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Effect of crude sugar ester fractions from wild *Nicotiana* sp. on tobacco aphid, *Myzus nicotianae* and bud worm, *Helicoverpa armigera*

J V Prasad, U Sreedhar and S Gunneswara Rao

Central Tobacco Research Institute, Rajahmundry - 533 105, East Godavari District, Andhra Pradesh, India.
E mail: prasadjv64@yahoo.co.in

Abstract

Sugar ester fractions from Nicotiana gossei, N. trigonophylla, N. repanda, N. benthamiana, N. glutinosa *and* N. plumbaginifolia *were evaluated against tobacco aphid,* Myzus nicotianae *and the bud worm* Helicoverpa armigera. *Crude sugar ester fractions from* N. gossei *and* N. trigonophylla *caused significant mortality of the tobacco aphid at all concentrations tested followed by the extracts from* N. glutinosa. *The highest mortality (90%) was recorded in case of 2% crude sugar esters from* N. trigonophylla. *The crude sugar ester fraction from* N. gossei *appeared to have growth regulatory properties against the larvae of* H. armigera *when incorporated into artificial diet at concentration of 2.5 mg/ g of diet though they did not exhibit significant toxicity. Highest oviposition deterrence (oviposition index 0.13) was recorded with the dichloromethane fraction of* N. trigonophylla *at 0.5% concentration followed by the same fraction at 0.25 % concentration, dichloromethane fraction of* N. gossei *at 0.5% and 0.25 % concentrations.*

Keywords: Sugar esters, wild *Nicotiana* sp, *Myzus nicotianae, Helicoverpa armigera*

Introduction

A group of natural sucrose and glucose esters from sugar ester isolates of *Nicotiana gossei* and other *Nicotiana* species have been demonstrated to be highly effective against nymphal stages of the greenhouse white fly, *Trialeurodes vaporariorum* and *Bemisia tabaci* (Neal *et al.*, 1990). These studies have aroused interest in the sugar ester isolates from different wild *Nicotiana* species and their ability to control soft bodied insects in different crops. The tobacco aphid, *Myzus nicotianae* is a major pest of tobacco and its infestation coincides with the priming of tobacco leaves during crop season. Use of chemical pesticides indiscriminately at this stage of the crop could result in the presence of pesticide residues beyond guidance residue levels in cured leaf. In order to contain reliance on chemical pesticides and thereby reduce the chances of residues, research emphasis on tobacco has always been given to methods of pest management using biological agents or bio-rational insecticides that are target pest specific and do not leave any harmful residues. In this direction, the present study is planned to isolate sugar ester fractions from select wild *Nicotiana* species and test their bioefficacy against two major pests of tobacco (*M. nicotianae* and *Helicoverpa armigera*) to achieve the objective of producing cleaner and pesticide residue free tobacco.

Materials and methods

The wild tobacco species *viz., N. gossei, N. trigonophylla, N. plumbaginifolia, N. repanda, N. glutinosa* and *N. benthamiana* were grown on a sandy loam soil following standard agronomic practices. The tobacco aphid, *M. nicotianae* was collected from field infested tobacco plants and directly used in the bioassays. The culture of *H. armigera* was reared on a bengal gram based artificial diet (Veerareddy and Bhattacharya, 1990).

Mature and fully expanded leaves of the wild *Nicotiana* species were harvested at blooming stage for extracting the sugar ester fractions. The leaves were dipped in dichloromethane to get the sugar esters into solution. After evaporating the solvent, the extract was dissolved in hexane and partitioned with acetonitrile. The acetonitrile fraction was again partitioned with 1N tartaric acid to remove the alkaloids and finally the acetonitrile fraction thus obtained was used as crude sugar ester fraction in bioassays against *M. nicotianae* (Severson *et al.*, 1991). Fifty fully grown apterous aphids were sprayed uniformly on their dorsal surface with specific concentration of the extracts using a chromatography sprayer and were later transferred on to a tobacco leaf confined in a 250 ml capacity ventilated plastic jar. The assay was replicated three times and mortality was

recorded after 24 hours. The sugar ester fraction from *N. gossei* was incorporated into the artificial diet of *H. armigera* larvae at concentrations from 0.25 to 2.5 mg / g of diet and three day old larvae of *H. armigera* were used for studying the effect of the extracts on the growth and development of the test insect.

The dry leaf powders of *N. gossei, N. trigonophylla* and *N. glutinosa* were extracted with hexane and dichloromethane using Soxhlet method and these extracts were tested for their oviposition deterrence activity against the adult moths of *H. armigera.* Ten tender twigs of bengal gram were sprayed with known concentration of the extracts and offered for egg laying to two pairs of *H. armigera* confined in 10 l capacity plastic jars. The jars were covered with white muslin cloth for aeration. Number of eggs laid on the bengal gram twigs and other surfaces on the jar and the muslin cloth were counted separately for calculating the oviposition index which is the ratio of eggs laid on treated surface and those laid on untreated surfaces. An ovipostion index between 0.3 and 0.6 indicates moderate deterrence whereas an index below 0.3 indicates strong deterrence (Ryan *et al.*, 2009).

Results and discussion

Effect of sugar ester fractions on *M. nicotianae*

Both the crude sugar ester fractions from *N.gossei* and *N. trigonophylla* brought about a significant mortality of the tobacco aphid at all concentrations tested (Table 1). The highest mortality was recorded in case of 2% crude sugar ester fraction from *N. trigonophylla.* Compared to the sugar ester fraction from *N. gossei,* the fraction from *N. trigonophylla* was observed to be more bioactive against tobacco aphid. The aphids died of desiccation after being sprayed with the crude sugar ester fraction. Between the two extracts tested, the sugar ester fraction from *N. glutinosa* was found to be more toxic to the aphids than the sugar ester fraction obtained from *N. plumbaginifolia* (Table 2). The highest mortality was recorded in case of 2% crude sugar esters from *N. glutinosa.* When the effect of crude sugar ester fraction from *N. benthamiana* and *N. repanda* was studied, it was observed that the sugar ester fraction from *N. repanda* was found to be more toxic to the aphids than the same obtained from *M. benthamiana.* The highest mortality of 61.5% was recorded in case of 2 % crude sugar ester fraction obtained from *N. repanda* (Table 3).

Cuticular constituents of tobacco have long been established as factors that govern insect and disease resistance in tobacco (Severson *et al.*,1985). Acyl sugars, consisting of sucrose and glucose esters, present in *Nicotiana* and secreted by trichomes deterred the green peach aphid, *Myzus persicae* from feeding on *Solanum berthaultii* (Neal *et al.*, 1990). The levels of sugar esters and mono-ols in certain

Table 1. The effect of crude sugar ester (SE) fractions from *N. gossei* (Ng) and *N. trigonophylla* (Nt) on tobacco aphid, *M. nicotianae*

Treatment	Mean % mortality
SE from Ng @ 0.25%	43.1(46.7)*
SE from Ng @ 0.50%	59.2(73.3)
SE from Ng @ 1.00%	72.3(86.7)
SE from Ng @ 2.00%	81.1(93.3)
SE from Nt @ 0.25%	55.0(66.7)
SE from Nt @ 0.50%	55.3(66.7)
SE from Nt @ 1.00 %	81.1(93.3)
SE from Nt @ 2.00 %	90.0(100.0)
Control with solvent	12.3(6.7)
Control (no spray)	0.0(0.0)
C D at 5%	16.6
C V %	17.6
SEM	5.6

* Figures in parentheses are original means.

Table 2 . The effect of crude sugar ester fractions from *N. glutinosa* (Ngl) and *N. plumbaginifolia* (Np) on tobacco aphid, *M. nicotianae*

Treatment	Mean % mortality
SE from Ngl @ 0.25%	31.0(26.7)*
SE from Ngl @ 0.50%	41.1(43.3)
SE from Ngl @ 1.00%	61.6(77.3)
SE from Ngl @ 2.00%	76.5(92.0)
SE from Np @ 0.25%	10.9(5.3)
SE from Np@ 0.50%	32.3(28.7)
SE from Np@ 1.00 %	39.6(40.7)
SE from Np@ 2.00 %	49.2(57.3)
Control with solvent	19.0(6.7)
Control (no spray)	1.0(0.0)
CD at 5%CV %	13.4
CV%	8.3
SEM	2.8

* Figures in parentheses are original means

Table 3. The effect of crude sugar ester fractions (SE) from *N. benthamiana* (Nb) and *N. repanda* (Nr) on tobacco aphid, *M. nicotianae*

Treatment	Mean % mortality
SE from Nb @ 0.25 %	15.1(7.0)
SE from Nb @ 0.50 %	30.6(26.0)
SE from Nb @ 1.00 %	39.4(40.3)
SE from Nb @ 2.00 %	39.2(40.0)
SE from Nr @ 0.25%	33.2(30.0)
SE from Nr @ 0.50 %	45.4(50.7)
SE from Nr @ 1.00 %	50.8(60.0)
SE from Nr @ 2.00 %	61.5(77.0)
Control with solvent	17.0(8.7)
Control (no spray)	0.0(0.0)
CD at 5 %	7.9
CV %	10.2
SEM	2.0

* Figures in parentheses are original means

tobacco types had significant positive association with aphid resistance in these types. More than the total quantity of sugar esters, quality of sugar esters and the presence of mono-ols had a role in governing aphid resistance in these tobacco types (Johnson *et al.*, 2001). By modifying the sugar and fatty acid composition in synthetic sugar esters Puterka *et al.*, (2003) observed that sucrose octanoate, high in monoester content had the highest activity against a range of arthropod pests at low concentrations of 1200-2400 ppm. All of the synthetic sugar ester materials that were examined in this study had superior insecticidal activity compared with insecticidal soap.

Effect of sugar ester fractions on *H. armigera*

Mean larval mortality of 22 and 29% was recorded with SE of *N. gossei* at concentrations of 1.25 and 2.5 mg / g of the diet, respectively. The weight of the larvae recorded at 5 days after treatment was significantly low in all the treatments compared to control with maximum reduction being recorded at 2.5 mg/g concentration (Table 4). Significant reduction in per cent pupation was observed only at concentrations of 1.25 and 2.5 mg / g of diet incorporated with SE of *N. gossei*. The mean larval period was significantly prolonged in the diets with sugar fractions ranging from 0.50 to 2.5 mg/g. Per cent emergence of adults was also significantly influenced in all the treatments compared to control. Thus the crude sugar fraction from *N. gossei* appears to have growth regulatory properties though they did not exhibit significant toxicity to the larvae of *H. armigera.* Prabhu *et al.*, (1981) reported that fractions of cytoplasmic polar lipids of *N. gossei* were toxic to *Spodoptera litura* and attributed toxicity to alkaloids and saponins in the leaf.

Oviposition deterrence of organic solvent extracts against *H. armigera*

Highest oviposition deterrence was recorded by the dichloromethane fraction of *N. trigonophylla* at 0.5% concentration followed by the same fraction at 0.25 % concentration, dichloromethane fraction of *N. gossei* at 0.5% and the same fraction at 0.25 % concentration (Table 5). The hexane and dichloromethane fractions of *N. glutinosa* exhibited very low oviposition deterrence even

Table 4. The influence of sugar ester (SE) fraction from *N. gossei* on the growth and development of 3 day old *H. armigera* larvae through diet incorporation

Treatment	% Mortality	Larval weight 5 DAT (mg)	% Pupation	Mean larval period (days)	% Adult emergence
SE @ 0.25 mg /g of diet	0.0(0.0)	294.0	80.8(95.0)	14.9	65.8(82.6)
SE @ 0.50 mg /g of diet	4.6(2.5)	289.0	74.1(90.0)	18.8	60.1(75.0)
SE @ 1.25 mg / g of diet	22.5(15.0)	260.0	58.6(72.5)	18.1	56.9(70.2)
SE @ 2.50 mg / g of diet	29.9(25.0)	207.8	42.1(45.0)	19.5	52.3(62.6)
Control	0.0(0.0)	396.0	85.4(99.4)	14.3	80.8(97.4)
CD	7.5	24.7	13.5	0.8	10.5
CV	42.9	5.57	14.1	3.1	10.8
SEM	2.4	8.05	4.5	0.3	3.4

* Figures in parentheses are original means; DAT = Days after treatment

Table 5. Oviposition deterrence activity of wild *Nicotiana* species against *Helicoverpa armigera*

Nicotiana species	Solvent used for extraction	Oviposition index (eggs laid on treated surface/ eggs laid on untreated surface)
N. gossei 0.25 %	Hexane	0.56
N. gossei 0.50 %	Hexane	0.48
N. gossei 0.25 %	Dichloromethane	0.36
N. gossei 0.50 %	Dichloromethane	0.29
N. trigonophylla 0.25 %	Hexane	0.49
N. trigonophylla 0.50 %	Hexane	0.40
N. trigonophylla 0.25%	Dichloromethane	0.24
N. trigonophylla 0.50 %	Dichloromethane	0.13
N. glutinosa 0.25%	Hexane	0.79
N. glutinosa 0.50 %	Hexane	0.73
N. glutinosa 0.25 %	Dichloromethane	0.80
N. glutinosa 0.50 %	Dichloromethane	0.67
SEM		0.03
CD 5 %		0.11
CV %		13.53

at 0.5% concentration. The leaf miner, *Liriomyza trifolii* is deterred from ovipositing on *L. pennellii* by acyl sugars (Hawthorne *et al.*, 1992).

The aphidicidal and other biological properties of trichome exudates and the variety of wild germplasm available offer promise in tobacco breeding programs to develop pest-resistant tobacco cultivars. A number of these tobaccos could be used for production of natural sugar ester biorationals or used in a breeding program for development of aphid resistant cultivars. The knowledge of metabolites, as well as the genes and proteins responsible for them in trichome exudates can assist in the classical breeding programs, as well as targeted genetic engineering, aimed to optimize trichome density and physiology to facilitate customization of metabolite production or to tune biocide activity to enhance crop protection.

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