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Feeding Deterrence and Inhibitory Effects of Bee Balm (*Monarda didyma*) Leaves on Fall Armyworm

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The fall armyworm [*Spodoptera frugiperda* (J.E. Smith)] is a serious pest of many field and horticulture crops. Because of the many advantages for the use of plant-derived pesticides, we tested whether bee balm (*Monarda didyma* L.) leaves could have feeding deterrence on fall armyworm. When *S. frugiperda* larvae (neonates and instars 3–4) were supplied with freshly harvested leaves of *Zea mays* L. (corn), bee balm, or *Melissa officinalis* L. (lemon balm) for 9 days, 94% of the insects survived on corn leaves, yet, only 0.96% and 48% of the insects survived on bee balm and lemon balm leaves, respectively. For the larvae that survived, their growth on bee balm and lemon balm was significantly reduced ($P < 0.001$) compared to larvae fed corn leaves. When a choice between corn and bee balm leaves was given, the insect preferred corn leaves. The nature of the feeding deterrence was likely phytochemical rather than structural since fall armyworm larvae reared on an artificial diet mixed with macerated bee balm leaves were significantly ($P < 0.05$) inhibited when compared to those reared on an artificial diet mixed with macerated corn leaves. Our results suggest that there is potential for the use of bee balm for fall armyworm control.

Fall armyworm [*Spodoptera frugiperda* (J.E. Smith)] is a serious pest of economically important crops especially in the subtropical and tropical regions of the Western Hemisphere. It is a migratory pest and populations can move northward each season to southern Canada (Sparks, 1979). Larvae of this polyphagous pest have a wide host range and voraciously consume the foliage of many field and horticultural crops (Capinera, 2014). Although many insecticides are available for controlling fall armyworm (Young, 1979), insecticide use is not without problems. Emerging resistance to insecticides (Carvalho et al., 2013; Yu, 1991; Yu et al. 2003) and potential environmental concerns associated with the use of insecticides (Wauchope, 1978) are making alternative approaches to control this pest attractive.

Botanical insecticides, deterrents, and repellents can be effective alternatives to chemical insecticides under appropriate circumstances (Isman, 2006). Phytochemicals with insecticidal and pest repellent properties could also provide lead structures for developing new insecticides. While there are no known botanical insecticide products that control fall armyworm, some limonoids from neem (*Azadirachta indica* A. Juss) (Koul et al., 2004; Mendel et al., 1993) and loline alkaloid derivatives from tall fescue plants (*Festuca arundinacea* Schreb.) (Riedell et al., 1991) were reported to be feeding deterrents for fall armyworm larvae.

When a greenhouse grown pepper (*Capsicum annuum* L.) crop in Florida was seriously damaged by a fall armyworm infestation, we observed that bee balm plants kept in close proximity to the pepper plants were not attacked, suggesting that bee balm may have chemical or physical repellency to the caterpillars. This observation, combined with the fact that *Monarda* essential oils have been shown to have diverse biological activities, including repellency to mosquitoes (Tabanca et al., 2013) and bean weevil (Weaver et al., 1995), led us to hypothesize that phytochemicals in *Monarda didyma* leaves may have feeding deterrence or inhibitory qualities on fall armyworm. If so, then perhaps *M. didyma* could be used as a "push" species in a push–pull integrated pest management system (Cook et al., 2007). In the "push–pull" strategy for integrated pest management, repellent stimuli are used to make the crop unattractive to the pest (push) luring the pest to an attractive source (pull). The objectives of this research were to: 1) determine if there would be weight and mortality differences in fall armyworm larvae fed bee balm, lemon balm, or corn leaves; 2) observe feeding preferences and behaviors when neonates or mid-instars of fall armyworm were offered a choice between corn and bee balm leaves; and 3) determine whether the feeding deterrence with bee balm was constitutive or structural.

Materials and Methods

We used laboratory-reared fall armyworm larvae in several bioassays. We tested: a) the effects on neonates (newly emerged larvae) and mid-instars when fed leaves of bee balm, lemon balm or corn, in a no-choice test; b) the behavior of fall armyworm when presented with both corn and bee balm leaves in a choice test; and c) the effect of supplementation of artificial diet with bee balm leaves compared to corn leaf supplementation.

FALL ARMYWORM COLONY MAINTENANCE. All fall armyworm cultures were maintained at the USDA–ARS Center for Medical, Agricultural and Veterinary Entomology (CMAVE) laboratory in

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Gainesville, FL. Insect culture procedures followed Stuhl et al. (2008). Briefly, adults were placed in cylindrical screen cages (28 cm in height, 21 cm in diameter) and supplied with a 2% sugar-honey solution for nourishment. Paper towels (Sparkle™, Georgia-Pacific, Atlanta, GA) were stretched at the tops of the cages as an oviposition substrate. Emerging neonates were placed in rearing trays (BioServ, Frenchtown, NJ) that contained a pinto bean artificial diet (Guy et al., 1985). After 23 ± 1 days, pupae were removed from the trays, sexed, and emerged adults were placed in the screen cages. Larvae and adults were reared in incubators or large rearing units at ≈23 °C, 70% RH, and 14 h/10 h (light/dark) photoperiod.

Bee balm and lemon balm maintenance. Seeds were acquired from Johnny's Selected Seeds (Winslow, ME,). The first group of plants was planted in Jan. 2014, in Fafard 2B Professional Growing Mix (Sungro Horticulture, Agawam, MA), fertilized with Peter's Professional (N-P-K 20-20-20) 2 $g \cdot L^{-1}$ every three weeks and watered to field capacity when needed. The second group of seeds was planted in Feb. 2015, in Miracle-Gro Organic Choice Potting Mix (N-P-K 0.10-0.05-0.05, Miracle-Gro, Marysville, OH), fertilized with Marine Cuisine (10N–7P–7K, Fox Farm, Arcata, CA) time release as a tea at the rate of 200 mL per 3.785 L of water and used to irrigated the plants at a rate of 200 mL of tea per 7.6 L pot. Corn (popcorn type) seedlings were grown in 10 cm plastic containers in Miracle-Gro Organic Choice Potting Mix and irrigated to field capacity when needed and fertilized with Peter's Professional (The Scotts Company LLC, Marysville, OH) 2 $g \cdot L^{-1}$ once a week.

Experiment 1: No choice test. Neonates from the "Hendry" corn strain colony (generations F-11 and F-12) were used in this experiment. Three trials were conducted using fresh bee balm, lemon balm and corn leaves. Polystyrene 60 mm x 15 mm petri dishes were used in Trial 1 (20 dishes per plant species). Fully-expanded leaves (1 leaf per dish) were placed on top of Whatman No. 1 filter paper, cut to fit, and moistened with 0.3 mL distilled water. One neonate per petri dish was carefully placed inside the dish and sealed with Parafilm strips (Pechiney Plastic Packaging, Menasha, WI). For Trial 2 and Trial 3, rearing trays BIO-RT-32 cell (Frontier, Newark, DE) with 16 cells per plant species were used. Cells were covered with gas permeable adhesive tabs (BIO-CV-4, Bio-Serv, Pitman, NJ). Moisture and plant material were replaced as needed. Dishes or trays were kept in an environmental chamber set at 24.8 °C, 70% RH, and 13 h/11 h (light/dark) photoperiod. Larval weight and mortality were recorded after 9 days.

Experiment 2: Choice test. Neonates from the "Hendry" corn strain colony (F-13) were used for Trial 1. Third and fourth instars from the "Hendry" (F-14) and "DRU" (F-56) corn strain colonies were used for Trial 2 and Trial 3, respectively. In all three trials, leaf disks punched (radius = 10.5 mm) from fresh plant material of bee balm and corn were placed in polystyrene 100 mm x 15 mm petri dishes. Leaf disks were placed on top of Whatman No. 1, 90 mm filter paper, moistened with 1.0 mL distilled water. In Trial 1, three treatments were composed of: a) 6 dishes of bee balm (2 disks in each dish) with corn (2 disks in each dish) and 20 neonates were placed in each dish; b) 6 dishes of corn only (4 disks of corn in each dish) with 20 neonates in each dish; and c) 2 dishes with corn and bee balm and no neonates (control, to check the quality of the leaf disks following the incubation). Dishes were assessed 24 and 48 h later to determine the number of insects feeding at the time of observation and the amount of leaf eaten. The latter was scored using a scale from 0 to 5, where 5 meant all the leaf had been eaten and 0 meant no leaf had been eaten. In Trial 2 and Trial 3, mid-instars (3rd and 4th instars) were used with five larvae per dish. All other protocols were as in Trial 1.

Experiment 3: Effect of leaves blended in artificial medium. Fresh leaves of *M. didyma* or *Z. mays* (620 g fresh weight) were macerated with 200 ml of distilled water using a blender (Nutribullet, Pacoima, CA). These blended materials were added to pinto bean diet (Guy et al., 1985) at concentrations of 50% (wt/wt), 20% (wt/wt) and 10% (wt/wt). The control was artificial pinto bean diet alone. One 32-cell rearing tray per *Z. mays* and one 32-cell tray per *M. didyma* was used with 8 cells per treatment. After the diets were allowed to gel overnight, "Hendry" colony corn strain fall armyworm (F-16) 8-day-old mid-instars were placed in individual cells and covered with gas-permeable lids. Weights and mortality were recorded at 9 days. When surviving larvae pupated, the pupae were placed in rearing cages to test for fecundity.

Statistics. All experiments were set up using the completely randomized design. Quantitative data were analyzed using analysis of variance with mean separation by Tukey's test using SAS statistical software version 9.4 (SAS Institute Inc., Cary, NC).

Results

Neonates and mid-instar larvae of fall armyworm were presented with corn, bee balm or lemon balm leaves in a no-choice experiment. While the insects fed well on corn leaves, they tried to move away from bee balm or lemon balm leaves, a behavior suggestive of repellency by these two species of Lamiaceae. In no-choice experiments, fall armyworm larvae fed on very small amounts of bee balm and lemon balm leaves and were visibly affected—in terms of differences in body color and size—even within one day of feeding (Fig. 1). When left to feed on these diets for several days, insect mortality was significantly greater in the bee balm and lemon balm leaf treatments compared to the corn leaf control. Following nine days, the larval survival

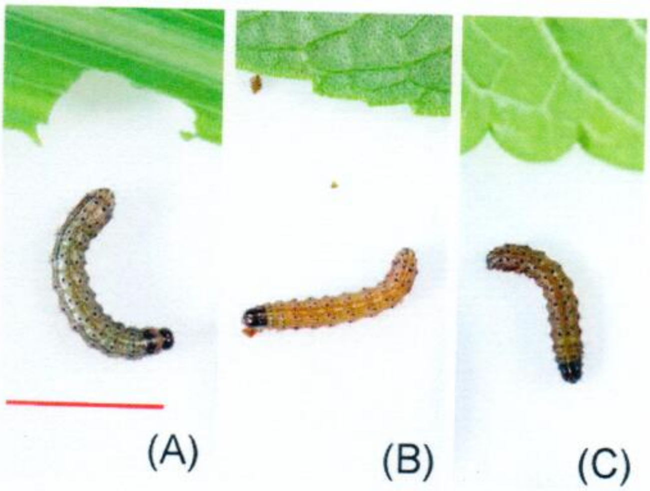

Fig. 1. The morphology of fall armyworm early third instar larvae following their feeding for 24 h either on leaves of corn (**A**), bee balm (**B**) or lemon balm (**C**), photographed under same magnification. The bar in (**A**) representing 9 mm also applies to (**B**) and (**C**).

rates were 94%, 48%, and 0.96% for corn, lemon balm and bee balm leaves, respectively. The larvae surviving on bee balm and lemon balm were significantly smaller than those fed corn leaves ($P < 0.01$) (Fig. 2).

When given a choice between bee balm and corn leaf disks in the same dish, fall armyworm larvae preferred corn over bee balm. In a choice experiment, five larvae in a dish were presented with both corn and bee balm leaf disks. After 48 h, only 1.6% of the larvae were observed to be on bee balm leaf, while 56.6% of the larvae were on corn leaves; 28.6% showed no preference at the time of observation and 13.2% died. Some of the deaths were observed to be due to cannibalism, a common feature of this pest.

When leaf consumption was scored using a 0 to 5 scale in a choice experiment, bee balm leaf consumption was significantly less than corn leaf consumption ($P < 0.01$) (Fig. 3). The insects consumed comparable amounts of corn leaf whether presented in the dish where bee balm leaves were also presented or corn leaf alone was presented. These results together suggest that bee balm has feeding deterrence for fall armyworm and the presence of bee balm leaf in the dish was not inhibiting the insect from eating corn leaf provided in the same dish.

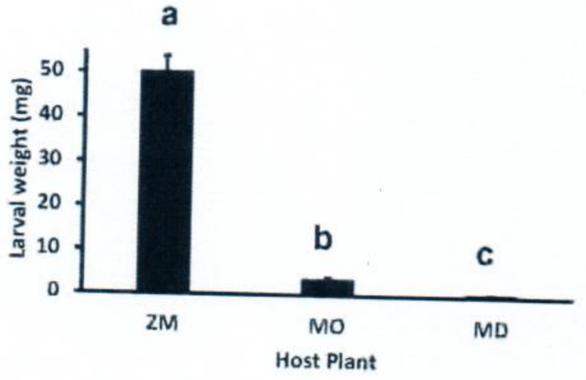

Fig 2. Effect on fall armyworm larval growth of a diet of leaves of corn (ZM), lemon balm (MO) or bee balm (MD) in a no-choice experiment. Bars represent mean larval weight (n = 3 to 19) and standard error. Neonates (n = 20 per host species) were tested and the larvae were weighed after 9 days of feeding. One of two experiments with similar results is shown. Bars marked by different letters indicate significant difference at $P < 0.01$ according to Tukey's test.

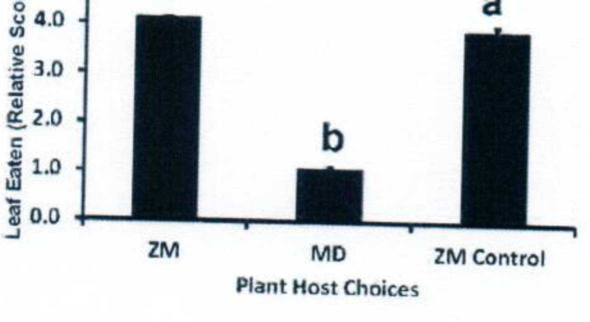

Fig. 3. Feeding behavior of fall armyworm larvae when presented with a choice between corn (ZM) and bee balm (MD) leaf disks. Amount of the leaf disk eaten over 48 h by a single third instar larva in a choice experiment was scored using a 0–5 scale (0 = no consumption and 5 = the entire leaf disk consumed). Bars represent means of 18 observations each and standard error. Bars marked by different letters indicate significant difference at $P < 0.01$.

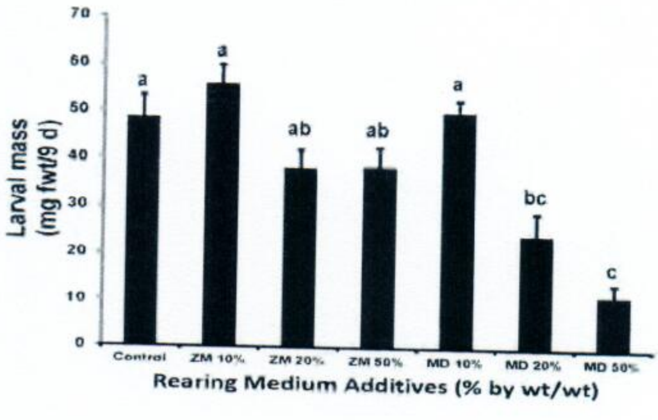

Fig.4. Effect of macerated leaves from corn (ZM) or bee balm (MD) when fed to fall armyworm larvae via artificial diet. Mean larval mass was measured nine days following feeding third instar larvae on artificial medium supplemented with 0 or 10% to 50% wt/wt of freshly macerated leaves. Means indicated with same letters are not significantly different at $P < 0.05$ as determined with Tukey's test.

To test whether feeding deterrence of bee balm was due to phytochemicals or simply by structural features, we tested the effect of a supplement of blended leaves of bee balm in an artificial medium on larval growth compared to media supplemented with blended corn leaves. While corn leaf extract up to 50% (wt/wt) had no significant effects on larval growth, bee balm leaf extract at 20% (wt/wt) and 50% (wt/wt) significantly reduced larval growth (Fig. 4). The concentration dependent reduction of larval growth by bee balm leaves in this experiment suggests a phytochemical basis for the inhibitory effects, although additional feeding deterrence due to structural features of the leaf in the feeding studies (Fig. 2, Fig. 3) cannot be ruled out.

Caterpillars developing from bee balm leaf diet from the experiment shown in Fig. 4, were qualitatively smaller compared to those from the corn diet. While 18 out of 18 caterpillars grown on corn diet pupated, only 11 out of 20 larvae reared on bee balm leaf diet pupated. The adults reared on bee balm leaf diet also had reduced egg laying capacity and the eggs from the bee balm diet-fed insects were sterile when observed under the microscope (data not shown).

Discussion

Monarda essential oils have been reported to have diverse biological activities including repellency to mosquitos (Tabanca et al., 2013) and bean weevil (Weaver et al., 1995). However, the current study is the first to record the feeding deterrence and inhibitory effects of *M. didyma* leaves on fall armyworm. In our study both the neonates and mid-instar larvae were significantly affected by a *M. didyma* diet (Fig. 1 and 2). When fed only bee balm leaves, the mortality of fall armyworm was high and the surviving larvae were severely inhibited (Fig. 2). Although fall armyworm prefers grass hosts, it is a pest in many dicot species as well (Sparks, 1979). In the choice experiments, the fall armyworm showed a clear preference to corn (Fig. 3), suggesting repellency by bee balm. Our results showing severe inhibition of larval growth by bee balm leaves and subsequent developmental effects are consistent with the hypothesis that phytochemicals in *M. didyma* leaves had both feeding deterrence and inhibitory effects. Inhibitory effects of macerated *M. didyma* leaves added to the artificial diet (Fig. 4) indicate that the major part of the inhibitory effects is from the phytochemicals rather than structural. The presence of deterrence and inhibitory activities in lemon balm (Fig. 2), but with lower potency than in *M. didyma*, suggests that it will be useful to screen additional members of the Lamiaceae for feeding deterrence and inhibitory effects against fall armyworm.

In addition to insect deterrence, *Monarda* herbage or essential oils have been shown to have a diverse range of bioactivities. These include antifungal activities against grey mold (*Botrytis cinerea*) (Adebayo et al., 2013), damping-off (*Rhizoctonia solani*) (Gwinn et al., 2010) and micromycetes of indoor environments (Mickiene et al., 2014); antibacterial activity (Li et al., 2014), acaricidal activity (Kaidarov et al., 1992) and phytotoxicity (Rolli et al., 2014), making this an interesting genus to explore for the preparation of botanical pest management products. In this regard, all twelve species of *Monarda* and their hybrids distributed in North America and Mexico (Bailey and Bailey, 1976) can be screened for specific bioactivities and chemical composition. So far, phytochemical investigations on some of the species of *Monarda* identified compounds potentially explaining their bioactivities, such as thymol, p-cymene, limonene, linalol and carvacrol as major components of essential oils (Carnat et al., 1991; Li et al., 2014). One study on antifungal activities found that *M. didyma* essential oils to be better than those from *M. fistulosa* L. (Adebayo et al., 2013), suggesting that various species of *Monarda* could potentially have varying levels of pest deterrence activities.

Currently we do not know the specific phytochemicals responsible for the feeding deterrence and inhibitory effects observed on fall armyworm larvae. Future bioassay-guided fractionation of *Monarda* leaf chemicals should identify the most active fractions and their composition. Our findings suggest that bee balm and related plants can play a role in an integrated approach to manage fall armyworm. Other than the pest management value, our study also provides a new model system to investigate plant-insect interaction in which both the plant host and the pest likely evolved in the same natural habitat, providing an opportunity to test the ecological and evolutionary significance of phytochemical defense against insect pests.

Literature Cited

Adebayo, O., A. Belanger, and S. Khanizadeh. 2013. Variable inhibitory activities of essential oils of three *Monarda* species on the growth of Botrytis cinerea. Can. J. Plant Sci. 93:987–995.

Bailey, L.H. and E. Bailey. 1976. Hortus Third. A concise dictionary of plants cultivated in the United States and Canada. Macmillan Publishing Co.: New York, NY. p. 737–738.

Capinera, J.L. 2014. Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Insecta: Lepidoptera: Noctuidae). IFAS Extension, University of Florida, EENY098. <http://edis.ifas.ufl.edu/in255>

Carnat, A., J.L. Lamaison, and A. Remery. 1991. Composition of leaf and flower essential oil from *Monarda didyma* L. cultivated in France. Flav. Frag. J. 6:79–80.

Carvalho, R.A., C. Omoto, L.M. Field, M.S. Williamson and C. Bass. 2013. Investigating the molecular mechanisms of organophosphate and pyrethroid resistance in the fall armyworm *Spodoptera frugiperda*. PLoS ONE 8(4): e62268. doi:10.1371/journal.pone.0062268.

Cook, S.M., Z.R. Khan, and J.A. Pickett. 2007. The use of push-pull strategies in integrated pest management. Ann. Rev. Entomol. 52:375–400.

Guy, R.N., N.C. Leppla, J.R. Rye, C.W. Green, S.L. Barette, and K.A. Hollien,. 1985. *Trichoplusia ni*. In: Singh, P. and R.F. Moore (eds.) Handbook of insect rearing, vol. 2. Elsevier, Amsterdam, p. 487–494.

Gwinn, K.D., B.H. Ownley, S.E. Greene, M.M. Clark, C.L. Taylor, T.N. Springfield, D.J. Trently, J.F. Green, A. Reed, and S.L. Hamilton. 2010. Role of essential oils in control of Rhizoctonia Damping-Off in tomato with bioactive *Monarda* herbage. Phytopathology. 100:493–501.

Isman, M.B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol. 51:45–66.

Kaidarov, K.M., O.V. Karavaeva, V.V. Kiseleva, M.O. Karryev, and I.G. Nikolaeva. 1992. Acaricidal activity of some preparations of plant origin. Izvestiya Akaemii Nauk Turkmenistana. Seriya Biologicheskikh Nauk 3:53–55.

Koul, O., G. Singh, R. Singh, J. Singh W.M. Daniewski, and S. Berlozecki. 2004. Bioefficacy and mode-of-action of some limonoids of salannin group from *Azadirachta indica* A. Juss and their role in a multicomponent system against lepidopteran larvae. J. Biosci. 29:409–416.

Li, H., T. Yang, F. Li, Y. Yao, and Z. Sun . 2014. Antibacterial activity and mechanism of action of *Monarda punctate* essential oil and its main components against common bacterial pathogens in respiratory tract. Intl. J. Clinical and Exp. Pathol. 7:7389–7398.

Mendel, M.J., A.R. Alford, M.S. Rajab, and M.D. Bentley.1993. Relationship of citrus limonoid structure to feeding deterrence against fall armyworm (Lepidoptera, Noctuidae) larvae. Environ. Entomol. 22:167–173.

Mickiene, R., B. Bakutis, A. Maruska, O. Ragazinskiene, and V. Kaskoniene. 2014. Effect of the volatile secondary metabolites of *Monarda didyma* L., *Lamium album* L., and *Myrrhis odorata* L. plants against micromycetes of indoor environments of animals. Veterinarija ir Zootechnika 68:48-54.

Riedell, W.E., R.J. Kieckhefer, R.E., R.J. Petroski, and R.G. Powell. 1991. Naturally-occurring and synthetic loline alkaloid derivatives-insect feeding-behavior modification and toxicity. J. Entomol. Sci. 26: 122–129.

Rolli, E., M. Marieschi, S. Maietti, G. Sacchetti, R. Bruni, and G. Sacchetti. 2014. Comparative phytotoxicity of 25 essential oils on pre- and post-emergence development of *Solanum lycopersicum* L: A multivariate approach. Indus. Crops Prod. 60:280–290.

Sparks, A.N. 1979. A review of the biology of the fall armyworm. Fla. Entomol. 62: 82–87.

Stuhl, C.J., R.L. Meagher, and R.N. Nagoshi. 2008. Genetic variation in neonate behavior of fall armyworm (Lepidoptera: Noctuidae). Florida Entomol. 91:151–158.

Tabanca, N., Bernier, U.R., Ali, A., Wang, M., Demirci, B., Blythe, E.K., Khan, S.I., Baser, K.H.C., and I.A. Khan. 2013. Bioassay-guided investigation of two *Monarda* essential oils as repellents of yellow fever mosquito *Aedes aegypti*. J. Agr. Food Chem. 61:8573–8580.

Wauchope, R.D. 1978. The pesticide content of surface water draining from agricultural fields—A review. J. Env. Qlty. 7:459–472.

Weaver, D.K., Phillips, T.W., Dunkel, F.V., Weaver, T., Grubb, R.T., and E.L. Nance. 1995. Dried leaves from Rocky-mountain plants decrease infestation by stored-product beetles. J. Chem. Ecol. 21:127–142.

Young, J.R. 1979. Fall armyworm: Control with insecticides. Florida Entomol. 62:130–133.

Yu, S.J., Nguyen, S.N., and G.E. Abo-Eighar. 2003. Biochemical characteristics of insecticide resistance in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). Pesticide Biochem. Physiol. 77:1–11.

Yu, S.J. 1991. Insecticide resistance in the Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith). Pesticide Biochem. Physiol. 39:84–91.