness\ mortality}\ X\ 100$$

Formula for finding the Abbott percentage of efficacy of survival and mortality.

Results and Discussion

Under experimental conditions, the adults of *P. persimilis* with the application by direct contact of the



polymer mesh present a mortality of 54%, while the application by treated surface shows a mortality of 24%, for the statistical analysis a transformation was made to the data by means of the y.transf.betareg function, so that the betareg function included in the analysis the data corresponding to 0 and 100, This was done for each of the treatments, so it was found that there are statistically significant differences between the treatments with the application by direct contact to the individuals (z= 7.11; Pr(>|z|) = 1.12e-12 P = 0.0001) (see Figure 3A), while there were no significant differences for treated area (z = 1.55; Pr(>|z|) = 0.12 P = 0.12) (see Figure 4A).

Figure 3 **-** Mean proportion and standard deviations (lines on each bar) of mortality in the direct contact application of each of the species. The different letters (a, b) indicate significant differences at p-value<0.05. **A.** Adults of *Phytoseiulus persimilis*, **B.** Nymphs of *Phytoseiulus persimilis*, **C.** Adults of *Neoseiulus californicus*, **D.** *Nymphs of* Neoseiulus californicus**,** E. *Adults of* Tetranychus urticae**,** F. *Nymphs of* Tetranychus urticae.

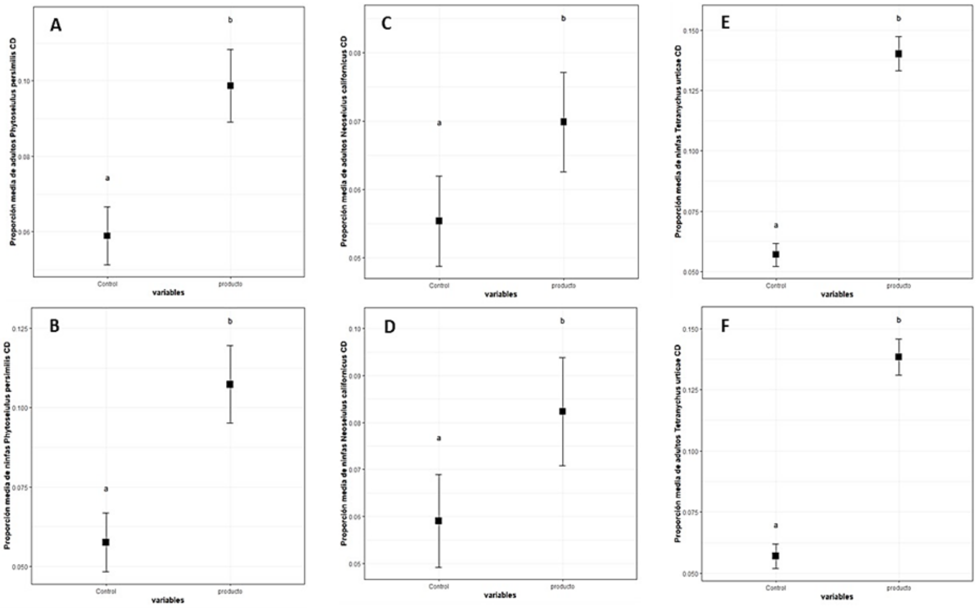


Figure 4 **-** Mean proportion and standard deviations (lines on each bar) of mortality in the application per treated area of each of the species. The different letters (a, b) indicate significant differences at p-value<0.05. **A.** Adults of *Phytoseiulus persimilis*, **B.** Nymphs of *Phytoseiulus persimilis*, **C.** Adults of *Neoseiulus californicus*, **D.** *Nymphs of* Neoseiulus californicus**,** E. *Adults of* Tetranychus urticae**,** F. *Nymphs of* Tetranychus urticae.

In the case of *P. persimilis* nymphs, a mortality rate of 64% was shown with direct contact application and 54% with treated surface application, at a significance level of p value at 0.05 and at p-value at 0.05 there were no significant differences between polymer application and control treatment in the treated surface input mode (see figure 4C). however, in the direct contact application, there is a difference between the treatments evaluated with a Pr(>|z|) value of 0.0009 (see Figure 3C).

In the case of the montages made for *N. californicus nymphs* , it is shown that with the application by direct contact to the individuals there is a mortality of 36%, which presents significant differences between the (Pr(>|z|) = 0.0005 P = 0.0005), on the other hand, with the application made by treated surface there are no differences between treatments with a mortality percentage of 6% and with a z-value of 0.95 it is rejected the null hypothesis (see Figures 3D and 4D).

The non-statistical analysis of efficacy with the Abbott test shows compatibility percentages greater than 70% in both forms of application of the silicone polymer and in both stages of development, however, 100% mortality, while in the application by treated surface a mortality of 16% of the individuals is shown, i.e. there are significant differences in the first (direct contact) and in the later (Treated surface) not (see figure 3E and 4E). According to the z-value found in direct contact in the case of adults (19.42) and nymphs (21.05), the null hypothesis is rejected, i.e. there is an effect of the product on individuals.

Percentage of mortality with the Abbott efficacy formula

Tabla 1 - Porcentaje de mortalidad con la fórmula de la eficacia de Abbott

Especie	*Phytoseiulus persimilis*		*Neoseiulus californicus*		*Tetranychus urticae*	
Estado de desarrollo	*Adultos*	*Ninfas*	*Adultos*	*Ninfas*	*Adultos*	*Ninfas*
Contacto directo	52	38,8	79,4	72,6	97,6	100
Superficie tratada	76,8	56,6	91,8	95,8	12,4	14,6

Discussion

The three-dimensional structure polymer mesh is a polymeric network known as 3D-IPNS TM (Three Dimensional – Immobilizing Polymeric Net Structure technology) technology (Marinković & Montforts, 2020; Slowing, *et al*., 2010). It is composed of silicone nanoparticles, silicone polymers, siloxanes and antioxidants which act exclusively mechanically or physically on individuals who have been applied by direct contact (Marinković & Montforts, 2020; Carcouet, 2014) (See Figure 5), because it forms a structural network of polymeric silicone on the body surface of arthropods, causing them to immobilize (Bekken, Pijnenburg & Aardbeiendag, 2019) (See Figure 6).

This mesh works and is effective as observed in the previous results by direct contact, this happens because, when the micro spraying is carried out, the droplets that are distributed come into contact with the individuals (Patrzałek et al., 2019), in this way the silicone polymers cross-link generating a condensation which forms a sticky structure (net) on the biological target (Marinković & Montforts, 2020), as a result of this, its vital physical functions are blocked quickly, to be more specific, it crushes the arthropod and blocks its trachea causing asphyxia (ICB pharma, 2020).

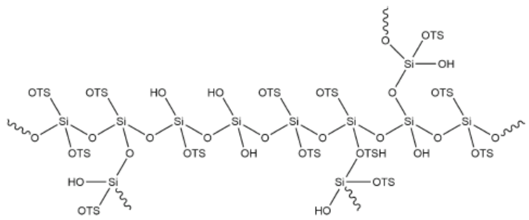


Figure 5 - Composition of the three-dimensional polymer mesh. (Marinković & Montforts, 2020).

Pesticide contamination and the generation of resistant populations has triggered the search for alternatives for pest control, as shown by Patrzałek et al. (2019), in their research in which they test the efficacy of physical control in *Tetranychus urticae* and *Aphis pomi* with the application of a modified trisiloxane, organ and a crosslinking agent in raspberry (*Rubus idaeus*) and blackcurrant plants (*Ribes nigrum*), which shows an effectiveness of more than 90% in the control of the populations of these pests and a significant decrease in other stages of development such as eggs and larvae, it also indicates a rapid action of the product, this agrees with what was found in the results of the polymer mesh efficacy test, in which the mortality percentages exceed 90% when the product is applied by direct contact to the target biological. Another research carried out by Marinković & Montforts, (2020), points out the immobilization of arthropods when applied with a product consisting of reactive monomers that polymerize on individuals, the above is related to what was observed in the research carried out with the silicone polymer in the adult and immature stages of *Tetranychus urticae*, because, This product generates the same immobilization effect on the biological target.

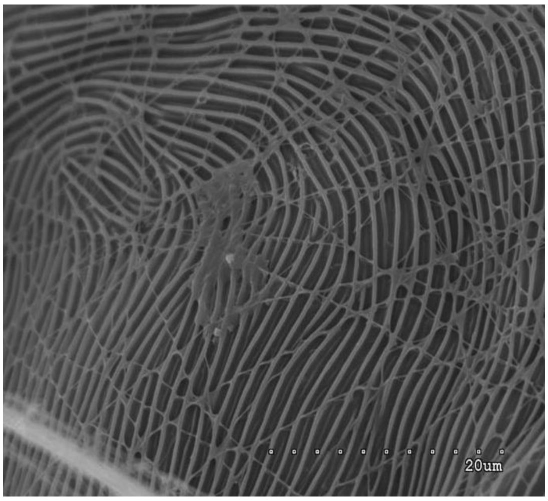


Figure 6 - Structure of the three-dimensional polymer mesh. Taken from Patrzałek *et al*., 2019.

Other methods of physical control in mites are reported by Gatarayiha et al. in 2010, in which they show the use of potassium silicate on adults and nymphs of *Tetranychus urticae*, the results show that the application of this product alone does not generate mortality in individuals, however, in combination with *Beauveria bassiana* It generates a high efficiency in the control of this pest and indicates that the application of this silicate provides nutrition to the plants and therefore protection against high infestations of *T urticae*. Several studies have shown that the use of granite rock dust in dry and aqueous formulations generates a repellent, insecticidal and anti-ovipositional action against the two-hammer mite (Faraone & Hillier, 2020), as shown by Faraone et al. (2020) in their research in which they suggest that mites are susceptible to rock dust with application by contact and by indirect interaction through plant feeding that were applied with the product. Finally, physical-mechanical control is mentioned with the application of nano insecticides (Stadler, Buteler & Weaver, 2010) and diatomaceous earths which have an insecticidal effect on various arthropods as mentioned by Korunic et al. (2019) in their work to reduce pest populations of stored grains such as *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum*, these have also been reported for the control of *T. urticae,* in which efficacy percentages close to 80% are indicated with the application by contact of this product that generates damage to the exocuticle of the arthropod (Vargas, 2013). This indicates that the application of physical and mechanical controls is an alternative and complementary tool to integrated pest management.

Pest control in recent years has been based on the integration of different tools for population reduction, as shown by Forero et al. (2010) in their study of compatibility with the predatory mite *Neoseiulus californicus*, in which they demonstrated the compatibility of three active ingredients (prochloraz (0.6 cm3/L), bupyrimate (1.0 g/L) and bifenazate (0.3 g/L)) and susceptibility to one (methomyl (0.5 g/L)), this is related to the data found with the application of the polymer mesh in which a compatibility with the application per treated surface is greater than 80% and with the application by direct contact a compatibility of 50%.

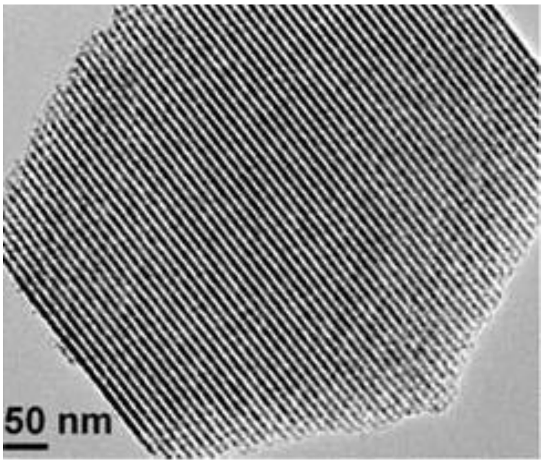


Figure 7 - Nanometric structure of the three-dimensional polymer mesh. Taken from Carcouet, 2014.

In the applications carried out by direct contact to the biological targets of study under laboratory conditions, they show significant differences and the susceptibility or compatibility with the product may be given in terms of difference in behavior, morphology and biology of the different organisms, for example there is an important differentiation between *Tetranychus urticae* in relation to its predators *Phytoseiulus persimilis* and *Neoseiulus californicus*, since the plague individual belongs to the suborder prostigmata and its predators belong to the mesostigmata group (Linsley, 1967), this refers to the fact that the prostigmata has the stigmata (fine orifices used in the respiratory system as tracheas for the circulation of oxygen) located at the base of the chelicerae (Ferragut, 2015), on the other hand, The mesostigmata or also called parasitiform have the stigmas located behind the coxae IV or they can also be in the coxae III and IV (Abelló, *et al*., 2004), so the mechanical process of asphyxiation of individuals can be less, because it crushes the individuals preventing breathing due to the stigmata. however, in the case of *Phytoseiulus persimilis* and *Neoseiulus californicus* , as they are located in the pleural part, they may not generate the same effect, in addition to their morphology in size and pear shape (Esteves, *et al*., 2010) allow them not to be immobilized by the product.

From an agroecological context, these types of tools become fundamental strategies that, linked to other processes, help to reduce pest populations and maintain a balance in agroecosystems

(Sarandon & Flores, 2014); To this end, we must take into account the three dimensions of agroecology, which consist of: 1) Having a scientific research approach for the holistic study of food systems and agroecosystems, 2) Generating practices of resilience and durability and 3) A new way of considering productive systems in relation to society (CISDE, 2018), as a consequence of this, the application of a siliconized polymer mesh must be regulated, since it is a polymer formed by nanoparticles of 50 to 2000 nm (See figure 7) it can be considered as a microplastic which will be exposed to workers, especially when the application is carried out in less than optimal conditions such as rain and high relative humidity and the drying process of the polymer is not fast and generate waste (Marinković & Montforts, 2020).

Conclusion

Based on the results obtained, the use of the three-dimensional polymer mesh is effective in immobilizing the adult and immature stages of *Tetranychus urticae* under laboratory conditions and can be considered as an alternative for the reduction of populations of this pest, being also of low ecological risk. The integration of natural enemies together with the physical-mechanical control is feasible, because due to the different modes of entry (treated surface and direct contact) they show a high compatibility and their interaction with the mesh is less compared to the pest mite. The application by the different modes of entry shows that the product has a greater efficacy with the application by direct contact, because the polymer mesh is directly in contact with the biological target, however, the dose to be applied must be taken into account to avoid generating toxicity in the plant or contamination in the environment. For future research, it is suggested to measure the efficacy of this product under field conditions, as well as to evaluate it in other biological targets of economic importance and on other biological control agents such as lacewings, anthocorids, coccinellids, among others, and it is recommended to determine the phytotoxic effects generated in plants by this type of polymers.

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