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Mosquitocidal and Oviposition Deterrent Effects in Medicinal and other Plant Extracts on *Culex pipiens* L.

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ABSTRACT. Mosquitocidal effect of neem seed kernels, *A .indica, R. stricta, H .bacciferum, S .aromaticum* cloves and orange peels extracts were tested on *Culex pipiens* L. All materials significantly deterred oviposition at 0.05 and 0.1% concentrations of water or methanol extracts. Up to 100% inhibition of oviposition was obtained in 0.05% *A .indica* and *R. stricta* aqueous or methanol extracts; 0.05% citrus peels methanol extract and 0.1% methanol extracts of all materials.

Acute LC_{50}s were 270, 950, 941 and 1131 ppm, water extract, for *R. stricta, A. indica, H. bacciferum* and *S. aromaticum,* respectively. Chronic toxicities caused by *R. stricta* at 0.02 and 0.04% concentrations, reached up to 70% and 100% larval mortality, respectively. All materials conferred significant negative influence on larval development, consequently reducing pupation and adult emergence. *R. stricta* and *S. aromaticum* caused severe impact on development where only 10 and 16.7% of the larvae reared in media containing 200 ppm of their water extracts completed their development to pupal stage, and none reached adulthood.

Application of such compounds, if proved environmentally safe, to mosquito breeding sites may have great practical significance on management of *C. pipiens* field populations.

Introduction

Most mosquito control programs target larval stages with larvicides at their breeding sites, because adulticides may only reduce adult populations temporarily allowing for rapid upsurges within few days. Kettle (1995) pointed out that control of immature stages of mosquitoes has traditionally been achieved by the application of chemical insecticides in solution in oils, as emulsions, wet-

table powders or dusts. Great risks are involved in treating peridomestic water, especially in rural areas, with insecticides as this water is used for domestic purposes, however, it provides excellent breeding sites for mosquitoes.

Moreover, due to the dramatic increase in resistance of mosquitoes to chemicals in absence of new alternative compounds, thoughts have been directed towards finding better alternative means of control. The urge for this has increased, especially after recent public awareness of potential hazards emanating from the widespread use of insecticides (Busvine and Pal 1969, Curtis and Pasteur 1981 & WHO, 1987). For example, environmental pollution has adverse effects on beneficial organisms particularly the environmentally safe forms used in biological control of pests.

One of these alternatives is the use of compounds extracted from plants such as botanical insecticides, being cheap, environmentally safe and have low mammalian toxicity (Arnason *et al.* 1989 & Isman 1994). A considerable number of plant derivatives have been shown to be effective against a wide array of insect species, including mosquitoes (Schumutterer 1990, Wilps 1995 Amorose 1995 & Elhag *et al.* 1996). Among these are seeds and leaves of the neem tree, *Azadirachta indica* A. Juss (Ascher 1993, Schumutterer 1990 & Stark *et al.* 1990) aerial parts of harmal, *Rhazya stricta* Decaisne; and ramram, *Heliotropium bacciferum* (Forssk.) (Migahid 1978, Elhag *et al.* 1996); cloves, Syzygeum *aromaticum* (L.) (Hassanali *et al.* 1990 & Caledrone *et al.* 1991); and orange peels (Don-Pedro 1985). *R. stricta* and *H. bacciferum* are two herbs widely distributed in Saudi Arabia (Migahid 1978), both being reputed in folk medicine, the former for treatment of syphlis, chronic rheumatism and similar types of pains, and the latter as emetic (Al-Yahya *et al.* 1990). Previous work with extracts of *R. stricta* (Elhag *et al.* 1996) has shown that it caused impaired egg hatching, larval mortality and complete suppression of pupation in *C. pipiens*. Chatterjee *et al.* (1974), Rahman and Fatima (1982), Ahmad (1983) and Hassan *et al.* (1977) reported on the rich alkaloidal contents of different classes in *R. stricta*. The oviposition deterrence produced by *R. stricta* treated chickpea seeds on *Callosobruchus maculatus* (F.) has recently been documented by Elhag (1998).

A large amount of literature on the effects of neem on insects has been published in the last two decades (Schmutterer 1990), yet little is known about its effect on mosquitoes. However, Amorose (1995) reported on the toxic effects of neem oil and deoiled neem cake on 3rd and 4th larval instars of *C. quinquefasciatus* Say. Both pure azadirachtin and crude extracts were reported to inhibit feeding, metamorphosis, fecundity, and ovipositional behavior in many insect species (Gaaboub and Hayes 1984, Schmutterer 1990, Ascher 1993). The deterrent effect of neem on oviposition of insects has been proved in insects

(Singh and Srivastava 1983, Chen *et al.* 1996, Elhag *et al.* 1998), however, mosquitoes were not included in such aforementioned studies.

In this study, the oviposition deterrence of both aqueous and methanol extracts from five plant species: two wild herbs, *R. stricta* and *H. bacciferum;* neem seed kernels; *S. aromaticum* cloves and orange peels on female *Culex pipiens* mosquitoes was investigated in the laboratory. Toxic and negative effects of water extracts from the first four plant species on larval growth and development were also studied.

Materials and Methods

Extracts

Test materials were collected from the central areas of Saudi Arabia. Aerial parts of *R. stricta* and *H. bacciferum,* neem seed kernels, orange peels and *S. aromaticum* cloves were air dried in the laboratory, ground to a fine powder and extracted by methanol and warm distilled water at ambient temperatures. A gentle warming to 35-40ºC was sometimes found necessary, especially when the solvent was taken straight from the refrigerator. The powdered material was mechanically stirred for 2-3 hr with the appropriate solvent and filtered. Then the solvent was carefully removed by slow evaporation.

Insects

Adult females and second instar larvae were obtained from a laboratory strain of *C. pipiens* maintained on pigeon blood and 10% sucrose solution, at the College of Agriculture and Veterinary Medicine Research Center, KSU, Meleida, Saudi Arabia. Larval food consisted of fine bread crumbs and yeast, served in aged tap water.

Test procedure

Stock solutions of the plant materials were prepared by redissolving the extracts in warm distilled water, *i.e.* 0.5 g/ 100 ml. Different concentrations of 200, 400, 500, 600, 800 and 1000 ppm were prepared from the stock solutions. Ten second instar larvae were transferred from the culture into plastic yogurt cups of 8-cm dia. 10 cm deep, each containing 30 ml of the desired concentration of *R. stricta, H. bacciferum,* neem seed kernels or *S. aromaticum* extracts. Each treatment was replicated four times, with a water control. Larvae were fed ad libitum and kept at laboratory conditions, *i.e*. a temperature of 25 ± 3ºC, RH 60 ± 5% and 12:12 L: D hr photoperiod. Larval mortalities were assayed at 48 hr, 96 hr and 10 days after treatment. Larvae were considered either alive if clearly moving normally, or dead when no movement and no response to gentle prodding were observed. Percentage of successful pupation and adult emergence were determined by monitoring on daily basis until all adults in the control have

emerged. Data were analyzed using maximum likelihood procedures and the probit analysis (Finney 1971), and the effectiveness was expressed as LC_{50} values.

For oviposition deterrence tests, two concentrations, *i.e.* 500 and 1000-ppm oviposition media in addition to a water control were used. Orange peels extract was also used in this test. Caged male and female mosquitoes were allowed to feed on pigeon blood over-night. Glass beakers, *i.e.* 100-ml each containing the oviposition media from one type of extract and controls were introduced into the cage the night after. Thus each material's water or methanol extract was tested at three different times against a water control, in a completely randomized design with three replications. Beakers were removed the next morning and egg batches and total eggs laid were determined. Data were analyzed using ANOVA and the treatment means were separated using Duncan's (1955) multiple range test.

Results and Discussion

Toxic effects

The LC_{50} and 95% confidence limits *i.e.,* CL for each plant extract are shown in Table 1. Significant differences were indicated by failure of 95% CL to overlap. Percentages of mortalities are given in Table 2. Two and 10 days after treatment the acute and chronic LC_{50} for second instar *C. pipiens* larvae exposed to *R. stricta* were 270.2 and 190.1, respectively. At the lowest concentration tested *i.e.,* 200 ppm, chronic toxicities reached 70%, and up to 100% at 400 ppm (Table 2). After 2 and 4 days in the 1000 and 600-ppm concentrations larvae suffered up to 100% mortalities, respectively.

TABLE 1. LC_{50} values and 95% confidence limits for *Culex pipiens* larvae reared in media containing aqueous extracts from four plant materials.

Material	Assay time (days)	Slope	LC_{50} (95% CL)	Prob.
Rhazya stricta	2	4.05	270.2 (223.9 - 325.9)	0.9
	4	5.76	241.3 (207.6 - 280.4)	0.9
	10	1.70	190.1 (170.3 - 222.3)	0.0
Azadirachta indica	2	2.50	949.9 (708.4 - 1386.9)	0.2
	4	2.17	568.6 (478.5 - 675.7)	0.5
	10	1.86	343.7 (260.5 - 489.8)	0.0
Heliotropium bacciferum	2	2.08	941.2 (676.8 - 1310.6)	0.2
	4	2.57	610.0 (502.0 - 741.3)	0.1
	10	2.47	429.9 (347.4 - 531.8)	0.3
Syzygeum aromaticum	2	2.86	1130.7 (838.9 - 1525.2)	0.0
	4	2.21	1075.5 (748.6 - 1547.5)	0.0
	10	2.19	889.4 (666.7 - 1187.7)	0.0

TABLE 2. Percentage of mortalities, successful pupation and adult emergence of *Culex pipiens* larvae reared in media containing water extracts from our plant materials.

Plant material	Conc. ppm	% Mortality after			% Successful pupation or adult emergence	
		2d	4d	10d	P	Ad
Rhazya stricta	200	30	33.3	70.0	10.0	0.0
	400	76.7	86.7	100	0.0	0.0
	600	90.0	100	100	0.0	0.0
	800	96.7	100	100	0.0	0.0
	1000	100	100	100	0.0	0.0
	Cont.	0.0	0.0	0.0	100	100
Azadirachta indica	200	10.0	13.3	46.7	20.0	13.3
	400	13.3	26.7	46.7	20.0	10.0
	600	26.7	46.7	50.0	13.3	6.7
	800	50.0	70.0	77.0	6.7	3.3
	1000	50.0	80.0	90.0	3.3	3.3
	Cont.	0.0	0.0	6.7	100	6.7
Heliotropium bacciferum	200	13.3	16.7	26.7	20.0	16.7
	400	16.7	23.0	43.0	26.7	10.0
	600	20.0	43.0	53.0	16.7	10.0
	800	53.0	67.7	80.0	10.0	0.0
	1000	57.7	77.0	86.7	3.3	0.0
	Cont.	0.0	0.0	3.3	100	100
Syzygeum aromaticum	200	3.3	10.0	13.3	16.7	0.0
	400	6.7	13.3	16.7	6.7	0.0
	600	10.0	16.7	20.0	3.3	0.0
	800	33.3	40.0	46.7	3.3	0.0
	1000	50.0	56.7	66.7	0.0	0.0
	Cont.	0.0	0.0	3.3	96.3	90.0

Extracts from *A. indica* were less toxic than *R. stricta*, where the acute and chronic LC_{50} values were higher, being 949.9 and 343.7, respectively. A maximum of 90% mortality was reached only in the 1000 ppm after 10 days. *H. bacciferum* evoked more-or-less similar effects comparable to those obtained for neem. The acute and chronic LC_{50} were 941.2 and 429.9, respectively. At the 1000 ppm the highest mortality (86.7%) caused by *H. bacciferum* occurred after 10 days.

The toxic effect of *S. aromaticum* cloves on *C. pipiens* larvae was moderate in comparison with other materials tested. Its acute and chronic LC_{50} values ranged between 889 and 1131 ppm, respectively. In the 1000-ppm concentration, the highest mortality (66.7%) was obtained after 10 days.

Extracts of the four plant species investigated in this work have shown remarkable bioactivity in relation to toxicity and retardation of growth and development of the larvae of *C. pipiens*. These effects were found to be most pronounced in the extracts of *R. stricta,* which caused highest rate of mortality, compared to neem, *H. bacciferum* or *S. aromaticum.* Thus the extract of *R. stricta* caused acute toxicity at 600 ppm concentration leading up to 90% larval mortality over a period of 48 hr, and 100% after 96 hr exposure. On the other hand, toxicity of the three others just increased above 50% larval mortality at even relatively high concentration of 900 ppm.

A very striking observation was that the length of exposure time of all plant extracts resulted in increased mortality until the 10th day, indicating that *C. pipiens* larvae cannot tolerate long exposures to such plant materials, especially *R. stricta* and neem.

Previous work with aqueous extracts of *R. stricta* (Elhag *et al.* 1996) has shown similar toxicity to *C. pipiens* in that it caused impaired egg hatching, larval mortality and complete suppression of pupation. Chatterjee *et al.* (1974), Rahman and Fatima (1982), Ahmad *et al.* (1983) and Hassan *et al.* (1977) reported on the rich alkaloidal contents of different classes in *R. stricta* such as rhazine, sewarine, strictalamine, rhazimal, rhazimol and others. This herb which is locally known as harmal is distributed throughout Saudi Arabia (Migahid 1978), and is reported to be used for the treatment of syphilis, chronic rheumatism and for other types of pains by the folk medicine practitioners (Al-Yahya *et al.* 1990). The phytochemical analysis of *R. stricta* (aerial parts) revealed the presence of alkaloids, flavonoids, tannins, sterols and/or triterpenes, volatile bases and volatile oil. Its ethanol and chloroform extracts were both highly toxic to Brine shrimps, with LC_{50} values of 62.10 ppm and 348.7 ppm, respectively (Al-Yahya *et al.* 1990). The present results in this respect are in agreement with these findings.

Little is known about the effect of neem on mosquitoes inspite of the vast amount of information on its effects on insects. However, Amorose (1995) reported LC_{50} values of neem oil and deoiled neem cake against 3rd and 4rth larval instars of *C. quinquefasciatus* as 0 .99 and 1.20 ppm; and 0.55 and 0.72 ppm, respectively. Stark (1996) suggested that different formulations of neem do not produce the same level of control of certain pest species when applied at equivalent levels of azadirachtin.

The toxic action of *H. bacciferum* was similar to that of the neem extract. Elhag *et al.* (1996) obtained $LC_{50} \approx 1000$ ppm of its aqueous extract against 3rd instar larvae of *C. pipiens.* This herb, locally known as Ramram, is widely distributed throughout the Central and Eastern parts of Saudi Arabia (Migahid 1978). Al-Yahia *et al.* (1990) reported that the folk medicine practitioners use it

for treatment of dog bite and skin diseases. Its aerial parts contain alkaloids, flavonoids, tannins, sterols and/or triterpenes, volatile oils and volatile bases. *S. aromaticum* cloves produced very low toxicity values with the acute and chronic LC_{50} values ranging between 1130.7 and 889.4 ppm; respectively. Hassanali *et al.* (1990) used cloves as grain protectants against weevil attack, depending on their repellency to adults. However, Caledrone *et al.* (1991) reported significant acaricidal properties in clove oil.

In respect of their toxicity, *R. stricta* and neem are the two most promising products that may have practical potential to manipulate mosquito larvae.

Effect on development

The data obtained on the effects of the four plant materials on growth and development of *C. pipiens* larvae, revealed that no further larval development took place beyond the second instar in concentrations of 400 ppm and above in the *R. stricta* extract. Only 10% successful pupation occurred in the 200 ppm, but no adult emergence was observed in any of the concentrations tested.

The proportion of individuals pupating in the neem extracts was relatively higher than in the *R. stricta* treatment, the lowest being 3.3% in the 1000-ppm concentration. Adult emergence in the neem treatments ranged between 3.3 and 13.3% in the highest and lowest concentrations, respectively.

H. bacciferum produced somewhat similar results as those of neem. However, it completely suppressed adult emergence at the 800 and1000 ppm levels.

S. aromaticum cloves gave better results than both neem and *H. bacciferum,* in percentage of successful pupation and adult emergence. In fact, development to the adult stage was completely arrested at all concentration levels, whereas only 6.7% pupation took place at clove concentrations as low as 400 ppm.

All four materials tested conferred significant negative influence on larval development, to varying degrees, consequently reducing pupation and inhibiting adult emergence. In their lowest concentrations, *R. stricta* and *S. aromaticum* cloves produced most remarkable impact on larval development, where only 10 and 16.7% of the larvae, respectively, completed their development to the pupal stage.

Of most interest, is the fact that they completely inhibited adult emergence in all concentrations. For *R. stricta* the results could be attributed to its toxic effects on the larvae, discussed above. Similar results were obtained by Elhag *et al.* (1996) who found that 100-ppm concentration of its aqueous extract caused larval mortality and affected pupation and adult emergence. The complete inhibition of adult emergence by *S. aromaticum* extracts, even at its lowest con-

centration, 200 ppm, inspite of its mild toxic action, is of interest because it suggests a different mode of inhibitory action, probably peculiar medium effects, disturbing normal sequence of development.

Neem extract affected pupal formation and adult emergence, but at higher concentrations. Stark *et al.* (1990) obtained complete inhibition of adult emergence of *Dacus dorsalis* Hendel and *Ceratitis capitata* Wied. At 14 ppm azadirachtin, and 10 ppm for *D. cucurbitae* Coq. when late 3rd larval instars or pupae were exposed to it. Larval growth of *C. capitata* was prolonged and pupal size was reduced when larvae were reared on a medium containing neem (Stephens and Schumutterer 1982). In this study, pupation and adult emergence were reduced by 80.0%, and 87%, respectively, when larvae of *C. pipiens* were reared in a medium containing as low as 200 ppm aqueous neem seed extract,. However, the corresponding toxicity after 10 days was only 47% mortality, as indicated by the data in Table 2. The negative effects of neem extracts on pupation and adult emergence may be due to its antifeeding or growth regulating effects on mosquito larvae, which resulted in arresting further development. Norris (1986) and Saxena (1987) explained that treating food materials with neem can disrupt insect feeding by making the treated material unattractive or unpalatable; and consequently, insect growth, survival and reproduction are adversely affected. Neraliya and Srivastava (1996) found that crude petroleum-ether-acetone extracts of 17 plants possess growth regulatory activity on late 3rd larval instars of *C. quinquefasciatus.* This activity severely affected molting and metamorphosis like production of larval-larval, larval-pupal and pupal-adult intermediates.

Effect on oviposition

Results on the oviposition deterrence effects caused by the aqueous and methanolic extracts from the five plant materials are given in Table 3. Oviposition was completely suppressed by both concentrations of water and methanol extracts of neem and *R. stricta,* and by the methanol extracts of all materials, except for the 500-ppm level of *H. bacciferum.* All material extracts and levels tested significantly reduced oviposition compared with the untreated controls.

Females oviposited insignificant numbers of eggs in media containing low concentrations of water and methanol extracts from *H. bacciferum,* and in both concentrations of the orange peels.

Remarkable results were obtained on oviposition deterrence by all materials tested. Up to 100% reduction in oviposition was obtained when water or methanol extracts from neem, *R.stricta* or cloves, in as low as 0.05% concentrations, were incorporated into the females' oviposition media. It is uncertain whether reduced oviposition rates on treated water have resulted from the repellent ac-

tion of the plant chemicals or from non-volatile components detected by the female's ovipositor as a stimulus to reduce oviposition. Chen *et al.* (1996) found that a concentration as low as 0.2% diethyl ether neem extract effectively deterred oviposition of *Bactrocera dorsalis* (Hendel) in treated guava fruits. They argued that the presence of ovipositor stings on the fruits implies that the neem extracts may act not only as olfactory cues for preventing landing of the flies on the treated fruits, but also as nonvolatile components detected by the ovipositor as a signal to reduce egg-laying. In this study, many adult mosquitoes were found dead on the liquid surface, including the controls, probably implying that detection of the chemical components in ovipositon substrates is a function of the ovipositor rather than due to olfactory cues that prevent landing.

TABLE 3. Oviposition deterrent effects of five plant aqueous and methanolic extracts on *Culex pipiens* Mosquitoes.

Material and Conc. (ppm)		No. eggs laid (Mean ± S.D.)	
		Aqueous	Methanolic
control		1290.0 ± 316.3 a (3.0)	1485.0 ± 170.9 a (3.7)
Azadirachta indica	500	0.0 d	0.0 b
	1000	0.0 d	0.0 b
Heliotropium bacciferum	500	256.7 ± 137.2 b (1.7)	43.7 ± 61.7 b (0.3)
	1000	0.0 d	0.0 b
Rhazya stricta	500	0.0 d	0.0 b
	1000	0.0 d	0.0 b
Syzygeum aromaticum	500	15.0 ± 21.2 (0.3)	0.0 b
	1000	0.0 d	0.0 b
Citrus peels	500	293.7 ± 74.4 b (1.3)	N.T.
	1000	98.7 ± 71.5 c (0.7)	N.T.

Numbers between parenthesis = No. of egg batches.
Means within a column followed by different letters are significantly different, $P < 0.05$, Duncan's Multiple Range Test.
N.T. = No tested.

In this current study, the plant materials having the greatest negative effects on *C. pipiens* mosquitoes' growth and development are *R. stricta* and *S. aromaticum.* Although the toxic mode of action of *R. stricta* in insects is not yet

known, it might be attributed to its growth retarding influence, due to its content of biologically active alkaloids. The action of *S. aromaticum* was manifested mainly in its growth and development inhibitory effects, and not in its toxic action as indicated by the low mortality rates evident in its extracts. Although antifeeding effects cannot be assayed in the case of mosquito larvae, yet it can not be ruled out in the case of *S. aromaticum,* neem and *H. bacciferum.* This is because even at low concentrations of 200 ppm, of all three materials, which caused very low 48 hr mortalities ranging between 3.3 and 30%, only 10-20% pupation and 0.0-16.7% adult emergence took place, compared with 96.3-100% and 90-100% in the control, respectively. The high delayed mortalities *i.e.,* after 10 days, in extracts of neem and *H. bacciferum* may be a consequence of antifeedant action, or interference with other vital processes. Among the materials tested, *R. stricta* and *S. aromaticum* may be considered the best suppressants of mosquito development that may have great potential in reducing outdoor larval populations.

Conclusion

The importance of this oviposition deterrence, toxic and/or growth and development retarding influence of the tested materials will have greater practical significance if such effects can be observed when applied to natural habitats. Such materials could then have practical application in mosquito larval breeding sites such as peridomestic water, rain-water collection areas, ditches, tree holes, artificial containers etc., in rural as well as urban localities where treatment areas are limited, and, therefore, manageable. Moreover, these materials are cheap, safe, act on a broad spectrum of insects, and much more easy to handle than synthetic insecticides. However, the broad-spectrum activity of these products is an undesirable trait when IPM programs are involved.

More investigations are needed to clarify the identity of their biologically active components, and mode of action on mosquitoes and proof for their human and environmental safety, notably on natural enemies. The adverse effects obtained by Lowery and Isman (1994) *i.e.,* 50-100% reduction in pupation and adult emergence in two aphid predators, *Coccinella undecimpunctata* (L.) and *Eupodes fumipennis* (Thom.) on canola plants treated with neem in greenhouse tests, is a striking example of the evil effects of these materials. Greenhouse and field trials may be necessary to determine the full impact of such products on beneficial insects.

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الأثر الرادع لمستخلصات نباتات طبية وغيرها على بعوض *كيوليكس بيبينز ل .*

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المستخلص . كان لمستخلصات بذور النيم ، *A. indica* ، الحرمل *R. stricta* ، الرمرام *H. bacciferum* ، القرنفل *S. aromaticum* وقشور البرتقال أثر معنوي مانع لوضع البيض عند تركيز ٠,٠٥ و ٠,١٪ من المستخلص المائي أو الميثانولي ، ووصلت نسبة المنع ١٠٠٪ عند تركيز ٠,٠٥٪ للمستخلصين المائي و الميثانولي للحرمل و النيم ، ٠,٠٥٪ للمستخلص الميثانولي لقشور البرتقال و ٠,١٪ للمستخلص الميثانولي لكل المواد .

بلغت قيمة ت. م ٥٠ (LC_{50}) عقب ٤٨ ساعة ٢٧٠ ، ٩٥٠ ، ٩٤١ و ١١٣١ ج. م أي جزء في المليون للحرمل ، النيم ، الرمرام و القرنفل ، على التوالي . و عند تركيزي ٠,٠٢ و ٠,٠٤٪ بعد ١٠ أيام من المعاملة كان لنسبة السمية المزمنة على اليرقات التي نتجت عن مستخلص الحرمل المائي تأثير ٧٠ و ١٠٠٪ موت ، على التوالي .

تسببت كل المواد المستخدمة في إنتاج تأثيرات سلبية معنوية على تنامي اليرقات ، و بالتالي في خفض نسبة التعذير و خروج الحشرات الكاملة .

هذا ولقد كان لمستخلصي الحرمل و القرنفل الأثر الأبلغ حيث نجحت ١٠ و ١٦,٧٪ فقط من اليرقات المرباة في تركيز ٠,٠٢٪ من مستخلصيهما المائيين في بلوغ طور العذراء ، بينما لم يبلغ أيًا منها طور الحشرة الكاملة .

إن إضافة مثل هذه المركبات ، بعد التأكد من سلامتها للبيئة ، إلى التجمعات المائية ومواقع التربية الطبيعية للبعوض فائدة عظيمة في الإقلال من تكاثره .