Impact of Joint Toxic Action of Mineral and Plant Oils on Efficiency Enhancement of Insect Growth Regulators and Indoxacarb against *Spodoptera Littoralis* (Boisd.)

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Abstract: The joint toxic action of Avaunt compound, IGRs (Dimilin and Cymax), plant oil (Orange) and mineral oils (KZ-Kapee) was investigated on laboratory strain of the 4th instar larvae of Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd). Mixtures of Avaunt/Dimilin showed highest synergistic action that was clearly observed with the combinations of the lowest concentration of Dimilin (LC_{10}+LC_{10}), (LC_{25}+LC_{10}) and (LC_{50}+LC_{10}), while, Mixtures of Avaunt/Cymax showed the highest synergistic action that was only observed with the combination of LC_{25}+LC_{10}. Mixtures of Avaunt with Kapee oil or with Orange oil at different concentrations showed the highest potential action that was clearly observed with the combinations (LC_{10}+LC_{10}), (LC_{25}+LC_{10}), (LC_{10}+LC_{25}), (LC_{25}+LC_{25}) and (LC_{10}+LC_{50}), also, slight synergism was clearly shown with the combination (LC_{50}+LC_{10}), while, mixtures of Avaunt / KZ oil produced different levels of antagonism, except with the combination (LC_{25}+LC_{10}) showing slight potentiation. Mixtures of Dimilin with KZ oil or Orange oil showed different levels of antagonism, except with (LC_{10}+LC_{25}) that was produced the highest level of synergism for Dimilin/KZ oil and mixture of Dimilin/Kapee oil showing different levels of additive and synergistic action. While Cymax was the highest synergistic action when mixed with KZ or Kapee oils at concentrations (LC_{10}+LC_{10}) and (LC_{10}+LC_{25}). The additive effective produced from mixtures Cymax/KZ and Cymax/Kapee oil with combinations (LC_{50}+LC_{25}) and (LC_{50}+LC_{50}) for both mixtures. Though, use mineral or plant oils, IGR (Cymax-Dimilin) and Avaunt compound in binary mixtures with low concentration, proved to be effective in control of *S. littoralis* is a well polyphagous as one of the most important agricultural pest in Egypt.

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1. Introduction

Consumption increasing of the synthetic pesticides in the developing countries has led to a number of problems such as adverse effects on the natural enemy populations and the development of resistance. The cotton leafworm *Spodoptera littoralis* (Boisd) (Lepidoptera, Noctuidae) is one of the major and wide spread pests, also, it is considered polyphagous key pest in Egypt, and it is active all year round without hibernation period and attacking cotton and many important vegetables and field crops (**Nasr *et al*., 1984).** Attention to find out another safer less expensive agents to control these insect. Among their compounds, Insect Growth Regulators (IGRs) are going significant importance in crop protection and also caused largely selectivity spare beneficial insects and suitable properties **(Raslan 2002) .**Oils have different effect on pest insects, the most important is that they block the air holes (spiracles) through which insects breathe, causing die from as phyxiation and may act as poisons, interacting with the fatty acids of the insect and interfering with normal metabolism **(Stadler and Buteler 2009).** Indoxacarb, the native ingredient insecticide, is a board spectrum Lepidoptera insecticide that also, has activity on selected other pests. It represents a new class of insecticides, the oxidizations that is considered an excellent toxicological and ecotoxicological profile.

To avoid the large quantity of chemical agents released in the environment, while, ensuring effective pest control is always desirable, so the synergism described as (the cooperative action of two components of a mixture, such that the total effect is greater.

The present study aimed to evaluate joint toxic action of mineral or plant oils, IGRs and Avaunt on a laboratory strain of S. *littoralis.* This can help in developing valid programmes for control of this pest, particularly when it infests cotton late in the growing season.

2. Materials and Methods

1. Tested insect:

Laboratory strain of the cotton leafworm *S. littoralis* were used in these search. Eggs were obtained from laboratory strain maintained at the cotton pest research department, Plant Protection Research Institute, Agricultural Research Center, Dokki; Giza. These eggs were kept in glass jar covered with gauze under laboratory condition of 25±2°C and 65±5% R.H. until hatching. The larvae were reared on fresh leaves of castor bean *Ricinus communis* for several generations without exposure to insecticides. This procedure continued till the fourth larval instar described by **El defrawi *et al*., 1964**.

***2.* Tested compounds:**

1. Oxadiazine insecticide: Indoxacarb (Avaunt 15% SC).
2. Insect growth regulators: Benzoylurea: Diflubenzuron (Dimilin 48% WP) and Lufenuron (Cymax 5% EC).
3. Mineral oils: Mineral oil (KZ oil 95% EC) and (Kapee oil 96.5% EC).
4. Plant oils: Prev-AM (Orange oil 60% SL).

***3.* Bioassay studies**

4^{th} instar larvae of *S. littoralis* stage, were used to determine the toxicity action of the tested materials, the tests were carried out by dipping castor bean leaves in solution at four concentrations tested of Avaunt, IGRs and previous oils for 10 seconds and left for air dryness. Four replicates of 10 larvae for each were made for the control and for each concentration in case of studying mortality regression lines and the joint toxic action of the different mixtures.

Mortality counts in case of Avaunt and mineral and plant oils either alone or mixtures were made 24 hours after treatment, while in case of IGRs either alone or mixtures were made 72 hours after treatment. All the experiments were carried out under conditions of (25±2°C and 65±5% R.H).

The data were corrected relatively to control mortality **(Abbott, 1925).** The data were subjected analysis by the method of **(Busvine 1971)**. The software program used to calculate the LC_{50}, LC_{25} and LC_{10} with their curves and slope values.

***4.* Experimental preparation:**

The LC_{50}, LC_{25} and LC_{10} values were determined for all previous materials and concentrations corresponding to these values were prepared. A total of 99 binary mixtures were applied on 4^{th} instar larvae of *S. littoralis* larvae in proportion to their toxicity of mineral and plant oils on IGRs and Avaunt. The binary mixtures studied were {(LC_{50}+LC_{50}), (LC_{50}+LC_{25}), (LC50+LC_{10})}, {(LC_{25}+LC_{50}), (LC_{25}+LC_{25}), LC_{25}+LC_{10})}, {(LC_{10}+LC_{50}), (LC_{10}+LC_{25}), LC_{10}+LC_{10})}, respectively.

***5.* Co-toxicity factors (CF) estimation:**

The combined action of the different mixtures was expressed as the Co-toxicity factors (CF) which estimated according to the equation given by **Mansour *et al*., 1966** as follow:

Co-toxicity factor (CF) = Observed mortality% – Expected mortality % X 100/Expected mortality %

Co-toxicity factor differentiate results in three categories as following: A positive factor of 20 or more is considered as potentiation, a negative of 20 or more is considered as antagonism, while intermediate value between (-20 and+20) indicate only additive effect.

3. Results and Discussion

Effect of joint action of tested formulation mixtures was studied on 4^{th} *S. littoralis* larvae. Data presented in Table (1) showed the calculated LC_{50}, LC_{25}, LC_{10} and slope values for Avaunt compound, two formulations of IGRs (Cymax, Dimilin), two mineral oil formulations (KZ,Kapee oils) and one plant oil (Orange oil). Data indicated that Dimilin was the most effective compound of IGRs, while KZ oil was more toxic effect oils. Likewise, Avaunt pesticide was more toxic effect (LC_{50} was 3.08).

The effect of joint toxic action for the response 11 binary insecticides, IGRs and (Mineral-plant) oil formulations mixtures on 4^{th} *S. littoralis* larvae is presented in Tables (2), (3), (4) and (5).

Table (1): Comparative to toxicity of different assayed materials against 4^{th} instar larvae of *S. littoralis.*

Treatment	Slope	LC_{50} in ppm (F.L)	LC_{25} in ppm (F.L)	LC_{10} in ppm (F.L)
Avaunt 15% SC	2.11	3.08(2.61-3.55)	1.47(0.83-1.91)	0.76(0.27-1.17)
Cymax 5% EC	0.95	197.37(126.8-273.1)	38.34(3.22-76.31)	8.77(0.09-29.44)
Dimilin 48% WP	1.34	52.32(8.90-85.5)	16.42(0.63-40.30)	5.78(0.04-20.76)
Orange oil 60% SL	1.73	1.55(1.20-1.85)	0.63(0.33-0.89)	0.28(0.10-0.47)
KZ oil 95% EC	1.36	1.54(1.07-1.92)	0.49(0.17-0.79)	0.176(0.029-0.37)
Kapee oil 96.5% EC	1.32	1.67(1.18-2.07)	0.51(0.17-0.82)	0.178(0.027-0.38)

As shown in Table (2), mixture of the pesticide Avaunt and the IGR Dimilin produced different levels of additive and synergistic action, the highest genrgistic action was that occurred in case of combinations (LC_{10}+LC_{10}), followed by (LC_{25}+LC_{10}), and (LC_{50}+LC_{10}), where CFs were +250, +150 and +62.5, respectively, while the lowest synergistic action was detected with the combinations (LC_{10}+LC_{25}), and (LC_{25}+LC_{25}), where CFs were +42.8 and +30, respectively. While antagonism was found with the mixture (LC_{10}+LC_{50}), (CF was -58.3) and (LC_{50}+LC_{50}), (CF was -35). An additive effect was only obtained with the mixture (LC_{50}+LC_{25}), (CF was +3.3).

As the mixture of the pesticide Avaunt and the IGR Cymax, the Co-toxicity factor obtained with (LC_{50}+LC_{10}) caused the zero value when the observed mortality reached 60% and indicated additive effect, while the most of mixtures produced a variable rates of antagonistic action occurred in case of the combinations (LC_{10}+LC_{50}), CF=-75, (LC_{10}+LC_{25}), CF=-71.4, (LC_{50}+LC_{25}), CF=-56.6, (LC_{10}+LC_{10}), CF= -37.5 and (LC_{50}+LC_{50}), CF= -25. The combination (LC_{25}+LC_{10}), showed only the highest synergism (CF= +128.5), on the contrary, (LC_{25}+LC_{25}), produce only additive effect (CF= +5).

Table (2): joint action and Co-toxicity factor of the insecticide Avaunt and two IGR compounds against 4th larval instar of *S. littoralis* after 24 hours.

Mixture	Avaunt/Dimilin			Avaunt/Cymax		
	Expect mortality %	Observe mortality %	CF*	Expect mortality %	Observe mortality %	CF*
LC_{50}+LC_{50}	100	65	-35	100	75	-25
LC_{50}+LC_{25}	75	77.5	+3.3	75	32.5	-56.6
LC_{50}+LC_{10}	60	97.5	+62.5	60	60	+0
LC_{25}+LC_{50}	75	62.5	-16.6	75	97.5	+30
LC_{25}+LC_{25}	50	65	+30	50	52.5	+5
LC_{25}+LC_{10}	35	87.5	+150	35	80	+128.5
LC_{10}+LC_{50}	60	25	-58.3	60	15	-75
LC_{10}+LC_{25}	35	50	+42.8	35	10	-71.4
LC_{10}+LC_{10}	20	70	+250	20	12.5	-37.5

CF* = Co-toxicity factor = Observed mortality% – Expected mortality % X 100 / Expected mortality %

In Table (3), the mixture of the insecticide Avaunt / the mineral oil Kapee produced the highest level of synergism as potentiation action occurred in all levels of combination, expect (LC_{50}+LC_{50}), which produced additive effects (CF was+0), followed by the mixtures of Avaunt / Orange oil produced the most effect was potentiating action, while in case of combinations (LC_{50}+LC_{50}), and (LC_{50}+LC_{25}) showed only antagonism (CF= -35 and -33.3, respectively. The mixture (LC_{25}+LC_{50}), produced additive effect (CF= +13.3). Finally, as for the mixtures of Avaunt / KZ oil, the most combinations produced different levels of antagonism except with the combination (LC_{25}+LC_{10}), showing only the highest potentiation (CF was +42.85), and the combination (LC_{25}+LC_{25}), producing only additive effect (CF was +0).

Table (3): joint action and Co-toxicity factor of the insecticide Avaunt, two mineral oils and one plant oil against 4th larval instar of *S. littoralis* after 24 hours.

Mixture	Avaunt / Kapee oil			Avaunt / orange oil			Avaunt / KZ oil		
	Expect mortality %	Observe mortality %	CF*	Expect mortality %	Observe mortality %	CF*	Expect mortality %	Observe mortality %	CF*
LC_{50}+LC_{50}	100	100	+0	100	65	-35	100	72.5	-27.5
LC_{50}+LC_{25}	75	92.5	+23.3	75	72.5	-33.3	75	65	-13.33
LC_{50}+LC_{10}	60	85	+41.6	60	95	+58.3	60	62.5	+4.16
LC_{25}+LC_{50}	75	97.5	+30	75	85	+13.3	75	57.5	-23.33
LC_{25}+LC_{25}	50	95	+90	50	97.5	+95	50	50	+0
LC_{25}+LC_{10}	35	100	+185.7	35	95	+171.4	35	50	+42.85
LC_{10}+LC_{50}	60	92.5	+54.2	60	80	+33.3	60	27.5	-54.16
LC_{10}+LC_{25}	35	92.5	+164.3	35	82.5	+135.7	35	22.5	-35.71
LC_{10}+LC_{10}	20	75	+275	20	70	+250	20	22.5	+12.5

CF* = Co-toxicity factor = Observed mortality% – Expected mortality % X 100 / Expected mortality %

Table (4) shows the case of the mixtures of Dimilin (IGR) with different oil formulations. All combinations of Dimilin with both of Orange oil and KZ oil formulations produced different levels of antagonism, expect with the combination Dimilin / KZ oil (LC_{10}+C_{25}), which caused the only highest synergism (CF +100).

The remaining mixtures of Dimilin / Kapee oil produced different levels of synergism, where the mixture (LC_{10}+LC_{25}), produced the highest level of synergism followed by (LC_{10}+LC_{50}), (LC_{50}+LC_{25}), and (LC_{25}+LC_{50}), (CFs were -107.1, +54.2, +26.7 and 26.7, respectively), while the combinations (LC_{25}+LC_{10}) and (LC_{50}+LC_{50}) produced slight rate of additive effect (CFs were +14.3 and -5 respectively). The remaining combinations produces different levels of antagonism, including (LC_{25}+LC_{25}), followed by (LC_{50}+LC_{10}), (LC_{10}+LC_{10}), (CFs were -70, -54.2 and -25, respectively). Finally, it is to be noted that the high levels of synergism attained were those obtained from mixing the low concentrations together, while rates of antagonism were less pronounced in mixtures including high concentrations. This indicates that in preparing binary insecticide mixtures, concentrations-ratio relationships should be considered.

Table (4): joint action and Co-toxicity factor of the IGR Dimilin and three mineral oils against 4th larval instar of *S. littoralis* after 72 hours.

Mixtures	Dimilin / Kapee oil			Dimilin / orange oil			Dimilin / KZ oil		
	Expect mortality %	Observe mortality %	CF*	Expect mortality %	Observe mortality %	CF*	Expect mortality %	Observe mortality %	CF*
$LC_{50}+LC_{50}$	100	95	-5	100	22.5	-77	100	15	-85
$LC_{50}+LC_{25}$	75	95	+26.7	75	12.5	-83.3	75	7.5	-90
$LC_{50}+LC_{10}$	60	27.5	-54.2	60	10	-83.3	60	22.5	-62.5
$LC_{25}+LC_{50}$	75	95	+26.7	75	7.5	-90	75	12.5	-83.3
$LC_{25}+LC_{25}$	50	15	-70	50	15	-70	50	22.5	-55
$LC_{25}+LC_{10}$	35	40	+14.3	35	15	-57.1	35	5	-85.7
$LC_{10}+LC_{50}$	60	92.5	+54.2	60	17.5	-70.8	60	10	-83.3
$LC_{10}+LC_{25}$	35	72.5	+107.1	35	17.5	-50	35	70	+100
$LC_{10}+LC_{10}$	20	15	-25	20	27.5	-37.5	20	17.5	-12.5

CF* = Co-toxicity factor = Observed mortality% – Expected mortality % X 100 / Expected mortality %

Table (5): joint action and Co-toxicity factor of the insecticide Avaunt, the IGR Cymax, two mineral oils and one plant oil against 4th larval instar of *S. littoralis* after 24 hours.

	Cymax / Kapee oil			Cymax / orange oil			Cymax / KZ oil		
Mixtures	Expect mortality %	Observe mortality %	CF*	Expect mortality %	Observe mortality %	CF*	Expect mortality %	Observe mortality %	CF*
$LC_{50}+LC_{50}$	100	92.5	-7.5	100	100	+0	100	100	+0
$LC_{50}+LC_{25}$	75	67.5	-10	75	100	+33.3	75	70	-6.6
$LC_{50}+LC_{10}$	60	95	+58.3	60	95	+58.3	60	47.5	-20.8
$LC_{25}+LC_{50}$	75	17.5	-76.7	75	67.5	-10	75	50	-33.3
$LC_{25}+LC_{25}$	50	45	-10	50	95	+90	50	65	+30
$LC_{25}+LC_{10}$	35	67.5	+92.9	35	65	+85.7	35	62.5	+78.6
$LC_{10}+LC_{50}$	60	95	+58.4	60	72.5	+20.8	60	50	-16.7
$LC_{10}+LC_{25}$	35	50	+42.9	35	72.5	+107.1	35	67.5	+92.9
$LC_{10}+LC_{10}$	20	47.5	+137.5	20	35	+75	20	72.5	+262.5

CF* = Co-toxicity factor = Observed mortality% – Expected mortality % X 100 / Expected mortality %

As shown in Table (5), the mixtures of Cymax (IGR) with KZ oil which produced high levels of potentiation were ($LC_{10}+LC_{25}$), ($LC_{25}+LC_{10}$), and ($LC_{25}+LC_{25}$), where CFs were +92.9, +78.6 and +30, respectively, while the combinations which produced additive were ($LC_{50}+LC_{50}$), ($LC_{50}+LC_{25}$), and ($LC_{10}+LC_{50}$), where CFs were +0, -6.6 and -16.7, respectively. Whereas antagonism effect was obtained with the mixtures ($LC_{50}+LC_{10}$), and ($LC_{25}+LC_{50}$) (CFs were -20.8 and -33.3, respectively. Likewise, the mixture of Cymax (IGR) with Kapee oil, the combination ($LC_{10}+LC_{10}$), showed the highest rate of synergism followed by ($LC_{25}+LC_{10}$), ($LC_{10}+LC_{50}$), ($LC_{50}+LC_{10}$), and ($LC_{10}+LC_{25}$). (CFs were+137.5, +92.9, +58.4, +58.3 and 42.9, respectively. An additive effect was obtained with the mixtures ($LC_{50}+ LC_{25}$), ($LC_{25}+LC_{25}$), and ($LC_{50}+LC_{50}$), (CFs were -10, -10 and -7.5, respectively).

The mixtures Cymax (IGR) / Kapee oil are similar with mixtures Cymax / Orange oil in effect on 4th instar of *S. littoralis* larvae after 72 hours. ($LC_{50}+LC_{50}$) for both mixture indicated that the zero value of Co-toxicity factor and the observed mortality reached 100%. In case of (LC_{25} Cymax / LC_{50} orange oil), produced additive effect (CF was -10). The same concentrations in the combination of Cymax / Kapee oil and ($LC_{25}+LC_{25}$), produced also additive effect (CF was -10).

It should be noticed that the high levels of synergism attained were those obtained from mixing the low concentrations together, while rates of potentiation were less obvious in the use of lower doses of the required pesticides and an environment that is less contaminated with pesticides. This approach may include the use of mineral and plant oils and insect growth regulators.

These results obtained by **Zidan *et al.*, (1987)**. Who found that when mineral oil used in combination with insecticides increased their efficiency on the cotton leafworm S. *littoralis* Boisd. Also, **EL-Deeb, 1993** found the joint action of mineral oil/insecticide combinations revealed different levels of synergism on adult of wheat aphid *Rhopalosiphum* padi (L.). Likewise, **Khalequzzaman and Chowdhury. (2003)** found that oils of neem, sesame, castor and soybean as synergist with pirimphos-methyl enhance the mortality of the adult *T. castanium*. Also, **Abd-EL-Razik *et al.*, (2012)** revealed that corn oil, sunflower oil and sesame oil when combined with pyridalyl and abamectin proved to have synergistic effect against Callosobruchus maculates adults. Also, these results agree with **Ghoneim et *al.*, 2012**, who assessed the mixtures of organophosphorus (OP) compounds with IGRs for their potentiation in resistant field population of the cotton leafworm S. *littoralis* Boisd. The results of co-toxicity factor against 4th instar larvae showed that the OP chlorpyrifos when mixed with the IGR hexaflumuron or triflumuron produced high synergism, while additive was produced when mixed with the IGRs chlorfluazuron, chromafenozide or tobufenozite. As for mixtures of the OP profenofos with IGRs, profenofos combined with hexaflumuron caused high synergism, however the mixtures of profenofos with triflumuron, lufenuron, chromafenozide, flufenoxuron, tobufenozite or chlorfluazuron produced additive effect.

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