Oviposition Deterrent Activities of *Pachyrhizus erosus* Seed Extract and Other Natural Products on *Plutella xylostella* (Lepidoptera: Plutellidae)

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**ABSTRACT.** An extract of a rotenone-containing plant yam bean, *Pachyrhizus erosus* (L.) Urban, seeds was tested against the diamondback moth, *Plutella xylostella* (L.) in a greenhouse to determine its potential as an oviposition deterrent and compared with coumarin and rutin, known as diamondback moth oviposition deterrent compounds, rotenone, and an extract of Peruvian cube root, at a concentration of 0.5% (w/v). Oviposition deterrent index (ODI) was used to determine effects of extracts or compounds in inhibiting oviposition of diamondback moth. Coumarin showed a stronger deterrent effect than the yam bean seed extract with a higher ODI value. On the contrary, rotenone, rutin, and the cube root extract, containing 6.7% (w/w) of rotenone, showed no significant deterrent effects having low or negative ODI values, suggesting that the deterrent effect of the yam bean seed extract is not due to rotenone content of the yam bean seeds. The extract of yam bean seed and coumarin partially deterred the moth from laying eggs on treated leaves in a concentration-dependent manner. The effective concentration for 50% deterrence of coumarin and the yam bean seed extract were 0.11 and 0.83% (w/v), respectively. However, the yam bean seed extract showed a residual deterrent effect on the moth even at 3 d after the treatment and is probably because of its low volatile nature. A long-term deterrence of the yam bean seed extract is an advantage over coumarins. Both the yam bean seed extract and coumarin deterred diamondback moth from laying eggs in total darkness, indicating their nonvisual deterrent effect. This made the extract an effective deterrent to diamondback moth in light and in darkness. To conclude, this study revealed the potential of the crude extract of the yam bean seed to prevent diamondback moth from ovipositing on its plant host.

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), has long been recognized as a cosmopolitan pest of cruciferous crops. The caterpillars are considered to be the most important factors limiting the successful production of cruciferous vegetables in South-East Asia (Finch and Thompson 1992) by greatly reducing both factors limiting the successful production of cruciferous crops. The caterpillars are considered to be the most important insect pests (Hansberry and Lee 1943, Lee and Hansberry 1945) of crucifers. The caterpillars are considered to be the most important insect pests (Hansberry and Lee 1943, Lee and Hansberry 1945). The yam bean, *Pachyrhizus erosus* (L.) Urban (Fabales: Fabaceae), is a leguminous shrub which is indigenous to Latin America (Broadbent and Shone 1963), but has a widespread distribution in tropical countries grown for its edible tubers (Chiu 1950, Broadbent and Shone 1963, Crosby 1971, Allen and Allen 1981), with notable success in Southeast Asia (Sorensen 1996). The yam bean plant has been reported as one among several plants that possesses broad spectrum pest control properties (Grainge and Ahmed 1988). The seeds of this plant have long been used as insecticides and fish poisons in various tropical countries (Hansberry and Lee 1943). Toxicity studies of yam bean seeds showed that it has a great value as botanical insecticides to some important insect pests (Hansberry and Lee 1943, Lee and Hansberry 1943, Plank 1944, Norton and Hansberry 1945, Hansberry et al. 1947, Chiu 1950, Tangchiphinithikan et al. 2007, Pangnakorn et al. 2011, Yongkhamcha and Indrapichate 2012). The mature seeds contain approximately 0.5% rotenone and 0.5% rotenoids and saponins.
The possibilities of utilizing the rotenone content of the seeds as a pesticide appear attractive (Sørensen 1996), especially as a low-cost insecticide with no residual effects for low-input, sustainable farming systems (Sørensen et al. 1994).

Rotenone is a crystalline substance produced mainly by extracting the roots and stems of the genera Lonchocarpus and Derris. Rotenoid compounds have previously been evaluated largely as insect toxicants and the doses used have been those required to cause high levels of mortality on target pests (Hassanali and Lwande 1989). It was found later that rotenone and other rotenoid compounds, and extracts of rotenoid-bearing plants were potent insect antifeedants and growth regulators (Bentley et al. 1987, Chiu 1989, Nawrot et al. 1989). Recent findings showed that oviposition by Monochamus alternatus and Callosobruchus maculatus was deterred by rotenone (Li et al. 2005, Belmain et al. 2012).

It is clear that some other potencies of rotenoid-bearing plants need to be studied in order to fully explore their biological activities. Hassanali and Lwande (1989) stated that it is necessary to re-evaluate the rotenoids as anti-insect natural products. Renewed effort in studying rotenoid bearing plants is clearly needed (Chiu 1989). Rotenoid-based insecticides may provide a basis for a new promising way of environmentally sound pest control within the framework of integrated pest management.

Natural organic compounds are probably the best sources of insect oviposition deterrents (Schoonhoven 1982). Testing extracts of unrelated, nonhost plants as deterrents against a particular insect, however, are the usual approach to find materials that are capable of deterring insects from laying eggs (Renwick 1988).

The objective of this study was to explore the potential of an extract of yam bean, P. erosus (L.) Urban, seed as an oviposition deterrent against adult diamondback moth, with a view to isolate subsequently the anti-oviposition compound(s) from the extract. There is a need for novel oviposition (and feeding) deterrents in insect pest management particularly with improved persistence and leaf surface compatibility and most likely of moderate polarity. An additional feature of any putative deterrent would be limited habituation to the effect under high pest pressure or repeated exposure. Thus, comparisons with known diamondback moth oviposition deterrent compounds, coumarin and rutin (Tabashnik 1985), and with pure rotenone and another rotenone-containing plant (Peruvian cube root or Lonchocarpus sp.), were also carried out in order to show the relative effectiveness of the yam bean seed extract. Rotenone has been found to deter the oviposition of M. alternatus Hope (Li et al. 2005) and C. maculatus (Belmain et al. 2012).

The diamondback moth was used in this study because the insect is considered the most destructive insect pest of cruciferous plants and is one of the world’s major insect pests (Talekar and Shelton 1993, Zalucki et al. 2012). A conservative estimate showed that total costs associated with diamondback moth management is US$4–5 billion (Zalucki et al. 2012).

Materials and Methods

**Extraction Procedure.** The yam bean seeds, collected in Bogor, West Java, Indonesia, were sun dried and ground in a coffee mill and this produced greenish-yellow, finely ground material for the extraction. The extraction was made by placing 25 g of the ground material in a single thickness Whatman cellulose extraction thimble (30 by 100 mm). The thimble was soxhlet-extracted with chloroform for 8–10 h at 75–80°C using a water bath. The extract was evaporated on a rotary evaporator at 40°C and further dried using a stream of oxygen-free nitrogen. The residue was the yam bean seed extract (6.34 g).

A similar extraction procedure was employed for the ground Peruvian cube root by placing 10 g of the material in the extraction thimble to produce 2.42 g of the cube root extract. The ground material, containing 6.7% (w/w) of rotenone, was supplied by Ford Smith & Co. Ltd., London, United Kingdom.

**Bioassay Procedure.** A cypermethrin susceptible strain of diamondback moth was obtained from The School of Biology, University of Birmingham, United Kingdom. This strain was collected by Paul Todd and Dr. Ray Thorhill in Thailand in 1982 and has been cultured in the University of Birmingham since then. For the experiment, the insects were reared at the greenhouse located at the Department of Agricultural and Environmental Science, Newcastle University, United Kingdom. The temperature was maintained at 20–25°C with a relative humidity of 50–60%.

The diamondback moth was reared in a nylon mesh cage (60 by 60 by 60 cm) located in the greenhouse. They were supplied with 12-wk-old potted Chinese cabbage (Brassica chinensis) plants for feeding the larvae and laying eggs. Plants bearing eggs were removed to other similar sized cages for rearing the larvae. New potted plants were introduced at least once a week to feed the larvae.

About 100–150 adult male and female insects (1–3-d-old) were introduced into a screen cage (45 by 30 by 30 cm). They were fed with 10% (w/v) honey solution provided on cotton wool in Petri dishes. Excised Chinese cabbage leaves from 12-wk-old plants, as oviposition substrates, were cut to give the laminae a standard circle of 7 cm in diameter. Their petioles were left intact and placed vertically into 250 ml Erlenmeyer flasks filled with water, so the laminae were exposed to diamondback moth adults. Each Erlenmeyer flask was considered as one experimental unit and each leaf represented one replicate. The laminae were previously dipped (for 10 s) into either a control or treated solutions and air dried under greenhouse conditions for about 1 h. The laminae were then exposed to the insects for 24 h. The formulation (i.e., the preparation of the test materials) contained 0.1% (w/v) of Triton X-114 and 10% (v/v) of methanol (for rutin), or 50% (v/v) of acetone (for rotenone), or 10% (v/v) of acetone (for coumarin and extracts of yam bean and cube) in water. The same preparation, without test materials added, was used as their controls. Triton X-114, rutin (quercetin 3–α–rutinoside trihydrate, ~95%), and coumarin (1,2-benzopyrone) were purchased from Sigma Chemicals Company. Rotenone, 97% pure, was obtained from Aldrich Chemical Company Ltd., Dorset, United Kingdom.

Two choice tests were used throughout the study. Both treated and control leaves were inserted in an Erlenmeyer flask and arranged in completely randomized designs. The numbers of eggs laid on each leaf were counted after exposing the leaves to the insects for 24 h.

**Tests of Plant Extracts and Natural Products.** To determine the potential of plant extracts (yam bean seed and cube root extracts) and natural products (coumarin, rotenone, and rutin) as oviposition deterrents, the materials were tested separately at a concentration of 0.5% (w/v). There were nine replicates for each experiment. The choice of concentration in this experiment was mainly based on the solubility of test materials. It was found previously that above 0.5% (w/v) the formulation containing rotenone showed undissolved material in the solution, so it was decided to choose 0.5% (w/v) in the experiment. Test materials that showed deterrence against adult moth oviposition were further tested.

**Response to Concentrations.** This experiment measured the effectiveness of test materials that showed deterrence at various concentrations. There were nine replicates for each concentration.

**Residual Effects.** In order to determine the residual activity of the materials that showed deterrence adult moths were exposed to the treated leaves at various days after treatment (DAT) ranging from 0 to 3 d. There were six replicates per treatment.

**Photoperiod Effects.** To determine the effectiveness of the deterrents according to photoperiod the materials that showed deterrence were also assayed in total darkness. Theoretically, a visual deterrence is only effective in the presence of appropriate light, whereas a nonvisual deterrence is effective in the presence of light and in darkness. There were nine replicates per treatment.

**Statistical Analyses.** The number of eggs laid on treated and control leaves was analyzed by t-tests for paired comparisons. Data were log-
transformed before analysis to meet the assumptions of parametric statistical tests. Oviposition deterrent indices (ODI), following Huang et al. (1995), were also calculated as follows:

$$ODI = \frac{100(C - T)}{C + T},$$

where $C$ and $T$ are the mean number of eggs laid on control and treated leaves, respectively. Data were not transformed for the calculation of the index. The effective concentration for 50% oviposition deterrent ($EC_{50}$) of test materials that showed deterrence was determined by Probit analysis (Finney 1971).

**Results**

**Tests of Plant Extracts and Natural Compounds.** In dual choice tests between treated and control leaves, it was shown that only the yam bean seed extract and coumarin significantly ($t = 5.00$, $df = 8$, $P = 0.001$, and $t = 14.53$, $df = 8$, $P < 0.001$, respectively) deterred the oviposition of the diamondback moth adults. The deterrent effect of coumarin was stronger (ODI = 78.4) than that of the yam bean seed extract (ODI = 29.0) at a concentration of 0.5% (w/v). In contrast, leaves treated with rotenone, rutin, and the cube root extract at a concentration of 0.5% (w/v) showed no deterrent effect on the moth ($t = 1.35$, $df = 8$, $P = 0.215$; $t = 0.07$, $df = 8$, $P = 0.949$; and $t = 2.22$, $df = 8$, $P = 0.057$, respectively). No deterrent effect of the test materials was indicated by their low or negative ODI values (Table 1).

**Concentrations Responses.** This study showed that the number of eggs laid by diamondback moth on treated and control leaves did not differ significantly at the lowest concentrations of the yam bean seed extract (0.1% w/v) and coumarin at a concentration of 0.025% (w/v) with $P$ values of 0.729 ($t = 0.36$, $df = 8$) and 0.454 ($t = 0.79$, $df = 8$), respectively. The ODI values for both materials were 3.0. At higher concentrations, however, significantly fewer eggs were laid by diamondback moth adults on leaves treated with the yam bean seed extract and coumarin, and the effects were concentration dependent. The ODI values by concentration are presented in Table 2, which generally show increasing activity as the concentration of the yam bean seed extract and coumarin increases. Neither the yam bean seed extract nor coumarin completely deterred oviposition by diamondback moth adults at any concentration used in the experiment. The highest ODI of the yam bean seed extract and coumarin in this study were 55.0 and 78.4 at a concentration of 2 and 0.5% (w/v), respectively. Leaves treated with coumarin at a concentration of 0.25 and 0.5% (w/v) showed phytotoxic symptoms, i.e., withered and slightly dry at the end of the experiments. The effective concentration for $EC_{50}$ of the yam bean seed extract and coumarin were 0.83 and 0.11% (w/v), respectively.

**Residual Effects.** The deterrent effects of the yam bean seed extract and coumarin on the oviposition of diamondback moth adults showed that ODI values of the yam bean seed extract were relatively unchanged at zeroth to third day, while that of coumarin declined with time, shown by a decrease of ODI value from 41.0 at zeroth day to 13.3 on the third day. In all experiments with the yam bean seed extract, significant differences (first day, $t = 6.02$, $df = 5$, $P = 0.002$; second day, $t = 5.26$, $df = 5$, $P = 0.003$; third day, $t = 2.83$, $df = 5$, $P = 0.037$) were evident in the number of eggs on treated and control leaves. Significant differences (first day, $t = 2.68$, $df = 5$, $P = 0.044$; second day, $t = 4.97$, $df = 5$, $P = 0.004$; third day, $t = 3.39$, $df = 5$, $P = 0.020$) were also found in the number of eggs on treated and control leaves in all experiments with coumarin (Table 3). The ODI values of leaves treated with the yam bean seed extract were relatively unchanged at zeroth to third DAT. Meanwhile, coumarin-treated leaves resulted in varied ODI

| Table 1. Oviposition by *P. xylostella* on cabbage leaves treated with plant extracts and natural plant products at a concentration of 0.5% (w/v) |
|----------------------------------|
| Plant extracts and natural products | Mean number of eggs laid (± SE) | ODI⁴ |
| Treated | Control | $P$ values⁵ |
|---|---|---|
| Yam bean seed extract | 60 ± 5 | 108 ± 10 | 0.001 | 29.0 |
| Cube root extract | 162 ± 10 | 144 ± 14 | 0.057 | -6.0 |
| Coumarin | 39 ± 5 | 322 ± 21 | <0.001 | 78.4 |
| Rotenone | 131 ± 6 | 118 ± 9 | 0.215 | -5.2 |
| Rutin | 163 ± 14 | 167 ± 22 | 0.949 | 1.2 |

⁴Average of nine replicates. SE = standard error. ⁵Statistical tests refer to t-test for pair comparison between treated and control leaves. ⁶ODI = oviposition deterrent index.

| Table 2. Oviposition by *P. xylostella* on cabbage leaves treated with the yam bean seed extract and coumarin according to concentration |
|----------------------------------|
| Concentration (% w/v) | Mean number of eggs laid (± SE) | ODI⁴ |
| Treated | Control | $P$ values⁵ |
|---|---|---|
| Yam bean seed extract | | |
| 0.1 | 189 ± 18 | 199 ± 19 | 0.729 | 3.0 |
| 0.25 | 35 ± 5 | 54 ± 5 | 0.026 | 21.3 |
| 0.5 | 60 ± 5 | 108 ± 10 | 0.001 | 29.0 |
| 1.0 | 50 ± 6 | 89 ± 10 | 0.014 | 28.0 |
| 2.0 | 24 ± 5 | 82 ± 11 | <0.001 | 55.0 |
| Coumarin | | |
| 0.025 | 128 ± 12 | 136 ± 9 | 0.454 | 3.0 |
| 0.05 | 85 ± 7 | 126 ± 6 | <0.001 | 19.4 |
| 0.1 | 121 ± 10 | 289 ± 23 | <0.001 | 41.0 |
| 0.25 | 87 ± 12 | 347 ± 26 | <0.001 | 60.0 |
| 0.50 | 39 ± 5 | 322 ± 21 | <0.001 | 78.4 |

⁴Average of nine replicates. SE = standard error. ⁵Statistical tests refer to t-test for pair comparison between treated and control leaves. ⁶ODI = oviposition deterrent index.

| Table 3. Oviposition by *P. xylostella* on cabbage leaves treated with the yam bean seed extract (1% w/v) and coumarin (0.1% w/v) at DAT |
|----------------------------------|
| DAT⁶ | Mean number of eggs laid (± SE) | ODI⁴ |
| Treated | Control | $P$ values⁵ |
|---|---|---|
| Yam bean seed extract | | |
| 0 | 50 ± 6 | 89 ± 10 | 0.014 | 28.0 |
| 1 | 77 ± 8 | 126 ± 9 | 0.002 | 24.1 |
| 2 | 29 ± 8 | 63 ± 9 | 0.003 | 37.0 |
| 3 | 59 ± 7 | 111 ± 13 | 0.037 | 31.0 |
| Coumarin | | |
| 0 | 121 ± 10 | 289 ± 23 | <0.001 | 41.0 |
| 1 | 246 ± 19 | 294 ± 17 | 0.044 | 9.0 |
| 2 | 118 ± 10 | 200 ± 16 | 0.004 | 26.0 |
| 3 | 159 ± 18 | 208 ± 4 | 0.020 | 13.3 |

⁶DAT = days after treatment. ⁷Average of nine replicates. SE = standard error; Statistical tests refer to t-test for pair comparison between treated and control leaves. ⁸ODI = oviposition deterrent index.
values that tended to decrease with increasing time (zeroth to third day) after treatment.

**Photoperiod Effects.** Both the yam bean seed extract and coumarin caused a significant reduction ($t = 9.52$, $df = 8$, $P < 0.001$, and $t = 6.12$, $df = 8$, $P < 0.001$, respectively) on the oviposition of diamondback moth adults in the absence of light. The ODI values of the yam bean seed extract increased from 28.0 to 42.0 and that for coumarin decreased from 41.0 to 27.0 when the photoperiods changed from 16:8 to 0:24 (L:D) h regimes (Table 4).

### Discussion

In this study, diamondback moth adults were inhibited to oviposit on cabbage leaves treated with the yam bean seed extract and coumarin. Other natural compounds, i.e., rotenone and rutin, and the cube root extract showed no deterrent effect on the oviposition of diamondback moth. The absence of deterrent effect of rutin was rather expected since this compound had been shown by Tabashnik (1985) to deter strongly diamondback moth adult oviposition only at a high concentration (0.1 M, equal to 6.3% w/v) and weakly at 0.01 M (0.63% w/v). The change of leaf color due to rutin was believed to be responsible for the visual deterrent effect of rutin to diamondback moth (Tabashnik 1985).

However, in this study, rutin did not change the color appearance of Chinese cabbage leaves, possibly because of the low concentration of rutin (0.5% w/v) used in the experiment. It was found that rutin and rotenone crystallized on leaf surfaces after the treated leaves were dry. The result reported here is in agreement with Jermy and Szentesi (1978), who also found that rutin did not deter the oviposition of adult *Pieris brassicae*.

The lack of deterrent effects of pure rotenone and the cube root extract containing 6.7% (w/v) of rotenone on the oviposition of diamondback moth adults suggesting that the deterrent effect of the yam bean seed extract is probably not due to rotenone content of the seeds (0.5%; Sorensen 1996). However, rotenone deterred the oviposition of Coleopteran *M. alternates* and *C. maculatus* (Li et al. 2005, Belmain et al. 2012). It is probable that a compound or compounds other than rotenone are responsible for the deterrent effects of the yam bean seed extract on the oviposition of the diamondback moth adults. The majority of compounds found in the yam bean seeds belong to the isoflavonoid group, i.e., 3-arylcoumarin or phenylfuranocoumarin, coumestan, isoflavone, isoflavanone, and rotenoids. Compounds other than isoflavonoids were also found in the yam bean seeds such as pachyrrhizid, saponins, and a dulcitol (Phrutivorapongkul et al. 2002, Barrera-Necha et al. 2004).

This study found that leaves treated with coumarin at a concentration of 0.25 and 0.5% (w/v) were withered and slightly dry after the 24-h experiment. The phytotoxicity of coumarin has been reported by many researchers (Razavi 2011), e.g., through total inhibition of seed germination, growth of roots and hypocotyls in *Lactuca sativa* and in *Lepidium sativum* at a low concentration of 0.01% (Wink and Twardowsky 1992). The phytotoxic effects on the coumarin-treated leaves might contribute to its high deterrent effects on the oviposition of diamondback moth adults. Nevertheless, the study showed that the deterrent effect of coumarin was stronger than the yam bean seed extract by inhibiting the oviposition of diamondback moth adults at lower concentrations. Their EC50 values showed that oviposition deterrent of coumarin was almost eightfold greater than by the yam bean seed extract.

The yam bean seed extract gave residual activity at three DAT, probably because of its low volatility. In contrast, the effect of the volatile compound coumarin (Tabashnik 1985) varied and decreased with increasing time (zeroth to third day) after treatment (Table 2). This fact could explain why most efforts to use oviposition deterrents as protective agents involve nonvolatile plant constituents (Renwick 1988). This result showed an advantage of using the yam bean seed extract as an oviposition deterrent against insect pests over volatile compounds, although this could be offset through controlled release formulation.

The ability of the yam bean seed extract to deter oviposition of diamondback moth adults in complete darkness indicates that the deterrent effect of the crude yam bean extract was likely mediated, partly or entirely, by nonvisual cues. The increase of its ODI in the absence of light supports the above suggestion. Previous studies showed that visual and nonvisual cues affected diamondback moth oviposition (Gupta and Thorsteinson 1960, Tabashnik 1985, Spencer et al. 1999, Badenes-Peres et al. 2004). Tabashnik (1985) suggested that when visual cues are not available, nonvisual cues become more influential in deterring oviposition of diamondback moth. The low volatility of the yam bean seed extract suggests that its nonvisual deterrence was because of contact cues rather than olfactory cues. Contact-chemoreception has already been known to be one among other sensory modalities involved in mediating diamondback moth oviposition (Gupta and Thorsteinson 1960, Tabashnik 1985) and was reported to be the major and most common sensory modality involved in moth oviposition (Ramaswamy 1988). The term contact-chemoreception refers to a definition given by Stadler and Hanson (1975), which includes taste or gustation and close range olfaction.

In case of the volatile compound coumarin, the experiment was unable to show that changing the photoperiod from 16:8 (L:D) h regimes to the total darkness increased the nonvisual (olfactory and contact) deterrence of coumarin. In contrast, Tabashnik (1985) demonstrated that coumarin was a more potent deterrent on diamondback moth oviposition in darkness than in light due to its little effect on the visual appearance of the plant. The low persistent effect of the volatile nature of coumarin, as shown by its wide range of ODI (Table 4), may be the reason for these variable results. As a volatile compound, the deterrent effect of coumarin is affected by various environmental factors, such as slight changes of temperature and relative humidity in the glass house.

It is concluded that the yam bean seed extract and coumarin partially deterred the diamondback moth adults from laying eggs on treated leaves in a concentration-dependent manner. The oviposition deterrent effect of the yam bean seed extract was not because of its rotenone content. Below its phytotoxic concentrations, coumarin showed a stronger oviposition deterrent effect than the yam bean seed extract. However, the deterrent effect of the yam bean seed extract was more stable than coumarin over a period of 72 h. Both the yam bean seed extract and coumarin deterred diamondback moth adults from laying eggs in total darkness, indicating their nonvisual deterrent effect. The stability of the yam bean seed extract and its ability to deter adult moth oviposition in total darkness seem promising to prevent high infestation by a nocturnal insect such as the diamondback moth, at least in the first 3 d of the oviposition period of the insect. This study was able to show the potential of using the crude extract of the yam bean seed to prevent diamondback moth adults from ovipositing its plant host. This finding should lead to a further study in an attempt to isolate and to test a compound or compounds that deterred the oviposition of the diamondback moth.
from the yam bean seed. The purified active compounds may very well enable higher activity on the lead surface and give efficacious oviposition deterrence. Identification of the active compound may yield a new route to oviposition deterrence and provide a new insight into biologically activities of rotenoid-containing plants.

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