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Full Length Research Paper

Bioactive fractions of n-hexane extracts from *Vigna* pods against the legume pod borer, *Maruca vitrata* Fabricius

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The identification of resistance factors in *Vigna* species has been a major constraint to the development of effective host-plant resistance against the major insect pests of cowpea. We studied the effects of some fractions of the n-hexane extract of green pods of three *Vigna* species namely *V. vexillata*, *V. oblongifolia* and *V. unguiculata* on the growth, development and fecundity of *Maruca vitrata* Fabricius. When incorporated in the artificial diet, the phenolic, acidic and basic fractions had no significant effect on larval growth and development. However, the neutral fraction from the three *Vigna* species showed antibiosis by significantly reducing larval weight, percentage pupation and adult emergence. There was no pupation on diet with *V. vexillata* neutral fraction. We conclude that neutral fractions of the green pods of wild *Vigna* species contain metabolites which can be exploited in the genetic engineering of cultivated cowpea for resistance against *M. vitrata*.

Key words: *Vigna* species, resistance, n-hexane extract, *Maruca vitrata*, larval growth, fecundity.

INTRODUCTION

The legume pod borer, *Maruca vitrata* Fabricius (Synonym: *Maruca testulalis* Geyer) is one of the major pests of cowpea in the tropics (Jackai and Daoust, 1985). Its control has been mainly by insecticide application, but the increasing cost, ecological impact and mammalian toxicity of these insecticides as well as development of insect resistance to them have necessitated the search for alternative control measures. Jackai and Adalla (1997) identified host plant resistance as a major compo-nent of an integrated pest management strategy against *M. vitrata* on cowpea. A proper understanding of the mechanisms and bases of resistance will help in identifying the components of resistance for use as markers in screening programmes and biotechnology.

The biochemical bases of resistance are especially useful in biotechnology because they involve metabolites and enzyme activities. Several compounds in plants are known to be detrimental to the growth, development and fecundity of insects. Many of such compounds have contributed to resistance in crop plants against insect pests.

Jackai (1995) and Jackai et al. (1996) found good levels of resistance among the wild *Vigna* species, *V. vexillata* A. Richard and *V. oblongifolia* A. Richard, in both field and laboratory experiments. Umoren (1995) found that protease inhibitors were not involved in this resistance, and lectins could not be associated with these wild *Vigna* resistances to *M. vitrata* (J. Machuka, Centre for Complimentary Medicine and Biotechnology, Kenyatta University, Nairobi, Kenya, personal communication). In a preliminary experiment, the n-hexane extracts of green pods of *V. vexillata* and *V. oblongifolia* showed antibiosis against *M. vitrata*. We then fractionated these extracts into acidic, basic, neutral and phenolic fractions for ease

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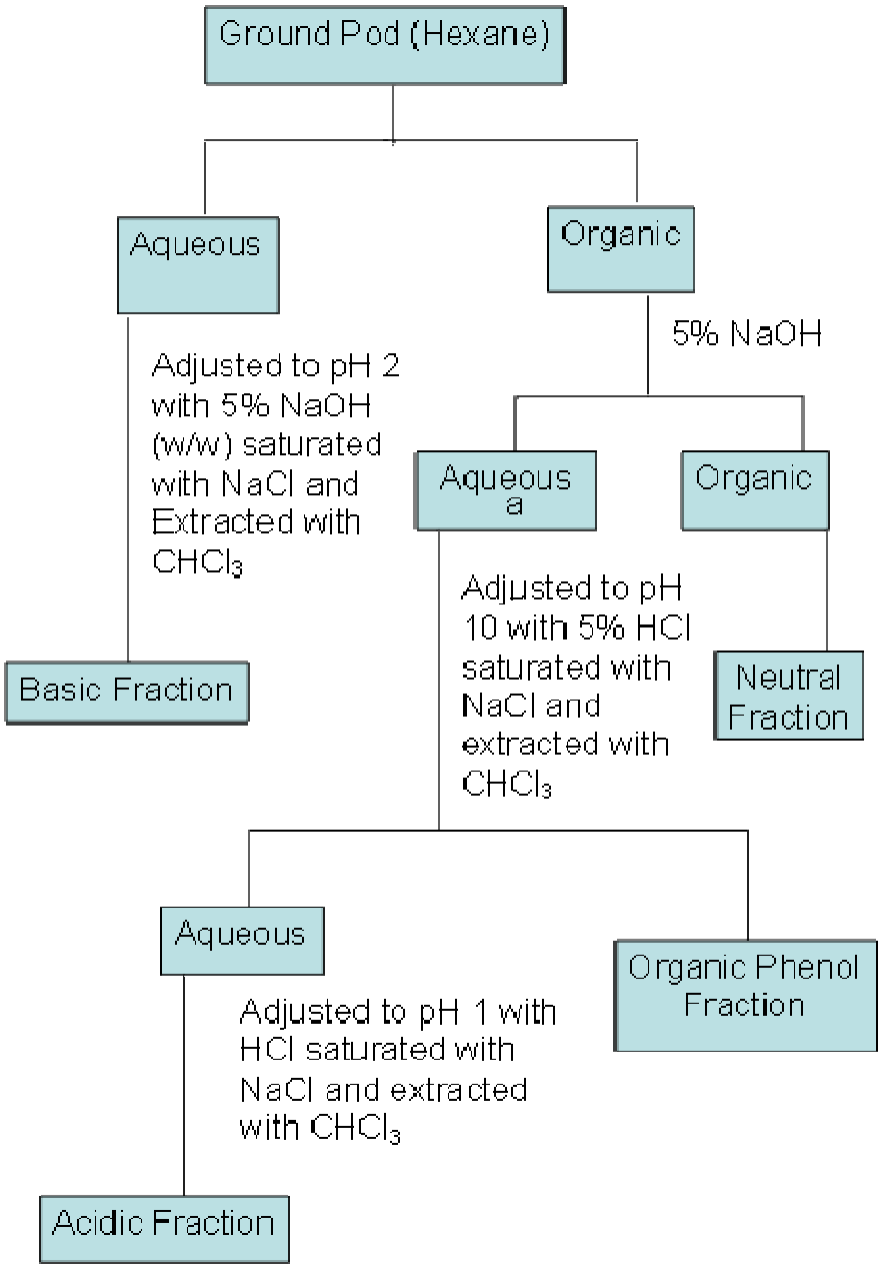

Figure 1. Extraction procedure for the various n-hexane fractions.

of purification and identification of the bioactive compounds. This paper reports on the effects of these fractions on the survival, growth, development and fecundity of *M. vitrata*.

MATERIALS AND METHODS

Preparation of extracts

About 100g of 8 – 10 days old (green) pods of *V. vexillata* (acc. TVnu 72), *V. oblongifolia* (acc. TVnu 42) and *V. unguiculata* (acc. IT84S-2246 – a susceptible cowpea cultivar) were freeze dried, blended and extracted in 150 ml n-hexane for 72 h in a cool, dark cupboard. The solvent was evaporated with a rotary evaporator to obtain the 100% crude extract. Acidic, basic, neutral and phenolic fractions were obtained using the procedure shown in Figure 1.

Feeding bioassay

A cowpea + wheatgerm artificial diet (Jackai and Raulston, 1988) was used for the bioassay. Each fraction was incorporated into the artificial diet contained in separate 50 ml plastic cups at a rate of 2.0% (w/w) using a cellulose carrier. They were dispensed into wells in plastic containers and infested with newly emerged *M. vitrata* larvae at a rate of three larvae per well. The control diets consisted of (i) artificial diet + cellulose, (ii) artificial diet +chloroform

Table 1. Survival, weight, pupation, growth index, adult emergence and fecundity of *M. vitrata* reared on artificial diet incorporated with acidic fraction of TVnu 42 pod extract.

Treatments	Larval survival (%)*	Larval weight (mg)*	Percentage Pupation	Pupal Weight (mg)	Growth index	Adult Emergence (%)	Eggs laid per female
TVnu 42	77.5 ± 1.56b	58.0 ± 1.25a	70.4 ± 2.61b	47.9 ± 0.34a	0.23 ± 0.002c	62.9 ± 2.74b	314.2 ± 6.33a
Diet + Chloroform	95.0 ± 0.61a	60.0 ± 0.82a	93.3 ± 1.41a	60.9 ± 4.20a	0.29 ± 0.002a	90.0 ± 1.61a	329.3 ± 5.62a
Diet + Cellulose	98.3 ± 0.37a	62.9 ± 0.53a	93.3 ± 1.41a	40.6 ± 0.39a	0.25 ± 0.003b	86.7 ± 1.72a	329.3 ± 8.85a
Diet alone	85.8 ± 0.91ab	77.0 ± 1.37a	88.9 ± 1.61a	47.8 ± 0.34a	0.27 ± 0.002ab	83.3 ± 1.76a	328.5 ± 5.83a

Means followed by the same letter(s) in columns are not significantly different (P = 0.05; SNK).
*Record taken 10 DAI.

Table 2. Survival, weight, pupation, growth index, adult emergence and fecundity of *M. vitrata* reared on artificial diet incorporated with basic fraction of TVnu 72 pod extract.

Treatments	Larval survival (%)*	Larval weight (mg)*	Percentage Pupation	Pupal Weight (mg)	Growth index	Adult Emergence (%)	Eggs laid per female
TVnu 72	81.7 ± 3.37a	32.4 ± 2.66b	75.0 ± 3.26a	46.7 ± 0.59a	0.18 ± 0.006b	68.3 ± 3.37a	285.9 ± 10.78a
Diet + Chloroform	90.0 ± 1.61a	62.7 ± 1.31a	93.3 ± 1.41a	44.8 ± 0.86a	0.29 ± 0.002a	90.0 ± 1.61a	329.3 ± 5.62a
Diet + Cellulose	96.7 ± 1.05a	62.7 ± 1.09a	93.3 ± 1.41a	46.2 ± 0.65a	0.25 ± 0.003a	86.7 ± 1.72a	329.7 ± 8.85a
Diet alone	85.0 ± 2.00a	77.3 ± 3.06a	90.0 ± 1.61a	48.3 ± 0.65a	0.27 ± 0.002a	83.4 ± 1.76a	328.5 ± 5.83a

Means followed by the same letter(s) in columns are not significantly different (P = 0.05; SNK).
*Record taken 10 DAI.

Table 3. Survival, weight, pupation, growth index, adult emergence and fecundity of *M. vitrata* reared on artificial diet incorporated with phenol fraction of TVnu 72, TVnu 42 and IT84S-2246 pod extract.

Treatments	Larval survival (%)*	Larval weight (mg)*	Percentage Pupation	Pupal Weight (mg)	Growth index	Adult emergence (%)	Eggs laid per female
TVnu 72	65.0 ± 3.47a	68.1 ± 1.55a	58.3 ± 2.97b	49.6 ± 1.35a	0.23 ± 0.008 a	51.7 ± 2.66b	338.3 ± 8.24a
TVnu 42	76.7 ± 3.44a	61.5 ± 1.20a	70.0 ± 3.22ab	45.1 ± 1.18a	0.25 ± 0.009a	66.7 ± 3.04ab	344.6 ± 5.38a
IT84S-2246	81.7 ± 2.00a	70.6 ± 1.49a	78.3 ± 1.93ab	54.6 ± 0.74a	0.28 ± 0.002a	75.0 ± 1.80a	349.8 ± 4.70a
Diet + Chloroform	90.0 ± 1.61a	62.7 ± 1.31a	93.3 ± 1.41a	44.8 ± 0.86a	.29 ± 0.002a	90.0 ± 1.61a	329.3 ± 5.62a
Diet + Cellulose	96.7 ± 1.05a	62.7 ± 1.09a	93.3 ± 1.41a	46.2 ± 0.64a	.25 ± 0.003a	86.7 ± 1.72a	329.7 ± 8.85a
Diet alone	85.0 ± 2.00a	77.3 ± 3.06a	90.0 ± 1.61a	48.3 ± 0.65a	.27 ± 0.002a	83.3 ± 1.76a	328.5 ± 5.83a

Means followed by the same letter(s) in columns are not significantly different (P = 0.05; SNK).
*Record taken 10 DAI.

Table 4. Survival, weight, pupation, growth index, adult emergence and fecundity of *M. vitrata* reared on artificial diet incorporated with neutral fraction of TVnu 72, TVnu 42 and IT84S-2246 pod.

Treatments	Larval survival (%)*	Larval weight (mg)*	Percentage Pupation	Pupal Weight (mg)	Growth index	Adult Emergence (%)	Eggs laid per female
TVnu 72	0.0 ± 0.00c	-	-	-	-	-	-
TVnu 42	60.0 ± 3.78b	48.9 ± 0.83a	60.0 ± 3.78b	48.9 ± 0.83a	0.25 ± 0.002b	50.0 ± 3.24b	216.4 ± 11.19b
IT84S-2246	45.0 ± 1.58b	29.8 ± 1.12b	45.0 ± 1.58b	29.8 ± 1.12b	0.21 ± 0.003c	20.0 ± 1.72c	148.8 ± 5.61b
Diet + Chloroform	93.3 ± 1.41a	44.8 ± 0.86a	93.3 ± 1.41a	44.8 ± 0.86a	.29 ± 0.002a	90.0 ± 1.61a	329.3 ± 5.62a
Diet + Cellulose	93.3 ± 1.41a	46.2 ± 0.65a	93.3 ± 1.41a	46.2 ± 0.65a	.25 ± 0.003b	86.7 ± 1.72a	329.7 ± 8.85a
Diet alone	90.0 ± 1.61a	48.3 ± 0.65a	90.0 ± 1.61a	48.3 ± 0.65a	.27 ± 0.002a	83.3 ± 1.76a	328.5 ± 5.83a

Means followed by the same letter(s) in columns are not significantly different (P = 0.05; SNK).
*Record taken 10 DAI.

and (iii) artificial diet alone. The wells were covered with a thin transparent nylon sheet using a warm electric iron. Holes were made on the sheet with an entomological pin to allow for ventilation. The experiment was replicated four times and arranged in a completely randomized design in trays. The trays were kept in the laboratory at ambient temperature (27 ± 2^{O}C) and relative humidity (65 – 85%). Larval survival and weight were taken 10 days after infestation. At pupation, pupal number and weight were recorded and pupae were transferred into plastic arena (9.0 cm diameter x 4.5 cm dept) lined with moist filter paper towel and covered with a ventilated lid. Percentage adult emergence, adult weight and fecundity were recorded. The data were analysed using analysis of variance (ANOVA). Means were separated with Student-Newman-Keuls Test (SNK) where the F-value was significant.

RESULTS AND DISCUSSION

The survival, feeding, weight of *M. vitrata* larvae and pupae reared on artificial diet incorporated with acid fraction of TVnu 42 extract as well as the adult emergence and fecundity are shown in Table 1. Acid fractions of TVnu 72 and IT84S-2246 extracts were too small to incorporate into the artificial diet. Table 2 shows similar results for the larvae reared on the diet incorporated with basic fraction of TVnu 72 extract. Basic fractions of TVnu 42 and IT84S-2246 were too small to make any meaningful replication. There was no difference between the treatments in the parameters assessed. The phenolic fractions of TVnu 72, TVnu 42 and IT84S-2246 pod extracts had no significant effect on the survival and weight of *M. vitrata* larvae, but this fraction of TVnu 72 extract had a significantly ($P<0.05$) lower percentage pupation and adult emergence compared with other treatments (Table 3). However, there was no difference in pupal weight, percentage adult emergence and fecundity between these treatments. The neutral fraction of TVnu 72 extract significantly ($P< 0.05$) reduced larval survival compared with the controls while larval weight was reduced by those of TVnu 72 and IT84S-2246 (Table 4). There was no pupation on diets containing TVnu 72 neutral fraction while pupation on diets containing those of TVnu 42 and IT84S-2246 was significantly ($P < 0.05$) lower than in the control diets.

Since over 60% of adults emerged from treatments with TVnu 42 acid and TVnu 72 basic fractions, these fractions do not appear to contain biochemical factors important in the resistance of these wild *Vigna* species to *M. vitrata*. Similarly, phenolic compounds in the green pods of the *Vigna* species are not important in their antibiosis against the insect. This confirms the report of Oghiakhe et al. (1993) who could not find any significant correlation between the phenolic concentration in pods of wild and cultivated *Vigna* species and damage by *M. vitrata*. However, phenol is known to play important defensive roles against some other cowpea pests (Baker et al., 1989), but this does not seem to apply to *M. vitrata*. That the neutral fractions of TVnu 72, TVnu 42 and IT84S-2246 showed strong antibiosis against *M. vitrata* larvae suggests that biochemical factors present in these fractions are important in *Vigna* resistance to the insect. Since all larvae died before pupation on diet treated with TVnu 72 neutral fraction, this fraction contains the most toxic compounds to *M. vitrata*. The factors responsible for antibiosis in IT84S-2246 neutral fraction are more potent than those of TVnu 42. Cowpea accession IT84S-2246 is known to be resistant to some insect pests such as aphids and bruchids (Singh et al., 1990). It will be necessary to purify and identify the compound(s) responsible for the antibiosis in these neutral fractions so that the gene coding for their biosynthesis could be identified, cloned and introduced into cultivated *Vigna*. This will go a long way in reducing cowpea damage in the field by *M. vitrata*.

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