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ENHANCING THE POTENCY OF VEGETABLE OILS BY COMBINING WITH PIRIMIPHOS-METHYL FOR PROTECTION OF STORED COWPEA AGAINST INFESTATION BY *CALLOSOBRUCHUS MACULATUS* (FAB.)

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ABSTRACT: The cowpea beetle *Callosobruchus maculatus* (Fab.) is a major insect pest of stored cowpea in Ghana. The efficacy of coconut, groundnut and soybean oils applied at 1, 2, 5 and 10 ml kg^{-1} and pirimiphos-methyl at 2.5, 5.0 and 10 μl active ingredient in 2 ml of water kg^{-1} of grain (1/16, 1/8 and 1/4 of the recommended dose) alone or in combination to adults and immature stages of *C. maculatus* and the persistence of the treatments in cowpea grain were evaluated in the laboratory. All the treatments caused significant mortality compared to untreated controls. Lower dosages of the oils and pirimiphos-methyl when combined were highly toxic to *C. maculatus*. The mixtures also completely inhibited the development of immature stages of the beetle. Only pirimiphos-methyl and the mixtures remained effective 60 days after application. Pirimiphos-methyl can be used at reduced rates if combined with lower dosages of vegetable oils to control the infestation of *C. maculatus* in stored cowpea.

Key words/phrases: ***Callosobruchus maculatus***, **cowpea, mortality, pirimiphos-methyl, potency**

INTRODUCTION

Cowpea *Vigna unguiculata* is an important source of protein in the tropics (Owusu-Akyaw, 1991). A major constraint to large-scale cowpea production is the attack by a complex of insect pests from the time of planting through harvesting and during storage. The cowpea beetle, *Callosobruchus maculatus* (Fab.) is the most serious pest of stored cowpea and if not controlled can cause a total loss in traditional stores in Ghana. Infestation by *C. maculatus* can begin in the field prior to harvesting when eggs are laid on maturing pods. Application of synthetic insecticides is the most widely used method of controlling insect pest infestation of durable stored products. However, several detrimental effects associated with indiscriminate use of these insecticides on the environment and health, development of genetically resistant strains, erratic supply and high cost of the recommended insecticides have become a major concern to governments, policy makers, researchers and the general public. These constraints have stimulated interest in the search for alternative methods of pest control to reduce the sole reliance on synthetic insecticides.

The use of locally available vegetable oils as grain protectants is an old practice (Pereira, 1983). In recent years, research on the efficacy of vegetable oils as stored grain protectants against insects has been intensified (Don-Pedro, 1987; 1989; Kumar and Okonronkwo, 1991; Jackai, 1993; Ivania *et al.*, 1995; Obeng-Ofori, 1995). A major limitation to the practical utilization of vegetable oils as grain protectants is the high rates (10 ml kg^{-1}) required to effectively disinfect grains (Don-Pedro, 1989). In practice, these high rates are costly to apply. Don-Pedro (1989) suggested the possibility of using reduced levels of oils in combination with synthetic insecticides in simple mixtures as a means of making their use more attractive and effective. Chander *et al.*, (1991) reported that a combination of 4 ml kg^{-1} of mustard oil and Turmeric powder at 20 g kg^{-1} gave the best protection of rice, completely suppressing progeny emergence by *S. oryzae* (L.). More recently, Tembo and Murfitt (1995) also showed that wheat treated with vegetable oils combined with pirimiphos-methyl at half the recommended dose was as effective as pirimiphos-methyl at the recommended dose against *Sitophilus granarius* (L.).

This paper extends the above approach by evaluating the potency and protectant potential of combining vegetable oils (Soybean, groundnut and coconut) and pirimiphos-methy (organophosphorus insecticide) in simple mixtures for protection of stored cowpea against infestation by *C. maculantus*.

MATERIALS AND METHODS

Culturing of cowpea beetle

C. maculatus was cultured in the laboratory maintained at 26 ± 2° C and 65–70% r.h. One hundred adults of mixed sex were obtained from a laboratory stock culture at the Department of Crop Science, University of Ghana, Legon and reared on 500 g of cowpea in glass jars. After two weeks of oviposition, the parent adults were removed by sieving the grains with a sieve of mesh size

2.0 mm. The beetles that subsequently emerged were used for the different bioassays.

Vegetable oils and insecticide used

Three locally prepared oils, groundnut, soybean and coconut were purchased from the Madina market, Accra, Ghana. Pirimiphos-methyl, a broad-spectrum organophosphorus insecticide was purchased from the Ministry of Food and Agriculture, Accra. It is manufactured by ICI, UK and the active ingredient is 0-0-dimethyyl-0 (2-diethyl amino-6-methyl-pyrimidine-4-yl) phosphorothioat (Gruszdyev *et al.,* 1983). It is formulated as liquid or dust and has contact, stomach and fumigant activity against a wide range of insects.

Adult mortality bioassay

Dosages of vegetable oils at 2, 5 and 10 m kg^{-1} and pirimiphos-methyl at 2.5, 5 and 10 μl in 2 ml water kg^{-1} of grain were used to test the efficacy of the oils and insecticide alone when applied to cowpea grains. Each dose was pipetted into separate samples of 200 g of dry cowpea grain in glass jars and mixed thoroughly with a glass rod. For the insecticide-oil combinations, 1, 2 and 5 ml kg^{-1} of each oil was mixed with pirimiphos-methyl at 5 μl (1/8 dose) and 2.5 μl (1/16 dose) active ingredient in 2 ml water kg^{-1} of grain. Samples of 200 g of cowpea were treated with each mixture. Twenty adults of mixed sex (2–5 days old) were introduced into glass jars containing the different treatments. Untreated grains in glass jars acted as control. Each treatment was replicated four times. Mortality counts were made after 24 hours. Insects were presumed dead if they did not move when touched with a pin.

Effect of oils and insecticide on immature stages

One hundred and fifty adults of *C. maculatus* of mixed sex (2–5 days old) were introduced onto a sample of cowpea for seven days to allow for oviposition, after which the parent adults were sieved out. The infested cowpea grains were divided into batches of 200 g and put into glass jars, which were covered with muslin cloth and held in place by rubber bands. One day after adult removal, one set of batches of 200 g cowpea were mixed with 2, 5 and 10 ml kg^{-1} of groundnut, soybean or coconut oil; 2.5, 5 and 10 μl pirimiphos-methyl alone or 1, 2 and 5 ml oil kg^{-1} of grain combined with pirimiphos-methyl at 2.5 and 5 μl to test the effectiveness of the oils and insecticide on the eggs. To determine the toxicity of the treatments on the early larval, late larval and pupal stages, the experiment was repeated one week, two weeks and three weeks after adult removal from the infested grain sample using batches of 200 g of infested cowpea in the different glass jars. After seven weeks the number of F1 progeny produced in the different treatments was counted. Each treatment was replicated four times.

Persistence of the products in grain

To assess the persistence of the treatments, oils alone (2, 5 and 10 ml kg^{-1}) and in combination with pirimiphos-methyl (1, 2 and 5 ml kg^{-1} plus 2.5 and 5 μl doses) were tested. Twenty *C. maculatus* adults (2–5 days old) were exposed to treated cowpea which had been stored for 15, 30 and 60 days. Mortality counts were carried out after 24 hour exposure.

RESULTS

The toxicity of oils and pirimiphos-methyl to adult insects

All the treatments comprising vegetable oils and, pirimiphos-methyl alone or when combined caused significant ($P < 0.001$) mortality of *C. maculatus* compared to untreated grains (Table 1). Grains treated with oils at the rate of 10 ml kg^{-1} or pirimiphos-methyl at 10 μl in 2.0 ml water kg^{-1} of grain (1/4 of the recommended dose) killed over 50 and 90%, respectively of *C. maculatus* exposed (Table 1). Lower doses of the vegetable oils and pirimiphos-methyl when applied alone were less effective against the insects than when mixed. The mixtures were highly toxic to the beetles (Table 1). For example, a mixture of 1 ml of the oils and 2.5 μl of pirimiphos-methyl killed over 80% of the weevils exposed within 24 hours.

Persistence in grain

The potency of the different treatments after different intervals of storage is shown in Table 2. When the products were applied singly, only pirimiphos-methyl at 1/4 of the recommended dose (10 μl) caused significantly ($P < 0.001$) higher mortality of *C. maculatus* than the untreated controls after treated grains had been stored for 15 days (Table 2). Each of the oils when applied alone was not toxic to the beetles exposed only 15 days after application. The biological activity of all the products decreased significantly with storage period. The mixtures, however, induced appreciable mortality, with the most potent combination (pirimiphos-methyl at 5 μl plus 5 ml kg^{-1} oil) killing nearly 50% of the beetles exposed 60 days after treatment (Table 2).

Table 1. Toxicity of different vegetable oils and pirimiphos-methyl against *C. maculatus* in stored cowpea.

Treatment	Dosage kg^{-1}	Adult mortality (%) after 24 h
Control	0	1^{e}
Coconut	2 ml	17^{d}
	5 ml	23^{d}
	10 ml	50^{c}
Groundnut	2 ml	22^{d}
	5 ml	30^{d}
	10 ml	56^{c}
Soybean	2 ml	16^{d}
	5 ml	20^{d}
	10 ml	52^{c}
Pirimiphos-methyl	2.5 µl	47^{c}
	5.0 µl	70^{b}
	10.0 µl	90^{a}
Pirimiphos-methyl	2.5 µl	
+ Coconut	+ 1 ml	87^{a}
	+ 2 ml	90^{a}
	+ 5 ml	94^{a}
Pirimiphos-methyl	5.0 µl	
+ Coconut	+ 1 ml	88^{a}
	+ 2 ml	96^{a}
	+ 5 ml	98^{a}
Pirimiphos-methyl	2.5 µl	
+ Groundnut	+ 1 ml	88^{a}
	+ 2 ml	90^{a}
	+ 5 ml	100^{a}
Pirimiphos-methyl	5.0 µl	
+ Groundnut	+ 1 ml	87^{a}
	+ 2 ml	99^{a}
	+ 5 ml	100^{a}
Pirimiphos-methyl	2.5 µl	
+ Soybean	+ 1 ml	90^{a}
	+ 2 ml	90^{a}
	+ 5 ml	98^{a}
Pirimiphos-methyl	5.0 µl	
+ Soybean	+ 1 ml	87^{a}
	+ 2 ml	100^{a}
	+ 5 ml	100^{a}

2.5 µl, 5 µl and 10 µl of pirimiphos-methyl in 2 ml water per kg of grain are equivalent to 1/16, 1/8 and 1/4 of the recommended dosage, respectively. All values are means from four replicates. Means followed by different letter (s) are significantly different, $P < 0.001$, LSD.

Table 2. Mortality of *C. maculatus* in cowpea grains treated with different combinations of vegetable oils and pirimiphos-methyl after different storage intervals.

Treatment	Dosage kg^{-1}	Adult mortality (%) days after treatment		
		15	30	60
Control	0	1^{e}	2^{e}	0^{e}
Coconut	2 ml	0^{e}	0^{e}	0^{e}
	5 ml	0^{e}	0^{e}	0^{e}
	10 ml	3^{e}	0^{e}	0^{e}
Groundnut	2 ml	2^{e}	0^{e}	0^{e}
	5 ml	3^{e}	0^{e}	0^{e}
	10 ml	4^{e}	0^{e}	0^{e}
Soybean	2 ml	0^{e}	0^{e}	0^{e}
	5 ml	2^{e}	0^{e}	0^{e}
	10 ml	3^{e}	0^{e}	0^{e}
Pirimiphos-methyl	2.5 μl	15^{cd}	10^{d}	3^{e}
	5.0 μl	26^{c}	10^{d}	3^{e}
	10.0 μl	30^{c}	12^{d}	3^{e}
Pirimiphos-methyl + Coconut	2.5 μl			
	+ 1 ml	20^{c}	19^{c}	3^{e}
	+ 2 ml	55^{b}	21^{c}	19^{c}
	+ 5 ml	70^{a}	49^{b}	48^{b}
Pirimiphos-methyl + Coconut	5.0 μl			
	+ 1 ml	25^{c}	20^{c}	3^{e}
	+ 2 ml	49^{b}	30^{c}	19^{c}
	+ 5 ml	75^{a}	50^{b}	47^{b}
Pirimiphos-methyl + Groundnut	2.5 μl			
	+ 1 ml	22^{c}	19^{c}	6^{e}
	+ 2 ml	54^{b}	26^{c}	20^{c}
	+ 5 ml	77^{a}	49^{b}	48^{b}
Pirimiphos-methyl + Groundnut	5.0 μl			
	+ 1 ml	25^{c}	21^{c}	8^{de}
	+ 2 ml	60^{b}	31^{c}	20^{c}
	+ 5 ml	78^{a}	51^{b}	49^{b}
Pirimiphos-methyl + Soybean	2.5 μl			
	+ 1 ml	30^{c}	22^{c}	5^{e}
	+ 2 ml	58^{b}	29^{c}	22^{c}
	+ 5 ml	75^{a}	53^{b}	48^{b}
Pirimiphos-methyl + Soybean	5.0 μl			
	+ 1 ml	23^{c}	23^{c}	6^{e}
	+ 2 ml	57^{b}	30^{c}	22^{c}
	+ 5 ml	79^{a}	55^{b}	48^{b}

Effect on immature stages

Adult emergence from pre-infested cowpea with eggs and early larval stages of *C. maculatus* was significantly ($P < 0.001$) reduced by oil and pirimiphos-methyl applied alone or in combination to cowpea seeds (Table 3). Only oils at the rate of 10 ml kg^{-1} significantly ($P < 0.001$) controlled the late larval stage *of C. maculatus*. The uncombined treatments were not toxic to the pupal stages of the beetles (Tables 3). The mixtures completely inhibited the development of eggs and larvae of *C. maculatus* hidden inside the cowpea grains (Table 3).

Table 3. Effect of vegetable oils and pirimiphos-methyl on the degree of infestation by *C. maculatus* as determined by the number of eggs, larvae and pupae that emerged from treated grains.

Treatment	Dosage kg^{-1}	Adult (no.) that emerged after treatment			
		Eggs	Early larvae	Late Larvae	Pupae
Control	0	259^{a}	260^{a}	255^{a}	253^{a}
Coconut	2 ml	17^{b}	55^{b}	245^{a}	256^{a}
	5 ml	16^{b}	59^{b}	240^{a}	250^{a}
	10 ml	5^{c}	48^{b}	142^{b}	249^{a}
Groundnut	2 ml	8^{b}	56^{b}	249^{a}	258^{a}
	5 ml	19^{b}	54^{d}	239^{a}	252^{a}
	10 ml	4^{c}	47^{b}	155^{b}	252^{a}
Soybean	2 ml	17^{b}	60^{b}	241^{a}	249^{a}
	5 ml	15^{b}	57^{b}	238^{a}	246^{a}
	10 ml	4^{c}	45^{b}	159^{b}	251^{a}
Pirimiphos-methyl	2.5 µl	15^{b}	55^{b}	160^{b}	250^{a}
	5.0 µl	17^{b}	53^{b}	149^{b}	249^{a}
	10.0 µl	3^{c}	43^{b}	157^{b}	256^{a}
Pirimiphos-methyl	2.5 µl				
+ Coconut	+ 1 ml	0^{c}	0^{c}	51^{c}	161^{b}
	+ 2 ml	0^{c}	0^{c}	48^{c}	154^{b}
	+ 5 ml	0^{c}	0^{c}	28^{d}	159^{b}
Pirimiphos-methyl	5.0 µl				
+ Coconut	+ 1 ml	0^{c}	0^{c}	50^{c}	158^{b}
	+ 2 ml	0^{c}	0^{c}	45^{c}	162^{b}
	+ 5 ml	0^{c}	0^{c}	29^{d}	149^{b}
Pirimiphos-methyl	2.5 µl				
+ Groundnut	+ 1 ml	0^{c}	0^{c}	55^{c}	153^{b}
	+ 2 ml	0^{c}	0^{c}	49^{c}	159^{b}
	+ 5 ml	0^{c}	0^{c}	31^{d}	157^{b}
Pirimiphos-methyl	5.0 µl				
+ Groundnut	+ 1 ml	0^{c}	0^{c}	53^{c}	158^{b}
	+ 2 ml	0^{c}	0^{c}	47^{c}	158^{b}
	+ 5 ml	0^{c}	0^{c}	31^{d}	155^{b}
Pirimiphos-methyl	2.5 µl				
+ Soybean	+ 1 ml	0^{c}	0^{c}	50^{c}	148^{b}
	+ 2 ml	0^{c}	0^{c}	45^{c}	147^{b}
	+ 5 ml	0^{c}	0^{c}	30^{d}	155^{b}
Pirimiphos-methyl	5.0 µl				
+ Soybean	+ 1 ml	0^{c}	0^{c}	47^{c}	154^{b}
	+ 2 ml	0^{c}	0^{c}	46^{c}	157^{b}
	+ 5 ml	0^{c}	0^{c}	30^{d}	152^{b}

DISCUSSION

The vegetable oils applied at 2, 5 and 10 ml kg^{-1} and pirimiphos-methyl at 1/4, 1/8 and 1/16 of the recommended dosage (10, 5 and 2.5 μl in 2.0 ml of water kg^{-1} of grain) caused significant mortality of *C. maculatus* within 24 hour exposure compared to untreated controls. Similarly, the mixtures of 1, 2 and 5 ml of each oil kg^{-1} and pirimiphos-methyl at 1/16 and 1/8 of the recommended dosage (2.5 and 5 μl) were highly toxic to *C. maculatus*. Dead insects from oil treated grains showed signs of rapid immobilization with their legs flexed and clinging to either the grain or the surface of containers whereas those from pirimiphos-methyl treated grains appeared paralysed with their metathoracic wings unfolded and stretched outside the elytra. Several pervious studies have demonstrated the effectiveness of different vegetable oils in protecting grains against major stored product insect pests (Don-Pedro, 1987; Kumar and Okonronkwo, 1991; Obeng-Ofori, 1995). Although, the mode of action of vegetable oils is not clearly understood, it has been suggested by Don-Pedro (1989) that insect death caused by oils is due to anoxia or interference in normal respiration resulting in suffocation (Schoonhoven, 1978).

The decrease in mortality with storage following treatment with vegetable oils obtained in the present study supports the above mechanism. The oils when applied alone were not toxic to the beetles exposed only 15 days after application. Pirimiphos-methyl and the mixtures, however, remained effective after treated seeds had been stored for 60 days. Presumably, with storage the oils were absorbed by the grains, thereby reducing its availability for pick up by the beetles (Tembo and Murfitt, 1995). Furthermore, the oils could also act as antifeedants or modify the storage micro-environment thereby discouraging insect penetration and feeding (Obeng-Ofori, 1995).

The efficacy of the oils and pirimiphos-methyl alone on the beetles was dose dependent with higher doses providing greater protection for a longer period. The practical utilization of vegetable oils as grain protectants is limited by the high rates required to disinfect grains (Don-Pedro, 1989). In this work, vegetable oils applied at the rate of 10 ml kg^{-1} gave the most effective control of the insects where oils alone were tested. Vegetable oils and reduced doses (1/4, 1/8 and 1/16) of the recommended dose of pirimiphos-methyl when applied alone were less effective than when combined.

The protectant potential of low doses (1 and 2 ml kg^{-1}) of the oil and reduced levels (1/8 and 1/16) of pirimiphos-methyl applied as mixtures were more potent than the oils alone at 10 ml kg^{-1} or the pirimiphos-methyl at 10 μl against *C. maculatus*. The mixing of the oils with pirimiphos-methyl enhanced chemical toxicity and persistence, hence, the higher mortality of adult beetles in cowpea grains treated with the mixtures. Oil and insecticide mixtures also completely inhibited the development of the egg, early and late larval stages of the beetles compared to the treatment with the oil or pirimiphos-methyl alone in which only the eggs and the early larval instars were killed. The reasons for the enhanced toxicity and persistence of the mixed products is probably because the oils increased uniformity of distribution of the toxicant over the grain surface thereby increasing the probability of insect contacting a lethal dose of the chemical (Salt and Ford, 1994). Furthermore, the oils can increase the rate of insecticide penetration into insect cuticle (Anderson *et al.*, 1986).

The application of oil/insecticide mixtures may minimize insecticide usage and hence reduce health hazards to applicators and reduce the amount of insecticides used to protect stored products. Furthermore, the use of reduced rates of oils (*e.g.*, 1 or 2 ml kg^{-1} when combined with insecticide) will make their utilization more economical and attractive. The mode of action of vegetable oils needs to be studied in more detail to promote the development of more potent fractions for use as grain protectants. Treatment of grains with vegetable oil/insecticide mixtures could have important practical applications in developing countries where insecticides are expensive, in short supply or where vegetable oils are readily available. We have initiated a comprehensive large-scale evaluation of the practical utilization of vegetable oil/insecticide mixtures to protect farm-stored grains in Ghana.

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