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Effectiveness of Three Plant Oils in Binary Mixtures with Pyridalyl, Abamectin, Spinosad and Malathion Against *Callosobruchus maculatus* (F.) Adults

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ABATRACT

Toxicity of pyridalyl, abamectin, spinosad and malathion and three plant oil sviz. corn oil (*Zea maize*) sun flower oil (*Helianthus annus*) and sesame oil (*Sesamum indicum*) either singly or as binary mixtures in different mixing ratios were investigated on the cowpea weevil *Callosobruchus maculates* (F.) adults through residual film bioassay. The LC_{50} of pyridalyl, abamectin, spinosad and malathion were 79663.06, 19964.04, 11380 and 884 ppm and that of corn oil sun flower oil and sesame oil were 4.452E+5, 5.064 E+5 and 17729.27 ppm, respectively after 9 days post treatment. The four insecticides were tested in binary mixtures with the three plant oils at mixing ratios of 99:195:5 and 90:10 (insecticide:oil). All plant oils when combined with pyridalyl and abamectin proved to have synergistic effect against *C. maculatus* adults except at 95:5 and 90:10 mixing ratios (abamectin+sun flower oil) exhibited antagonistic effect. On the other hand antagonistic effect was achieved when spinosad and malathion were mixed with the tested plant oils except at 95:5 mixing ratio for spinosad+sesame oil which evoked synergistic effect.

Key words: Plant oils, toxicity, insecticide, malathion, pyridalyl, abamectin, spinosad

INTORDUCTION

Cowpea is an essential food legume in the tropics where it has high dietary protein content and an important nitrogen fixing legume in tropical cropping system. Post harvest grains storage however is a major constraint for cowpea expansion and year-round availability.

Among several insect pests that attack this crop the cowpea beetle *Callosobruchus maculatus* (F.) (Coleoptera: Burchidae) is a worldwide pest of cowpea *Vigna unguiculata* (L.) and destructive primary insect pest of stored legumes (Dimetry *et al.*, 2007). Infestation of cowpea by this bruchid can occur in the field before mature seeds are invested (Huignard, 1985) and then *C. maculatus* multiplies rapidly in storage where it causes very high post harvest losses reaching 60% without control (Pereira, 1983; Ouedraogo *et al.*, 1996; Sanon *et al.*, 1998).

Plant products have played an important role in the traditional methods of protection against crop pest in Africa (Gaby, 1988; Poswal and Akpa, 1991). Farmers in Egypt use ashes sand plant organs or extracts to preserve stored cowpea from insect damage. These treatments are only efficient in domestic situations but small-scale farmers use few conventional insecticides because of their cost and lack information qualified advisor or adequate materials. Chemical control using synthetic insecticides has dominated control tactics against cowpea and bruchids insect pests. However, use of insecticides in stored products is facing restrictions such as a high mammalian toxicity and expensive costs. In addition pest populations are developing resistance to chemical insecticides (Phillips *et al.*, 2000).

Though there remains a need to continue to investigate safe and effective chemical control strategies for *C. maculatus* on stored products. However, new molecules that meet environmental and food safety requirements are needed to control *C. maculatus* in cowpea. In this respect spinosada biopesticide in Naturalytes family of insecticides is a promising alternative to other comerically available pesticide for control of storage-insect pests. Spinosad has been successfully used against some insect pests of stored corn and rice (Fang *et al.*, 2002a, b). This biopesticide has been reported to have fewer hazards for the environment and mammals than many other currently available pesticides (Thompson *et al.*, 2000). In additionpyridalyl(S-1812) represent another new effective insecticide having new unique mode of action different from pyrethroids organophosphates carbamates and IGRsand being effective against resistant strains of various insect pests while having less destructive effects against beneficial insects (Cook *et al.*, 2004).

However, since the recent interest originated from the need for pesticide products or/and new approaches with potentially less negative environmental and health impacts than when use the highly effective synthetic pesticidesit was felt necessary to study the effect of integrating these new insecticides with some plant oils to know if it will be of positive value or not.

A major limitation to the practical utilization of vegetable oils as grain protectants is the high rates (10 mL kg^{-1}) required to effectively disinfest grains (Don-Pedro, 1987). These rates are impractical and costly to apply. Don-Pedro (1989) suggested the possibility of using reduced levels of oils in combination with synthetic insecticides in simple binary mixtures as a means of making their use more attractive and effective. In this respect Tembo and Murfitt (1995) showed that wheat treated with vegetable oils combined with primiphos-methyl at half the recommended dose was as effective as primiphos-methyl at the recommended dose against *Sitophilus granarious* (L.).

Accordingly the aim of this study was to find out whether plant oils (corn oil, sun flower oil and sesame oil) can be integrated with acceptably safe insecticide (spinosad, pyridalyl, abamectin and malathion) to increase their potentiality against the cowpea weevil *Callosobruchus maculatus* (F.) adults and consequently reduce costs and environmental pollution.

MATERIAL AND METHODS

Test insect: Cowpea beetle *C. maculatus* stock cultures maintained in 1 L glass jars containing a cowpea seeds *V. unguiculata* (Walp) and incubated in a laboratory control conditions at temperature 30±2 and 60±5°C RH% in the Department of PesticideEl-Menuifya University Shibin El-Kom Cairo Egypt.

Insecticides: Four groups of formulated insecticides were chosen for investigation either alone or in mixtures. These insecticides include:

- Naturally derived insecticides: Spinosad (spintor 24%SC)
- Abamectin (vertemic 1.8% EC)
- The conventional insecticides: Malathion (malathion 57%EC)
- Newly developed insecticide pyridalyl (pleoS1812 50% EC)

In addition to three oils sunflower, corn and sesame oils were included in tests either alone or in mixtures.

Bioassay: For assessment the toxicity of each insecticide was tested separately or in binary mixtures stock solution of each insecticide and oil alone or mixture of two components were prepared fresh daily on the basis of active ingredient (w/v). The tested mixing ratios of each mixture were 99:195:5 and 90:10 on the basis of active ingredient for both components (insecticide/oil).

Stock solutions of either separate insecticide and oil or mixture were serially diluted with water to 5-7 concentrations resulting in10-90% mortality which was used to calculate LC_{50} value.

Ten sexed pairs of *C. maculatus* adults (0-24 h old) were released in glass petri dishes (11 cm diameter) containing 20 g cowpea seeds and allowed to lay eggs for 3 days. On the fourth day the adults were removed. The number of eggs laid on seeds in each dish were counted and then sprayed up to the wetting point with 2 mL of appropriate concentration of each tested compound separately or in mixture using hand atomizer sprayer (20 mL). Seeds sprayed with water alone served as a control. At the end of the sprayed operation all samples were left overnight in open petri dishes to dry. Three dishes (replicates) were used for each concentration. At 9 days post-treatment the number of unhatched eggs was recorded and corrected for control response according to Abbott's formula (Abbott, 1925).

To calculate LC_{50} values for either mixtures of insecticide and the three oils or their components separately probit analysis (Finney, 1971) were adoptedusing the corrected mortality percentages. The co-toxicity coefficient based on LC_{50} values was calculated according to method of Sun and Johnson (1960) using toxicity index (Sun, 1950) by the following equation:

$$\text{Actual T.i of mixture M (using A as standard)} = \frac{LC_{50} \text{ of A}}{LC_{50} \text{ of M}} \times 100$$

$$\text{Thoretical T.i. of mixture M} = \text{T.i. of A} \times \text{\% of A in M} + \text{T.i. of B} \times \text{\% of B in M}$$

$$\text{Co-toxicity coefficient of mixture} = \frac{\text{Actual T.i. of a mixture}}{\text{Theoretical T.i. of mixture}} \times 100$$

where, T.i. = Toxicity index.

When the co-toxicity coefficient of a mixture is 100 the effect of this mixture indicates probability of similar action. If the mixture gives a coefficient significantly greater than 100 it indicates synergistic action. An independent action usually should give a coefficient less than 100.

RESULTS AND DISCUSSION

Results: In toxicity tests with pyridalyl the LC_{50} has been calculated as 79663.06 ppm. On the other hand the LC_{50} values for cornsunflower and sesame oils were 4.452 E+5, 5.064 E+5 and 17729.27 ppm, respectively (Table 1). When pyridalyl was mixed with corn oil at 99:195:5 and 90:10 (insecticide/oil) the obtained LC_{50} values were 2887.32, 6233.04 and 10127.01 ppm and the calculated co-toxicity coefficient values were 2781.88, 1332.86 and 856.91 at 99:195:5 and 90:10 ratio, respectively, at 9 days of treatment. It was clear that the co-toxicity coefficient values were remarkably high at all mixing ratios, suggesting high synergistic effect.

Table 1: LC_{50} co-toxicity coefficient and synergistic ratio due to the effect of pridalyl and different fixed oils mixtures on egg of *C. maculatus* after 9 days of treatment

		95 % confidence limits		
Mixture ratio Pyr.:Oil	LC_{50} ppm	Lower	Upper	Co toxicity coefficient
Pyridalyl+corn oil				
0:1	4.452 E+5	28998.375	8.263 E+9	-
99:1	2887.32	646.337	3.034 E+17	2781.88
95:5	6233.04	-	-	1332.86
90:10	10127.01	1237.278	1.351 E+15	856.91
1:0	79663.06	8021.68	1.536 E+8	-
Pyridalyl+sun flower oil				
0:1	5.0644 E+5	35224.074	3.608 E+9	-
99:1	6866.45	-	-	1170.01
95:5	8679.88	1279.630	9.503 E+26	958.13
90:10	22799.92	8229.525	1.227 E+5	381.57
1:0	79663.06	8021.68	1.536 E+8	-
Pyridalyl+sesame oil				
0:1	17729.27	4811.376	2.880 E+5	-
99:1	14502.68	1261.211	6.661 E+11	530.60
95:5	41846.67	-	-	162.03
90:10	12476.19	-	-	473.19
1:0	79663.06	8021.68	1.536 E+8	-

Regarding pyridalyl+sunflower oil mixtures it was obvious that relatively lower co-toxicity coefficient values were achieved recording 1170.01, 958.13 and 381.57 at mixing ratios of 99:195:5 and 90:10 (insecticides/oil), respectively. Similarlythe co-toxicity coefficient values were also high and came next to mixtures of corn oil.

As for mixtures of pyridalyl and sesame oil the obtained values of co-toxicity coefficient exhibited relatively lower synergistic action and recorded 530.6, 162.03 and 473.19 at 99:195:5 and 90:10 mixing ratios, respectively. However, the higher synergistic action was recorded generally at mixing ratios of 99:1 (insecticide+oil) for all oil tested.

As shown in Table 2 mixtures of abamectin and corn oil suggested remarkably high synergistic activity at all mixing ratios, recording co-toxicity coefficient values reached 7720.83, 8665.16 and 9500.24 for 99:195:5 and 90:10, respectively. Similarly the mixtures of abamectin and sesame oil revealed high synergistic effect recording co-toxicity coefficient of 1631.52, 1621.45 and 555.98 at mixing ratios of 99:195:5 and 90:10, respectively. On contrary abamectin and sunflower oil combinations evoked moderate antagonistic effect at mixing ratios of 95:5 and 90:10 recording co-toxicity coefficient of 57.20 and 85.65, respectively whereas, 99:1 mixing ratio resulted in slight synergistic effect achieving co-toxicity coefficient of 163.45.

The joint action data in Table 3 illustrate that spinosad in combinations with corn sunflower and sesame oil revealed severely antagonistic effect at all mixing ratios recording co-toxicity coefficient values of 17.89, 20.79 and 22.49, 35.84, 29.68 and 26.54, 38.5 and 98.76, respectively. In contrast spinosad+sesame oil (90:10) revealed high synergistic effect recording co-toxicity coefficient of 503.40.

As shown in Table 4 mixtures of malathion with the three oils at 99:195:5 and 90:10 mixing ratios exhibited remarkably high antagonistic action where the co-toxicity coefficient were 1.331.38

Table 2: LC_{50} co-toxicity coefficient and synergistic ratio due to the effect of abamectin and different fixed oils mixtures on egg of *C. maculatus* after 9 days of treatment

		95% confidence limits		
Mixture ratio Aba.:Oil	LC_{50} ppm	Lower	Upper	Co toxicity coefficient
Abamectin+corn oil				
0:1	4.452 E+5	28998.375	8.263 E+9	-
99:1	261.08	52.551	2766.227	7720.83
95:5	241.96	61.910	2056.009	8665.16
90:10	232.33	29.676	54447.172	9500.24
1:0	19964.04	1444.301	1.00000 E+38	-
Abamectin+sun flower oil				
0:1	5.064 E+5	35224.07	3.608 E+9	-
99:1	12332.46	6272.933	33881.104	163.45
95:5	36663.68	-	-	57.20
90:10	25786.07	2697.607	2.415 E+29	85.65
1:0	19964.04	1444.301	1.00000 E+38	-
Abamectin+sesam oil				
0:1	17729.27	4811.376	2.880 E+5	-
99:1	1222.08	6272.933	33881.104	1631.52
95:5	1223.48	397.653	9.070 E+6	1621.45
90:10	3545.92	-	-	555.98
1:0	19964.04	1444.301	1.00000 E+38	-

Table 3: LC_{50} co-toxicity coefficient and synergistic ratio due to the effect of spinosad and different fixed oils mixtures on egg of *C. maculatus* after 9 days of treatment

		95 % confidence limits		
Mixture ratio Spn:Oil	LC_{50} ppm	Lower	Upper	Co toxicity coefficient
Spinosad+corn oil				
0:1	4.452 E+5	28998.375	8.263 E+9	-
99:1	64212.23	119115.830	4.852 E+5	17.89
95:5	57532.47	5964.980	2.115 E+10	20.79
90:10	56063.20	18791.172	3.239 E+5	22.49
1:0	11380.52	4727.916	47668.774	-
Spinosad+sun flower oil				
0:1	5.064 E+5	35224.070	3.608 E+9	-
99:1	32069.04	13954.850	1.356 E+5	35.84
95:5	40317.58	14595.980	2.016E+5	29.68
90:10	47508.74	12573.550	5.684E +5	26.54
1:0	11380.52	4727.916	5.684 E+5	-
Spinosad+sesam oil				
0:1	17729.27	4811.376	2.880 E+5	-
99:1	29669.77	3132.990	3.790 E+5	38.50
95:5	11732.50	-	-	98.76
90:10	2344.67	471.200	1.231 E+14	503.40
1:0	11380.52	4727.916	47668.774	-

and 80.39; 2.431.44 and 6.01; 2.137.00 and 3.24 for malathion when combined with corn sun flower and sesame oil at prementioned mixing ratios, respectively.

Table 4: LC_{50} co-toxicity coefficient and synergistic ratio due to the effect of malathion rent fixed oils mixtures on egg of *C. maculatus* after 9 days of treatment

		95 % confidence limits		
Mixture ratio Mal.:Oil	LC_{50} ppm	Lower	Upper	Co toxicity coefficient
Malathion+corn oil				
0:1	4.452 E+5	28998.375	8.263 E+9	-
99:1	67314.94	4180.240	4.056 E+28	1.33
95:5	68086.20	-	-	1.38
90:10	1229.35	431.820	1.652 E+6	80.39
1:0	889.56	713.314	115.700	-
Malathion+sun flower oil				
0:1	5.064 E+5	35224.070	3.608 E+9	-
99:1	36850.65	3221.940	4.671 E+12	2.43
95:5	64760.91	-	-	1.44
90:10	16449.85	6191.420	1.073 E+5	6.01
1:0	889.56	713.314	115.700	-
Malathion+sesam oil				
0:1	17729.27	4811.376	2.880 E+5	-
99:1	42095.53	-	-	2.13
95:5	13327.44	3823.750	4.796 E+5	7.00
90:10	30358.67	4619.340	1.811 E+15	3.24
1:0	889.56	713.314	115.700	-

DISCUSSION

Regarding the efficacy of insecticides when tested alone it was obvious that abamectin was effective in inducing mortality of *C. maculatus* and therefore, proved to be a good insecticide for control of insect. This result corroborates the findings of Mohamed *et al.* (2009) which found that the hatching rate of eggs of *C. maculatus* decreased dramatically as the concentration of avermectin increased.

The avermectin bioinsecticide showed more protection to the seeds due to its potential effect on the fecundity of *C. maculatus* female and resulted in the reduction of the deposite eggs number which averaged 80.6 eggs compared to 135 eggs in lufenuron treatments. However, avermectin affect as a nerve poison which inhibit both nerve and nerve to muscles communication to the insect (Hink *et al.*, 1991). Generallyavermectin treatment gave more protection of the legume seeds than lufenuron (Mohamed *et al.*, 2009).

In our study spinosad was not effective against *C. maculatus* this agree with (Sanon *et al.*, 2010) which found that the insecticidal activity of spinosad on *C. maculatus* adults in the laboratory was lower than expected based on the higher mortality rated reported for *Rhyzophertha dominica* (F.) (Toews *et al.*, 2003; Nayak *et al.*, 2005) and *Tribolium* sp. (Toews *et al.*, 2003). However, these higher rates of mortality were obtained after exposure periods that were longer than those in the laboratory experiment. Spinosad seems to be less effective than deltamethrin at least for short (24 h) exposure periods. As reported for other insecticides (Arthur, 1997, 1998) the efficacy of spinosad depends on the duration of insect exposure. In contrary Rajput (2010) found that among the insecticides tested for their efficacy as seed treatment spinosad 45 SC was found to be most toxic against S. *oryzae C. chinensis* and *C. maculatus* with LC_{50} values of 0.08 ppm 0.24 ppm and 0.05 ppm, respectively as compared to treated with malathion (12.67, 23.13 and 34.33 ppm).

Fenvalerate 20 EC and dichlorvos 76 EC were least toxic against *S. oryzae*, *C. chinensis* and *C. maculatus*, respectively.

Reviewing the efficacy of oils as seed protectantsthere were differences in oils efficacy at the doses tested under different experimental conditions. Several earlier studies have demonstrated the effectiveness of different vegetable oils in protecting grains against major stored-product insect pests (Shaaya *et al.*, 1976; Don-Pedro, 1987, 1989; Obeng-Ofori, 1995). Singh *et al.* (1978), Messina and Renwick (1983), Pandey *et al.* (1983), Pereira (1983) and Don-Pedro (1989) have shown that oil coating is effective in controlling *C. maculatus* and present results are accordance with them. 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 oils could also acts as antifeedants or modify the storage micro-environment thereby discouraging insect penetration and feeding (Obeng-Ofori, 1995).

As for the efficacy of oils alone the present results are in agreement with those of Ali *et al.* (1983) who reported the toxic effect of neem, coconut, rapeseed, mustared, sesame, dalda and palm oil on the pulse beetle *Callosobruchus chinensis*. The results are also similar to the findings of Mueke (1989) and Mondal and Akhtar (1992) who reported the insecticidal properties of castor oil against *C. maculatus*. Also Aktar and Mondal (1996) observed that sesame oil is effective against larva of *T. confusum*.

Similarly, Pacheco *et al.* (1995) evaluated refined soybean and crude castor oils for the control of infestation of *Callosobruchus maculatus* (F.) and *C. phaseoli* (Glyienhol) in stored chick pea (*Cicer arietinum* L.) and observed that both oils inhibited population growth of the two insect species as compared to untreated seeds.

Castor oil was more effective than soybean oil. Later on Visetson *et al.* (2003) found that sesame oil showed good synergism with cypermethrin yielding Synergistic Ratios (SR) that ranged from 1.54-6.33 in contact testing method and 2.04-5.88 in no-choice leaf dipping method and were comparable to using PB which showed SR's of 6.33 and 6.71, respectively.

Both PB and sesame oil mixed together with cypermethrin inhibited monooxygenase activity by approximately two-thrid but induced glutathione -S- transferases ca. 2.3 fold in both methods. The synergists had no effect on esterase activity (C.F ca 1.2). However, Khalequzzaman and Chowdhury (2003) found that oils of neem, sesame, castor and soybean as synergist with pirimiphos-methyl enhance the mortality of the adult *T. castanium* and the maximum synergism was at 1:10 ratio. Likewise, the present study shows that the tested plant oils have relatively low insecticidal activity when tested alone but some such as corn oil when combined with pyridalyl and abamectin or sesame oil when combined with abamectin exhibited considerably high synergistic activity.

Recently Law-Ogbomo (2007) studied the efficacy of three plant oils (rubber seed oil, palm oil and palm kerned oil) in reducing post harvest loss caused by *Callosobruchus maculatus* F. adults. Exposing adult weevils to levels of 02.55 and 10 mL kg^{-1} admixed with cowpea grains resulted in significantly high mortality of adults (70-96%) at 7 days after all plant oil treatments. The plant oils gave appreciable percentage reduction in weight loss (0.1-1.7%) significant reduction in punctured grain (0-32%) versus (48.2%) and (93.2%) in control, respectively in addition that oils treatments had no adverse effects on seed viability. A combination of rubber seed oil with cowpea seeds at 10 mg kg^{-1} grain gave the most effective reduction in *Callosobruchus* population and damage.

As for testing oils in binary mixtures with insecticides Sridevi and Dhingra (1996, 1999, 2000) evaluated the variation in the efficacy of deltamethrin formulated alone and in combination with five non-toxic vegetable oils viz., sesame, karanj, neem and citronella oil in four rates (1:11: 21:41: 8) against susceptible and resistant strains of *T. castanium* by direct spray and residue film methods and observed that all the vegetable oils proved additive when combined with deltamethrin except neem oil which showed antagonistic effect.

Though use plant oils in binary mixtures (admix) with low amounts of insecticides proved to be effective in control of *Callosobruchus maculatus* F. weevil when these products (oils) are relatively available and cheep when compare with the conventional insecticides alone as possible alternative protectants of stored grains (Enobakhare and Law-Ogbomo, 2002). In addition plant oils have no adverse effects after storage on cooking and taste. It also eliminates the possibility of food contamination when insecticides are mixed with grain in storage.

Accordingly the most effective strategy and economically reasonable in prevent the post harvest loss of the small-scale farmers could obtained by integrating the tested plant oils with low amount of effective and safe insecticides.

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