

EFFECT OF WILD GINGER, *ZINGIBER PURPUREUM* ROSCOE. ON COWPEA SEED BRUCHID, *Callosobruchus maculatus* (F.) IN STORED COWPEA

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ABSTRACT

In our search for botanicals with insecticidal properties, a series of laboratory experiments were conducted to investigate the effects of wild ginger, *Zingiber purpureum* (Zingiberaceae) against cowpea seed bruchid, *Callosobruchus maculatus* (Coleoptera:Bruchidae) in stored cowpea. The rhizome and roots of wild ginger reduced the adult longevity, oviposition, egg hatchability and F₁ adult emergence of the bruchid. The LC₅₀ values of dry and fresh rhizome powder on adult bruchid were estimated as 46719 and 48632 ppm respectively, while that of the standard insecticide, pirimiphos methyl was estimated as 435 ppm. The fresh rhizome powder had contact and fumigant toxic effect on bruchids. But in the process of drying the fumigant activity appears to be reduced. As the seed penetration activity of the active constituent of wild ginger is poor, the developing stages in the seed were not affected. The rhizome powder could prevent bruchid infestation of cowpea seeds up to 40 days after treatment. Furthermore, wild ginger preparations had no effect on the germination of cowpea seeds. These findings indicate that wild ginger preparations could be used to protect stored seed cowpea from bruchid infestations. However, further studies on its active compound(s) need to be carried out before making recommendations.

KEY WORDS: Bruchid, *Callosobruchus maculatus*, Cowpea, *Zingiber purpureum*

INTRODUCTION

Grain legumes constitute the main source of protein for the developing countries where per capita consumption of animal protein is very low. The seeds are often stored for long periods and insect infestations of seeds cause weight and quality losses that lead to the reduction of commercial values and seed germination (Talekar, 1987). Seed bruchid, *Callosobruchus* spp. are considered as the most important storage pest of grain legumes (Hall, 1970; Hill, 1983).

At present a number of methods are being used for the control of these storage pests and chemical control is the most widely practised method. But use of insecticides to protect grain legumes in storage has serious limitations on small

farmers (Talekar, 1987). Use of non - chemical methods such as proper drying, and use of vegetable oils, ash, and resistant varieties are always not successful. Therefore, a suitable alternative strategy which provide safe, effective and economical pest control is urgently needed. Higher plants offer an excellent source of biologically active natural products which can be exploited as sources of insecticides (Alkofahi *et al.*, 1989).

Family Zingiberaceae consists of over 40 genera and about 1000 species of plants. Out of 36 species of Zingiberaceae plants found in Sri Lanka, 12 species are endemic (Burt and Smith, 1983). Some species of Zingiberaceae plants have been used for medicinal purposes and some species have been reported to have biologically active compounds (Ohsawa *et al.*, 1994; Grainge and Ahmed, 1987). Wild ginger, *Zingiber purpureum* (Zingiberaceae) is being cultivated throughout tropical Asia for medicinal purposes (Burt and Smith, 1983). The environmental conditions prevailing in the mid- and up- country wet zone of Sri Lanka are favourable for the cultivation of this plant. Thus, this plant can be cultivated extensively in these areas, if necessary. However, the insecticidal properties of this plant have not been exploited.

In this study, an attempt was made to investigate the possible use of wild ginger for the control of cowpea bruchid, *Callosobruchus maculatus* on stored seed cowpea (*Vigna unguiculata*).

MATERIALS AND METHODS

The investigations were carried out during 1990-91 at the Division of Entomology, Central Agricultural Research Institute (CARI), Gannoruwa. All experiments were carried out in a completely randomized block with 4 replications under the prevailing environmental conditions ($27 \pm 3^{\circ}\text{C}$ and $80\% \pm 5 \text{ RH}$). Except for the experiment testing seed viability, all other experiments were conducted in transparent plastic vials (8.0 cm high and 4.0 cm diam.). The vial opening was covered with a porous nylon mesh to prevent the escape of introduced bruchids. Cowpea seed (cv. Bushitavo) was used as the source of food for the bruchids.

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Test Insects

Cowpea bruchid was reared in transparent plastic bottles (1 litre) each containing 50 g of cowpea seeds. A circular window (2 cm diam.) was made in the middle of the lid of each bottle to provide ventilation to the bruchids. This window was made by making a hole to which a fine steel gauze (25 mesh per cm) was fixed. One-day-old bruchid adults were used in all experiments. To obtain one-day-old adult bruchids, culture bottles with emerging adults were selected and all the adults were removed 24 h before each experiment. The adults which emerged within 24 h period prior to the experiment were considered as one-day-old adults and used in the experiments. Their sex was determined as described by Southgate *et al.* (1957).

Plant materials

Wild ginger plants were grown in the field at CARI, Gannoruwa. Rhizome and roots were removed from the plants and cleaned by rinsing with tap water. Four different preparations of rhizome and two different preparations of roots were tested against the bruchid, to determine the most suitable preparation for seed treatment. The six different preparations and their preparation methods were as described below. Shade-dried rhizome/root and fresh rhizome/root were separately ground using a domestic grinder to obtain dry rhizome powder, dry root powder, fresh rhizome powder and fresh root powder. Pieces (1 mm³) of dry rhizome and fresh rhizome were obtained by chopping the respective plant parts with a knife.

In these studies, the different preparations of powder and pieces were compared for their activity against the bruchids. If pieces are equally active as powders, use of pieces has some advantages over powders. If a volatile active compound is present in the plant material, its emission is faster from the powders than from the pieces. Therefore the pieces should be able to retain their fumigant activity for a longer period than the powders thereby acting as a slow release insecticide formulation. Further, the rhizome and roots contain a yellow dye which on contact stains the seeds. The contact with seeds can be minimized by using pieces and this will help to reduce the staining of seeds. The dry and fresh plant parts were used to investigate the effect of drying on the insecticidal activity against the bruchids.

Comparative efficacy of different preparations of wild ginger and pirimiphos methyl against bruchid infestation

Forty grams of cowpea seed were separately mixed with the six preparations of wild ginger (3% w/w) and pirimiphos methyl (Actellic 2% D.) (0.05% w/w). The treated seeds were then transferred into separate vials and five pairs of one-day-old bruchids were introduced. Another 40 g of cowpea seeds without any treatment was used as the untreated control. The bruchid mortality was recorded at 3, 4, 5, and 6 days after their introduction (DAI). Two weeks after the bruchid introduction, a sample of 25 seeds was randomly selected from each vial and the number of hatched and unhatched eggs was counted. The seeds were replaced after counting. When F_1 adults emerged, the number of emergence holes in 40 g seed sample was recorded.

Estimation of LC_{50} values

Cowpea seeds (40 g) were separately mixed with five different concentrations of dry and fresh rhizome powder (0,2,3,5,10% w/w) and were transferred into plastic vials. Then, five pairs of one-day-old bruchid adults were introduced. The bruchid mortality was recorded 96 h after introduction. The LC_{50} values were estimated using the Probit analysis. LC_{50} value of pirimiphos methyl on adult bruchids was also estimated using a similar method with 0.002, 0.003, 0.005 and 0.01 % concentrations of the treatment.

Effect of dry rhizome powder on different growth stages of bruchid

Bruchids were confined to culture bottles with untreated cowpea seeds for 24 h and removed to obtain one-day-old bruchid eggs. Then, a set of vials each containing 25 g of cowpea seeds with one-day-old eggs (1 egg/seed) was prepared. The vial opening was covered with fine nylon mesh to prevent further infestation of seeds. Dry rhizome powder (3% w/w) was added to the vials separately at 1,4,8,16 and 21 days after egg deposition. Then, the vial content was carefully mixed. For each treatment a vial of untreated cowpea seeds (25 g) with same development stage of bruchid was used as the untreated control. The number of emergence holes in each seed sample was counted, after all adults had emerged.

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Determination of persistency of activity

Five pairs of bruchid adults were introduced into plastic vials each containing 40 g of cowpea seeds which were separately treated with dry and fresh rhizome powders (3% w/w) at 1, 7, 14, 21, 30 and 40 days prior to the experiment. The number of emergence holes on a 40 g seed sample was recorded after the emergence of all adults.

Determination of mode of action

Contact toxicity

Five pairs of one-day-old adult bruchids were introduced into two vials each containing 40 g of cowpea seeds treated with dry rhizome powder (5% w/w). The opening of one vial was covered with a fine nylon mesh which provides proper ventilation to identify contact toxicity. Opening of the second vial was covered with an air-tight lid to identify the combined activity of contact and fumigant toxicity.

Fumigant toxicity

The plastic vials were divided into two compartments (low and upper) using a wire mesh (25 mesh per cm). Two grams of dry rhizome powder were placed at the bottom compartment and 40 g of untreated cowpea seeds were placed in the upper compartment. Then, five pairs of one-day-old bruchid adults were introduced into the upper compartment of each vial. The opening of one vial was covered with fine nylon mesh while the opening of another vial was covered with an air-tight plastic lid. The same procedure was followed to test the fumigant toxicity of fresh rhizome powder on the bruchid. Two plastic vials with similar arrangement but without plant parts were used as untreated controls.

Effect of wild ginger on seed viability

Fifteen cowpea seeds mixed with different preparations of wild ginger (3% w/w) and pirimiphos methyl (0.05% w/w) were separately spread on a moistened filter paper kept in a glass petri dish (9 cm diam.). Water was added to the filter paper when necessary to provide sufficient moisture for the germinating seeds. The number of seeds germinated in each petri dish was recorded 10 days after placement of seeds.

RESULTS AND DISCUSSION

Comparative efficacy

There was no significant difference in adult mortality among the different treatments and control up to 3 DAI. However, at 4 DAI, all the wild ginger preparations and pirimiphos methyl treatment caused significantly higher mortality to bruchid adults when compared with the control (Table 1).

Table 1. Effects of different preparations of wild ginger and pirimiphos methyl on *C. maculatus* in stored seed cowpea

Treatment	Mortality (%)				Oviposition/ 25 seeds	% egg hatchability	No. emerged/ 40 g seeds
	DAI						
	3	4	5	6			

Dry rhizome powder	28	47 bc	75 ab	100 a	05 bc	09 bc	02 b
Dry rhizome pieces	28	35 c	82 ab	100 a	09 b	06 bc	02 b
Fresh rhizome powder	31	45 bc	85 ab	100 a	09 b	06 bc	02 b
Fresh rhizome pieces	09	32 c	80 ab	85 a	11 b	09 bc	03 b
Dry root powder	22	40 bc	80 ab	87 a	10 b	13 b	02 b
Fresh root powder	13	37 c	75 ab	92 a	23 b	12 b	03 b
Pirimiphos methyl	30	62 a	95 a	100 a	11 b	20 b	05 b
Control	01	02 d	07 c	32 b	37 a	97 a	156 a
CV (%)	ns	13.3	16.4	8.7	15	21.2	5.7

In each column, values followed by the same letter do not differ significantly according to DMRT at $p=0.05$; DAI=days after introduction of bruchids

Although, at 4 DAI pirimiphos methyl caused significantly higher mortality of adult bruchids than the wild ginger preparations, the difference disappeared at 5 and 6 DAI. The results indicate that different preparations of wild ginger were as toxic as pirimiphos methyl against the bruchid adults. However, the toxic effect of wild ginger preparations act on the bruchid slower than that of pirimiphos methyl. The egg deposition, egg hatchability and F_1 adult emergence of bruchids were also significantly low in seeds treated with pirimiphos methyl and wild ginger preparations when compared with the control. The low egg deposition on treated seeds may be attributed to the presence of few live adults in treated vials. But the low percentage

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of egg hatchability may be due to some egg sterilizing effect of treatments, specially in the rhizome materials of wild ginger. The significantly low F_1 adult emergence on treated seeds over the control may be due to the combined activity of all of these adverse effects on the bruchids. The results indicated that different preparations of rhizome and root of wild ginger were equally effective as pirimiphos methyl for the control of bruchids in stored seed cowpea.

Estimation of LC_{50} values

The LC_{50} values of dry rhizome powder, fresh rhizome powder and pirimiphos methyl on adult bruchids were 46719, 48632 and 435 ppm respectively (Table 2). These results indicated that pirimiphos methyl was more toxic than the rhizome preparations of wild ginger. However, the experiment should be conducted using the active ingredient of wild ginger to compare their relative toxicity.

Table 2. Relative toxicity of dry and fresh rhizome powder of wild ginger and pirimiphos methyl on *C. maculatus* adults

Insecticide	LC_{50} of adult bruchids* on treated cowpea seed (ppm)
Dry rhizome powder	46719
Fresh rhizome powder	48632
Pirimiphos methyl	435

* Average of 40 adults

Effect on growth stages

Cowpea seeds mixed with dry rhizome powder of wild ginger at 1 day and 4 days after egg deposition significantly reduced the adult emergence of bruchids when compared with the control, where 100% adult emergence was observed (Table 3). However, the treatment of seeds with dry wild ginger rhizome powder at 9, 16 and 20 days after egg deposition failed to show any significant difference in adult emergence over the control. Raina (1970) showed that the incubation period of this bruchid is 4 days at 30°C and 70% RH. The present experiments were also conducted under similar environmental conditions and it was observed that within 4-5 days after egg deposition, the eggs hatched and 1st instar larvae penetrated into the seed. This indicates that rhizome powder of wild

Table 3. Effect of dry rhizome powder of wild ginger on different growth stages of *C. maculatus*

<i>Time of treatment</i>	<i>Number of adults emerged / 25 eggs</i>
One day after egg deposition (One day old eggs)	0.50 b
Four days after egg deposition (Egg-larval stages)	0.75 b
Nine days after egg deposition (Larval stage inside the seed)	24.75 a
Sixteen days after egg deposition (Larval-pupal stages inside the seed)	24.50 a
Twenty days after egg deposition (Pupal stage before emergence of adults)	24.50 a
Control	25.00 a
CV (%)	8.9

Values followed by the same letter do not differ significantly according to DMRT at $p=0.05$

ginger is effective only on the eggs and 1st instar larvae before entering into the seeds. The rhizome powder had no effect on rest of the development stages which occur in the seeds. The failure of the rhizome materials to cause any significant toxicity on the developing stages in the seed may be attributed to the poor penetration activity of the active compound(s).

Persistency of the insecticidal activity

Cowpea seeds treated with dry or fresh rhizome powder of wild ginger significantly reduced the seed damage from fresh infestations of bruchids up to 40 days after treatment (DAT), when compared with the control (Table 4). However, there was no significant difference in the insecticidal activity between dry and fresh rhizome powder preparations of wild ginger. Thus, both fresh and dry rhizome powder of wild ginger equally reduced seed damage against bruchid and the activity persisted up to 40 DAT.

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Table 4. Persistency of rhizome powder of wild ginger against the infestation of *C. maculatus* on stored cowpea seeds

<i>Time of bruchid infestation (DAT)</i>	<i>Form of rhizome</i>	<i>Seed damage (Emergence holes / 40 seeds)</i>
01	dry	1 b
	fresh	1 b
07	dry	1 b
	fresh	1 b
14	dry	1 b
	fresh	2 b
21	dry	2 b
	fresh	2 b
30	dry	2 b
	fresh	2 b
40	dry	3 b
	fresh	3 b
Control	-	158 a
CV (%)		10.5

Values followed by the same letter do not differ significantly according to DMRT at $p=0.05$;
DAT=days after treatment

Mode of action

Seed damage in the control treatment under ventilated and air-tight conditions was similar (Table 5). This indicates that the oxygen content in the vials was sufficient for the bruchids during the experimental period. All treatments with wild ginger significantly reduced seed damage when compared with the control. However, there was no significant difference in the seed damage between seed mixed with dried and fresh rhizome powder and kept under air-tight or ventilated conditions. This indicates that both dry and fresh rhizome powder have similar contact toxicity against the bruchids.

But when seeds and dry or fresh rhizome powder were kept separately in the same vial under air-tight or ventilated conditions, the treatment with fresh rhizome powder under air-tight conditions showed significantly low seed damage

Table 5. Mode of action of dry/fresh rhizome powder of wild ginger on *C. maculatus*

<i>Treatment</i>	<i>Stored condition</i>	<i>Number of emergence holes/40 g seeds</i>
Seed mixed with dry powder	air tight	2 f
	ventilated	2 f
Seed mixed with fresh powder	air tight	3 f
	ventilated	3 f
Seed and dry powder kept separately	air tight	64 d
	ventilated	83 c
Seed and fresh powder kept separately	air tight	3 f
	ventilated	53 e
Control	air tight	142 ab
	ventilated	158 a
CV (%)		7.4

Values followed by the same letter do not differ significantly according to DMRT at $p=0.05$

compared to the other treatments. This indicates that in addition to contact toxicity, the fresh rhizome powder has fumigant toxic effect on the bruchid. The low fumigant toxic effect of dry rhizome powder under the similar air-tight conditions may be due to decomposition of the volatile active compound in the process of drying. Furthermore, under air-tight conditions, the fumigant present in the rhizome could become concentrated inside the vial causing higher toxicity to the bruchid than the similar treatment under ventilated conditions.

Effect on germination

There was no significant difference in the germination of cowpea seeds mixed with different preparations of wild ginger and pirimiphos methyl when compared to the control (Table 6). This indicates that different preparations of wild ginger and pirimiphos methyl have no affect on the germination of cowpea seeds.

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Table 6. Effect of different preparations of wild ginger on germination of treated seed cowpea

<i>Treatment</i>	<i>% Germination</i>
§ Dry rhizome powder	93 ab
Dry rhizome pieces	95 ab
Fresh rhizome powder	90 b
▪ Fresh rhizome pieces	92 b
§ Dry root powder	88 b
Fresh root powder	90 b
Pirimiphos methyl	93 ab
Control	100 a
‡ CV (%)	3.0

Values followed by the same letter do not differ significantly according to DMRT at $p=0.05$

CONCLUSIONS

Rhizome and roots of wild ginger reduced adult longevity, oviposition, egg hatchability and F_1 adult emergence of the seed bruchid in stored cowpea. Its residual activity can prevail up to 40 days after treatment in stored cowpea. The LC_{50} values of fresh (48632 ppm) and dry (46719 ppm) rhizome powder on adult bruchids were found to be much higher than that of the standard insecticide, pirimiphos methyl (435 ppm).

The rhizome has contact and fumigant toxic activity on bruchids. But in the process of drying the fumigant activity is reduced. However, seed penetration of the active constituent of wild ginger appears to be poor and rhizome powder did not affect the developing stages inside the seeds. Although rhizome powder has comparatively slow toxicity, it can prevent bruchid infestation up to 40 days after treatment.

Wild ginger preparations do not affect the germination of cowpea. Therefore, this plant can be used for the protection of stored seed cowpea from bruchid infestation. However, further studies are needed to identify the active compound(s) and their effect on non-target organisms before recommending its use.

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