VOL. 7, NO. 11, NOVEMBER 2012 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

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EVALUATION OF *Melia azedarach* AS A BOTANICAL PESTICIDE AGAINST BEET ARMYWORM (*Spodoptera exigua*)

Travis Lee M. and Ken Mix
Department of Agriculture, Texas State University, San Marcos, Texas, USA
E-Mail: liveinlove4@hotmail.com

ABSTRACT

Far less research has been conducted on insecticidal characteristics of *Melia azedarach*, compared to *Azadirachta indica*. With the negative impact chemical pesticides have on the environment and a population looking for alternative methods of controlling insect populations, *M. azedarach* may prove useful. Fruits and roots were collected from local *M. azedarach* trees. Volatiles were cold extracted from the fruits, root cambium and root cortex using water and methanol. The extracts were evaporated to obtain a concentrate and remove methanol. Neonate *Spodoptera exigua* were fed extract mixed into larval diet at a 1:30 (extract: diet). Neonates were placed in 5ml containers. Individual 1st instars were given either a diet treated with a single extract or untreated diet (control). This equaled 4 treatments and each paired with untreated diet. For each treatment or paired control n=10, for a total of 80 individual neonate containers. At 9 days the mean mortality and mean weight were obtained for each container and nonparametric Wilcoxan test was used to determine the level of significance for mortality and weight gain between each treatment and control.

Keywords: *Melia azedarach*, *Spodoptera exigua*, biopesticide, *Azadirachta indica*, root cambium, root cortex.

INTRODUCTION

The use of persistent chemical insecticides has often resulted in ecological backlash. Ecological backlash is a counter-response of the ecosystem that causes the managed system to be less manageable than prior to control applications. Specifically with insect pest populations it manifests in forms of resistance, resurgence or replacement. Resistance is when the population becomes less susceptible to control measures. The result can be a pest population that is larger and harder to control, hence more destructive. Resurgence is the rapid and injurious appearance of a pest population after a broad-spectrum pesticide killed off the natural enemies of the pest that usually kept its population down. Replacement is when pests are killed and another pest fills the niche vacated and injures the crop. One mechanism that leads to backlash is the use of environmentally persistent synthetic chemical pesticides that lead to longer and sometimes low level exposure. Further, they may kill a broader range of pests as well as non-pests. Non-pests mortality is frequently collateral damage of beneficial insect populations. Loss of these beneficial insects can reduce predatory pressure on pests thus exacerbating the backlash by allowing them to feed unperturbed and over populate.

The focus on biodegradable or more selective pesticides, that are less persistent and suitable for use in integrated pest management are becoming more important for the safety of the environment and society. For this reason there has been growing interest in alternative botanical insecticides for use in sustainable agriculture (Klocke *et al*., 1991). Many plant derived products meet this criterion and have potential for more research (Guleria *et al*., 2009). For example, selected marigolds (*Tagetes spp*) have been interplanted with crops susceptible to nematodes to decrease nematode population (Ploeg, 2002). Pleog (2002) planted tomatoes immediately after growing *Tagetes patula* (Single Gold Marigold) in that same area. The study showed that tomato production increase by 50% and nematode damage and population decreased significantly. Studies utilizing *Artemisia capillaris* extracts have shown that it can be used as a biopesticide against the *Sitophilus zeamais* (maize weevil) (Liu *et al*., 2010). *Azadirachta indica* is one botanical insecticide on which extensive work has been conducted. For example, *A. indica* seed extracts fed to grasshopper nymphs under field conditions proved to be effective in reducing grasshopper populations to an acceptable level (Baumgart, 1995). Another study showed that *A. indica* oil was effective in controlling mosquito larva under natural field conditions with 100% larva control after 7 days (Dua *et al*., 2009) Andrade-Coelho *et al*. (2009) fed *Lutzomyia longipalpis* (sand fly) larvae *A. indica* fruits and leaves. The result was 70% mortality among larva fed fruit and leaves while the control only had 11.1% mortality at 14 days. In this same study *M. azedarach* fruit and leaves were used similarly as *A. indica* and showed on day 14 mortality increased to 95.6% while the control was only 14% mortality (Andrade-Coelho *et al*., 2009).

A. indica is a close relative to *M. azedarach* (Ahmed, 1985) and therefore a similar bioactivity as a biopesticide may be expected. *A. indica* and *M. azedarach* are so closely related that the rootstock of *M. azedarach* has been used to graft *A. indica* scions for production in cooler climates (Forim *et al*., 2010). Similar to *A. indica*, *M. azedarach* is a native of south Asian regions and studies have been conducted on its use as a biopesticide (Nardo *et al*., 1997, Valladares *et al*., 1997, Schmidt *et al*., 1998, Borges *et al*., 2003). Nardo *et al*. (1997) found that *Bemisia tabaci* (white fly) fed *M. azedarach* extract died at the same rate as unfed (starved) *B. tabaci*. Valladares *et al*. (1997) studied the effects of ethanolic extracts of *M. azedarach* fruits with concentrations of 2,5 and 10% deterred feeding by both larva and adults of

Xanthagalleruca luteola (elm leaf beetle). Schmidt *et al*. (1998) found that extract from *M. azedarach* fruit effects the neuroendocrine control on *Spodoptera littoralis* (egyptian cotton leafworm) and *Agrotis ipsilon* (black cutworm). Similarly, Borges *et al*. (2003), immersed *Boophilus microplus* (tick) larvae and engorged females in *M. azedarach* decreasing concentrations of 0.25% to 0.015% mortality was evaluated after 24, 72, and 168 hours after treatment and found it had significant mortality rate on larva and reduced production in females.

JUSTIFICATION

This study investigates the use of extracts from *M. azedarach* as a potential botanical pesticide. This particular relative of Neem has been largely overlooked. A few studies, however, indicated potential similar to *A. indica*. Most of them focus solely on extracts from berries and leaves (Valladaras *et al*., 1997, Bordges *et al*., 2003). Those studies have shown significant results as a pesticide. In this study, however, root cortex was utilized along with fruits for comparison. Few, if any, studies investigate the use of extracts from *M. azedarach* root cortex as a potential botanical pesticide.

METHODS AND MATERIALS

Extracts from *M. azedarach* berries and roots were fed to *Spodoptera exigua* (beet armyworms) larval for 9 days. Local *M. azedarach* berries and roots were collected. Ripe berries were collected in October, 2010. The roots were collected post-abscission in December, 2010, a time more likely for the plant to have sequestered secondary compounds in the roots. The plants were collected in San Marcos, TX, USDA hardiness zone 8b.

The specific soil the roots were located in is a rocky hillslope soil in the Comfort-Rock series, which are heavy clay soils dominated by limestone cobbles and boulders.

Methanol and aqueous extracts where made from 400 ml crushed berries (included flesh, seed and shell) and 300 ml of root cortex shavings. The berries and solvents were left to sit for a total of ten days. The extracts were then filtered through cheesecloth to remove large debris then through a coffee filter paper to remove smaller sediments. Following this the methanol and water were evaporated from the extracts to make a liquid concentrate. *S. exigua* laboratory diet was prepared according to directions for control diets and treated diets were prepared using 1 ml extract per 30 ml of diet. The approximate volume of extract concentrate in the diet was 33, 000 ppm.

The treatment block consisted of 10 containers with multiple *S. exigua* 2^{nd} instar larva in each fed diet with extract and 10 similar containers fed control diet. The samples were then placed randomly within each treatment block. Larvae were fed diet for 9 days. Mortality was counted at days 4 and 7. On the 9th day a mean weight was obtained for surviving larva in each container to compare mean weight gain between treatments and control. Each container was considered a sample and means of larval mortality and weight were developed for each (Table 1 and 2, respectively).

RESULTS AND DISCUSSIONS

The results of analyses indicated that mortality at 4 days was not significantly different ($p < 0.05$) for larva fed diets with aqueous or methanolic extracts of berry or root cortex, compared to larva fed control diets (Table-1, Figure-1).

Table-1. Mortality difference between berry and root cortex on days 4 and 7 relevant to their solvent.

		Berry			Control				
Extract	**Days**	**n**	**Mean**	**StD**	**n**	**Mean**	**StD**	**dif**	**p-value**
Methanol	4	10	0.119	0.120	10	0.065	0.105	0.054	0.1502
	7	9	0.372	0.274	9	0.250	0.273	0.122	0.1790
Aqueous	4	10	0.165	0.108	10	0.113	0.089	0.052	0.1285
	7	9	0.315	0.217	8	0.191	0.198	0.124	0.1200

		Cortex			Control				
Extract	**Days**	**n**	**Mean**	**StD**	**n**	**Mean**	**StD**	**dif**	**p-value**
Methanol	4	10	0.069	0.087	10	0.053	0.088	0.016	0.3372
	7	8	0.256	0.076	10	0.157	0.165	0.099	0.0679
Aqueous	4	10	0.117	0.109	10	0.062	0.106	0.055	0.1213
	7	10	0.309	0.239	10	0.160	0.123	0.149	0.0483

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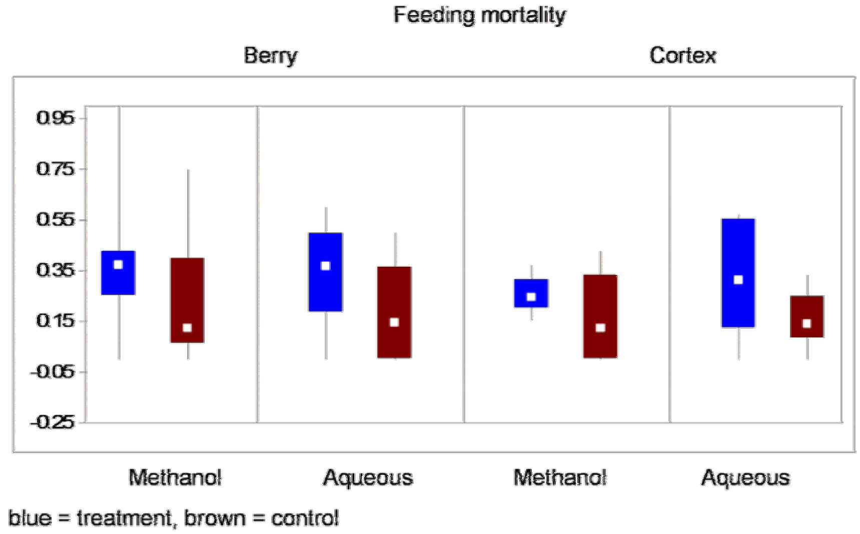

Figure-1. Graph illustrates mortality on day 7. The central open square shows the mean, the box is the interquartile range and the whiskers represent the minimum and maximum mortality of each extract.

Similarly, at 7 days, there was no significant difference in mortality for larva fed berry aqueous or methanolic extracts and those fed methanolic root cortex extracts, compared to controls. However, those fed diets with aqueous extracts of root cortex did have a significantly higher rate of mortality compared to controls at $p < 0.05$ (Table-1, Figure-1). This is similar to other studies of *M. azedarach* extracts fed to insects; although extract concentrations vary between studies. Juan *et al*. (2006) found 2nd instar *Sesamia nonagrioides* fed a diet laced with seed and fruit methanolic extracts of *M. azedarcach* at 2000 ppm resulted in an antifeedent effect. Borges *et al*. (2003) utilized hexane, $CHCl_3$ and ethanol as extracting solvents in concentrations between 0.25% and 0.015%. The results indicated contact with all extracts of *M. azedarach* caused mortality of *B. microplus*. The mortality rate ranged from 50-100%. It was highest, however, with the 12.5% $CHCl_3$ extract (effectively 125, 000ppm) solution and 7 day exposure. Ethanolic extracts nearly always caused the lowest mortality. Comparatively, the 30,000 ppm aqueous extract used in this study resulted in lower mortality at 7 days, with maximum mean mortality of 0.309 individual 1st instars container-1. The lower mortality rate may be a result of either the lower concentration or extract used; however, it was significantly higher than the mortality in containers containing untreated diet. Nardo *et al*. (1997) reported a higher mortality rate from using an aqueous extract without evaporating the extract into a concentrate. They found that spraying the direct extract solution on *Bemisia tabaci* produced near 100% mortality within 4 days.

The results of the weight gain analyses indicated the extracts of *M. azedarach* influenced weight gain. When samples were weighed and analyzed, it was found weight gain for individual 1st instars fed any extract was significantly lower ($p < 0.05$) when compared to weight gain in samples fed untreated diets (Table-2, Figure-2).

Table-2. Represents the average minimum and maximum weight per container on day 9.

	Water			Control				
Source	**n**	**Mean**	**StD**	**n**	**Mean**	**StD**	**dif**	**p-value**
Berry	10	0.00021	0.00006	10	0.00064	0.00017	0.00043	0.0001
Cortex	9	0.00018	0.00012	9	0.00044	0.00016	0.00026	0.0010

	Methanol			Control				
Source	**n**	**Mean**	**StD**	**n**	**Mean**	**StD**	**dif**	**p-value**
Berry	8	0.00007	0.00004	10	0.00049	0.00023	0.00042	0.0001
Cortex	9	0.00006	0.00007	9	0.00066	0.00021	0.00060	0.0009

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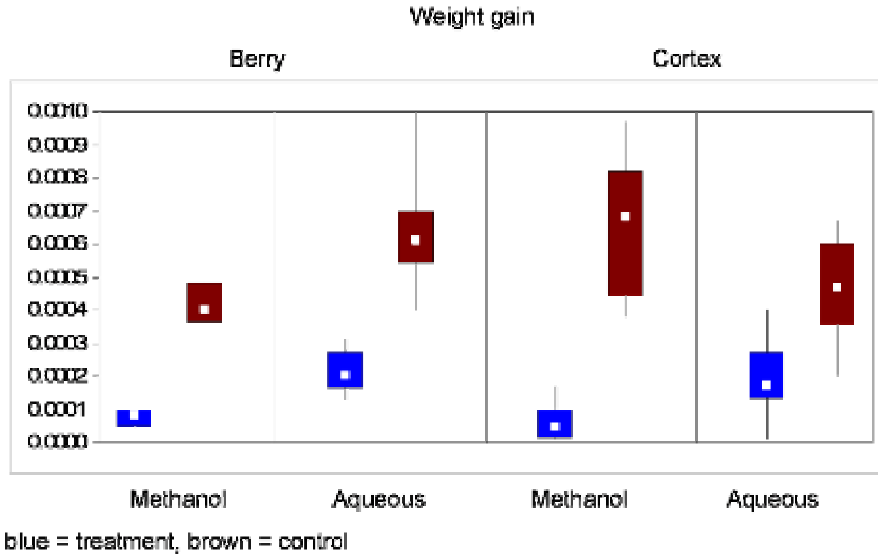

Figure-2. Weight on day 9. The central open square shows the mean, the box is the interquartile range and the whiskers represent the minimum and maximum weight for each extract.

The mean weight gain for larva fed treated diet was between 30% and 10% lower than control larva weight gain (Table-2). Similar reductions of weight gain were noted by Valladares *et al*. (1997) using *M. azedarach* ethanolic fruit extracts of 2, 5 and 10% concentration on *Xanthogaleruca luteola*. The test showed consumption on treated food was more than four times lower than consumption on the control food (Valladares *et al*., 1997).

Each source for extracts, root cortex or berry extract, of *M. azedarach* had a significant effect on *S. exigua* weight gain. There was no significant difference in weight gain between berries or root cortex, however extract solvent did have different effects. Larvas fed methanolic extracts were significantly lower in weight compared to those fed aqueous extracts (Table-3).

Table-3. This table shows the difference in weight between the extracts and source on.

Water			Methanol				
n	Mean	StD	n	Mean	StD	dif	p-value
19	0.00110	0.00084	14	0.00027	0.00026	0.00083	0.0064

Berry			Cortex				
n	Mean	StD	n	Mean	StD	dif	p-value
17	0.00094	0.00087	16	0.00050	0.00060	0.00044	0.1433

In contrast to extract, Juan *et al*. (2000) study showed that the seed extracts had more pronounced effects on the *S. nonagrioides* than fruit extracts. Therefore, different parts of the plant may have more bioactive compounds or higher concentration than other parts, but there is no indication berries or roots were more effective in this study presented here.

The reduction in weight gain potentially leaves larva unfit to molt or reproduce, thus reducing reproduction opportunities and keeping their population to a minimum. The results of this study suggest that extracts of *M. azedarach* may show promise as a botanical pesticide against *S. exugia*. Similarly, Nardo *et al*. (1997) found that when feeding *B. tabaci* aqueous extracts also interfered with the transmission of the golden bean mosaic virus. This is probably because feeding was inhibited therefore the insects were unable to transmit the virus. Similarly, feeding inhibition may be the cause of low body weight or mortality in the study presented here. Nonetheless, the results show *M. azedarach* extracts interfere with growth and though may not cause immediate mortality likely reduces fitness by virtue of low body weight.

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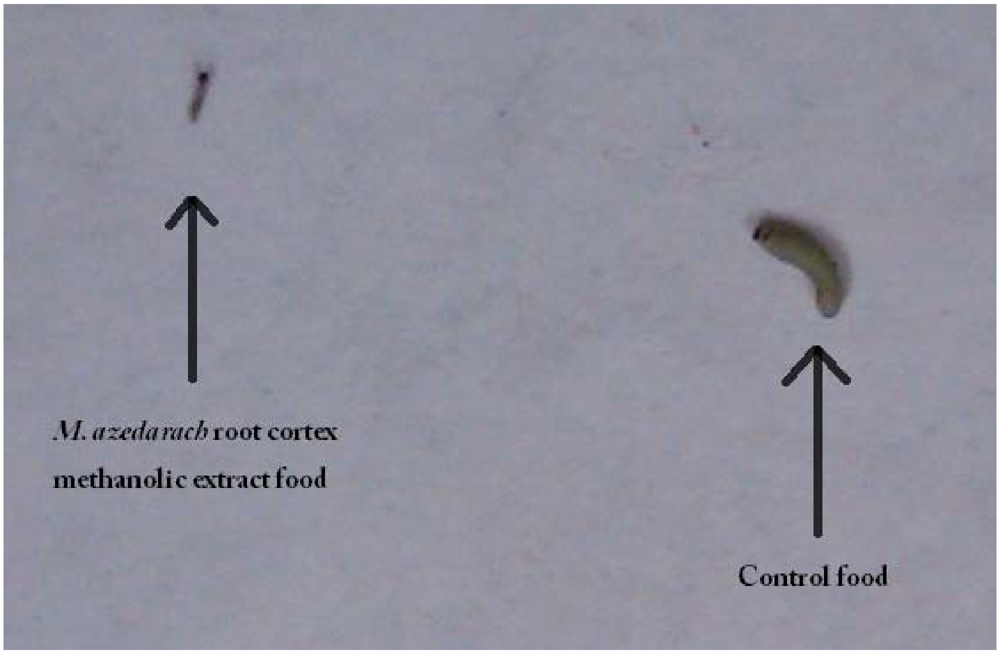

Figure-3. Difference of weight gain between methanolic extract food and control food.

CONCLUSIONS

Solvents types appear to have impacted the extracts effectiveness on mortality and weight gain. Aqueous extracts of root cortex did cause a significant increase in mortality. The root cortex may have a stronger concentration of bioactive compounds or different, more lethal aqueous bioactive compound than fruits. These results are significant in that aqueous extracts are more affordable for the average gardener or small-scale farmer, particularly in developing regions. More interestingly, populations showed significant differences in weight gain. Treated populations did not gain as much weight as untreated populations. This may eventually reduce their ability to molt or reproduce, thus keeping their populations suppressed. The methanolic root extract also had a significant impact on the *S. exigua*, weight gain; though seasonality may influence root extract bioactivity.

The results showed that the *M. azedarach* fruit and root cortex extracts can potentially be used as a botanical insecticide. *M. azedarach* produce a large amount of fruit potentially making it an easily accessed insecticide for home gardeners, small farms or developing regions. Roots are much more difficult to collect, possibly making it less economical unless further research shows they are more effective. More work should be conducted to see if seasonality of the root cortex changes results, if the mortality or morbidity was due to aversion or toxicity of extract.

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