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Source: Florida Entomologist, 97(1) : 269-271

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.097.0137>

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# POTENTIAL OF TERPENOIDS AND MEALYBUG EXTRACT TO DETER THE ESTABLISHMENT OF *DACTYLOPIUS OPUNTIAE* (HEMIPTERA: DACTYLOPIIDAE) CRAWLERS ON *OPUNTIA FICUS-INDICA*

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The prickly-pear cactus, *Opuntia ficus-indica* (L.) Mill. (Caryophyllales: Cactaceae), is a socially and economically important plant for Mexico, as the young cladode is used as a vegetable and forage, and its fruit (*Tuna*) fetches a good price in the domestic market. Further, a highly appreciated natural dye based on carminic acid is obtained from cultivated *Dactylopius coccus* Costa (Hemiptera: Dactylopiidae) reared on the cladode.

The mealybugs *D. coccus* and *D. opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) grow on *O. ficus-indica* and can co-exist in the same area and plant (Llenderal & Campos 2001). Dye producers know that *Opuntia* plants infested with *D. coccus* can support a high population of this insect for up to 7 yr while a high population of *D. opuntiae* can kill an *Opuntia* plant in 6 months (Llenderal & Campos 2001). *Opuntia* growers consider *D. opuntiae* as pest because they feed on the comestible part of the plant, and make it unmarketable. Similarly, dye producers consider *D. opuntiae* as an undesired insect in their crops because of lower dye production per insect and shorter plant survival (Llenderal & Campos 2001).

Mealybug nymphs or crawlers are dispersed by the wind. Once on a suitable host, the crawlers will fix to a cladode and remain there for the rest of their lives. Once they reach maturity, a coat of wax will cover the whole insect providing protection from insecticides, the environment and predators (Pretorius et al. 1992; Llenderal & Campos 2001). In Mexico, control of *D. opuntiae* is achieved by continuous use of synthetic insecticides (Vigueras et al. 2009), or is attempted by manually brushing off the early infestations. However, the latter is labor intensive and effective only at the early stage of the infestation.

The objective of this paper was to test terpenoids known for their repellency or toxicity to insects, such as Eugenol 99% (Across Organics, Mexico), 1,8-Cineol 99% and Menthol 99% (Sigma Aldrich, Mexico) as an alternative to prevent crawlers from establishing on non-infested cladodes under shade house conditions. As carminic acid is known to be repellent to mealybug predators (Eisner et al. 1980) and as we observed the apparent repellency of *D. opuntiae* hemolymph on crawler fixation, an aqueous extract of this insect was also tested. All treatments were evaluated at 0.002, 0.02 and 0.2% in a 50% water-ethanol solu-

tion, plus a mixture of water-ethanol (50%) as a control treatment. The *D. opuntiae* extract was obtained according to Gibaja (1998) and Saénz et al. (2006). All tested compounds had good solubility in the water-ethanol mixture.

One side of a healthy-uninfested cladode of *Opuntia ficus-indica* var. 'Milpa Alta' was sprayed with the test compound, the other side received no application and served as the control. Thirty crawlers were deposited on each side of the cladode after the application of the compound. We sprayed 9 cladodes per treatment. To test for the effect of the treatments on the first generation (50 days after application), we counted the number of crawlers fixed on each side of the cladode and compared counts on each side by a paired *t* test. Later, the data were transformed into standard scores (Quinn & Keough 2002) prior to an ANOVA and the LSD procedure. To test any effect of the compounds on the second generation (107 days after treatment) and because of the large number of insects fixed on the cladode and the difficulty of identifying single individuals at this time, we measured the area covered by the insects and compared the 2 cladode sides by a paired *t* test. The total area for each cladode was obtained from a digital picture by the open source ImageJ software (Schneider et al. 2012).

Fifty days after treatment, the mean ( $\pm$  SEM) numbers of crawlers fixed on the cladode sprayed with eugenol at 0.2% ( $5.33 \pm 1.9$ ), cineol at 0.002% ( $7.77 \pm 1.35$ ) and extract of *D. opuntiae* at 0.02% ( $8 \pm 1.46$ ) were significantly less than those fixed on the unsprayed side ( $14.55 \pm 3.0$ ,  $14.88 \pm 1.7$  and  $12.88 \pm 1.5$ , respectively; paired *t* = 2.7, df = 8, *P* = 0.02 for eugenol at 0.2%, paired *t* = 3.02, df = 8, *P* = 0.01 for cineol at 0.002% and paired *t* = 2.33, df = 8, *P* = 0.048 for *D. opuntiae* extract). These numbers represent reductions of 63, 47 and 37% in the number of fixed crawlers, respectively. No difference in count was observed in the ethanol-water control treatment ( $5.44 \pm 0.55$  and  $9.22 \pm 1.77$ , sprayed and un-sprayed respectively; paired *t* test = 1.7, df = 8, *P* = 0.1). ANOVA analysis showed that eugenol at 0.2%, *D. opuntiae* extract at 0.002% and menthol at 0.02% produced significant reductions of 71, 67 and 63%, respectively, on the numbers of crawlers fixed relative to the water-ethanol treatment (*F* = 4.47; *gl* = 12, 104; *P* < 0.0001) (Fig. 1).

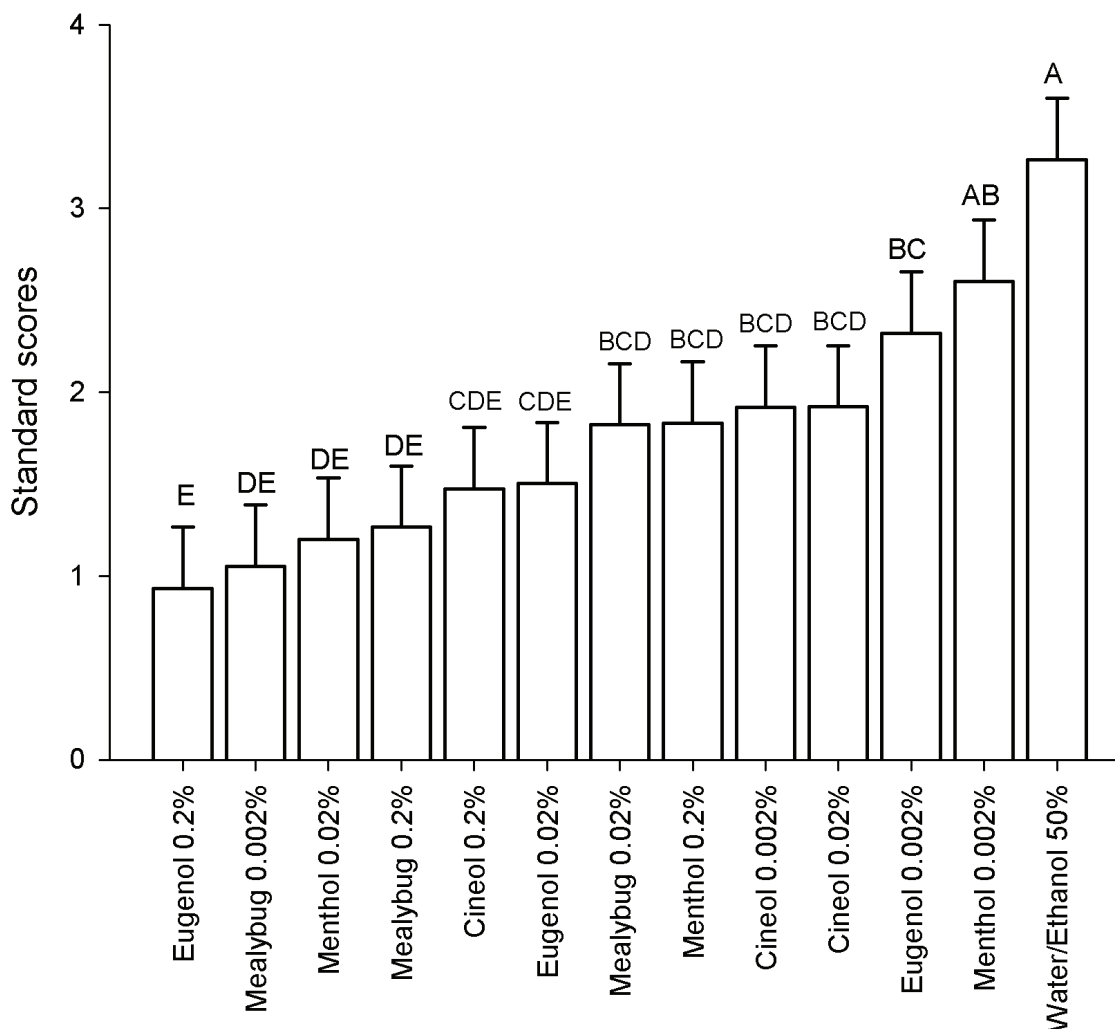


Fig. 1. Mean ( $\pm$  SEM) no. of *Dactylopa opuntiae* crawlers fixed on *Opuntia ficus-indica* cladodes fifty days after the treatment with varying doses of plant essential oils and mealybug extract in a water/ethanol mix. Bars followed by the same letter are not significantly different (LSD,  $P > 0.05$ ).

At 107 days post-treatment, the cladodes sprayed with menthol at 0.002% and cineol at 0.2% presented less surface covered by the insects ( $12.84 \pm 4.28$  and  $25.2 \pm 19.79$  cm<sup>2</sup>) than those unsprayed ( $52.85 \pm 8.04$  and  $39.04 \pm 10.29$  cm<sup>2</sup>, respectively) ( $t_{\text{paired}} = 2.53$ ,  $P = 0.04$  for menthol 0.002% and  $t_{\text{paired}} = 2.71$ ,  $P = 0.03$  for cineol 0.02%); hence reduction of 75.7 and 35.4%, respectively. There was no reduction in the area covered by the mealybugs in the water-ethanol treatment ( $21.48 \pm 7.17$  and  $25.03 \pm 7.75$  cm<sup>2</sup>,  $t_{\text{paired}} = 1.38$ ,  $P = 0.2$ ) at 107 days post-treatment.

Our results corroborate those of Viguera et al. (2009) and Vázquez-García et al. (2011) who investigated the role of plant extracts on insect repellency. The former reported that *Mentha piperita* L. (Lamiales: Lamiaceae), *M. viridis* L.,

*Tagetes erecta* L. (Asterales: Asteraceae) and *Chenopodium ambrosioides* L. (Caryophyllales: Chenopodiaceae) were toxic for first and second instars nymphs (crawlers) of *D. opuntiae* suggesting that the terpenoids present in the extract may be responsible for the toxicity. Vázquez-García et al. (2011) reported that essential oils of *Ocimum basilicum* L. (Lamiales: Lamiaceae), *Mentha spicata* L., *Cymbopogon winterianus* (Jowitt) (Poales: Poaceae) and *Lippia graveolens* Kunth (Lamiales: Verbenaceae) were toxic to 1st instar *D. opuntiae*. Cineol is toxic to *Spodoptera litura* (Fabricius) (Noctuidae), *Brevicoryne brassicae* (L.) (Aphididae) *Musca domestica* L. (Muscidae) and repellent to *Tribolium confusum* du Val (Tenebrionidae). Eugenol is toxic to *M. domestica*, *S. litura*, *Sitophilus granarius* L. (Curculionidae)

and repellent to *T. confusum*. Menthol has been reported toxic for *M. domestica* and repellent for *T. confusum* (Adler et al. 2000; Koul et al. 2008; Palacios et al. 2009). Cineol applied on *Brassica oleraceae* L. var. *capitata* (Brassicaceae, cabbage) repelled 96% of the aphids, *Myzus persicae* (Sulzer) and *B. brassicae* (Ricci et al. 2010). Carminic acid, the principal component of the *D. opuntiae* extract is water soluble (Gibaja 1998) and is a feeding deterrent for predators of *Dactylopius* spp. (Eisner et al. 1980). Repeated application of the mealybug extract warrants further exploration.

#### SUMMARY

Eugenol, menthol and *D. opuntiae* extract reduced the number of *D. opuntiae* crawlers fixed to healthy *Opuntia* cladodes by 71, 63, and 67% respectively. Furthermore, 107 days into the experiment, a reduction of 75% of crawlers fixation was observed on cladodes treated with menthol. There is great potential for the use of *D. opuntiae* extract as a control measure as it is easy to obtain and to apply, and it is available to the farmer.

Key Words: eugenol, carminic acid, menthol, cladode

#### RESUMEN

Eugenol y mentol así como el extracto de *D. opuntiae* redujeron en un 71, 63 y 67%, el número de ninfas que se fijaron a cladodios sanos de *Opuntia*. Mas aún, 107 d después de iniciado el experimento, se observó una reducción del 75% de ninfas fijadas en los cladodios tratados con mentol. El extracto de *D. opuntiae* tiene gran potencial para ser usado como método de control ya que es fácil de obtener y aplicar y está disponible para el campesino.

Palabras Clave: eugenol, ácido carmínico, mentol, cladodio

#### ACKNOWLEDGMENTS

APR was funded by a Master Scholarship from the Mexican National Council of Science and Technology. This research was founded by SIP grant 20110675 to AJP. We thank Georgina Sánchez-Rivera, María Magdalena Callado-Galindo and Daniel Antonio Vázquez-Covarrubias for help during data collection. We thank the New Zealand Institute for Plant and Food Research Limited for providing facilities for writing the manuscript, and Lloyd Stringer (PFR, NZ) for proofreading and improving the English. AJP acknowledges support from Consejo Nacional de Ciencia y Tecnología through

a Sabbatical Year Fellowship (ref. 175805). FCA and AJP are Comisión de Operación y Fomento de Actividades Académicas and Estímulo al Desempeño de los Investigadores fellows.

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