# Toxicity and Spraying Schedules of a Biopesticide Prepared from *Piper guineense* against Two Cowpea Pests

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**Abstract**

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The toxicity of three concentrations (5%, 10% and 20% w/v) and spraying schedules (2, 4 and 6 weekly applications) of an extract from West African black pepper, *Piper guineense*, for managing two major post-flowering pests of cowpea, *Vigna unguiculata,* was investigated in two cropping seasons at the Research Farm of the Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria. The insect pests were the larvae of *Maruca vitrata* (Lepidoptera: Pyralidae) and the cowpea coreid bug, *Clavigralla tomentosicollis* (Hemiptera: Coreidae). The higher concentrations (10% and 20% w/v) and more frequent applications (4 and 6/week) significantly ($P < 0.05$) reduced the numbers of the two insect pests compared to the untreated control in both years. Pod damage was significantly reduced and grain yields consequently increased in treated plots compared with the other extract treatments and the untreated control. West African black pepper extract applied at higher concentrations and more frequently could play an important role in integrated management of pests' infestations on field cowpea managed by limited resource farmers in third world countries.

**Keywords**: toxicity; *Piper guineense* extract; *Maruca vitrata*; *Clavigralla tomentosicollis;* spray schedules; cowpea

Infestation by insect pests on cowpea, *Vigna unguiculata* (L.) Walp., particularly at the post-flowering phase of plant growth, remains the major hindrance to its production (Jackai *et al*. 1992). The three main pests of cowpea in Nigeria include the flower bud thrips *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae), the legume pod borer *Maruca vitrata* Fab. (Lepidoptera: Pyralidae) and the pod sucking bug complex mainly pre-dominated by *Clavigralla tomentosicollis* Stal. (Hemiptera: Coreidae). The larvae of *M. vitrata* feed on flowers causing flower abortion. *Maruca* larvae also and *C. tomentosicollis* (adults and nymphs) feed on immature pods causing premature drying and abscission of pods and seed abortion (Jackai *et al*. 1992) resulting in considerable losses in grain yield and quality. Damage may amount to 80–100% where modern control measures are absent. Currently, these measures rely heavily on the use of synthetic insecticides (Jackai & Oyediran 1991). However, the indiscriminate use of these chemicals has given rise to a number of problems including genetic resistance of pest species (Champ & Dyke 1976; Georghiou & Lagunes-Tejeda 1991), accumulation of toxic residues in treated grains (Snelson 1987), health hazards (to warm-blooded animals), environmental contamination, increased costs of application which causes a serious drain on the fragile economy of third world countries. These problems have necessitated the search for alternative and effective biodegradable insecticides, which have greater selectivity. The re-evaluation and use of traditional botanical pest control agents (powder, water extracts, oil and wood ash) that lo-

cal farmers have been using over the past decades though without much success, seem to provide a clue to local sourcing of pest control strategies. Fortunately, Nigeria has an array of landraces of herbal plants with medicinal and/or insecticidal properties, which could be harnessed for the production of biopesticides for pest management on limited resource farmers' farms.

Most researches conducted worldwide in the past two or three decades using plant products as crop protectants were done on storage pests (Ivbijaro 1984; Don-Pedro 1989; Dike & Mbah 1992; Oparaeke & Dike 1996; Oparaeke 1997). However, crude extracts of some spices and herbs, which possess insecticidal activity, have been evaluated mainly in laboratory studies to control field pests of arable crops. Extracts of *Nicotiana tabacum* L., *Derris elliptica* Linn, *Lonchocarpus uruca* Linn (Matsumura 1975), *Melia azaderach* Linn, *Argemone mexicana* Linn (Pandey *et al*. 1981), *Chrysanthemum cinerariaefolium* Linn (Stoll 1986), *Azadirachta indica* A. Juss (Olaifa & Adenuga 1988; Jackai & Oyediran 1991; Tanzubil 1991; Jackai *et al*. 1992), *Syzygium aromaticum* (L.) Merr. & Perr., *Allium sativum* L. and *Monodora myristica* (Gaertn.) Dunal (Oparaeke *et al.* 2000a, 2001), have been reported to possess insecticidal activity against a number of insect pest species.

In Nigeria, there is a dearth of information on the use of naturally growing plants as biopesticides (Olaifa *et al.* 1987). The present work was undertaken to establish the effectiveness of different concentrations and spraying schedules of water extracts of West African black pepper to control post-flowering insect pests of cowpea.

## MATERIALS AND METHODS

Dried fruits of West African black pepper, *Piper guineense* Schum. & Thonn, were purchased in markets at Aba in Abia State of Nigeria, dried in an oven at 80°C to stabilize the moisture content and ground into powder using an electric hammer mill. Different amounts (250 g, 500 g and 1000 g representing 5%, 10% and 20% w/v, respectively) of the powder were weighed separately into 10 l plastic buckets containing 3 l of tap water, stirred vigorously for 10 min and allowed to stand for 12 hours. The mixtures were filtered using a double folded muslin cloth with additional 1.5 l of water to obtain crude extracts of different concentrations, 250 ml of 20% soap (50 g bar soap flakes dissolved in 250 ml of boiling water) and starch (Robin®) solutions each were added to give the extracts a slightly sticky and emulsifiable characteristic. The extracts were then labeled and ready for application.

The trials were conducted under rain fed conditions for the periods 1999 and 2000 on the research farm of the Institute for Agricultural Research, Samaru, Zaria (11°11' E and 07°38' N), situated in the northern Guinea Savanna of Nigeria. The field used for each trial had an area of about 0.4 ha. It was sprayed with glyphosate at 2.5 kg a.i./ha and the weeds allowed to wilt for 21 days before disc harrowing and ridging at 0.75 m spacing. The treatments consisted of black pepper extracts applied at three rates and spraying schedules as indicated below. There were also two checks, the synthetic insecticide Uppercott® and an untreated control. The main plots (3.75 m × 54 m) consisted of three concentrations of black pepper extract, Uppercott and the untreated control. Sub plots (3.75m × 6.0 m) were treated in three different application schedules. Each plot/treatment (consisting of five ridges: three main and two discard ridges) was replicated three times and was separated by a 2 m wide border along the ridge and two unplanted ridges.

The cowpea cv. Sampea 7 was used for planting; it is of medium duration (80–85 days) with semierect growth habit and highly susceptible to the major post-flowering pests whose peak populations synchronize with the growth period of the plant in the savanna ecological zone where this study was conducted. The seeds were dressed with Apron Plus 50D (one satchet/2 kg seeds) before sowing. Three seeds were sown per hole between August 3 and 7 in both years with intra-row spacing of 25 cm, giving 24 plants per row. The preemergence herbicide Galex (Metalachlor 250 a.i. + Metabromuron 250 a.i./l) was applied at 1.5 kg a.i./ha immediately after sowing to control growth of weed seeds. Seedlings were thinned to two plants per hole 2–3 weeks after sowing (WAS) and NPK (15:15:15) fertiliser was used to top-dress the seedlings at the rate of 35 kg a.i./ha. The plots were weeded with a hoe once at 6 WAS to ensure a clean environment for crop growth. Beginning from the fourth week after sowing, a tank mixture of benomyl and mancozeb was applied at the rate of 0.30 kg a.i./ha every week for 4 weeks to control fungal diseases. Beginning at flower bud formation or onset of flowering (6 WAS), three levels of

black pepper extract 5%, 10% and 20% (w/v) were applied 2, 4 or 6 times in weekly intervals, using a pressurised air low volume knapsack sprayer at a discharge rate of 150 l/ha. Uppercott was sprayed once weekly for four weeks at the rate of 1 l/ha, while the untreated control remained unsprayed.

The insect pests were sampled before each week's spraying between 6.30 am and 8.30 am, and on the three inner rows of the plots. *Maruca* pod borer larvae were assessed by randomly picking 20 flowers per plot, placing them in vials containing 30% alcohol (Amatobi 1994), and next day the flowers were dissected in the laboratory and the insects found were identified and counted. *Maruca* larvae were also sampled by randomly picking 12 pods per plot from six plants located in three 1 $m^2$ quadrants randomly selected within the main rows, while the pod sucking bug (*C. tomentosicollis*) was visually counted on 20 plants in the same quadrants. Damage to pods, such as pods with holes, wrinkled, twisted, and shriveled or poorly developed, was calculated using the formula:

$$\% \text{ pod damage} = \frac{\text{total pods produced/plant} - \text{number of undamaged pods/plant}}{\text{total pods produced/plant}} \times 100 \quad (1)$$

Twenty plants were randomly selected per plot and inspected for phytotoxicity (wilting, discoloration, leaf shedding, stunting of terminal buds and death of plant). Grain yields were taken from dried pods harvested from the main rows, threshed, winnowed and weighed.

Data obtained were subjected to square root or arcsine transformation (for percentage data) before analysis of variance (ANOVA) while treatment means were separated using Student Newman Keuls test ($P < 0.05$) (SAS Institute 1990).

## RESULTS

The mean number of *Maruca* pod borers (MPBs) and pod sucking bugs (PSBs) infestations on cowpea flowers and pods in both seasons were significantly lower ($P < 0.05$) on plots sprayed with 20%, 10% and 5% w/v extracts of *P. guineense* than on the unsprayed control plots. When applied at 6 and 4 weekly intervals, the 20% and 10% rates significantly reduced the test insects at 8 WAS

Table 1. Effect of three concentrations and spraying schedules of black pepper extract on the populations of larvae of *Maruca vitrata* and of *Clavigralla tomentosicollis* on cowpea in the cropping seasons 1999 and 2000

| | Mean number of insects | | | |
|---|---|---|---|---|
| | *Maruca* larvae/flower and/or pod | | *C. tomentosicollis*/plant | |
| | 1999 | 2000 | 1999 | 2000 |
| Control (0.0) | 3.48$^{a}$ | 2.84$^{a}$ | 5.42$^{a}$ | 7.22$^{a}$ |
| $C^1$ $R^1$ | 1.72$^{b}$ | 1.42$^{b}$ | 2.27$^{b}$ | 3.28$^{b}$ |
| $C^1$ $R^2$ | 1.54$^{bc}$ | 1.20$^{bc}$ | 1.80$^{bc}$ | 2.83$^{bc}$ |
| $C^1$ $R^3$ | 1.28$^{bcd}$ | 1.12$^{bcd}$ | 1.52$^{cd}$ | 2.38$^{cd}$ |
| $C^2$ $R^1$ | 1.40$^{bcd}$ | 1.06$^{bcd}$ | 1.28$^{cd}$ | 2.17$^{cd}$ |
| $C^2$ $R^2$ | 1.08$^{cd}$ | 0.78$^{cd}$ | 0.95$^{def}$ | 1.78$^{def}$ |
| $C^2$ $R^3$ | 1.06$^{cd}$ | 0.78$^{cd}$ | 0.95$^{def}$ | 1.78$^{def}$ |
| $C^3$ $R^1$ | 1.14$^{bcd}$ | 1.03$^{bcd}$ | 0.98$^{def}$ | 1.85$^{def}$ |
| $C^3$ $R^2$ | 0.86$^{d}$ | 0.68$^{d}$ | 0.83$^{ef}$ | 1.56$^{ef}$ |
| $C^3$ $R^3$ | 0.74$^{d}$ | 0.60$^{d}$ | 0.60$^{f}$ | 1.03$^{f}$ |
| Uppercott | 0.70$^{d}$ | 0.48$^{d}$ | 0.38$^{f}$ | 0.60$^{f}$ |
| S.E ± | 0.22 | 0.17 | 0.19 | 0.21 |

Means followed by the same superscript(s) in a column do not differ significantly ($P < 0.05$; Student Newman Keuls test)
$C^1$ = 5% w/v, $R^1$ = 2 weekly sprayings; $C^2$ = 10% w/v, $R^2$ = 4 weekly sprayings; $C^3$ = 20% w/v. $R^3$ = 6 weekly sprayings

Table 2. Effect of three concentrations and spraying schedules of black pepper extract on pod damage per plant and grain yield of cowpea in the cropping seasons of 1999 and 2000

| | Mean values of yield parameters | | | |
|---|---|---|---|---|
| | pod damage (%) | | grain yield (kg/ha) | |
| | 1999 | 2000 | 1999 | 2000 |
| Control (0.0) | 90.8[a] | 91.8[a] | 125.5[a] | 150.7[a] |
| $C^1$ $R^1$ | 43.1[b] | 42.6[b] | 362.7[b] | 378.1[b] |
| $C^1$ $R^2$ | 41.2[c] | 40.6[c] | 419.2[c] | 427.9[c] |
| $C^1$ $R^3$ | 39.7[c] | 39.6[c] | 434.8[c] | 478.9[c] |
| $C^2$ $R^1$ | 29.6[d] | 29.0[d] | 508.4[d] | 578.7[d] |
| $C^2$ $R^2$ | 19.2[e] | 18.4[e] | 952.0[e] | 968.7[e] |
| $C^2$ $R^3$ | 18.2[e] | 17.1[e] | 966.9[e] | 988.0[e] |
| $C^3$ $R^1$ | 17.8[e] | 16.5[e] | 971.2[e] | 992.3[e] |
| $C^3$ $R^2$ | 15.6[f] | 14.7[f] | 1009.9[f] | 1086.4[f] |
| $C^3$ $R^3$ | 13.1[g] | 13.5[fg] | 1139.2[g] | 1122.7[g] |
| Uppercott | 12.4[g] | 12.4[g] | 1172.6[g] | 1178.3[g] |
| S.E ± | 0.39 | 0.42 | 6.35 | 7.94 |

Means followed by the same superscript(s) in a column do not differ significantly ($P < 0.05$; Student Newman Keuls test)
$C^1$ = 5% w/v, $R^1$ = 2 weekly sprayings; $C^2$ = 10% w/v, $R^2$ = 4 weekly sprayings; $C^3$ = 20% w/v, $R^3$ = 6 weekly sprayings

compared with the 5% rate, but they were not significantly superior to Uppercott in efficacy (Table 1). However, the 20% rate with 6 weekly applications was not significantly superior to 4 weekly applications that gave a similar reduction of the pests' population. Pod damage was significantly reduced ($P < 0.05$) on plots sprayed with 20% and 10% w/v extracts with 6 or 4 weekly applications, while grain yields were also highest and similar at these rates and spray schedules as compared to the 5% rate at all schedules and the untreated control in both years (Table 2). Grain yields from plots treated with the 20% extract at 6 or 4 weekly applications compared favourably with that from plots sprayed with Uppercott. There were no signs of phytotoxicity on plots treated with black pepper extracts, except for a whitish substance observed on plants 2 dayd after spraying, which disappeared after a light shower of rain.

## DISCUSSION

The three rates and spraying schedules of *P. guineense* extracts caused various degrees of reduction of the pests species tested and offered various levels of protection to both flowers and pods as well as increased grain yield in sprayed plots throughout the period compared to the unsprayed plots. This investigation ranked 20 % and 10 % *P. guineense* extracts with 6 or 4 weekly applications superior to the 5 % rate at all spray schedules. These findings corroborated an earlier work by Ekesi (2000) who reported that crude extracts of *P. guineense*, garlic bulbs and neem extracts at 15%, 10% and 5 % rates (w/v) significantly reduced egg viability in MPB larvae and PSBs. Crude extract, essential oil and powder of *P. guineense* have been evaluated and found effective against boll weevil, *Anthonomus grandis* Boh. (Scott & McKibben 1978); European corn borer *Ostrinia nubilalis* Hubn. (Ewete *et al.* 1996); thrips, MPBs, PSBs (Oparaeke *et al.* 2001); cowpea bruchid, *Callosobruchus maculatus* Fab. (Ivbijaro & Agbaje 1986; Oparaeke 1997); bambarra groundnut bruchid, *C. subinnotatus* Pic. (Oparaeke & Bunmi 2001) and rice weevil, *Sitophilus oryzae* L. (Su 1977).

*P. guineense* contains Piperine and Chavicine which are insecticidal to crop pests (Oliver 1959; Su 1977) and its mode of action was reported to be contact toxicity (Olaifa *et al.* 1987). The plant extract also contains phenyl-propanoid, myristicine (sarisan, safrole and elemeicin) and 51-mono-sesquiterpenoids (Oliver 1959). Olaifa and Akingbohungbe (1986) reported antifeedant and insecticidal effects of the passive extracts of *P. guineense* and other plants on some pest species. The essential oils of

*P. guineense*, *Monodora tenuifolia* and *Lantana adoensis* have exhibited acute toxicity to larvae of *Acrae eponina* Cramer, to the adult cotton stainer *Dysdercus superstitiousus* Fab., flea beetle *Ootheca mutabilis* Sahlberg, and the pod sucker *Riptortus dentipes* Fab. (Olaifa *et al.* 1987).

However, Oparaeke *et al.* (2000b) in a field study using 10% and 20% crude extracts of *P. guineense* observed a systemic effect on thrips and MPB larvae in cowpea flowers while direct contact with *Maruca* larvae and PSBs (adults and nymphs) was found to be highly lethal. These combined properties of West African black pepper are useful for any insecticide to be considered as a potential candidate in the effective management of noxious insect pests of arable crops. Another important attribute of an effective insecticide is its persistence and rain fastness. In this study, 20% soap and starch solution each was mixed with the crude extracts to serve as an emulsifier and sticker, respectively. These materials proved effective in improving the ability of the extract to stay on cowpea plants for a long time without being washed off by the very frequent rains characteristic of this tropical environment. None of these materials have so far been proved to have insecticidal properties, and they are readily available in local markets around Zaria (Nigeria) and could be accessed by both large and small-scale farmers alike.

The present study has shown the efficacy of 20% and 10% w/v extracts of *P. guineense* with 6 or 4 weekly sprayings for control of post-flowering pests of cowpea. In Nigeria, four weekly applications of insecticides are common, no matter the concentration or type of insecticide used on cowpea. Although yields are the ultimate data for judging the effectiveness of an insecticide, the grain yields obtained from plots treated with the 20% extract of *P. guineense* at four weekly intervals did not significantly differ from that of six weekly applications with the 20% extract. The former schedule would appear to be more cost effective for production of cowpea of medium duration such as cv. Sampea 7l, grown in this ecological zone where water is scarce.

Investigations should be intensified on the use of this material and other hitherto untested plants whose bioactivity is currently restricted to storage pests and screen-house investigations, for the control of field pests of crops. Further tests are also necessary using the essential oil of *P. guineense* in ultra low volume (ULV) formulation, which could reduce the amount of water required for crude extract preparation in some areas where water is deficient. *Piper guineense* is a tropical plant of West African origin and is available in most markets in Nigeria. A powder or water extract from it are used traditionally for prophylactic or therapeutic treatment of various body disorders and as condiment in food preparations. Thus, preparations made from this material are unlikely to pose any health hazard to man and livestock. More importantly, the extraction procedure is simple, inexpensive and easily adaptable by limited resource farmers in a developing country such as Nigeria.

The results presented in this study have shown that increasing the concentration of black pepper extract to 20% w/v with four weekly applications is effective, safe and cheap for the control of post-flowering insect pests of cowpea without degrading the environment.

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