Seed extract shows promise in leafminer control

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An extract from seeds of the Neem tree may offer an alternative means of controlling the leafminer, *Liriomyza trifolii* (Burgess), a pest of chrysanthemum, gerbera, and numerous bedding plants. During the past four years, reliance primarily on synthetic chemical insecticides for control has contributed to destruction of beneficial insects, development of insecticide resistance by the leafminer, resurgence of primary and secondary pests, and hazards to humans and the environment.

The Neem tree, *Azadirachta indica* A. Juss (family: Meliaceae), is a fast-growing, evergreen, tropical species. The active principles of the seed extract have complex aromatic structures that could reduce the possibility of resistance development. Neem is also biodegradable, very low in mammalian toxicity, and potentially compatible with natural enemies.

Neem

During the past decade, Neem has gained increasing attention as a "natural" insecticide, and its activity has been evaluated against many economically important insect species. It has been used primarily as a repellent or antifeedant but rarely as a conventional insecticide. Neem also has many other commercially exploitable properties, several of which are medicinal.

The seeds contain most of the active materials (azadirachtin is the most active component) that show feeding and egg-laying repellency and growth-regulating properties. It has been reported to exert insecticidal action by disrupting growth, possibly by interfering with the concentration of molting hormones in the insect. Because of the unique properties and great potential of this compound, we undertook studies to evaluate its repellent and insecticidal properties with *L. trifolii* as the target pest on chrysanthemum.

Repellency trials

We evaluated three concentrations of Neem seed extract (in ethanol) — 0.1, 0.2, and 0.4 percent — with two spreader-stickers — Triton B-1956 and Tween 20 — for ability to repel feeding and egg-laying by the adult leafminer. Host plants were standardized chrysanthemums (three leaves, one month old) of the cultivar 'White Hurricane'.

In an initial experiment, chrysanthemum plants received a single application of the extract as a foliar spray at one of the three concentrations with Triton B-1956. Control plants received an application of water only. After the leaves had dried, we divided the plants into four groups; each group contained one plant from each treatment and was exposed on a different day to large colonies of leafminers for two hours. This ensured that egg-laying, subsequent egg hatch, and larval development in all plants would be synchronous.

Exposure occurred soon after the initial application (day 0) and on days 1, 3, and 5. Plants were exposed to fly colonies on a rotating stage (turning a quarter turn every 15 minutes) to reduce any positional bias. There were 10 plants for each exposure for each treatment. Plants were then kept in an environmental chamber at 26.7°C, 50 to 60 percent relative humidity, with a 14.5-hour photophase. Feeding punctures and developing mines (eggs laid) were counted on the third day after exposure to the fly colony

Neem did not completely repel adult leafminers but did deter them from feeding and laying eggs during the five-day observation period. A steady decline in deterrence was apparent at all concentrations tested.

Phytotoxicity was a problem in this trial; leaves on treated plants developed brown necrotic lesions the day after spraying. Damage was greatest in the 0.4 percent treatment and least at 0.1 percent. To reduce phytotoxicity, we tried Tween 20 as a spreader-sticker in place of Triton B-1956; deterrent effects were similar without any signs of phytotoxicity.

A second experiment evaluated the effect of multiple Neem applications on leafminer feeding and egg-laying. On day 0, all the plants used in one trial were sprayed with water only (control) or with Neem seed extracts at 0.1, 0.2 and 0.4 percent concentrations with Tween 20 as the spreader-sticker. Successive exposures of the treated plants to leafminers, as in the first experiment, were on day 0, 1, and 3. On day 4, all plants that had not been exposed received a second application of the treatments; these were exposed to the fly colonies on days 4, 5, and 7 A third application was made to the remaining unexposed plants on day 8 with exposures to fly colonies following on day 11 and day 15. There were five plants for each exposure for each treatment.

The extract from the seed of the fast-growing, evergreen, tropical Neem tree has been tested as a natural insecticide against many insect species.

In this second experiment, Neem deterred the leafminer from feeding and laying eggs on treated plants on the day the extract was applied (day 0, 4, and 8) and on the day after application (table 1). Three days after Neem applications, however, most of the treatments showed no significant differences.

Efficacy against larvae

In tests against early and late larval stages, we exposed standardized chrysanthemum plants to large colonies of leafminers for two hours, and three to four days later, made pretreatment counts of mines. These were followed immediately by applications of Neem seed extracts at 0.1, 0.2, and 0.4 percent to plants for efficacy trials with early larval stages. For applications against late larval stages, the same treatments were made when late third-stage larvae were present in the plants (four to five days after egg hatch). Before larvae emerged from leaves, each plant was tipped over a petri dish filled with sand. Pupae were collected from the sand, counted, and placed in plastic vials for adult emergence. Adults were counted as they emerged in the vials.

In the Neem 0.4 percent treatment, significantly fewer pupae were obtained from plants containing young leafminer larvae than in the other treatments (table 2). Adult emergence was significantly lower from all Neem-treated plants compared with the control. In addition, a large number of pupae emerging from Neem-treated plants were deformed, which supports previous data suggesting that Neem has growth-regulating properties. Although all larval stages treated with Neem successfully pupated, the growth-regulating effect was evident when adult emergence was examined: significantly fewer emerged after application of 0.2 and 0.4 percent Neem than in the control or 0.1 percent Neem treatment.

Neem seed extract at concentrations tested did not control leafminers in an economic sense, but its growth-regulating properties caused deformity of pupae (bottom row) and inhibited emergence of adults. Top row shows normal adult *L. trifolii* and pupae.

TABLE 1. The effect of single and multiple applications of Neem seed extract on feeding and egg-laying (mines) of the leafminer, *Liriomyza trifolii*

	Day after treatment								
Treatment	0	1	3	4†	5	7	8†	11	15
	*Mean percent feeding punctures per plant**								
Control	65.2a	40.6a	29.2a	45.1a	40.1a	30.6a	46.2a	9.6ab	24.8a
Neem 0.1%	17.3b	25.2b	25.4a	36.2a	25.1b	25.7a	32.6b	39.1a	29.9a
0.2%	12.8b	20.2bc	25.6a	13.3b	23.5b	25.0ab	15.6c	21.3bc	21.1a
0.4%	4.6b	14.0c	19.8a	5.5b	11.2c	18.8b	5.6d	10.1c	24.2a
Total mean no. feed punctures per day	330.5	300.9	225.0	227.7	624.9	620.1	467.8	184.8	449.4
	Mean percent mines per plant*								
Control	37.9a	31.9a	28.2a	35.0a	28.1ab	27.6a	34.3a	30.3a	29.1a
Neem 0.1%	29.7a	23.6b	19.6a	32.9a	23.0ab	28.8a	30.0a	30.5a	31.9a
0.2%	22.7ab	25.2ab	30.4a	21.8b	31.2a	20.1b	23.2b	23.1ab	21.2ab
0.4%	9.7b	19.7b	21.8a	10.4c	17.8b	23.6ab	12.4c	16.0b	17.8b
Total mean no. mines per day	91.8	75.4	45.2	87.4	249.5	311.7	217.4	60.5	176.2

NOTE: Neem applied to chrysanthemums with Tween 20 as spreader-sticker.
* Within each section, means in the same column followed by the same letter do not differ significantly ($P = 0.05$), Duncan's new multiple range test. No data transformations were performed. N = 10 for all treatments.
† Indicates another application of all treatments was made.

TABLE 2. Toxicity of Neem seed extract* when appled to early larval stages of *L. trifolii* in chrysanthemum.

Treatment	Pretreatment count** (larvae/plant)	Posttreatment count**	
		Pupae/plant	Adults/plant
Control	38.8 a	27.2 a	21.0 a
Neem 0.1%	37.8 a	26.0 a	7.3 b
0.2%	40.3 a	21.4 a	0.9 c
0.4%	41.3 a	7.6 b	0.1 c

NOTE: Applied to chrysanthemums with Tween 20 as spreader-sticker.
** Means in the same column folowed by the same letter do not differ significantly ($P = 0.05$), Duncan's new multiple range test. No data transformations were performed. N = 60, 50, 40, 60 plants, respectively, in each treatment column.

Potential

Neem seed extracts at the concentrations tested would not effectively deter *L. trifolii* in an economic sense; that is, the leafminer was not completely inhibited from either feeding or laying eggs for more than 24 hours. Also, because the viability of these eggs was not affected by the highest Neem concentration (0.4 percent), even with multiple applications, larvae developed to at least the second stage, which would decrease the aesthetic value of chrysanthemums and reduce their marketability.

The efficacy of Neem as a foliar spray applied against early- and late-stage larvae was surprisingly high, which suggests that this material penetrates the chrysanthemum leaf to reach the mining larvae. In addition, many of the larvae that survived to pupate did not emerge as adults.

Because Neem has a complex structure that may reduce development of resistance, is biodegradable, is compatible with nontarget organisms, may be compatible with parasites, and has low mammalian toxicity, it is a strong candidate for inclusion in an integrated pest management program for control of the leafminer on chrysanthemum. Its growth-regulating activity can have a long-term effect in reducing leafminer populations and could augment other control strategies designed to keep pest populations below damaging levels. Researchers with the U.S. Department of Agriculture in Beltsville, Maryland, have demonstrated that Neem also has good systemic properties when applied as a soil drench for leafminer control.

Registration of this material presently is being pursued at the federal level. Problems of a standardized formulation must still be resolved in addition to the use of an appropriate spreader-sticker. Our results demonstrate that phytotoxicity may be a problem unless compatible spreader-stickers are used.

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