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The efficacy of enhanced aqueous extracts of *Melia azedarach* leaves and fruits integrated with the *Camptotylus reuteri* releases against the sweetpotato whitefly nymphs

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Abstract

Bioassays with aqueous extracts of different growth stages of *Melia azedarach* L. (chinaberry) leaves and fruits were conducted against 3rd - 4th instar nymphs of *Bemisia tabaci* (Gennadius) on tomato plants. Mortality of instars was assessed 15 days post-treatment. Results illustrated that every *Melia* extract produced a significant greater mortality of *B. tabaci* than the controls. Extracts combined with the surfactant were significantly more toxic than those applied alone. Extracts with Tween-20 caused a mortality range of 34.6 to 67.9% and 53.5 to 74.1% for leaves and fruits, respectively. These was parallel to 15.6 and 31.4% and 11.4 to 18.7% for leaf and fruit extracts, lacking the surfactant, respectively. The synchronous application of one indigenous adult mirid predator, *Camptotylus reuteri* (Jakovlev) introduced at 30 min after spraying the *Melia* fruit extract, for a period of 3 days, was not synergistic with the botanical extract. The potential insecticidal activity of *Melia* extracts appears to be enhanced by Tween-20, and they are safe to integrate with *C. reuteri* biocontrol agent as the extracts did not cause any mortality of the predator.

Key words: *Melia azedarach*, *Bemisia tabaci*, *Camptotylus reuteri*, Tween-20, botanical insecticide.

Introduction

Bemisia tabaci (Gennadius) (Homoptera Aleyrodidae), variously known as the cotton, tobacco or sweetpotato whitefly, is a highly polyphagous pest recorded from more than 500 host plant species (Greathead, 1986) representing more than 60 plant families (Mound and Halsey, 1978). All stages are difficult to control with conventional insecticides because of their high reproductive rate and their preferred habitat on the undersurface of leaves (Cahill *et al*., 1996). *B. tabaci* nymphs and adults cause direct damage by piercing and sucking cell contents and in excreting huge amounts of honeydew that promotes sooty mould fungal development and reduces the photosynthetic efficiency of the plant and thus yield. Indirect damage is manifested by the transmission of more than 50 geminiviruses including the tomato yellow leaf curl virus (TYLCV) attacking tomatoes grown commercially worldwide (Markham *et al*., 1996). *B. tabaci* is notorious for its resistance to an array of pesticides extending from the pyrethroids, organophosphates (OPs) and carbamates (Cahill *et al*., 1994) to the insect growth regulators buprofenzin and pyriproxifen (Horowitz *et al.,* 1994). Hence, considerable effort has been made towards the discovery and development of alternatives for management of this insect with reduced hazards to the environment and human health.

Botanicals offer a potentially safe, environmentally sound and effective tool to suppress some threatening pests. Among botanicals, preparations from the neem tree *Azadirachta indica* A. Juss (Meliaceae), with its potent active ingredient, azadirachtin, have been thoroughly studied as crop protectants. Azadirachtin is a tetranortriterpenoid with behavioural and physiological bioactivities in more than 200 species of insects, extremely low mammalian toxicity, some selectivity favoring biocontrol agents and systemic action in a variety of important crop plants (Isman, 1997). Botanicals such as neem or chinaberry contain a mixture of active constituents, which might diffuse the selection process, mitigating the development of resistance compared to that expected with a single active ingredient (Feng and Isman, 1995).

Provided it is compatible with biocontrol agents, the Persian lilac or Chinaberry tree, *Melia azedarach* L. (Meliaceae), a close relative of neem, presents a promising approach for integrated pest management. Depending on the source material and the pest in question, the insecticidal potency of *M. azedarach* extracts could be equivalent to that of neem extracts (Champagne *et al*., 1989; Lee *et al*., 1991).

M. azedarach is abundant in Lebanon and other Mediterranean countries where it is mainly planted for shade. The main objective of our study was to explore the utilization of a local plant resource for the management of *B. tabaci* on tomato by testing aqueous extracts of *M. azedarach* leaves and fruits on the 3rd - 4th instars of *B. tabaci*. We also examined the effect of integrating a local mirid predator *Camptotylus reuteri* (Jakovlev) (Heteroptera Miridae) with *M. azedarach* fruit extracts.

Materials and Methods

Insect Material

A laboratory colony *B. tabaci* biotype A, more than five years old, originally collected from field populations on beans and cucumbers grown in coastal areas of Lebanon was reared on broccoli *Brassica oleraceae* L. cv. Calabrese Natalino and eggplants *Solanum melon-*

gena L. cv. Blackbeauty. Whiteflies were maintained in a cage (140 × 85 × 130 cm) entirely covered with fine mesh screen (270 × 770 µm) in a glasshouse at 25 ± 2 °C, 80 ± 10 % RH, and a 16:8 L:D photophase, located on the campus of the American University of Beirut (AUB), Beirut, Lebanon. A continuous supply of new plants was provided as needed for the colony replenishment. The colony was supplemented annually with leaves highly infested with 3rd - 4th instars of *B. tabaci* collected from the field; only emerging adults from the latter were introduced because of the menace of contamination with other intruder parasitoids and arthropods if nymph infested leaves were to be supplied to the colony.

Adults and nymphs of *C. reuteri* were collected from a field of eggplants infested with *B. tabaci*, at Choueifat (in the middle coastal area of Lebanon) during September-October, 1998. The mirids were removed by a manual aspirator, transferred in a cooler from the field to the glasshouse compartment and released in an insect proof cage (74 × 145 × 95 cm). The cage was placed in a glasshouse at an average temperature of 27 ± 3 °C, 65 ± 20% RH and continuous light. *C. reuteri* were exposed to the above conditions for four days before experimental use. They were maintained on whitefly-infested eggplants and *Myzus persicae* (Sulzer) (Homoptera Aphididae) collected from the AUB campus and introduced into the colony. Sponges soaked in distilled water were placed in the colony to provide water for the predator.

Plant Material

Tomato plant preparation

Tomato seeds (*Lycopersicon esculentum* L., cv. Ronda 44) were planted in trays containing a soil mixture of peatmoss:perlite (3:1). Seedlings with the first true leaves were drenched with fungicides Rhizolex® (Tolclofos-methyl) and Tachigaren® (Hymexazole) to avoid damping-off disease and then transplanted into 12 cm plastic pots containing the same potting mixture. Each seedling was placed in a cylindrical insect proof plastic cage (30 cm high by 20 cm diameter) covered with mesh (270 × 770 µm) firmly held with a plastic lid at the top. Aeration in the cage was enhanced by drilling four holes each of 7.5 cm diameter in the body of the cage that were covered with mesh. An opening of 2.5 cm in diameter was also drilled at the level of the soil in the pot for watering during the experimental period. Ten adult whiteflies were collected from the rearing colony by a manual respirator and then introduced in a glass vial into each cage. Two days later, plants were checked for oviposition. Two weeks later, forty 3rd - 4th instars of *B. tabaci* were counted at random per plant and were marked on one or more tomato leaves.

Preparation of *M. azedarach* extracts

Source of plant material

Leaves and fruits of *M. azedarach* trees located on the AUB campus were collected during June 1996 (leaves and green fruits) and November 1996 (mature fruits). The leaves were classified into different age classes according to their maturity: old, from the lowest part of the whole branch, medium, from the middle part, and young, from the tip of the main branch. Each plant part was weighed and stored in a plastic bag at -20°C. When needed, the frozen plant material was removed from the freezer, soaked for 15 minutes in distilled water then dried at ambient laboratory temperature for 6-12 hours.

Extraction of plant material

Lots of 100g dry weight of each sample of leaves or fruits and 70g of mature fruits only for *C. reuteri* bioassay, were ground with distilled water in a blender (16,000 rpm; Sorvall Omnimixer, USA) at a ratio of 1:5 (w/v), and soaked for 48 hours at room temperature. The extracts were filtered through cheesecloth, followed by vacuum filtration through filter paper (Whatman no. 40). Tween-20 was added as an adjuvant to the *Melia* extracts at a rate of 0.5%. The extracts were applied to the whitefly-infested plants till runoff using SPRA-Tool® (Crown, Illinois, USA) sprayers.

Experimental Set-up

All bioassays were performed in the glasshouse at 25 ± 2°C, RH of 80 ± 10%, and 16:8 L:D photoperiod. Each plant received an average of 18.2 ml of the *Melia* extract. The treatments included 6 extracts of each of the leaf and fruit material with and without Tween-20 (0.5%), distilled water plus Tween-20 (0.5%) and distilled water alone as controls. Mortality was assessed by counting insects with melanotic spots in the late third instars or leaky symptom/oozing in the late fourth instars or incomplete emergence of adults from the nymphal exuviae.

Five treatments with the introduction or absence of the predator included: mature fruit extracts with and without Tween–20 (0.5%), *Melia* extract with adjuvant in the presence of the predator, distilled water with adjuvant in presence of the predator and an unsprayed plant to which *C. reuteri* was introduced. Each plant in treatments including *Melia* extracts received an average of 25 ml of the solution. The mirid was introduced on the sprayed plant 30 minutes after spraying. The experimental duration was 72 hours. Each treatment was replicated three times and the entire experiment was repeated twice. The observed feeding symptom of the adult *C. reuteri* on *B. tabaci* instars was in the form of a small hole in the body of each instar.

Statistical analysis

The statistical package MSTAT-C (Anonymous, 1991) was used for data analysis. For the bioassays with various *M. azedarach* aqueous extracts, the data was collected in the form of percent mortality of the 3rd - 4th instars of *B. tabaci*, corrected with respect to the distilled water control based on Abbott's (1925) formula, then transformed into arcsin √x, x being the percent mortality of nymphs, to normalize the mortality. The design was a completely randomized design (CRD) and the analysis of variance was performed over the factor *Melia* age and presence or absence of Tween-20. The data assembled from the integration of mature *Melia* fruit extracts and the adult predator on the 3rd - 4th instars of *B. tabaci* was transformed into arcsin √x, x be-

ing the percent mortality to normalize the data. The experiment was laid out as a completely randomized block design (RCBD) with multiple observations. All means were separated by Fisher's (1960) LSD test if significant F values were obtained (Gomez and Gomez, 1984).

Further analysis for the association between the *Melia* extract and the adjuvant Tween-20, and the additive and non-additive interactions between the predator and the adjuvant were determined separately using a modified procedure by McVay *et al*. (1977) and Salama *et al.* (1984) for probit analysis. The expected additive proportional mortality (Me) of the *Melia* extract-Tween-20 combinations was calculated by: Me = Mn + Mi (1 - Mn), where Mn and Mi are the observed proportional mortalities caused by the *Melia* extract and Tween-20 alone, respectively. Results from the chi-square test, $\chi^2 = (Mni - Me)^2 / Me$, where Mni is the observed mortality due to *Melia* extract and Tween-20 combinations, were compared to a chi-square table for 1 df, $\alpha = 0.05$. If the calculated chi-square value exceeded the table value, a non-additive effect (i.e. synergistic or antagonistic) was assumed (Finney, 1964). Furthermore, if the difference Mni - Me = D had a positive value, a significant interaction was considered synergistic; if D had a negative value, a significant interaction was considered antagonistic.

Results and discussion

Bioassay of the *M. azedarach* aqueous extracts on the 3rd - 4th instar nymphs of *B. tabaci* indicated that there were significant differences in percent mortality among treatments (F = 5.25; 11, 24; P<0.05). The percent mortality caused by the extracts with Tween-20 ranged from 34.6 to 67.9% for leaves, and from 53.5 to 74.1% for fruits. Mortality caused by the extracts alone (without Tween-20), ranged from 15.6 to 31.4% for leaves and from 11.4 to 18.7% for fruits (figure 1). There were no significant differences in percent mortality of instars among leaf or fruit extracts of different maturity periods in the absence of Tween-20. These extracts were comparable in their effects on *B. tabaci* nymphs to distilled water plus Tween-20 (figure 1). All plant extracts with Tween-20 were significantly more effective than the distilled water control (figure 1). The high activity of these extracts against *B. tabaci* instars was verified by the determined synergistic effect of Tween-20 with each extract (table 1). The enhancement of *Melia* activity by Tween-20 can be explained by the fact that Tween-20 likely facilitates the absorption of the active principles in *M. azedarach* through the waxy cuticle of the whitefly nymphs, and/or facilitates the movement of the active principles into the parenchymal cells of the host plant, from which they are ingested by nymphal whiteflies.

In this study, three symptoms due to *Melia* toxins were observed on the whitefly instars and were used to assess mortality. The first symptom, on late fourth instars, was exudation of body fluids through tissue disruption or "oozing" symptom noted three to four days after treatment. The second symptom was manifested by adults that could not emerge from the pupal exuvia, represented by 2.5 - 7.5% of dead instars. The last symptom was 3rd instars that turned brownish due to dried insect body. Schlüter (1995) proposed the cellular mechanism that underlies the oozing or leaky symptom caused by azadirachtin 1 - 2 days after application as fat cells starting to separate from intact neighbour cells. He further attributed the dry "brown pupa" symptom observed in the third instar to melanization, a defense reaction leading to massive tissue degeneration

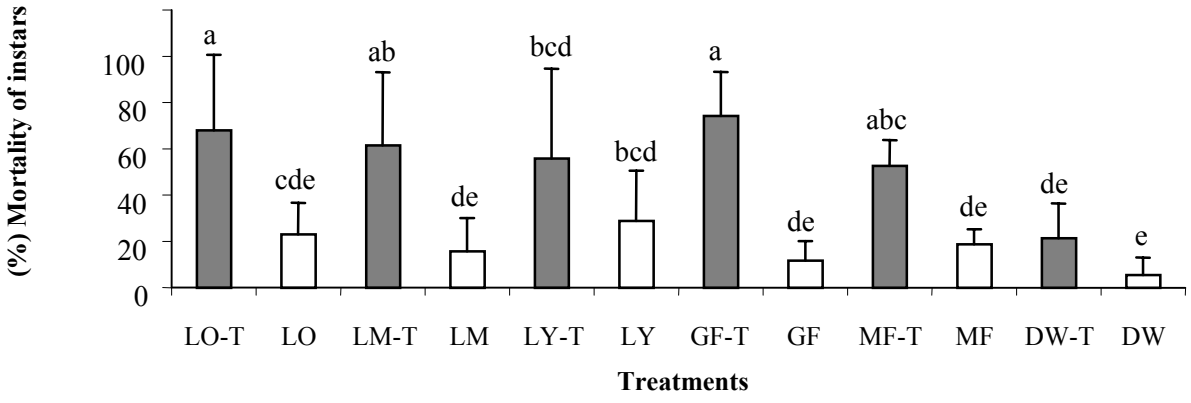

Figure 1. Effect of semiqualitative age classes of *M. azedarach* aqueous extracts on 3rd - 4th instars of *B. tabaci.* Means were separated by LSD test (P < 0.05). Columns headed by the same letters are not significantly different. Treatments included: LO-T = Leaf old + Tween; LO = Leaf old; LM-T =Leaf medium + Tween; LM = Leaf medium; LY-T = Leaf young + Tween; LY =Leaf young; GF-T = Green fruits + Tween-20; GF = Green fruits; MF-T = Mature fruits + Tween; MF = Mature fruits; DW-T = Distilled water + Tween; DW = Distilled water; T = Tween-20 added at 0.5% to some extracts.

Table 1. Determination of the synergistic effect* between semiqualitative age classes of *M. azedarach* leaves and fruits and Tween-20 (0.5%) against *B. tabaci* **.

Melia extracts	Proportion Mortality				χ^2 calculated	χ^2 tabulated	D
	Mn	Mi	Mni	Me			
Old leaves	0.1887	0.1633	0.6661	0.3212	0.3704	0.0039	0.3449
Medium leaves	0.1121	0.1633	0.5956	0.2571	0.4457	0.0039	0.3385
Young leaves	0.2401	0.1633	0.7408	0.3642	0.3849	0.0039	0.3766
Green fruits	0.0648	0.1633	0.7257	0.2175	1.1873	0.0039	0.5082
Mature fruits	0.1358	0.1633	0.4860	0.2792	0.1526	0.0039	0.2064

*Finney's (1964) method modified by McVay *et al*. (1977) and Salama *et al*. (1984)
**All mortality means were adjusted with respect to the control, distilled water (5.6% mortality) according to Abbott's (1925)
Mn = Observed proportional mortality caused by each *Melia* extract alone
Mi = Observed proportional mortality caused by Tween-20 (0.5%)
Me = Expected proportional mortality for the *Melia*-Tween-20 combination
Mni = Observed proportional mortality due to the *Melia*-Tween-20 combination
χ^2 calculated = $(Mni-Me)^2/Me$
χ^2 tabulated (df =1, P = 0.05)
D = Mni-Me (D is positive implies synergism; D is negative implies antagonism)

and loss of body contents ensuing in drying. Ascher *et al*. (1995) related the "half emerging pupae" symptom to the presence of meliacin and meliacarpin compounds that were detected in the methanolic extracts of green chinaberry fruits, whereas Isman (1997) explained this symptom by disturbance of hormonal balance as release of ecdysteroid causing ecdysal failure when the adult emerges from the nymphal exuvia. The similarity in symptoms between *M. azedarach* and neem can be related to the analogy of some *Melia* toxins to azadirachtin that were isolated from fruits of *M. azedarach* (Kraus *et al*., 1987). The ecdysis-inhibiting properties of neem might be present in *M. azedarach* compounds as suggested by both Valladares *et al*. (1997) and Cabral *et al*. (1996). This fluctuation in chemistry of *M. azedarach* leaves and fruits (ripe and unripe) was also noted by Valladares *et al*. (1999) testing the repellent and insecticidal properties against eggs and nymphs of *Triatoma infestans* Klug (Heteroptera Reduviidae), the unripe fruit being the most effective.

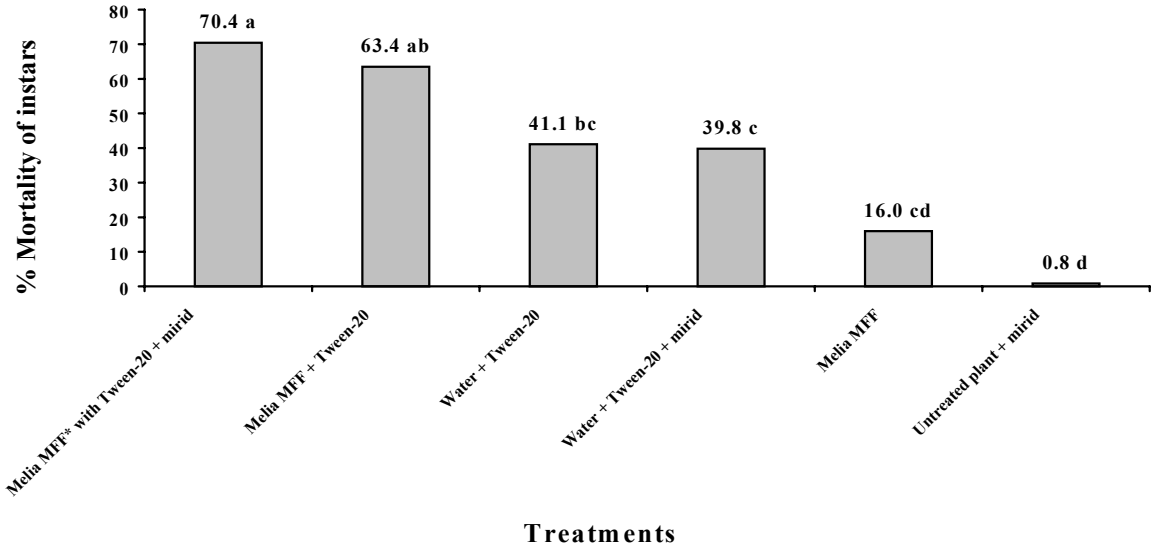

Figure 2. Effect of *M. azedarach* fruit extract and the indigenous mirid *C. reuteri* on the 3rd - 4th instars of *B. tabaci*. Means were separated by LSD (P<0.05) test. Columns headed by the same letters are not significantly different.
* MFF = Mature fruit frozen.

Table 2. Determination of the synergistic effect between *Melia* fruit extract and Tween-20 (0.5%) against *B. tabaci.*

Proportion Mortality				χ^2 calculated	χ^2 tabulated	D
Mni	Mi	Mn	Me			
0.6343	0.4109	0.1597	0.5050	0.0331	0.0039	1.293

*Finney's (1964) method modified by McVay *et al*. (1977) and Salama *et al*. (1984)
**All mortality means were adjusted with respect to the control, distilled water (5.6% mortality) according to Abbott's (1925)
Mn = Observed proportional mortality caused by each *Melia* extract alone
Mi = Observed proportional mortality caused by Tween-20 (0.5%)
Me = Expected proportional mortality for the *Melia*-Tween-20 combination
Mni = Observed proportional mortality due to the *Melia*-Tween-20 combination
χ^2 calculated = $(Mni-Me)^2/Me$
χ^2 tabulated (df =1, P = 0.05)
D = Mni-Me (D is positive implies synergism; D is negative implies antagonism)

Mature fruit extracts of *M. azedarach* combined with the mirid species on the 3rd - 4th instar nymphs of *B. tabaci* led to significant differences ($F = 9.76$; $df = 5, 23$; $P < 0.05$) in nymphal mortality among treatments. However, there was no significant difference in mortality of nymphs between the integrated treatment, i.e. *Melia* extract with Tween-20 and the mirid predator *C. reuteri*, and treatment with the extract and Tween-20 alone (figure 2). In no case did *C. reuteri* enhance nymphal mortality in conjunction with an extract or Tween-20 alone, nor was the mirid effective by itself (figure 2). This result verifies that Tween-20 can have a higher detrimental effect on the instars compared to the mirid effect alone (0.83%) under the tested experimental conditions. The percent mortality due to the mirid feeding in the combined treatment was 5.7% compared to 31.6% due to Tween-20.

Our observations showed that the dead instars due to *Melia* treatment were not fed upon by the mirid and the *Melia* treatments were not detrimental to the predator but might have repelled it from feeding. The insect was often found on new foliage or partially treated foliage as a mean to sustain itself. Our results agree with those of Valladares *et al*. (1997) who found that both larvae and adults of *Xanthogalleruca luteola* (Muller) (Coleoptera Chrysomelidae) were deterred from feeding on leaves treated with ethanolic extracts of *M. azedarach* mature fruits. They noted that the antifeedant effect was independent from the concentration and was apparent even at the lowest concentration tested. Antifeedant effects of *M. azedarach* extracts are known for some other insects (Chiu, 1984; Saxena *et al*., 1984; Ascher, 1987; Kraus *et al*., 1987; Lee *et al*., 1991). *C. reuteri* behaviour could be explained by the fact that host selection is governed by the responses of the insect's gustatory and olfactory sensilla to some compounds acting on feeding behaviour (Blaney and Simmonds, 1996; Nicol *et al*., 1995). This is also supported by the avoidance of paper refuge treated with *M. azedarach* extracts shown by *T. infestans* (Palacios *et al*., 1993). Feeding deterrence without previous contact with the compound has also been reported in experiments with neem (Saxena, 1989).

Similarly, significant difference in the mortality of instars between *Melia* extracts with Tween-20 and *Melia* extracts alone was detected in the experiment with the mirid. This could be related to the oily nature of Tween-20, which either promotes the penetration of aqueous *Melia* extracts through the storage protein and dense fat bodies of the instars or facilitates the absorption of hydrophilic active constituents of the *Melia* fruit extracts through the waxy surface of the epidermal layer of the leaf into the parenchyma tissue from which the insects take it up during feeding. This is suggested by the synergy detected between the *Melia* fruit extracts and the emulsifier Tween-20 (table 2). Also, it is unlikely that adults emerging from *Melia*-treated pupae would be able to produce the same damage as normal ones, for example in terms of oviposition rate (Abou-Fakhr Hammad *et al*., 2001). According to Shmutterer (1990), the fecundity of homopterous insects is strongly influenced by neem extracts or azadirachtin. Coudriet *et al*. (1985) found that *B. tabaci* confined to cotton treated with neem seed extracts deposited > 80 % fewer eggs compared with controls up to 7 d after treatments. Also Lowery and Isman (1996) found that exposure to azadirachtin reduced the fertility (live offspring per aphid per day) and the fecundity (live + dead offspring per aphid per day) of adults green peach aphid *M. persicae*, lettuce aphid *Nasonovia ribisnigri* (Mosley), and strawberry aphid *Chaetosiphon fragaefolii* (Cockerell), in a linear concentration-dependent manner. Thus the insecticidal effect of *Melia* should not be underestimated by considering emerging adults as having escaped control. Valladares *et al*. (1999) noted that the nymphs of *T. infestans* reared in refuge treated with ethanolic extracts of different age classes of leaves and fruits of *M. azedarach* were noticeably smaller and lighter in color than respective controls after exposure to the extract for one instar, suggesting that the overall effects could be dramatic if accumulated through the insect's life cycle.

Conclusions

We have demonstrated that extracts of *Melia* leaves were as effective as those of fruits in causing mortality of *B. tabaci* 3rd - 4th instars. In addition, efficacies of both the leaf and fruit extracts are significantly enhanced by the emulsifier Tween-20, verified by the synergistic effect. Owing to the degradation of the active principles in these extracts, repeated applications may be required for effective suppression of *B. tabaci* popu-

lations. The efficacy of the *Melia* extract could be enhanced by using a more proficient extraction procedure, because the quantity of the biologically active compounds varies with the extraction method (Flint and Parks, 1989). The lipophilic portion of the *Melia* extract i.e., oil, may be a valid approach, especially against insecticide resistant pests. The limited impact of *C. reuteri* in our study may be attributed to intrinsic factors related to the predator or to the fact that the insect was exposed to infested tomato plants in its rearing colony for only a few days. A conditioning period might be necessary to enhance predator activity under the experimental conditions. Furthermore, the adequate timing of the synchronous implementation of a botanical insecticide enjoying a repellent activity such as neem or *Melia* with a natural enemy is of primordial importance delineating the impact of the natural enemy in a sound pest management tactic.

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