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TOXICOLOGICAL AND BIOLOGICAL EFFECT OF *Calendula officinalis* EXTRACTS ON THE COTTON LEAF WORM, *Spodoptera littoralis* (Bosid.)

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ABSTRACT

The present investigation was conducted to evaluate the insecticidal and delayed effects of flowers, leaves and stems extracts of *Calendula officinalis* plant on the 4*th* instar larvae of *Spodoptera littoralis* (Boisd). The four crude extractions (chloroform, dichloromethane, ethanol and hexane) for each part of the plant proved high insecticidal activity against the cotton leaf worm larvae by using the residual thin film technique. All extracts had no toxic effect on the same larvae with leaf-dipping technique. Residual thin-film treatment of larvae with all extracts produced high larval and total mortality, low percentage of adult emergence and sterility. Feeding treatments reduced the pupal weight, fecundity of emerged adult females, and hatchability of deposited eggs by these females, which was reflected in high percentage of sterility. These delayed latent effects were more pronounced in treatments of larvae with dichloromethane extracts.

Negligible phytotoxicity of the formulation of dichloromethane and chloroform extracts on cotton plants. The second instar larvae were more susceptible to the effect of the formulated extracts than that of the fourth instar.

Keywords: Plant extracts, *Calendula officinalis*, *Spodoptera littoralis*, insecticidal activities, delayed latent effects.

INTRODUCTION

The cotton leaf worm *Spodoptera littoralis* (Boisd.) play an important role in cotton production in Egypt, so the success in controlling such insect will result in improving cotton production.

Organophosphorous, carbamate and pyrethroid insecticides have been used successfully for the control of the cotton leaf worm. These synthetic pesticides are expensive, hazardous to human health and to the environment, also *S. littoralis*, have highly resistance to several synthetic insecticides (Georghiou and Lagunes-Tejada 1991).

To date, there are many studies on using inexpensive plant derivatives that could serve as substitutes for toxic insecticides. Neem seeds kernel was discovered as a feeding deterrent of locusts (Pradhan *et al.*, 1967). Several workers in India reported it to be an effective antifeedant against various crop pests such as *Spodoptera litura* (F.) on castor (Mane, 1968). Extracts of neem fruits, seeds, seed kernels and bark possess insect repellent, antifeedant, growth inhibitor and other insecticidal properties (Jacobson, 1989; Saxena, 1989 and Schmutterer, 1990).

Pesticidal activity of Egyptian plant extracts were investigated by Saleh *et al.*, 1986 (a & b); Salem, 1995; Amer *et al.*, 2001; Soliman, 2001 and Hassanein *et al.*, 2004.

The present work aimed to study the toxicological and biological latent effects of chloroform, dichloromethane, ethanol and hexane extracts of flower, leaf and stem of *Calendula officinalis* plant on the cotton leaf worm, *Spodoptera littoralis* larvae.

MATERIALS AND METHODS

1. Preparation of *Calendula officinalis* extracts:

a. Collection and preparation of Plant Material:

Calendula officinalis was collected from the Tenth of Ramadan City in May and June 2003. The plant was divided into three parts (flowers, leaves and stems), then left to dry in fresh air for one month. Every part of plant was milled well by a mixer miller machine to get a powder sample.

b. System of extraction:

Ground flowers, leaves and stems were extracted according to Kato-Noguchi *et al.*, 1994. Weight of 750 grams from each sample was shacked in a dark vessel with 2.25 liters of (hexane, chloroform, ethanol and dichloromethane) for 8 hours. Each extract was filtered in dark vessel purified and evaporated under vaccum with rotary evaporator at 40° C to dryness. Residues of different extracts were diluted with acetone and made up to 80, 40, 20, 10 and 5 mg/ml.

2. Formulation of the extracts:

Active ingredients are biologically active in extremely small quantity, so the chemical has to be prepared in a form that is convenient to use and to spread over large areas. The preparation of the active ingredient in a form suitable for use is referred to as a formulation.

The biological performance of active ingredient is frequently affected by the choice of formulation. The most common formulation is emulsifiable concentrate, which represent the largest volume of all pesticide formulation in terms of consumption. Emulsifiable concentrates are made from active ingredient, solvent and surfactants. Surfactants emulsifiers were added to these formulations to ensure spontaneous emulsification with good emulsion stability properties in the spray tank. Careful selection of balanced pair emulsifier blend (ionic and nonionic surfactant) is frequently necessary to ensure that emulsion dilution stability is maintained over widely differing climatic conditions and degree of water hardness.

Emulsifier decrease surface tension of spray solution where droplet makes spreading on the surface of the leaves and easily penetrates inside leaves surface.

3. Insects:

Larvae of *Spodoptera littoralis* were reared on castor bean leaves under laboratory conditions (25 ± 2°C and 70 ± 5% R.H.) for several years away from insecticidal contamination in Central Agricultural Pesticides Laboratory.

4. Treatments:

4.1. Crude extracts:

Concentrations of 80, 40, 20, 10 and 5 mg/ml were prepared from chloroform, dichloromethane, ethanol and hexane extracts of flowers, leaves

and stems of *C. officinalis*. Two methods were used to evaluate the insecticidal activities of twelve extracts:

4.1.I. Residual thin-film technique:

One ml from each concentration was evenly distributed on the Petri-dish surface (9 cm in diameter).The solvent was completely evaporated under laboratory conditions until thin film of plant extract concentration was formed. Control was treated with solvent only of each extract. Ten *4th* instar larvae were exposed to the residual thin film of each concentration for 6 hrs (Brady, 1966). Each treatment was replicated six times. Mortality counts were estimated, the LC_{50} and LC_{90} values were calculated as Finny (1971) and the toxicity index as Sun (1950).

4.1.II. Leaf dipping technique: (Feeding method)

Castor bean leaves were dipped in each concentration of all extracts for 20 sec., and then the leaves were left for 30 min. to dry, control leaves were dipped in solvents. Ten *4th* instar larvae were fed on treated and control leaves and six replicates were used for each concentration and control. Mortality counted was after 24 hrs then LC_{50}, LC_{90} and toxicity index were estimated.

The remaining survival larvae from treatment with two techniques were reared and certain of their biological activities were recorded. Larval, pupal and total mortality, pupal weight, fecundity of emerged adult female, hatchability of laying eggs and sterility percentages were recorded. Mean ± S.E. and significance of difference from control (at $P<0.05$ & 0.01) were calculated.

4.2 Formulations:

4.2.I. Phytotoxic effect:

Phytotoxicity of chloroform and dichloromethane extract formulations were determined one week after spraying of cotton plants grown in pots and left under field conditions.

4.2.II. Insecticidal activities:

Second and fourth instar larvae of *S. littoralis* were treated with several concentrations (75000, 37500, 18750, 9375, 4687.5 and 2343.8 ppm.) of formulated extracts by using leaf-dipping technique. Mortality was counted after 24 hrs., LC_{50}, LC_{90} and toxicity index were calculated also.

RESULTS AND DISCUSSION

Data concerning the toxic effect of chloroform, dichloromethane, ethanol and hexane crude extracts of flower, leaf and stem of *C. officinalis* plant against *4th* instar larvae of *S. littoralis* by using residual thin – film technique are shown in Table (1). The obtained results proved that flower ethanolic extract was more effective than hexane, dichloromethane and chloroform extracts of flower. Dichloromethane extract of leaves gave more potent insecticidal action than ethanol, hexane and chloroform leaf extracts. Chloroform extract of stems was more highly toxic on larvae than those of dichloromethane, ethanol and hexane. The tested extracts could be arranged according to their insecticidal action (LC_{50} value and toxicity index)

as follows: chloroform of stems (4.3 mg/ml and 100) > dichloromethane of leaves (5.9 mg/ml and 72.9) > dichloromethane of stems (15.2 mg/ml and 28.3) > ethanol of stems (24.1 mg/ml and 17.8) > ethanol of leaves (30.02 mg/ml and 14.3) > ethanol of flowers (43.3 mg/ml and 10) > hexane of flowers (57.7 mg/ml and 7.5) > hexane of leaves (64.3 mg/ml and 6.7) > dichloromethane of flowers (101.5 mg/ml and 4.2) > chloroform of leaves (101.7 mg/ml and 4.2) > chloroform of flowers (124.8 mg/ml and 3.5) > hexane of stems (229.7 mg/ml and 1.9).

From the above mentioned results we can conclude that the crude extracts of *C. officinalis* stems were more effective than crude extracts of leaves and flowers. Chloroform extract of stems and dichloromethane extract from leaves and stems exhibited higher toxicity against *S. littoralis* larvae than other extracts. The activity of these extracts was due to the hydorlyzable tannins (condensed) and phenolic glycosides in chloroform extract of stems and dichloromethane extracts of stems and leaves of *C. officinalis* plants (Hussein, 1985 and El-Sheikh *et al.*, 2004). On the other hand, the leaf dipping technique gave no toxic effect on *S. littoralis* larvae with all extracts.

Table (1): Insecticidal effect of flowers, leaves and stems extracts of *Calendula officinalis* on *4th* instar larvae of *Spodoptera littoralis* by using residual thin film technique:

Crude Extract		LC_{50} (mg/ml) [Fiducial Limits]	LC_{90} (mg/ml) [Fiducial Limits]	Slope	Toxicity Index
Flowers	Chloroform	124.8 [79.9 – 403.9]	488.6 [208.1 – 6311.7]	1.29	3.5
	Dichloromethane	101.5 [72.6 – 160.9]	305.7 [160.9 – 1866.03]	1.35	4.2
	Ethanol	43.3 [32.7 – 63.4]	190.9 [112.6 – 508.5]	1.31	10.0
	Hexane	57.7 [40.2 – 106.0]	358.9 [167.9 – 1810.4]	1.40	7.5
Leaves	Chloroform	101.7 [65.9 – 258.3]	514.7 [217.4 – 4383.5]	1.12	4.2
	Dichloromethane	5.9 [2.5 – 9.1]	49.6 [30.5 – 135.6]	1.25	72.9
	Ethanol	30.02 [23.96 – 38.6]	97.3 [68. 5 – 172.2]	1.18	14.3
	Hexane	64.3 [41.5 – 149.7]	530.9 [216.5 – 6246.9]	1.22	6.7
Stems	Chloroform	4.3 [1.4 – 7.2]	38.2 [23.8 – 103.6]	1.46	100
	Dichloromethane	15.2 [11.6 – 19.3]	56.03 [39.97 – 96.3]	1.24	28.3
	Ethanol	24.1 [18.4 – 32.2]	107.7 [69.6 – 228.2]	1.33	17.8
	Hexane	229.7 [103.7– 943.7]	1834.4 [401.97 – $6.46x10^5$]	1.42	1.9

Data in Tables (2 – 6) showed the latent biological effects of twelve extracts on *S. littoralis* larvae by using residual – thin film and leaf dipping techniques.

Table (2) reveals the delayed latent effects of chloroform extract of flowers, leaves and stems of *C. officinalis* on *4th* instar larvae.High

percentage of initial mortality (mortality of larvae after 24 hrs) was produced with thin film treatment of stems extract (55 – 100 %) at different concentration (5 – 80 mg/ml), but flowers and leaves extracts produced very low percentage of mortality (0 – 33.3% and 0 – 20% respectively). Morality increased during the moulting of larvae reached 80, 86.7 and 66.7% with the low concentration (5 mg/ml) of stems, leaves and flowers extract respectively at the end of larval stage. All extracts produced high total mortality and low emergence percentages which reached 86.7 and 13.3% for leaves, 83.3 and 16.7% for stems and 70 and 30% for flowers at low concetration compared with control 8.3 and 91.7% respectively. No adult emerged from high concetrations of leaves and stems extracts, while adult female moths emerged from high concentration of flower extract could not lay any egg.

In spite of no initial mortality, flower extract produced a higher percentage of larval and total mortality (ranging from 55 – 85% and 65 – 100%) at different concentrations than the other two extracts with leaf-dipping technique (feeding method). The emergence percentage ranged from 0 to 35%, 48.3 – 85% and 55 – 90% for flowers, stems and leaves extracts respectively at concentrations from 80 – 5 mg/ml, while that of control reached 93.3%.

A significant decreasing effect in pupal weight was produced with high concentraion of leaf dipping technique by leaf extract (44.8 and 44.2%), flower extract (40.1 and 41.7%) and stem extract (33 and 33.2%) for female and male respectively as compared with control (Table 6). Insignificant increase in pupal weight was produced by low concentration of the three extract especially with residual thin film technique. High significant reducing effect in fecundity (mean number of eggs laied by female moth) was produced by leaf extract (74.7% at 80 mg/ml) and flower extract (66.3% at 10 mg/ml) with feeding treatments. Also the same effect was produced by residual thin film treatment with flower extract (63.2%) at the high concentration (40 mg/ml). All concentations of the three extracts produced significant reduction in fecundity with two methods except the low concentration of leaf extract. Insignificant decrease in hatchability percentage was pronounced with all treatments. A significant difference was shown in flower extracts treatment (41.7% at 40 mg/ml concentration). The reduction in fecundity and hatchability was reflected as a high percentage in sterility of adult moths which ranged from 37.6 – 78.6% and 65.8 – 75.6% for flower extract, 28.7% and 21.8 – 80.5% for leaf extract and 15.3 – 50.8% and 42.2 – 68.9 for stem extract with residual thin film and leaf dipping method respectively at different concentrations. Flower extract produced a higher percentage of sterility at the lower concentration (5 mg/ml) with two methods.

Data in Table (3) clearly indicate that the treatment with dichloromethane extract of flowers, leaves and stems produced deleterious effects on cotton leaf worm development by using residual thin film and leaf-dipping techniques. High mortality percentages of larvae were produced with three extracts at different concentration by two methods of treatment. The total mortality ranged from 86.7 – 100% and 70 – 100% for leaf extract, 80 – 100% for stem extract and 53.3 – 80% and 55 – 90% for flower extract with thin film and feeding methods at concentrations from 5 – 80% mg/ml

respectively as compared with those of control (6.6 – 3.4%). A significant decreasing effect in pupal weight resulted from feeding treatment with leaf extract (48.6 & 50.2, 46.2 & 51.6 and 37.5 & 47.5%) and stem extract 43.4 & 38.1, 41.8 & 38.6 and 39.8 & 40.8%) at concentrations of 20, 10 and 5 mg/ml for female and male pupae respectively (Table 6). High concentrations (80, 40 and 20 mg/ml) of flower extract gave the same effect (45 & 44, 37.5 & 45.7 and 32.7 & 40.8%) on female and male pupae. The three extracts produced low percentage of adult emergence and reduced their fecundity. The reduction in number of deposited eggs was significantly pronounced with all concentrations of extracts by residual thin film, but feeding method produced a highly significant reduction effect expanded from high to low concentration which reach to 64.5, 69.5 and 71.2% for flower, leaf and stem extracts at 5 mg/ml concentration. Very low percent of egg hatchability 44.4% was produced by stem extract at the lower concentration of feeding treatment compared with control (85.7%). Also, a higher sterility percentage 85% was produced by this extract. Flower and leaf extract produced low percentages in hatchability (63.1 and 48.7%) and high sterility (73.7 and 82.5%) at the same concentration. Dichloromethane extractration of stem proved a superior toxic and delayed effects on *S. littoralis* larvae by two methods of treatment.

Table (4) revealed that the residual thin film treatment with flowers, leaves and stems produced high percentages of larval mortality and total morality and reduced the pupal weight, emergence and fecundity of adult moths. The reduction in fecundity of females was highly significant with flower and stem extracts treatment (81.9 and 72.6%) at concentration 20 mg/ml (Table 6). This effect was produced also, by feeding treatment of the three extracts at all concentration. A significant reduction in hatchability percentage was produced by feeding treatment with flower extract (39.9% at 20 mg/ml) and leaf extract (58.4, 57.2, 47.1 and 45.6% at 40, 20, 10 and 5 mg/ml) and stem extract (60.5, 57.9, 45.1, 42.3 and 34.7 at 80, 40, 20, 10 and 5 mg/ml). High percentages of sterility resulted from flower extract treatment (95.4, 69.9 and 60.6% at concentrations of 20, 10 and 5 mg/ml) with residual thin film, while leaf extract produced the same effect (88, 86.3, 82.6 and 78.9% at 40, 20, 10 and 5 mg/ml concentration) with feeding method treatments. Ethanol extraction of leaves was the most potent than that of flower and stem.

The delayed latent effects of hexane extract of flowers, leaves and stems on *4th* instar larvae of cotton leafworm are revealed in Table (5). All extract produced high total mortality percentages ranging from 76.6 – 96.7%, 66.7 – 93.4% and 60 – 93.3% for leaves, flowers and stems respectively at different concentrations (5 – 80 mg/ml with residual thin film treatments) compared with the value of control (6.7%). Insignificant decrease in pupal weight was presented in these treatments. Feeding method produced certain signifcant decrease in male and female pupal weight with high concentrations of leaf and flower extracts. Two treatments produced reduction in egg laying, but this effect reached high percentages with leaf extract (80 & 66.4%) at high concentrations (40 & 20 mg/ml) with flower extracts (75.5, 71.1, 64.5 and 57.4%) and stem extract (75, 73.7, 64.7, 63.3 and 55.1%) at all

concentrations of feeding treatments. Hatchability percentages decreased with the increase of the three extracts concentration, by feeding treatments. A higher percentage of sterility produced by high concentrations of the three extracts by feeding, but with treatment of low concentration, flower extract proved superior in increasing the sterility, which reached to 33.1% and 69.2% with residual thin film and feeding method treatments.

Hassanein Amal *et al.*,(2004) reported that the four crude extracts (hexane, chloroform, ethyl acetate and ethanol) of *Anthemis melapodia* and *Artmesia monosperma* plants caused high total mortality, high reduction in egg laying rate (for the adult female moths resulting from survival larvae) and egg hatchability, which was refelected in sterility percentages. The effects were more pronounced in residual thin film than leaf-dipping technique particularly with the higher concentration used.

From the above mentioned results we it may be concluded that the residual thin film treatments of *C. officinialis* extracts produced high initial total mortality and low pupal weight, adult emergence and sterility. The feeding treatments caused low initial mortality, decrease pupal weight, female fecundity and hatchability of deposited eggs and high percentages of sterility. Two methods of treatment produced malformation in larvae, pupae and adult which caused the appearance of intermediate phases. These delayed effects were proportional to increasing of concentration of all extracts. Dichloromethane extract of flowers, leaves and stems produced a deleterious effect on the development of *S. littoralis* larvae, than that of chloroform, ethanol and hexane.

Saleh *et al.*, (1986 a) reported that petroleum ether extract of *Argemone mexicana*, *Poinciana regia*, *Tagetes erecta* and *Tagetes patula* plants inhibited the growth of cotton leafworm and delayed the metamorphosis process, also, these extracts produced marked ratio of deformed adults. Although, these plant extracts did not have direct killing effect on the studied insect, they showed a definite interaction with one or more of the physiological process of insect which may result in possible population control of insects.

Negligible phytotoxicity were observed one week after the successive spraying of cotton plants (grow in pots under field conditions) by the formulated dichloromethane and chlorofrom extracts of *C. officinalis* flowers, leaves and stems.

Data presented in Table (7) reveal the toxic effect of the formulated dichloromethane and chloroform extracts of flowers leaves and stems of *C. officinalis* on *2nd* and *4th* instar larvae of *S. littoralis*. The tested formulations are arranged according to their efficiency (LC_{50}'s value and toxicity index) as follows: dichloromethane extract of; stems (8695.8 & 12664.3 ppm and 100 T.I.) leaves (11600.7 & 18042.2 ppm and 75 & 70.2 T.I) and flowers (13711.2 & 20305.6 ppm and 63.4 & 62.4 T.I.) chloroform extract of flowers (15368.2 & 21743.3 ppm and 56.6 & 58.3 T.I.), leaves (23410.9 & 31021.2 ppm and 37.1 & 40.8 T.I.) and stems (35191.6 & 45421.1 ppm and 24.7 & 27.9 T.I.) for *2nd* and *4th* instars larvae respectively. These results reveal that the *2nd* instar larvae were more, susceptible to the effect of dichloromethane and chloroform extract formulations than larvae of *4th* instar.

Table (2): Latent effects of chloroform extracts of *Calenduia officinalis* on *Spodoptera littoralis* larvae by using Residual thin film and leaf dipping techniques

Biological Effects		Concentrations (mg/ml) 80 F	80 L	80 S	40 F	40 L	40 S	20 F	20 L	20 S	10 F	10 L	10 S	5 F	5 L	5 S	Control
Mortality after 24 hrs (%)	R.T.	33.3	20.0	100.0	13.3	15.0	83.3	6.7	10.0	75.0	0.0	0.0	70.0	0.0	0.0	55.0	0.0
	L.D.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Average larval mortality (%)	R.T.	83.3	100.0	100.0	73.3	96.7	100.0	70.0	96.7	96.7	70.0	90.0	90.0	66.7	86.7	80.0	5.0
	L.D.	85.0	40.0	31.7	80.0	28.3	30.0	68.3	20.0	25.0	65.0	16.7	18.3	55.0	10.0	15.0	6.7
Average Pupal Mortality (%)	R.T.	3.3	0.0	0.0	6.7	3.3	0.0	6.7	3.3	3.3	6.7	3.3	3.3	3.3	0.0	3.3	3.3
	L.D.	15.0	5.0	20.0	11.7	10.0	13.3	3.3	6.7	8.3	0.0	5.0	5.0	10	0.0	0.0	0.0
Mean Pupal weight (gm) ± S.E.	R.T. ♀	0.172 ± 0.057	-	-	0.186 ± 0.051	-	-	0.218 ± 0.011	-	-	0.234 ± 0.029	0.246 ± 0.023	0.250 ± 0.008	0.241 ± 0.022	0.255 ± 0.021	0.252 ± 0.007	0.226 ± 0.035
	L.D. ♂	0.165 ± 0.073	-	-	0.170 ± 0.034	0.155 ± 0.007	-	0.205 ± 0.040	0.201 ± 0.005	0.313 ± 0.016	0.211 ± 0.039	0.215 ± 0.011	0.230 ± 0.002	0.216 ± 0.033	0.243 ± 0.006	0.239 ± 0.028	0.209 ± 0.023
	R.T. ♀	0.127 ± 0.012	0.117 ± 0.014	0.142 ± 0.010	0.143 ± 0.028	0.124 ± 0.023	0.156 ± 0.020	0.157 ± 0.035	0.135 ± 0.028	0.161 ± 0.023	0.169 ± 0.043	0.152 ± 0.024	0.203 ± 0.035	0.185 ± 0.032	0.167 ± 0.039	0.221 ± 0.026	0.212 ± 0.052
	L.D. ♂	0.116 ± 0.016	0.111 ± 0.020	0.133 ± 0.190	0.135 ± 0.012	0.113 ± 0.009	0.140 ± 0.019	0.139 ± 0.017	0.120 ± 0.022	0.147 ± 0.028	0.136 ± 0.025	0.137 ± 0.031	0.191 ± 0.032	0.153 ± 0.029	0.139 ± 0.013	0.207 ± 0.028	0.199 ± 0.027
Total Mortality (%)	R.T.	86.6	100.0	100.0	80.0	100.0	100.0	76.7	100.0	100.0	76.7	93.3	93.3	70.0	86.7	83.3	8.3
	L.D.	100.0	45.0	51.7	91.7	38.3	43.3	71.6	26.7	33.3	65.0	21.7	23.3	65.0	10.0	15.0	6.7
Emergence (%)	R.T.	13.3	0.0	0.0	20.0	0.0	0.0	23.3	0.0	0.0	23.3	6.7	6.7	30.0	13.3	16.7	91.7
	L.D.	0.0	55.0	48.3	8.3	61.7	56.7	28.4	73.3	66.7	35.0	78.3	76.7	35.0	90.0	85.0	93.3
Fecundity Mean no. of eggs/female ± S.E.	R.T.	-	-	-	229.3 ± 15.6	-	-	275.0 ± 49.4	-	-	331.0 ± 27.2	-	427.0 ± 22.0	408.2 ± 55.8	450.0 ± 29.5	620.0 ± 00.0	622.5 ± 38.6
	L.D.	-	150.0 ± 20.4	250.0 ± 23.0	-	225.2 ± 45.6	261.6 ± 27.4	-	267.6 ± 34.2	282.0 ± 36.2	200.0 ± 18.2	360.0 ± 50.0	350.5 ± 12.5	233.3 ± 29.6	502.2 ± 39.4	380.0 ± 25.2	593.2 ± 72.4
Hatchability (%)	R.T.	-	-	-	52.5	-	-	72.7	-	-	84.3	-	64.6	85.8	88.9	76.6	90.1
	L.D.	-	66.7	64.0	-	73.3	68.8	-	78.9	71.0	82.5	80.6	79.9	75.0	79.7	77.9	86.3
Sterility (%)	R.T.	-	-	-	78.6	-	-	64.4	-	-	50.3	-	50.8	37.6	28.7	15.3	
	L.D.	-	80.5	68.9	-	67.8	64.8	-	58.8	61.0	75.6	43.3	45.3	65.8	21.8	42.2	

F: Flowers, L: Leaves, S: stems, R.T.: Residual Thin Film technique, L.D.: Leaf Dipping technique, S.E. Standard Error.

Table (3): Latent effects of Dichloromethane extracts of *Calendula officinalis* on *Spodoptera littoralis* larvae by using Residual thin film and leaf dipping techniques

Biological Effects		Concentrations (mg/ml) 80 F	80 L	80 S	40 F	40 L	40 S	20 F	20 L	20 S	10 F	10 L	10 S	5 F	5 L	5 S	Control
Mortality after 24 hrs (%)	R.T.	33.3	100.0	100.0	23.0	100.0	100.0	0.0	100.0	80.0	0.0	60.0	26.7	0.0	45.0	25.0	0.0
	L.D.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Average larval mortality (%)	R.T.	73.3	100.0	100.0	60.0	100.0	100.0	56.7	100.0	100.0	50.0	86.7	83.3	43.3	86.7	73.3	3.3
	L.D.	80.0	100.0	100.0	66.7	100.0	100.0	50.0	80.0	95.0	43.3	73.3	81.7	40.0	56.7	60.0	1.7
Average Pupal Mortality (%)	T	6.7	0.0	0.0	3.3	0.0	0.0	3.3	0.0	0.0	6.7	6.6	6.7	10.0	0.0	6.7	3.3
	L.D.	10.0	0.0	0.0	10.0	0.0	0.0	18.3	11.7	0.0	16.7	10.0	5.0	15.0	13.3	20.0	1.7
Mean Pupal weight (gm) ± S.E.	R.T. ♀	0.185 ± 0.035	-	-	0.215 ± 0.029	-	-	0.224 ± 0.033	-	-	0.274 ± 0.007	0.212 ± 0.002	0.273 ± 0.013	0.270 ± 0.031	0.244 ± 0.027	0.277 ± 0.016	0.237 ± 0.041
	L.D. ♂	0.173 ± 0.047	-	-	0.187 ± 0.004	-	-	0.201 ± 0.021	-	-	0.212 ± 0.012	0.198 ± 0.009	0.234 ± 0.023	0.234 ± 0.012	0.225 ± 0.004	0.241 ± 0.028	0.219 ± 0.053
	R.T. ♀	0.138 ± 0.019	-	-	0.157 ± 0.012	-	-	0.169 ± 0.010	0.129 ± 0.008	0.142 ± 0.016	0.182 ± 0.024	0.135 ± 0.013	0.146 ± 0.016	0.187 ± 0.019	0.157 ± 0.028	0.151 ± 0.019	0.251 ± 0.042
	L.D. ♂	0.125 ± 0.012	-	-	0.121 ± 0.033	-	-	0.132 ± 0.015	0.111 ± 0.013	0.138 ± 0.018	0.163 ± 0.007	0.108 ± 0.026	0.137 ± 0.019	0.155 ± 0.020	0.117 ± 0.018	0.132 ± 0.006	0.223 ± 0.033
Total Mortality (%)	R.T.	80.0	100.0	100.0	63.3	100.0	100.0	60.0	100.0	100.0	56.7	93.3	90.0	53.3	86.7	80.0	6.6
	L.D.	90.0	100.0	100.0	76.7	100.0	100.0	68.3	91.7	95.0	60.0	83.3	86.7	55.0	70.0	80.0	3.4
Emergence (%)	R.T.	20.0	0.0	0.0	36.7	0.0	0.0	40.0	0.0	0.0	43.3	6.7	10.0	46.7	13.3	20.0	93.4
	L.D.	10.0	0.0	0.0	23.3	0.0	0.0	31.7	8.3	5.0	40.0	16.7	13.3	45.0	30.0	20.0	96.6
Fecundity Mean no. of eggs/female ± S.E.	R.T.	263.3 ± 25.6	-	-	278 ± 37.6	-	-	290.8 ± 12.1	-	-	284.0 ± 57.0	270.0 ± 16.0	0.0	299.5 ± 81.5	430.0 ± 27.0	524.5 ± 59.8	562.7 ± 59.3
	L.D.	-	-	-	-	-	-	145.6 ± 35.2	0.0	0.0	198.2 ± 36.4	163.0 ± 11.8	0.0	221.8 ± 25.2	191.0 ± 18.5	180.2 ± 9.4	625.4 ± 85.2
Hatchability (%)	R.T.	55.7	-	-	73.2	-	-	77.3	-	-	82.0	77.8	-	88.0	83.1	76.3	87.3
	L.D.	-	-	-	-	-	-	52.9	-	-	54.5	46.0	-	63.1	48.7	44.4	85.2
Sterility (%)	R.T.	70.2	-	-	58.6	-	-	54.2	-	-	52.6	57.2	-	46.4	23.9	18.5	
	L.D.	-	-	-	-	-	-	85.5	-	-	79.7	85.9	-	73.7	82.5	85.0	

F: Flowers, L: Leaves, S: stems, R.T.: Residual Thin Film technique, L.D.: Leaf Dipping technique, S.E. Standard Error.

Table (4): Latent effects of Ethanol extracts of *Calendula officinalis* on *Spodoptera littoralis* larvae by using Residual thin film and leaf dipping techniques

Biological Effects		Concentrations (ppm)															Control
		80			40			20			10			5			
		F	L	S	F	L	S	F	L	S	F	L	S	F	L	S	
Mortality after 24 hrs (%)	R.T.	80.0	80.0	83.3	55.0	70.0	70.0	20.0	33.3	45.0	16.0	15.0	20.0	0.0	0.0	10.0	0.0
	L.D.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Average larval mortality (%)	R.T.	100.0	100.0	96.7	93.3	100.0	90.0	83.3	96.7	86.7	81.7	96.7	76.7	63.3	96.7	76.7	3.3
	L.D.	81.7	35.0	40.0	70.0	25.0	35.0	56.7	21.7	30.0	55.0	20.0	25.0	45.0	10.0	20.0	3.3
Average Pupal Mortality (%)	R.T.	0.0	0.0	0.0	3.3	0.0	6.7	3.3	3.3	0.0	1.7	3.3	3.3	6.7	0.0	3.3	0.0
	L.D.	5.0	16.7	5.0	15.0	25.0	8.3	18.3	20.0	10.0	13.3	5.0	15.0	13.3	15.0	5.0	1.7
Mean Pupal weight (gm) ± S.E.	R.T. ♀	-	-	0.269 ± 0.022	0.261 ± 0.011	-	0.208 ± 0.018	0.237 ± 0.020	-	0.246 ± 0.037	0.225 ± 0.012	0.307 ± 0.006	0.254 ± 0.032	0.240 ± 0.025	0.363 ± 0.010	0.268 ± 0.010	0.247 ± 0.031
	L.D. ♂	-	-	0.250 ± 0.008	0.316 ± 0.037	-	0.181 ± 0.032	0.210 ± 0.039	0.150 ± 0.048	0.229 ± 0.017	0.201 ± 0.018	0.229 ± 0.021	0.225 ± 0.008	0.217 ± 0.022	0.232 ± 0.024	0.233 ± 0.024	0.221 ± 0.045
	R.T. ♀	0.130 ± 0.020	0.126 ± 0.023	0.135 ± 0.019	0.157 ± 0.030	0.133 ± 0.013	0.142 ± 0.013	0.165 ± 0.017	0.137 ± 0.012	0.146 ± 0.022	0.203 ± 0.042	0.183 ± 0.025	0.151 ± 0.006	0.176 ± 0.028	0.184 ± 0.019	0.180 ± 0.031	0.249 ± 0.022
	L.D. ♂	0.117 ± 0.050	0.121 ± 0.016	0.116 ± 0.021	0.123 ± 0.011	0.119 ± 0.023	0.119 ± 0.010	0.144 ± 0.021	0.128 ± 0.008	0.118 ± 0.017	0.162 ± 0.037	0.142 ± 0.003	0.129 ± 0.019	0.167 ± 0.033	0.136 ± 0.027	0.143 ± 0.012	0.210 ± 0.004
Total Mortality (%)	R.T.	100.0	100.0	96.7	96.6	100.0	96.7	86.6	100.0	86.7	83.4	100.0	80.0	70.0	96.7	80.0	3.3
	L.D.	86.7	51.7	45.0	85.0	50.0	43.3	75.0	41.7	40.0	68.3	25.0	40.0	58.3	25.0	25.0	5.0
Emergence (%)	R.T.	0.0	0.0	3.3	3.4	0.0	3.3	13.4	0.0	13.3	16.6	0.0	20.0	30.0	3.3	20.0	96.7
	L.D.	13.3	48.3	55.0	15.0	50.0	56.7	25.0	58.3	60.0	31.7	75.0	60.0	41.7	75.0	75.0	95
Fecundity Mean no. of eggs/female ± S.E.	R.T.	-	-	-	-	-	-	87.5 ± 13.2	-	132.5 ± 10.0	170.7 ± 22.3	-	421.0 ± 17.2	213.5 ± 9.3	-	527.7 ± 9.3	482.8 ± 79.2
	L.D.	-	-	200.0 ± 52.5	-	177.4 ± 14.2	237.6 ± 32.2	187.5 ± 22.5	196.6 ± 27.0	243.4 ± 41.8	312.5 ± 12.5	211.0 ± 31.2	261.2 ± 23.5	350.2 ± 49.6	238.2 ± 57.4	303.3 ± 10.3	613.0 ± 93.8
Hatchability (%)	R.T.	-	-	-	-	-	-	42.9	-	89.6	84.0	-	86.5	87.8	-	87.8	98.6
	L.D.	-	-	37.5	-	39.5	40.0	57.1	40.7	52.2	70.4	47.9	54.8	80.0	51.7	62.0	95.0
Sterility (%)	R.T.	-	-	-	-	-	-	95.4	-	75.1	69.9	-	23.5	60.6	-	18.6	-
	L.D.	-	-	87.1	-	88.0	83.7	81.6	86.3	78.2	62.2	82.6	75.4	51.9	78.9	67.7	-

F: Flowers, L: Leaves, S: stems, R.T.: Residual Thin Film technique, L.D.: Leaf Dipping technique, S.E. Standard Error.

Table (5): Biological effects of Hexane extracts of *Calendula officinalis* on *Spodoptera littoralis* larvae by using Residual thin film and leaf dipping techniques

Biological Effects		Concentrations (mg/ml)															Control
		80			40			20			10			5			
		F	L	S	F	L	S	F	L	S	F	L	S	F	L	S	
Mortality after 24 hrs (%)	R.T.	60.0	54.0	70.0	40.0	33.3	56.7	16.6	30.0	50.0	11.7	15.0	26.7	6.7	3.3	25.0	0.0
	L.D.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Average larval mortality (%)	R.T.	81.7	96.7	83.3	80.0	93.3	73.3	66.7	73.3	63.3	63.3	70.0	63.3	56.7	66.7	46.7	6.7
	R.T.	80.0	21.7	53.3	55.0	16.7	50.0	53.3	15.0	30.0	40.0	10.0	25.0	25.0	10.0	20.0	3.3
Average Pupal Mortality (%)	R.T.	11.7	0.0	10.0	3.3	0.0	6.7	3.3	16.7	16.7	6.7	13.3	6.7	10.0	10.3	13.3	0.0
	L.D.	10.0	16.7	11.7	6.7	15.0	5.0	5.0	13.3	0.0	15.0	15.0	6.7	20.0	10.0	10.0	6.7
Mean Pupal weight (gm) ± S.E.	R.T. ♀	0.171 ± 0.032	0.252 ± 0.021	0.212 ± 0.019	0.179 ± 0.023	0.206 ± 0.011	0.219 ± 0.037	0.216 ± 0.043	0.214 ± 0.040	0.234 ± 0.008	0.224 ± 0.066	0.228 ± 0.027	0.225 ± 0.042	0.258 ± 0.012	0.234 ± 0.015	0.254 ± 0.004	0.230 ± 0.077
	L.D. ♂	0.150 ± 0.007	0.231 ± 0.011	0.208 ± 0.023	0.163 ± 0.070	0.187 ± 0.009	0.211 ± 0.031	0.187 ± 0.077	0.192 ± 0.053	0.223 ± 0.002	0.183 ± 0.038	0.200 ± 0.013	0.219 ± 0.036	0.234 ± 0.017	0.213 ± 0.008	0.237 ± 0.008	0.222 ± 0.051
	R.T. ♀	0.154 ± 0.012	0.133 ± 0.019	0.163 ± 0.023	0.169 ± 0.016	0.141 ± 0.013	0.177 ± 0.008	0.174 ± 0.021	0.147 ± 0.026	0.179 ± 0.030	0.177 ± 0.046	0.159 ± 0.022	0.186 ± 0.029	0.184 ± 0.033	0.172 ± 0.007	0.198 ± 0.022	0.227 ± 0.038
	L.D. ♂	0.132 ± 0.019	0.123 ± 0.021	0.155 ± 0.011	0.137 ± 0.018	0.112 ± 0.024	0.158 ± 0.025	0.142 ± 0.027	0.131 ± 0.012	0.167 ± 0.027	0.140 ± 0.034	0.141 ± 0.008	0.171 ± 0.012	0.166 ± 0.009	0.143 ± 0.012	0.180 ± 0.006	0.203 ± 0.022
Total Mortality (%)	R.T.	93.4	96.7	93.3	83.3	93.3	80.0	70.0	90.0	80.0	70.0	83.3	70.0	66.7	77.0	60.0	6.7
	L.D.	90.0	38.4	65.0	61.7	31.7	55.0	58.3	28.3	30.0	55.0	25.0	31.7	45.0	20.0	30.0	10.0
Emergence (%)	R.T.	6.6	3.3	6.7	16.7	6.7	20.0	30.0	10.0	20.0	30.0	16.7	30.0	33.3	23.0	40.0	93.3
	L.D.	10.0	61.6	35.0	38.3	68.3	45.0	41.7	71.7	70.0	45.0	75.0	68.3	55.0	80.0	70.0	90.0
Fecundity Mean no. of eggs/female ± S.E.	R.T.	-	-	233.0 ± 9.5	-	406.0 ± 15.5	295.8 ± 23.4	271.4 ± 35.2	411.0 ± 20.0	396.5 ± 42.3	292.3 ± 43.0	452.3 ± 30.6	450.0 ± 47.2	379.0 ± 25.2	467.0 ± 29.0	564.3 ± 12.6	539.5 ± 86.4
	L.D.	-	-	150.2 ± 11.4	141.6 ± 16.2	120.3 ± 8.6	158.0 ± 23.0	174.0 ± 25.5	201.8 ± 18.0	212.2 ± 15.4	213.2 ± 41.4	352.0 ± 23.0	220.6 ± 10.2	256.0 ± 21.2	377.8 ± 33.0	270.0 ± 19.0	601.2 ± 54.6
Hatchability (%)	R.T.	-	-	95.0	-	87.7	87.5	84.0	91.0	84.9	97.9	89.2	98.3	84.2	92.5	87.6	88.4
	L.D.	-	-	26.6	56.5	49.9	63.3	59.8	59.5	80.1	68.0	73.9	79.3	66.4	71.5	77.8	91.7
Sterility (%)	R.T.	-	-	53.6	-	25.3	45.8	52.2	21.6	29.4	40.0	15.4	7.3	33.1	9.4	3.7	-
	L.D.	-	-	92.8	85.5	89.1	81.9	81.1	78.2	69.2	73.7	52.8	68.3	69.2	51.0	61.9	-

F: Flowers, L: Leaves, S: stems, R.T.: Residual Thin Film technique, L.D.: Leaf Dipping technique, S.E. Standard Error.

Table (6): Change Percent in biological activities *Spodoptera littoralis* treated with four extracts of flower, leaf and stem of *Calendula officinalis*.

				Flower Extracts				Leaf Extracts				Stem Extracts			
				Chl.	Dich.	Eth	Hex	Chl.	Dich.	Eth	Hex	Chl.	Dich.	Eth	Hex
Pupal Weight	80	R.T.	♀	(-) 23.9*	(-) 21.9*	-	(-) 25.7**	-	-	-	(+) 9.6*	-	-	(+) 8.9*	(-) 7.8*
			♂	(-) 21.1*	(-) 21.0*	-	(-) 32.4**	-	-	-	(+) 4.1*	-	-	(+) 13.1*	(-) 6.3*
		L.D.	♀	(-) 40.1**	(-) 45.0**	(-) 47.8**	(-) 32.2**	(-) 44.8**	-	(-) 49.4**	(-) 41.4**	(-) 33.0**	-	(-) 45.8**	(-) 28.2*
			♂	(-) 41.7**	(-) 44.0**	(-) 44.3**	(-) 35.0**	(-) 44.2**	-	(-) 42.4**	(-) 39.4**	(-) 33.2**	-	(-) 44.8**	(-) 23.7*
	40	R.T.	♀	(-) 17.7*	(-) 9.3*	(+) 5.7*	(-) 22.2*	-	-	-	(-) 10.4*	-	-	(-) 15.8*	(-) 4.8*
			♂	(-) 18.7*	(-) 14.6*	(+) 43.0**	(-) 26.7*	(-) 25.8*	-	-	(-) 15.8*	-	-	(-) 18.1*	(-) 5.0*
		L.D.	♀	(-) 32.6**	(-) 37.5**	(-) 37.0**	(-) 25.6*	(-) 41.5**	-	(-) 46.6**	(-) 37.9**	(-) 26.4*	-	(-) 43.0**	(-) 22.0*
			♂	(-) 32.2**	(-) 45.7**	(-) 41.4**	(-) 32.5**	(-) 43.2**	-	(-) 43.3**	(-) 44.8**	(-) 29.7*	-	(-) 43.3**	(-) 22.2*
	20	R.T.	♀	(-) 3.5*	(-) 5.5*	(-) 4.1*	(-) 6.1*	-	-	-	(-) 7.0*	-	-	(-) 0.4*	(+) 1.7*
			♂	(-) 1.9*	(-) 8.2*	(-) 5.0*	(-) 15.8*	(-) 3.8*	-	(-) 32.1**	(-) 13.5*	(+) 49.8**	-	(+) 3.6*	(+) 0.5*
		L.D.	♀	(-) 25.9*	(-) 32.7**	(-) 33.7**	(-) 23.4*	(-) 36.3**	(-) 48.6**	(-) 45.0**	(-) 35.2**	(-) 24.1*	(-) 43.4**	(-) 41.4**	(-) 21.2*
			♂	(-) 30.2**	(-) 40.8**	(-) 31.4**	(-) 30.1**	(-) 39.7**	(-) 50.2**	(-) 39.1**	(-) 35.5**	(-) 26.1*	(-) 38.1**	(-) 44.0**	(-) 17.7*
	10	R.T.	♀	(+) 3.5*	(+) 15.6*	(-) 8.9*	(-) 2.6*	(+) 8.9*	(-) 10.5*	(+) 24.3*	(-) 0.9*	(+) 10.6*	(+) 15.2*	(+) 2.8*	(-) 2.2*
			♂	(+) 0.96*	(-) 3.2*	(-) 9.1*	(-) 17.6*	(+) 2.9*	(-) 9.6*	(+) 3.6*	(-) 10.0*	(+) 10.1*	(+) 6.9*	(+) 1.8*	(-) 1.4*
		L.D.	♀	(-) 20.0*	(-) 27.5*	(-) 18.5*	(-) 22.0*	(-) 28.3*	(-) 46.2**	(-) 26.5*	(-) 30.0**	(-) 4.3*	(-) 41.8**	(-) 39.4**	(-) 18.1*
			♂	(-) 31.7**	(-) 26.9*	(-) 22.9*	(-) 31.0**	(-) 31.2**	(-) 51.6**	(-) 32.4**	(-) 31.0**	(-) 4.0*	(-) 38.6**	(-) 38.6**	(-) 15.8*
	5	R.T.	♀	(+) 6.6*	(+) 13.9*	(-) 2.8*	(+) 12.2*	(+) 17.3*	(+) 3.0*	(+) 47.0**	(+) 1.7*	(+) 11.5*	(+) 16.9*	(+) 8.5*	(+) 10.4*
			♂	(+) 3.3*	(-) 6.9*	(-) 1.8*	(+) 5.4*	(+) 16.3*	(+) 2.7*	(+) 5.0*	(-) 4.1*	(+) 14.4*	(+) 10.0*	(+) 5.4*	(+) 6.8*
		L.D.	♀	(-) 12.7*	(-) 25.5*	(-) 29.3*	(-) 19.0*	(-) 21.2*	(-) 37.5**	(-) 26.1*	(-) 24.2*	(+) 4.3*	(-) 39.8**	(-) 27.7*	(-) 12.8*
			♂	(-) 23.1*	(-) 30.5**	(-) 20.5*	(-) 18.2*	(-) 30.2**	(-) 47.5**	(-) 35.2**	(-) 30.0**	(+) 4.0*	(-) 40.8**	(-) 31.9**	(-) 11.3*

R.T. = Residual thin film technique L.D.= Leaf dipping technique (-) Decrease (+) Increase
Chl = Chloroform Dich = Dichloromethane Eth = Ethanol Hex = Hexane
* = Not significant (P>0.05) ** = Significant (P<0.05) *** = Highly significant (P<0.01)

(Continue) table (6): Change Percent in biological activities *Spodoptera littoralis* treated with four extracts of flower, leaf and stem of *Calendula officinalis*.

			Flower Extracts				Leaf Extracts				Stem Extracts			
			Chl.	Dich.	Eth	Hex	Chl.	Dich.	Eth	Hex	Chl.	Dich.	Eth	Hex
Fecundity	80	R.T.	-	(-) 53.2**	-	-	-	-	-	-	-	-	-	(-) 56.8***
		L.D.	-	-	-	-	(-)74.7***	-	-	-	(-) 57.9**	-	(-) 67.4***	(-) 75.0***
	40	R.T.	(-) 63.2***	(-) 50.6**	-	-	-	-	-	(-) 24.8*	-	-	-	(-) 45.2***
		L.D.	-	-	-	(-) 76.5***	(-) 62.0***	-	(-) 71.1***	(-) 80.0***	(-) 55.9**	-	(-) 61.2***	(-) 73.7***
	20	R.T.	(-) 55.8**	(-) 48.3**	(-) 81.9***	(-) 50.0**	-	-	-	(-) 23.8*	-	-	(-) 72.6***	(-) 26.5*
		L.D.	-	(-) 78.7***	(-) 69.4***	(-) 71.7***	(-) 54.9**	-	(-) 67.9***	(-) 66.4***	(-) 52.5**	-	(-) 60.3***	(-) 64.7***
	10	R.T.	(-) 46.8**	(-) 49.5**	(-) 64.6***	(-) 45.8**	-	(-) 52.0**	-	(-) 16.2*	(-) 31.4**	-	(-) 12.8*	(-) 16.6*
		L.D.	(-) 66.3***	(-) 68.3***	(-) 49.0**	(-) 64.5***	(-) 39.3**	(-) 73.9***	(-) 65.6***	(-) 41.5**	(-) 40.9**	-	(-) 57.4**	(-) 63.3***
	5	R.T.	(-) 34.4**	(-) 46.8**	(-) 55.8**	(-) 29.8*	(-) 27.7*	(-) 20.0*	-	(-) 13.4*	(-) 0.4*	(-) 6.8*	(+) 9.3*	(+) 4.6*
		L.D.	(-) 60.7***	(-) 64.5***	(-) 42.9**	(-) 57.4**	(-) 15.3*	(-) 69.5***	(-) 61.1***	(-) 37.2**	(-) 35.9**	(-) 71.2***	(-) 50.5**	(-) 55.1**
Hatchability	80	R.T.	-	(-) 36.2**	-	-	-	-	-	-	-	-	-	(+) 7.5*
		L.D.	-	-	-	-	(-) 22.7*	-	-	-	(-) 25.8*	-	(-) 60.5***	(-) 71.0***
	40	R.T.	(-) 41.7**	(-) 16.2*	-	-	-	-	-	(-) 0.8*	-	-	-	(-) 1.0*
		L.D.	-	-	-	(-) 38.4**	(-) 15.1*	-	(-) 58.4**	(-) 45.6**	(-) 20.3*	-	(-) 57.9**	(-) 31.0**
	20	R.T.	(-) 19.3*	(-) 11.5*	(-) 56.5**	(-) 5.0*	-	-	-	(+) 2.9*	-	-	(-) 9.1*	(-) 4.0*
		L.D.	-	(-) 37.9**	(-) 39.9**	(-) 34.8**	(-) 8.6*	-	(-) 57.2**	(-) 35.1**	(-) 17.7*	-	(-) 45.1**	(-) 12.7*
	10	R.T.	(-) 6.4*	(+) 6.1*	(-) 14.8*	(+) 10.8*	-	(-) 10.9*	-	(-) 0.9*	(-) 28.3*	-	(-) 12.3*	(+) 11.2*
		L.D.	(-) 27.6*	(-) 36.0**	(-) 25.9*	(-) 25.9*	(-) 6.6*	(-) 46.0**	(-) 47.1**	(-) 19.4*	(-) 7.4*	-	(-) 42.3**	(-) 13.5*
	5	R.T.	(-) 4.8*	(+) 8.0*	(-) 11.0*	(-) 4.8*	(-) 1.3*	(-) 4.8*	-	(+) 4.6*	(-) 15.0*	(-) 12.6*	(-) 11.0*	(-) 0.9*
		L.D.	(-) 13.1*	(-) 25.9*	(-) 15.8*	(-) 27.6*	(-) 7.7*	(-) 42.8**	(-) 45.6**	(-) 22.0*	(-) 9.7*	(-) 47.9**	(-) 34.7**	(-) 15.2*

R.T. = Residual thin film technique L.D.= Leaf dipping technique (-) Decrease (+) Increase

Chl = Chloroform Dich = Dichloromethane Eth = Ethanol Hex = Hexane

* = Not significant (P>0.05) ** = Significant (P<0.05) *** = Highly significant (P<0.01)

The formulations of dichloromethane extract of stems, leaves and flowers proved a highly toxic effect on *S. littoralis* larvae than those of chloroform extract.

These results may have practical implication in providing long-term control of cotton leafworm in the field. In addition, natural pesticides are safer than synthetic to the beneficial organisms and environment (Schmutterer 1995).

Table (7): Insecticidal effect of formulated Dichloromethane and Chloroform extracts of flowers, leaves and stems of *Calendula officinalis* on *2nd* and *4th* instar larvae of *Spodoptera littoralis* by using thin film technique:

Larval instar	Formulated Extract		LC_{50} (mg/ml) [Fiducial Limits]	LC_{90} (mg/ml) [Fiducial Limits]	Slope	Toxicity Index
2nd	Dichloro.	Flowers	13711.25 [11397.4 – 16515.9]	1.17×10^5 [82184 – 1.88×10^5	1.38	63.4
		Leaves	11600.7 [9623.2 – 13915.6]	96505.8 [64160.9 – 1.5×10^5]	1.39	75
		Stems	8695.8 [7137.5 – 10429.5]	70762 [51962.7 – 1.07×10^5]	1.41	100
	Chloro.	Flowers	15368.2 [13760.7 – 18616.6]	1.35×10^5 [93507.99 – 2.23×10^5]	1.36	56.6
		Leaves	23410.9 [19357.3 – 28977.5]	2.04×10^5 [1.35×10^5 – 3.58×10^5]	1.4	37.1
		Stems	35191.6 [28210.6 – 46137.9]	3.54×10^5 [2.13×10^5 – 2.27×10^5]	1.28	24.7
4th	Dichloro.	Flowers	20305.6 [17049.3 – 24530.05]	1.5×10^5 [1.05×10^5 – 2.4×10^5]	1.48	62.4
		Leaves	18042.2 [15090.8 – 21808.9]	1.43×10^5 [99893.9 – 2.32×10^5]	1.43	70.2
		Stems	12664.3 [10387.9 – 15408.1]	1.26×10^5 [86102.7 – 2.13×10^5]	1.28	100
	Chloro.	Flowers	21743.3 [18512.5 – 26514.9]	1.7×10^5 [1.46×10^5 – 3.6×10^5]	1.43	58.3
		Leaves	31021.2 [25828.3 – 38365.2]	2.14×10^5 [1.46×10^5 – 3.6×10^5]	1.53	40.8
		Stems	45421.1 [36731.96 – 59180.1]	3.33×10^5 [2.11×10^5 – 6.3×10^5]	1.48	27.9

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التأثير الإبادي والبيولوجي لمستخلصات نبات الكالنديولا اوفيشانااليس على يرقات دودة ورق القطن

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تتعلق هذه الدراسة بتوضيح التأثيرات الإبادية والمتأخرة لمستخلصات أزهار وأوراق وسيقان نبات الكالنديولا اوفيشانااليس على العمر اليرقي الرابع لدودة ورق القطن. حيث كان لمستخلصات كل جزء في المذيبات الأربعة (كلوروفورم – داي كلوروميثان – ايثانول – هكسان) نشاط إبادي مرتفع ضد دودة ورق القطن باستخدام طريقة الغشاء السطحي في حين أن هذه المستخلصات لم تعطي أي تأثير إبادي سريع باستخدام طريقة غمر الورق (التغذية). حيث أن معاملة الغشاء السطحي للمستخلص تحدث نسبة موت عالية في اليرقات والعدد الكلي وأيضا تقلل نسبة خروج الحشرات الكاملة وتحدث نسبة عقم قليلة بين تلك الفراشات إلا أن طريقة المعاملة بالتغذية تقلل وزن العذارى وعدد البيض التي تضعه الفراشات الإناث وأيضا تقلل نسبة فقس البيض الموضوع وهذا ينعكس على إحداث عقم بنسب مرتفعة. وكان لمستخلص الداي كلوروميثان التأثير الأكثر فاعلية على اليرقات المعاملة.

وعند رش مستحضرات مستخلصات الداي كلوروميثان والكلوروفورم على نبات القطن لم يكن لها تأثير يذكر في حين أن هذه المستحضرات أبدت كفاءة إبادية عالية على يرقات دودة ورق القطن وكانت يرقات العمر الثاني أكثر حساسية من يرقات العمر الرابع.