

Joint action between some vegetable oils and their methylated form against the 4th larval instar of *Spodoptera littoralis* (Boisd.)

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ABSTRACT

The toxicity and the joint action of some vegetable oils and their methylated products were evaluated in laboratory studies against the 4th instar larvae of *Spodoptera littoralis*. Thin film technique was used as a method of application in this investigation. Cumin oil was the most effective at the LC₅₀ level whereas nigella oil was the least toxic one. Their LC₅₀ values were 0.08 and 0.2 µl / cm², respectively. The other four oils, garlic, caraway, thyme and castor bean had LC₅₀ values ranged from 0.12 to 0.16 µl / cm². The methylation of the tested oils appeared change in the efficiency, either decrease or increase against 4th larval instar. The methylation process increased the efficacy of two oils (garlic and nigella) while decreased it, in another two oils (caraway and cumin) at LC₅₀ and LC₉₀ level. On the other hand, the methylation of castor bean oil decreased the LC₉₀ value from 0.79 to 0.66 µl / cm², while its LC₅₀ value increased from 0.16 to 0.34 µl / cm². Three experiments were conducted to study the joint action of the binary mixtures of vegetable oils and methylated vegetable oils against 4th larval instar. The first experiment was mixing LC₂₅ of two vegetable oils together, the second one was mixing a vegetable oil with a methylated one and the third one was mixing the methylated oil with other methylated one. Results indicate that out of 49 tested mixtures; only 38 pairs showed potentiation, 7 pairs produced additive effect and 4 pairs disclosed antagonistic effect.

Keywords: Joint action, vegetable oils, methylation, *Spodoptera littoralis*

INTRODUCTION

Chemical control methods using synthetic insecticides had been favoured so far because of their speedy action and ease of application.

However, the public concern over the harmful effect of chemical insecticides on the environment and human health has enhanced the search for safer and environmentally friendly control alternatives where plant oils seem to be relevant and had great promise as alternative to the use of chemicals. Soybean oil at 5 % concentration controlled San Jose scale when applied at dormant stage. Cotton seed oil was less effective than petroleum oil in controlling European red mite in both lab and field conditions (Zhiguo et al., 2000).

When compared oils with synthetic pesticides or fungicides, the efficacy of plant oils is much lower and therefore, higher concentrations or repeated applications are often required to maintain control. Horticulture oils have not been found to lead to insect resistance (Thomson, 1983). Because of their mode of action, horticulture oils do not pose the high exposure risk that chemical pesticides do. Horticulture oils have relatively low mammalian toxicity (Grossman, 1990) while petroleum based horticulture oils can cause skin and eye irritation (Olkowski, 1991).

Many commercially formulated pesticides made from botanical oils contain synthetic chemical synergists. These synergists have no insecticidal effect of their own, but serve to enhance the efficacy of chemicals. Spray oil concentrate consumption in the United States is estimated at 7-10 million gallons annually, of which 6-8 million gallons are petroleum oils. Methylated vegetable oil use is estimated at 0.8 - 1.2 million gallons and straight vegetable oils at 0.2 - 0.5 million gallons annually. In pesticide application, spray oil concentrates are used at relatively high volumes to replace much or all of the water normally used as a carrier for pesticides. Formulations normally consist of 80 to 90 % oil and 10 to 20 % surfactant. Use of vegetable oils has been proven to improve the efficiency of some pesticides, but research has shown little or no difference in efficiency based on the type of vegetable oil used Anonymous (1997).

The present study was undertaken to determine the efficacy of six vegetable oils namely; cumin, garlic, caraway, thyme, castor bean and nigella as well as their methylated form against the 4th larval instar of *Spodoptera littoralis*. The joint action effect between each two mixed vegetable oils, vegetable oils with their methylated form and each two mixed methylated against the 4th larval instar of *Spodoptera littoralis* was also investigated.

MATERIALS AND METHODS

Test insects: The laboratory strain of the cotton leafworm *Spodoptera littoralis* which was reared for several years under laboratory conditions at 25 ± 2 °C and 65 + 5 R.H. away from insecticidal contamination served as a laboratory test insect. The larvae were fed on castor bean leaves as described by EL- defrawi *et al.*, (1964).

Experimental vegetable oils: The six vegetable oils used in this study were (cumin) *Cuminum cyminum*, (garlic) *Allium sativum*, (caraway) *Carum carvi*, (thyme) *Thymus ulgaris*, (castor bean) *Ricinus communis* and (nigella) *Nigella sativa*.

Methylation of vegetable oils: The previous vegetable oils were converted to methyl form using ethereal solution of diazomethane. Oils were dissolved in 0.5 ml of anhydrous diethyl ether and methylated by drop wise addition of diazomethane solution until the yellow colour (Vogel, 1975). The mixture was then left at room temperature for 15 min and the solvent was evaporated on a water bath maintained at 60 °C. Finally the methylated oils were dissolved in pure solvent and an aliquot of this solution were subjected to bioassay test.

Method of application: Thin film technique was used as a method of application in this investigation (Asher and Mirion, 1981), where the tested concentrations were applied through acetone or benzene to the surface of 9 cm in Petri-dish. One ml of each concentration of the tested oils was spread on the inner surface of a Petri dish, by moving the dish gently in circles. The Petri dish used as control was treated with 1ml acetone or benzene only. The solvent was evaporated under room condition in few minutes leaving a thin film of oil on the surface of Petri-dish.

Twenty of one day-old fourth instar larvae of cotton leafworm were exposed for six hrs in each Petri-dish, then transferred to clean glass containers and fed on fresh castor bean leaves. Five replicates of each concentration and the control were made. Percentage mortality was calculated after 24 hrs and corrected by Abbott's formula (1925). The slope, LC₅₀ and LC₉₀ values for each oil were calculated according to Finney (1971). To determine the joint action of candidate vegetable oils with each

other and their methylated form, the LC₂₅ of each oil was mixed with the LC₂₅ of the another vegetable oil.

The co-toxicity factor (CF) was applied to determine the joint action using the equation reported by Mansour *et al.*, (1966) as follows:

$$CF = \frac{\text{Observed mortality \%} - \text{Expected mortality \%}}{\text{Expected mortality \%}} \times 100$$

This factor was used to classify results into three categories. A positive factor 20 or more is considered potentiation, a negative factor 20 or more means antagonism and intermediate values between -20 and +20 indicate only additive effect.

RESULTS AND DISCUSSION

Toxicity of vegetable oils to 4th larval instar of cotton leafworm: The aim of this study was to determine the efficacy of six oils namely cumin, garlic, caraway, thyme, castor bean, and nigella against 4th instar larvae of *S. littoralis*. The LC₅₀ and LC₉₀ values are tabulated in Table (1), with their corresponding slopes, toxicity index and the relative potencies. The result in Table (1) shows the efficiency of the tested oils against 4th instar larvae of *S. littoralis*. Cumin oil was the most effective at the LC₅₀ level, whereas that of the nigella oil was the least active, against the 4th larval instar of *S. littoralis*.

The tested six oils could be classified into three categories at LC₅₀ level. The first category included cumin oil only which gave the highest effect against 4th larvae instar of *S. littoralis* with LC₅₀ 0.08 $\mu\text{l} / \text{cm}^2$. The second category included four oils garlic, castor bean, thyme and caraway which had LC₅₀ values 0.12, 0.13, 0.14 and 0.16 $\mu\text{l} / \text{cm}^2$, respectively.

The third categories include the least oil nigella with LC₅₀ value 0.21 $\mu\text{l} / \text{cm}^2$. Based on LC₉₀ value, the order of efficiency was differ than that at LC₅₀ values. The garlic, cumin and thyme oils gave the lowest LC₉₀ values 0.2, 0.23 and 0.35 $\mu\text{l} / \text{cm}^2$, respectively. Nigella and caraway oils had the same LC₉₀ values (0.42 and 0.43 $\mu\text{l} / \text{cm}^2$), while the castor bean oil had the highest LC₉₀ value 0.79 $\mu\text{l} / \text{cm}^2$.

Table (1): Toxicity of different vegetable oils against 4th instar larvae of cotton leafworm, *S. littoralis*.

Oils	LC ₅₀ μl/cm ²	LC ₉₀ μl/cm ²	Slope	Index	Folds
Cumin	0.08	0.23	2.79	100	2.64
Garlic	0.12	0.29	3.34	66.6	1.74
Caraway	0.13	0.44	2.49	61.5	1.61
Thyme	0.14	0.35	3.18	57.1	1.52
Castor bean	0.16	0.79	1.86	50.0	1.31
Nigella	0.21	0.42	4.38	38.1	1.0

The toxicity index obtained by comparing the toxicity or efficiency of the tested compounds, at a fixed level (LC₅₀ or LC₉₀) to their most effective compound. While, in contrast, relative potency was determined as a given level (such as LC₅₀) by comparing the number of folds or times of potency of the test oils with that of the least toxic one. Since cumin was the most toxic oil among the tested ones, it was used as a standard in calculating the toxicity index at LC₅₀ level, whereas the thyme was used as a standard in calculating the relative folds. On basis of the different criteria used (i.e. LC₅₀ and LC₉₀) toxicity index and relative folds, the toxicity index (Table 1) indicated that garlic, caraway, thyme and castor bean were 66.6 %, 61.5 %, 57.1 % and 50 % as effective as cumin at LC₅₀ value, while nigella oil was 38.1 %. Comparing the slope values of the toxicity lines of these oils, data in Table (1) show that nigella had the steepest toxicity line with 4.38 slope value. Garlic, thyme, cumin and caraway had 3.34, 3.18, 2.79 and 2.49, while the least one castor bean oil had the flattest LC-p line with the lowest slope value 1.86.

These results are in agreement with many investigators. Olkowski (1991), reported that horticulture oils are effective in controlling aphids, adelgids, spider mites, mealy bugs, sawfly larvae, whiteflies, plant bugs, caterpillars, scales and some plant diseases like rusts and mildews .They flood insects breathing pores which lead to prompt asphyxiation and suffocation. Oils also kill an insect when it touches the outer body, or cuticle, of an insect leading to dehydration and death of the pest. Bhargava and Meena (2002) who found that castor bean, mustard, groundnut, sesame,

coconut and sunflower oils caused significant mortality adults of pulse beetle, *Callosobruchus chinensis* (Linn.) on cowpea after three days of treatment. Also, Bunker and Bhargava (2002) determined the effect of vegetable oils on the eggs of *Corcyra cephalonica*. The treatments were castor bean *Ricinus communis*, coconut (*Cocos nucifera*), groundnut (*Arachis hypogaea*), Indian mustard (*Brassica juncea*), sesame (*Sesamum indicum*), and sunflower (*Helianthus annuus*) oils at 0.5, 1.0, 2.0, 3.0 and 5.0 %. All the vegetable oil concentrations were significantly superior than the control in reducing egg hatchability. The percentage of egg hatch inhibition in all the treatments increased with an increase in concentration. Castor oil was the most effective in reducing egg hatchability followed by Indian mustard, groundnut, sesame, coconut, and sunflower oils.

Qing *et al.*, (2003) tested the insecticidal activity of the crude extract of *Peganum harmala* against *Lipophis erysimi*, and *Spodoptera littoralis*. The methanolic extract of *P. harmala* at 1000 µg / ml recorded 93.07 % and 96.36 % mortality of the turnip aphid (*L. erysimi*) at 24 and 48 hrs after the treatment, respectively. Using the leaf disk method, the antifeedant rates of methanolic extract of *P. harmala* at 10000 µg / ml were 90.80 % and 72.13 % respectively, against the 5th instar larvae of *P. rapae* and the 3rd instar larvae of *S. littoralis* after 24 hrs. More recently, Yadav *et al.*, (2004) investigate the effect of vegetable oils on the orientation and oviposition of pulse beetle *Callosobruchus maculatus* on green gram during storage. Sesame, coconut, mustard, groundnut and soybean, and non-edible oils (mahua, castor, karanj and neem) were mixed with seeds at 10 ml / kg seed. Vegetable oils reduced beetle incidence on seeds to 5.91-7.50 beetles, compared to the control (16 beetles). Among vegetable oils, mahua oil was the most effective. Oviposition was reduced by vegetable oil treatment. Neem oil reduced the number of oviposited eggs to 3.58 eggs, compared to untreated control (91.25 eggs).

Toxicity of methylated vegetable oils to 4th larval instar of cotton leafworm *S. littoralis*: Data in Table (2) Show the efficiency of the methylated tested oils against the 4th instar larvae of *S. littoralis*. The methylated garlic oil was the most effective at LC₅₀ level whereas castor bean oil was the least active with LC₅₀ 0.07 and 0.34 µl/cm², respectively. The cumin, caraway and nigella gave LC₅₀ values 0.15, 0.15 and 0.19 µl / cm², respectively.

Data in Tables (1 and 2) show that the methylation of the tested oils appeared change in the efficiency decreasing or increasing against 4th larval instar of *S. littoralis*.

Table (2): Toxicity of some vegetable oils methylated against 4th larvae instar of cotton leafworm *S. littoralis*.

Oils	LC ₅₀ μl/cm ²	LC ₉₀ μl/cm ²	Slope	Index	Folds
Garlic	0.07	0.24	2.52	100	4.55
Cumin	0.15	0.49	2.54	46.6	2.20
Caraway	0.15	0.54	2.34	46.66	2.20
Nigella	0.19	0.37	4.39	36.8	1.76
Castor bean	0.34	0.66	4.39	20.5	21.96

The methylation process increased the efficiency of two oils, garlic and nigella, and decreased the efficiency of another two oils caraway and cumin. The LC₅₀ and LC₉₀ values decreased from 0.12 and 0.29 for garlic oil to 0.07 and 0.24 for methylated garlic oil, respectively. In the case of nigella the LC₅₀ and LC₉₀ decreased from 0.21 and 0.42 for the non- methylated oil to 0.19 and 0.37 for the methylated oil. On the other hand in the case of castor bean oil the methylation decreased the LC₉₀ value from 0.79 to 0.66 while LC₅₀ value increased from 0.16 to 0.34. This can be seen in the change of the slope from 1.86 for the non- methylated oil to 4.39 for the methylated oil.

The percent contribution of LC value of oil to the corresponding LC value of the methylated oil as toxicity index was calculated as follows:

$$\frac{\text{LC of oil}}{\text{LC of methylated oil}} \times 100$$

These were 171.4 % and 120.1 % for LC₅₀ and LC₉₀ of garlic and for nigella were 110.5 % and 113.5 % for LC₅₀ and LC₉₀, respectively.

The toxicity index for caraway was 86.7 % and 83.3 % at LC₅₀ and LC₉₀ levels respectively. The corresponding values for cumin were 53.3 % and 46.9 %. On the other hand, in the case of castor bean oil, the toxicity index was 47.1 % at LC₅₀ while it was 119.7 % at LC₉₀ level.

Holloway *et al.*, (2000) studied the effects of 10 commercially available tank- mix adjuvants on the retention and coverage of aqueous sprays on foliage. Retention enhancement was also achieved using the mineral oil, vegetable oil, methylated vegetable oil and phospholipids ECs and the organosilicone surfactant. The best EC was the methylated vegetable oil which also had the highest emulsifier content. Also, good coverage could still be achieved by adding the water soluble surfactants, as well as the methylated vegetable oil and phospholipids ECs. Moustafa *et al.*, (2002) studied the efficiency of three types of oils against *Aphis fabae* and *Tetranychus urticae*. Refined paraffinic oil, vegetable oil and esterified seed oil emulsified with ethoxylated fatty alcohol. The results showed that half-dose 80 % EC was the most toxic against the adults of both faba bean aphid and red mite at LC₂₅, LC₅₀ and LC₉₀ levels. Alpha-z 96.4 % EC came next in toxicity and the vegetable oil was the least effective. All candidate oils exhibited 100 % mortality against aphid adults after 24 hours from treatment at the concentrations 200, 100 and 50 ppm. Complete inhibition of egg hatchability of the red mite *T. urticae* was obtained at 200 and 100 ppm for all oils. Proper mineral oils, esterified seed oils and vegetable oils of consistent quality can contribute in the development of sound integrated pest management programmes, since they are environmentally compatible, possess unique modes of action, novel tools for resistance management and reduce impact on beneficial insects.

Recently, Mei *et al.*, (2005) measured the effect of 8 methylated vegetable oil adjuvants including methylated rapeseed oil methylated cottonseed oil methylated groundnut oil methylated maize oil methylated soybean oil methylated sunflower oil methylated flaxseed oil and methylated castor oil on 4 herbicides in common usage for controlling barnyard grass and redroot pigweed. The results showed that all the methylated vegetable oils had some enhancement effect on the 4 herbicides.

The joint action effect: Three experiments were conducted to study the joint action of the binary mixtures of vegetable oils and methylated vegetable oils against the 4th larval instar of cotton leafworm. The concentration of each oil, giving LC₂₅ was mixed that of the mix causing 50 % mortality. The first experiment was mixing two vegetable oils together. The second one was mixing vegetable oils with a methylated one. The third one was mixing methylated oils with another methylated oils.

Data presented in Table (3) indicate that out of the 14 mixtures tested only 5 pairs showed potentiation, 5 pairs produced additive effect and 2 pairs disclosed antagonistic effect. The joint action of candidate vegetable oils tested listed in Table (3) could be summarized as follows:

Cumin oil: the potentiation effect was observed only in case of garlic and caraway oils. The co- toxicity factor indicate that only two oils nigella, and castor bean showed additive effect , while the antagonistic effect in case of cumin oil occurred with thyme oil.

Table (3): Joint action for two mixed vegetable oils at LC₂₅ levels of each as tested against the 4th instar larvae of *S. littoralis*.

No	Treatment	Observed mortality	Co- toxicity factor	Type of interaction
Cumin				
1	+ Garlic	83.0	66.0	potentiation
2	+ Nigella	51.7	3.4	additive
3	+ Caraway	71.6	42.0	potentiation
4	+ Thyme	38.3	-23.4	antagonism
5	+ Castor bean	46.6	-6.8	additive
Caraway				
1	+ Garlic	90.0	80.0	potentiation
2	+ Nigella	71.6	43.2	potentiation
3	+ Castor bean	50.0	0.0	additive
4	+ Thyme	37.5	-25	antagonism
Garlic				
1	+ Nigella	83.3	66.6	potentiation
2	+ Castor bean	86.6	73.2	potentiation
3	+ Thyme	80.0	60.0	potentiation
Nigella				
1	+ Castor bean	46.6	-6.8	additive
2	+ Thyme	48.3	-3.4	additive

Castor				
1	Thyme	63.3	26.6	potentiation

Caraway oil: the potentiation effect was observed in three oils, garlic, nigella and cumin. In case of caraway oil with castor bean showed an additive effect, and with thyme showed an antagonistic effect.

Garlic oil: mixtures of garlic oil with all tested vegetable oils, cumin, caraway nigella, castor bean and thyme showed a high potentiation effect. The co- toxicity factor ranged from + 60 to + 80.

Nigella oil: the additive effect was observed in three oils cumin, thyme and castor bean while the potentiation effect was observed in garlic and caraway

Castor bean oil: gave potentiation effect with thyme and garlic and additive effect with other tested oils.

Data of the second experiment, in which vegetable oil mixed with methylated oil tabulated in Table (4). Data indicate that out of the 25 mixtures tested 21 pairs showed potentiation effect only 2 pairs produced additive effect and 2 pairs produced antagonistic effect. The joint action of candidate vegetable oils and methylated vegetable oils tested listed in Table (4) could be summarized as follows:

Cumin oil: mixtures of cumin oil with all tested methylated vegetable oils showed a potentiation effect. The co- toxicity factor was 33.2, 33.2, 56.6, 63.2 and 73.2 from methylated caraway, nigella, cumin, garlic and castor bean, respectively.

Caraway oil: the co- toxicity factor indicate 4 binary mixtures of caraway oil with methylated vegetable oils showed potentiation effect and ranged from 40 to 46.6. The additive effect was obtained in caraway with methylated nigella oil.

Garlic oil: the garlic oil gave potentiation effect with all tested methylated vegetable oils the co- toxicity factor ranged from +63.2 to + 86.6.

Nigella oil: the co- toxicity factor indicated that 3 pairs showed potentiation effect, nigella with methylated cumin, garlic and castor bean oils. In the case

of nigella with methylated caraway gave additive effect, while nigella with its methylated oil gave antagonistic effect with co- toxicity factor -25.

Table (4): Joint action for vegetable oils with methylated vegetable oils at LC₂₅ level of each as tested against 4th larval instar *S. littoralis*.

No	Treatment	Observed mortality	Co- toxicity factor	Type of interaction
Cumin				
1	+ M cumin	78.3	56.6	potentiation
2	+ M caraway	66.6	33.2	potentiation
3	+ M garlic	81.6	63.2	potentiation
4	+ M nigella	66.6	33.2	potentiation
5	+ M castor bean	86.6	73.2	potentiation
Caraway				
1	+ M cumin	70.0	40.0	potentiation
2	+ M caraway	73.3	46.6	potentiation
3	+ M garlic	70.0	40.0	potentiation
4	+ M nigella	53.3	6.6	additive
5	+ M castor bean	71.6	43.2	potentiation
Garlic				
1	+ M cumin	88.3	76.6	potentiation
2	+ M caraway	86.6	73.2	potentiation
3	+ M garlic	81.6	63.2	potentiation
4	+ M nigella	88.3	76.6	potentiation
5	+ M castor bean	93.3	86.6	potentiation
Nigella				
1	+M cumin	75.0	50.0	potentiation
2	+ M caraway	55.0	10.0	additive
3	+ M garlic	62.5	25.0	potentiation
4	+ M nigella	37.5	-25	antagonism
5	+ M castor bean	60.0	20.0	potentiation
Castor				
1	+ M cumin	81.6	63.2	Potentiation
2	+ Caraway	66.6	33.2	Potentiation
3	+ M garlic	83.0	66.0	Potentiation
4	+ M nigella	65.0	30.0	Potentiation

5	+ M castor bean	20.0	-60	antagonism
M= methylated				

Castor bean: The potentiation effect was observed in 4 pairs. The antagonistic action was when castor bean oil mixed with its methylated oil.

Data presented in Table (5) indicate 10 mixtures tested, when mixing one methylated oil with another methylated oil. The co- toxicity factor gave potentiation effect with all tested methylated oil with another methylated oil. The co- toxicity factor ranged from + 23 to + 85 (Table 5).

Seume and O'Brien (1960) reported that antagonism could be produced if one compound interfered with the activation of other by retarding it so that maximal effects were not arrived simultaneously. The detoxifying enzymes then have more chance of acting on the less toxic percent compound. Barakat *et al.*, (1984) studied the joint action of binary mixtures of ten plant extract and five pesticides against adult female of *Tetranychus utricae*. Results indicate that out of 100 mixtures tested, only 56 pairs showed potentiation, 12 pairs produced additive effect and 32 pairs disclosed antagonistic effect.

The potentiation of insecticides as a result of some plant extracts may be attributed to the affinity of some of their components and/or their fast reaction with detoxifying enzyme, forming a relatively stable substrate enzyme complex that block the detoxifying enzyme.

Tripathy and Singh (2005) studied the synergistic effect of some commonly available vegetable oils (mustard oil, sesame oil, linseed oil, niger oil, neem oil, castor oil, cottonseed oil, custard apple seed oil, pongamia oil, sunflower oil, soyabean oil, groundnut oil and [Strychnos nux-vomica] seed oil, all applied at 100 micro g per larvae) with cypermethrin (0.1 μ . g.) and fenvalerate (0.2 μ g) on *Helicoverpa armigera* larvae. Cotton eed oil alone and custard apple seed oil + cypermethrin or fenvalerate gave the highest larval mortality.

Table (5): Joint action for tow mixed methylated vegetable oils against 4th larval instar *S. littoralis*.

No	Treatment	Observed mortality	Co- toxicity factor	Type of interaction
Cumin methylated oil with				
1	+ M caraway	61.66	23.32	potentiation
2	+ M garlic	78.33	56.66	potentiation
3	+ M nigella	65.0	30.0	potentiation
4	+ M thyme	63.3	86.6	potentiation
Caraway methylated oil				
1	+ M caraway	85.0	56.6	potentiation
2	+ M nigella	78.3	43.2	potentiation
3	+ M castor bean	90.0	80.0	potentiation
Garlic methylated oil				
1	+ M nigella	66.6	33.2	potentiation
2	+ M castor bean	86.6	73.2	potentiation
Nigella methylated oil				
1	+ M Castor bean	92.5	85.0	potentiation

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