CONTROL OF THE COWPEA BEETLE *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: BRUCHIDAE) ON STORED COWPEA USING VEGETABLE OILS

By

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DECLARATION

I, hereby declare that, except for references to other people's work which have been duly cited, this work is the result of my own research and this thesis has not been presented either in whole or in part for any degree elsewhere.

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ABSTRACT

Coconut oil and two types of palm oil (Palmin oil and Frytol oil) were evaluated for the control of the cowpea beetle, *Callosobruchus maculatus* (F.) on stored cowpea. The oils were applied at the rate of 4, 6 and 10 ml per kg cowpea. Oil treatment reduced oviposition and progeny emergence but did not affect the longevity of the adult insects. The highest dosage of 10 ml per kg seed protected the seeds for five months. Palmin oil appeared superior to coconut and frytol oil. The oils had no adverse effect on seed viability, cooking time and taste of the cooked beans after six months storage. Mould growth was, however, detected in infested oil-treated seeds stored in cotton sacks. Nutritional analysis showed that the oils did not affect the nutritional content of the oil-treated seeds and the oils did not also go rancid after the 6 months storage.

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DEDICATION

To my parents Mr. E. A. Osekre and Madam Faustina Okai Okang.

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CHAPTER ONE

1.0 INTRODUCTION

Pulses are a major source of protein in Asia and West Africa (Pereira, 1982) and most developing countries. The cowpea, *Vigna unguiculata* (L.) Walps., is one of such important grain legumes in West Africa and a major source of protein (Stanton, 1966). Singh and Rachie (1985) reported that the crop is grown extensively in sixteen countries including Nigeria, Niger, Burkina Faso, Uganda, Ghana, etc. Its widespread cultivation depends largely on the fact that in Africa, cowpea represents the legume of choice for many homes, in the face of the high cost of animal protein. In Ghana, annual production of cowpea is estimated to be about 30,000 tons (Rawnsley, 1964; PPME, 1989), and it is the second most important food legume cultivated in Ghana after groundnut (Tanzubil, 1986). Efforts to increase the production of cowpea are hampered by insect pests, encountered in the field and during the storage of the harvested produce.

The most important pest of stored cowpea is the cowpea beetle, *Callosobruchus maculatus* (F.), which has a world-wide distribution (Singh *et al.*, 1978). Singh and Rachie (1985) reported that farm storage of cowpea for six months is often accompanied by about 30% loss in weight, with up to 70% of the seeds being damaged, rendering them virtually unfit for consumption. Grain damage could also reach 100% if the insects are not controlled (Owusu-Akyaw, 1991).

The serious damage caused to the grain by the pest has led to extensive use of synthetic insecticides to control it. In West Africa where the bulk of food consumed is produced by small-scale farmers using traditional methods, there is widespread misuse of insecticides with serious consequences (Ahmed *et al.*, 1984). Tembo and Murfit (1995) also expressed concern about the development of resistant strains, toxic residues, workers' safety, erratic supply and the prohibitive cost of synthetic insecticides.

These problems associated with the use of synthetic insecticides, have stimulated over the years, interest in finding alternatives to their use. Vegetable oils are reported as one of such viable alternatives to control some insect pests of stored products, including *C. maculatus (*Singh *et al.*, 1978; Don-Pedro, 1987; 1989).

The potential of these oils for the control of this pest had prompted several trials to determine their efficacy as protectants of cowpea against pest damage (Mital, 1971; IITA, 1975, Singh *et al.*, 1978; Golob and Webley, 1980; Messima and Renwich, 1983; Anon, 1985).

However, the effectiveness of the oils for the control of the pest has varied from one worker to the other. There are also some contradictory reports on the effect of oils on seed viability, taste and flavour of oil-treated grains. There was therefore the need to re-evaluate the efficacy of the oils for protection of cowpea against pest infestation. It was against this background that this study was initiated with the following objectives;

1. re-evaluation of the effectiveness of some vegetable oils commonly available in Ghana.
2. determination of the optimum dosage level of the oils that can give adequate protection and the appropriate duration for which they can be effective.
3. identification of the particular stage(s) of the insect which are affected by oils.
4. determination of any undersirable properties, if any, (for example mouldiness) that the oils may impart to the treated seeds under storage conditions.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 The cowpea beetle. *Callosobruchus maculatus* (F.) (Bruchidae)

Callosobruchus maculatus (F.) is a Coleopteran belonging to the family Bruchidae. The family contains many field and storage pests. The larvae of most species develop in the seeds of leguminous crops. The most valuable feature in the recognition of bruchid species is the arrangement of teeth and ridges on the hind femur (NRI, 1991).

C. maculatus, which does not attack *Phaseolus* spp (in the strict sense), is a small beetle, about 4-5 mm long. The body, which is clothed in short hairs, is compact. The elytra cover all but (unlike other similar insects) the last abdominal tergum (called the pygidium) and the antennae are relatively long.

It has a pair of distinct ridges (inner and outer) on the ventral side of each hind femur and each ridge bears a tooth near the apical end. The inner tooth is triangular, and equal to (or slightly longer than) the outer tooth. The antennae of both sexes are slightly serrated. Females often have strong markings on the elytra, consisting of two large lateral dark patches mid-way along the elytra and smaller

patches at the anterior and posterior ends, leaving a pale brown cross-shaped area covering the rest. The males are much less distinctly marked.

The adult beetles, which do not feed on stored products, are short-lived (usually no more than 12 days under optimum conditions) and during this time the females lay many eggs (up to 115), although oviposition may be depressed in the presence of previously infested seeds (NRI, 1991). The optimum temperature for oviposition is 30-35°C. As the eggs are laid, they are firmly glued to the surface of the host seeds, smooth-seeded varieties being more suitable for oviposition than rough-seeded varieties with oval flat bases. When newly laid they are small, grey and inconspicuous. Upon hatching, the larva bites through the testa of the seed and into the cotyledons. The developing larva feeds entirely within a single seed, excavating a chamber within the cotyledons as it grows. The optimum development conditions are around 32°C and 90% r.h. and the minimum development period of *C. maculatus* breeding on cowpea seeds is about 36 days, pupation taking place within the seed 26 days after oviposition (NRI, 1991).

The infestation of cowpea by the insect may start from the field, where eggs are laid on pods. As the pods dry, the pest's ability to infest them decreases. Thus dry cowpeas stored in pods are less susceptible to attack than threshed cowpeas. The insects reduce the food value, cause loss of grain weight, loss of market value and loss of viability of the seeds when they attack the grain. Singh and Rachie (1985) reported that the insects often cause about 30% loss in weight of stored cowpea seed, with up to 70% of the seeds rendered virtually unfit for consumption after farm storage for six months.

2.2 Grain protection with plant oils

Plant products including oils have played an important part in traditional methods of protection against crops pests and disease vectors in Africa (Stoll, 1988; Poswal and Akpa, 1991). The precise strategy used by different communities varies from place to place and appears to depend partly on the type and efficacy of suitable plant materials available in different locations (Hassanali *et al.*, 1983). The use of plant oils in the control of storage insect pests is an ancient practice (Yun-tai and Burkholder, 1981) and is particularly appropriate in small-scale storage systems. Pereira (1983) in support of this claim, added that mixing oils obtained from locally available plants with pulses has been an ancient Indian method of protection against bruchid attack. Treacy *et al.*, (1991) observed that vegetable and petroleum oils are commonly used as diluent adjuvants for insecticides to enhance their activity. In recent years, attention has been given to the use of vegetable oils as stored grain protectants against insects (Golob and Webley, 1980; Qi and Burkholder, 1981; Pereira, 1983; Ivbijaro *et al.*, 1984; Signamony *et al.*, 1986; Don-Pedro, 1989; Hall and Harman, 1991; Stamopoulous, 1991). Several other workers have reported that vegetable oils provided sufficient protection to cowpea for varying durations, and Golob and Webley (1980) concluded that it is now well established that vegetable oils are very effective in controlling certain species of bruchids on pulses by their ovicidal effect. The widely used oils are palm oil, palm kernel oil, coconut oil, sunflower oil and soybean oil.

2.2.2 Effective oil levels against the insect

Most workers recommended oil dosage levels of between 5-10 ml per kilogram of the grains for adequate protection and at the recommended highest dose of 10 ml/kg protection lasted for more than 5 months. It appears dosage levels lower than 5 ml/kg were not high enough to effect good coating to give adequate protection for more than 16 weeks of storage. In an efficacy test on four different oils, for example, Singh *et al.*, (1978), showed that levels of 1 ml/kg did not give adequate protection after six months storage (Table 1).

Table 1. Percent protection (uninfested cowpea seeds) provided by various edible oils against the cowpea beetle, *C. maculatus* after six months storage (After Singh *et al.*, 1978)

Edible Oil	Oil dosage (ml/kg)			
	1	3	5	8
Groundnut	81.5	94.5	100.0	100.0
Castor oil	77.0	90.0	99.0	100.0
Coconut oil	63.3	82.0	95.0	100.0
Palm kernel oil	59.8	76.8	92.0	100.0

Anon (1980) reported that oil applied at 4 ml/kg of seed recorded a 6.8% seed damage at the end of 18 weeks infestation period while the control recorded a 100% seed damaged Barreto *et al.*, (1983) noted that soybean oil offered protection against *Acanthoscelides obtectus* (Say) in beans at the doses of 2 ml and 4 ml/kg for only four months. Obeng-Ofori (1995) also reported that at 1 ml/kg, none of cotton seed, soybean, corn, groundnut and palm oils had a significant effect on adult mortality of *Cryptolestes pusillus* (Schonherr) and *Rhyzopertha dominica* (Fabr.).

2.2.3 As stated earlier, the effectiveness of these oils for the control of beetles vary from one worker to the other. Taylor and Evans (1980) reported that using 5 ml of palm and groundnut oils protected cowpea for 8 months. Pereira (1983) also reported that after three months storage, neem oil at 3 ml/kg cowpea significantly reduced the expected emergence of the progenies of *C. maculatus* and this activity was retained even after six months storage. In furtherance of this assertion, he maintained that even at 1 ml/kg of bambara, groundnut oil significantly reduced the expected emergence after 3 and 6 months storage. Pandey *et al.*, (1981) reported that neem oil at 3% provided complete protection for up to 135 days and 1% neem oil gave good protection for up to 90 days.

Neem seed oil showed 100% control of *Callosobruchus chinensis* for 5 months when applied at 10 ml/kg of cowpea (Das, 1986; Das and Karin 1986; Das, 1987). Findings at the International Institute of Tropical Agriculture (IITA, 1975) are confirmed by the reports of Taylor and Evans (1980), when they noted that 5-10 ml/kg could adequately protect the grains for several months.

2.2.4 Effect of oils on oviposition

Several workers have reported that plant oils cause significant reduction in oviposition in *C. maculatus*, *Sitophilus zeamais* and *Prostephanus truncatus* (Singh *et al.*, 1978; Pereira, 1983; Sujatha and Punnaiah, 1985; Yadav, 1985; Babu *et al.*, 1989; Don-Pedro, 1989; Pacheco *et al.*, 1995). Singh *et al.*, (1978) found that the mean number of eggs laid by *C. maculatus* on groundnut oil treated seeds was significantly less than that on the untreated control. Don-Pedro (1989) however explained that although oils had no significant effect on oviposition in *S. zeamais*, groundnut oil, applied at 14 ml/kg significantly reduced total oviposition. In another experiment, X-ray radiographic study showed that the oils had no signficant effect on oviposition in *S. zeamais* in non-choice experiments. No reasons were, however, given for this observation. Pereira

(1983) reported that 8 ml neem oil/kg seed significantly reduced oviposition of *C. maculatus* on cowpea, while on bambara groundnut, neem oil, palm oil and industrially extracted groundnut oil significantly reduced oviposition, even at 3 ml oil/kg seed, and karite oil was effective at 5 ml/kg seed. In contrast, Pacheco *et al.*, (1995) found that oviposition of *C. phaseoli* (Gyllenhal) was not affected by treatment of the seeds with castor and soybean oils.

2.2.5 <u>Toxicity of oil</u>

There appears, however, to be less agreement on the effect of oils on insect mortality or longevity. For example, while some workers (Singh *et al.*, 1978; Tikku *et al.*, 1981) reported that vegetable oils had no effect on mortality or longevity of adult bruchids, others (Hill and Schoonhoven, 1981; Ivbijaro *et al.*, 1985) reported a high level of toxicity of oils against adult bruchids and *Sitophilus oryzae* (L.). In a similar study, using adult *S. zeamais,* Don-Pedro (1989) noted that freshly applied oils caused appreciable mortality only at 14 ml/kg or more. This was in agreement with the findings of Qi and Burkholder (1981) who demonstrated that significant mortality occurred only when adult *S. granarius* (L.) were exposed to grains freshly treated with 10 ml/kg of groundnut oil or more. This confirmed the work of Don-Pedro (1989) that oils have no contact chemical toxicity against active adults unless these were trapped in large oil droplets. Similarly, Salas (1985) using 10 ml castor oil per 1 kg of maize obtained 100% mortality of *S. oryzae* within 3 months. Ketkar (1987) also obtained 100% mortality of cowpea beetle in cowpea seeds treated with coconut oil at 10 ml/kg grain. Salas and Hernandez (1985) also observed 100% mortality of *A. obtectus* within one hour of treatment with castor oil applied at 10 ml/kg of pigeon pea seeds.

2.2.6 Effect of oil on insect development

Singh *et al.*,(1978), Pereira (1983), Babu *et al.*, (1989), Don-Pedro (1989), Khaire *et al.*, (1992) and Pacheco *et al.*, (1995) all noted that oils caused inhibition of insect development. For instance, Pereira (1983) reported that after three months storage, neem oil, at 3 ml/kg cowpeas or bambara groundnuts, significantly reduced the expected emergence of *C. maculatus* and this activity was retained even after six months storage. It was further observed that, with cowpea treated with 5 ml neem oil/kg seed, no adults emerged after 90 days storage. However, when the testa was peeled off 5 and 90 days after treatment, most of the expected adults emerged from seeds treated with castor, neem and karanj oil at all concentrations, suggesting that the oils offered protection by acting as an ovicide on the surface of the grains, but did not penetrate the grain to affect other stages of the insects. They further reported that 66 days after treatment, neem oil at less than 0.75% and 1.00% levels were the most effective with no emergence of adult progeny. Don-Pedro (1989) noted that neither groundnut nor traditional coconut oils at 14 and 17.5 ml/kg had a significant ($P>0.05$) effect on adult emergence when applied to grains containing late stage larvae or pupae of *C. maculatus*.

2.3 Studies on other aspects of oil treatment

Effect of oil treatment on mould growth, taste and flavour and viability of seeds had also been investigated by various workers (Mummigatti and

Ragunathan, 1977; Singh *et al*., 1978; Cruz and Cardona, 1981; Hill and Schoonhoven, 1981b; Rashid, 1985; Babu *et al*., 1989; Khaire *et al*., 1992; Obeng-Ofori, 1995 and Pacheco *et al*., 1995).

On mouldiness, Babu *et al*., (1989) noted that applying neem oil to grain with an unacceptably high moisture content before storage could lead to the production of aflatoxin formation, since high humidity situation would be created in the grain mass and thus enhance fungal and bacterial colonization.

On seed viability, while Mummigatti and Ragunathan (1977), Singh *et al*., (1978), Varma and Pandey (1978), Adu (1986) and Obeng-Ofori (1995) observed that oils did not reduce seed viability after storage Echendu (1991), Yun-tai and Burkholder (1981), and Tembo and Murfitt (1995) reported that oil treatment reduced germination of wheat. Khalique *et al*., (1988) observed that, for the protection of stored chick-pea, *Cicer arietinum* L., against *C. chinensis* the application of mustard oil at a dose of 10 ml/kg was effective for five months storage, and did not affect seed viability, but the oil caused complete inhibition of germination of the Kabuli type of the peas.

The effect of oils on flavour, nutritive and cooking properties of oil-treated seeds had been investigated by several workers. Cruz and Cardona (1981), Hill and Schoonhoven (1981) and Pacheco (1995) reported that oils did not alter these aspects of the seeds. The nutritive value of the treated seeds was retained when prepared for consumption. Funke (unpublished) found that soybean, corn and peanut oils did not alter flavour, texture or cooking time of beans stored for 90 days after treatment. On the other hand, Cruz and Cardona (1981) reported that soybean and corn oil improved the palatability of cowpea.

2.4 Effects of plant oils on insects

Many workers have assigned various effects of the oils on insects that may have led to less or no damage to grains. These effects have ranged from physical, through repellent action, to chemical toxicity to all stages of insect development. Green *et al.*, (1987) noted that the oils may affect insects by flooding their spiracles causing asphyxiation. Hewlett (1975) and Schoonhoven (1978) suggested that the toxicity of oils may be due to the oils entering the trachae of the insects and thereby suffocating them. However, other effects other than oxygen starvation probably also play a role (Schoonhoven, 1978; Shaaya and Ikan, 1978). Varma and Pandey (1978) and Babu *et al.*, (1989) noted that oils act as repellents and ovicides. Su *et al.*, (1972) attributed mortality to toxic and physical effect, which cause changes in surface and oxygen tension in the eggs. Interestingly, Punj *et al.*, (1990) explained that oils retarded development of the insects. Mital (1971) reported that the effects of groundnut and palm kernel oils were due to their peroxide content and high surface tension. Pereira (1983), Qi and Burkholder (981) and Zhang and Zho (1983), suggested reduced oviposition, or egg hatch or both.

It was also thought that oils exert some lethal action on developing embryos or first instar larvae, for example, by the reduction in the rate of gaseous exchange due to a 'barrier' and/or direct toxicity by penetrated oil fractions (Don-Pedro, 1989). Credland (1992) presented a hypothesis that occlusion of the funnel by some oils is the reason for their ovicidal and perhaps larvicidal effect. Another hypothesis is that oil infiltration under the operculum may block respiration or disrupt the water balance of eggs and developing embryo (Messina and Renwich, 1983). Shaaya *et al.*, (1976), postulated that fatty acid chain lengths are related to the amount of insect control. Hill and Schoonhoven (1981 a, b,) also attributed the insecticidal activity of oils to the triglyceride and oleic acid content. Obeng-Ofori (1995) postulated that oils could also act as an anti-feedant or modify the storage micro-environment, thereby discouraging insect penetration and feeding.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of cowpea seeds and oils

The cowpea variety (Black-eye) was used for this study because of its popularity in the country, and also its susceptibility to the cowpea beetle. Insecticide-free and undamaged seeds were obtained from the Ghana Food Distribution Corporation, Accra..

Three vegetable oils, coconut and two types of palm oil ("Palmin" oil and "Frytol" oil) were used. Palmin oil is an industrially manufactured palm oil while Frytol is a refined and deodorised palm oil. These two types of oils were chosen because they are becoming more popular and gradually replacing the traditionally prepared ones, especially groundnut oil. They were both purchased from a distributor of Lever Brothers Ghana Limited, the manufacturers. Coconut oil was bought from the Makola Market in Accra. They were stored in a room maintained at 25±2°C and 65-70% r.h.

3.2 Culturing of experimental insects

500 ml Kilner jars and cowpea grains were kept in oven at 60°C for 2 hours for sterilization. These were used for the experiments. 750g of the sterilized cowpea grains were put in 10 separate previously cleaned 2-litre Kilner jars. One hundred adult cowpea beetles *C. maculatus* previously obtained from heavily infested cowpea from the market, were introduced into each of the jars and covered with muslin held in place by the covers of the jars. This ensured that aeration was maintained in the jars and it also prevented the escape of introduced

insects . The jars were placed in plastic trays containing industrial oil so as to restrict movement of mites and other insects that might attempt to invade and contaminate the cultures. The cultures were maintained in a laboratory at 25±2°C and 65-70% r.h. On the 20th day after the cultures were set up, the jars were emptied and all the insects (both dead and alive) were removed and the jars refilled with the same grains. From the 30th day of each culture the first adults began to emerge and these were used for the different experiments described below.

3.3 Effect of the oils on oviposition

Eight replicates of 0.02 ml, 0.03 ml and 0.05 ml each of coconut, frytol, and palmin oil were coated separately on 5g undamaged cowpea grains. These were equivalents of 4 ml, 6 ml and 10 ml/kg, respectively. Three pairs of 0-1 day old adults of the insects from the cultures were introduced into the Kilner jars covered with muslin held in place by the jar covers. Also, three pairs each of 0-1 day old adults were put into eight jars containing 5 g cowpea grains which have not been treated with the oils to serve as the control. Taking into consideration the fact that the adults are short-lived and therefore the female lays eggs during the early days, and also, the eggs take about six days to hatch, the grains from each jar were picked individually and the eggs (which are whitish in colour after hatching) were counted seven days after infestation. The inability of the insects to lay many eggs on the oil-treated grains prompted further investigation as to whether the insects mated or did not mate at all.

For this study, a pair of freshly-emerged adults were put into 25 separate small vials which had the open end sealed with cellotape. Four holes were punctured in the cellotape on each vial to ensure aeration within the vials. The paired insects were closely observed until each pair had mated. Twenty grams of cowpea seeds were admixed with 0.10 ml of palmin oil and 5 g of the oil-treated seeds were put into 4 separate petri dishes. Five already mated females were introduced into each of the petri dishes and the other five introduced into a petri dish containing 5 g untreated seeds. These were kept for 8 days, and the eggs in each dish were counted.

3.4 Effect of the oils on emergence of F_1 progeny

The experimental set-up described above for oviposition was maintained for 30 days after which F_1 progenies started to emerge. On the 40^{th} day the emerged adults in each jar were counted. These numbers expressed over the number of eggs laid in the respective jars, gave the percentage emergence. The efficacy of each treatment was determined by comparing numbers of F_1 progenies in the treated grains with those in the untreated control.

3.5 Effect of oils on the longevity of insects

A separate set-up using similar quantities of grain, dosage levels, number of replicates (Section 3.3) and five pairs of 0-1 day old adults was maintained. Based on the mortality rate of the insects obtained from a previous investigation, mortality counts of the insects were done 4, 7 and 14 days after the insects had been introduced into petri dishes containing either oil-treated or untreated seeds (control treatment). Thus the petri dishes were inspected daily, and insects that had died in each petri dish were removed. Insects which did not move or had uncoordinated movement when probed with a hair brush were recorded as dead. A cumulative number of insects found dead was taken on the specified days.

3.6 **Effect of Palmin oil on eggs**

Palmin oil which was easier to work with, because it took a longer time to solidify, and therefore made it easier for 1 µl of it to be taken, was chosen for this study. One hundred (100) undamaged cowpea grains were put in two separate Kilner jars, and 20 pairs of the insects were introduced into each jar. Grains with eggs glued to them in each jar were removed, grouping the eggs into 1-, 2-, 3- and 4- days age groups after they were laid. The eggs were identified with the aid of a microscope, as eggs less than five days old are normally not noticeable with the naked eyes. Because Singh *et al.*, (1978) had reported that the part of the egg in contact with the oil was important for this investigation, the anterior and posterior ends of each egg was determined by using a microscope. Ten eggs were picked for each treatment from the four age groups. For one set of the grains, approximately 1 µl of Palmin oil was carefully placed at the anterior ends of the eggs laid on untreated grains of the specified age groups. On each occasion, after placing the oil, the egg on the grain was observed under the microscope for some few minutes. The eggs were kept for 30 days after which new adults started to emerge.

3.7 **Effect of Palmin oil on larvae**

200 g whole cowpea grains were put into Kilner jars, and twenty 0-1 days old adult beetles were introduced into each jar. As eggs were laid, 20 grains with

egg(s) glued to them were individually picked and kept in petri dishes (88 mm diameter x 14 mm high). Five sets of these were maintained. Three experimental set-ups were maintained for 15, 17 and 19 days, respectively, taking first day of observation of egg on the grain as day zero, before they were transferred into the petri dishes. On these specified days the grains in each set of petri dishes were individually picked, cut open along the longitudinal plane and approximately 1 μ 1 of palmin oil was deposited on the developing larvae inside. The two halves of each grain were neatly patched together into one grain using paste made from ground cowpea mixed with water. A control treatment, where the grains were cut open the same way but no oil was deposited on the larvae before patching up, was also maintained (Fig. 1). It must be explained that it was noticed during preliminary investigations that it was very difficult to locate larvae earlier than the 15 days after oviposition, when longitudinal sections were made. On the 40th day the petri dishes were put into oven to kill all insects. All the emerged but dead insect in each case, were counted. All the grains were re-opened again and observations made on the effect of the oil on the insect as compared to the control, by looking at the larvae inside the grains.

Fig.1: Cowpea seeds rejoined into individual whole seeds; (left) - control; (right) - oil deposited on larvae

3.8 Protection offered bv the oils against *C. maculatus* on stored cowpea under various conditions

Cowpea seeds were treated with 1.0, 1.5 and 2.5 ml of each oil per 250 g cowpea which are the equivalents of 4, 6 and 10 ml/kg. The test seeds were exposed to cowpea beetle under four different storage conditions. Each treatment was replicated five times. In test one, treated and untreated seeds were kept in Kilner jars, covered with muslin held in place by jar covers and placed in trays containing industrial oil. 15 pairs of 0-1 day old beetles were used to infest each treatment. These were left for 6 months without interruption. In test two, the same procedure as outlined above was undertaken, but, in this case, the treatments were re-infested with 15 pairs of the insects after every forty days intervals.

In test three, in which only palmin oil was chosen because it appeared to be slightly superior to the other two oils, the treated and untreated seeds were kept in 11 cm x 20 cm x 1 cm cotton sacks and infested with 15 pairs of 0-1 days old insects. The bags were tied and after every forty days the sacks were untied and the grains re-infested with another 15 pairs of the insects. This test was designed to find out what would happen to the grains in such a container, as farmers normally store their produce in sacks.

In test four, which was designed to simulate farm storage conditions where insects move freely in farm store to infest produce, the palmin oil treated seeds and the untreated control were kept in 11 cm x 20 cm x 1 cm and infested with 15 pairs of 0-1 day old beetles. The sacks were loosely tied with twine in such a way that the insects could easily escape and/or enter every sack through the tied ends. It must be stated that this situation was actually observed to have taken place, as after the second month of initial infestation, the insects were seen invading all the sacks. No re-infestation was intentionally applied to this test, and they were stored for five months and percent infestation was determined. All grains with one or more holes were counted as infested.

3.9 Effect of oil on cowpea seed viability

Cowpea seeds were treated with the different oils at rate of 10 ml/kg. This dosage level was chosen as that is reported to offer adequate protection for at least five months (Taylor and Vickery, 1974; Varma *et al.*, 1976; Singh *et al.*, 1978; Golob and Webley, 1980). Five seeds were collected from each of 36 jars containing either oil-treated seeds or seeds not treated with oils (control) and bulked according to the three oils. Four replicates were maintained for each treatment. The bulked samples were placed separately on moist filter paper in petri dishes. After five days they were inspected and the number of grains that germinated in each dish were counted. The remaining treated and untreated seeds were put in Kilner jars, covered with muslin held in place by jar covers and left free from infestation for five months. Viability test using the method already described was conducted.

3.10 Cooking properties of the treated grains

500 g undamaged seeds treated with 5 ml of the different oils were kept in three Kilner jars for six months. The same quantity of the untreated seeds was also kept for the same period. They were then separately boiled, and the time taken to cook well was recorded. The cooked grains were served to four people (two females and two males) to consume and to comment on flavour and taste.

3.11 **Mouldiness of treated grains**

All experimental set-ups maintained were critically examined and random samples of the grains were taken with a spoon from the bottom and the top of the grain mass in the jars and observed under the microscope for development of mould.

3.12 **Effect of oil-treatment on nutritive content of the seeds**

500 g of whole seeds were treated with 5 ml of palmin oil and stored in Kilner jar. The same quantity of seeds without oil treatment was also maintained as control. No insects were introduced into the jars. These were maintained for six months. 200 g each of the oil-treated and untreated seeds were ground into powder using a mill and were used for nutritional analysis.

3.12.1 **Determination of total ash**

Four silica dishes (about 7 cm diam.) were put in an oven at 100°C for eight hours after which they were removed. After cooling, they were weighed. 2 g of each sample (ground untreated or ground oil-treated grains) were weighed into one silica dish. The dishes and their contents were placed in a cold muffle furnace fitted with a temperature controller. The furnace was then switched on and left overnight. The following day, the dishes were removed and kept in a desiccator. After cooling, they were removed and the dishes with their contents reweighed. The weight of ash in each case was then calculated as follows;

$$\%\ \text{Ash} = \frac{W_A - W_T}{W_S - W_T} \times 100$$

Where W_A = weight of crucible and ash
W_T = weight of cruicible
W_S = weight of crucible and ground sample

3.12.2 Determination of crude protein

Crude protein was determined by first determining the percentage nitrogen in each sample, before determining the percentage protein using a conversion factor. For the percentage nitrogen determination, six Kjeldahl digestion flask were thoroughly cleaned. One filter paper each was put into two of the Kjeldahl digestion flasks which were used for blank titration. One gramme of the oil-treated ground grains was weighed into two small filter papers. The filter papers were wrapped around the samples and dropped to the bottom of two other Kjeldahl digestion flasks. The same quantity of untreated grains was weighed into two separate filter papers. They were also wrapped and dropped to the bottom of the other two Kheldahl flasks. To lumps of copper sulphate and 40 ml concentrated sulphuric acid were added to each flask.

Each flask with contents was heated gently in an inclined position for the contents to start frothing, and heating continued until frothing ceased. The flasks were removed and placed on burners for their contents to boil briskly for 2 hours, after which they were removed. After cooling, approximately 200 ml water and 25 ml sodium thiosulphate solution were added to each flask and stirred gently for the contents to mix. A piece of zinc was placed in each flask and sufficient 0.1M sodium hydroxide solution was carefully poured down the side of each flask to make the contents strongly alkaline. The flasks were connected in turns to a distillation apparatus incorporating an efficient splash head and condense (Fig. 2).

A delivery tube which drips just below the surface of a pipette volume of standard acid contained in a conical flask receiver was fitted to the condenser. By the operation of the distillation apparatus, the contents of the digestion flasks mixed, then boiled until at least 150 ml of each sample had distilled into the receiver. Five drops of methyl red indicator were added to each of the two flasks designated for blank titration. Each was then titrated with 0.1M hydroxide solution. The same titration procedure was applied to the other four samples. The percentage crude protein was calculated using the formular below:

$$\% \quad \text{Nitrogen} = \frac{\text{Titre value x Normality of acid x 0.014}}{\text{weight of sample}} \text{ x } 100$$

$$\% \quad \text{Protein} = \% \text{ Nitrogen x } 6.25$$

Where 6.25 is a conversion factor for cowpea

3.13.3 Determination of crude fibre

A more selective procedure using 3% sodium lauryl sulphate without acid to determine Neutral Detergent Fibre (NDF) was used. This gives a better yield of dietary fibre because of greater recovery of celluose, hemicellulose and lignin (Van Soest and Wine, 1967). One gramme each of air-dried sample ground to pass through 0.40 mm mesh was weighed into a bezeious beaker. 100 ml of cold neutral detergent solution, 2 ml decahydronaphthalene and 0.5 g sodium sulphite were added in that order. Each sample was heated to boil for 5-10 minutes, reducing heat as boiling began, to avoid foaming. Each sample was refluxed for 60 minutes, timing from onset of boiling. Each beaker was swirled, and contents poured into previously weighed crucible. Vacuum was then admitted until after crucible had been filled, using low vacuum at first, increasing it only as more fibre was needed. Samples were separately rinsed into crucible with 50 ml of hot (80-90°C) distilled water. Vacuum was removed, mat broken up, and crucible filled with hot water. The resulting liquid was filtered and washed repeated for each sample. Acetone was then used to wash samples twice and sucked dry. Each crucible with contents was dried at 100°C overnight, cooled in an desiccator and weighed. The yield of recovered neutral detergent fibre was calculated as:

$$\% W_C = \frac{W_t - W_o}{1} \times 100$$

Where Wc = cell wall constituents (% crude fibre)
Wt = weight of crucible + cell wall constituents
Wo = weight of crucible

3.13 Rancidity of the oils (Coconut, Palmin and Frytol oils)

Rancidity of the oils was investigated by first extracting the oil which had coated the grains. 250 g of oil-treated and untreated grains which had been stored for six months were used. Each sample was divided into two portions and subjected to the following treatments in turns. Each sample was poured into

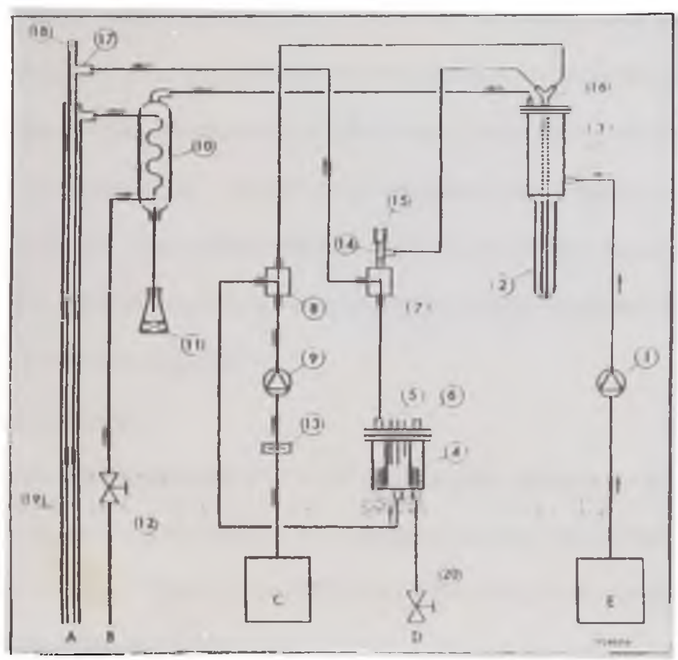

FIG 2: A bioassay system for the determination of protein content of the grains.

1. Alkali pump. 2. Test tube. 3. Splash head. 4. Steam generator. 5. Level pin. 6. Over heat protector. 7. Steam valve. 8. Water valve. 9. Water pump. 10. Condenser. 11 Receiver flask. 12. Water saving valve. 13. Water filter. 14. T-joint. 16. Non-return valve. 16. Y-joint. 17. T-joint. 18. Non-return valve. 19. Waste tube. 20. Drain valve. A. Drain B. Cooling water inlet C. Water tank D. Drain Steam Generator E. Alkali tank.

separating funnel and 100 ml of chloroform and 50 ml of methanol were added and shaken vigorously for two minutes, then 100 ml of water added and shaken again. The chloroform column was separated from the water column by first collecting the water portion into a flask and the chloroform portion into another flask. The chloroform layer was supposed to collect lipid soluble constituents into a mixture, that is, extract the oil together with any other related constituents from the grains. The extracts were bulked according to the treatments. Each bulked extract was concentrated into a vial using a rotary vacuum distillator, then dried using nitrogen gas. Distilled water was added to each sample to make the volume to 30 ml. Each sample (admixture of extracted oil from the treated grains and water, and solution from untreated grains) was shaken vigorously and the p^H of each determined using a p^H meter.

3.14 **Statistical analysis**

The data were transformed to either square-root values or Arcsin (in cases where values were in percentages) and analysis of variance was performed on the transformed data. Treatment means were compared using Fisher's protected least significant difference (LSD).

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Effect of oils on oviposition

The results of this experiment showed that the oils deterred oviposition. There were significant differences in the percentage reduction in oviposition of the beetle by the different oils (Table 2). There was also significant reduction in oviposition as the oil dosage increased. These results agree with those reported by other workers (Singh *et al*, 1978; Messina and Renwich, 1983; Sujatha and Punnaiah, 1985; Yadav, 1985, Babu *et al.*, 1989; Pacheco *et al.*, 1995), but contradict the observation made by Don-Pedro (1989) that, in a no-choice experiment oils had no significant effect on total oviposition. The reduction in oviposition on the oil-treated seeds may be due to the presence of oil film on the seeds, which presented an unsuitable surface for oviposition. Don-Pedro (1989) demonstrated that oils had no significant effect on total oviposition in a no-choice experiment, but a contrary observation was made in a two-way choice chamber. This suggests that the insects prefer clean seeds to oil-treated ones when they have a choice, but where there is no alternative, they still lay eggs on oil-treated seeds.

Table 2: Percentage reduction in oviposition of *C. maculatus* by different oil treatments.

	Dosage ml/kg		
Treatments	4	6	10
Palmin oil	42.8±1.02^{a}	58.7±0.38^{bc}	88.6±0.0^{d}
Coconut oil	48.0±0.78^{b}	60.3±0.42^{c}	86.7±1.16^{d}
Frytol oil	54.0±0.62^{b}	57.2±0.81^{bc}	65.3±2.53^{c}

Values followed by the same letter are not significantly different (P>0.05)

Means ± SEM. SEM = Standard error of mean.

4.2 <u>Effect of oils on progeny emergence</u>

The results obtained showed that the three oils reduced progeny emergence. Percentage reduction in progeny emergence offered by the different oil treatments was significantly different and there was also significant reduction in progeny emergence as the oil dosage increased (Table 3). These results are in agreement with the observations made by Singh *et al*, (1978), Pereira (1983), Babu et al., (1989); Don-Pedro (1989), Khaire *et al.*, (1992) and Pacheco *et al.*, (1995), that oils significantly reduced the expected emergence of *C. maculatus*. However seeds treated with the oils at all the concentrations (seven days after eggs were laid), resulted in emergence of adults. This shows that the oils are only effective when applied before eggs are laid or before eggs hatch into larvae, since the eggs take about six days to hatch. This confirms the findings of Don-Pedro (1989) that groundnut and traditionally prepared coconut oils applied at 14 and 17.5 ml/kg had no significant ($P>0.05$) effect on adult emergence when applied to seeds containing late stage larvae/pupae. The oils primarily affect the eggs rather than the larvae or pupae, which live mainly inside the seed, where they may not come into contact with the oil.

Table 3: Percentage reduction of *C. maculatus* F1 progeny emergence by different oil treatments.

Oil	Dosage ml/kg 4	6	10
Palmin oil	55.5±0.31[a]	100±0.0[c]	100±0.0[c]
Coconut oil	69.9±0.20[a]	81.5±0.16[b]	100±0.0[c]
Frytol oil	65.2±0.21[a]	75.3±0.12[b]	100±0.0[c]

Values followed by the same letter(s) are not significantly different ($P>0.05$)

Mean ± SEM   SEM = Standard error of mean

4.3 Effect of Palmin oil on eggs

The results presented in Table 4 showed that Palm oil has ovicidal effect depending on the part of the egg which comes into contact with the oil. In all situations where the oil was deposited at the anterior end of the egg, the embryo developed normally and penetrated the seed. However, when the drop of oil was deposited at the posterior end of the egg, they did not hatch into larvae. Closer observation under the microscope showed that the embryo "coagulated" within the egg. This activity of the oil at the posterior end of the egg suggests that oil penetrates into the egg to attack the developing embryo through the micropyle which is situated at that end.

Singh *et al.* (1978) also offered similar suggestion and added that the action of the oil might be due to changes in surface tension (which could lead to protoplasm coagulation) and in oxygen tension within the egg. Don-Pedro (1989) hypothesized that the oils exert some lethal action on the developing embryo or first instar larvae, for example, by the reduction in the rate of gaseous exchange due to a "barrier" effect and/or direct toxicity by penetrated oil fractions. These suggestions support the earlier observation that the destructive effect of the oil on the egg is one of the most important control activity against the proliferation of the insect. However, considering the fact that this activity also depends on the area of the egg that comes into contact with the oil, and the volume of oil that is adequate to effect good protection, admixture of the oil and seeds should be done properly so that the whole surface of the seed is coated. This may explain why smaller dosages are not very effective as protectants.

Table 4: Number of larvae that hatched from 10 eggs after 1 μl of Palmin oil had been placed separately on each end of the egg

	Age of Egg (days)			
Position of Egg	1	2	3	4
Anterior end	10^a	10^a	9^a	10^a
Posterior end	0^b	0^b	0^b	0^b

Means followed by different letter(s) are significantly different

4.4 Effect of Palmin oil on larvae

The results of this experiment showed that direct topical application of the oil was toxic to every stage of the larva (Table 5). The fact that over 90% of the larvae in the control treatment continued their development and emerged showed that normal development processes were not adversely affected when the grains were cut open and closed up again. The oil did not also stop the development of the larval or pupal stages of the insect when the seeds were treated with oil after the larvae had already bored into the seed. This confirms the observation made by Pereira (1983) that when the testa was peeled away 5 and 90 days after treatment, most of the expected adults emerged from the seeds treated with castor, neem and karanj oils at all dosages. The results showed that since larvae of *C. maculatus* develop inside the grain, topical application of oil, though very effective, is of little practical application if larvae had already bored into the grains.

Table 5: Number of larvae that developed into adults from 100 larvae after 1 μl of Palmin oil had been placed separately on the larvae inside the grain.

Treatment	Age of larvae (days) 10	12	14
Palmin oil	0.00^{b}	0.00^{b}	0.00^{b}
Control	95^{a}	90^{a}	96^{a}

Means followed by different letter(s) are significantly different

4.5 Effect of oils on longevity of the insects

The oils had no effect on the longevity of adult *C. maculatus*. In the control, the percent mortality of 67.5% was highest compared to 65.0%, 40.0% and 65.0% recorded for palmin, coconut and frytol oils respectively, after 4 days of storage (Figure 3). After 7 days 86.25% mortality was obtained in the control treatment, and nearly 90%, by the fourteenth day. These results confirm the findings by Singh *et al.*, (1978), Tikku *et al.*, (1981) and Don-Pedro (1989), that vegetable oils are not toxic to short-lived adult beetles. The contradictory reports by Hill and Schoonhoven (1981) and Ivbijaro *et al.*, (1984) that oils had effect on the longevity of the insect, was possibly due the insects drowning in large quantities (<10 ml/kg) of free surface oil, which, as explained by Singh *et al.*, (1978), could cause death of the insects by flooding their spiracles and causing asphyxiation, or entering the tracheae causing anoxia (Hewlett, 1975). In addition, it is not economical to use such high levels of the oils. These results confirm the point that control measures should aim at the egg which are more susceptible to the oil.

4.6 Protection offered by the oils against *C. maculatus* on stored cowpea under various condtiions

The results of these experiments showed similar degree of infestation to the seeds, whether in Kilner jars or in cotton sacks. There was significant reduction in infestation of the oil-treated seeds as the oil dosage increased (Tables 6 & 7). The insects caused more infestation when less than 10 ml of the oils per kg of seeds was used. When the oil-treated seeds were stored in Kilner jars, 100% infestation was recorded for all three types of oils using 4 and 6 ml/kg, and over 10% infestation, using 10 ml/kg (Table 6), as compared to

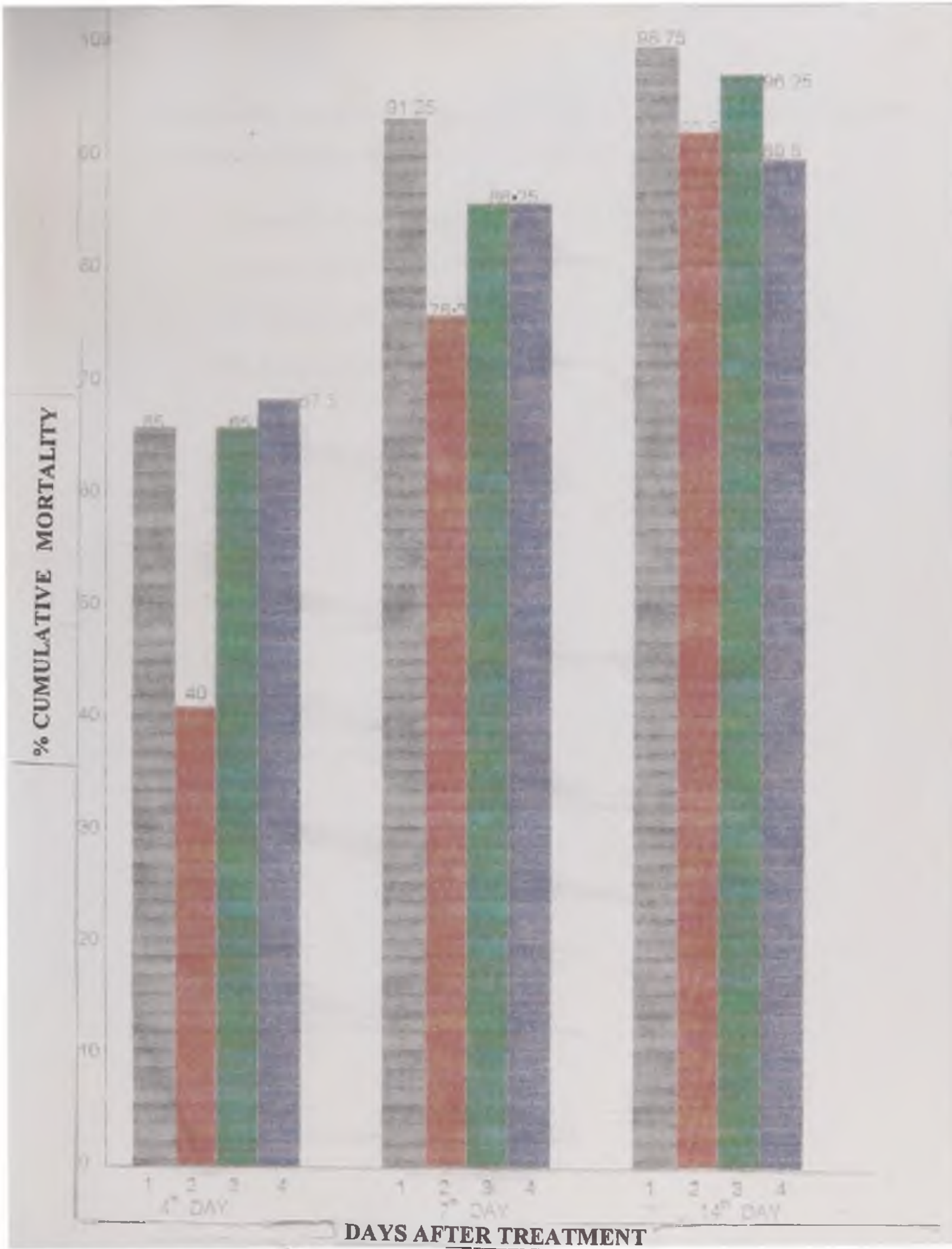

FIG 3: PERCENT CUMULATIVE MORTALITY OF *C. MACULATUS* ON OIL-TREATED COWPEA SEEDS

KEY:

1 - PALMIN OIL
2 - COCONUT OIL
3 - FRYTOL OIL
4 - CONTROL

no infestation recorded when seeds were stored after only the initial infestation . Two main conclusion can be drawn from the results in Tables 6 & 7:

(a) the recommended dosage of 10 ml/kg could effectively protect the seeds for six months.

(b) the different oils offered similar protection to the grains throughout the storage period.

Table 6: Percentage reduction in *C. maculatus* infestation of cowpea treated with plant oils after six months storage in Kilner jars under various infestation regimes.

Infestation regime/oil	Dosage ml/kg 4	6	10
Artificial re-infestation every 40 days after initial infestation with 15 adult insects/			
Palmin oil	0^{a}	0^{a}	89.7^{c}
Coconut oil	0^{a}	0^{a}	90.2^{c}
Frytol oil	0^{a}	0^{a}	90.0^{c}
No artificial re-infestation after initial infestation with 15 adult insects/			
Palmin oil	0^{a}	16.2^{b}	100^{d}
Coconut oil	0^{a}	15.1^{b}	100^{d}
Frytol oil	0^{a}	17.2^{b}	100^{d}

Values followed by the same letter(s) are not significantly different ($P>0.05$)

Table 7: Percentage reduction in *C. maculatus* infestation of cowpea treated with Palmin oil and stored for 5 months in cotton sacks under various infestation regimes.

Infestation regime	Dosage ml/kg 4	6	10
Artificial re-infestation every 40 days after initial infestation with 15 adult insects	0^{a}	7.8^{b}	98.8^{c}
Exposed to natural infestation in farm store after initial infestation with 15 adult insects	0^{a}	11.4^{b}	100^{c}

Values followed by the same letter(s) are not significantly different ($P>0.05$)

4.7 Effect of oil treatment on cowpea seed viability

Oil treatment had no effect on the viability of the grains (Table 8). This confirms the report by Mummigatti and Ragunathan (1977), Singh *et al.*, (1978), Varma and Pandey (1978), Adu (1986), Obeng-Ofori (1995) and contradicts Tembo and Murfitt (1995). Yun-tail and Burkholder (1981) reported that oil treatment reduced germination of wheat. Khaire *et al.*, (1992) also showed that using mustard oil at the same dose of 10 ml/kg, did not affect the viability of one variety of chickpea but caused complete inhibition of germination another variety, when the oil was applied and stored the same way. It appears, then, that differences in percentage germination of oil-treated seeds observed by different workers may be attributed to differences in oil type, oil extraction method or technique employed and the type or variety of grain. Even if oil treatment does adversely affect grains and thus reduce their viability, they can be used to protect grains which are to be used for consumption.

Table 8. Effect of oil treatment on germination of cowpea seeds stored for 5 months.

Treatment	Initial % germination	Final % germination
Frytol oil	84	83
Coconut oil	87	84
Palmin Oil	82	80
Control	86	83
LSD (5%)	NS	NS

4.8 Mould growth on oil-treated cowpea grains stored in Kilner jars and cotton sacks after six months storage

Mouldy seeds were observed in all treatments except in the oil treated seeds at 10 ml/kg kept in Kilner jars, where there was no insect infestation or very low infestation levels. Mould growth was observed to have started in grains at the bottom of the containers where insect activity was highest. Every seed sampled from the bottom of the jars was mouldy. Only a few seeds from the top were, however, affected by mould. All the seeds in the cotton sacks were affected by mould. Mouldiness seems to relate to insect population, the type of material used for storage and moisture content of stored cowpea.. High mouldy conditions at the bottom of containers may have resulted from respiratory activites of the insects.

4.9 Effect of oil treatment on nutritive content of cowpea seeds

The results of this experiment showed that oil treatment did not affect the nutritive content of the grains (Table 9). Similarly crude protein, total ash and crude fibre contents were obtained for both oil-treated and untreated seed. These findings agree with the report by Cruz and Cardona (1981), Hill and Schoonhoven (1981b) and Pacheco *et al.*, (1995), that oils do not alter the nutritive content of grains. These findings are also of particular importance considering the fact that cowpea is the main source of protein in most developing countries.

Table 9: Percent crude protein, total ash and crude fibre content of palmin treated cowpea seeds after six months storage.

Treatment	Crude Protein	Total ash	Crude fibre
Palmin oil	23.5	2.8	21.5
Control	23.7	2.8	23.8
LSD (5%)	NS	NS	NS

4.9.1 **Rancidity of the oils after the storage period**

The results obtained indicated that the oils as at the end of six months of storage did not go rancid (Table 10).

Table 10: pH of oil extracted from oil-treated cowpea seeds stored for six months.

Treatment	pH of extracted oil
Palmin oil	9.6
Coconut oil	9.6
Frytol oil	9.8
Control	9.6
LSD (5%)	NS

4.9.2 Cooking properties of the oil-treated grains

Cooking properties of the grains, such as time taken to cook well, flavour and taste, were not altered after six months storage when oil-treated grains were compared to untreated grains kept in sealed containers and stored for the same period. (Funke (unpublished) observed that soybean, corn and peanut oils did not alter flavour, texture or cooking time of beans stored for 90 days, and Cruz and Cardona (1981) found that soybean and corn oils improved the palatability of cowpea.

CHAPTER FIVE

5.0 SUMMARY AND CONCLUSION

The results of these experiments have shown that the cowpea beetle *C. maculatus* can be effectively controlled by admixing 10 ml of vegetable oils with 1 kg of cowpea seeds for at most five months. For this to be achieved, proper admixing of the oil and the grains should be done to ensure uniform coating. The admixture should also be done before the seeds are infested since larvae and pupae inside the seeds are not affected by oil. Oil-treated seeds should be stored in a room with low relative humidity. The use of vegetable oils should be encouraged on small farm storage, as the cost of these oils is low when compared with the losses incurred in untreated seeds. Furthermore, vegetable oils do not adversely affect cooking properties and taste of the seeds. The problem of food contamination encountered when synthetic insecticides are used as protectants of stored seeds is also prevented.

The use of vegetable oils for the protection of stored cowpea would help the small-scale producers of the crop reduce cost incurred in protecting cowpea seeds when synthetic insecticides are used. Additionally, more seeds would be available for use as food and seed grains and for sale by the farmer as seed infestation would be reduced. Consumers would also get more value for their money as well as enjoy seeds free of the cowpea beetle throughout the year. The nation would also benefit by the increase in volume of the grains that would be available for consumption as a result of the reduction in post-harvest losses caused by the beetle. For instance, a 10% reduction in post harvest loss of 30% of 30,000 tons of cowpea produced, would make available extra 3,000 tons for consumption.

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APPENDIX 1

Oviposition of *C. maculatus* on cowpea seeds treated with vegetable oils

Anova: Single-Factor			Untransformed Data			
Summary						
Groups	Count	Sum	Average	Variance		
PO.0.02	8.000	63.000	7.875	8.696		
PO.0.03	8.000	36.000	4.500	3.714		
PO.0.05	8.000	0.000	0.000	0.000		
CO.0.02	8.000	89.000	11.125	26.411		
CO.0.03	8.000	28.000	3.500	6.000		
CO.0.05	8.000	1.000	0.125	0.125		
FO.0.02	8.000	97.000	12.125	34.125		
FO.0.03	8.000	41.000	5.125	4.125		
FO.0.05	8.000	28.000	3.500	5.714		
ANOVA						
SOURCE OF VARIATION						
	SS	Df	MS	F	P-value	Fcrit
Treatment	1193.278	8.000	149.160	8.343	0.000	2.089
Error	1126.375	63.000	17.879			
Total	2319.653	71.000				

APPENDIX 2

Oviposition of *C. maculatus* on cowpea seeds treated with vegetable oil

ANOVA					
SOURCE OF VARIATION					
	df	SS	MS	F-value	P-value
Treatment	8	49.233	6.154	10.894	0.0001
Residual	63	35.589	.565		

Dependent: Compact Variable 1

Mean Table
Effect: TREATMENTS
Dependent: Compact Variable 1

Groups	Count	Mean	Std. Dev.	Std. Error	
PO.0.02	8	2.672	1.408	.498	
PO.0.03	8	2.314	.407	.144	
PO.0.05	8	1.000	0.000	0.000	
CO.0.02	8	3.352	1.000	.357	
CO.0.03	8	2.004	.642	.227	
CO.0.05	8	1.052	.146	.052	
FO.0.02	8	3.514	.943	.334	
FO.0.03	8	2.507	.497	.176	
FO.0.05	8	2.048	.590	.209	

APPENDIX 3

Mean number of eggs laid by *C. maculatus* on cowpea grains treated with vegetable oils at different dosage rates.

	Dosage ml/kg		
Treatments	4	6	10
Palmin oil	2.67 ± 0.49^{bc}	2.31 ± 0.14^{b}	1.00 ± 0.00^{a}
Coconut oil	3.35 ± 0.38^{cd}	2.00 ± 0.23^{b}	1.05 ± 0.05^{a}
Frytol oil	3.51 ± 0.33^{d}	2.04 ± 0.18^{b}	2.05 ± 0.21^{a}
Control		7.52 ± 0.42^{e}	

Values followed by the same letter in columns are not significantly different ($P>0.05$).

Means ± SEM. SEM = Standard error of mean.

APPENDIX 4

Percentage reduction in oviposition of *C. maculatus* by different oil treatments

ANOVA					
SOURCE OF VARIATION					
	df	SS	MS	F-value	P-value
Treatment	2	357.5	178.7	9.64	0.0003
Residual	12	222.5	18.5		
Total	14	580.0			

ANOVA					
SOURCE OF VARIATION					
	df	SS	MS	F-value	P-value
Treatment	2	1525.3	762.6	27.75	0.000
Residual	12	329.7	27.5		
Total	14	1855.0			

Dependent: Compact Variable 1

Mean Table
Effect: TREATMENTS
Dependent: Compact Variable 1

Groups	Count	Mean	Std. Dev.	Std. Error
PO.0.02	5	52.968	6.216	1.02
PO.0.03	5	55.985	3.451	0.38
PO.0.05	5	90.000	0.000	0.00
FO.0.02	5	46.123	3.536	0.62
FO.0.03	5	58.158	3.891	0.81
FO.0.05	5	87.178	8.472	2.53
CO.0.02	5	44.192	2.123	0.78
CO.0.03	5	54.826	3.621	0.42
CO.0.05	5	67.338	3.265	1.16

APPENDIX 5

Emergence of F1 progenies from eggs laid by C. maculatus on oil-treated cowpea.

Anova: Single Factor			Untransformed Data			
Summary						
Groups	Count	Sum	Average	Variance		
PO.0.02	8.0000	26.0000	3.2500	19.0714		
PO.0.03	8.0000	0.0000	0.0000	0.0000		
PO.0.05	8.0000	0.0000	0.0000	0.0000		
CO.0.02	8.0000	25.0000	3.1250	5.2679		
CO.0.03	8.0000	5.0000	0.6250	1.9821		
CO.0.05	8.0000	0.0000	0.0000	0.0000		
FO.0.02	8.0000	31.0000	3.8750	5.8393		
FO.0.03	8.0000	4.0000	0.5000	0.8571		
FO.0.05	8.0000	0.0000	0.0000	0.0000		
ANOVA						
SOURCE OF VARIATION						
	SS	Df	MS	F	P-value	Fcrit
Treatment	172.8611	8.0000	21.6076	5.8898	0.000	2.0892
Error	231.1250	63.000	3.6687			
Total	403.9861	71.000				

APPENDIX 6

Emergence of F1 progenies from eggs laid by *C. maculatus* on oil treated cowpea.

ANOVA					
SOURCE OF VARIATION					
	df	SS	MS	F-value	P-value
Treatment	8	14.018	1.752	9.005	.0001
Residual	63	12.259	.195		

Dependent: Compact Variable 1

Mean Table
Effect: TREATMENTS
Dependent: Compact Variable 1

Groups	Count	Mean	Std. Dev.	Std. Error
PO.0.02	8	1.826	.872	.308
PO.0.03	8	1.000	0.000	0.000
PO.0.05	8	1.000	0.000	0.000
CO.0.02	8	1.959	.574	.203
CO.0.03	8	1.206	.441	.156
CO.0.05	8	1.000	0.000	0.000
FO.0.02	8	2.137	.594	.210
FO.0.03	8	1.183	.339	.120
FO.O.05	8	1.000	0.000	0.000

APPENDIX 7

Emergence of F1 progenies from eggs laid by *C. maculatus* on oil-treated cowpea (square root transformed data).

Dosage (ml)

Treatment	0.02	0.03	0.05
Palmin oil	1.826[b]	1.000[a]	1.000[a]
Coconut oil	1.959[b]	1.206[a]	1.000[a]
Frytol oil	2.137[b]	1.183[a]	1.000[a]
Control		5.054[c]	

Values followed by the same letter in columns are not significantly different (P>0.05).