EFFECT OF NEEM AND MEE OIL TREATMENT ON EGG MORTALITY, SEX RATIO, FECUNDITY AND LONGIVITY OF *CALLOSOBRUCHUS MACULATUS* (F.) AND GERMINATION OF COWPEA SEED

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ABSTRACT

A series of experiments was conducted to find out the effect of neem and mee oil on *Callosobruchus maculatus* and germination of cowpea seeds. Neem oil at the rate of 7.5 ml/kg was found to be equally effective as mee oil at the same rate, in egg mortality when applied to egg already laid on cowpea seeds. However, neem oil was superior in egg mortality up to 5 days old. Although both oils at this rate were equally effective in inhibiting adult emergence up to 8 days after egg laying, neem oil and mee oil at the rate of 2.5 ml/kg was effective in inhibiting adult emergence only up to 6 and 4 days after egg laying respectively. The total number of eggs laid by a female bruchid on cowpea seeds did not significantly vary between untreated (97 ± 3.8 eggs/ female) and treated samples with mee oil at the rate of 7.5 ml/kg (89 ± 4.7 eggs/female) and neem oil at the rate of 5.0 ml/kg (91 ± 5.2 eggs/female) at 6 months after treatment. However, both oils at these rates were effective in inhibiting adult emergence. Female to male ratio of *C. maculatus* after the treatments was around 2:3 when the total progeny was considered. However, this ratio was comparatively high at the beginning of adult emergence and low at the later stages. Adult longevity of both male and female *C. maculatus* was around 8.5 days with the neem oil treatment and the untreated control. But, female adult longevity of 7.7±0.37 days was significantly different from that of male (8.8±0.20 days) when they were exposed to cowpea seeds treated with mee oil at the rate of 7.5 ml/kg, 6 months earlier. Neem oil and mee oil treated separately at the rates of 7.5, 5.0 and 1.0 ml/kg on cowpea seeds did not affect the germination of the cowpea seeds at 12 and 36 weeks after treatment.

KEY WORDS: *Callosobruchus maculatus*, Cowpea, Mee oil, Neem oil

INTRODUCTION

Callosobruchus maculatus is an important pest in cowpea in the storage in Sri Lanka (Ranasinghe and Dharmasena, 1987). Most of the farmers in the dry zone of this country apply toxic insecticides in controlling this pest (Dharmasena, 1995). Present recommendation by the Department of Agriculture is to pretreat the gunny bags with a mixture of pirimiphos-methyl at the rate of 27 ml per 10 l of water and sun dry them before use (Pesticide Recommendation of the Dept. of Agriculture, Sri Lanka.1997. Unpubl.)

There are numerous reports which illustrated the possibility of using edible and non-edible seed oils for bruchid control in legumes (Bhaduri *et al.*, 1990; Lienard *et al.*, 1993; Ketkar, 1986). However, information about the use of edible mee oil is scanty. Mee trees are found in many homegardens in the dry zone of Sri Lanka (Dharmasena, 1993). It is an old tradition among peasant farmers in the dry zone of Sri Lanka to extract oil from mee and neem seeds (Karunaratne, 1986). Ranasinghe and Dharmasena (1987) reported that treating cowpea seeds with mee oil at the rate of 15 ml/kg is effective in protecting cowpea seeds from damage by *Callosobruchus maculatus*. However, effect of this edible oil under low concentrations, and the effective

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period under storage and viability of treated seeds after long term storage under Sri Lankan conditions have not been studied. Therefore, a series of experiments were carried out to obtain additional information about its use before recommending mee oil for the control of bruchids.

MATERIALS AND METHODS

Effect of neem and mee oil applied to the eggs of *C. maculatus* at different times after egg laying

This experiment was conducted to assess whether the age of the egg on the seeds will affect its susceptibility to neem or mee oil. This experiment also investigated the effect of the different concentrations of the oils on egg mortality. The effect of ambient weather conditions prevailing in the dry zone of Sri Lanka on bruchid egg development was an additional objective of this part of the study.

Twenty-five cowpea seeds each bearing an average of 4 eggs were treated with either neem oil or mee oil at the rate of 2.5 or 7.5 ml/kg at 0.25, 1, 2, 3, 4, 5, 6, 7, 8, and 9 days after egg laying. The number of live and dead eggs were counted on the 12th day after egg laying. Whitish eggs were considered as live eggs while transparent eggs were considered as dead eggs. The number of adult emerging were taken from the 19th day after egg laying up to 35th day after egg laying. Temperature and humidity were recorded daily throughout the experiment. This experiment was conducted in a split-plot design with oil type and dosage as the main plot and time after egg laying as the sub-plot. Each variable had five replications.

Effect of neem and mee oil and age of the female on fecundity, longevity, sex ratio and development time of *C. maculatus* on cowpea seeds, six months after treatment

Cowpea seeds (cv.Waruni) treated separately with neem oil (5.0 ml/kg) and mee oil (7.5 ml/kg) were stored in insect-proof glass jars for 6 months. Different rates of application were selected because neem oil at the rate of 5.0 ml/kg and mee oil at the rate of 7.5 ml/kg were equally effective in inhibiting adult emergence of *C. maculatus* up to eight months after treatment (Dharmasena, 1995). Four seeds treated either with neem oil or mee oil were placed in a glass vial (dia 2.5 cm, height 7.5 cm) and covered with a plastic lid with a window for ventilation. Untreated seeds stored in the same way were also placed in similar containers. Un-mated newly emerged (0-9 h-old) adults (1 female+1 male) were placed into each vial. This experiment was conducted in a completely randomized design with 10 replications per treatment. Adult bruchids were transferred to treated and untreated seeds daily until they died. The number of dead females and males were counted daily. The number of adult females and males emerging from the seeds was recorded from the first day of adult emergence up to 35th day after egg laying. In order to avoid overlapping with the next generation, the adult count was taken only up to 35 days.

Effect of neem and mee oil on germination of cowpea seeds at 12 and 36 weeks after treatment

This experiment was carried out at the Seed Laboratory, Maha Illuppallama, according to the Hilter method (Fuch, 1981). Polythene boxes (9.5cm x 6.5cm x 8.0 cm) half-filled with coarse sand (particle size of 2-3mm) were used in the test. The sand was washed and sterilized before the test, in order to avoid infestations with any microorganism which might otherwise affect the germination of the seeds. Treated and untreated cowpea seeds soaked in water for 24 h were planted in the sand culture, with 25 seeds in each box and covered by a transparent lid. Temperature and relative humidity were maintained at 25±2°C and 60±5 %, respectively, in the chamber where the boxes were placed. The number of plants germinated was counted 5 days after planting. This experiment was carried out 3 and 9 months after treatment in a completely randomized design with eight replications per treatment.

RESULTS

Effect of neem and mee oil applied to the eggs of *C. maculatus* at different times after egg laying

Neem oil as well as mee oil were effective in the control of *C. maculatus* when applied to the seeds bearing eggs from different age groups but the effectiveness was dependent on the age of the eggs (Table 1; Fig.1). Overall, the neem oil was more effective than mee oil at reducing the number of larvae emerging from the eggs into the seeds (Table 1). Young eggs were more susceptible to mortality than the neonate larvae. For example, seeds with bruchid eggs older than 5 days would have had neonate larvae penetrating into the testa and cotyledon. The results show that both rates of neem oil and the higher rate of mee oil caused a significant decrease in the number of larvae penetrating into the seeds when the oils were applied to seeds bearing eggs less than 5 days old (Table 1). Percentage egg mortality from 5-9 days after egg laying was not significantly different from 16% to 11% in the untreated controls (Fig.1).

The numbers of adults emerging from seeds in all the treatments, except the treatment with mee oil at the rate of 2.5 ml/kg at 8 and 9 days after egg laying, were significantly lower than the number emerging from seeds in the untreated control (Table 1). The results also show that neem oil is more effective at lower concentrations than mee oil in decreasing the number of adults emerging from seeds bearing bruchid eggs. Furthermore, the oils are more effective against eggs than neonate larvae. The numbers of adults emerging per seed in the treatment with neem oil at the rate of 7.5 ml/kg at 0.25, 1, 2, 3, 4, 5, 6, and 7 days after emergence were negligible.

Table 1. Effect of neem and mee oil applied at different days after egg laying on eggs of *C.maculatus* laid on cowpea seeds

DAE	*Number of larvae per seed ± S.E.*				*Number of adults per seed±S.E.*			
	Neem oil		*Mee oil*		*Neem oil*		*Mee oil*	
	2.5 ml/kg	*7.5 ml/kg*	*2.5 ml/kg*	*7.5 ml/kg*	*2.5 ml/kg*	*7.5 ml/kg*	*2.5 ml/kg*	*7.5 ml/kg*
0.25	0.2±0.1 hi	0.0±0.0 i	1.9±0.2 ef	0.2±0.0 i	0.0±0.0 e	0.0±0.0 e	*0.1±0.0 e*	0.1±0.1 e
1	0.2±0.1 i	0.0±0.0 i	2.2±0.2 e	0.0±0.0 i	0.0±0.0 e	0.0±0.0 e	0.8±0.3 d	0.0±0.0 e
2	0.8±0.2 gh	0.2±0.1 h	2.0±0.2 e	0.0±0.0 i	0.3±0.1 e	0.2±0.1 e	0.6± 0.1 d	0.0± 0.0 e
3	1.1±0.4 g	0.2±0.0 h	2.6±0.2 cd	0.2±0.2 e	0.1±0.0 e	0.0±0.0 e	0.1±0.0 e	0.0±0.0 e
4	1.2±0.3 fg	0.2±0.1 h	3.0±0.1 abc	0.2±0.2 e	0.2±0.1 e	0.0±0.0 e	0.0±0.0 e	0.0±0.0 e
5	3.0±0.3 ab	2.0±0.3 e	3.4±0.1 a	3.3±0.1 ab	0.2±0.1 e	0.0±0.0 e	0.7±0.0 d	0.0±0.0 e
6	3.3±0.3 ab	2.5±0.2 cd	3.6±0.1 a	3.6±0.1 a	0.1±0.0 e	0.0±0.0 e	2.3±0.2 ab	0.1±0.1 e
7	3.1±0.3 abc	2.5±0.2 cd	3.5±0.1 a	3.4±0.1 a	1.2±0.2 c	0.0±0.0 e	2.3±0.2 ab	0.4±0.1 e
8	3.2±0.2 ab	2.4±0.2 de	3.4±0.1 a	3.5±0.1 a	1.4±0.2 c	0.4±0.3 e	2.6±0.2 a	0.4±0.1 e
9	3.2±0.3 ab	2.9±0.2 bcd	3.6±0.1 a	3.6±0.1 a	2.0±0.3 b	1.9±0.3 b	2.7±0.1 a	1.4±0.3 c
Cont	3.4±0.3		3.6±0.1		2.6±0.3		2.8±0.2	
F value (Oil type)			446 ***		F value (Oil type)		48.0 ***	
F value (DAE)			249.3 ***		F value (DAE)		79.9 ***	
F value (Dosage)			298.0 ***		F value (Dosage)		188.2 ***	
F value (Oil type x Dosage)			22.0 ***		F value (Oil type x Dosage)		9.6 **	
F value (DAE x Dosage)			15.9 **		F value (DAE x Dosage)		44.4 ***	
F value (Oil type x DAE x Dosage)			15.2 **		F value (Oil type x DAE x Dosage)		6.5 **	
CV(%) =16.1					CV(%) = 38.3			

DAE = Days after eggs laying; Five seeds with 4 eggs/seeds in one replication; All figures are means of five replications. Cont = Control; Figures followed by the same letter are not significantly different at P = 0.05 level; * Significant at 5%; ** Significant at 1%; *** Significant at 0.1%

Effect of neem and mee oil, and age of the female on fecundity, longevity, sex ratio and development time of *C. maculatus* on cowpea seeds, six months after treatment

The fecundity of *C. maculatus* on the untreated seeds was 97±3.8 eggs per female which did not differ significantly from 89±4.7 and 91±5.2 eggs per female on cowpea seeds treated with neem oil and mee oil at the rates of 5.0 and 7.5 ml/kg, respectively (Table 2). However, the oils caused a significant decrease in the adults emerging from the seeds, relative to the control. Thirty-three adults per female emerged in the untreated control, compared to 10 and 3 adults per female in the mee and neem oil treatments, respectively (Table 2). The longevity of female adults in the neem and mee oil treatments was 8.7±0.37 and 7.7±0.37 days, respectively, compared to 8.1±0.35 in the control (Table 2). Male adult longevity in the neem and mee oil treatments as well as the untreated control was around 8.7 days (Table 2). In comparison with the control, the sex ratio of emerging adults (1:1.5) was not influenced significantly by the mee oil though in the neem oil treatment female:male ratio was 1:1.1. This differance

may be due to two factors. Firstly, due to diferences in the development period. Sex ratio (F:M) in the control when development period was 19 and 20 days was 1:2.3 while in the neem oil treatment when the development period was 21 and 22 days, female : male ratio was 1:1.6. Secondly lack of enough adults in the neem oil treatment may have some impact on sex ratio (Table 3). In the oil treatments and the control, more males emerged in the first three days of emergence and more females emerged subsequently. The development period of *C. maculatus* ranged from 19-25 days in the control. Similarly, it ranged from 19-24 days in the mee oil treatment. However, in the neem oil treatment the range of development period (21-28 days) was comparatively high (Table 3).

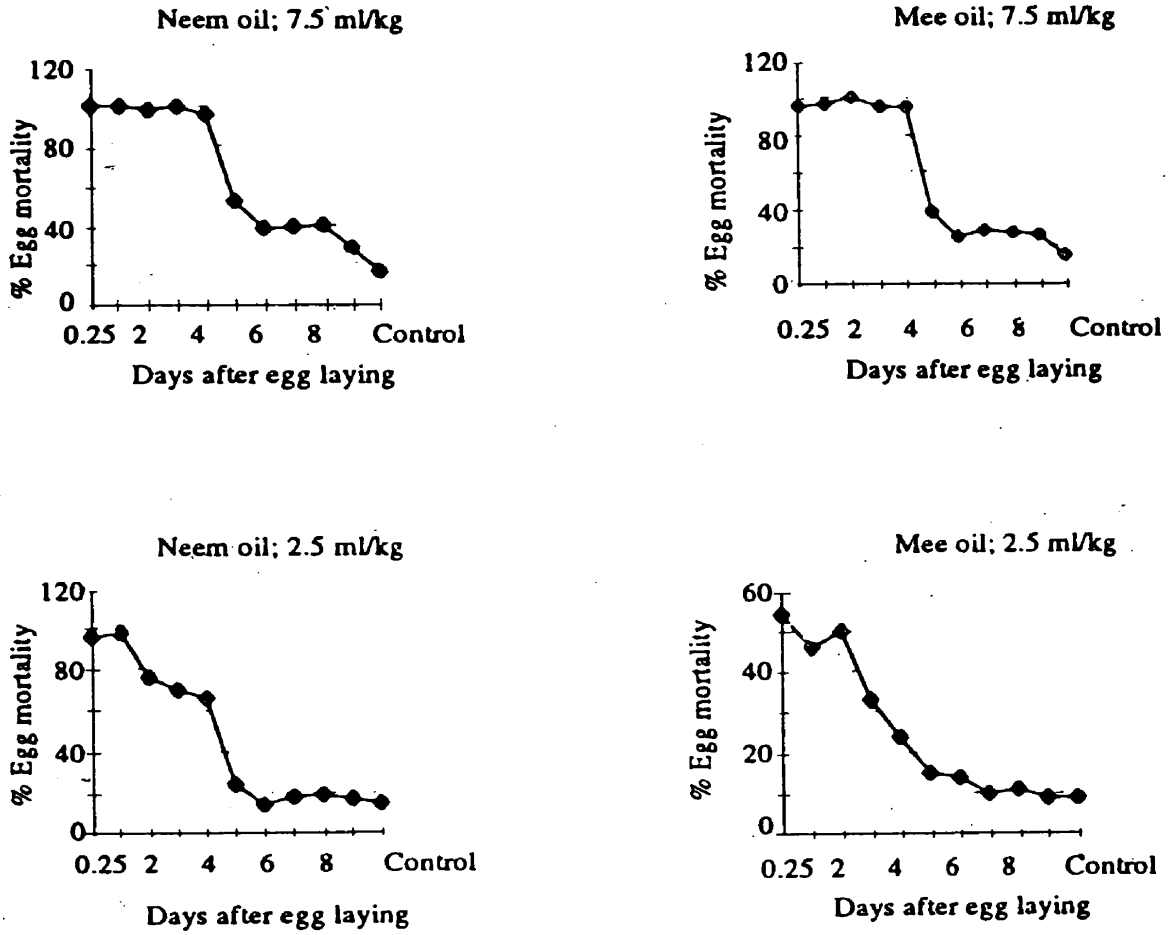

Fig.1. Effect of neem and mee oil treated cowpea seeds with eggs of *C. maculatus* at different rates on egg mortality

Table 2. Adult longevity, fecundity and F1 progeny of *C. maculatus* on cowpea seeds treated 6 months earlier with neem and mee oil

Treatment	*Dosage (ml/kg)*	*Average adult longevity (days ± S.E.) Female:Male*	*Number of eggs per female ± S.E.*	*Number of F1 progeny per female ± S.E.*
Control	—	8.1±0.35 8.7±0.40	97±3.8	33±3.3
Mee oil	7.5	7.7±0.37 8.8±0.20	91±5.2	10±1.0
Neem oil	5.0	8.7±0.37 8.7±0.24	89±4.7	3±0.7
F value			0.8 ns	59.7 ***
CV (%)			15.5	37.7
LSD (0.05)			—	5.8

ns = Not significant ; *** Significant at p = 0.1%

Table 3. Fecundity and sex ratio of *C. maculatus* on cowpea seeds (cv.Waruni) treated with neem and mee oil

DAE	*Untreated control*				*Mee oil 7.5 ml/kg**				*Neem oil 5.0 ml/kg **			
	Number of adults				*Number of adults*				*Number of adults*			
	No.of eggs	*19&20 # F:M*	*24&25 @ F:M*	*Total+ F:M*	*No.of eggs*	*19&20 # F:M*	*23&24 @ F:M*	*Total+ F:M*	*No.of eggs*	*21&22# F:M*	*27&28 @ F.M*	*Total+ F:M*
1	278	29:48	1: 0	57:99	353	0:3	3:4	12:25	335	3:8	-	4:8
2	260	13:7	1:6	43:74	257	0:2	3:6	12:21	259	3:4	4:1	8:5
3	118	0:8	2:1	40:42	178	-	4:5	8:11	166	1:1	-	2:2
4	132	-	1:1	22:25	77	-	2:0	3:1	76	-	-	-
5	58	-	1:0	3:1	25	-	1:0	1:0	41	-	-	-
6	26	-	0:1	0:1	16	-	1:0	1:0	14	-	-	-
7	-	-	-	7	-	-	-	-	2	-	-	-
Total	972	42:97	6:9	165:252	913	0:05	14:15	37:57	893	8:13	4:12	14:15

Total number of adults emerged in the first two days from 10 replicates; @ Total number of adults emerged in the last two days in 10 replicates; + Total number of egg laid in 10 replicates; * Treated 6 months earlier; F= Female; M= Male

The number of eggs laid by females decreased as the females got older, irrespective of the oil treatmernt (Table 3). Futhermore, the proportion of viable eggs laid by females decreased as the female aged. This relationship is clearly seen in the untreated control.

Effect of neem and mee oil on germination of cowpea seeds at 12 and 36 months after treatment

The percentage germination of cowpea seeds at 12 and 36 weeks after treatment with neem and mee oil at the rates of 7.5, 5.0 and 1.0 ml/kg did not differ significantly from that of the untreated control (Table 4). The percentage germination in all the treatments was more than 90%.

Table 4. Effect of neem and mee oil on germination of cowpea seeds at 12 and 36 weeks after treatment

Treatment	*Dosage (ml/kg)*	*% Germination (12 WAT)*	*% Grmination (36 WAT)*
Neem oil	7.5	92 ± 2.4 (9.6 ± 0.07)	94 ± 1.4 (9.7 ± 0.7)
Neem oil	5.0	94 ± 0.0 (9.7 ± 0.05)	94 ± 0.7 (9.7 ± 0.04)
Neem oil	1.0	96 ± 1.4 (9.8 ± 0.08)	94 ± 0.7 (9.7 ± 0.04)
Mee oil	7.5	91 ± 1.1 (9.5 ± 0.06)	90 ± 1.8 (9.5 ± 0.09)
Mee oil	5.0	94 ± 1.1 (9.7 ± 0.06)	90 ± 1.3 (9.5 ± 0.09)
Mee oil	1.0	94 ± 1.8 (9.7 ± 0.09)	95 ± 1.2 (9.7 ± 0.08)
Control	—	95 ± 1.3 (9.7 ± 0.08)	94 ± 1.2 (9.7 ± 0.08)
F value		1.4 ns	1.7ns
CV(%)		2.4	4.8

All figures are means of five replications; ns = not significant; WAT = weeks after treament; Figures within parenthesis are square root values

DISCUSSION

The results from this study suggest that farmers could pretreat their seeds just after harvesting in January and protect them from bruchid damage up to 6 months. If they wanted to keep their seeds for a longer period, then they could inspect their seeds for the presence of bruchid eggs and can decide whether it was necessary to treat their seed again or not. The ovicidal action of the second application would inhibit adult emergence if there were any live eggs on the seeds. Das (1987) reported that neem oil applied at the rate of 8.0 % (w/w) to chickpea seeds bearing 12-day-old eggs of *C. chinensis* caused 64% mortality compared to 3% in the control. In the present study, the decrease in protection recorded when seeds were treated with 2.5 ml/kg of mee oil could be due to the fact that the small amount of oil in these lower concentrations was not enough to cover the seed surface completely.

Khaire *et al.* (1992) reported that neem oil applied at the rate of 5.0, 7.5 and 10.0 ml/kg did not affect germination of pigeonpea up to 100 days. According to the present study, neem and mee oil treated separately at the rate of 1.0, 5.0 and 7.5 ml/kg can be used to store cowpea seeds up to 9 months without any effect on germination. Dharmasena (1995) reported that neem oil and mee oil at the rate of 1.5% (v/w) did no affect germination of cowpea (variety MI 35) up to 1 month after treatment, although they did report that neem oil affected germination 10 days after treatment.

Van Huis (1991) reported that it is imperative to take socio-economic and cultural aspects into account in the development of an effective control programme as farmers need to be informed about the implementation of control measures. Neem oil-treated seeds cannot be consumed because of the bitter taste left by the neem (Ketkar, 1986). Therefore, neem oil can only be applied to seeds meant for planting. Mee is an edible oil and does not taint the seed flavour; therefore the seeds treated with mee oil could be consumed.

This study has shown that both neem and mee oil do not influence the fecundity of *C. maculatus* at 6 months after treatment. Fecundity of the Sri Lankan strain of *C. maculatus* on untreated cowpea seeds at 28.8°C (24.8-34.8°C) and 65% R.H. (51-80%) was 97±3.8. Fecundity on the neem and mee oil treatments under the same conditions were 89±4.7 and 91±5.2, respectively. These figures are in accordance with results obtained by earlier workers (Howe and Currie, 1964). Michell (1991) reported that fecundity over a 10 year-period of a South Indian strain of *C. maculatus* ranged from 47.8±2.4 to 92.0±2.21. The present study has also shown that the number of eggs laid per day varies. More than 80% of the total number of eggs were laid in the first three days after emergence. According to the present study, neem oil has no effect on female or male longevity of *C. maculatus*. However, female longivity of 7.7 day on mee oil treatment was significantly lower than that of male (8.8 days) on the same treatment. Ketkar (1986) reported that adult longevity remained unchanged, even if cowpeas were treated with 1.0% (v/w) neem oil.

It appears that oil treatments had not affected the sex ratio of emerging *C. maculatus*. However, more males emerged at the beginning of the emergence per seed. Nevertheless, the development period of females was not longer than that of males. Howe and Currie (1964) reported that there were no signs of sex differences in the rate of development for *C. maculatus* in cowpeas but on garden peas males appear to develop more rapidly. Howe and Currie (1964) reported that the maximum development period for *C. maculatus* on cowpea was 23 days which is similar to that recorded in this study. However, Parsai *et al.* (1990) reported that groundnut and mustard oil treatments in urdbean did increase the development period of *C. chinensis*.

Don-Pedro (1989) reported that first instar larvae of *C. maculatus* developed in the egg and then on the third day started boring through the seed testa. The present study has established that the incubation period of the egg of *C. maculatus* Sri Lankan strain is four days. Thus the high level of larval mortality caused by neem and mee oil recorded at 5 or 6 days after egg laying could be partly due to the mortality of the first instar larvae. This is in accordance with the results of Lienard *et al.*(1993). This implies that first stadium larvae are very susceptible to oil treatments.

Overall, neem oil caused higher egg and larval mortality than mee oil. This was probably due to the direct toxic effect of compounds in the neem oil. Devaraj (1990) reported that the neem tree contains a number of pesticidal constituents such as azadirachtin, salannin and meliantriol. The oils will also contain fatty acids which

could kill the eggs and the first instar larvae (Don-Pedro, 1990; Lienard *et al.*,1993). Oleic and linoleic acids were highly effective as ovicides against *C. maculatus* (Don-Pedro, 1990; Lienard *et al.*,1993). Karunaratne (1986) reported that mee oil contains oleic and linoleic acids at 45% and 14 %, respectively. Therefore, the high egg mortality with mee oil may be due to these two fatty acids. The level of fatty acids in the neem oil has not been documented.

It would be very important to study the pesticidal compounds in mee oil, especially since Hall and Harman (1991) reported that saturated fatty acids which remain on the seed surface were effective in killing eggs of *Zabrotes subfasicatus* on dry bean (*Phaseolus vulgaris*). In order to ensure a satisfactory period of protection, it is necessary to re-apply these seed oils 6 months after initial treatment. Neem and mee oil when applied to seeds at the rate of 2.5 ml/kg were effective in reducing adult emergence up to 7 - 9 days after egg laying and higher rates of these oils (7.5 ml/kg) would be sufficient to destroy eggs already laid on these seeds. When neem oil was applied to seeds at the higher rate it totally inhibited adult emergence and did not impair seed germination. Thus this dose could be used to protect seeds that would be used by farmers for planting. It may not be necessary to apply neem oil again if the treated seeds are stored in a proper manner (Dharmasena, 1995). The results from this study suggest that mee oil at the rate of 7.5 ml/kg could be recommended for use by farmers to protect seeds meant for consumption.

CONCLUSIONS

Mee oil at the rate of 7.5 ml/kg can be recommanded as a long term protectant against *C. maculatus* of cowpea seeds meant for consumption. If bruchid eggs are present after 6 months in the storage then mee oil could be used to kill them and protect the seeds for another six months. Neem oil at the rate of 5.0 ml/kg is effective in controlling the same pest for more than 6 months. Repeated application of neem oil kill bruchid eggs and can be used as a preservative for cowpea seeds meant for planting purposes.

Under the conditions prevailing in the dry zone of Sri Lanka, fecundity of *C. maculatus* ranged from 89 - 97 and longivity on untreated seeds is arround 8.5 days. Incubation period of eggs of *C. maculatus* is four days while male to female ratio is 3:2.

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