

Feeding and Mortality of Western Flower Thrips, *Frankliniella occidentalis* Pergande, 1895 (Thysanoptera: Thripidae) in Response to Botanical Extracts

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Abstract: The effects of commercially available oil and extracts from *Azadirachta indica* A. Juss. (neem) seed kernels and dried leaves of *Parthenium hysterophorus* L. and *Datura alba* (Nees) on Western flower thrips (WFT) mortality and feeding damage were investigated. On healthy, untreated leaves, female thrips caused more feeding damage than male thrips at all densities tested (1, 5 and 10 thrips). Our analysis also revealed that as densities of thrips increased, the amount of feeding damage/individual decreased, suggesting that interactions between thrips were occurring that limited their feeding. Our investigation showed that commercially available *A. indica* oil and all of the crude extracts tested have potential in reducing WFT feeding and causing significant mortality using foliar applications, although none were as effective as Conserve® (spinosad), a commercial pesticide, in causing short-term mortality. All the extracts tested increased western flower thrips mortality significantly 48 hours post-exposure when compared to the control. *Azadirachta indica* was most effective in causing WFT mortality at 24 hours, and there were no significant differences among the extracts at 48 hours, with *A. indica* and *D. alba* causing more than 40% WFT mortality. All the botanical extracts similarly reduced WFT feeding after 24 hours; however, after 48 hours the most effective reduction in feeding was in response to *A. indica*. We conclude that all the botanical extracts we tested potentially could be used as a part of an integrated pest management program for WFT. The efficacy against multiple pests and the commercial availability of *A. indica* extracts makes it an especially good choice for further studies on its use as a management strategy for WFT. There is considerable interest in Pakistan in the use of *P. hysterophorus* and *D. alba* as management strategies for thrips, in particular *T. tabaci*. These plants occur in abundance locally and extracts are inexpensive and easy to produce. Our findings suggest that botanical extracts from both of these species are worthy of further exploration using different formulations and application methods.

Key words: Western flower thrips (WFT), *Azadirachta indica*, neem oil, neem seed, botanical extracts, *Parthenium hysterophorus*, *Datura alba*.

Introduction

The western flower thrips (WFT), *Frankliniella occidentalis* Pergande, 1895 (Thysanoptera: Thripidae), is an important pest, causing damage directly through feeding and indirectly by transmitting viruses (WHITFIELD et al. 2005). Over the past three decades, the WFT have become increasingly serious pests worldwide (REITZ 2009). The WFT feeds

in a piercing-sucking manner, leaving silvery areas on leaves, flowers and fruits where cells have been emptied. WFT feeding involves puncturing epidermal and mesophyll cells and subsequently ingesting the cell contents via the feeding tube formed by the maxillary stylets (STAFFORD et al. 2012, CHISHOLM & LEWIS 1984). Feeding behaviour is sexually dimor-

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phic and typically female WFT feed a great deal more than male WFT (STAFFORD et al. 2011, HARREWIJN et al. 1996, ULLMAN et al. 1989). During feeding, the WFT acquires and ultimately transmits viruses in the genus *Tospovirus* (family Bunyaviridae). The tospoviruses are a rapidly emerging group of viruses and the WFT causes crop losses worldwide by transmitting multiple tospoviruses to more than 1,000 plant hosts (PAPPU et al. 2009, WHITFIELD et al. 2005). The minute size, thigmotactic behaviour and high fecundity of the WFT, combined with rapid development of pesticide resistance, make this insect very difficult to manage (GERIN et al. 1999). Many traditional pesticides may also kill its natural enemies leading to a resurgence of the insect pest, as well as causing residual effects, resulting in ground-water contamination, environmental problems and impacts on human health (WABALE & KHARDE 2010). For these reasons, there has been considerable interest in developing environmentally friendly pesticides as part of integrated pest management (IPM) practices. Some alternative pesticides, such as botanical extracts from *A. indica* (neem), are considered promising because they do not harm extensively beneficial organisms (parasitoids and predators) and have short residual activity (SAXENA 2006, MILLER & UETZ 1998). Neem extracts have been shown to reduce feeding, deter pests, inhibit the growth of insects and affect oviposition activity (GUJAR 1992). *Azadirachta indica* extracts have also been shown to act as feeding deterrents when used for controlling thrips (JENSEN et al. 2002). In general, botanically derived insecticides are thought to be ecologically acceptable and safe, while offering effective control when used in conjunction with other IPM practices (NATHAN et al. 2004). For these reasons, there is a keen interest in exploration of new botanical extracts for management. Our objective was to evaluate the efficacy of extracts from *A. indica* seeds and leaves of *Parthenium hysterophorus* and *Datura alba* for reducing WFT survival and feeding.

Materials and Methods

Rearing of WFT: The WFT were collected from onion, *Allium cepa*, in a field located at the University of California, Davis and were reared in the laboratory on green beans, *Phaseolus vulgaris*, using previously described methods (ULLMAN et al. 1992). Rearing containers were held at room temperature in a cage (1.83m x 0.61m x 1.22m) covered with thrips-proof screening in a laboratory in Storer Hall, University of California, Davis, CA (approximately 23.2 °C ±1).

Leaf material: All bioassays were conducted using leaf discs (1.2 cm diameter) cut from *Emilia sonchifolia* leaves. For each bioassay, 32 ml plastic snap-top vials were used. Each leaf disc was embedded into a 1% agar solution on the inside of the vial cap. This kept leaf discs moist and prevented thrips from feeding on the lower leaf disc surface. Young adult WFT, 4-8 days post adult eclosion, were used in all experiments. These were collected from the colony and held on ice to immobilise the insects. A fine brush was used to move appropriate number and gender of thrips into the body of the snap-top vial. The vial was then turned upside down and snapped onto the lid containing the agar-embedded leaf disc. These were then held under lights on the laboratory bench at room temperature (approximately 23 ± 1 °C).

Measurement of Mortality and Feeding

Damage: At the end of each leaf disc assay (24-48 hours depending upon the experiment), thrips were removed from the vials and the number of living and dead thrips counted. Then digital images of the leaf discs were captured with a Leica MZ FLIII fluorescence stereomicroscope with an attached digital camera (Diagnostic Instruments, Inc. Model #7.3 Three Shot Color). The feeding damage was then measured using Image J software (ONEAL et al. 2002) and converted to mm² for statistical analyses.

Comparison of male and female WFT feeding:

To determine whether male or female thrips should be used to test the efficacy of botanical extracts, we examined feeding responses of males and females on healthy, untreated leaves. In this experiment, we assessed feeding damage caused by 1, 5 and 10 male and female WFT per leaf disc. Each treatment was replicated five times.

Efficacy of botanical extracts on WFT feeding damage and mortality:

Assays were done using four materials: commercially available, pure *A. indica* oil (Bulk Apothecary, California, USA), crude extracts from *A. indica* seed, as well as from dried, crushed foliage of *P. hysterophorus* and *D. alba*. Pure *A. indica* oil was diluted in Tween (0.01%) to create concentrations of 1%, 3%, 5% and 10%. *Azadirachta indica* seed and leaves of *P. hysterophorus* and *D. alba* were collected in Peshawar Khyber Pakhtunkhwa, Pakistan. *Azadirachta indica* seed was crushed and ground to approximately 2 mm particles. *Parthenium hysterophorus* and *D. alba* leaves were dried in an oven for 72 hours at 60°C and also ground into approximately 2 mm particles as previously described (HAZARA et al. 1999). Five hundred grams of each botanical sample was wrapped in Muslin cloth and placed in 5 litres of boiling water, along with 10 grams of Ariel (detergent powder) as an

adjuvant, for 24 hours to make a 10% concentration (weight: volume). This stock solution was diluted in water to make a 5% concentration. The insecticide “Conserve®” (spinosad), an effective biopesticide against WFT (JONES et al. 2005), was used at 0.05% (212ul/400ml of water) as a non-botanical pesticide control. Two negative controls were included in each experiment, distilled water and 0.01% Tween.

All assays were done using five female thrips/leaf disc. Bioassays of commercially available *A. indica* oil were replicated seven times with ten leaf discs/replication. Experiments comparing crude extracts from *A. indica* seed and leaves of *P. hysterophorus* and *D. alba* were replicated five times with six leaf discs/replication. Leaf discs were prepared by dipping into the extracts at the appropriate dilutions. They were then allowed to dry in a fume hood before being embedded on agar in the snap-top vial lid as described earlier.

Statistical analyses: Statistix 8.1 statistical program was used. Differences between extract treatments were tested by ANOVA. A means separation test was conducted using the least significant difference (LSD) for each experiment. The impact of male and female thrips density on feeding damage was analysed using linear regression. To determine whether thrips individuals reduced their feeding in presence of other thrips, feeding damage caused by 5 or 10 male and female thrips was divided by that caused by an individual. A factorial analysis was then performed using Statistix 8.1 software.

Results

Comparison of male and female western flower thrips (WFT) feeding damage on untreated plant material: Our results showed that at all densities female thrips fed more and caused more feeding damage than males (Figs. 1a and 1b). Feeding damage caused by male and female thrips increased as the number of insects/leaf disc was increased, with the correlations between number of thrips and feeding damage being stronger for females ($R^2 = 0.8916$ and 0.9423 , respectively). The greatest amount of feeding damage was caused by 10 females (mean = 5.87 mm^2), more than five times the damage caused by 10 male WFT (mean = 0.707 mm^2). Although feeding damage increased with thrips density (Fig. 1), the amount of feeding damage did not correspond to that expected based on the feeding damage caused by an individual (Fig. 1b). For both males and females feeding damage/individual decreased significantly when density was increased from one to five insects, while there were no further significant

decreases when thrips density was increased to ten insects (Fig. 1b). These data suggest that interactions between thrips on healthy, untreated leaves may occur that reduce individual feeding. The very significant difference between the quantity of female and male feeding damage guided our choice to conduct all future experiments with female WFT.

Effect of commercially available *A. indica* on WFT mortality and feeding damage: All the concentrations of *A. indica* oil tested were significantly less effective than Conserve® which caused 86% WFT mortality (Fig. 2). Concentrations of 1, 3 and 5% *A. indica* oil did not differ significantly from one another with regards to WFT mortality, but were significantly less effective than the 10% concentration. None of the thrips placed on leaf discs treated with Tween or water died. Although *A. indica* oil was not as effective as Conserve® in causing WFT mortality, all the concentrations were as effective as Conserve® (spinosad) in significantly reducing WFT feeding when compared to controls treated with Tween or water (Fig. 3). Notably, the low amount of feeding damage on Conserve® treated leaf discs occurred due to high WFT mortality, while *A. indica* oil reduced thrips feeding although mortality was much lower than when treated with Conserve®.

Effect of crude botanical extracts on WFT mortality and feeding damage: None of the botanical extracts tested was as effective as Conserve® in causing mortality (Fig. 4). Conserve® caused 100% mortality within 24 hours, while amongst the botanical extracts only *A. indica* caused mortality (7%) in the same timeframe. For all three botanical extracts, mortality increased significantly 48 hours post-treatment and at this point in time the treatments did not differ significantly from one another. There was no mortality on leaf discs treated with water. In contrast, all the botanical extracts significantly reduced WFT feeding when compared to the water controls (Fig. 5). As expected, given the very high mortality caused, the least feeding damage was found on Conserve®-treated leaf discs.

Discussion

Our results showed that botanical extracts had potential in reducing WFT feeding and causing significant mortality using foliar applications, although they were not as effective in causing short-term mortality as the commercial insecticide Conserve® which caused 80-100% mortality. The reduction in feeding damage and the mortality we observed make these botanicals worthy of further consideration as control measures, especially when the ecological advantag-

es and benefits for integrating natural enemies into control programs are considered.

In our study, female WFT were shown to cause significantly more feeding damage than males (Figs. 1a & 1b), a finding that supported our choice to conduct all of our experiments evaluating botanical extracts using female WFT. Our findings that male WFT cause less feeding damage than females are consistent with previous studies (STAFFORD et al. 2011, WETERING et al. 1998). Although male and female feeding damage increased as the number of insects increased, the increased quantity of feeding damage did not correspond to what would be expected based on mean feeding damage caused by

individual male and female insects. The decreased feeding damage/individual as thrips density increased suggests that the insects may interact with one another in ways that actually limit their feeding. Little is known about thrips interactions with one another; however, males, not females, are known to exhibit swarming behaviour occasionally (MATTESON & TERRY 1992). In our experiment, interactions related to insect density apparently occurred amongst males and females. This interesting finding warrants further future investigation and should be taken into consideration when behavioural studies, virus transmission experiments and investigations of WFT feeding damage are conducted.

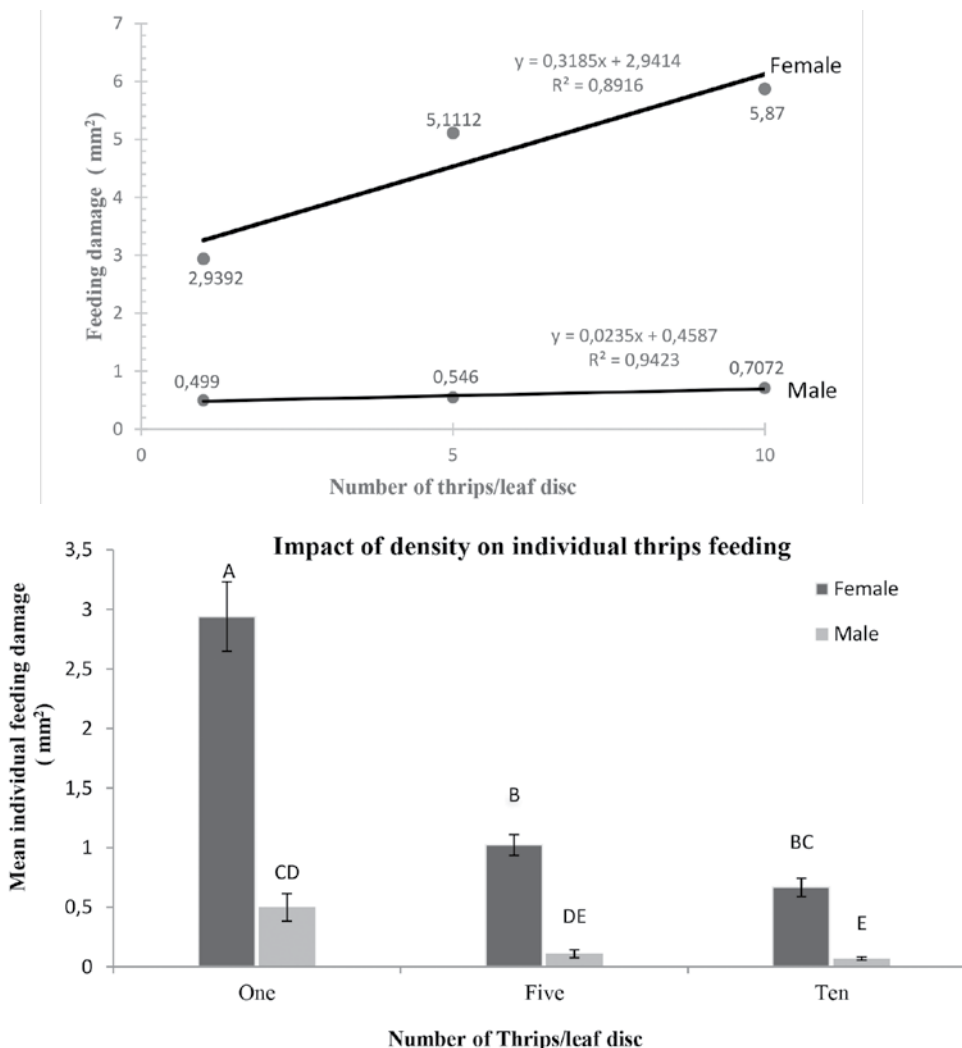


Fig. 1. A Effect of insect density (1, 5 and 10 male and female insects) on feeding damage (mean mm²) caused by western flower thrips (WFT), *Frankliniella occidentalis*. **A)** Linear regression analysis shows increased feeding damage is associated with increased density of male and female thrips. **B)** Individual feeding damage shows that females cause significantly more feeding damage (mean mm²/individual) than males. Feeding damage caused by individual male and female adult western flower thrips (WFT), *Frankliniella occidentalis*, decreases significantly as insect density increases to 5 thrips, without further decreases at a density of 10 thrips. Standard errors are shown with lines on each bar. Bars marked with the same letter are not statistically different ($P \leq 0,05$, ANOVA, LSD multiple means comparison). (n=6 leaf discs/experimental replication X 5 experimental replications)

We have shown that all the botanical extracts in our study significantly reduced female feeding when compared to plain water or Tween treated control leaves, with *A. indica* having the most significant effect. Although *P. hysterothorus* only caused 20% mortality in our studies, it was similar to *A. indica* in reducing WFT feeding, while *D. alba* was significantly less effective. With regard to short-term mortality, our data suggest that foliar applications of *A. indica* and *D. alba* have greater potential for reducing WFT populations than *P. hysterothorus*, both causing greater than 40% mortality after 48 hours of exposure, although these differences were not statistically significant (Fig. 4). Mortality did not vary significantly at 1, 3 and 5% applications of *A. indica*

oil, but did increase significantly to greater than 40% mortality at a 10% concentration. Similarly, crude extracts of *A. indica* seed caused greater than 40% mortality at 48 hours post-exposure. While this level of mortality is probably not adequate as a control measure in most field settings, it may be adequate if used in conjunction with other management strategies or if a more effective method of application could be used. E. g., studies of systemic effects of *A. indica* on WFT following soil applications, reported mortality of 50-93% depending on the soil substrate (THOEMING et al. 2003). *Azadirachta indica* products have been shown to reduce survival and damage caused by other insects, such as *Stenchaetothrips biformis*, in rice (PILLAI & PONNIAH 1988). Mortality

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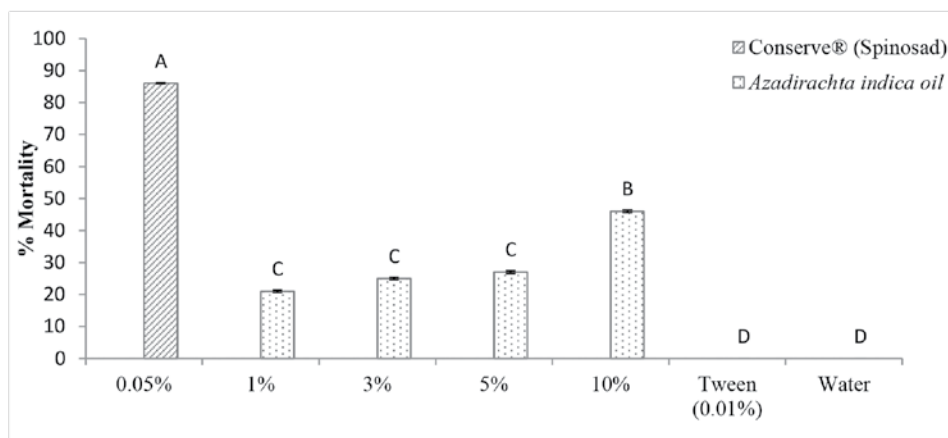


Fig. 2. Mean mortality of female western flower thrips (WFT), *Frankliniella occidentalis* in response to Conserve® (spinosad), four concentrations of commercially available *Azadirachta indica* oil (1, 3, 5, and 10%) and controls (Tween and water), (n=7 leaf discs/experimental replication, 5 thrips/leaf disc, 10 experimental replications). Standard errors are shown with lines on each bar. Bars marked with the same letter are not statistically different ($P \geq 0.05$, ANOVA, LSD multiple means comparison)

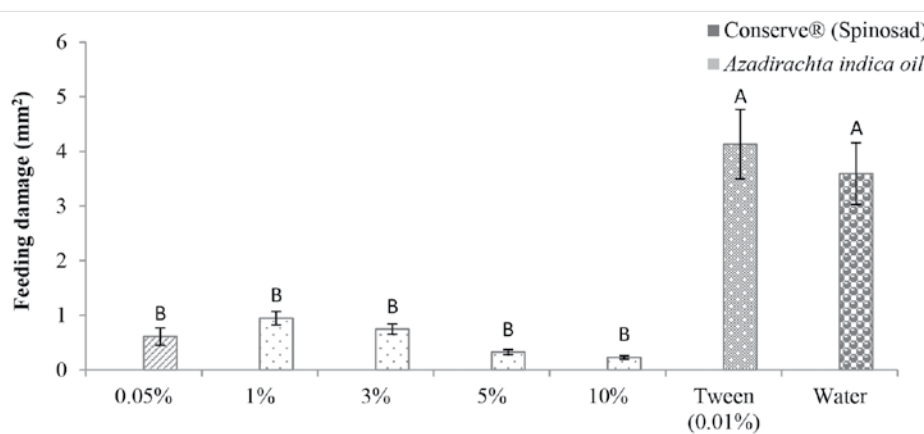


Fig. 3. Mean feeding damage (mm²) by female adult western flower thrips (WFT), *Frankliniella occidentalis* in response to Conserve® (spinosad), four concentrations of commercially available *Azadirachta indica* oil (1, 3, 5, and 10%) and controls (Tween and water). Bars represent means (n=7 leaf discs/experimental replication, 5 thrips/leaf disc, 10 experimental replications). Standard errors are shown with lines on each bar. Bars marked with the same letter are not statistically different ($P \geq 0.05$, ANOVA, LSD multiple means comparison)

and decreased developmental rate have also been shown for *Diaphorina citri* (WEATHERSBEE III & MCKENZIE 2005). Our study is the first to show mortality and reduced feeding by WFT in response to foliar treatments of *P. hysterophorus* and *D. alba*, although the application of these botanical extracts, as well as those from *A. indica* and *Citrullus colocynthis* have already been shown to cause a significant decline in the population of *Thrips tabaci* (KADRI & BASAVANA GOUD 2010, MISHRA et al. 2007; MALIK et al. 2003). Indeed, *D. alba* was more effective than

A. indica against *T. tabaci* under field conditions (KHAN et al. 2013). Two of the botanicals in our study, *A. indica* seed and *P. hysterophorus* leaves, have also been shown by others to inhibit development of woolly aphids *Ceratovacuna lanigera* Zehntner (WABALE & KHARDE 2010).

Taken together, the reduced WFT feeding and increased mortality we observed suggests that botanical extracts of *P. hysterophorus*, *D. alba*, and *A. indica* potentially could be used in integrated pest management of WFT. Efficacy against multiple pests

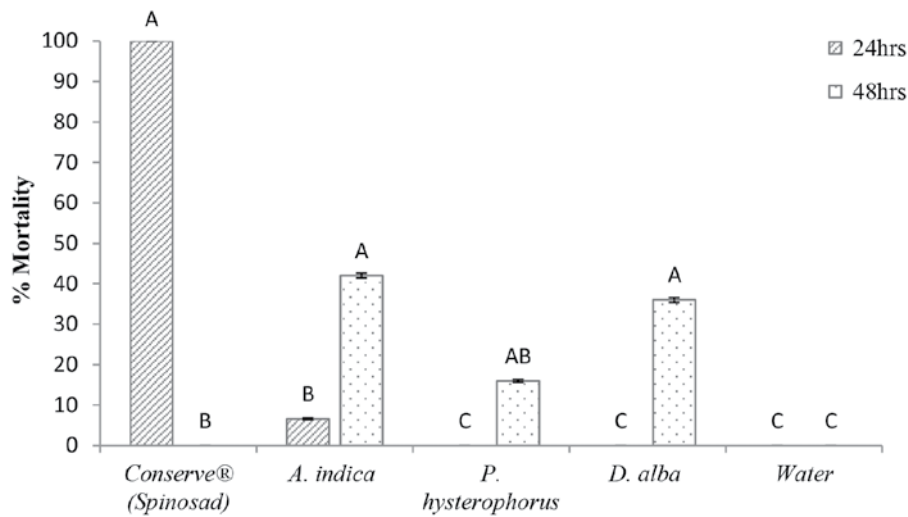


Fig. 4. Mean mortality of female adult western flower thrips (WFT), *Frankliniella occidentalis* in response to Conserve® (spinosad) and 5% solutions of three crude botanical extracts, *Azadirachta indica*, *Parthenium hysterophorus*, *Datura alba* and a water control. Bars represent means (n=5 leaf discs/experimental replication, 5 thrips/leaf disc, 6 experimental replications). Standard errors are shown with lines on each bar. Bars marked with the same letter are not statistically different ($P \geq 0.05$, ANOVA, LSD multiple means comparison)

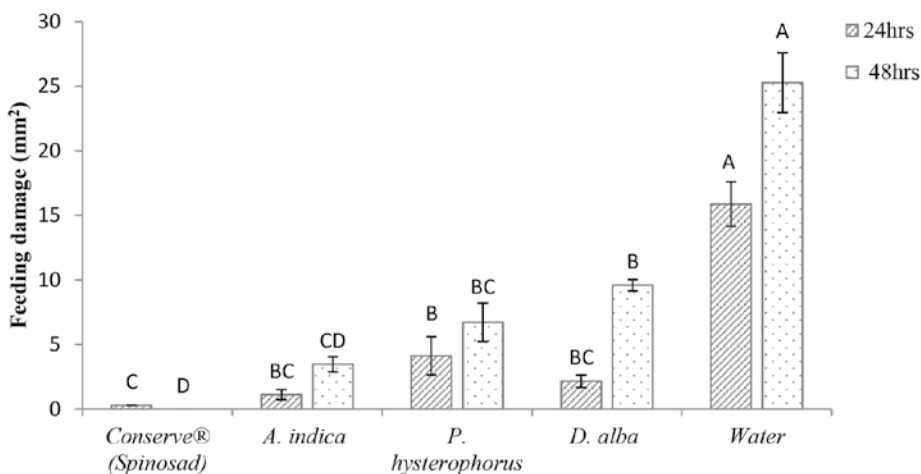


Fig. 5. Comparison of feeding damage (mm²) by female adult western flower thrips (WFT), *Frankliniella occidentalis* in response to Conserve® (spinosad), 5% dilutions of three crude botanical extracts: *Azadirachta indica*, *Parthenium hysterophorus*, *Datura alba* and a water control (n=5 leaf discs/experimental replication, 5 thrips/leaf disc, 6 experimental replications). Standard errors are shown with lines on each bar. Bars marked with the same letter are not statistically different ($P \geq 0.05$, ANOVA, LSD multiple means comparison)

and the commercial availability of *A. indica* extracts makes it an especially good choice for further studies on its use as a management strategy for WFT. There is considerable interest in Pakistan in the use of *P. hysterophorus* and *D. alba* as management strategies for thrips, in particular *T. tabaci*. These plants occur in abundance locally and extracts are inexpensive and easy to produce. Our findings suggest that botanical extracts from both of these species are wor-

thy of further exploration, including different application methods and evaluation of potential impacts on thrips development.

Acknowledgement: This study was supported by Higher Education Commission of Pakistan (HEC) and Agricultural Food Research Initiative, National Institute of Food and Agriculture, Coordinated Agricultural Project Grant #2012-68004-20166. We thank Michael Parrella for thrips rearing space, Jay Rosenheim for statistical advice and Michelle Early for technical support.

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Received: 19.02.2016

Accepted: 19.12.2016