Colegio de Postgraduados

Control of the whitefly *Bemisia tabaci* (Genn.) in greenhouses

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ABSTRACT

A formulation based on neem oil (*Azadirachta indica*), chamomile (*Matricaria chamomilla*), lechuguilla (*Agave lechuguilla* Torrey) and cactus pectin extracts at different concentrations was used in greenhouses to control the whitefly *Bemisia tabaci* (Genn.). For moth monitoring, ecological water traps were used to which Noctovi® dipteran pheromones and a food component based on 50% sugar and 20% powdered yeast were added. The work was carried out in six tomato and strawberry production greenhouses located in the city of Durango, in northern Mexico, from August to November 2014. Plant extracts formulated with lechugilla surfactant based on chicalote (*Argemone mexicana* L.), skunk epazote (*Chenopodium glaucum*) and higuerilla (*Ricinus communis*) were used as controls and sprayed at intervals of 15 days each. Field results indicate that the formulation based on neem oil, nopal pectin, chamomile and lechuguilla extracts presented a moth mortality rate of 60% in tomato crops and 62% in strawberry crops.

Keywords: Neem oil, greenhouse, whitefly.

INTRODUCTION

Tomato (*Lycopersicum solanum*) is among the most economically important crops worldwide, with China and the USA being the main producers in the world, yielding more than 198 million tons (López and Estrada, 2005). Mexico is the main international exporter, sending the product to the USA, Canada, and El Salvador; in 2011 alone, 1,872,000 tons were produced (PRODUCE, 2012). In the state of Durango, Mexico, there is an approximate area of 150 hectares of greenhouse structures, 268 hectares of shade netting and 23 hectares of shade house (SIAP, 2014), within these areas, horticultural products such as: pumpkin, onion, strawberry, bell pepper, tomato and broadleaf vegetables are grown. These crops can be attacked by insects such as whiteflies, aphids, thrips and paratrioza, which are vectors of virus, bacterial and fungal diseases that cause, among other ailments, rotting of the stem, root, and fruit, reducing the quality and quantity of production by up to 60% (García and González, 2006). *Bemisia tabaci* (Geen.) has a reproductive cycle consisting of egg, four nymphal stages, pupa, and adult, which lasts approximately 28 days at average temperatures of 20-22 °C. Adults and nymphs are located on the underside of leaves, especially in the apical buds. The whitefly (Hemiptera: Aleyrodidae) is a phytophagous insect that causes damage to plants; as an imago, it feeds on the phloem by sucking the assimilated photos with its stylet (Rosell *et al.*, 1995). Greenhouses possess temperature and relative humidity conditions for the optimal growth of pests such as the whitefly and other diseases (Monroy, 2010).

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Among the organic alternatives to counteract the harmful effect caused by whiteflies are neem extracts (*Azadirachta indica* Juss.). This biorational has given good results, exhibiting several advantages such as high biodegradability and low risk to human health (Lannacone and Lamas, 2002). A pest control method used by producers is to use systemic action insecticides, based on organochlorine, organophosphorus and pyrethroid compounds; on average 2.0 liters per hectare are applied, a situation that is causing environmental and health deterioration to the final consumers due to the residual nature of the product in the plant tissue. The objective of this work is the biological control of the whitefly, *Bemisia tabaci* (Genn.) in protected environments through bioformulates with the use of biorational extracts of neem, chicalote, skunk epazote, higuerilla and chamomile at different doses and concentrations.

MATERIALS AND METHODS

Field work was performed from August to December 2014, in six greenhouses with a size of 10 meters wide by 200 meters long where vegetables, mainly tomato and strawberry (Table 1) are produced. Additionally, bioassays were conducted in a CR® brand bioclimatic chamber with controlled temperature and humidity. T1: bioformulate based on neem extract (*Azadirachta indica*), chamomile (*Matricaria chamomilla* L.), nopal pectin and lechuguilla extract (*Agave lechuguilla* Torrey) in a proportion of 25, 50, 15, 10%. T2: based on skunk epazote extract (*Dysphania graveolens*) and lechuguilla extract at a ratio of 70, 30%. T3: based on extract of chicalote (*Argemone mexicana*) and lechuguilla extract at a ratio of 70, 30%. T4: based on higuerilla (*Ricinus communis*) seed extract and lechuguilla extract at a ratio of 70, 30%. Three replicates were carried for each treatment under a completely randomized design for a total of 12 experiments, when significant differences were detected in the analysis of variance at a $p<0.05$ a comparison of means was performed by DMS using the Olivares, 1994 statistical software. In each experiment, 50 adult whitefly specimens were placed in a plastic container measuring 8×15 cm, bioformulates were

Table 1. Location of the coopertaing greenhouses.

Greenhouse	Location
Santa Rosa	Km 22 México Highway. 24.013459 N, −104.436055 W
Santa Cruz	Km 52 Nombre de Dios-Poanas Highway Durango. 23.907906 N, −104.132659 W.
Municipales	Eastern wastewater treatment plant PTAR. 24.026888 N, −104.602761W.
Diez de Santa Lucia	Km 2 Málaga Highway Durango. 24.133390 N, −104.543595 W.
Gabino Santillán	Gabino Santillán town Durango. 23.982207 N, −104.573153 W.
Diego Berlanga	Gabino Santillán town Durango. 23.982207 N, −104.573153 W.
Vermi Orgánicos de Durango	Parral km 2.5 Highway Durango. 24.099605 N, −104.696247 W.

sprayed twice a day, percentage of mortality was then evaluated. The bioformulation was developed in five stages:

First: extraction of cactus pectin, which is used to obtain an organic emulsifier in a proportion of 20 grams in 100 milliliters of water, until boiling for 10 minutes. The technique consists of: using fresh nopal of any variety, unstemmed and without thorns, preferably cut the same day or refrigerated to avoid desiccation and loss of volatile compounds, for every kilogram of fresh nopal, approximately 20 grams of filtered, purified nopal pectin are obtained, the objective of using this pectin is to emulsify the neem oil and make it soluble in water. For the formulation of the chicalote, epazote de zorrillo and higuerilla extracts, 100 grams of fresh leaves were placed separately in two liters of water, the surfactant based on lechuguilla was added in a mixture of 30% each in liquid form, then placed in 5.0 liter containers and stored under refrigeration.

Second: obtaining the surfactant, the process consists of using the leaves of lechuguilla (*Agave lechuguilla* Torrey), which is defibrated and using the residue of the carving called guishe, which contains up to 50% surfactant.

Third: obtaining chamomile extracts, the process consists of boiling the chamomile with six sachets per 1.0 liter of water for 10 minutes, strain and let cool.

Fourth: Use of commercial organic neem oil in 1.0 liter presentation.

Fifth: Elaboration of the trap, which consists of a 15×20 cm sheet of blue color for diptera and yellow color for lepidoptera with the following characteristics: suitable with a solar led bulb that works for 8 hours at night and is charged with light during the day. An open container with a mixture of Noctovi® pheromones and food composed of 50% sugar, 20% yeast and water.

RESULTS AND DISCUSSION

After different laboratory tests to achieve an effective emulsification and proven efficiency in terms of mortality in greenhouses, the mixture employed for this study was composed as follows: 40% *Agave lechuguilla* extract, 10% neem oil, 10% emulsifier (cactus pectin) and 10% chamomile extract, 5 ml per plant without direct dilution were applied. The results obtained for the control of whitefly, aphids, and thrips in two greenhouses employing this mixture are shown in Figure 1, where it can be observed that the percentage of whitefly mortality was 60% in the tomato crop in the Gabino Santillan greenhouse and 62% in the strawberry crop in the Vermi Organicos greenhouse, respectively. As for thrips (*Frankliniella occidentalis*). The formulated product reduced the presence of this pest by 15% in the tomato crop and by 10% in the strawberry crop. The results regarding the analysis of variance for each of the treatments used in a bioclimatic chamber with a controlled environment are shown in Table 2.

The ANVA results show that there is statistical significance among the treatments (formulations), the comparison of means is shown in Table 3.

Table 3 shows that the formulation based on neem oil, with cactus pectin, chamomile and lechuguilla agave extract, exhibited the best efficiency in whitefly mortality with 71.3%, 6% more compared with the other three formulations that statistically presented the

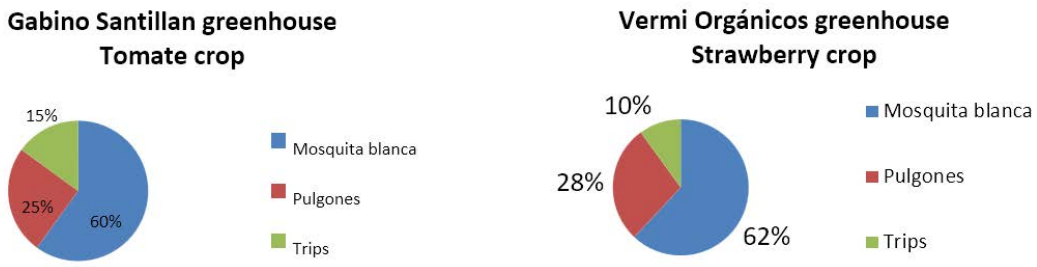

Figure 1. Results obtained in pest control, using a formulation based on neem oil, nopal pectin, chamomile extract and lechuguilla extract.

Table 2. ANVA of the bioclimatic chamber bioassays.

FV	GL	SC	CM	F	P>F
Treatments	3	189.582031	63.194012	11.1512	0.004
Error	8	45.335938	5.666992		
Total	11	234.917969			

P<0.05

Table 3. Comparison of means obtained from bioassays with *Bemisia tabaci* in bioclimatic chamber.

Formulate	Mortality (%)
Neem oil, chamomille extract, cactus pectin, lechuguilla extract	71.3 a
Epazote de zorrillo	65.0 b
Chicalote	62.3 b
Higuerilla	61.0 b

DMS: 4.482. Means with different letters in the column are statistically distinct (p<0.05).

same efficiency, so its use depends on the costs and the availability of plant material, since these plants can only be obtained in the wild during the summer. Aldás, 2014, mentions that applying neem oil from commercial formulations with 4.5 ml l^{-1} of water sprayed every 14 days produced 57.5% mortality of whitefly in open field chard crops. In this work, the field application was 5 ml of non-diluted formulated mixture per plant, achieving 60 and 62% efficiency in mortality in tomato and strawberry crops, respectively. López and Estrada, 2005, demonstrated that with the use of Oleo Nim 80 CE®, Neo Nim 60 CE®, Cuba Nim T®, Cuba Nim SM® and Foliar Nim HM® it is possible to effectively control damage caused by pests such as *Bemisia tabaci* (Genn.) in beans and tomato. The biological effectiveness achieved in these experiments ranged between 75 and 100%, which confirms the feasibility of the integration of these bioinsecticides in integrated pest management (IPM) for sustainable agriculture. In this work, it was necessary to emulsify the neem oil with nopal pectin and a lechuguilla-based adherent, which demonstrated greater adherence of the formulation on tomato leaves. Muñiz *et al.*, 2016, found that the products that showed the highest mortality were Neem Oil Spray and PHC® Neem, at a concentration of 0.6 mg ml^{-1}. The best repellent effect was from Neem Oil Spray (82.6%), PHC® Neeem (72.3%), Biosave® Neem (70.8%), and Neemix® 4.5 (59.9%); the first two showed greater

persistence with similar effects after 3 days. In this study, mortality effects on whiteflies appeared after 72 hours of exposure to the formulation in a bioclimatic chamber and after 8 hours in greenhouse conditions. Santiago *et al*., 2009, evaluated the essential oils of cinnamon (*Cinnamomum zeylanicum* Breyne (Lauraceae)), orange (*Citrus sinensis* L.), clove (*Eugenia caryophyllata* Thumb) and thyme (*Thymus vulgaris* L), and conducted bioassays in greenhouses using the acrylic cylinder method, where 20 adults were exposed to a bean leaf disc treated with essential oil. Repellency was measured by the difference between insects perched and not perched on the disc at three, four, five, six and twenty-four hours after application. Cinnamon and thyme essential oils at 1% concentration showed the highest repellency (91 and 93%, respectively), clove essential oil was not very efficient, while orange essential oil did not cause repellency. Thyme oil was stable up to 24 hours. In this work, the spraying was done directly in a bioclimatic chamber as well as in a greenhouse, the application was at an undiluted concentration and applied directly. Santiago *et al*., 2013, used ethanolic extracts based on neem oil, chicalote, higuerilla and epazote of zorrillo to eliminate whitefly from tomato leaves and found that any of the extracts used exhibited a mortality of 76%, in this work however; none of the extracts surpassed 65% mortality in greenhouse conditions. Lopez and Estrada, 2005, used mixtures with water and different concentrations of neem oil against whitefly in cucumber crops in greenhouse conditions and reported mortality above 75% using a 20% concentration. Neem oil mixed with water did not emulsify properly in this work, it was necessary to apply an emulsifier. Cruz, 2009, mentions that ethanolic extracts showed insecticidal effects against whitefly eggs and nymphs, but not against adults. In eggs, ethanolic extracts of *Annona squamosa*, *Cryptandra myriantha*, *Petiveria*, *Alliacea* and *Tamarix arborea* when applied at 5 mg ml^{-1} caused more than 80% mortality.

In whitefly nymphs, ethanolic extracts of *Eugenia winzerlingii* and *P. alliacea* at 2.5 mg ml^{-1} caused more than 80% mortality. On the other hand, aqueous extracts only showed insecticidal effects against whitefly eggs, with extracts from *A. squamosa* and *E. winzerlingii* at a concentration of 0.75% causing more than 80% mortality. These results suggest that *A. squamosa* and *E. winzerlingii* species could be used in the future for natural control of *Bemisia tabaci*. No ethanolic extracts were used in this study; extraction was conducted with hot water in all cases, with the exception of neem oil, which was used as a commercial product.

CONCLUSIONS

Any form of extract prepared from neem oil, chicalote or skunkweed epazote, exhibits good results for the control of white mosquito in tomato and strawberry greenhouses, mixture preparation should be as described in this article. The bioformulation based on neem oil, chamomile extract and guishe extract, used periodically on vegetable crops in protected agriculture, represents a considerable reduction in the use of agrochemicals for the control of whiteflies, thrips and other leaf pests. The differences found in the percentage of mortality in greenhouses and in bioclimatic chambers are due to the fact that in the latter, photoperiod, relative humidity and temperature can be controlled more effectively.

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