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Evaluation of Mixtures of Plant Oils as Synergists for Pirimiphos-methyl in Mixed Formulations Against *Tribolium castaneum* (Herbst)

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Abstract: The variation in the efficacy of pirimiphos-methyl, neem, sesame, castor and soybean oil either alone against the adults of CTC-12, pH-1, Kano and Black strains of ***Tribolium castaneum*** (Herbst) and in combination of four ratios of pirimiphos-methyl and oil (1:1, 1:2, 1:5, 1:10) against CTC-12 strain of the beetle was evaluated by film residue method. The results showed that the LD_{50} of pirimiphos-methyl was 0.304, 0.355, 0.264 and 0.317 $\mu g\ cm^{-2}$ for CTC-12, PH-1, Kano and Black strains, respectively. The same value was 56.58, 23.07, 26.70 and 35.60 $\mu g\ cm^{-2}$ from neem oil; 11.71, 2.89, 44.71 and 7.97 $\mu g\ cm^{-2}$ for sesame oil; 14.85, 9.48, 42.97 and 23.40 $\mu g\ cm^{-2}$ for castor oil and 19.86, 20.95, 22.96 and 67.27 $\mu g\ cm^{-2}$ for soybean oil in the same order of strains, respectively. All oils proved additive when combined with pirimiphos-methyl except neem and soybean oils which showed an antagonistic effect against the CTC-12 strain of ***T. castaneum*** at 1:1 ratio. The maximum synergism of pirimiphos-methyl was observed at 1:10 ratio having the highest co-toxicity coefficient value for neem oil (4908.53) followed by sesame oil (434.11), castor oil (295.24) and soybean oil (232.93).

Key words: ***Tribolium castaneum***, pirimiphos-methyl, plant oils, synergism, co-toxicity coefficient, LD_{50}

Introduction

The use of pesticides is one means of preventing some losses during storage. However, the choice of pesticides for storage pest control is very limited because of the strict requirements imposed for the safe use of synthetic insecticides on or near food. The continuous use of chemical pesticides for control of stored-grain pests has resulted in serious problems such as insecticide resistance (Pacheco ***et al.***, 1990; Sartori ***et al.***, 1990). Furthermore, the efficacy of insecticides against storage pests varies greatly after treatment (Suchita ***et al.***, 1989; Pinto ***et al.***, 1997). Chemicals used for control of stored product pests, or as protectants need also to be compared with the suitability and effectiveness of alternative methods of control. Non-chemical methods are attractive since they neither leave chemical residues in the commodity nor do they cause resistance in insects. The public awareness and concern for environmental quality, has led to more focused attention on research and development of biological agents, either as alternatives or in integrated programmes (Hidalgo ***et al.***, 1998). The need of evaluation of pest management strategies based on non-insecticidal chemicals having behavioural or physiological activity with insecticidal potential with or without selective insecticide deployment has been recognized as an ecological imperative for well over a decade now. However such chemicals are often highly specific, biodegradable of low persistence and their control potential is sophisticated and long term. Although ecologically desirable, these very characteristics of non-insecticidal chemicals weigh against their economic feasibility and reduce their appeal to the primary users i.e- farmers (Sharma ***et al.***, 1983).

Synthetic insecticides are expensive for subsistence farmers and they may pose potential risks owing to the lack of adequate technical knowledge related to their safe use. One alternative to synthetic insecticides is insecticidal plants; African and Asian farmers are traditionally familiar with them (Thiam and Ducommun, 1993). In ancient times, oils obtained from locally available plants were used for stored grain protection against insect attack. In recent years, attention has been given to use of vegetable oils as stored grain protectants against insects (Verma and Pandey, 1978; Oca ***et al.***, 1978; Santos ***et al.***, 1981; Pandey ***et al.***, 1981; Qi and Burkholder, 1981; Messina and Renwick, 1983; Pereira, 1983; Ivbijaro, 1984 a, b; Ivbijaro ***et al.***, 1984; Pierrard, 1986; Saim and Meloan, 1986; Sighamony ***et ai.***, 1986; Ahmed ***et al.***, 1988; Don-Pedro, 1989; Kumari ***et al.***, 1990; Hall and Harman, 1991; Stamopoulos, 1991; Pacheco ***et al.***, 1995; Shaaya ***et al.***, 1997). Oils extracted from plants have been extensively used in tropical countries for crop protection (Singh ***et al.***, 1978; DabireÂ, 1993; Rajapakse and van Emden, 1997). The mode of action of these oils is yet to be confirmed (Tembo and Murfitt, 1995), but most appear to cause death of insect egg, larva or adult by suffocation (Hewlett, 1975; Ivbijaro ***et al.***, 1984; Don-Pedro, 1989). The fact that most studies on the use of plant oils as proctectants of stored grain against insects have shown their action to be mainly against eggs and early larval stages restricts their use. Don-Perdo (1989) indicated that vegetable oils used alone were less effective than commercial insecticides and suggested the possibility of using vegetable oils in combination with synthetic insecticide in simple mixture as a mean of making their use more attractive and effective. In line with this hypothesis, earlier studies by Ahmed and Gardiner (1967) showed that dilute malathion in oil was more effective than concentrated malathion topically applied on the desert locust.

Tribolium species are major pests of stored grains and grain products in the tropics (Howe, 1965). Control of these insects 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 (Jembere ***et al.***, 1995; Okonkwo and Okoye, 1996). The objective of this study was to investigating the effect of pirimiphos-methyl and plant oils combined in simple mixtures treated on adult ***Tribolium castaneum***.

Materials and Methods

Test insect

Four strains of the flour beetle, *T. castaneum* viz., CTC-12, PH-1, Kano and Black strains were used for this study, the stock of which were collected from the Crop Protection Lab. of the Department of Zoology, Rajshahi University and successfully reared at the Entomology Laboratory of Carmichael College, Rangpur. The experiments were carried out during June to December, 2000. Mass cultures were maintained in jars (1000 ml) and subcultures were in beakers (500 ml) with food medium and kept in an incubator at 30±0.5°C. A standard mixture of whole-wheat flour with powdered dry yeast in a ratio of 19:1 (Park and Frank, 1948; Park, 1962; Zyromska-Rudzka, 1966; Khalequzzaman ***et al.***, 1994) was used as food medium throughout the experimental period.

Toxicity tests

Residual film method (Busvine, 1971) was used to test the mortality of the adults of *T. **castaneum***. 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). Technical grade of the test insecticided was taken as sample from ACI Limited. This 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 with the doses 0.944, 0.472, 0.236 and 0.118 μg cm^{-2} for CTC-12, PH-1, Kano and Black strains. 1 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.

Neem oil was diluted in acetone and different doses were prepared. The doses were 361.13, 180.56, 90.28, 45.14 and 22.57, μg cm^{-2} for CTC-12 and Black strains and 180.56, 90.28, 45.14, 22.57, 11.29 and 5.65 μg cm^{-2} for PH-1 and Kano strains. The doses of sesame oil were 213.78, 106.89, 53.44, 26.72, 13.36 and 6.68 μg cm^{-2} for CTC-12; 106.89, 53.44, 26.72, 13.36, 6.68 and 3.34 μg cm^{-2} for PH-1 and Black strains; 427.56, 213.78, 106.89, 53.44, 26.72 and 13.36 μg cm^{-2} for Kano strain. In castor oil the doses were 218.03, 109.02, 54.51, 27.25, 13.63 and 6.81 μg cm^{-2} for all strains. The doses of soybean oil were 397.48, 198.74, 99.37, 49.68, 24.84 and 12.42, μg cm^{-2} for CTC-12 and Black strains; 198.74, 99.37, 49.68, 24.84 and 12.42 μg cm^{-2} for PH-1strain; 794.95, 397.48, 198.74, 99.37, 49.68 and 24.48 μg cm^{-2} for Kano strain.

Application of pirimiphos-methyl as combined dose with oils was made on adult CTC-12 strain of *T. **castaneum***. In mixtures 1.179, 0.059, 0.295 and 0.148μg cm^{-2} of pirimiphos-methyl was mixed with neem oil in mass ratios of 1:1, 1:2, 1:5, 1:10 and thus four doses were made for each ratio and a control. In the similar way 0.615, 0.307, 0.154 and 0.077 μg cm^{-2}; 0.622, 0.311, 0.156 and 0.078μg cm^{-2} ; and 1.185, 0.593, 0.296, 0.148 and 1.074μg cm^{-2} of pirimiphos-methyl was mixed in mass ratios of 1:1, 1:2, 1:5, 1:10 with sesame oil, castor oil and soybean oil separately. Then the combined doses were applied on to the petri dish to study film residue bioassay in the similar way as individual bioassay with pirimiphos-methyl or oil.

Analysis of Data

The mortality of adult beetles was recorded after 24 h of treatment. Corrected mortality percentage was calculated using Abbott's formula (Abbott, 1925); probit analysis was done according to Finney (1947) and Busvine (1971) 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 and Johnson (1960 a,b). 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 and it indicates a synergistic action. On the other hand, when a mixture gives a co-toxicity coefficient less than 100 and the effect of the mixture indicates an antagonistic action.

Results

Toxicity of pirimiphos-methyl and different oils treated alone to *T. castaneum*

In toxicity tests with pirimiphos-methyl the LD_{50} value has been calculated as 0.304, 0.355, 0.264 and 0.317 μg cm^{-2} for CTC-12, PH-1, Kano and Black strains, respectively. Here Kano strain was the most tolerant to pirimiphos-methyl followed by CTC-12, Black and PH-1 showed most susceptible (Table 1).

In plant oils the order of toxicity was different for different oils and the strains studied. The LD_{50} value of neem oil was 56.58, 23.07, 26.70 and 35.60 μg cm^{-2} for the above strains, respectively. The same was 11.71, 2.89, 44.71 and 7.97 μg cm^{-2}; 14.85, 9.48, 42.97 and 23.40 μg cm^{-2} and 19.86, 20.95, 22.96 and 67.27 μg cm^{-2} when treated with sesame, castor and soybean oil for CTC-12, PH-1, Kano and Black strains, respectively.

Dead insects from oil treatment showed signs of rapid immobilization with their legs flexed and clinging to the surface of the petri dishes, whereas those from pirimiphos-methyl treated appeared paralysed with their metathiracic wings unfolded and stretched outside the elytra.

Combined action of pirimiphos-methyl and different oils on CTC-12 strain of *T. castaneum*

As the CTC-12 strain of the red flour beetle *T. castaneum* is a resistant strain against organophosphorus insecticide (Lloyd and Ruczkowski, 1980) hence this strain was selected for combined action of pirimiphos-methyl with different plant oils used in mass ratios of 1:1, 1:2, 1:5 and 1:10. The obtained mortality data were analyzed and expressed as combined LD_{50}, segregated LD_{50}'s of toxicant and oil.

Pirimiphos-methyl and neem oil was used as mixtures in the mass ratios of 1:1, 1:2, 1:5 and 1:10 ratio and the combined LD_{50} was calculated as 0.626, 0.785, 1.224 and 0.068 μg cm^{-2}, respectively. In the same ratios of pirimiphos-methyl and sesame oil the combined LD_{50} was 0.339, 0.338, 0.621 and 0.769 μg cm^{-2}, respectively. In mixtures of pirimiphos-methyl and castor oil the combined LD_{50} was 0.411, 0.712, 1.796 and 1.132 μg cm^{-2} and for mixture of pirimiphos-methyl and soybean oil it was 0.637, 0.677, 1.284 and 1.428 μg cm^{-2} in 1:1, 1:2, 1:5 and 1:10 ratios, respectively (Table 2).

Table 1: LD_{50}, 95% confidence limits and regression equations of pirimiphos-methyl and oils to adult *T. castaneum* after 24 h of treatment

Insecticides	Insect Strain	LD_{50} ($\mu g\ cm^{-2}$)	95% confidence limits Lower ($\mu g\ cm^{-2}$)	Upper ($\mu g\ cm^{-2}$)	Regression equations	X^2(df)
Pirimiphos-mehthyl	CTC-12	0.304	0.230	0.401	Y=3.47445+3.160826X	1.25(2)
	PH-1	0.355	0.265	0.474	Y=3.355314+2.989912X	0.67(2)
	Kano	0.264	0.176	0.394	Y=4.148195+2.021863X	0.38(2)
	Black	0.317	0.227	0.442	Y=3.754982+2.480811X	0.18(2)
Neem oil	CTC-12	56.58	37.32	85.81	Y=2.757289+1.279562X	3.57(3)
	PH-1	23.07	14.15	37.59	Y=3.736476+0.9270168X	7.59(4)
	Kano	26.70	19.70	36.18	Y=2.74127+1.583456X	1.77(4)
	Black	35.60	25.05	50.60	Y=2.786119+1.426986X	2.57 (4)
Sesame oil	CTC-12	11.71	7.16	19.14	Y= 3.681136 + 1.234376X	1.10(4)
	PH-1	2.89	1.37	6.09	Y= 4.477584 + 1.133158X	0.93(4)
	Kano	44.71	21.10	94.73	Y= 3.226101 + 1.074846X	11.31(4)
	Black	7.97	5.05	12.59	Y=3.939747+1.175808X	1.65(4)
Castor oil	CTC-1	14.85	9.52	23.17	Y=3.527855+1.256423X	3.40(4)
	PH-1	9.48	4.66	19.31	Y=4.082174+0.9394229X	0.64(4)
	Kano	42.97	32.66	56.55	Y=1.847224+1.930438X	0.82(3)
	Black	23.40	17.18	31.87	Y=2.790454+1.613723X	4.55(4)
Soybean oil	CTC-12	19.86	11.19	35.26	Y=3.571706+1.100389X	1.53(4)
	PH-1	20.95	13.31	32.98	Y=3.160791+1.391983X	0.21(3)
	Kano	22.96	8.39	62.84	Y=3.927785+0.7878612X	0.31(4)
	Black	67.27	48.49	93.32	Y=2.028839+1.625514X	3.41(3)

Table 2: LD_{50}, 95% confidence limits and regression equations of pirimiphos-methyl (Actelic): in mass ratios of different oils to adult *T. castaneum* after 24 hours of treatment

Pirimiphos-methl: Plant oil	Ratio	LD_{50} ($\mu g\ cm^{-2}$)	95% confidence limits Lower ($\mu g\ cm^{-2}$)	Upper ($\mu g\ cm^{-2}$)	Regression equations	X^2(df)
Neem oil	1: 1	0.626	0.461	0.850	Y = 2.700127+2.885509 X	0.680(2)
	1: 2	0.785	0.555	1.111	Y = 3.024173+2.206962X	0.126(3)
	1: 5	1.224	0.865	1.731	Y = 2.550592+2.251408X	0.499(3)
	1: 10	0.068	0.000	15.273	Y = 5.131612+0.7880261X	0.130(2}
Sesame oil	1: 1	0.339	0.218	0.528	Y = 4.0211+1.843696X	0.057(2)
	1: 2	0.338	0.158	0.723	Y = 4.31794 +1.288594X	0.712(2)
	1: 5	0.621	0.377	1.022	Y = 3.468274+1.930142X	0.283(2)
	1: 10	0.769	0.320	1.846	Y = 3.598245+1.581325X	0.039(2)
Castor oil	1: 1	0.411	0.272	0.621	Y = 3.814597+1.928488 X	0.082(2)
	1: 2	0.712	0.524	0.967	Y = 2.610553+2.801626X	0.238(2)
	1: 5	1.796	0.937	3.441	Y = 3.475114+1.215675X	0.211(2)
	1: 10	1.132	0.535	2.392	Y = 3.502298+1.421169X	0.471(2)
Soybean oil	1: 1	0.637	0.441	0.922	Y = 3.38839+2.002935X	1.091(3)
	1: 2	0.677	0.465	0.984	Y = 3.301746+2.044187X	0.267(3)
	1: 5	1.284	0.823	2.003	Y = 3.156049+1.663222X	1.177(3)
	1: 10	1.428	0.804	2.536	Y = 3.231912+1.530884X	0.450(3)

Synergistic effect of pirimiphos methyl and different plant oils

The mass mixtures of pirimiphos-methyl and different plant oils increased the mortality percentage of the beetles than when it was used alone. The combined LD_{50} values have been segregated as ratio and the co-toxicity coefficient values were calculated and are presented in Table 3. It was observed that in most of the cases used oils acted as synergist with pirimiphos-methyl to ***T. custaneum*** having co-toxicity coefficient values greater than 100.

The results shows that pirimiphos-methyl was best synergised at 1:10 ratio having the highest co-toxicity coefficient value for neem oil (4908.53) followed by sesame (434.11), castor (295.24) and soybean (232.93). Other ratios for all the studied oils were also proved to synergised pirimiphos-methyl at different levels having co-toxicity value above 100 excepting 1:1 ratio of neem and soybean oils which proved antagonistic having the same value less than 100.

Discussion:

The LC_{50} of pirimiphos-methyl against newly emerged and 15-day old adult beetles of Pak strain was 4508 ppm and 3.22 ppm. On the other hand, CTC-12 strain had LC_{50} value of 5821 ppm and 81 ppm, respectively (Mujeeb and Shakoori, 2000). Insecticides of plant origin have been reported to be effective against many insect pests (Jacobson, 1958, 1975). There are numerous reports that vegetable oils including rice bran and peanut, coconut, safflower, groundnut, palm, maize, soybean, cottonseed, mustard, castor, sunflower and neem oil are effective protactants against insect pests (Mummigatti and Ragunathan, 1977; Sangappa, 1977; Schoonhoven, 1978; Pandey ***et al.***, 1981; Don-Pedro, 1989; Malek and Wilkins, 1995; Aktar and Mondal, 1996; Islam, 1987). Tembo and Murfitt (1995) observed that vegetable oils (groundnut, rape seed and sunflower) at 10 ml^{-1} kg were tested alone and in combination with pirimiphos-methyl at ½, 1/3 or 1/4 recommended

Table 3:Co-toxicity coefficient of oils with tested pirimiphos-methyl applied in deferent mass ratio on adult *T. castaneum*

Oils	Pirimiphos-methyl: Oils	Combined LD_{50} ($\mu g\ cm^{-2}$)	Toxicant LD_{50} ($\mu g\ cm^{-2}$)	Cotoxity coefficient
Neem oil	1:1	0.626	0.313	96.96
	1:2	0.785	0.261	116.00
	1:5	1.224	0.204	148.88
	1:10	0.068	0.006	4908.53
Sesame oil	1:1	0.339	0.169	178.94
	1:2	0.338	0.112	269.43
	1:5	0.621	0.103	293.22
	1:10	0.769	0.069	434.11
Castor oil	1:1	0.411	0.205	147.56
	1:2	0.712	0.237	127.90
	1:5	1.796	0.299	101.49
	1:10	1.132	0.102	295.24
Soybean oil	1:1	0.637	0.318	95.28
	1:2	0.677	0.225	134.58
	1:5	1.284	0.214	141.94
	1:10	1.428	0.129	232.93

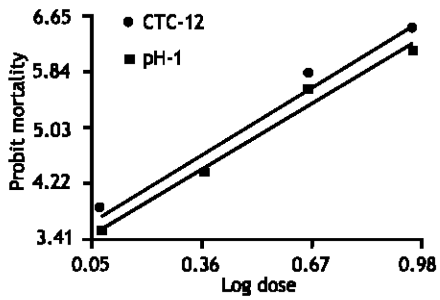

Fig. 1: Probit-regression lines of pirimiphos-methyl on CTC-12 and pH-1 strains of *T. castaneum*

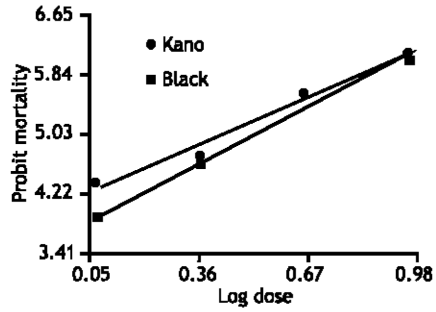

Fig. 2: Probit-regression lines of pirimiphos-methyl on Kano and Black strains of *T. castaneum*

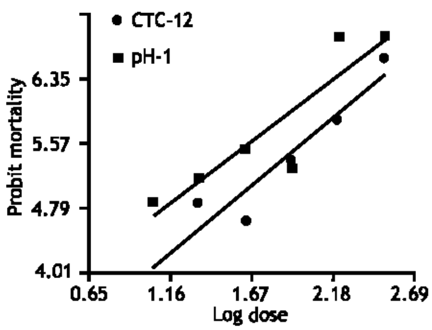

Fig. 3: Probit-regression lines of neem oil on CTC-12 and pH-1 strains of *T. castaneum*

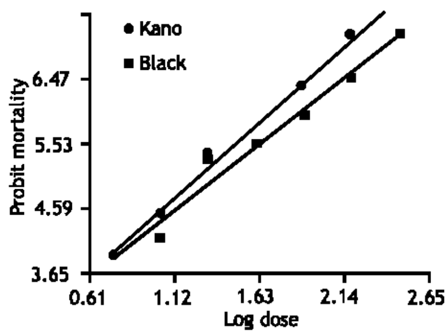

Fig. 4: Probit-regression lines of neem oil on Kano and Black strains of *T. castaneum*

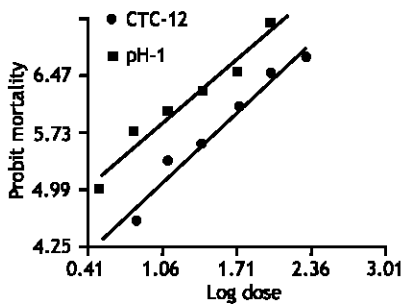

Fig. 5: Probit-regression lines of sesame oil on CTC-12 and pH-1 strains of *T. castaneum*

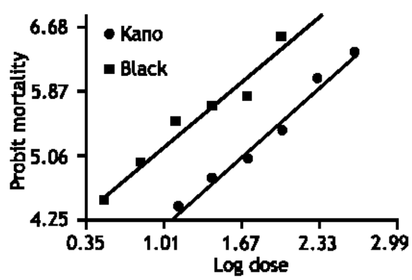

Fig. 6: Probit-regression lines of sesame oil on Kano and Black strains of *T. castaneum*

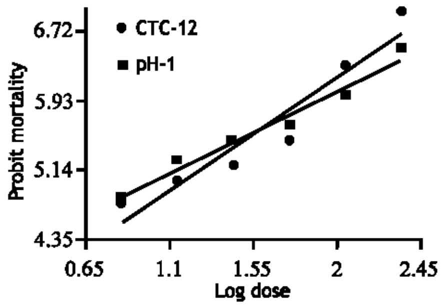

Fig. 7: Probit-regression lines of castor on CTC-12 and pH-1 strains of *T. castaneum*

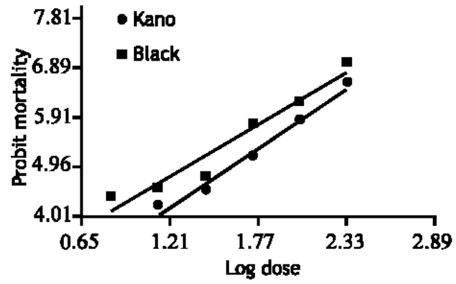

Fig. 8: Probit-regression lines of castor oil on Kano and Black strains of *T. castaneum*

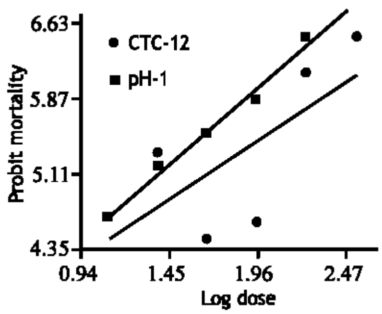

Fig. 9: Probit-regression lines of soybean oil on CTC-12 and pH-1 strains of *T. castaneum*

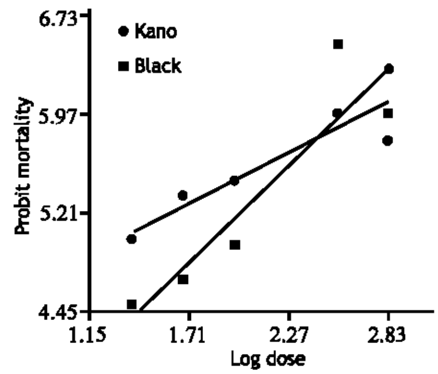

Fig. 10: Probit-regression lines of soybean oil on Kano and Black strains of *T. castaneum*

dosage against ***Sitophilus granarius*** (L.) caused significant mortality compared to controls (untreated grain) and treatments with vegetable oils combined with pirimiphos-methyl at half the recommended dose were as effective as pirimiphos-methyl at the recommended dose. Pacheco ***et al.*** (1995) used refined soybean and crude castor oils were evaluated for the control of infestations of ***Callosobruchus maculatus*** (F.) and ***Callosobruchus*** phaseoli (Gyllenhal) 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.

The fumigant toxicity of a large number of essential oils extracted from various spices and herb plants was assessed against several major stored-product insects (Kéïta ***et al.***, 2000; Kéita, 2001; Raja ***et al.***, 2001, Papachristos and Stamopoulos, 2002 a,b). ***T. castaneum*** (Herbst) was found to be the most resistant, compared with ***Sitophilus oryzae*** (L.) ***Rhyzopertha dominica*** (F.) and ***Oryzaephilus surinamensis*** (L.) to most essential oils tested. 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 (***Pongamia pinnata***), neem and citronella

(***Cymbopogon nardus***) oil in four ratios (1:1, 1:2, 1:4, 1:8) against the adults of susceptible and resistant strains of ***T. castaneum*** by direct spray and film residue methods and observed that all the vegetable oils proved additive when combined with deltamethrin except neem oil which showed an antagonistic effect against the S-strain of ***T. castaneum***.

The present results are in-agreement with those of Jilani and Malik (1973) and Ali ***et al.*** (1983) who reported the toxic effect of neem, coconut, rapeseed, mustard, 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 Aktar (1992) who reported the insecticidal properties of castor oil against ***C. maculatus*** and ***T. castaneum*** respectively. Aktar and Mondal (1992) observed that sesame oil is effective against larvae of ***T. confusum***.

From the present experiments it is very much clear that oils of neem, sesame, castor and soybean as synergist with pirimiphos-methyl enhance the mortality of the adult ***T. castaneum***. The maximum synergism was at 1:10 ratio. The reason for the enhanced toxicity and persistence of insecticide when combined with plant oils were not examined in the experiments, but a possible explanation is that oils increased the pick-up of the insecticide, which is in line with the findings of Ahmed and Gardiner (1967) and Tembo and Murfitt (1995), increase the rate of penetration of the insecticide into the insect as suggested by Benezet and Forgash (1972). The determination of joint toxicity of insecticides is a complicated problem. A simple additive effect based on the mortalities produced by the components applied separately and jointly is rather misleading because the linear relationship between doses and mortality is not based on an arithmetic scale but on a log probit scale. If the toxicity of a mixture is calculated by a simple additive effect, apparent synergistic action can be bound by designing an experiment. So that the concentration of each two (or more) components in the mixture is so adjusted to produce a low but significant mortality.

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