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ABSTRACT

Bio-assay of Diver® for mealy-bug and its parasitoid, *A. bambawalei*, was carried out during 2010-2011 in the IPM Laboratory, Department of Agri. Entomology, University of Agriculture, Faisalabad. All the concentrations of Diver®, which were applied in laboratory conditions, were effective against mealy-bug. Diver® is the best mineral oil to control mealy-bug when compared to other synthetic pesticides. On the numerical basis of data, which were recorded on mortality basis, the highest concentration of Diver® was most effective against mealy-bug, followed by the other concentrations. All the concentrations of Diver® on the parasitoid of mealy-bug swerve less and their effects were increased day by day, e.g. in 3 days the effect was minimal and in 21 days the effect of mortality was at its maximum because the residual toxicity of Diver® was present for a long time.

Keywords: cotton, mealy bug, parasitoids, Diver®, Hemiptera.

INTRODUCTION

Agriculture is the primary and leading sector of Pakistan and has a vital role in Pakistan's economy. Its contribution to GDP is more than 22% and it provides direct employment to 45% of the total labor. Most of the population of the Pakistan is located in rural areas that have a direct or indirect relationship with agriculture. Agriculture is much more important as it provides raw material to the industry. Cotton is the most important cash and fiber crop of Pakistan. Pakistan is the fifth largest producer of cotton, but almost 40% of cotton is consumed domestically.Leftover cotton is exported. Pakistan is the third largest exporter of raw cotton on the globe. Cotton and its products contribute up to 10% to the country's GDP (Anonymous, 2007).

The mealybug, *Phenacoccus solenopsis*, has recently emerged as a severe pest of cotton in Pakistan (Ahmed *et al*., 2012).Itwas first found in Asia (Pakistan) in 2005 (Abbas *et al*., 2005; Zaka *et al*., 2006)

and was recorded from India soon afterwards (Yousuf *et al*., 2007). It spread in Asia from Pakistan and India because these countries have been infested for a longer period of time (from 2005 to onward) where outbreaks of this species severely affected the cotton industries. In the cotton-producing belt of Pakistan (totaling 3.27 million ha in Punjab and Sindh), over 60,700 ha have been seriously damaged by *P. solenopsis*. Reportedly, 12% of the crop was lost in 2006; whereas, in 2007, almost 40% of the cotton in Punjab was damaged (Kakakhel, 2007).

In Pakistan, mealy bug has been reported on 154 species of plants including field crops, vegetables, fruits, ornamentals, weeds, bushes and trees (Arif *et al.,* 2009; Saini *et al.,* 2009). According to Afzal *et al*. (2009) and Aheer *et al*. (2009), mealybug feed on nearly 149 plant species, suck plant sap and cause leaves to distort and fall.

Mineral oils are aromatic oily liquids with good insecticidal properties (Konstantopoulou *et al.,* 1992; Karpouhtsis *et al*., 1998). Mineral Oils are remarkably less toxic and are safe for humans as compared to synthetic pesticides. They have very little effect on non-target insects, natural enemies, and on the environment. Mineral Oils enter into the insect body through integument, as well as through the tracheal system, cover the insect and plug the spiracles, thus causing suffocation and ultimately death (Stadler and Buteler, 2009). Resistance development is very rare in the case of mineral oils. Oils are also less expensive as compared to the synthetic pesticides (Weidhaas and Johnson, 1988). The present study is aimed to evaluate the percentage mortality of the mealy bug and its parasitoid with different concentrations of Diver®.

METHODOLOGY

Collection of mature females and immature males of cotton mealy bugs were taken from the infested plants of the shoe flower (*Rosa chinensis*), along with infested twigs, and brought into the IPM Laboratory, Department of Agri. Entomology, University of Agriculture Faisalabad. Cotton mealy bugs were collected with a camel hair brush and placed in glass cages made up of transparent plastic (20×30×30 cm). Fresh pumpkin fruits were provided in the cages as food material for the adult female mealy bugs; whereas cotton soaked in a honey and water solution was plugged in the Petri dish. This solution worked as food material for adult male mealy bugs. These foods were replaced with fresh food when necessary. For mass rearing of mealy bugs, these cages were retained under laboratory conditions (35±5ºC, 60±5 R.H. and DL period 10:14).

Mass-rearing of Mealybug Parasitoid, A. bambawalei

The endophagous parasitic wasp *Aenasius bambawalei* hayat was reared in the laboratory on the colonies of its host *Phenacoccusso lenopsis* Tinsly. Mummies of the cotton mealy-bug were collected in plastic jars with the help of a camel hair brush directly from the cotton field and vegetables (e.g. Tomato, Okra, eggplant and pumpkin) located on thecampus of the University of Agriculture, Faisalabad.Adult parasitoid individuals were collected with the help of an aspirator from the infested plants of the shoe flower. This collection was made on a weekly basis and added to the cages to maintain our laboratory population. The collected parasitoids were identified on the basis of morphological characteristics by making comparisons with the available literature. The cotton mealy-bug infested pumpkin was picked from the

cage in which the mealy-bug was cultured, transferred to the cage of parasitoid individuals, and kept there overnight so that adult parasitoids parasitized and mummified the mealybug. The cotton plugs soaked with the honey and water solution was provided in the cages that had the parasitoid population. It served as food for adult parasitoids. For mass rearing of parasitoid individuals, laboratory conditions were maintained at 35±5^{o}C, 60±5 R.H and 10:14 DL period.

Preparation of Diver® test Solutions/Dilutions

Overall, ten test solutions (dilutions) of Diver®, i.e. 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, and 6.4%, were prepared. A stock solution of 6.4% was prepared and the rest of the concentrations were prepared from the stock solution by using the dilution method described by Ahmad *et al.* (2012). A control treatment having only water was also used.

Application of test solution on mealybugs

A leafy twig was dipped in test solution of each concentration of Diver® and control test solution. The treated twigs were dried for ten minutes and inserted through the small hole made in the centre of the circular thermo-pore disc. The circular thermo-pore disc was then adjusted in the plastic glass cups, which have the nutritive solution, in such, a way that the lower end of stem remained dipped in the solution. Thirty-three such treatment units for the evaluation of ten concentrations and a control against each life stage of the mealy bug was prepared and divided into three sets, each having eleven such units. A counted number of reproductive life stages of the maybug were inoculated on the treated twigs and the mortality response was recorded on a daily basis after 3, 7, 14 and 21 Days Post Treatment (DPT). The mortality was then transformed into percentage corrected mortality by the formulae described by Henderson and Tilton (1955).

Exposure of treated adult mealybug to adult parasitoid

A counted number of adult females were treated on the twigs as described above and then exposed to counted pairs of adult parasitoids to determine the rate of parasitism and mortality of adult parasitoids, on a daily basis. The data regarding mortality and parasitism of the adult parasitoid on treated mealy bugs was recorded. The mortality data was transformed into corrected mortality as described by Henderson and Tilton (1955).

Application of test solution on mummies

For the bioassay of Diver® on mummies, the counted mummies were pasted on spherical adhesive/tape which was fixed in a petri dish. Thirty such petri dishes were prepared in three sets/replications, each having ten such petri dishes. Nine concentrations and one control were applied on the pasted mummies with atomizer. After application, the space among treated mummies was dusted with sand/saw dust to avoid the direct contact of emerging parasitoid adults with the residues on the adhesive tape. The adults emerging from the mummies were counted and their mortality was recorded. The percent corrected mortality of emerging adult parasitoids was calculated as described by Henderson and Tilton (1955).

Exposure of Adult Parasitoid to test solution

One side of two glass slides was sprayed with test solutions and then set inside the plastic-glass cups having plastic-

glass lids. Thirty such units were prepared and divided into three sets, each having ten such units. Three sets served as replicates; ten units of two slides with ten concentrations of Diver®. A control was also prepared in the same way with a water treatment. The cotton plug soaked with parasitoid adult food was plugged in the hole made in the lid of each unit. The mortality of the adult parasitoid was observed daily after 24 and 48 hours and percent corrected mortality was calculated by the formula described by Henderson and Tilton (1955). After 48 hours, the surviving adults in pairs were released into newly prepared units that had adult female mealy bugs pasted on glass slides. The rate of parasitism was calculated by the following formula:

$$\textbf{Rate of Parasitism} = \frac{\textbf{\% parasitism}}{\textbf{Total Time (Days)}}$$

Statistical Analysis

Finally the data regarding mortality was analyzed by using the probate analysis technique (Finney, 1971) to find out LC_{50}. Mortality rate and parasitism was analyzed using the ANOVA technique to determine the significance of various concentrations of Diver®.Means of mortality and rate of parasitism of significant results were compared by using the Tuckey Honestly Difference Test as done by Danho *et al.* (2002).

RESULTS

Bioassay Studies

In bioassay studies, different concentrations of Diver® are used against cotton mealy bugs and its parasitoid, *Aenasius* species. The data regarding mortality and results are given below:

Percent Mortality of Adult Female Mealybug at Different Concentrations of Diver®

It is evident from Figure1 that the results were significant. The percent mortality of adult female mealy bugs varies from 0.000% to 35.00% after 3 days of Diver application, and after 7 days, the percent mortality varies from 0.000% to 41.667%. After 14 days,percent mortality of the treatments varied from 0.000% to 50.00%, and after 21 days, the percent mortality of adult female mealy bugs with diver concentrations varied from 0.000% to 61.667%. All data results were significant.

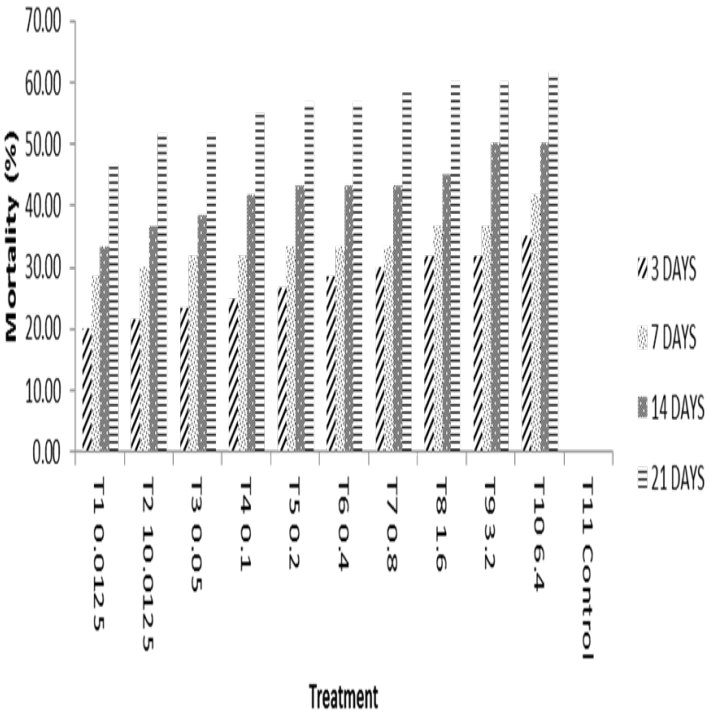

Figure 1: Percent mortality of mealy bug adult female at different concentration of Diver® at 3, 7, 14 and 21 days interval.

Percent Mortality of Adult Male Mealy-bug at Different Concentration of Diver®

It is evident from Figure 2 that the results were significant. The percent

mortality of adult male mealybugs varied from 0.000% to 35.00% after 3 days of Diver® application. After 7 days, the percent mortality varied from 0.000% to 43.333%. After 14 days, percent mortality from the treatments varied from 0.000% to 48.333%. After 21 days, the percent mortality of adult male mealy bugs with Diver® concentrations varied from 0.000% to 55.00%. All data results were significant.

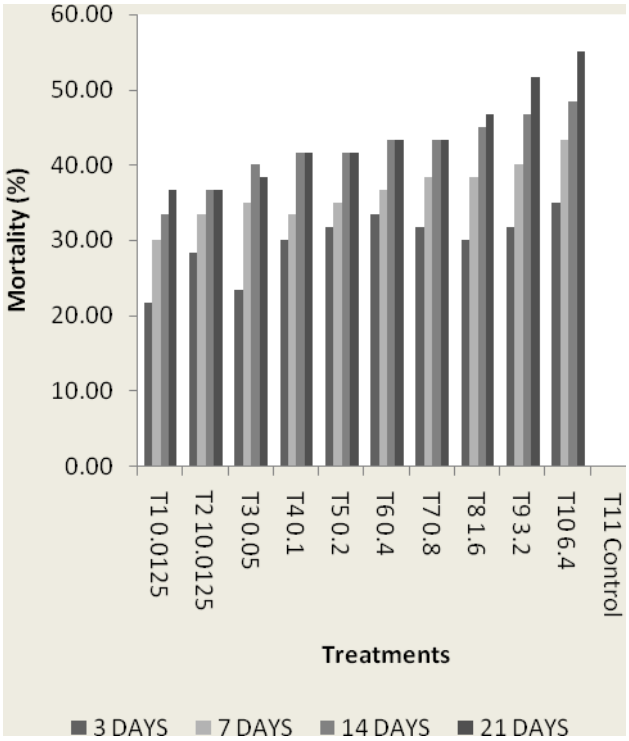

Figure 2: Percent mortality of mealy bug adult male at different concentrations of Diver® at 3, 7, 14 and 21 days interval.

Percent Mortality of First Instar Mealybug at Different Concentrations of Diver®

It is evident from Figure 3 that the results were significant. The percent mortality of 1st instars of mealy bugs varied from 0.000% to 31.667% after 3 days of Diver® application. After 7 days, the percent mortality varied from 0.000% to 38.333%. After 14 days, the percent mortality from the treatments varied from 0.000% to 48.333%. After 21 days, the percent mortality of the 1stinstars of mealybug with Diver®, concentrations varied from 0.000% to 60.00%. All the data showed significant results.

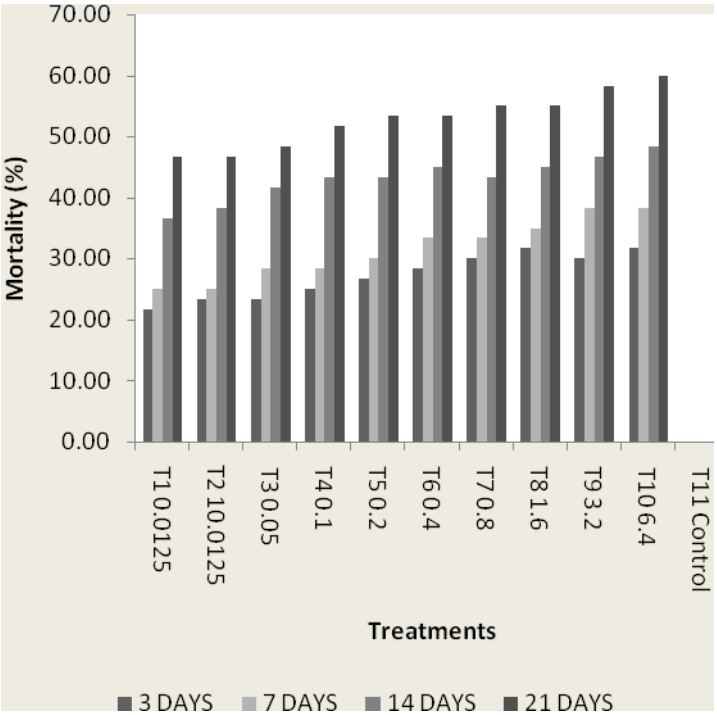

Figure 3: Percent mortality of mealy bug first instars at different concentrations of Diver® at 3, 7, 14 and 21 days interval.

Percent Mortality of Second Instar Mealybug at Different Concentrations of Diver®

It is evident from Figure4 that the results were significant. The percent mortality of 2nd instars of mealy bugs varied from 0.000% to 31.667% after 3 days of Diver® application. After 7 days, the percent mortality varied from 0.000% to 33.333% After 14 days, the percent mortality from the treatments varied from 0.000% to 38.333%. After 21 days, the percent mortality of the 2nd instars of mealy-bugs with Diver® concentration varied from 0.000% to

56.667%. All the data showed significant results.

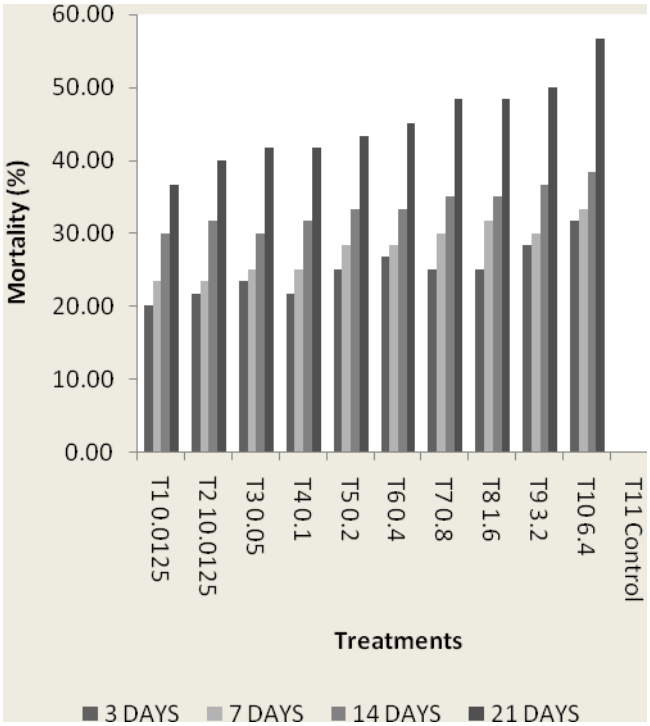

Figure 4: Percent mortality of mealy bug second instars at different concentrations of Diver® at 3, 7, 14 and 21 days interval.

Percent Mortality of Third Instar Mealybug at Different Concentrations of Diver®

It is evident from Figure5 that the results were significant. The percent mortality of the 3rd instars of mealy bugs varied from 0.000% to 35.00% after 3 days of Diver® application. After 7 days, thepercent mortality varied from 0.000% to 35.00%. After 14 days, the percent mortality from the treatments varied from0.000% to 40.000%. After 21 days, the percent mortality of the3rd instars of mealy-bugs with Diver® concentration varied from 0.000% to 53.333%. All the data showed significant results.

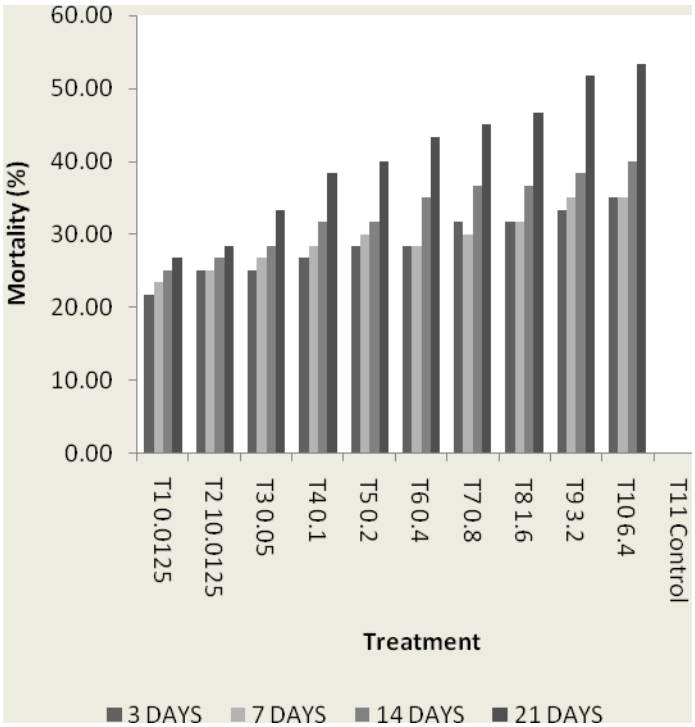

Figure 5: Percent mortality of mealy bug third instars at different concentrations of Diver® at 3, 7, 14 and 21 days interval

Percent Mortality of Adult Parasitoid Emerged From Treated Mummies at Different Concentrations of Diver®

It is evident from Figure 6 that the results were significant. The percent mortality of adult parasitoids varied from 0.000% to 33.333% after 24 hours of Diver® application. After 48 hours, the percent mortality varied from 0.000% to 55.000%. All the data showed significant results.

Percent Mortality of Adult Parasitoid at Different Concentrations of Diver®

It is evident from Figure 7 that the results were significant. The percent mortality of adult parasitoids varied from 0.000% to 33.333% after 24 hours of Diver® application. After 48 hours, the percent mortality varied from 0.000% to

51.667%. All the data showed significant results.

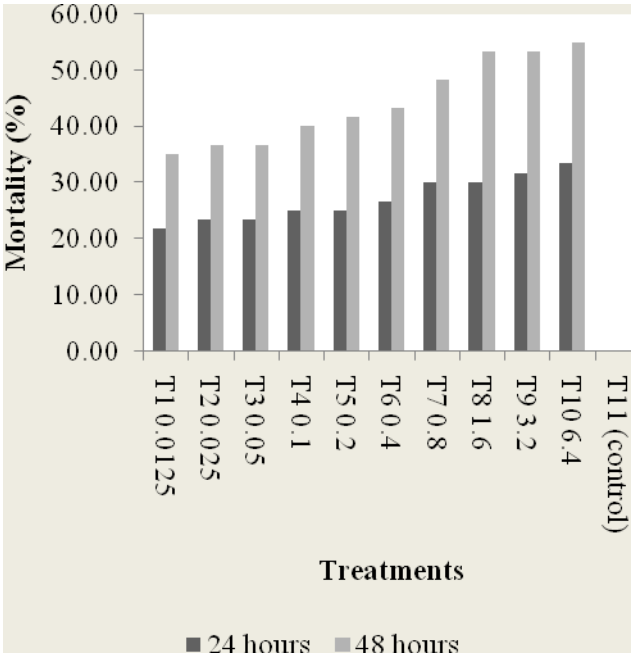

Figure 6: Percent mortality of adult parasitoid emerged from treated mummies at different concentrations of Diver®.

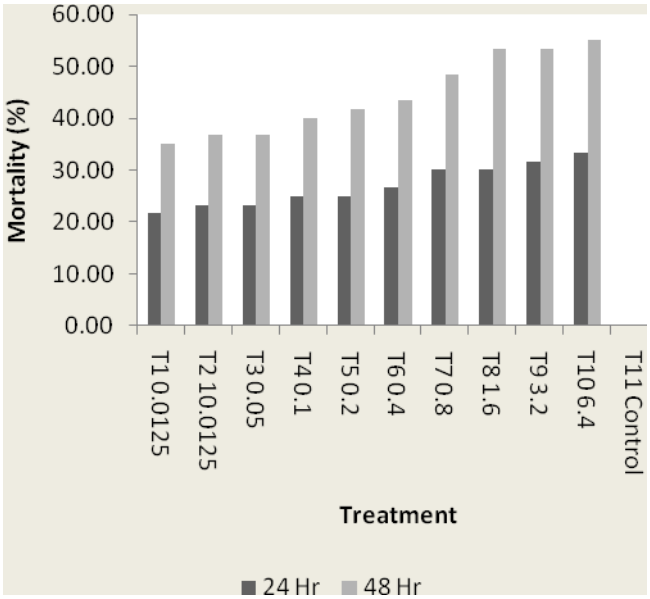

Figure 7: Percent mortality of parasitoid adults at different concentration of Diver® at 24 and 48 hours interval.

DISCUSSION

The main objective of the bioassay studies was to check the percentage mortality of mealy-bugs and their parasitoids with different concentration of Diver®. For this purpose, 10 different concentrations and one control was used against mealy-bugs and their parasitoid. Mortality responses of mealy bugs we rerecorded after 3, 7, 14 and 21 days and parasitoid mortality response was recorded after 24 and 48 hours. The results revealed that with all the treatments, maximum mortality was after 21 days and minimum mortality was recorded after 3 days. Control gave no results. Mortality also increased as the dose was increased. T10 (6.4) gave the best results from all treatments. Maximum mortality was observed after 21 days with T10 (6.4) and no mortality was observed with T11 (control). In the case of parasitoids, maximum mortality was observed after 48 hours with T10 (6.4), while control gave no results. Maximum parasitism was observed with non-treated parasitoids and treated parasitoids showed significantly less parasitism.All these results were supported by Kio and Patil *et al*. (2010), who investigated the insecticidal activity of oils against *Sitophilus zeamais.* They said that development of a progeny was greatly reduced by the application of oils. Studies revealed that oils show a subsequent mortality, however, the fecundity rate was not disturbed. Oils show best results against eggs and early stage larvae.Tomkins *et al.* (1996), who conducted a field trial to control insect pest with mineral oil, also supported these results. They evaluated different doses of mineral oil against insects and mites that attack kiwifruit. They showed that all

treatments reduced the infestation on leaves and fruit. Fruit damage and caterpillar infestation was also reduced. Thrips attack is also decreased with the application of mineral oil. The 2% treatment gave the best results and also controlled mites, although his experiment was in field. Our results contrast with Choi *et al*. (2003) who evaluated the insecticidal activity of 53 essential oils on different plants against different stages of *Trialeurodes vaporariorum.* This bioassay is without direct contact to the oils. Different results are disclosed according to oil, dose and stage of insect. Bay, caraway, clove, lemon and eucalyptus oils proved greatly effective against all three stages: adult, nymph, and eggs of the insect. The oils which gave the best results were than studied as a fumigant to control the same insect. This contrasts due to different application locations and our use of controlled conditions of a laboratory.

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