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Evaluation of Plant Oils as Synergists in Suppression of Malathion Resistance in *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst.)

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A B S T R A C T

The present study was carried out to determine the synergistic effects of edible oils (Palm, sunflower, cotton seed, rice bran, castor, sesame) and non-edible oils (Neem, pongamia, citronella, rosemary, thyme, jatropha and lemongrass) in the suppression of malathion resistance *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst.) by adopting residual film method. Among edible oils, sesamum exhibited high level of malathion resistance suppression in *S. oryzae* with SR of 74.48, followed by pongamia, rosemary, rice bran, thyme and neem oils exhibited moderate level of synergistic activity with suppression rate (SR) of 45.99, 45.99, 43.16, 42.61 and 35.87 per cent, respectively. The moderate level of malathion resistance suppression was observed from both non-edible and edible oils against *T. castaneum.* Among non-edible oils, pongamia, rosemary, thyme and neem recorded SR of 47.54, 42.57, 35.81 and 28.93 per cent respectively. The edible oils, sesame, rice bran and cotton seed recorded SR of 42.17, 33.34 and 33.34 per cent respectively. However, none of the plant oil was toxic when applied alone.

Introduction

More than 20,000 species of pre- and post-harvest pests destroy approximately one-third of the world's food production, valued annually at more than $100 billion, among which the highest losses (43% of potential production) occur in developing Asian and African countries (Ahmed and Grainge, 1986). Stored insect pests are the most damaging and the very difficult to control due to their small size, feeding behaviour and ability to attack grain before harvest. In India, losses caused by insects accounted for 6.5% of stored grains (Raju, 1984). The incidence of pesticide resistance is a growing problem in stored-product protection. Resistance to one or more pesticides has been reported in at least 500 species of insects and mites (Georghiou, 1990). An insecticide resistance problem in different stored-product insects has been reported from many countries, stored product insect pests were found to be resistant against several insecticides including bioresmethrin, carbaryl, chlorpyrifo, chlorpyrifos-methyl, cyanophos, cyfluthrin, cyhalothrin, cypermethrin, DDT, deltamethrin, diazinon, dichlorvos, ethylene dibromide, fenitrothion, lindane, malathion, methyl bromide, permethrin, phosphine, phoxim, pirimiphos-methyl, promecarb, propoxur, pyrethrins, temephos, tetrachlorvinphos (DARP, 2003).

Plant oils can play an important role in stored product protection and reduce the need for, and risks associated with, the use of conventional insecticides. 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 (Shaaya *et al.*, 1997). Two major constituents of the essential oil of garlic (*Allium sativum* L.), methyl allyl disulfide and diallyl trisulfide, were found to be potent contact toxicants, fumigants and feeding deterrents against *S. zeamais* and *T. castaneum* (Huang *et al.*, 2000). Xie *et al.*, (1995) reported the repellency and toxicity effects of azadirachtin and neem extracts on *C. ferrugineus, S. oryzae* and *T. castaneum*. Obeng-Ofori and Reichmuth (1997) reported the high toxicity and protectant potential of eugenol against *S. granarius, S. zeamais, T. castaneum* and *P. truncatus*. The essential oil of *Artemisia annua* L. was found to be toxic and repellent against *T. castaneum* and *C. maculatus* (Tripathi *et al.*, 2000).

Many of the stored product insects have developed resistance under field condition to insecticides especially to malathion, lindane and to the fumigant phosphine (Taylor, 1989). In India, so far eight insect species have been reported to have developed resistance to DDT, BHC, malathion and lindane (www.pesticideresistance.org).

An ideal insecticide synergist should be cheap, environmentally safe and effective at low doses (Raffa and Priester, 1985). The commonly available edible and non edible oils possessing these properties could be ideal candidates to be used as synergists. Some oils of plant origin were reported to reduce the insecticide resistance in several insects. At present, the stored product insects surviving repeated treatments with malathion and aluminium phosphide are subjected to further selection pressure of both the chemicals. To the sustained use of these chemicals, the status on the level of resistance is essential which will help to evolve appropriate resistance management strategies. Keeping in this view, present study aims to evaluate edible and non-edible oils as synergists in the suppression of malathion resistance in two stored pests were carried out.

Materials and Methods

Insects were collected from the Tamil Nadu Food Corporation of India godowns from grain stacks of rice, milled rice and wheat, preferably from the oldest stack present in the godown during at the time of visit and reared in the laboratory maintaining a succession of rearing containers for the regular supply of adults of known age for the bioassay tests (Bhatia *et al.*, 1990).

Edible oils like, Palm (*Elaeis guinensis* Jacq.), Sunflower (*Helianthus annus* L.), Cotton seed (*Gossypium hirsutum* L.), Rice bran (*Oryza sativa* L.), Castor (*Ricinus communis* L.), Sesame (*Sesamum indicum* L.) and among non-edible oils like, Neem (*Azadirachta indica* A. Juss), Pongamia (*Pongamia pinnata* L.), Citronella (*Cymbopogon nardus* L.), Rosemary (*Rosmarinus officinalis* L.), Thyme, (*Thymus vulgaris* L.), Jatropha (*Jatropha curcas* L.) and Lemongrass (*Cymbopogon citratus* Spreng.) were tested for their synergistic activity if any, to break down the resistance of malathion. All the oils were extracted from seeds except citronella, rosemary, thyme and lemongrass oils which were obtained from leaves and rice bran oil from rice bran.

The discriminating dose (DD) fixed by Insecticide Resistance Action Committee (IRAC) based on LC_{99} values obtained for the susceptible population was used in the study. The DD values for malathion for *S. oryzae* and *T. castaneum* were 1.5 and 0.5 ppm, respectively.

All the oils were evaluated at different concentrations of 10, 20, 40, 60, 80 and 100 ppm with and without malathion by residual film method (Busvine, 1971). The oils diluted to required concentrations with acetone and were bioassayed. The population was bioassayed with the DD od malathion plus non edible and edible oils separately at different concentrations. A control, without insecticide, was also used. The DD of malathion plus the different concentrations of oils were diluted with acetone and 1.0 ml of this solution was run onto a 2 cm petri dish, with four replications. The petri dish were left to dry before releasing the insects. Ten, 1-3 day old adults of malathion resistant *S. oryzae* and *T. castaneum*, were released into each replication. Observations were recorded 24 h after treatment. The mortality and suppression of resistance (SR) percentages were calculated.

The per cent corrected mortality in laboratory studies was worked out using Abbot's (Abbot, 1925) formula,

$$\text{Per cent corrected mortality} = \frac{(\text{Per cent test mortality}) - (\text{per cent control mortality})}{(100 - \text{per cent control mortality})} \times 100$$

The suppression of resistance (SR) was worked out by the following formula,

$$SR = \frac{(\text{Survival in insecticides}) - (\text{Survival in insecticides + synergist})}{\text{Survival in insecticides}} \times 100$$

Results and Discussion

None of the plant oil (non-edible and edible) tested was toxic when applied alone at a concentration upto 100 ppm to both the insects *S. oryzae* and *T. castaneum.*

Synergistic activity of edible and non edible oils with malathion against *S. oryzae*

Among the edible oils, sesame at 100 ppm concentration exhibited high level of suppression with a SR of 74.48 per cent followed by rice bran oil which suppressed malathion resistance at moderate level with SR of 43.16 per cent (Table 1). Haller *et al.*, (1942) observed that crystalline and non crystalline fraction of sesame oil with pyrethrin resulted in mortality of 85 and 89 per cent, respectively compared to 20 per cent in pyrethrin alone in houseflies. Similarly, Saxena (1987) reported synergistic effect of sesame oil with pyrethrum against *S. oryzae*. Sesame oil synergized fluvalinate up to 48 fold at LC_{90} against citrus thrips, *Scirtothrips citri* (Mout.) (Immaraju *et al.*, 1990).

Palm, sunflower and cotton seed oils have low suppression rate against *S. oryzae* (Table 1). Synergistic effect of sesame oil with various insecticides was reported in *H. armigera* (Sundramoorthy and Chitra, 1992; Manoharan and Uthamasamy, 1993; Gowda, 1996; Rajesekhar *et al.*, 1996). Combination of fenvalerate + sesame oil caused 69.26 per cent mortality in eggs of *P. xylostella* (Vastrad *et al.*, 2004).

Table.1 Synergistic activity of edible oils with Discriminating Dose (DD) of malathion against *S. oryzae*

Oil Dose	Palm		Sunflower		Cotton seed		Rice Bran		Castor		Sesamum	
	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)
0	99.18±1.17 (85.77)^{d}	-	99.18±1.17 (85.77)^{b}	-	98.50 ±1.58 (83.27)^{d}	-	96.77 ± 2.30 (80.34)^{e}	-	99.18±1.17 (85.77)^{c}	-	98.00±1.82 (82.05)^{f}	-
10	98.50 ±1.58 (83.27)^{d}	0.68	98.50 ±1.58 (83.27)^{b}	0.68	95.00±2.84 (77.12)^{cd}	3.55	95.00 ±2.84 (77.12)^{de}	1.82	95.41±2.73 (78.43)^{bc}	3.80	95.00±2.84 (77.12)^{f}	3.06
20	98.33±1.67 (82.67)^{c}	0.85	96.67±2.34 (80.34)^{a}	2.55	95.00±2.84 (77.12)^{cd}	3.55	95.00 ±2.84 (77.12)^{e}	1.82	94.00±3.09 (77.93)^{bc}	5.22	86.67±4.43 (69.04)^{e}	11.56
40	96.67±2.34 (80.34)^{c}	2.53	95.00±2.84 (77.12)^{a}	3.23	90.00±3.91 (73.40)^{bc}	8.62	85.00 ±4.65 (67.57)^{cd}	12.16	93.34±3.25 (76.73)^{bc}	5.88	70.00±5.97 (56.81)^{d}	28.57
60	91.67±3.60 (76.39)^{b}	7.57	93.34±3.25 (76.73)^{a}	4.88	80.00±5.21 (63.76)^{ab}	18.78	80.00 ±5.21 (63.76)^{bc}	17.32	90.00±3.91 (73.40)^{abc}	9.25	58.34±6.42 (49.83)^{c}	40.46
80	88.34±4.18 (71.21)^{b}	10.92	90.00±3.91 (73.40)^{a}	9.25	75.00±5.64 (60.35)^{a}	23.85	70.00 ±5.97 (56.81)^{ab}	27.66	85.00±4.65 (67.57)^{ab}	14.29	48.34±6.51 (44.05)^{b}	50.67
100	76.67±5.51 (61.55)^{a}	22.69	88.34±4.18 (71.21)^{a}	10.92	70.00±5.97 (56.81)^{a}	28.93	55.00 ±6.48 (47.87)^{a}	43.16	78.34±5.36 (62.31)^{a}	21.10	25.00±5.64 (30.00)^{a}	74.48

Figures in the parentheses are arcsin transformed values.

Means followed by common letter in a column are not significantly different at five percent level by LSD.

PR – Per cent Resistance; SE – Standard Error; SR – Suppression of Resistance; S – significant; NS – non significant

Table.2 Synergistic activity of non edible oils with Discriminating Dose (DD) of malathion against *S. oryzae*

Oil Dose	Neem		Pongamia		Citronella		Rosemary		Thyme		Jatropha		Lemon grass	
	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)
0	98.77±1.44 (84.93)^{b}	-	98.77±1.44 (84.93)^{c}	-	98.73±1.46 (83.56)^{d}	-	98.77 ± 1.45 (83.63)^{f}	-	98.76 ± 1.44 (83.63)^{d}	-	98.77±1.44 (84.93)	-	98.75 ±1.45 (83.97)^{e}	-
10	96.67±2.34 (80.34)^{b}	2.12	95.00±2.84 (77.12)^{c}	3.81	98.33±1.67 (82.67)^{d}	0.40	93.94 ± 3.11 (76.15)^{ef}	5.47	94.29 ± 3.02 (76.59)^{cd}	4.52	96.67±2.34 (79.50)	2.12	98.33±1.67 (82.67)^{de}	0.42
20	93.34±3.25 (76.73)^{b}	5.49	85.00±4.65 (67.57)^{b}	13.94	96.67±2.34 (79.50)^{cd}	2.08	86.67 ± 4.43 (69.04)^{de}	12.23	90.00 ± 3.91 (73.40)^{c}	8.86	95.00±2.84 (77.12)	3.81	96.67±2.34 (79.50)^{cd}	2.10
40	91.67±3.60 (73.67)^{b}	7.18	83.34±4.85 (66.02)^{b}	15.62	95.00±2.84 (77.12)^{cd}	3.77	83.34 ± 4.85 (66.02)^{cd}	15.60	86.67 ± 4.43 (69.04)^{bc}	12.24	93.34±3.25 (76.15)	5.49	93.34±3.25 (76.73)^{cd}	5.47
60	90.00±3.91 (73.40)^{b}	8.87	80.00±5.21 (63.76)^{b}	19.00	88.34±4.18 (71.21)^{bc}	10.52	76.67 ± 5.51 (61.34)^{bc}	22.35	80.00 ± 5.21 (63.76)^{b}	18.99	91.67±3.60 (76.39)	7.18	88.34±4.18 (71.21)^{bc}	10.54
80	76.67±5.51 (61.55)^{a}	22.37	65.00±6.21 (53.84)^{a}	34.19	80.00±5.21 (63.76)^{ab}	18.97	66.67 ± 6.14 (54.87)^{b}	32.48	66.67 ± 6.14 (54.87)^{a}	32.49	88.33±4.18 (70.03)	10.57	78.34±5.36 (62.79)^{ab}	20.66
100	63.34±6.27 (52.75)^{a}	35.87	53.34±6.49 (46.92)^{a}	45.99	73.34±5.76 (58.94)^{a}	25.71	53.34 ± 6.49 (46.92)^{a}	45.99	56.67 ± 6.45 (48.84)^{a}	42.61	85.00±4.65 (67.57)	13.94	71.67±5.87 (57.86)^{a}	27.42

Table.3 Synergistic activity of edible oils with Discriminating Dose (DD) of malathion against *T. castaneum*

Oil Dose	Palm		Sunflower		Cotton seed		Rice Bran		Castor		Sesamum	
	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)
0	99.18±1.17 (85.77)^{c}	-	98.50±1.58 (83.27)^{c}	-	97.51 ±2.03 (81.46)^{e}	-	97.51± 2.03 (81.46)^{d}	-	98.75±1.45 (83.97)^{d}	-	98.00±1.82 (82.05)^{c}	-
10	98.33±1.67 (82.67b)^{c}	0.85	98.33±1.67 (82.67)^{bc}	0.17	94.29±3.02 (76.59)^{de}	3.30	93.46±3.22 (75.60)^{cd}	4.15	98.75±1.45 (83.97)^{d}	0.00	96.67±2.34 (80.34)^{c}	1.35
20	95.00±2.84 (77.12)^{bc}	4.21	96.67±2.34 (80.34)^{bc}	1.85	92.50±3.43 (75.44)^{de}	5.13	90.00±3.91 (73.40)^{c}	7.70	96.67±2.34 (80.34)^{c}	2.10	93.34±3.25 (76.73)^{c}	4.75
40	91.67±3.60 (76.39)^{b}	7.57	95.00±2.84 (77.12)^{b}	3.55	90.00±3.91 (73.40)^{cd}	7.70	80.00±5.21 (63.76)^{b}	17.95	93.34±3.25 (76.73)^{bc}	5.47	80.00±5.21 (63.76)^{b}	18.36
60	88.34±4.18 (71.21)^{ab}	10.92	93.34±3.25 (76.73)^{ab}	5.23	80.00±5.21 (63.76)^{bc}	17.95	75.00±5.64 (60.35)^{ab}	23.08	91.67±3.60 (76.39)^{ab}	7.16	68.34±6.06 (55.85)^{ab}	30.26
80	86.67±4.43 (68.76)^{ab}	12.61	85.00±4.65 (67.57)^{ab}	13.70	75.00±5.64 (60.35)^{ab}	23.08	70.00±5.97 (56.81)^{ab}	28.21	88.34±4.18 (71.21)^{ab}	10.54	60.00±6.38 (50.83)^{a}	38.77
100	75.00±5.64 (60.35)^{a}	24.37	80.00±5.21 (63.76)^{a}	18.78	65.00±6.21 (53.74)^{a}	33.34	65.00±6.21 (53.74)^{a}	33.34	86.67±4.43 (68.76)^{a}	12.23	56.67±6.45 (48.84)^{a}	42.17

Table.4 Synergistic activity of non edible oils with Discriminating Dose (DD) of malathion against *T. castaneum*

Oil Dose	Neem		Pongamia		Citronella		Rosemary		Thyme		Jatropha		Lemon grass	
	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)	PR ± SE	SR (%)
0	98.50±1.58 (83.27)^{d}	-	98.50±1.58 (83.27)^{d}	-	98.50±1.58 (83.27)^{d}	-	98.68 ± 1.49 (83.42)^{d}	-	98.68 ± 1.49 (83.42)^{c}	-	98.50±1.58 (83.27)	-	98.50±1.58 (83.27)^{c}	-
10	96.67±2.34 (80.34)^{cd}	1.85	95.00±2.84 (77.12)^{cd}	3.55	98.33±1.67 (82.67)^{d}	0.17	94.85 ± 2.88 (77.39)^{cd}	3.88	93.74 ±3.15 (75.92)^{bc}	5.00	96.67±2.34 (80.34)	1.85	98.11±1.77 (82.13)^{bc}	0.39
20	93.34±3.25 (76.73)^{cd}	5.23	91.67±3.60 (76.39)^{c}	6.93	96.67±2.34 (80.34)^{cd}	1.85	90.00 ±3.91 (73.40)^{c}	8.79	93.34 ±3.25 (76.73)^{bc}	5.41	95.00±2.84 (77.12)	3.55	95.00±2.84 (77.12)^{bc}	3.55
40	90.00±3.91 (73.40)^{bc}	8.62	80.00±5.21 (63.76)^{b}	18.78	95.00±2.84 (77.12)^{cd}	3.55	80.00 ± 5.21 (63.76)^{b}	18.92	86.67 ±4.7 (68.76)^{b}	12.17	93.34±3.25 (76.73)	5.23	93.34±3.25 (76.73)^{b}	5.23
60	83.34±4.85 (66.02)^{ab}	15.39	65.00±6.21 (53.79)^{a}	34.01	93.34±3.25 (76.73)^{bc}	5.23	76.67 ±5.51 (61.55)^{b}	22.30	84.21 ±4.75 (67.16)^{b}	14.66	91.67±3.60 (76.39)	6.93	91.39±3.65 (76.26)^{b}	7.26
80	76.67±5.51 (61.55)^{a}	22.16	55.00±6.48 (47.87)^{a}	44.16	88.33±4.18 (70.03)^{ab}	10.34	70.00 ±5.97 (56.81)^{b}	29.06	70.00 ±5.97 (56.81)^{a}	29.06	88.33±4.18 (70.03)	10.32	88.33±4.18 (70.03)^{ab}	10.32
100	70.00±5.97 (56.81)^{a}	28.93	51.67±6.51 (45.96)^{a}	47.54	80.00±5.21 (63.76)^{a}	18.78	56.67 ±6.45 (48.84)^{a}	42.57	63.34 ±6.27 (52.83)^{a}	35.81	85.00±4.65 (67.57)	13.70	80.00±5.21 (63.76)^{a}	18.78

Sesame oil also showed good synergism with cypermethrin against larvae of *P. xylostella*. Both PBO and sesame oil played the same role in the inhibition of the enzyme, mono oxygenase (Visetson *et al.*, 2003). Among the Non-edible oils *viz.*, pongamia, rosemary, thyme and neem oils exhibited significantly moderate level of synergism with malathion to *S. oryzae* with corresponding SR of 45.99, 45.99, 42.61 and 35.87 per cent respectively (Table 2). Saxena (1987) observed that neem oil with pyrethrin, DDT and lindane at 1:5 exhibited synergism against *S. oryzae*. Similarly, Gowda (1996) reported that pongamia oil acted as MFO inhibitor in suppressing the resistance of fenvalerate and cypermethrin in *H. armigera*.

Synergistic activity of edible and non edible oils with malathion against *T. castaneum*

In case of *T. castaneum,* among edible oils, sesame, rice bran and cotton seed oils showed moderate level of resistance suppression with SR of 42.17, 33.34 and 33.34 per cent, respectively at concentration of 100 ppm with malathion (Table 3). Sasame oil as a natural synergist to phosphate insecticides has been demonstrated in *T. castaneum* (Khalequzzaman and Chowdhary, 2003). Pongamia oil synergized carbaryl, antagonized lindane and showed additive effects with DDT, malathion and dichlorvos against *Cylas formicarius* Fab. (Dhingra and Sarup, 1979) and against both normal and lindane resistance strains of *T. castaneum* (Dhingra and Sarup, 1981a and 1981b).

The non edible oils *viz*., pongamia, rosemary, thyme and neem oils showed moderate level of synergism at 100 ppm concentartion with SR of 47.54, 42.57, 35.81 and 28.93 respectively (Table 4). The neem oil with malathion at 1:5 exhibited synergism against *T. castaneum* (Parmer and Datta, 1986). The pongamia oil was able to suppress the malathion resistance almost similar to piperonyl butoxide (PBO) strongly suggest that pongamia oil also might be acting as the inhibitor of MFO enzyme system in *T. castaneum*.

From this study it is found that with the use of edible oil sesame with malathion against *S. oryzae* resistance level reduced from 98.00 to 25.00 per cent with a SR of 74.48 per cent. It is concluded that, among the enzymes, the MFO and carboxyl esterase play an important role in the resistance development in *S. oryzae* to malathion. Sesame oil suppressed insecticide resistance to higher level by inhibition effect of these enzymes.

Therefore, rather using insecticides alone, these types of oils can be used as synergist for effective control of stored grain pests even under insecticide resistance development situation. Further use of such oils also cost effective, eco-friendly and even they act as synergist at very low dose.

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