
RESULTS
AND
DISCUSSION

Results and Discussion

I) Laboratory Experiments:

1- Efficacy of volatile oils "Apilife VAR" for controlling wax moths larvae *Achoria grisella*:

1.1- Effect of feeding on diets treated with mixture of volatile oils "Apilife VAR" on larval mortality of the lesser wax moths *Achoria grisella*:

The efficacy of volatile oils expressed as percentage of wax moths larval mortality was investigated during this work. Results presented in Table (1) and Fig. (1) indicated that, the average mortality of the wax moths-larvae was significantly affected by volatile oils treatment. The average % of mortality was obviously increased with increasing doses of "Apilife VAR" as well as by increasing time after treatment.

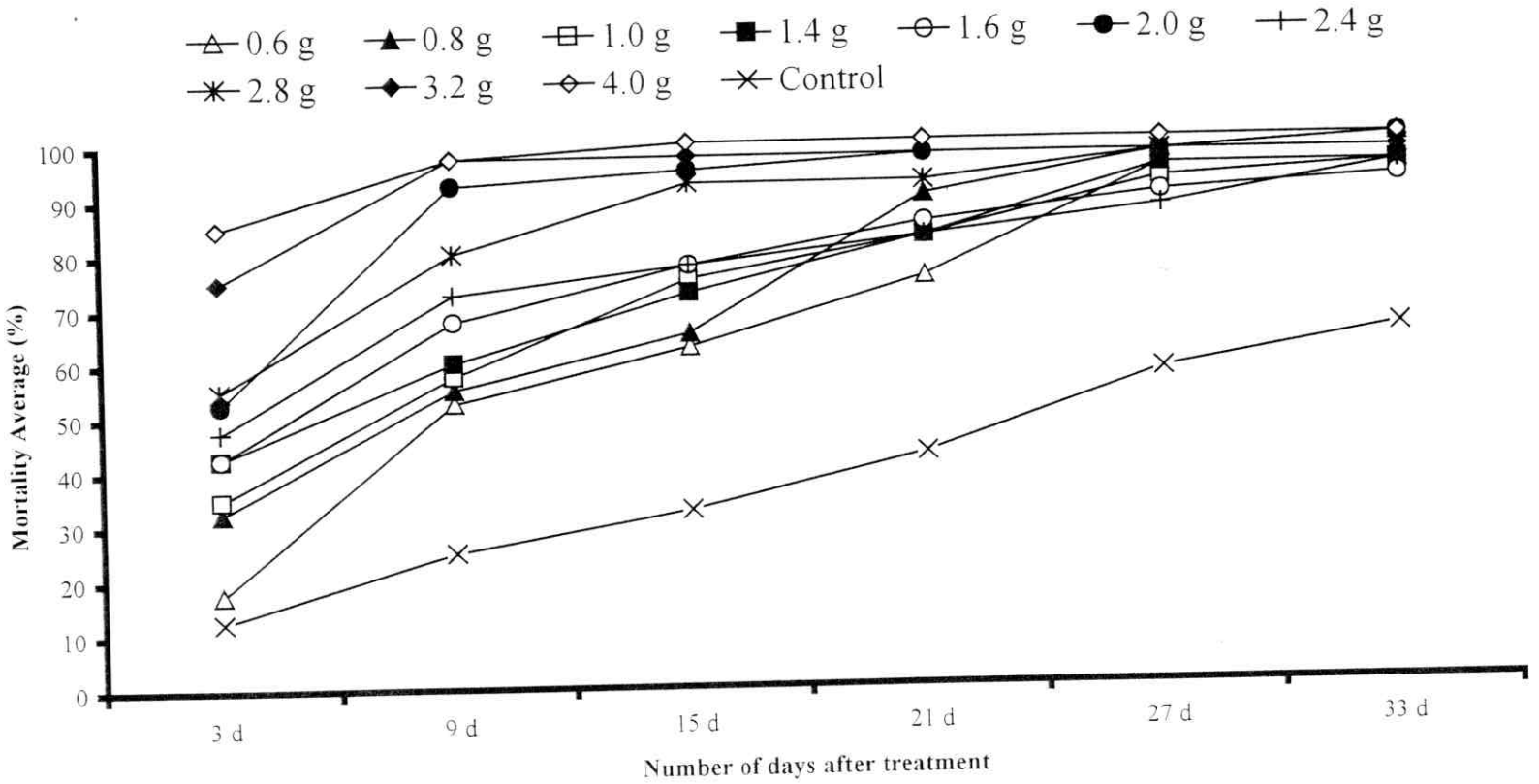
In control treatment (untreated-larvae) the recorded average mortality was 39.17%. However, the mixture of plant volatile oils "Apilife VAR" increased the average mortality to 71.07%, 77.14%, 76,79%, 78.21%, 79.29%, 90.71%, 80.36%, 87.86%, 94.64%, and 97.5%, by increasing the doses i.e. 0.6, 0.8, 1.0, 1.4, 1.6, 2.0. 2.4, 2.8, 3.2, and 4.0 g per 100 g artificial diet (medium), respectively. The average mortality was increased also by time elapsed after treatment. After 3, 9, 15, 21, 27, 33, and 39 days after treatment the recorded averages of mortality were 48.5%, 73.25%, 81.5%, 88.5%, 95.0%, 96.75%, and 100%, respectively. After 3 and 33 days the morality averages recorded in untreated-larvae (control) were 12.5% and 65.0%, respectively. The wax-moths larval mortality averages

reached to about 97.5% and 100% by 15 days after applying the volatile oils "Apilife Var" at rate of 3.2 and 4.0g/100 g diet medium, respectively.

Table (1): Effect of different doses (g/100 g diet) of volatile oils (Apilife VAR) added to growth medium on average % mortality of wax-moths larvae.

Doses "in g"	Average % mortality after "days"						Average (%)
	3 d	9 d	15 d	21 d	27 d	33 d	
0.6 g	17.5	52.5	62.5	75	95	95	71.07
0.8 g	32.5	55	65	90	97.5	100	77.14
1.0 g	35	57.5	75	82.5	92.5	95	76.79
1.4 g	42.5	60	72.5	82.5	95	95	78.21
1.6 g	42.5	67.5	77.5	85	90	92.5	79.29
2.0 g	52.5	92.5	95	97.5	97.5	100	90.71
2.4 g	47.5	72.5	77.5	82.5	87.5	95	80.36
2.8 g	55	80	92.5	92.5	97.5	97.5	87.86
3.2 g	75	97.5	97.5	97.5	97.5	97.5	94.64
4.0 g	85	97.5	100	-	-	-	94.16
Average	48.5	73.25	81.5	87.2	94.4	96.4	80.20
Control	12.5	25	32.5	42.5	57.5	65.0	39.17
Significance	***	***	***	**	***	***	
L.S.D.	25.46	28.00	26.12	24.09	15.51	15.14	

Fig.1: Effect of different concentrations of volatile oils (Apilife VAR) added to growth medium on the mortality % of wax-moths larvae



1.2- Effect of feeding on diets treated with volatile oils "Apilife VAR" on the life cycle duration of the lesser wax moths *Achoria grisella*:

The results presented in Table (2) and illustrated in Fig. (2) indicated clearly that the life cycle of *A. grisella* was adversely affected by rearing larvae on artificial diet treated with the volatile oils "Apilife VAR" mixture.

It could noticed also that the percentages of alive pupae, emerged adults as well as laying eggs per female adult were sharply decreased by increasing dose of the Apilife VAR in artificial diet compared with control treatment. No adults were emerged when volatile oils mixture was used at rate of 2.8 g per 100 g of the feeding artificial diet. Meanwhile, the few adult females emerged on diet treated with volatile oils mixture at rate of 2.0 or 2.4 g/100 gram diet failed to laying eggs.

The increased mortality of the wax moths- larvae in control treatment might be attributed to the cannibalism phenomenon. On the other hand, increasing in larval mortality as well as decreasing in percentages of pupae and adults emerged after treatment with the volatile oils "Apilife VAR" could be due to the fact that this mixture of volatile oils may be contain certain toxic compounds influences as antifeedant, toxic activity or lead to vital disturbance which is often lethal to insects. In general, the obtained results are in harmony and could be supported by the findings of folowing investigators.

Table (2): Effect of volatile oils "Apilife VAR" on the life cycle of the lesser wax moths *Achoria grisella* after 35 days from treatment.

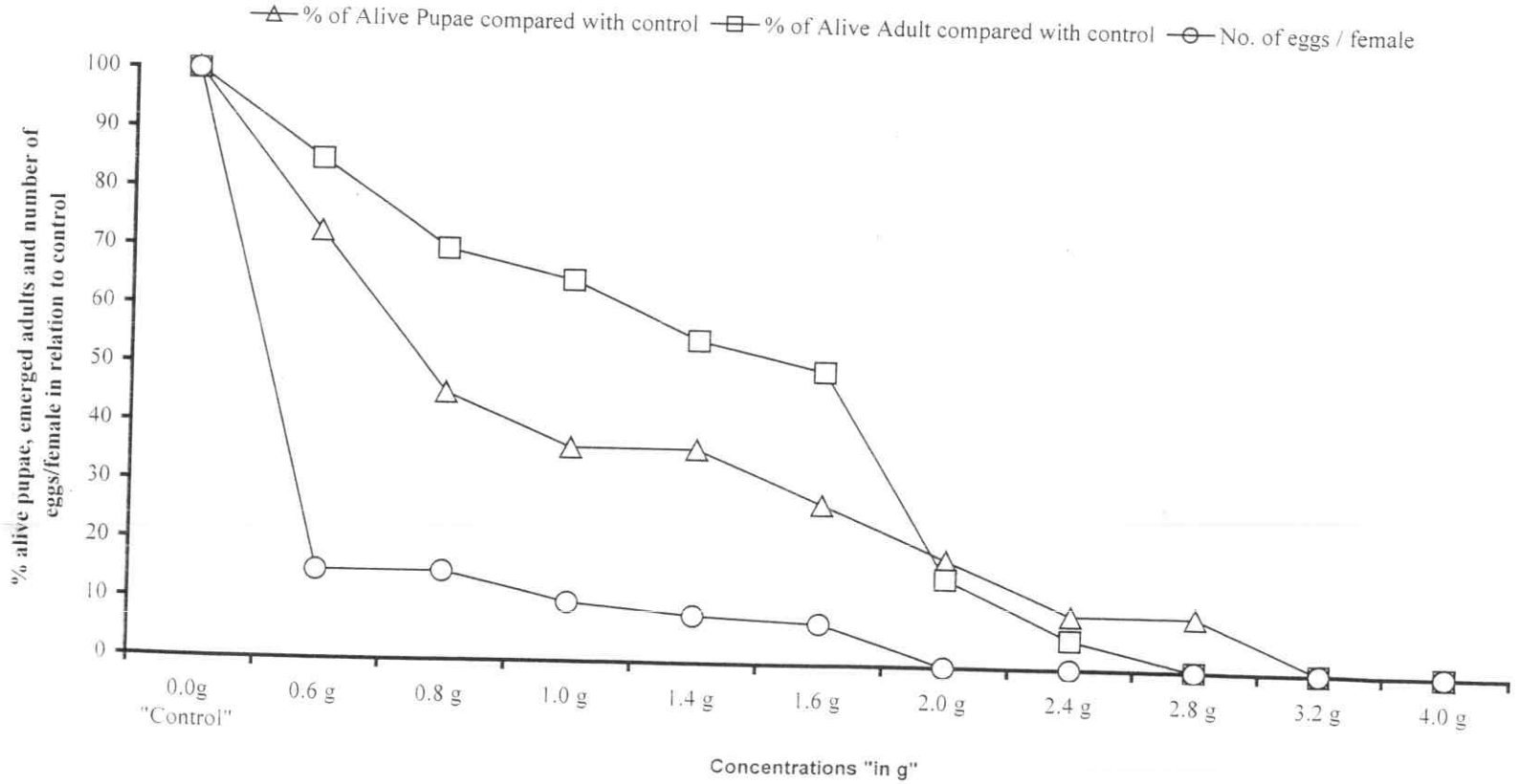
Doses "in g"	Alive Pupae		Alive adult		Laying Eggs	
	Number	% of control	Number	% of control	No. of eggs / female	% of control
0.6 g	8	72.7 *	17	85.0	58	23.2
0.8 g	5	45.5	14	70.0	39	15.6
1.0 g	4	36.4	13	65.0	25	10.0
1.4 g	4	36.4	11	55.0	18	7.2
1.6 g	3	27.3	10	50.0	10	4.0
2.0 g	2	18.2	3	15.0	0	0.0
2.4 g	1	9.1	1	5.0	0	0.0
2.8 g	1	9.1	0	0.0	0	0.0
3.2 g	0	0.0	0	0.0	0	0.0
4.0 g	0	0.0	0	0.0	0	0.0
Control	11	100%	20	100%	250	100.0%

$$* \% \text{ of Control} = \frac{\text{Number in particular treatment}}{\text{Number in control}} \times 100$$

Ellis and Simonds (1987) found that all adult *Acarapis woodi* infesting honeybees were killed when these bees were exposed to menthol fumes for 36 h (24 h at 35°C, 12 h at 38°); over 95% of nymphs were also killed. Most of the eggs present did not develop. Shorter exposure times at 35° were less effective.

Srivastava and Krishna (1990) found that eggs of *Dysdercus koenigii* held in eucalyptus oil odour regimes for 48 h at 18-20 or 23-26°C completely failed to hatch. After treatment

Fig. (2): Effect of volatile oils "Apilife VAR" on the life cycle of the lesser wax moths *Achoria grisella* after 35 days from treatment



for 24 h at the higher temperature, some eggs hatched but <50% of the resulting nymphs became adults (after a prolonged period of postembryonic development).

Pathak and Krishna, (1991) found that postembryonic development and adult emergence of *Corcyra cephalonica* were adversely affected when reared for the first 2 weeks of larval life or for a similar duration from the 16th day of their lives in an environment of eucalyptus oil volatiles. A marked decline in the reproductive potential, in terms of egg output and egg hatchability, was observed when larvae were reared for the first 15 days in the presence of eucalyptus oil volatiles or when the parents were exposed for 5 min to such an environment during adult life.

Srivastava and Krishna (1992) found that exposure of 1st-instar nymphs of *Dysdercus koenigii* between 24 and 48 h old to Eucalyptus oil odour for 2 h resulted in greater mortality in the 3rd instar. Adults that did emerge were reduced in size. More males emerged than females. Females that had undergone the treatment laid fewer eggs, of reduced viability, as compared with untreated females. If the insects were exposed to the Eucalyptus oil during embryogenesis, this reduction in breeding potential did not occur.

Imdorf *et al.*,(1995) mentioned that caged groups of 100 honey bees with 20 *V. jacobsoni* were kept for 72 h in an air stream containing one of the volatile test substances; dead and live bees and mites were then counted. Nearly 100% of mites (*V. jacobsoni*) were killed by a dose of 5-15 µg thymol / liter of air. Similar results were obtained with 50-150 µg camphor / liter or

20-60 µg menthol / liter. Eucalyptol was not effective in killing mites until applied at a dose of 240 µg / liter, and then it also killed 25% of bees.

Nelson(1995) evaluated the menthol for the control of honey bee tracheal mites (*Acarapis woodi*). All menthol treatments reduced mite prevalence (% of bees infested with mites) to < 1% by late summer, compared with control colonies (26%, from an initial 12%). Some treatments showed decreased brood rearing (though the difference was not significant) and significantly lower honey production. Brood mortality was seen in most treatments for a few days after application.

Srivastava *et al.*, (1995) stated that exposure of differently aged nymphs of *Dysdercus koenigii* to eucalyptus oil vapours for a brief period once during rearing variously affected their mortality in the course of development.

2- Efficacy of *Datura stramonium* and *Melia azaderachta*-powder for controlling wax moths larvae:

2.1- Effect of plant powder on the mortality of wax moths larvae *Achoria grisella*:

In this experiment, the plant powder obtained from *Datura stramonium* and *Melia azedrachta* plants were used at different concentration for testing their effect on the mortality percentage of wax-moths larvae. The obtained results are illustrated in Table (3) and Fig. (3).

The obtained results proved that powder of *Datura stramonium* plant was more effective in general than powder of *Melia azedrachta*. The averages of wax-moths mortality caused by these two plant powders were 68.36% and 59.79%,

respectively. Increasing concentration of both plant powders resulted in significant increase of average mortality. The mortality average of wax-moths larvae was increased from 36.86% (in control treatment) to 59.14%, 67.14%, 70.57%, and 76.57% when powder of *Datura* plant was used at 1g, 4g, 8g and 20g, respectively. However, the average of mortality caused by using powder of *Melia azedrachta* plant at the same four concentrations were 54.0%, 59.43%, 59.71%, and 66.0%, respectively.

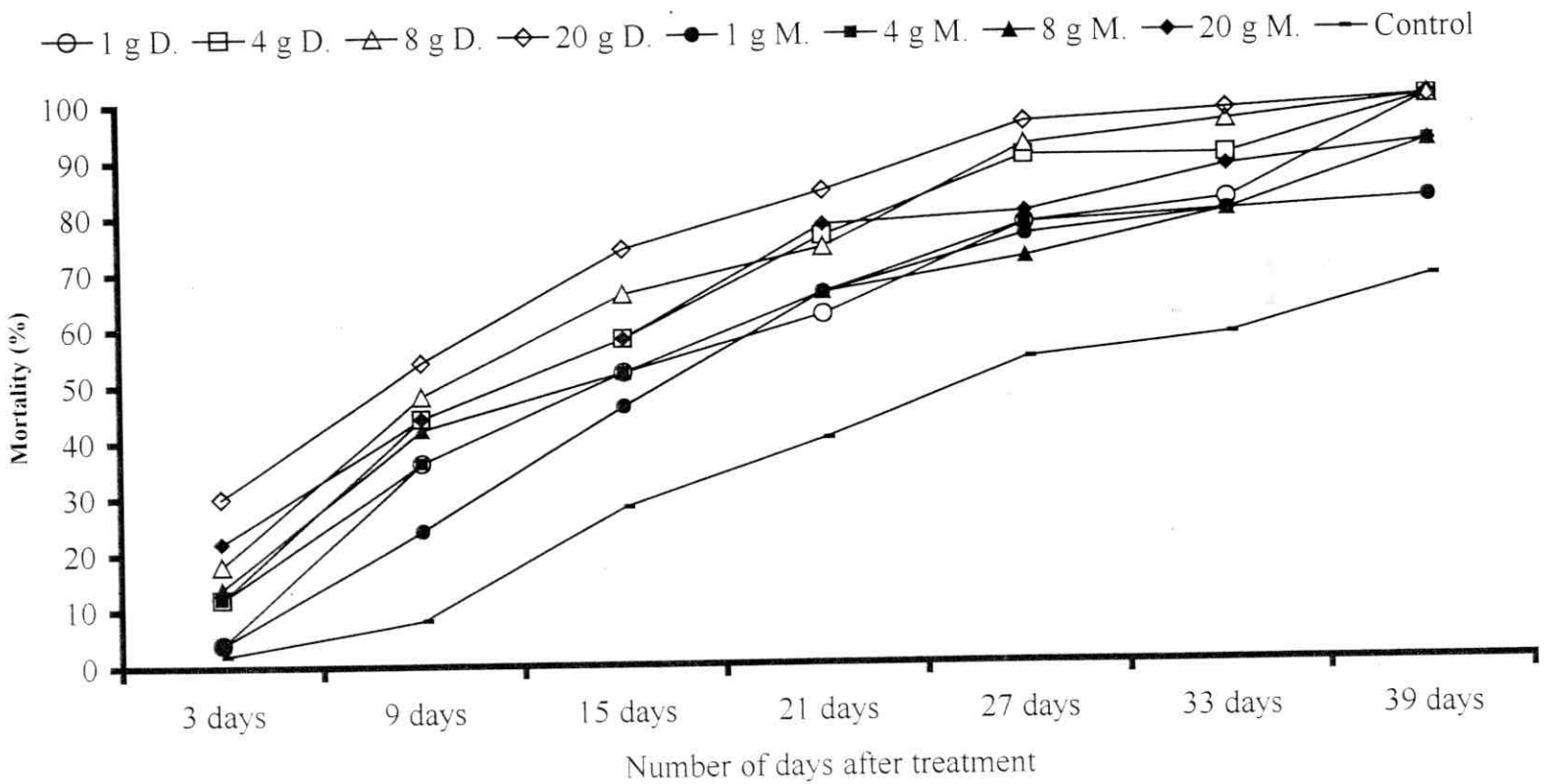
The tested plant powders seemed to have prolonged toxic effect as the average of wax-moths mortality was obviously increased by increasing time after plant powder-treatment. After 3, 9, 15, 21, 27, 33, and 39 days from the treatment of Datura plant powder application the mortality averages were 16.0%, 45.5%, 62.5%, 74.0% 89.0%, 91.5%, and 100.0%, respectively.

In the case of using the powder of *Melia*-plant the mortality averages recorded at the above mentioned periods were 13.0%, 36.5%, 52.0%, 69.0%, 76.5%, 82.0%, and 89.5%, respectively. In general, the obtained results indicated that, the *Datura stramonium* plant powder was more effective in controlling wax-moths larvae than powder of *Melia azedrachta*.

Table (3): Effect of different concentrations of *Datura stramonium*- and *Melia azedrachia*-plant powders on the larval mortality % of wax-moths after different periods.

Source of powder	Conc. "ing"	Mortality average after "days" (in %)							Mean
		3	9	15	21	27	33	39	
Datura stramonium	1 g	4	36	52	62	78	82	100	59.14
	4 g	12	44	58	76	90	90	100	67.14
	8 g	18	48	66	74	92	96	100	70.57
	20 g	30	54	74	84	96	98	100	76.57
Mean		16.0	45.5	62.5	74.0	89.0	91.5	100	68.36
Melia azedrachia	1 g	4	24	46	66	76	80	82	54.00
	4 g	12	36	52	66	78	80	92	59.43
	8 g	14	42	52	66	72	80	92	59.71
	20 g	22	44	58	78	80	88	92	66.00
Mean		13	36.5	52	69	76.5	82	89.5	59.79
Control		2	8	28	40	54	58	68	36.86
Significanc		N.S.	**	***	**	***	***	***	
L.S.D. at 0.01		23.91	28.89	23.00	25.12	18.75	16.81	10.73	

Fig.3: Effect of Datura stramonium "D" and Melia azedrachia "M" powder on mortality % of wax-moths larvae.



2.2- Effect of plant powder on the life cycle duration of the lesser wax moths *Achoria grisella*:

It could notice from data Tabulated in Table (4) and illustrated in Fig. (4) that the powders of both plants *D. stramonium* and *M. azadarachta* exerted deleterious effect on life cycle of the lesser wax moths *Achoria grisella* when added to their diets. Such adverse effect was increased by ncreasing concentration of a given plant powder. The highest percentages (100%) of alive pupae, emerged adults and eggs per female were noticed in control treatment.

Using powder of *Datura* plant at rates of 1, 4, 8, and 20 g / 100 g diet decreased percentages of alive pupae to 41.7, 25.0, 16.7 and 8.3%, respectively meanwhile, powder of *Melia* plant decreased percentages of alive pupae to 91.7, 83.3, 33.3 and 16.7%, for the same concentrations respectively.

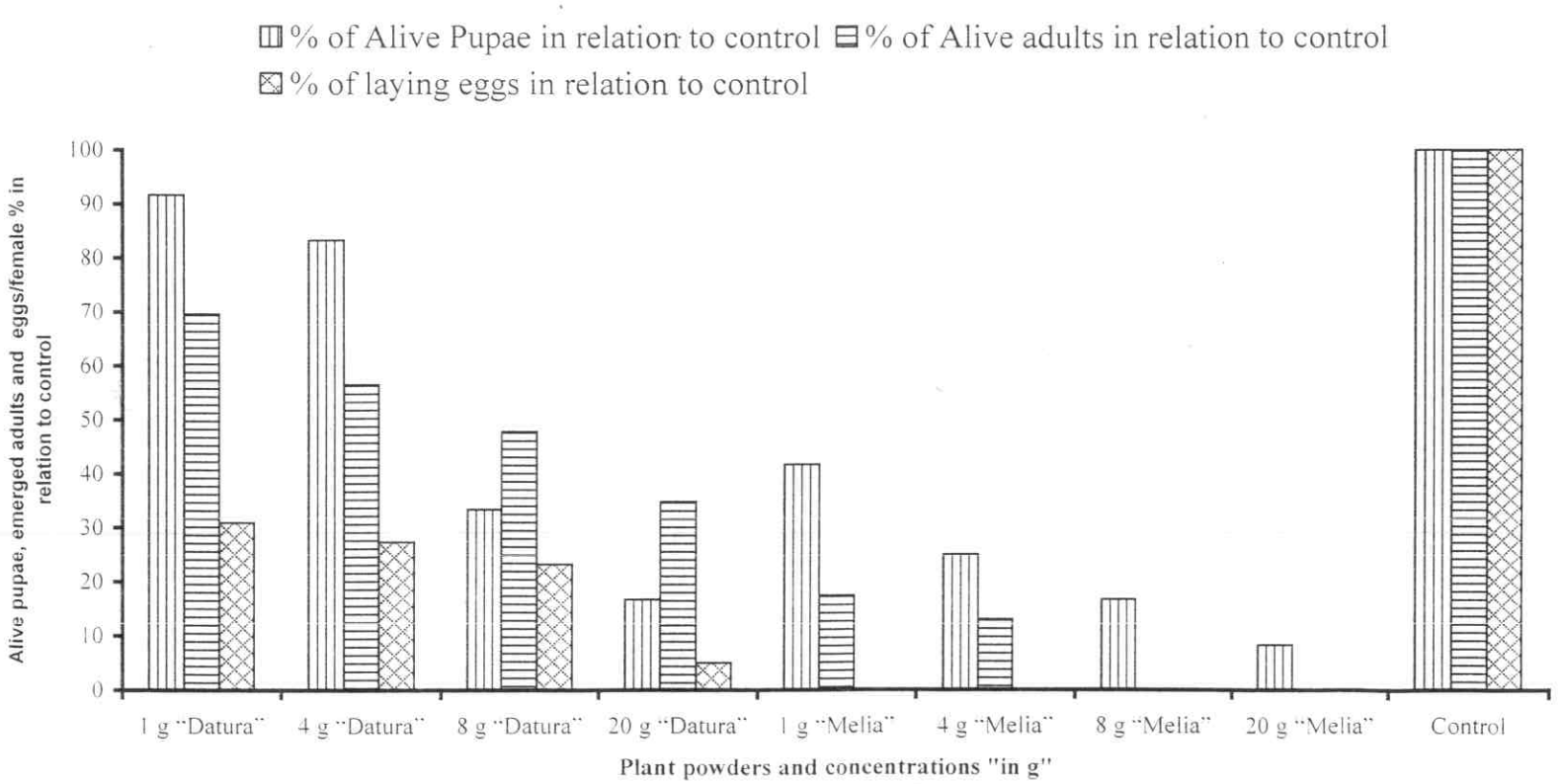
The above trend was noticed also in case of emerged adults. In this regard, powder of *Datura* plant was more effective in decreasing % of emerged adult than powder of *melia* plant. No adults were emerged by increasing rate of application of *datura* plant powder above 4.0 g / 100 g diet. On the other hand, applying powder of *Melia* plant at rates of 1, 4, 8 and 20 g / 100 g diet decreased the percentages of emerged adults to 69.6, 56.5, 47.8, and 34.8 % of those emerged in check one.

Table (4): Effect of *Datura stramonium* and *Melia azadarachta* powder on the life cycle of the lesser wax moths *Achoria grisella* during 35 days.

Plant powder and concentration "in g"		Alive Pupae		Alive Adult		Laying Eggs	
		Number	% of control	Number	% of control	No. of Eggs / female	% of control
Datura stramonium	1 g	5	41.7 *	4	17.4	0	0.0
	4 g	3	25.0	3	13.0	0	0.0
	8 g	2	16.7	0	0.0	0	0.0
	20 g	1	8.3	0	0.0	0	0.0
Melia azadirachta	1 g	11	91.7	16	69.6	68	30.9
	4 g	10	83.3	13	56.5	60	27.3
	8 g	4	33.3	11	47.8	51	23.2
	20 g	2	16.7	8	34.8	11	5.0
Control		12	100.0	23	100.0	220	100.0

$$* \% \text{ of Control} = \frac{\text{Number in particular treatment}}{\text{Number in control}} \times 100$$

Fig (4): Effect of *Datura stramonium* and *Melia azadarachta* powder on the life cycle of the lesser wax moths Achoria grisella during 35 days.



Increasing rate of application of powder of Melia plant gradually decreased the oviposition. Using powder of *Melia* plant at rates of 1, 4, 8 and 20 g / 100 g diet reduced number of eggs/female to 68.0, 60.0, 51.0, and 11.0%, respectively. However, powder of *Datura* plant resulted in complete suppress of oviposition even at the lowest tested rate (1g / 100 g diet).

2.3- Effect of different concentrations of some plant extracts on mortality of the wax moths larvae (*Galleria mellonella*).

This experiment was conducted to evaluate efficacy of some plant extracts against wax moths larvae (*Galleria mellonella*). The wax moths larvae were allowed to fed on pieces of beeswax treated with extracts of different three plants. The average of larval mortality was determined 3 days after treatment. The obtained results were tabulated in Table (5) and Fig. (5).

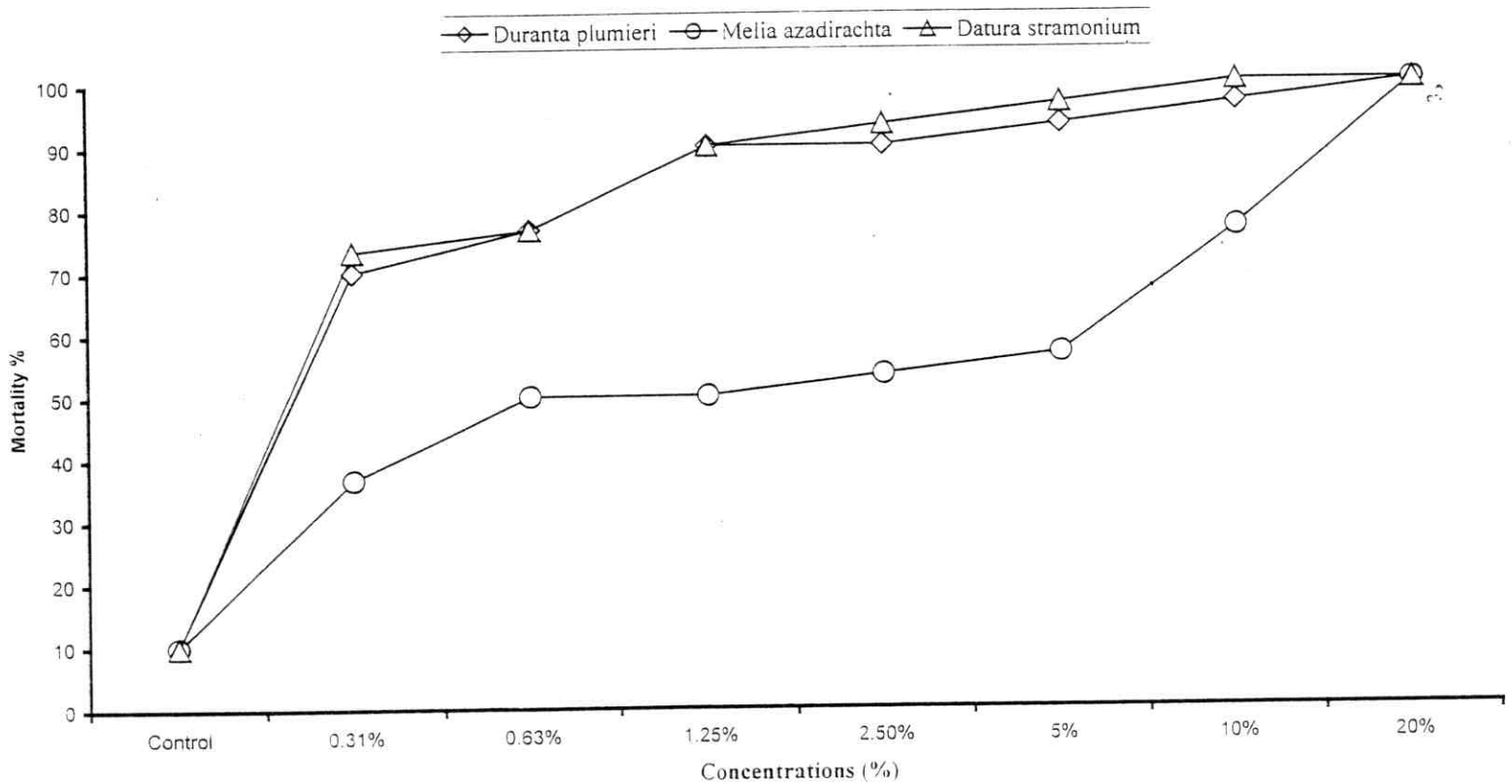
Table (5): Effect of different concentrations* of some plant extracts on % mortality of the wax moths larvae (*Galleria mellonella*), 3 days after treatment.

Concentrations of plant extracts	% of mortality, 3 days after treating with extract of		
	Duranta plumieri	*Melia azadirachta*	*Datura stramonium*
0.3125 %	70.0	36.7	73.3
0.625 %	76.7	50.0	76.7
1.25 %	90.0	50.0	90.0
2.5 %	90.0	53.3	93.3
5 %	93.3	56.7	96.7
10 %	96.7	76.7	100.0
20 %	100.0	100.0	100.0
Control	10.0	10.0	10.0

* Acetone extract

The obtained results illustrated that, *Datura stramonium* plant extract was the superior for killing wax moths larvae (*Galleria mellonella*) followed by *Duranta plumieri* and *Melia azadirachta* plants, respectively. After 3 days from application of *D. stramonium*, *D. plumieri* and *M. azadirachta* plant extracts at rate of 0.3125% the larval mortality was increased from 10% (in control treatment) to 73.33%, 70.0% and 36.6%, respectively. At rate of application 10 % percentage mortality was increased to 100.0%, 93.33% and 76.7% for the three plant extracts, respectively. At the highest tested rate (20%) average mortality reashed to 100% for the three plant extracts.

Fig. 5: Effect of different concentrations* of some plant extracts on mortality % of the wax moths larvae (*Galleria mellonella*), 3 days after treatment.



The effect of the plant powders or extracts could be due to the fact that these powders contain toxic compounds (terpenoids or similar compounds) which possess antifeedant, toxic activity or lead to moulting disturbance which is often lethal to insects **(Champagen et al., 1989)**.

The obtained results are in parallel with those reported by other researchers. **Xie et al.,(1995)** studied the repellency and toxicity effects of azadirachtin and 3 neem extracts to 3 stored-product insects in the laboratory and recorded that, significant negative correlations were found between insect settling response and extract concn. However, the neem extracts are slightly more active than pure azadirachtin when applied at equivalent azadirachtin concn, indicating that azadirachtin is not the only active compound in neem. Also, **Chakraborti and Chatterjee(1996)** studied the effects of 4 neem products on eggs and larvae of *Corcyra cephalonica* and stated that toxicity, mortality, concentration and growth inhibition established a dose-response relationship while feeding inhibition and growth disturbance were found to be independent events. Azadirachtin interfered with ecdysteroid [moulting hormone] titres and produced morphogenetic effects. It also adversely affected the reproductive potential of the initial generation.

In general, the obtained data are in harmony with the finding of several investigators on other lepidopterous insects **(El-Sayed 1985a; Khadr *et al.*, 1986; Abdul-Kadir and Connolly, 1991; Rashad *et al.*, 1991; Osman, (1993); Osman and Bradley 1993; Hashem *et al.*, 1994; Liu. 1995; Gerard and**

Ruf, 1995; Xie *et al.*, 1995; Su-TianYun *et al.*, 1999 and Valladares *et al.*, 1999).

3-Effect of carbon dioxide and phosphine on controlling of wax-moths larvae:

3.1- Efficacy of different concentrations of carbon dioxide for controlling the larvae of wax-moths (*Galleria mellonella*):

In this experiment, an attempt for controlling wax-moths larvae was carried out by exposure to carbon dioxide at different concentrations i.e. 0% (control), 25%, 50%, 75%, and 100% at two temperatures (20 and 30 °C) was investigated. The obtained results expressed in term of mortality of wax-moths larvae were summarized in Table (6) and Fig. (6).

Table (6): Effect of applying carbon dioxide at different concentrations on % mortality of the first larvae of wax-moths (*Galleria mellonella*):

Conc. %	No. of larvae used	% mortality after exposure for						Mean
		3 days at		6 days at		12 days at		
		20 °C	30 °C	20 °C	30 °C	20 °C	30 °C	
0 % *	30	10.0	6.7	10.0	6.7	16.7	23.3	12.23
25 %	30	16.7	23.3	26.7	23.3	30.0	36.7	26.12
50 %	30	20.0	53.3	36.7	56.7	45.0	86.7	49.73
75 %	30	23.3	60.0	43.3	70.0	53.3	90.0	56.65
100 %	30	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Mean		34.0	48.7	43.3	51.3	49.0	67.3	48.84

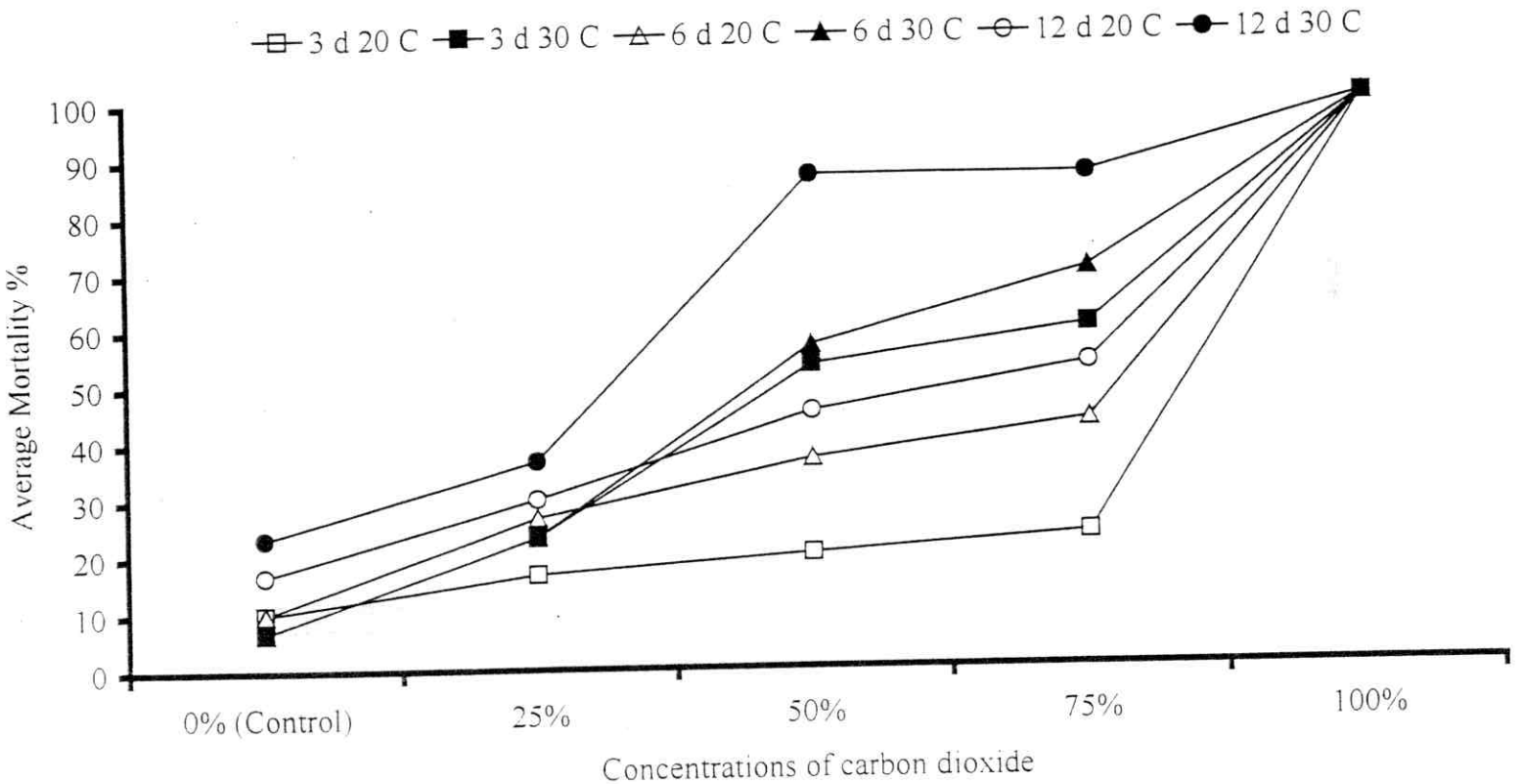
- Control.

L.S.D. at 0.01 for:

CO2 conc.	24.55	21.42	12.72
Temperature	N.S.	N.S.	N.S.
Interaction	N.S.	N.S.	N.S.

The obtained results showed wide variation in susceptibility and response of larvae to carbon dioxide at the two temperature. The average mortality of wax-moths larvae was increased significantly by increasing concentrations of carbon dioxide from 0% to 100%, prolonging exposure from 3 to 12 days and by elevating temperature from 20 °C to 30 °C. After exposing for 3 days at 20 °C, average % mortality was gradually increased from 10.0% (in control treatment) to 16.7%, 20.0%, 23.3% by increasing concentrations of carbon dioxide from 0% (in control treatment) to 25%, 50% , and 75%, respectively.

Fig. 6: Effect of treating with different concentrations of carbon dioxide at two temperature degrees on average % mortality of wax-moths larvae after 3, 6, and 12 days from treatment.



After 3 days at 30 °C, the same concentrations of carbon dioxide resulted, however, in 6.7%, 23.3%, 53.3%, and 60.0% mortality of wax-moths larvae, respectively. The same trend of the larvae mortality was noticed after 6 and 12 days. The averages mortality in larvae treated with 25%, 50%, and 75% of carbon dioxide and exposed at 30 °C were 36.7%, 86.7% and 90.0%, respectively.

It could be concluded that the response of wax-moths larvae to the different concentration of CO2 pressure tests (in term of % mortality) was more pronounced at the higher temperature (30 °C) than the lower one (20 °C). This

means that applying the highest concentrations of CO_2 i.e. 75% and 100% are the suitable conditions for controlling of wax-moths larvae especially in the hot weather or in the summer season.

These present results are in agreement with those obtained by **George et al., (1972)** who reported that all life stages of the greater wax-moth, *Galleria mellonella* (L.) were killed during 5 h. or less when they were exposed to a concentration of 97.2% CO2 at 100 °F. Also, 3-or 4-day exposures to 95.7% CO2 at 50, 60, 70 and 82 °F resulted in high larval moralities. The data obtained in the laboratory tests indicated that the applications of CO_2 to control the greater wax-moth should be made at/or slightly below 100 °F for optimum results. They also stated that, the use of CO_2 to control the greater wax-moth (*G. mellonella*) drops off slightly just below 1000F, but longer exposure should compensate for this shower action. Although, if this high temperature cannot be obtained, 50 to 60 °F should be used.

Also, **Edward *et al.*, (1972)** stated that, twelve semitrailer van loads of comb honey were treated with carbon dioxide (CO_2) for wax moth control. An average concentration 98.6% CO_2 was held for periods of 10 or 12 hrs., and the average amount of CO_2 used was 1, 337.5 Ib (0.52 Ib per cubic foot) per treatments. An average mortality of 97.8% of caged wax moths was achieved during treatments.

3.2- Efficacy of applying phosphine alone or mixed with different concentrations of carbon dioxide for controlling the wax-moths larvae:

In this experiment, wax-moths larvae were exposed for to a fixed concentration of phosphine (PH_3) either alone or mixed with different concentrations of CO_2 for 24, 48 or 72 h at two different temperatures (20 or 30 °C). Untreated wax-moths larvae, kept at the same temperatures for the same periods, was served as control. The obtained results are listed in Table (7) and Figs (7).

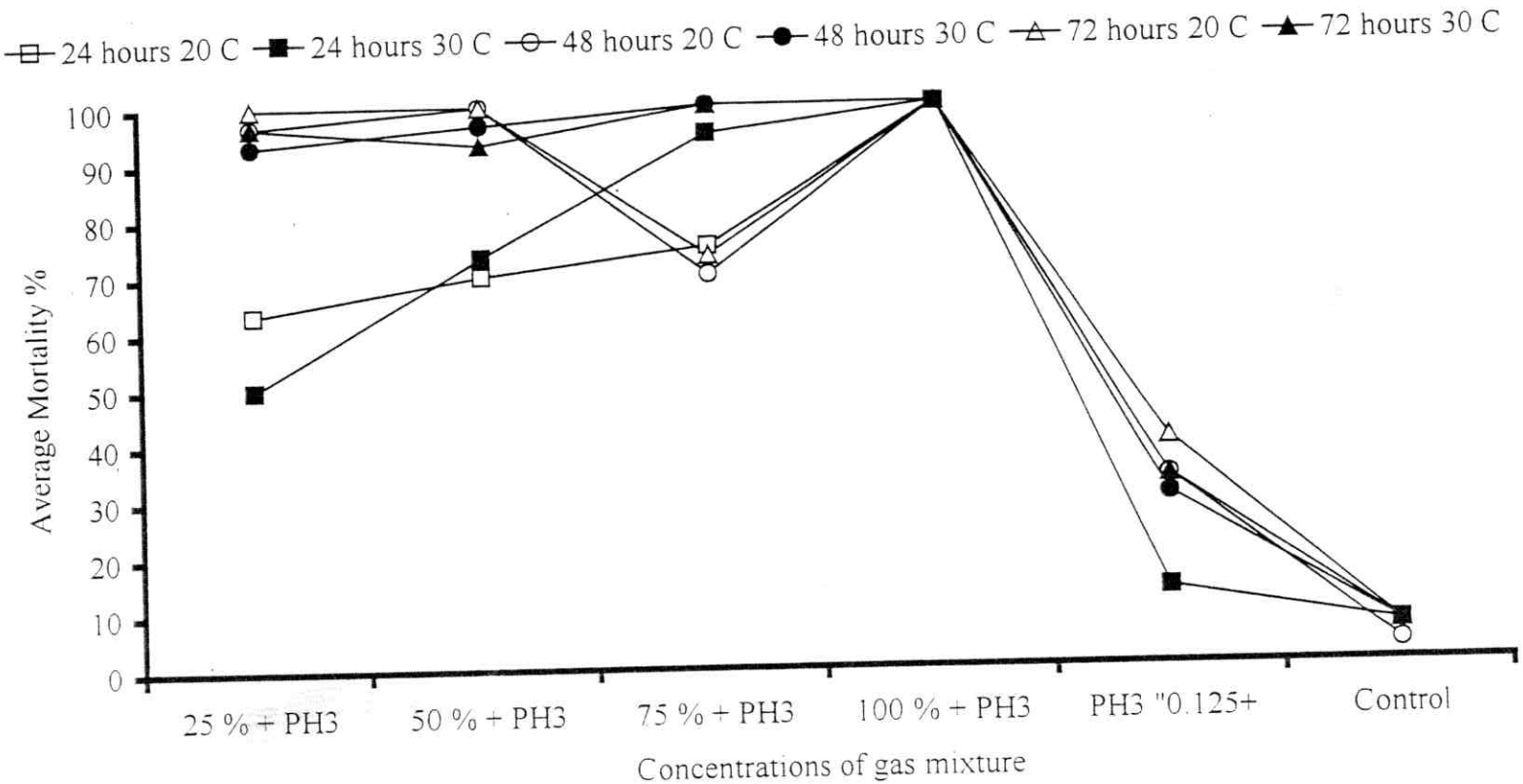
Table (7): Effect of phosphine (PH_3) at rate of 0.125 g alone or mixed with different concentrations of CO_2 on controlling the first larvae of wax moths.

Treatment	% mortality after exposure for						Mean
	24 hours		48 hours		72 hours		
	20 ºC	30 ºC	20 ºC	30 ºC	20 ºC	30 ºC	
Control	6.7	6.7	3.3	6.7	6.7	6.7	6.13
PH_3 "0.125 g"	13.3	13.3	33.3	30.0	40.0	33.3	27.20
25 % CO_2 + PH_3	63.3	50.0	96.7	93.3	100.0	96.7	83.33
50 % CO_2 + PH_3	70.0	73.3	100.0	96.7	100.0	93.3	88.88
75 % CO_2 + PH_3	75.0	95.0	70.0	100.0	73.3	100.0	85.55
100 % CO_2 + PH_3	100.0	100.0	100.0	100.0	100.0	100.0	100.00
Mean	53.8	55.6	67.2	71.1	70.0	71.7	64.90

L.S.D. at 0.01 for			
Gas Concentration	24.37	11.42	12.33
Temperature	N.S.	N.S.	N.S.
Interaction	N.S.	16.50	17.44

The present results indicated that, applying phosphine mixed with increasing concentrations of carbon dioxide was more effective in controlling wax-moths larvae than phosphine alone. The average mortality in case of applying phosphone alone was 27.2% compared with 6.13% in case of control treatment. However, phosphine mixed with 25%, 50%, 75%, and 100% carbon dioxide, increased averages of mortality to 83.33%, 88.88%, 85.55 and 100.0%, respectively. As for exposure temperatures, the same results showed no clear variations in the average mortality at the tested treatments.

Fig. 7: Effect of different concentrations of carbon dioxide-phosphine mixture on controlling the first larvae of wax-moths



It is worthy to state that mixture containing both phosphine and CO_2 accelerated the death of wax-moths larvae than phosphine or CO_2 each alone. The average mortality reached its maximum (100%) after 48 h only by using mixture containing phosphine + 50 CO_2 at 20 C or phosphine + 75 CO_2 30 C. Such level of mortality could be never obtained when CO_2 was used alone at these concentrations (see Table 3). Thus, the mixture containing phosphine and 50% or 75% of CO_2 could be recommended for controlling the wax moths larvae in stored bees-wax combs.

In light of results in Tables 6 and 7 we could concluded that phosphine gas increased efficacy of CO_2 in controlling wax-moths larvae. These results are in agreement with those obtained by **EL-Lakwah et al., (1989a)** who compared efficacy of phosphine with mixtures of phosphine gas plus carbon dioxide against diapause larvae of *Tragoderma granarium*. They showed that addition of varying concentrations of CO_2 (20, 50, 75 and 100%) to fixed PH_3 concentration had a negative influence on phosphine efficacy against diapause larvae of Khapra Beetle at 30 °C for short exposure periods of 18 and 24 hours. On the other hand, it was found that for longer exposure periods of 48 and 72 hours, the addition of CO_2 to phosphine induced significantly higher larval mortality than that by PH_3 alone, whereby an additive effect was proved for mixtures contained PH_3+75% and 100% CO_2. The addition of 20% and 50% CO_2 to PH_3 at 20 °C did not show any increase in larval mortalities of the mixtures for exposure periods between 24-96 hours. The larval mortalities obtained for an atmosphere contained 50% CO_2

alone at 20 and 30 °C was (0-6%) and (10-40%), respectively, after two days exposure period.

II) Applied Experiments:

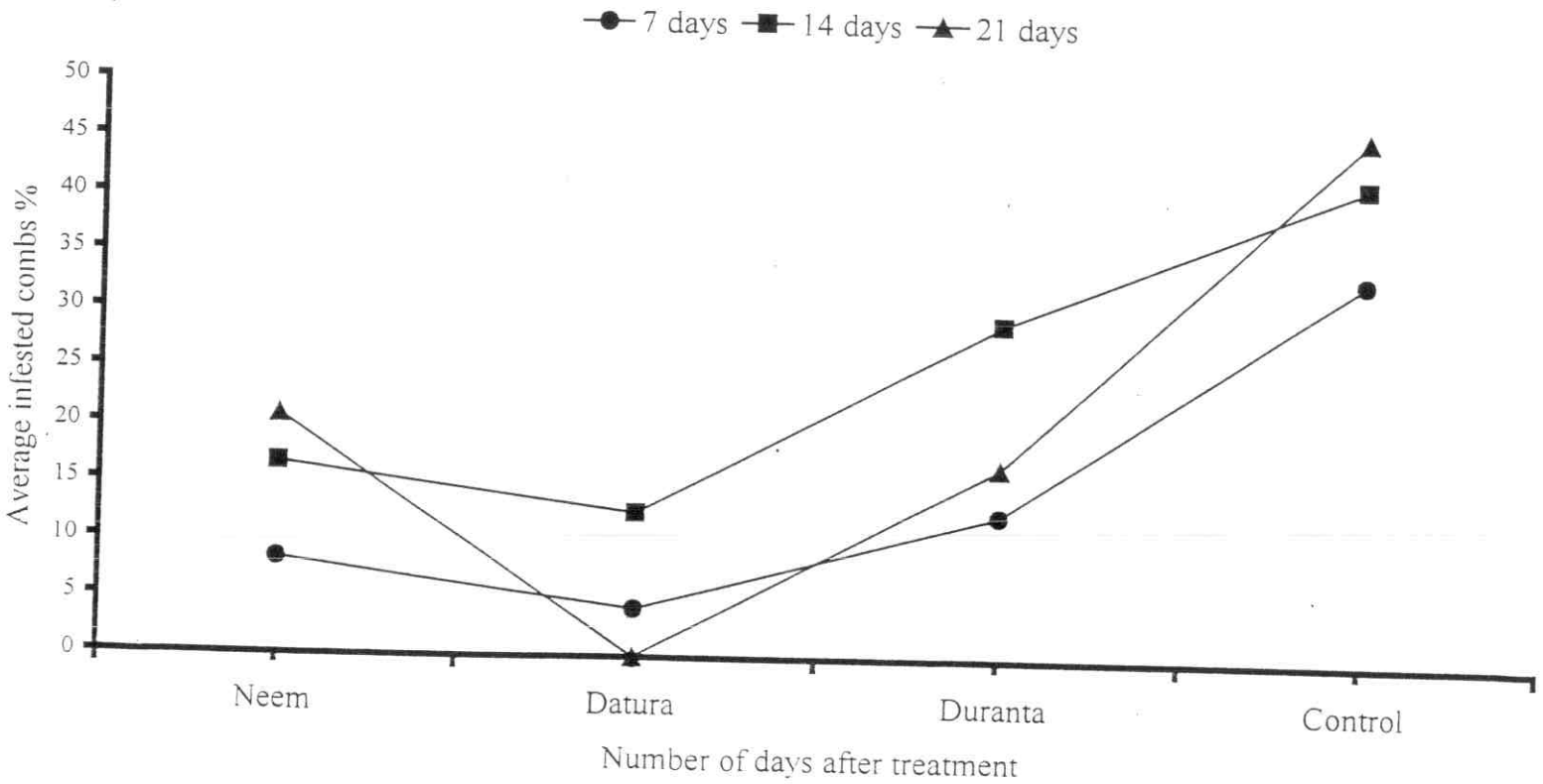
1-Effect of treating healthy wax-combs in stored hive boxes with different plant powders on controlling the wax-moths larvae:

In this field test, healthy combs (8 combs) were stored with other combs infested with wax-moths larvae (2 combs). Healthy combs only were dusted with powders prepared from leaves of *Datura stramonium*, *Duranta plumieri*, and *Melia azadarachia* plants. Percentages of infested combs as affected by plant powders were determined weekly for 3 successive weeks after treatment 7, 14 and 21 days after treatment. The obtained results are recorded in Table (8) and illustrated in Fig. (8).

Table (8): Effect of treating combs with different plant-powders on percentage of combs infected with wax-moths larvae.

Treatment	% infested combs after "days"			Mean
	7 days	14 days	21	
Neem	8.33	16.67	20.83	13.54
Datura	4.17	12.50	0.00	6.25
Duranta	12.50	29.17	16.67	18.75
Control	33.33	41.67	45.83	38.54

Fig. 8: Effect of treating combs with different plant-powders on percentage of combs infected with wax-moths larvae



The obtained results indicated clearly that all tested plant powders were effective and better for reducing percentages of infested combs than control treatment (average 38.54%). In this regard, powder of *Datura*-leaves came first (average 6.25%) followed by Neem-leaves (average 13.54%), and *Duranta*-leaves (average 18.75%).

Infestation with wax moths larvae was developed more quickly on untreated wax combs (control treatment) as it was reached to 33.33%, 41.67%, and 45.83% after 7, 14, and 21 days, respectively. In contrast, it was developed more slowly in plant powder-treated wax combs. In this respect powder of *Datura*-plant was the only treatment which lead to freeing wax combs completely from infection with wax moths larvae 21 dys after treatment (0.0%). In case of powder of Neem-plant leaves comb infestation were 8.33%, 16.67% , and 20.83% after 7, 14, and 21 days, respectively.

These results are in harmony with the findings of several investigators (**El-Sayed 1985a**; **Khadr *et al.*, 1986**; **Abdul-Kadir** and **Connolly, 1991**; **Rashad *et al.*, 1991**; **Osman, 1993**; **Osman** and **Bradley 1993**; **Hashem *et al.*, 1994**; **Liu. 1995**; **Gerard** and **Ruf, 1995**; **Xie *et al.*, 1995**; **Su-TianYun *et al.*, 1999** and **Valladares *et al.*, 1999**).

2-Effect of applying Formic acid, Methyl salicylate and Acetic acid with two different methods in controlling wax-moths larvae in stored combs:

In this experiment, solutions of the organic compounds: Formic acid (FA), Methyl salicylate (MS) and Acetic acid (AA) were used by two different methods for controlling wax-moths larvae in stored combs. In the first method healthy wax combs were sprayed (with solution of any of these organic compounds) for the first time (initial application) directly before storing in hive boxes. The same wax-combs were re-sprayed again 14 days latter after the initial application. In the second method, varro-form apparatus provided with any of these solutions was placed inside hive boxes containing healthy in infested combs. Results about efficacy of these treatments expressed in term of % infested combs after 2, 4, and 6 weeks from the initial treatment were recorded in Table (9) and illustrated in Fig. (9).

The obtained results showed that, all tested organic compounds were effective in controlling wax-moths larvae in stored combs compared with control treatment (average 40.45% infested comb). Using atomizer for spraying was more effective and better than using the varro-form apparatus for applying solution of any of the tested organic compounds in minimizing infestation of combs with wax-moths larvae. By using spraying method for applying MS, FA, and AA the average percentages of infested combs were 12.5%, 4.17%, and 5.56%, respectively.

In case of applying the varro-form apparatus the average percentages of infested combs were 12.5%, 31.94%, and 22.22% for these three compounds, respectively. The observed reduction

in efficacy of these compounds when they were applied by using the varro-form apparatus might be attributed mainly to the smallest amounts of solution that evaporated through the varro-form apparatus placed inside hive boxes. Along 6 weeks, this apparatus allowed only to evaporate 2 ml, 32 ml, and 39 ml from MS, FA, and AA, respectively. Reasonably it could attribute the observed increase in efficacy of these compounds when they were used as spray (by atomizer) to the fixed large amounts of these organic compounds that used for spraying healthy wax-combs (15 ml / comb).

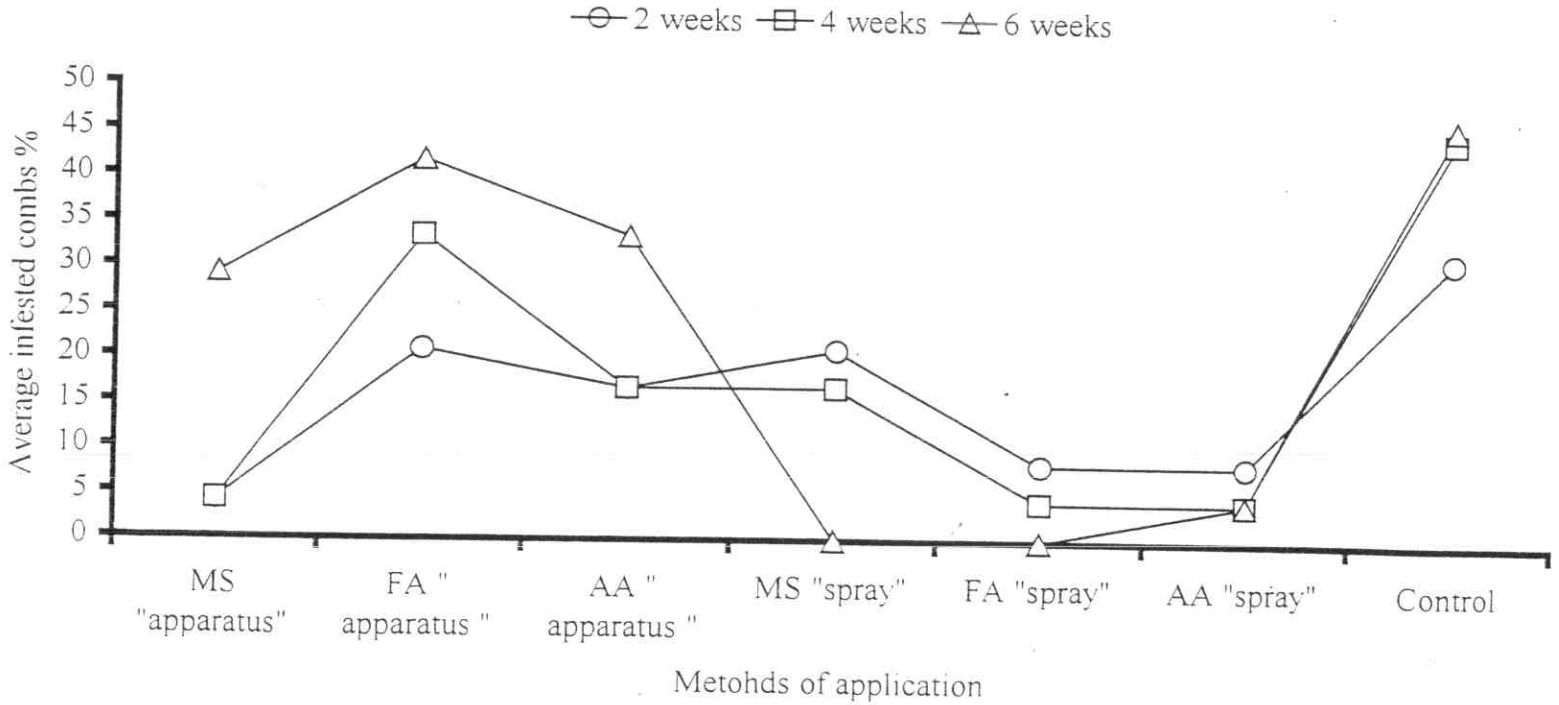
Table (9): Effect of using apparatus or spraying methods for applying solutions of Formic acid "FA", Acetic acid "AA", Methyl Salicylate "MS", for controlling wax-moths larvae (Combs were stored in hive boxes).

Tested compound	Method of application	Total volume used along experiment	% infested combs after "weeks"			
			2 ** weeks	4 weeks	6 weeks	Mean
Methyl Salicylate	Apparatus	2	4.17	4.17	29.17	12.50
	Spraying	720	20.83	16.67	0.00	12.50
Formic Acid	Apparatus	32	20.83	33.33	41.67	31.94
	Spraying	720	8.33	4.17	0.00	4.17
Acetic Acid	Apparatus	39	16.67	16.67	33.33	22.22
	Spraying	720	8.33	4.17	4.17	5.56
Mean			13.19	13.20	18.06	14.81
Control			31.20	44.33	45.84	40.45

* healthy combs sprayed two times at 2 weeks interval.

** the time of the second application.

Fig. 9 Effect of using apparatus or spraying methods for applying solutions of Formic acid "FA", Acetic acid "AA", Methyl salcilate "MS", for controlling wax-moths larvae (Combs were stored in hive boxes).



With method depending on using varro-form apparatus, MS compound was the superior for controlling wax-moths larvae in stored combs. During the first 4 weeks, after placing the apparatus containing MS inside hive box, % infested combs remained low (4.17%) but it was sharply increased during the latter 2 weeks to 29.17%. Efficacy of the MS solution in apparatus may be decreased by time and replacing solution of this compound in varro-form apparatus, at least every 3-4 weeks may be necessary.

With spraying method (using atomizer), both MS and FA resulted in complete eradication of comb-infestation with wax-moths larvae (0.0%) after 6 weeks from the initial spray. Pointing to cost of treatment, and because the large amounts used in spraying method, the cheap FA may be more preferable for controlling wax-moths larvae than the expensive MS compound.

The above results concluded that infestation of wax-combs with the wax-moths larvae on stored combs could be lowered or completely eradicated by applying solutions of the organic compounds MS (by using varro-form apparatus placed inside hive boxes) or using atomizer spraying healthy combs every two weeks with FA solution.

In general, the obtained results are in harmony with those reported by several investigators (Maneshasa 1987; Nelson 1995; Gretti et al., 1992; Buhlmann 1991; Sharma et al., 1994; Barbattini et al., 1994; Nelson 1995; Frilli et al., 1992; Greatti et al., 1993; Eischen 1998; and Sharma et al., 1999) who used one

or more of the tested chemicals for controlling other pests of honey bee.

3- Effect of spraying bees wax combs with mixture of volatile oils (Apilife VAR) on controlling wax moths larvae during storage:

3.1- Effect of spraying healthy combs only:

This study was aimed to investigate efficacy of spraying healthy beeswax combs with different concentrations of a mixture of volatile oils (Apilife VAR) on controlling infestation with wax-moths larvae during storage. The results are listed in Table (10) and Fig. (10).

The obtained results indicated significant efficacy of the mixture of volatile oils in controlling wax comb infestation with wax moths larvae even at its lowest concentration. It was noticed also that, % infested combs in control treatment was gradually increased by increasing storage period up to 7 weeks (average 43.29%). The opposite trend was noticed in combs treated by the volatile oils. Treating wax combs before storage resulted in gradual decrease in percentages of wax combs infested with moths larvae, by increasing time of storage. Average percentage of infested combs (during 7 weeks) was decreased to 19.17%, 5.81%, 4.60%, 3.52%, 1.67%, 0.48% (with an average 5.88%) by spraying healthy beeswax combs, before storage, with watery solution containing 6%, 8%, 10%, 12%, 14% and 20% Apilife VAR, respectively.

The same results stated also that, infestation with wax moths larvae was completely eradicated (0.0 %) after 7, 5 and 3

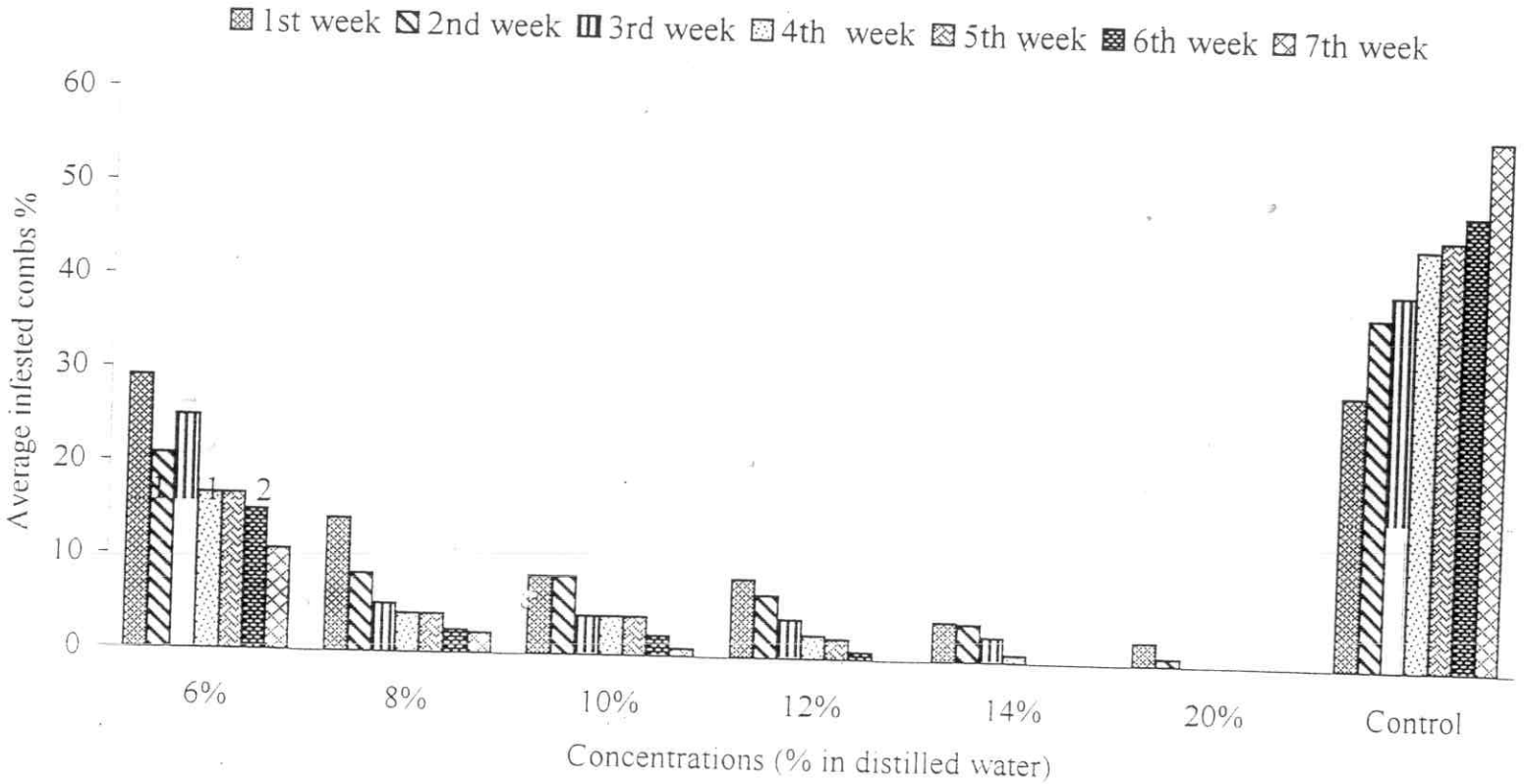
weeks from spraying healthy beeswax combs by Apilif VAR at concentrations of 14%, 16%, and 20%, respectively.

Table (10): Effect of treating healthy combs only with different concentrations "% in distilled water" of volatile oils mixture (Apilife VAR) on % combs infected with wax-moths larvae.

Apilife VAR conc. "%"	Periods "in weeks" after treatment							Mean
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	
6	29.17	20.83	25.00	16.67	16.67	15.00	10.83	19.17
8	14.17	8.33	5.17	4.17	4.17	2.50	2.17	5.81
10	8.33	8.33	4.17	4.17	4.17	2.17	0.83	4.60
12	8.33	6.67	4.17	2.5	2.17	0.83	0.00	3.52
14	4.17	4.00	2.67	0.83	0.00	0.00	0.00	1.67
20	2.50	0.83	0.00	0.00	0.00	0.00	0.00	0.48
Mean	11.11	8.17	6.86	4.72	4.53	3.42	2.17	5.88
Control	29.17	37.50	40.00	45.00	46.00	48.67	56.67	43.29

- Each figure is an average of infested combs in 3 boxes, each contain 8 healthy + 2 infected combs.

Fig. 10: Effect of treating healthy combs only with different concentrations "% in distilled water" of volatile oils mixture (Apilife VAR) on % combs infected with wax-moths larvae.



3.2- Effect of spraying both healthy and infested combs:

The obtained results illustrated in Table (11) and Fig. (11) go parallel with those previously mentioned in Table (10). However, percentage of beeswax combs infested with wax moths larvae reached to Nil (0.0%) 2, 5, 6, and 7 weeks after treating with Apilife VAR at concentrations 20%, 14%, 12% and 10%, respectively.

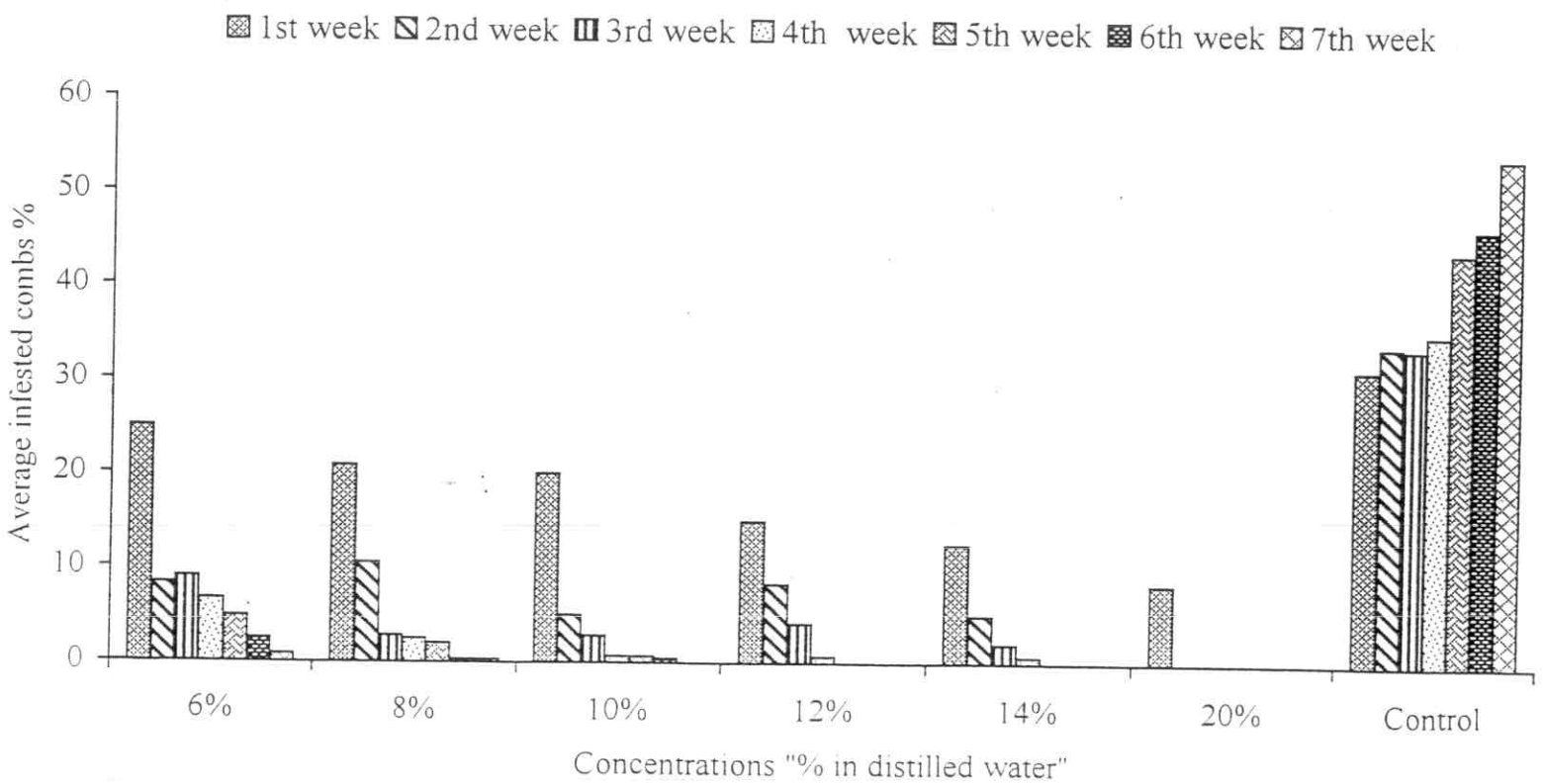
The remarkable reduction in percentage of combs infested with wax moths larvae which was observed after spraying with the Apilfe VAR

(Tables 10 and 11) might be due to the fact that these oils contain certain toxic compounds.

Table (11): Effect of treating both healthy and infested combs with different concentrations of volatile oils mixture (Apilife VAR) on % combs infected with wax-moths larvae.

Apilife VAR conc. "%"	Periods "in weeks" after treatment							Mean
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	
6	25.00	8.33	9.00	6.67	4.83	2.50	0.83	8.17
8	20.83	10.50	2.83	2.50	2.00	0.33	0.33	5.62
10	20.00	5.00	2.83	0.67	0.67	0.50	0.00	4.24
12	15.00	8.33	4.17	0.67	0.33	0.00	0.00	4.07
14	12.50	5.00	2.00	0.67	0.00	0.00	0.00	2.88
20	8.33	0.00	0.00	0.00	0.00	0.00	0.00	1.19
Mean	16.94	6.19	3.47	1.86	1.25	0.56	0.19	4.36
Control	31.25	33.75	33.50	35.00	43.75	46.25	53.75	39.61

Fig. 11: Effect of spraying both healthy and infested combs with different concentrations (% in distilled water) of volatile oils mixture (Apilife VAR) on % combs infected with wax-moths larvae.



In general, the above mentioned results are in harmony with the findings of several investigators who working on other insects. **Mutinelli et al.,(1996)** reported that, efficiency of ApiLife VAR applied at various doses and in different formulations to kill *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies was ranged between 51.5% and 68.7%. **Rickli et al., (1991)** reported also that, 96.4% and 99.0% of mites (*Varroa jacobsoni*) were killed in colonies treated with Apilife "VAR" for 38 and 96 days, respectively. **Imdorf et al., (1994 and 1995)** stated that Apilife-VAR had an effectiveness against *Varroa jacobsoni* in honey bee colonies. It is concluded that it can kill more than 95% mites when used to treat a colony in a single-box Swiss hive but is less effective if there is more than one box and also in Dadant hives. Values obtained for residues in honey are compared. However, the high efficiency of Apilife VAR in controlling *Varroa jacobsoni* infestations in honeybee colonies is not always guaranteed. They added that, there is no accumulation of residues in beeswax with extended use of Apilife VAR.