GLIRICIDIA SEPIUM AS A POSSIBLE SOURCE OF SEMIOCHEMICALS DETERRING OVIPOSITION OF *PLUTELLA XYLOSTELLA* (L.) (LEPIDOPTERA: YPONOMEUTIDAE)[1]

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ABSTRACT

No-choice, multiple-choice and field cage experiments have been conducted to test farmer's reports on the oviposition deterrency effect of madre de cacao, **Gliricidia sepium** (JACQ.) STEUD (Leguminosae) to the Diamondback moth **Plutella xylostella** (L.) (Lepidoptera: Yponomeutidae). The methods of preparation compared were: 1. ethanolic extract (20 mg/ml, 10 mg/ml), 2. three cups water extracts (20%, 10% on dry weight basis) and mulching (chopped, whole leaves).

The ethanolic extract at 20 mg/ml produced a reduction of oviposition by 70% compared to the control in the no-choice, multiple-choice and field cage experiments. At a concentration of 10 mg/ml, the ethanolic extract produced an oviposition deterrency of 25% only, compared with the control.

Water extracts produced in a no-choice experiment, comparing concentrations of 30, 20, 15, 10, 5 and 0 % a reduction of oviposition to 1.3; 8.1; 4.0; 15.2 and 31.8 %, respectively. In the field cage experiment, the 20 and 10 % water extracts produced a reduction of oviposition to 7.1 and 13.6 % compared to the control.

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Mulching both with chopped and whole leaves produced in all three test types ascertain but unreliable reduction ranging from 62% in the no-choice test to 110% in the field cage experiment. This method of application is not effective.

INTRODUCTION

The potential use of oviposition deterrents to protect crop plants from insects at a stage before any feeding damage does occur, is of great interest for todays attempts to develop integrated plant protection strategies.

Semiochemicals mediate host finding and host selection of many insects via olfactory cues and contact chemoreception. In Diamondback moth (DBM) *Plutella xylostella*, allyl isothiocyanate or sinigrin stimulates oviposition of the female DBM in the presence of a favorable physical and possibly other chemical stimulus (REED et al. 1989). In general, antennal receptors are responsible for perception of volatile stimuli (olfaction) and contact receptors are involved for nonvolatile cues. In most cases, olfactory cues mediate behavior before contact with a plant, whereas contact chemoreception comes into play after landing (MORGAN, E.D.,MANDAVA, N.B. 1990)

Gliricidia sepium is a plant species which is long known to Filipino farmers for its pest control properties. In a survey conducted in Central Luzon and Iloilo Provinces, *G. sepium* was the most popular species reported (ADALLA, C.A.,ATIENZA, E.C. 1989). Eighty two percent of farmers who had experience with plant pesticides claimed that the leaves of this plant when broadcast into the paddy, would drive away pests, particularly stemborer, but also rodents.

In the research program "Integrated Studies in Botanical Pesticides for the small Farmers", coordinated by PHILIPPINE COUNCIL FOR AGRICULTURE AND RECOURCES RESEARCH AND DEVELOPMENT (PCARRD) 1989 *G. sepium* was identified as having ovicidal activity of 96.7% as petroleum ether extract at 200 mg/ml and insecticidal activity as ethanolic extract with a mortality of 93.3% at 200 mg/ml and 86.7% at 100 mg/ml.

It has been reported that farmers from the south of the Philippine Island of Cebu, are knowledgeable about the oviposition deterrent effect of leaves of the leguminous shrub *G. sepium* to DBM when used as mulch. It was therefore decided to test their local knowledge of the allelochemical effect of this plant species with scientific methods.

Research proved that this non-host plant species, the leguminous shrub *G. sepium* (Fam. Leguminosae) possesses semiochemicals that act as oviposition deterrent to *P. xylostella* as water extract.

MATERIALS AND METHODS

Insect and plant culture

Test insects were taken from a laboratory culture which had been refreshed by field-collected pupae of *P. xylostella* about six months prior. The rearing took place on 7-8 week old cabbage plants, var. Resist Crown at 22-28°C, a RH at 50-70% and a 12/12 L/D photoperiod.

For the experiments, 0-48 hours-old adults were used. In the no-choice bioassays, 40 adults were introduced, in the multiple choice test and in the field cage experiment, 1,000 adults were introduced. The duration of oviposition was 48 hours for each type of experiment.

Preparation of plant materials and extracts

Water extracts

Initial bioassay and multiple-choice test in big cage:
13 g of fresh leaves were extracted in 100 ml distilled water for 24 hours

Bioassay comparison of concentration:
5, 10, 15, 20 and 30 g of dried plant powder were extracted in 100 ml distilled water for 24 hours

Field cage-experiment: 10 and 20 mg/ml:
10 and 20 g of dried plant powder were extracted in 100 ml distilled water for 24 hours

Ethanolic extracts

Field-cage experiment: 10 and 20 mg/ml

Mulching

Bioassay: 5 chopped leaves on paper collar around plant neck
5 whole leaves on paper collar around plant neck
5 whole leaves around plant placed on cagefloor

Field cage-experiment: 500 fresh leaves around 10 seedings

Spraying was conducted with a perfume atomizer until the seedling was completely coated, but not to run-off.

EXPERIMENTAL METHODS

No-choice bioassay

The test unit was a cage with a wooden frame and screened walls, measuring 50x50x50 cm. The test plants were 5-6 weeks old cabbage seedlings. In the mulching treatment, 5 leaves were used for test unit. In the no-choice test comparing different concentrations individual leaves were used instead of seedlings. These leaves were inserted into test tubes (2x20 mm) which were placed vertically into drinking cups of 200 cc volume filled with soil and covered with a paper collar. Five replications over time were conducted for each type.

Multiple-choice experiment

The test unit was a cage with a wooden frame and screened walls, measuring 150x100x50 cm. Five 5-6 weeks old cabbage seedlings were used per each treatment with 4 replications within the cage. The experimental design was arranged as a completely randomized design. The experiment was repeated 5 times.

Field cage experiment

The experimental unit was a field cage, walled with nylon screen with the measurements of 4.5x4.5x2.0 m. The experimental design was a Complete Randomized Block Design using 4 blocks. Each block was divided into 2 times 4 treatments. Each treatment consisted of 10 6-weeks old cabbage seedlings. Spraying was conducted as under the no-choice test.

Experiments conducted for the determination of oviposition deterrency

Experimental Method	Objectives	Treatments
No-choice Test	Initial bioassay	water extract, whole leaves, chopped leaves
	Comparison of concentration	water extract at 30, 20, 15, 10 and 5%
Multiple choice test	Verification of results of no-choice test in a multiple choice design	water extract, whole leaves, chopped leaves
Field cage experiment	Probing of results from no-choice and multiple-choice tests under semi-field conditions	water extract, ethanolic extract, whole leaves, chopped leaves

RESULTS

Initial bioassay

Table 1. Oviposition (%control) of *P. xylostella* on cabbage seedlings treated with leaves of *G. sepium* as water extract and as mulch in a no-choice cage bioassay.

Treatment	R1	R2	R3	R4	R5	R6	Mean	CV
Water extract	9.9	24.2	46.2	27.8	12.5	31.0	25.3 a	52.1
Chopped neck	15.3	2.6	120.0	70.7	108.4	90.0	67.8 b	72.0
Whole neck	16.5	17.2	113.5	28.7	104.9	92.9	62.3 b	73.9
Whole floor	55.8	30.8	118.9	160.5	117.2	93.8	96.2 bc	48.7
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0 c	-

Based on five seedlings/treatment and replication and 40 adult DBM. In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

In this experiment, the water extract of *G. sepium* was significantly different from all other treatments with only 25.3% of eggs laid compared to the untreated control. The mulching did not produce any pronounced effect worthwhile pursuing.

Comparison of concentration

Table 2. Oviposition of *P. xylostella* on cabbage leaves sprayed with water extracts of *G. sepium* at different concentrations 48 hours after release of adults in a no-choice test, expressed as absolute numbers.

Concentration (%)	R1	R2	R3	R4	R5	Mean	CV
30	37	2	0	29	3	14.2	122.8
20	16	161	4	110	69	88.0	60.7
15	82	34	23	34	25	39.6	61.2
10	434	99	241	30	8	162.4	108.4
5	756	335	43	150	340	324.8	83.8
0	1737	1101	653	790	573	970.8	48.8

Based on 5 leaves/test unit and 40 DBM adults

Table 3. Oviposition of *P. xylostella* on cabbage leaves sprayed with water extracts of *G. sepium* at different concentrations 48 hours of exposure in a no-choice test in percent from control.

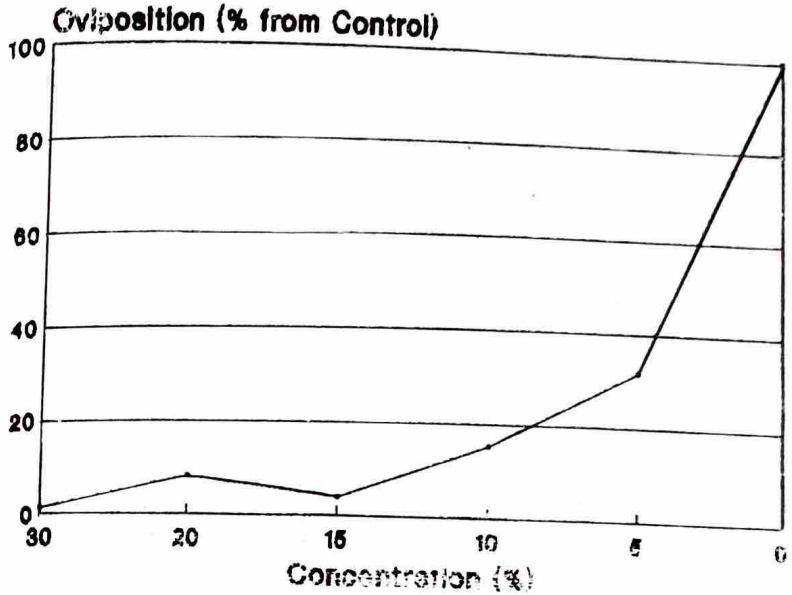
Concentration (%)	R1	R2	R3	R4	R5	Mean	CV
30	2.7	0.2	0.0	3.7	0.5	1.3	129.4
20	0.6	14.6	12.8	13.9	12.0	8.1	69.6
15	4.7	3.1	3.5	4.3	4.4	4.0	16.9
10	24.9	9.0	36.9	3.8*	1.4	15.2	20.0
5	43.5	30.4	6.6	19.0	59.3	31.8	12.9
0	100.0	100.0	100.0	100.0	100.0	100.0	-

Based on 5 leaves/test unit and 40 DBM adults

Table 2 and 3 show a clear dependency between concentration and oviposition deterrency.

Table 3 shows, that in the 30% water extract, only 1.3% eggs are deposited compared to the control. Event in the 5% water extract, only 31.8% eggs compared to the control are laid.

Figure 1. Relationship between concentration of aqueous extracts of *G. sepium* and oviposition by *P. xylostella* on treated cabbage leaves.



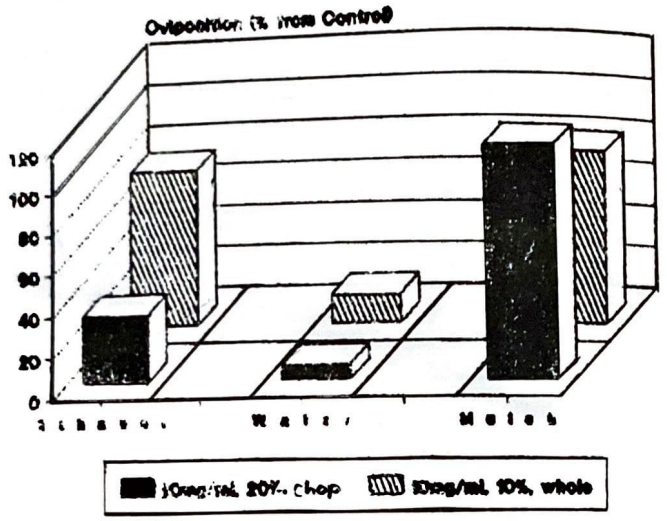
Field cage experiment

Table 4. Oviposition of DBM in a field cage experiment comparing different treatments with *G. sepium*, expressed in absolute numbers.

	Number of eggs laid (48 hours)					CV
	B1	B2	B3	B4	Mean	
Extract EtOH						
20 mg/ml	282	303	51	280	229.0	44.6
10 mg/ml	435	453	552	645	521.3	23.6
Extract H2O						
20%	35	9	32	51	31.8	102.6
10%	92	84	52	35	65.8	25.8
Mulching						
chopped	450	449	755	564	535.5	59.0
whole	862	419	656	205	554.5	15.4
Control						
EtOH	664	689	596	850	702.0	-
H2O	863	583	825	273	637.3	-

Based on 10 6-week old seedlings/treatment and block. In a column, means followed by a common letter are not significantly different at the 5% level of significance by DMRT.

Figure 2. Comparison of the oviposition deterrency effect of different methods of preparation and concentrations of *G. sepium* to *P. xylostella* in a field cage trial.



In Table 4 results obtained in the former experiments confirm the higher oviposition deterrency power of the water extract of *G. sepium*. In the treatment using 20% water extract, the oviposition per block was 31.8 eggs and for the 10% water extract 65.8 compared to 702 eggs for the ethanol control and 637.3 for the water control.

Table 5. Oviposition of *P. xylostella* in field cage experiment, comparing different *G. sepium*, expressed in % from control

	Number of eggs laid (48 hours)					CV
	B1	B2	B3	B4	Mean	
Extract EtOH						
20 mg/ml	42.5	43.9	8.6	32.9	31.98 b	44.6
10 mg/ml	65.5	65.8	92.6	75.9	74.98 c	23.6
Extract H2O						
20%	4.1	1.5	3.9	18.7	7.05 a	102.6
10%	10.7	14.4	6.3	12.8	13.63 a	25.8
Mulching						
chopped	65.9	77.0	91.5	206.6	110.25 c	59.0
whole	99.9	71.9	79.5	75.1	81.60 c	15.4
Control						
EtOH	100.0	100.0	100.0	100.0	100.0 c	-
H2O	100.0	100.0	100.0	100.0	100.0 c	-

Based on 10 6-week old seedlings/treatment and block. In a column, means followed by a common letter are not significantly different at the 5% level of significance by DMRT.

DISCUSSION

These data clearly indicate the superiority of the water-based extracts over all the other treatments with an oviposition of 7.05% for the 20% water extract at 20 mg/ml of the no-choice experiment could be confirmed with this field cage experiment. Ethanolic extracts at 10 mg/ml however, show very minor effects. Also mulching, using whole leaves and chopped leaves showed again very small effects.

PROSPECT OF USING *G. SEPIUM* IN INTEGRATED CONTROL SYSTEMS FOR *P. XYLOSTELLA*

At present, this plant species is being promoted as part of sustainable agricultural systems due to its positive effects in soil improvement, erosion control but also as feed source for livestock. The increasing cultivation of this species contributes also to the increasing availability of plant material.

The identification of a semiochemical for reducing the oviposition of *P. xylostella* has just begun. At the moment, there is no information yet on the chemical aspects such as optimal solvent, responsible compounds, optimal concentration but also persistence in the environment, etc. It is known to be water soluble. This makes it potentially interesting for farmers who are interested in preparing their own extracts.

This potential candidate as oviposition deterrent may be another prospective component for an integrated control strategy for *P. xylostella* presently being pushed for.

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