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Combined mode toxicity of different crude extracts of plants to mango mealybug *Rastrococcus invadens* Williams

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ABSTRACT: Six acetone extracts of *Annona senegalensis* root-bark; *Tephrosia vogelii* root-bark; stem-bark, leaf and seed; *Tephrosia candida* seed; and two aqueous residue extracts of neem seed kernel and *Annona muricata* seed kernel were tested for bioactivity against *Rasdtrococcus invadens* Williams at different concentrations with three replications. The six acetone extracts were highly toxic to adult female *R. invadens* in the combined mode experiment. *T. vogelii* stem-bark, followed by its seed; *A. senegalensis* root-bark and *T. candida seeds extracts, was most insecticidal to the mealybug.* Respective 48-h LD_{50} values for *T. vogelii* stem-bark and seed, *A. senegalensis* root bark, *T. vogelii* leaf and *T.candida* seed extracxts were 4.6, 4.6, 11.1, 11.3 and 15.6 µg/insect. Generally, mortalities by each effective extract increased with increase in concentrations. Knowledge of specific mode of action of individual insecticidal extracts of plants is required for developing botanical insecticide for field control of the mealybug pest.

Key words: Mango mealybug; *Ratrococcus invadens*; Adult mortality; plant extracts; combined mode toxicity.

Introduction

Several reports on the exotic mango mealybug *Restrococcus invadens* Williams (13, 21) from Togo and Ghana (2), Benin (17), Nigeria (10) and Gabon (7) in West Africa confirmed it a polyphagous pest of different species of fruit trees, fruit and leafy vegetables, weeds and ornamentals.

Mango, followed by citrus, is the most preferred host. As a result of its exotic origin (native to South-east Asia), polyphagy, rapid reproductive rate and the rate of spread, the method of control widely adopted by scientists in the mealybug infested zones has been that of classical Biological control (17, 11). Although, various reports thus far in Togo, Ghana, Benin, Congo and Nigeria have shown successful establishment of the exotic parasitoid *Gyranusoidea tebygi* Noyes on *T. invadens* (18, 14) it has not been able to reduce the meanlybug population below sub-economic level in certain ecological zone(s) as in Guinea Savanna of Nigeria. Low reduction in number of *R, invadens* by *G. tebygi* have been attributed to hyperparasitism of the parasitoid by the indigenous secondary parasitoids (3, 16).

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As a result of very low impact of the parasitoid on the meanlybug in some zones (19) an alternative or supplementary method of control is necessitated. Daramola et al., (8) advocated a short-term control of *R. invadens* on the infested shrubs or seedling plants of low heights and flowers by application of some synthetic insecticides. Although some synthetic insecticides recommended were tried and found effective, they are not environmentally safe enough for regular application compared to existing natural pesticides. In most ecology, natural (or botanical) insecticides offer a safer, environmentally friendlier and efficient alternative to synthetic insecticides (5). Low toxicity of crude extracts to beneficial insects could be of great value for their utilization in biologically oriented pest management programmes (9) against the meanlybug. Application of crude extracts that have been proved adequate for the control of this meanlybug on the infested plants of low height could be of major advantage to both rural and urban dwellers who are benefiting from such plant species serving as alternate hosts to the meanlybug.

In view of the dearth of knowledge on the effects of natural pesticides on the mango meanlybug, this paper considers bioactivity of some components of plants including *Tephrosia vogelii, Tephrosia candida, Annona senegalensis, Azadirachta indica* and *Annona muricata* for the future field control of the meanlybug pest.

Materials and Methods

Effects of crude extracts of plants on R. invadens

Extracts from *Annona senegalensis, Tephrosia vogelii, Tephrosia candida, Azadirachta indica* and *Annona muricata* were prepared and their insecticidal actgivity *Rastrococcus invadens* Williams investigated. This experiment was ppurposely set up to identify the crude extracts that could be used for future control of *R. invadens* on the infested shrubs or seedling plants of low height.

Preparation of crude extracts of A. senegalensis and Tephrosia spp.

Method of Aku et al., (4) was adopted to obtain extracts from the three plant species. Root-bark of matured stands of *A. senegalensis* growing in some parts of Makurdi Local Government Area was obtained. While the seeds from pods of *T. candida* were obtained from established stands in Ibaji Local Government Area, Kogi State, the seeds *T. vogelii* collected from the same ecological zone in the Guinea Savanna Area were planted and nurtured into mature stands between May 2000 and August 2001 at the University Teaching and Research Farm. Root-bark, stem-bark, leaf and seed were removed from the matured stands of *T. vogelii* in October 2001.

Eaach plant material obtained was washed in tap-water and air-dried initially for period of 3-4 weeks, and then sun-dried for the period of 3 days. Each dried material was milled to fine powder (300mm particle size) in a milling machine. In order to obtained crude extract from each powdered material, 10g of each powdered product was homogenized in 50 ml of methanol; water (4:1) mixture for 5 minutes. The liquid content was then filtered with Whatmann No. 2 filter paper. Each filterate was allowed to evaporate at room temperature until one tenth ($1/10^{th}$) of its initial volume was obtained. The filterate was then acidified with 2m sulphuric acid (H_2SO_4). The acidified filterate was weighed in each case and then dissolved in sufficient acetone so that 2, 5 and 10% solutions of the extracts were prepared.

Preparation of crude extracts of A. indica and A. muricata

The method of Valladares et al., (20) was modified in the preparation of extracts from seeds of *A. indica* and *A. muricata*. While the mature fruits of *A. indica* were obtained directly from the stands in Makurdi metropolis, mature fruits of *A. municata* were ppurchased from railway market in Makurdi town. The seeds of both fruits were removed from their fruits, washed and allowed to dry at room temperature. They were subsequently sun-dried for a period of 3-days. Each seed material was crushed and milled by use of electric milling machine. The fat content of each material was separated by addition of small quantity of n-hexane. This was followed by use of ethanol to obtain extract by means of soxhlet. The solvent was then evaporated to obtain the oil. Different weights (1,2,5 and 10g) of *A. indica* residue were

dissolved in 100ml of water to form different concentrations of the aqueous extract. Also, different concentrations of *A. muricata* residue extracts were equally prepared as described for *A. indica.*

Test Insects

Seedlings of mango plant and cuttings of *Ficus umbrellata* were raised in plastic pots. Four potted plants of mango (3-4 months old) and two potted plants of *F. umbrellata* (40 – 50 days old) were placed in individual wooden sleeve-cages with the dimension 50cm x 62cm x 70cm (LxWxH). The cages were placed on a plat-form 40cm above the ground in the insectary. Five larvipositing females of *R. invadens* were removed from naturally infested mango tree and transferred to five leaves (one meanlybug per leaf) of individual caged plant species. The adult females were left to produce crawlers which developed to adults. In this manner, *R. invadens* was maintained in the caged plants. The culture maintained in the insectary throughout the experiment and continued to serve as source of supply of the text insects.

In this study, only the adult females under one week old served' as test insects in all the experiments. The experiments, were carried out under fluctuating relative humidity (70-80%) and temperature (30±°C) of the environment. A mixture particular solvent and distilled water used for the extraction of each extract served as a standard control while distilled water alone served as an untreated control.

Toxicity of different extracts of R. invadens

The extracts from five(5) different plant species were tested on adult female *R. invadens* for their insecticidal effect.

A total of 30 plastic Petri-dishes each with 11.0cm diameter were used for combined toxicity study. Top lid or cover of each plastic Petri-dish had a nylon mesh aperture. Fresh mango leaves used in Petri-dish during the experiment were folded in each case for proper enclosure in the dishes.

A total of 10 adult female meanlybugs were removed from the culture and placed on the under surface of a detached fresh mango leaf. Each leaf was placed on a wet filter paper in the basal plate of plastic Petri-dish (11.0cm diameter). After the meanlybugs were settled, each leaf was turned upside down. The leaf was sprayed with determined dosage of emulsion of an extract by use of a micro-syringe under a uniform pressure. The leaf was then turned the right side up and the base of the petiole inserted into a moistened cotton wool to retard dessication. The basal plate of each Petri-dish was covered with a tight-fitting plastic lid that contained window of fine gauze. The experiment was replicated three times for each extract treatment. Treatment with water and solvent mixture were included as control. Moribund insects were determined and mortality counts recorded 48 hours after treatments (HAT). The minimum effective concentration of each extract was determined by application of series of increasing concentration of the extracts.

Data Analysis

In analyzing data, Abbott's formula was used for mortality in control (1) before probit analysis was carried out. The data obtained were arcsine-transformed, and then subjected to Analysis of Variance (ANOVA). Significantly different treatment means were separated by DNMRT at P = 0.05.

Results

Effects of crude extracts of plants on R. invadens

Toxicity effects of different plant extracts to adult female R. invadens

Studies on the combined effect of the individual plant extracts showed that most adult female *R. invadens* died within 12 hours period after treatment with those extracts that proved effective. Toxic effects observed from combined treatments of individual plant extract: root-bark (*A. senegalensis*), seed residues (*A. muricata* and *A. indica*) and seed (*T. candida*) on adult female *R. invadens* are shown in Table 1. Mortality of adult female meanlybug increased witn increase in concentration of acetone root-bark

extract of *A. senegalensis* and acetone seed extract *T. candida*. Percentage mortality differed significantly ($P<0.05$) among the treatments for the two named extracts. Adult mortalities caused by *A. senegalensis* root-bark extract at 5% and 10% treatment levels were significantly ($P<0.05$) higher than those recorded at 1 and 2% levels. Percent adult mortalities at different concentrations of *T. candida* seed extract were significantly different from one another. Numbers of dead females recorded 48 HAT for each concentration level of *A. senegalensis* root-bark and *T. candida* seed extracts were significantly ($P<0.05$) higher than those observed for the controls.

Table 1: Mean percentage mortality of adult female *R. invadens* sprayed with four different plant extracts in combined mode treatment.

Extract Concentration (%)	**Percentage Mortality of Adults Treated With Extracts**			
	Root-bark (*A. senegalensis*)	**Seed residue (*A. muricata*)**	**Seed residue (*A. indica*)**	**Seed (*T. candida*)**
1	61.1b	13.3a	10.0a	46.2d
2	68.9b	10.0a	6.7a	69.5c
5	88.07a	10.0a	13.3a	78.6b
10	89.1a	6.7a	6.7a	95.5a
Standard control	14.2c	14.2a	13.3a	17.6e
Control	9.6c	9.2a	3.3b	4.6f

Means within each column followed by the same letter are significantly different at 5% level (DNMRT).

Table 2: Mean percentage mortality of adult female *R. invadens* treated with extracts of different parts of *T. vogelii*.

Extract Concentration %	**Percentage Mortality of Adults Treated With Extracts of**			
	Leaf	**Root bark**	**Seed**	**Stem bark**
1	76.2b	41.0b	68.7b	83.4b
5	91.7a	81.3a	80.7b	86.7b
10	93.3a	85.0a	89.7a	94.7a
Standard control	22.9c	20.3c	25.7c	28.0c
Control	23.3c	18.1c	11.9d	11.5d

Means within each column followed by the same letter are not significantly different at 5% level (DNMRT).

The aqueous extracts of seed residue of *A. muricata* and *A. indica* had no significant ($P>0.05$) toxic effect on the adult female when compared with the controls.

Mortalities of adult females resulting from toxic effects of three concentration levels of acetone extracts of *T. vogelii* were significantly different ($P<0.05$) (Table 2). While mortalities at 5% and 10% concentrations were not significantly ($P>0.05$) different for leaf and root-bark extracts, data for stem-bark and seed extracts at the same concentration levels were significantly different ($P<0.05$). Adult mortality observed at 10% concentration for each candidate extract was significantly higher compared with that recorded at 2% level. Toxic effects on the adult observed at different concentrations for *T. vogelii* extract was significantly higher than those recorded for the controls. Mortality was significantly higher for standard control than for control with the stem-bark and seed extracts.

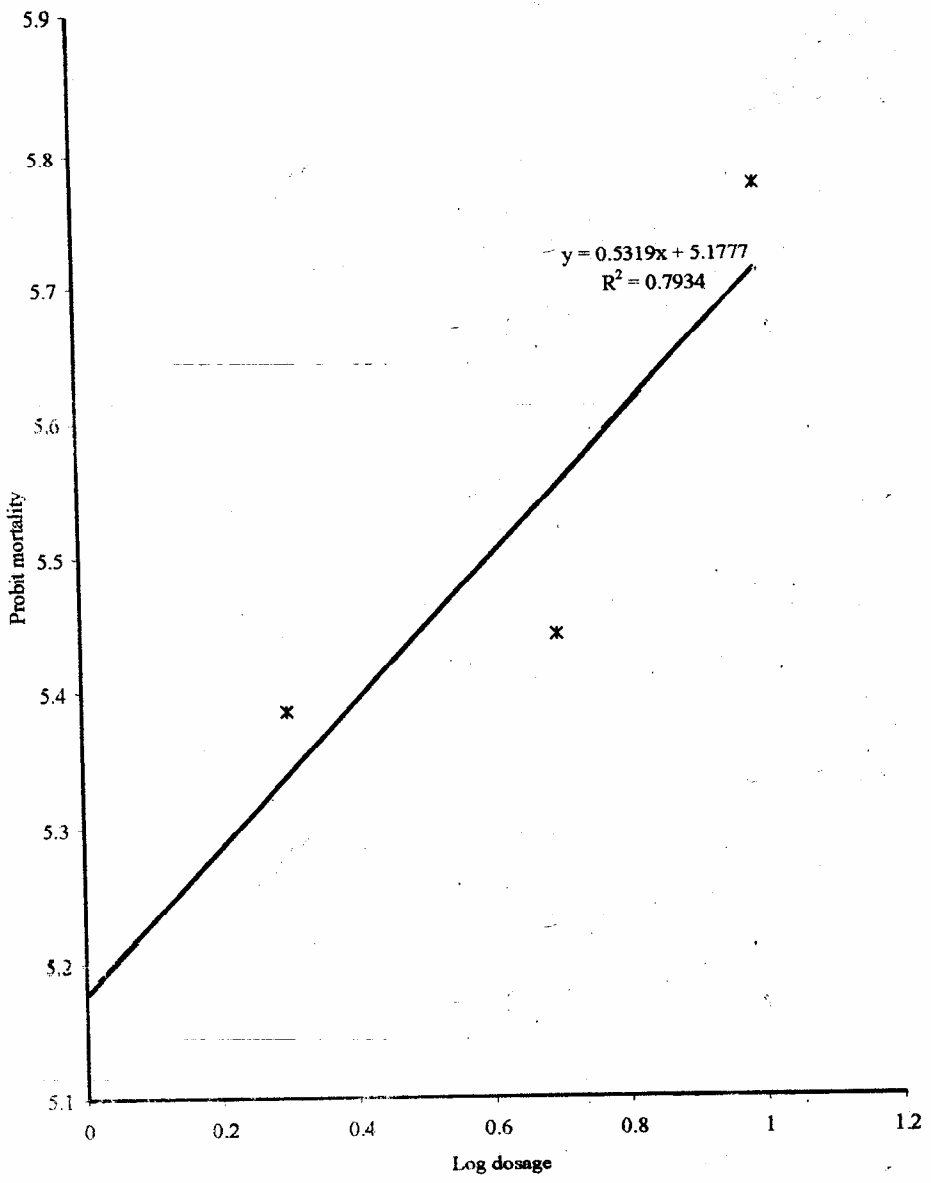

Fig. 1: Forty-eight-hour dosage mortality regression line for crude extract from *T. vogelii* stem bark (combined toxicity test) against adult female *R. invadens*, dosage in µg/insect.

Figure 1 shows the sample of probit regression line for 48-h data. The high significant R^2- value (explained variation in mortality caused to insect by the extract) indicates a good fit of the data to the regression. The steepness of each line indicates that the meanlybugs were quite susceptible to *T. vogelii* stem-bark extract.

LD_{50} value varied among different plant extracts. In a combined mode experiment, *T. vogelii* seed and stem-bark extracts each showed the lowest 48-h LD_{50} value of 6.4 µg/insect, followed by that of 11.1 µg/insect for *A. senegalensis* root-bark, 11.3 µg/insect for *T. vogelii* leaf and 15.6 µg/insect to *T. candida* seed extracts.

Discussion

Over 75% of crude extracts of plants screened against adult female *R. invadens* during the experiments were highly insecticidal. The choice of adult female among the life stages as test insect for investigation of the efficacy of the extracts was to allow ease of handling and more so that the relatively small immature stages were killed faster than the adult female by different solvents used for the preparation of the extracts. Perhaps, it might be justifiable to say that each of the bioactive extracts that significantly killed the adult females if capable of eliminating more of the pre-adult stages which have more relatively exposed and soft body compared to adult female with wax cover. Mortality of adult *A. craccivora* Koch was significantly lower compared to immature stages treated with the same dosage levels of *Acorus calamus* rhizome and *Glycosmis mauritiana* root-bark extracts (6).

The examination of the bioactivity of *T. vogelii* has indicated that all the four components screened were highly insecticidal to the meanlybug. Hence, when screening therefore for bioactivity, it is important to obtain as many different parts of the plant as possible for extraction. This high insect mortalities recorded at higher concentrations of both *T. candida* and *T. voglii* extracts has confirmed the report that several species of *Tephrosia* have insecticidal properties (15). High percent mortality observed at 5% and 10% concentrations for seed extract of *T. candida*; stem-bark and leaf extracts of *T. vogelii* were not significantly different.

In this study, aqueous residue extracts from neem seed kernel and *A. muricata* seed kernel were not insecticidal to the meanlybug. The non-effectiveness of the aqueous seed residue extracts may be an indication that the active principles insecticidal to the meanlybug were not present in the extracts. However, water and alcohol extracts of de-oiled neem seed kernel and aqueous seed kernel were reported to have demonstrated very good anti-feedant properties to un-named insect species (12). Whether the active principles were lost from the residue extracts in the process of extracting the oil is not known. However, active principles like azadirachtin and other active compounds were once obtained from neem seed kernels and some neem seed oil.

Further studies should be focused on mode of action of each effective crude extract of plants tested on the meanlybug. The knowledge of mode of action will help in development of the effective extracts into botanical insecticides for the control of *R. invadens* and other related homopterous insect pests.

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