INSECTICIDAL ACTIVITY OF THREE PHYTOCHEMICALS AGAINST THE EGYPTION COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD.) NOCTUIDAE : LEPIDOPTERA

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Abstract

The toxic effect of three phytochemicals which isolated from three plants, *Tephrosia apollinea* , *Ferula vesceritensis* and *Daucus carota* against the second and fourth instar larvae of the cotton leafworm ,S.littoralis The experiments were carried out in the laboratory to evaluate the molecular formula for these phytochemicals were C24H20O7, C22H30O4 and C24H30O3, respectively. The second and fourth instar larvae were allowed to contact for 24h. with the residual film of each phytochemical at different concentrations. The third one C24H30O3 was the most toxic against the second and fourth instars. The LC50 values were 56 and 100 ppm for 2nd and 4th larvae instars, respectively) , while the second compound (C22H30O4) was the least toxic. The LC50 values were 108 and 480ppm against both instars. The development of the treated larvae was affected, whereas, it increase the larval and pupal period , and decrease pupation and adult emergence percentages and pupal weight . Also ,the fecundity , fertility , Longevity were decreased ,and sex ratio of the adult stage treated as 4th instar larvae with the three phytochemicals was affected. Pupal and adults malformation percentages were produced as a result of the larval treatment of 4th instar with the three phytochemicals at the LC50 values.

INTRODUCTION

The cotton leafworm ,*Spodoptera littoralis* (Boisd) is one of the major pests. In addition, this insect cause a considerable damage to many of the important vegetables and field crops in Egypt. However, the increasing consumption of the synthetic pesticides in the developing countries has lead to a number of problems such as environmental pollution , adverse effects on non-target organisms and the development of resistance . The use of natural products of plant origin is a new trend that preserve the environment from contamination with harmful toxicants. The most familiar one of these chemicals in the recent years is azadirachtin that isolated from the fruits of Melia azedarach and seeds of *Azadirachta indica* (Meliaceae). Azadirachtin exhibits extremely low acute mammalian toxicity yet it is very effective as control agent for many insect groups (Champagne et. al. 1989). Besides azadirachtin, other plant derived extracts and phytochemicals have been shown to possess insecticidal

activity and some morphogenetic effects. The insecticidal activity of several phytochemicals was evaluated against several Lepidoptous insects . Some of these chemicals were tested against S. littoralis were reported by several workers, (Abdel-Aziz ,1994 ; Amr et al.,1995).Also some morphogenetic effects from some plant derived chemicals against several Lepidoptera species were recorded (Abo- El -Ghar et.al.,1994)

The prenciple aim of these present study was to evaluate the insecticidal activity and morphogenetic activities of some plant derived chemicals against the egyptian cotton leafworm , *S. littoralis* .

MATERIALS AND METHODS

1- Insect rearing

The cotton leaf worm, *S. littoralis* was reared in the laboratory for several generations at room temp. ranged between 25 - 28 C° and 60 -65% R.H. Larvae were fed on castor bean leaves, *Ricinus communis* (L.) in a wide glass jars until pupation period and adults emergence . The newly emerged adults were mated inside glass jars supplied with a piece of cotton wetted 10% sugar solution as feeding source for the emerged moths and branches of Tafla (*Nerium oleander* L.) or castor bean leaves as an ovinosition site (El- Defrawi *et al.*,1964). Egg masses were kept in plastic jars until hatching.

2- Material used.

Three phytochemicals were isolated from three plants, *Tephrosia apollinea*, *Ferula vesceritensis* and *Daucus carota* by chemistry division, Faculty of science, Minia University. These compounds were evaluated in laboratory tests against 2nd and 4th instar larvae of *S. littoralis*. The molecular formula for these phytochemicals were $C_{24}H_{20}O_7$, $C_{22}H_{30}O_4$ and $C_{24}H_{30}O_3$, respectively.

- The chemical structure of the first compound as following:

Molecular weight: 420mg

Molecular formula: $C_{24}H_{20}O_7$

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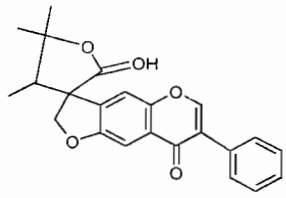
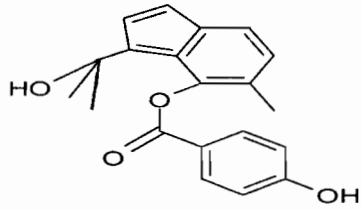


- The chemical structure of the second compound as following

Molecular weight: 358mg

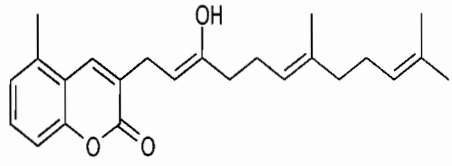
Molecular formula: $C_{22}H_{30}O_4$



- The chemical structure of the third compound as following:

Molecular weight: 366mg

Molecular formula: $C_{24}H_{30}O_3$



3- Test procedures.

A series of different concentrations were prepared on the active ingredient basis (p.p.m) by diluting the formulated chemical in the solvent. About 5- 8 concentrations, i.e. 1600 - 25 ppm for each of the three tested compounds were prepared. Thin film residues layer technique was used as a method of application, where the tested concentrations were applied through ethanol to the surface of a 0.9 cm diameter Petri – dish. One ml of each concentration of the tested plant extracts was spread on the inner surface of the Petri dish, by moving the dish gently in circles. The Petri dish used as control was treated with 1 ml ethanol only. The ethanol was evaporated under room temperature. Ten larvae of 4^{th} or 2^{nd} instar of cotton leafworm were exposed about 24 hours in each perti dish , then transferred to clean glass containers and fed fresh castor leaves until the pupation period (according to Ahmed,1985). Five replicates in each concentration were prepared. The larval mortality percent of both 2^{nd} and 4^{th} instar were recorded and corrected according to the check by using Abbott formula. (Abbott,1925) .The data analyzed by using probit analysis (Finney,1971), The LC_{50} values were estimated for each tested compound . The biological parameters of the foregoing three compounds were evaluated as larval and pupal period, the pupation and adult emergence percentages, pupal weight. Fecundity, fertility, longevity and sex ratio of the adult stage treated as 4^{th} instar larvae with LC_{50} each of the phytochemical compound were also considered. . Also, the observed malformations were recorded and photographed.

4- - Separation technique:

Air dried species of the aerial parts were extracted successfully with CH_2CL_2 at room temperature. After removal of solvent ,the residue was subjected to column chromatography using silica gel and eluded with n - hexane , EtOAC and MeOH in increasing order of polarity . Purification of the fractions preparative was made by TLC, HPLC and Sephadex Columns.

RESULTS AND DISCUSSION

1-Toxic effect:

Data illustrated in Table (1) showed the toxic effect of three phytochemicals which isolated from three plants; *Tephrosia apollinea*, *Ferula vesceritensis* and *Daucus*

carota against 2nd and 4th instar larvae of *S. littoralis*. The third compound ($C_{24}H_{30}O_3$) was the most toxic one against both 2nd and 4th instar larvae. The LC_{50}values were 56 and 100 ppm, respectively).While the second compound ($C_{22}H_{30}O_4$) had the least effect . The LC_{50} values showed 108 and 480 ppm against both 2nd and 4th instar larvae, while, the LC_{50} values for the first compound were 100 and 120 ppm ,respectively.

Table 1 . Insecticidal activity of tested phytochemicals against *Spodoptera littoralis* treated as 2nd and 4th instar larvae.

Compounds	2nd instar				4th instar			
	LC_{50} values ppm	Slop function	95% confidence limit Upper	Lower	LC_{50} values ppm	Slop function	95%confidence limit Upper	Lower
$C_{24}H_{20}O_7$	100	1.5	128	92	120	1.5	144	100
$C_{22}H_{30}O_4$	108	9.5	120	80	480	4.5	520	440
$C_{24}H_{30}O_3$	56	1.1	60	52	100	2.6	120	80

These results are agreement with those obtained by Pizzal ,et.al (1997) demonstrated the antioxidant and insecticidal activity of some activity components which were separated from essential oils that isolated from 20 different plants and analyzed by capillary gas chromatography _mass spectrometry(GC_MS) using two columns with different polarities(SE 52 and Carbowax).The antioxidant activity of these oils was evaluated with two methods : Crocin and Rancimat tests. Activity components were Carotol ,cuminaldehyde, cumic alcohol ,fenchone and trans anethol ,only found in many oils are peculiar for Carrot,(Daucus carota) Urinova et al.(1989) recorded a toxic activity of six complex esters isolated from Ferula spp. in Uzbekistan against the cotton pests, Aphis craccivora and Agrotis segetum. Blenty et al.(1987) bioassayed the two isolated antifeedants; isopongaflavone and tephrosin from methanol extracts of Tephrosia elote seeds. Tephrosin displayed height activity against Spodoptera exempta .

2. Latent effect:

2.1. Larval and pupal periods:

Data in Tables (2 and 3) indicated that the larval treatment of both 2nd and 4th. instar of *S. littoralis* with the three tested compounds at LC_{50} values, significantly difference ($p<0.001$) increased the larval period. The effect was more pronounced with the third compound ($C_{24}H_{30}O_3$),where the larval periods averaged 22.1and19days for the larvae treated at both 2nd and 4th instar , respectively as compared with 17.1 and 14 d. larval periods for 2nd and 4th instar ,respectively of the check. While with the

second compound the larval period showed 20 and17.7d. as larvae treated at 2nd and 4th instar ,respectively.

Tables (2 and 3)showed that the larval treatment of both 2nd and 4th instar with the three tested compounds at LC_{50} values highly significantly difference ($p<0.01$) increased the pupal periods of the resulting pupae. The effect was more pronounced with the third ($C_{24}H_{30}O_3$) and first ($C_{24}H_{20}O_7$) compounds, where the pupal period prolonged to about 14, 13.7 and 13, 11d.for the resulting pupae treated as 2nd and 4th instar larvae ,respectively. Whereas, the second compound ($C_{22}H_{30}O_4$) had the least effect on the pupal period, where it averaged 12 and 11d. for the resulting pupae treated as 2nd and 4th instar larvae ,respectively ,as compared with that of the check (8.7 and 7.5d).of pupae produced from both untreated 2nd and 4th instar larvae, respectively.

These results are agreement with those obtained by Mahmoud (2002), who demonstrated an increase in the larval and pupal periods of *A. ipsilon* as a result of the larval treatment of 4th instar larvae with *C. fistula* , *A. maritima* extracts by a contact method . Also, Osman(1999) found that *A. maritima* extract increased the larval and pupal periods of *A. ipsilon* following the larval feeding on this extract by a contact method. *Antonious et.al.*(1992) recorded that the longest pupal period was obtained when the larvae of *S. littoralis* treated with phytochemicals extracted by methanol , benzene or hexane of *Dieffenbachia maculata* and *Adhatada vasica* .

2.2. Pupation and Pupal weight:

Data in tables (2 and 3) demonstrated that the larval treatment of 2nd and 4th instar with the three tested compounds at LC_{50} values highly significantly ($p<0.01$) difference reduced the pupation percent . The third compound ($C_{24}H_{30}O_3$) had the highest effect on the pupation, it gave 41 and 57% pupation for pupae treated as 2nd and 4th instar larvae, respectively. Whereas, the first ($C_{24}H_{20}O_7$) and second ($C_{22}H_{30}O_4$) compounds reduced the pupation at a range from 54 to 71 % for pupae treated as 2nd and 4th instar larvae ,respectively, as compared to 100% pupation of the control.

Likewise, the larval treatment of 2nd and 4th instar larvae with the three tested compounds at the LC_{50} values highly significantly ($p<0.01$) reduced the pupal weight of the resulting pupae . The third compound ($C_{24}H_{30}O_3$) was the most suppressive one on the pupal weight, it averaged 0.259 and 0.264gm. for pupae treated as 2nd and 4th instar larvae , as compared to 0.341 and 0.383 gm., of pupal weight produced from untreated 2nd and 4th instar larvae, respectively. Whereas, the second compound ($C_{22}H_{30}O_4$) had the least effect on the pupal weight, it averaged 0.308 and 0.344gm. for pupae treated as 2nd and 4th instar larvae ,respectively ,as compared with that of the control.

Table 2. Effect of some phytochemicals on the larval and pupal development of *Spodoptera littoralis* treated as 2nd instar larvae with LC50 values.

Compounds	Larval periods (days) ± SD	% Pupation ± SD		Pupal period (days) ± SD	Pupal weight (gm) ± S.E	% Moth emergence ± SD	
		Normal	Malfo.			Normal	Malfo.
$C_{24}H_{20}O_7$	21 ± 1.6*	56.3±2.5**	2.6	13 ± 0.8**	0.295 ± 0.09**	62.2 ± 0.9**	1.5
$C_{22}H_{30}O_4$	20 ± 3.8*	54.3±3.7**	2.5	12.3 ± 1.2*	0.308 ± 0.07**	64 ± 0.3**	2.1
$C_{24}H_{30}O_3$	22.1 ± 0.5**	41.3±4.3**	3.9	14 ± 0.8**	0.259 ± 0.1**	61± 0.7**	2.5
Check	17.1 ± 1.4	100±0.0	0.0	8.7 + 0.9	0.341+ 0.02	94 ± 3.0**	0.0
F value	6.949	144.15		9.89	8.93	90.3	
P- value	0.022	0.0005		0.009	0.01	0.002	
L.S.D at 5 %	2.78	7.43		2.55	0.03	5.57	
1%	4.21	11.15		3.87	0.05	8.62	

** = Highly Significant (p<0.01) * Significant (p<0.05)

Table 3 . Effect of some phytochemicals on the larval and pupal development of *Spodoptera littoralis* treated as 4th instar larvae with LC50 values.

Compounds	Larval periods (days) ± SD	% Pupation ± SD		Pupal period (days) ± SD	Pupal weight (gm) ± S.E	% Moth emergence ± SD	
		Normal	Malfo.			Normal	Malfo.
$C_{24}H_{20}O_7$	19 ± 2.0**	70.7 ± 1.5**	1.7	11± 3.4**	0.313 ± 0.01*	66.8 ± 3.0**	2.4
$C_{22}H_{30}O_4$	17.7 ± 0.6*	63.7 ± 5.3**	0.7	10.8±0.5**	0.344 ± 0.01**	74.4 ± 0.6**	2
$C_{24}H_{30}O_3$	19 ± 2.0**	57.3 ± 5.6**	3.4	13.7± 3.0**	0.264 ± 0.03**	65.3 ± 1.2**	2.5
Check	14.4 ± 0.7	100 ± 0.0	0.0	7.5 + 0.5	0.383 ± 0.01	92.6±3.1	0.0
F value	7.8	36.4		34.2	16.9	71.6	
P- value	0.02	0.0003		0.0003	0.002	0.0004	
L.S.D at 5 %	2.7	8.4		1.5	0.03	5.13	
1 %	4.1	14.6		2.3	0.05	7.8	

** = Highly Significant (p<0.01) * Significant (p<0.05)

These results are similar with that obtained by Mahmoud(2002) who reported that the larval treatment of *A. ipsilon* with *C. fistula* , *A. maritima* and *T. tipu* extracts highly significantly (p<0.01) reduced the pupation percent to about 50% than of the control , and also decreased the pupal weight of the resulting pupae .Likewise, Osman (1999) recorded a decrease in the pupal weight of *A. ipsilon*

following the larval treatment with chloroform extract of *A. maritima* using a contact method .

Table 4 . Effect of some phytochemicals on the adult of *Spodoptera littoralis* treated as 4th instar larvae with LC50 values.

Compounds	Fecundity	Fertility	Longevity	Adult sex ratio(%)	
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D. (days)	Male	Female
$C_{24}H_{20}O_7$	133.6 ± 27**	126 ± 25.2**	5.3 ± 2*	37.9	62.1
$C_{22}H_{30}O_4$	222 ± 9.1**	209.4 ± 8.5**	5 ± 1.2*	47.7	52.3
$C_{24}H_{30}O_3$	119.1± 1.4**	112.4 ± 1.3**	6 ± 3 n.s	50	50
Check	736.7 ± 171.3	685.8 ± 170.3	8 ± 2	37	63
F value	15241.2	10342	4.69		
P- value	0.00004	0.00002	0.06		
L.S.D at 5 %	8.9	7.8	2.3		
1 %	12.46	11.69	3.54		

** = Highly Significant ($p<0.01$) * Significant ($p<0.05$)

2.3. Moths emergence:

Data in Tables (2 and3) indicated that the larval treatment of both 2nd and 4th instar larvae with the three tested compounds induced significantly ($p<0.01$) reduction in the moths emergence. The third ($C_{24}H_{30}O_3$) compound was the most effective one on the adult emergence ,where it averaged 61 and 65.3 % for adults treated as 2nd and 4th instar larvae ,respectively ,as compared to 94 and 92.6 % adult emergence of the untreated (adults emerged from untreated 2nd and 4th instar larvae, respectively). Whereas ,the second compound ($C_{22}H_{30}O_4$) had the least effect on the adult emergence ,it averaged 64and 74.4% for adults treated as 2nd and 4th instar larvae ,respectively .

The obtained results are agreement with those obtained by Mahmoud(2002) who recorded that the larval treatment of *A. ipsilon* with *A. maritima* extract induced the highest reduction in the adult emergence by a contact method .Also ,Abo_El - Ghar *et.al.*(1994) demonstrated a decrease in the adult emergence of *A .ipsilon* treated as instar larvae with petroleum ether extracts of *L. cylindrica* ,*A. majus* ,C. elegans and *V. rosea* ,as compared to control .Antonious *et.al* (1992) indicated a decrease in the adult emergence of *S .littoralis* after the larval treatment with ethanolic or methanolic extracts of *D. maculata* and *A. vasica* plants.

2.4. Adult fecundity and fertility:

Data presented in Table (4) demonstrated that the treatment of 4th instar of *S. littoralis* larvae with the three tested compounds at the LC_{50} values highly significantly ($p<0.01$) decrease of the fecundity of the emerged moths. The third compound

($C_{24}H_{30}O_3$) had the strongest effect on the fecundity, where it reduced the fecundity to average 119.1 eggs/f ,as compared to 736.7eggs/f of the check. While the second compound ($C_{22}H_{30}O_4$) had the least effect on the adult fecundity ,where it reduced the fecundity to average 222eggs/f .

Likewise, the larval treatment of 4th instar of *S. littoralis* with the three tested compounds at the LC_{50} values demonstrated a highly significantly ($p<0.01$) decrease of the eggs fertility (Table 4).The third compound ($C_{24}H_{30}O_3$) had the highest effect on the eggs fertility to average112.4eggs/f, as compared to 685.8 eggs/f of the check. Whereas, the second compound ($C_{22}H_{30}O_4$) had the least effect on the eggs fertility, it averaged 209.4eggs/f.

These results are agree to those obtained by Mahmoud(2002) who demonstrated a significant decrease in the adult fecundity and fertility of *A. ipsilon* treated as 4th instar larvae with both *A. maritima* and *C. fistula* extracts by a contact method. Also, the same results was obtained by Osman(1999)on *A. ipsilon* treated as larvae with chloroform extracts of *A .maritima* ,via oral adiministrations.

2.5. Adult longevity:

Table (4) showed that the larval treatment of 4th instar larvae of *S. littoralis* caused a significant ($p<0.05$) decrease in the longevity of the emerged adults, as compared with that of the check. All the three tested compounds decreased the adult longevity to average from 5 to 6 days., as compared to 8 days adult longevity of the check.

These results are similar with those obtained by Mahmoud (2002) who demonstrated a significant decrease in the adult longevity of *A. ipsilon* by the larval treatment of 4th instar with *A. maritima* and *T.tipu* extracts by a contact method .On the contrary ,Shukla *et.al.*(1997) found that adult longevity of *E. vittella* did 'nt differ significantly among the treatments of the plant leaves (,*A. indica* ,*O .basillicum* ,*E. rostrata*, *L. camara* , *A. sativum*)and the control.

2.6. Adult sex ratio :

Data in Table (4) indicated that the larval treatment of 4th instar of *S.littoralis* with the three tested compounds at theLC_{50} values shifted the sex ratio as it increased the males and decreased the females than that of the check. The effect was more pronounced with the third compound ($C_{24}H_{30}O_3$), where it reached to 50% for both males and females, as compared to 38 and 63% for males and females of the check. While the first compound did 'nt affect the sex ratio, it reached 37.9 and 62.1% for both males and females ,respectively .

2.7. Morphogenetic effects:

Data presented in Tables (2 and 3) demonstrated that the larval treatment of both 2nd and 4th instar larvae with the three tested compounds at the LC_{50} values induced a significant ($p<0.01$) increase in the pupal malformations ,as compared to the check .The third compound ($C_{24}H_{30}O_3$) induce the highest percent ,it reached to 3.9 and 3.4% for pupae treated, as 2nd and 4th instar larvae ,respectively ,as compared to 0% pupal malformation of the check. Whereas, the second compound ($C_{22}H_{30}O_4$) induced. the least percent as it reached to 2.5 and 0.7% pupal malformations for pupae treated as 2nd and 4th instar larvae respectively.

In Tables (2 and 3) it found that the larval treatment of 2nd and 4th instars causes adult malformation percent . The third compound ($C_{24}H_{30}O_3$) induce the highest adult malformations as it reached to 2.5% for adults treated as both second and fourth instar larvae ,as compared to 0% of the check. Whereas, the first and second compounds induced adult malformations at range from 1.5 to2% adult malformations.

These results are similar to those obtained by Mahmoud (2002),Abo- El - Ghar *et.al.*(1994), Antonious *et.al.*(1992) . Malformations of *S. littoralis* pupae resulting from the larval treatment of 2nd and 4th instars in the present work, mostly appeared as a malformed pre- pupa failed to cast the old cuticle with complete blackening of the body leading to death (Fig.1) ,or pupa with vestiture of larval skin and undersized pupa (Fig.2) ,or abnormal pupa showing body shrinkage (Fig.3) ,or a larval _pupal monstrosity with larval cuticle patches ,head capsule and thoracic legs; posterior half of the body has the pupal properties (Fig.4). Adult malformations often appeared as a moth failed to emerge from the pupal cast (Fig.5) or a moth showing poorly developed and deformed twisted wings (Fig.6 and 7),as compared to normal pupae and adults (Fig. 8 and 9)

(Fig 1) mostly appeared as a malformation pre_pupa failed to cast the old cuticle with complete blackening of the body leading to death

(Fig 2) or pupa with vestiture of larval skin undersized pupa

(Fig 3) or abnormal pupa showing body shrinkage

(Fig 4) or larval_pupal monstrosity with larval cuticle patches , head capsul and thoracic legs; posterior half of the body has the pupal properties

(Fig 5) Adult malformations often appeared as a moth failed to emerge from the pupal

(Fig 6 and 7) or a moth showing poorly developed and deformed twisted wings

(Fig 8 and 9) normal pupa and adults

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النشاط الابادى لثلاث مشتقات نباتية ضد دودة ورق القطن الكبرى

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اجريت هذه الدراسة بغرض تقيم التأثير السمى لثلاث مشتقات نباتية عزلت من نباتات العمى والقنة والجزر واختبرت معمليا ضد يرقات العمر الثانى والرابع لدودة ورقة القطن الكبرى . كانت الصيغة الجزئية لهذه المشتقات النباتية بالترتيب $C_{24}H_{20}O_7$, C24H30O3 ,C22H30O4. تركت يرقات العمر الثانى والرابع فى تلامس لمدة 24 ساعة مع فيلم المتبقيات الخاصة بكل مركب نباتى عند التركيزات مجال الدراسة، اظهرت النتائج ان المركب الثالث كان له التأثير السمى الآقوى والغالب .حيث بلغت قيمة التركيز النصفى له (56,10 ppm) لكلا العمرين الثانى والرابع على التوالى . بينما كان المركب الثانى له التأثير الآقل حيث بلغت قيمة التركيز النصفى (108,480 ppm) ضد كلا العمرين على الترتيب. تاثر النمو لليرقة المعاملة وادى الى زيادة فى طول العمر اليرقى والعذرى ونقص فى نسب التعزير والاختراق ونقص في الوزن العذرى ومعدل وضع البيض ودرجة الخصوبة كما ادى الى نقص فى العمر للحشرة الكاملة واختلاف فى النسب الجنسية للذكور والاناث للحشرات الكاملة المعاملة كيرقات عمر رابع بالمشتقات النباتية الثلاثة .كما احدثت المعاملة ليرقات العمر الثانى والرابع بالمركبات الثلاثة نسب من التشوهات فى طور العذراء والحشرة الكاملة.