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Toxicity of prirmiphos-methyl and three essential oils, alone and in combination against *Callosobruchus maculatus* (Fab.)

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Abstract: Toxicity of pirimiphos-methyl and three essential oils, viz. cardamom [*Elettaria cardamomum* (L.) Maton.], cinnamon (*Cinnamomum aromaticum* Nees) and clove [*Syzygium aromaticum* (L.) Merr. & Perry]; either singly or in mixture in different ratios were investigated on the cowpea weevil, *Callosobruchus maculatus* (F.) adults, through residual film bioassay. The LD_{50} of pirimiphos-methyl was 0.011 μg cm^{-2} and that of cardamom, cinnamon and clove oils were 20.68, 12.38 and 16.89 μg cm^{-2} respectively after 24 h post-treatment. Pirimiphos-methyl and tested essential oils were used as mixture of 1:1, 1:2, 1:5, 1:10 and 1:20 and the LD_{50} values were calculated. All essential oils when combined with pirimiphos-methyl proved to be antagonistic effect against *C. maculatus* adults except cardamom oil which offered synergistic effect at the 1:20 ratio with pirimiphos-methyl.

Key words: pirimiphos-methyl, cardamom, cinnamon, clove, $LD_{50,}$ synergism.

Introduction

Essential oils are also called volatile or ethereal oils (Guenther, 1948), derived mostly from vegetable sources, occur as a rule, in small concentrations in special cells of plant and distributed or distributed over many particular parts, e.g., leaves (citronella, eucalyptus), barks (cinnamon), roots, rhizomes (turmeric, ginger, galangal), flowers (bergamot tree and tuberose), fruits (all spice, anise, star anise) or seeds (nutmeg). They are volatile and can act like fumigants offering the prospect for use in stored-product protection (Huang *et al*., 2000; Lee *et al*., 2004), contact insecticides (Ngamo *et al*., 2007), antifeedent or repellent effects (Park *et al.,* 2003; García *et al*., 2005) and may also affect some biological parameters such as growth rate, life span and reproduction (Raja & William, 2008). The toxicity of a large number of essential oils and their constituents has been evaluated against a number of stored product insects (Asawalam, 2006; Chaubey, 2008).

Cardamom spice consisting of whole or ground dried fruit, or seeds, of *Elettaria cardamomum* (L.) Maton. an herbaceous perennial of the family Zingiberaceae. A perennial, reed-like herb, cardamom grows wild and is cultivated in India and Ceylon. The essential oil occurs in large parenchyma cells underlying the epidermis of the seed coat. Oil is extracted from the fruits is used both in pharmacy and perfumery. Its main constituents are 1,8-cineole (eucalyptol) and alpha-terpinyl acetate. Cinnamon (*Cinnamomum aromaticum* Nees) is a bushy evergreen tree of the family Lauraceae, native to Sri Lanka, India, and Myanmar and also cultivated in South America and the West Indies. The most abundant component in the stem bark oil is 1,8-cineole, which constitutes 30% of the oil. p-Cymene (20.7%), linalool (13,7%), α-terpineol (5.1%) and terpinen-4-ol (2.9%) were among the major constituents identified (Ali & Jantan, 1999). The clove tree [*Syzygium aromaticum* (L.) Merr. & Perry] is endemic in the North Moluccas (Indonesia) and was of old cultivated in the islands of Termate, Tidore, Bacan and the West coast of Hamahera. The Dutch extended cultivation to several other islands in the Moluccas, but only after the end of the Dutch monopoly (18th Century), clove trees were introduced to other countries. The oil itself is dominated by eugenol (70 to 85%), eugenol acetate (15%) and β-caryophyllene (5 to 12%), which together make up 99% of the oil (Bauer *et al.,* 2001). Pirimiphos-methyl (Actellic), an organophosphate, registered in the USA for treating stored corn and sorghum (USEPA, 2003) has lower mammalian toxicity showed to be effective on wheat against several stored-product insects (Huang & Subramanyam, 2005).

Callosobruchus species are major pests of stored grains and grain products in the tropics (Prevett, 1961). Over 90% of the insect damage to cowpea seeds is caused by *Callosobruchus maculatus* (F.) (Caswell, 1981). The insect lays its eggs on the seeds of cowpea, which hatch and produced larvae that bore into the seed cotyledons on which they feed (Onuh & Onyenekwe, 2008). Infestation may reach 100% within 3–5 months of storage (Braga *et al*., 2007). Control of *C. maculatus* relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms in addition to direct toxicity to users. The present study was to investigate the effect of pirimiphos-methyl and essential oils applied singly or combined in simple mixtures on adult *C. maculatus*.

Materials and Methods

Test insect and essential oils: C. maculatus from the groceries of Shaheb Bazar, Rajshahi. The cowpea, *Vigna unguiculata* (L.) Walp. seeds were used as food medium throughout the experiments. The essential oils, cardamom, cinnamon and clove were purchased as pure oil (India) from an essential oil shop. The oils were further dehydrated in a vacuum rotary evaporator. The oils were then collected in sealed glass containers and refrigerated in the dark at 4°C until they are used.

Toxicity tests with single dose: Residual film method (Busvine, 1971) was used to test the mortality of the adult *C. maculatus*. Technical grade of Pirimiphos-methyl (0-2-(diethylamino)-6-methylpyrimidin-4-yl) 0,0-dimethyl phosphorothioate (*C.A.*) 0-[2-(diethylamino)-6-methyl-4-pyrimidinyl] 0, 0-dimethyl phosphorothioate) was used as insecticide, collected from ACI Limited. The insecticide was diluted in acetone and different doses were prepared. At first an ad-hoc experiment was made. After having a clear picture about mortality of beetles, the final experiments were set up. The doses used for pirimiphos methyl were 0.047, 0.037, 0.028, 0.019 and 0.009 μg cm^{-2}, whereas the doses of cardamom, cinnamon and clove oils were 56.586, 47.155, 37.724, 28.293 and 18.862 μg cm^{-2}, 37.724, 31.436, 25.149, 18.862 and 12.575 μg cm^{-2}, and 47.155, 39.296, 31.436, 23.575 and 15.718 μg cm^{-2} respectively. One ml of liquid from each dose was dropped on petri dishes (90 mm) separately, covering uniformly the whole area of the petri dish. They were then kept open for sometimes to dry-up. Four plastic rings (30 mm) were placed inside a petri dish and 10 adult beetles were released within each ring. The rings within the petri dish were served as replications. The doses were calculated by measuring the actual amount of active ingredient (μg) present in one ml of the solvent divided by the surface area of the petri dish. One batch of control was maintained in which only acetone was applied for each strains, respectively.

Toxicity tests with combined doses: Pirimiphos-methyl was combined in mass ratios (1:1, 1:2, 1:5, 1:10 and 1:20) with the essential oils used and applied on C. *maculatus* adults. The doses of pirimiphis-methyl and cardamom oil in mass ratios were 0.051, 0.041, 0.031, 0.021 and 0.011 μg cm^{-2} for 1:1 ratio; 0.099, 0.079, 0.059, 0.039 and 0.019μg cm^{-2} for 1.2 ratio; 1.202, 0.962, 0.721, 0.481 and 0.240 μg cm^{-2} for 1:5 ratio; 1.587, 1.270, 0.952, 0.634 and 0.317 μg cm^{-2} for 1:10 ratio and 0.790, 0.632, 0.474, 0.316 and 0.158 μg cm^{-2} μg cm^{-2} for 1:20 ratio. In case of pirimiphos and cinnamon oil the combined doses were 0.130, 0.104, 0.078, 0.052 and 0.026 μg cm^{-2} for 1:1 ratio; 0.247, 0.198, 0.148, 0.099 and 0.049 μg cm^{-2} for 1.2 ratio; 10.601, 0.481, 0.361, 0.240 and 0.120 μg cm^{-2} for 1:5 ratio; 10.798, 0.638, 0.479, 0.317 and 0.159 μg cm^{-2} for 1:10 ratio and 0.790, 0.632, 0.474, 0.316 and 0.164μg cm^{-2} for 1:20 ratio. The doses of pirimiphos-methyl and clove oil in mass ratios were 0.108, 0.086, 0.065, 0.043 and 0.022 μg cm^{-2} for 1:1 ratio; 0.206, 0.165, 0.123, 0.082 and 0.041 μg cm^{-2} for 1.2 ratio; 0.250, 0.200, 0.150, 0.100 and 0.050 μg cm^{-2} for 1:5 ratio; 1.588, 1.270, 0.953, 0.635 and 0.317 μg cm^{-2} for 1:10 ratio and 2.369, 1.896, 1.422, 0.948 and 0.474 μg cm^{-2} for 1:20 ratio.

Analysis of Data: The mortality of adult beetles was recorded after 24h of treatment. Corrected mortality percentage was calculated using Abbott's formula (Abbott, 1925); probit analysis was done according to Finney (1947) using a software developed in the Department of Agricultural and Environmental Science, University of Newcastle upon Tyne, UK. If the probability was greater than 5% an automatic correction of heterogeneity was introduced. Co-toxicity coefficient was calculated as for Sun & Johnson (1960).

Results

Single bioassay: The LD_{50} of pirimiphos-methyl has been calculated as 0.011 μg cm^{-2} with the 95% confidence limits was 0.007 to 0.017 μg cm^{-2}. The LD_{50} of cardamom, cinnamon and clove oil was 20.68, 12.38 and 16.89 μg cm^{-2} respectively with the

95% confidence limits were 15.90 to 26.90, 8.65 to 17.73 and 12.98 to 21.97μg cm^{-2} respectively. The regression lines are presented in Fig.1.

Combined bioassay: Pirimiphos-methyl and cardamom oil was used as mixture of 1:1, 1:2, 1:5, 1:10 and 1:20 and the LD_{50} was 0.027, 0.048, 0.393, 0.418 and 0.201μg cm^{-2} respectively. In the same ratios of pirimiphos-methyl and cinnamon oil the LD_{50} was 0.079, 0.140, 0.327, 0.241 and 0.294 μg cm^{-2}; and pirimiphos-methyl and clove oil the LD_{50} was 0.077, 0.086, 0.100, 0.488 and 0.661 μg cm^{-2} (Table 1).

Table 1. LD_{50}, regression equation, co-toxicity coefficient and synergistic ratio due to the effect of pirimiphos-methyl and different essential oil mixtures on adult *C. maculatus* after 24 hours of treatment.

Mixture	Ratio	LD_{50} (μg cm^{-2})	95% confidence limits		Regression equations	χ^2 (3 df)	Cotoxicity coefficient	Synergistic ratio
			Lower (μg cm^{-2})	Upper (μg cm^{-2})				
PM:CA	1:1	0.027	0.020	0.037	Y = 4.418004± 1.348146X	0.61	84.44	0.422
	1:2	0.048	0.035	0.066	Y = 4.120276± 1.292739X	0.81	71.70	0.239
	1:5	0.393	0.251	0.614	Y = 4.302636± 1.173538X	0.76	17.40	0.029
	1:10	0.418	0.252	0.694	Y = 4.228422± 1.242516X	1.66	30.08	0.027
	1:20	0.201	0.112	0.358	Y = 4.661647± 1.120396X	0.30	120.00	0.057
PM:CI	1:1	0.079	0.053	0.120	Y = 4.087872± 1.013522X	1.11	28.72	0.144
	1:2	0.140	0.096	0.205	Y = 3.763572± 1.077843X	0.44	24.41	0.081
	1:5	0.327	0.207	0.515	Y = 4.543817± 0.8873508X	1.44	20.96	0.035
	1:10	0.241	0.124	0.466	Y = 4.680056± 0.8396521X	0.29	52.06	0.047
	1:20	0.294	0.192	0.448	Y = 4.472741± 1.127512X	0.07	82.01	0.039
PM:CL	1:1	0.077	0.051	0.116	Y = 4.000392± 1.125478X	1.43	29.53	0.148
	1:2	0.086	0.060	0.124	Y = 3.881017± 1.195447X	0.02	39.72	0.132
	1:5	0.100	0.070	0.144	Y = 3.762725± 1.235348X	0.14	68.26	0.114
	1:10	0.488	0.342	0.694	Y = 3.912558± 1.580527X	1.70	25.73	0.023
	1:20	0.661	0.488	0.894	Y = 3.354421± 2.00696X	1.65	36.31	0.017

PM = Pirimiphos-methyl; CA = Cardamom oil; CI = Cinnamon oil; CL = Clove oil

In case of mixture of pirimiphos-methyl and cardamom oil the cotoxicity coefficient value has been calculated as 84.44, 71.70, 17.40, 30.08 and 120.00 at 1:1, 1:2, 1:5, 1:10 and 1:20 ratios respectively after 24 h of observation. The cotoxicity coefficient was also highest at 1:20 ratios suggesting the synergistic effect in this ratio. However, synergistic ratio was 0.422, 0.239, 0.029, 0.027 and 0.057 followed the order 1:1 > 1:2> 1:20> 1:5>1:10. The cotoxicity coefficient of the mixture of pirimiphos-methyl and cinnamon oil in 1:1, 1:2, 1:5, 1:10 and 1:20 ratios has been calculated as 28.72, 24.41, 20.96, 52.06 and 82.01 respectively. Similarly the cotoxicity coefficient is highest at 1:20 ratio but no co-toxicity coefficient value was greater than 100, therefore with this oil no synergistic effect was significant. The synergistic ratio was 0.144, 0.081, 0.035, 0.047 and 0.039 follows the order 1:1 > 1:2 > 1:5 > 1:10 > 1:20. For the mixture of pirimiphos-methyl and clove oil the cotoxicity coefficient values were 29.53, 39.72, 68.26, 25.73 and 36.31 at 1:1, 1:2, 1:5, 1:10 and 1:20 ratios respectively for 24 hours after treatment. The highest cotoxicity coefficient is at 1:5 ratio. The cotoxicity coefficient, however, did not exceed the mark 100 in any ratio. The synergistic ratio was 0.148, 0.132, 0.114, 0.023 and 0.017 in the above mentioned ratios of mixtures respectively.

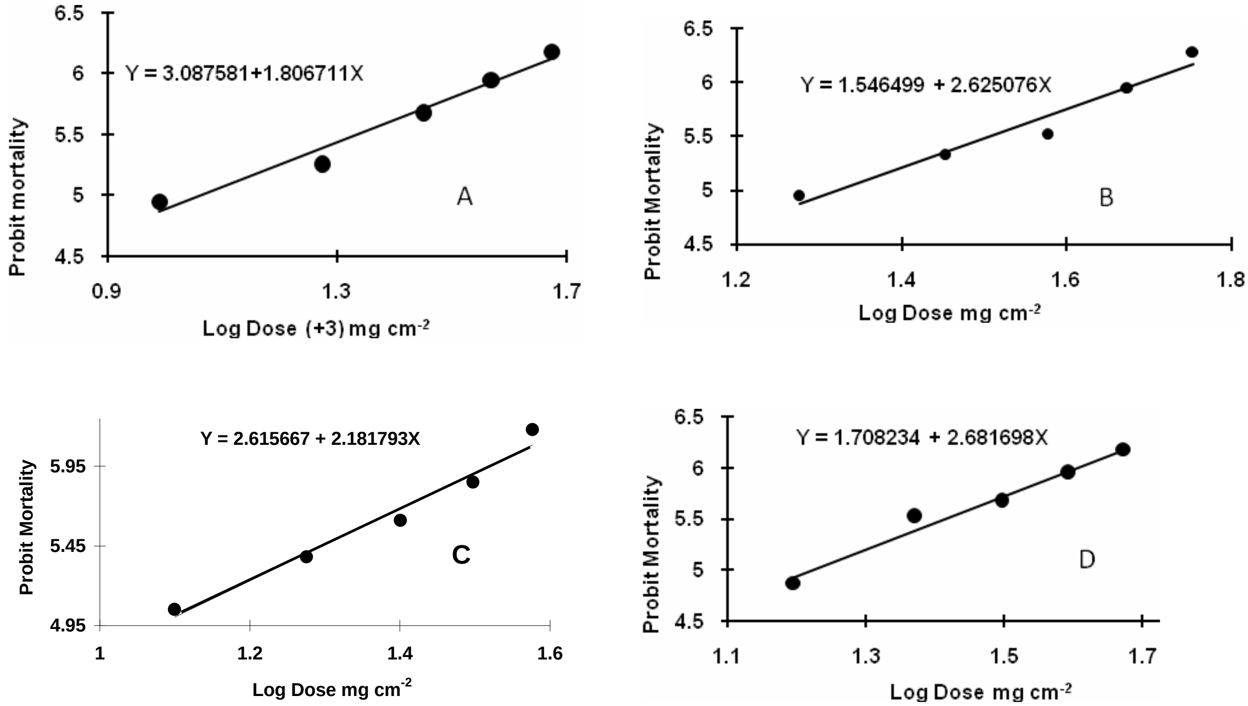

Fig. 1. Regression lines of log dose of pirimiphos-methyl (A), cardamom oil (B), cinnamon oil (C) and clove oil (D) on the probit mortality of adult *C. maculatus* after 24 hours of residual film treatment.

Discussion

Cinnamom oil was found to be highly effective in inducing mortality of *C. maculatus*. Clove and cardamom oils were also found to be effective as their LD_{50} values are very close. Pirimiphos-methyl also proved to be a good insecticide for control of *C. maculatus*. Swella & Mushobozy (2007) evaluated the effectiveness to control *C. maculatus* in cowpea by the synthetic insecticide Actellic dust (pirimiphos-methl), and by natural protectants ash, coconut oil, powdered cloves and black pepper. Seeds treated with Actellic dust and black pepper powder had significantly low percentages of damaged seeds. There were differences in oil efficacy at the doses tested under different experimental conditions. The present results corroborates the findings of Jilani & Malik (1973) who also reported the toxic effect of neem oil, coconut oil, rapeseed oil, mustard oil, sesame oil, dalda and palm oil on *C. chinensis*.

Cinnamaldehyde, the main constituent of cinnamon oil, exerted equal contact toxicity to both *Tribolium castaneum* and *Sitophilus zeamais* (Huang *et al*., 2000). Effect of three selected essential oils, viz. cardamom, cinnamon and clove oils on *C. maculatus* aduls evaluated in the present investigation exhibited that they induce mortality. Therefore, it was thought reasonable to study the combined action of these oils as synergist with pirimiphos-methyl. The results of the combined action revealed that the mixture of all three oil produced antagonistic effect in both 24 h after treatment. Only 1:20 ratio dose of pirimiphos-methyl showed some synergistic action having the co-toxicity coefficient value 120.00. The review of literature, however, revealed that no information is available on the combined action of mixture of essential oils for the control of *C. maculatus* and other stored grain pests. Kabeh & Jalingo (2007) studied the insecticidal potency of dust from leaves of *Vernonia amygdalina* was compared with pirimiphos-methyl powder on the larvae of *C. maculatus* and *S. zeamais* and observed that both toxicants gave substantial control. Similar results have been reported by Rajapakse & Rathnasekera (1998) who noted enhanced mortality and persistence of pirimiphos-methyl in causing significant mortality to *C. maculatus* and by Khalequzzaman and Chowdhury (2003) to *T. castaneum* when combined with vegetable oils.

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