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The Effect of Some Plant Extracts on Mosquito *Aedes aegypti* (L.)

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In this study, the biological effects of various concentrations of *Melia azedarach, Rhazya stricta, Jatropha curcas, Artemisia herba alba, Calotropis procera, Matricharia chamomella* and Diflubenzuron were assayed on an *Aedes aegypti* (L.) test population under controlled laboratory conditions. Concentration levels of responses were evaluated. Characteristics such as IC_{50} and IC_{90} the susceptibility of immature stages to these plant extracts and insect growth regulator and their accumulation effects were studied. The percentage mortality of the fourth instar of *Ae. aegypti* larvae increased significantly with latex concentrations, indicating a direct relationship between the concentration and different effects. The larval mortalities ranged between low or moderate. According the mode of action of different plant extracts and Diflubenzuron did not appear to give high percentage of mortality against larval stages, although in most cases a clearly delayed inhibition of adult emergence was noted. The survival pupae percentage that produced from treated with different concentrations indicated that increased significantly of pupal survival due to decreasing the concentrations. There were significantly larval mortality and inhibition adult emergency percent in the treated groups compared to the control group. The characteristics investigated here indicate that this plant extracts and insect growth regulators are effective alternatives for controlling the dengue vector.

Key words: *Aedes aegypti*; Juvenile Hormone; Plant Extracts; Insect Growth Regulators.

Mosquito (Diptera: Culicidae) presents an array of insects which more than any other group poses the greatest challenge to human and veterinary health as vectors of diseases (Guzman *et al*., 2010). One such insects, which share a close ecological niche with man is the mosquito, *Aedes aegypti* (L.). Worldwide, mosquitoes are a major public health problem. They are estimated to transmit diseases to more than 700 million people annually and are predicted to be currently responsible for the deaths of about one in 17 people (WHO, 2005). *Aedes aegypti* (*Stegomyia aegypti* sensu Reinert *et al*., 2004) is considered to be a vector of dengue fever, a disease endemic to South East Asia, Africa, and the Americas (Maillared *et al*., 1993; Amarasinghe and Letson; 2012, Aziz *et al*., 2014). The incidence of dengue fever has increased fourfold since 1970, and nearly half the world's population is now at risk. In 1990, almost 30% of the world population (1.5 billion people) lived in regions where the estimated risk of dengue transmission was greater the 50% (Hales *et al.*, 2002).

Control measure against this vector in the short-term is the use of conventional insecticides (Cao *et al*., 2006; Malik *et al*., 2007). This chemical

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control depends mainly on application organophosphates and IGR (Yang *et al*., 2002). Although application of insecticides to control dengue mosquito vector has shown knock-out effect during the outbreak, several environmental and health issues have been emerged during application of insecticides (Rahuman *et al*., 2009). This includes toxicity of non-target organisms as well as increasing the burden to the environmental and human health (Lee *et al*., 2001). On the other hand, the mosquitoes always develop resistance to the applied insecticides (Macedo *et al*. 1997, (Mahyoub, 2011). Eventually, this issue urges searching for novel and natural insecticides. Therefore, developing natural insecticides will help in reducing the adverse effect of chemicals on the environmental and human health (Ansari *et al*. 2000).

In this context, several researchers have conducted experimental studies on application of alternative insecticides resources which have minimal or absent undesirable effect on environment and human health. The extracts and/ or essential plant oils have been tested against insects and mosquito vectors. The biological agents are easily degradable into less or nontoxic compounds and proven to be safely used for mosquito control programs. In the literature, several experiments were carried out to examine the effect of plant extracts or essential oils against mosquito larvae and showed positive results (see Sharma *et al*. 2006; Rasheed *et al*. 2005; Amer and Mehlhorn 2006a, b; Rahuman *et al*. 2008a, b, c, d).

Based on recent reports on dengue from the Middle East, there is noticeable growing in the dengue incidence especially in Saudi Arabia. According to Aziz *et al*. (2014), a total number of 4411 dengue cases were reported in this area. This high number of cases is associated with remarkable failure in recent control strategies. Therefore, the present study aims to examine the biological effects of different alcoholic novel plant extracts as juvenile hormone analogues comparing with Diflubenzuron on growth stages of the mosquito *Ae. aegypti*.

MATERIALS AND METHODS

Test trials experiments were carried out at dengue mosquito research station in King Abdulaziz University, Jeddah (Saudi Arabia).

Mosquito culture

A field strain of *Ae. aegypti* was used in the present study. The parental strain was raised from wild larvae, collected from Al-Balad, Jeddah governorate, Saudi Arabia, and maintained under laboratory conditions of 27±1°C and 70±5% R.H., with 14:10 (L:D). The larvae were fed on a diet of fish food or dried bread powder and dried milk in the ratio of 1:1. Newly formed pupae were transferred from the trays to a cup containing water and placed in screened cages (60×30×45 cm) where the adult emerged. The adults were continuously provided with 5% sucrose solution mixed with zincovit vitamin drops in a jar with a cotton wick. On day four post emergence the adult females were deprived of sugar for 12 hours then provided with a shaved pigeon placed in resting cages overnight for blood feeding. Wet filter paper was placed on the corners for egg laying and the lifecycle was repeated. The larvae were reared until pupation and adult emergence took place for maintaining the stock culture (Morlan *et al.*, 1968).

Plant extraction

Fresh leaves or peels of some plants (*Melia azedarach, Rhazya stricta, Jatropha curcas, Artemisia herba alba, Calotropis procera, Matricharia chamomella*) were washed and shade dried at room temperature and prepared to extract. The effective ingredients were calculated using standard methods according to Eidi *et al*. (2005). Forty to sixty grams of leave/peels tissues were finely ground and loaded to a 250 ml glass stoppered of Soxhlet apparatus. Absolute acetone (200 ml) was added to the glass and the extraction was performed for 6 hours. The extracts were concentrated using a rotary evaporator to become semi-dry material .The extracted components was kept at -10°C until be used for testing against selected insect stages. Extraction was carried out according to the procedure of Warthen *et al.* (1984).

Preparation of stock solution

The stock solution of each plant extract was prepared by adding 1 ml of it to 99 ml of distilled water containing 0.5% triton X-100 as an emulsifier to ensure complete solubility of the extract in water. Series of concentrations (ppm) were prepared in distilled water according the following formula:

$$\textbf{Ppm} = \frac{\text{Conc.} \times \text{Weight}}{\text{Volume}} \times 10^6$$

Larval bioassay

Larval susceptibility tests were conducted according to the method of WHO (2005)**.** Treatments were carried out by exposing early fourth instar larvae of *A. aegypti* to various concentrations of the test compounds, in groups of waxed paper cups (400 ml capacity) containing 300 ml of tap water. Five replicates of 20 larvae per concentration, and so for the control trials were set up. The larvae were given the usual larval food during the tests. Cumulative mortalities of larvae and pupae as well as the adult emergence were recorded daily.

Statistical analysis

Larval mortalities were recorded daily. Live pupae were transferred to untreated water in new beakers for further observation. Partially emerged adults or these found completely emerged but unable to leave the water surface were recorded and scored as dead. Therefore the biological effect of the test plant extract and IGRs were expressed as the percentage of larvae that do not develop into successfully emerging adults, or the inhibition of adult emergence (WHO, 2005) The inhibition of adult emergence – concentration – probability line (IC – p line) was drawn for each extract using the method of (Finney, 1972). The criterion used to evaluate the biological effects of these plant extracts and IGRs were the median inhibitory concentration of adult formation (IC_{50}).

RESULTS AND DISCUSSION

The data presented in Tables (1 and 2), exhibit that the effects of the leaves alcoholic extract of *C. procera* against the 4th larval instars of *Ae. aegypti* with different concentrations (30-150 ppm). The percentage mortality of fourth instar larvae of *Ae. aegypti* increased significantly with concentration of the extract. The larval mortalities ranged between 15 to 68% according the concentrations from 30-150 ppm, respectively. According the mode of action of *C. procera* tested plant extract did not appear to give high percentage of mortality against larval stages, although in most cases a clearly delayed inhibition of adult emergence was noted as follow. Therefore, in the present work, cumulative mortality during larval development to pupae and adults has been taken as a criterion for evaluating the efficacy of such compounds as they have more juvenilizing effect than toxic mode of action (WHO, 2005).

The survival pupae percentage that produced from treated with different concentrations (30-150 ppm) of alcoholic extract of *C. procera* indicated that increased significantly of pupal survival due to decreasing the concentration of the extract. The survival pupae percentage reached 85, 69, 62, 49 and 32 as results of treated with 30, 60, 90, 120 and 150 ppm, respectively (Table 1). The obtained data indicated that the adult hatched percent ranged between 7-82 with different concentrations which ranged between 30-150 ppm, also the adult hatched percent increased with decreased the plant extract concentrations (Table, 1). Inhibition of adult emergency percent reached to 92.55 when using with 150 ppm, while reached 12.77 with 30 ppm. The obtained results are harmony with those obtained by Ramos *et al.* (2006) indicated that *C. procera* (Asclepiadaceae) is a well-known medicinal plant with leaves, roots, and bark which cause 100% mortality of 3rd instars of *Ae. aegypti* within 5 min with whole latex. Both fractions (water-soluble dialyzable and non-dialyzable rubber-free materials) were partially effective to prevent egg hatching and most of individuals growing under experimental conditions died before reaching 2nd instars or stayed in 1st instars. Whereas, were very toxic to 3rd instars causing 100% mortality within 24 h.

The required values, i.e. IC_{50} and IC_{90} are presented in Table 1 and Figure 1. Data given summarized the susceptibility of field strains of the 4th instar larvae of *Ae. aegypti* to the tested plant extracts. The results clearly showed that *C. procera* had IC_{50} of 70.8909 ppm, while LC_{90} was 160.8194 ppm against field strain. The slope of line is useful to known the homogeneity of stages of *Ae. aegypti* population, which reared under laboratory conditions. When the population of mosquito is similar in homogeneity or the degree of resistant meaning the slope is big or increase in regression, also, when tabulated $(Chi)^2$ larger than calculated at 0.05 level of significance indicates the homogeneity of results. Data in Table 1 and Figure 1 show that the slope of field stain of the 4th larval stages of *Ae. aegypti* population when using *C. procera* was 3.6062. Also results indicated that the tabulated X^2 $(Chi)^2$ was 6.7516, while calculated

Table 1. Biological effects of different leaves alcoholic extracts on different growth stages of the mosquito *Ae. aegy*pti.

Conc. (ppm)	Larval mortality (%)	Survival pupae (%)	Adults hatched (%)	Inhibition of adults emergency (%)	
a) Calotropis procera					
30	15	85	82	18	12.77*
60	31	69	60	40	36.17
90	38	62	43	57	54.26
120	51	49	16	84	82.98
150	68	32	7	93	92.55
Control	3	97	94	6	-
b) Melia azedarach					
20	4	96	80	20	20
30	7	93	58	42	42
40	9	91	36	64	64
50	12	88	25	75	75
60	16	84	9	91	91
Control	3	97	96	4	0.0
c) Rhazya stricta					
200	8	92	86	14	14
400	11	89	68	32	32
600	19	81	34	66	66
800	22	78	21	79	79
1000	36	64	7	93	93
Control	2	98	96	4	0.0
d) Artemisia herba alba					
300	15	85	78	22	22
500	23	77	58	42	42
700	41	59	41	59	59
900	60	40	22	78	78
1200	78	22	5	95	95
Control	3	97	96	4	0.0
e) Matricharia chamomilla					
100	16	84	70	30	30
150	33	67	46	54	54
200	40	60	32	68	68
250	52	48	21	79	79
300	69	31	10	90	90
Control	2	98	97	3	0.0
f) Jatropha curcas					
500	9	91	75	25	25
800	14	86	52	48	48
1100	20	80	25	75	75
1300	28	72	13	87	87
1500	73	27	6	94	94
Control	3	97	96	4	0.0
G) Diflubenzuron					
0.0001	3	97	75	25	19.35
0.0003	13	87	40	60	56.99
0.0006	16	84	26	74	72.04
0.0009	21	79	18	82	80.65
0.002	30	70	8	92	91.40
Control	3	97	93	7	0.00

Used 5 replicates (20 larvae/replicate).

*Used the equation Abbott (Abbott, 1987) to correct the percentage of inhibition in treatments, according to those in control (untreated).

X^2 $(Chi)^2$ was 7.8.

M. azedarach was tested with different concentrations (20-60 ppm) against the 4th larval instars of *Ae. aegypti*. The percentage mortality of the 4th instar larvae were very low ranged between 4 to 16% according the concentrations from 20-60 ppm. It is evident that all concentration of extract showed low larvicidal effect. The survival pupae percentage of *M. azedarach* indicated that increased significantly of pupal survival due to decreasing the concentration of the extract. The survival pupae percentage reached 96, 93, 91, 88 and 84 as results of treated with 20, 30, 40, 50 and 60 ppm, respectively (Table 1). The obtained data indicated that the adult hatched percent ranged between 9-80 with different concentrations which ranged between 20-60 ppm meaning the adult hatched percent increased with decreased the plant extract concentrations (Table 1). Inhibition of adult emergency percent reached to 91 when using 60 ppm, while reached 20 with 20 ppm.

The required values, i.e. IC_{50} and IC_{90} are presented in Tables (1) and Fig. (1). Data given summarized the susceptibility of field strains of the 4th instar larvae of *Ae. aegypti* to the tested plant extract. The results clearly showed that *M. azedarach* gave IC_{50} 32.6223 ppm, while IC_{90} was 64.9666 ppm against field strain. The slope of regression line on field stain of the 4th larval stages of *Ae. aegypti* population when using *M. azedarach* was 4.2838. Also results indicated that the tabulated X^2 $(Chi)^2$ was 2.5048, while calculated X^2 $(Chi)^2$ was 7.8. The obtained results are agreed with those obtained by Selvaraj and Mosses (2011) indicated that both seed and leaf extracts of *M. azedarach* gave significant larval mortality in all larval stages of *Ae. aegypti*. The first and second instar larvae were more susceptible to all concentrations of leaf and fruit extracts. When compared to the first two instar stages, the third and fourth instar larvae of the three mosquito species exhibited lower mortality when exposed to

Table 2. Susceptibility of the 4th larval stage of *Aedes aegypti* (L.). to different juvenile hormone analogues

Analogue	Effective concentrations (ppm)	Larval mortality (%)[a]	Statistical parameters[b]				
			LC_{50} (ppm)	LC_{90} (ppm)	Slope	X^2 $(Chi)^2$ C	T
***C**alotropis procera*	30-150	15-68	70.8909	160.8194	3.6026	9.7516	7.8
Melia azedarach	20-60	4-16	32.6223	64.9666	4.2838	2.5048	7.8
Rhazya stricta	200-1000	8-36	461.6986	1050.159	3.5910	7.7536	7.8
Artemisia herba alba	300-1200	15-78	542.5483	1214.099	3.6636	7.3041	7.8
Jatropha curcas	500-1500	9-73	753.2824	1445.174	4.5292	4.1193	7.8
Matricharia chamomilla	100-300	16-69	142.1649	325.8848	3.5573	1.10144	7.8
Diflubenzuron	0.0001-0.002	3-30	0.0003	0.0016	1.7108	1.7661	7.8

a: Five replicates, 20 larvae each; control mortalities ranged from 0.0%-3.0%. b: Litchfield and Wilcoxon (1949). When tabulated $(Chi)^2$ larger than calculated at 0.05 level of significance indicates the homogeneity of results
C = Calculated, T= Tabulated

all the concentrations (10, 20, 30, 40 and 50 ppm). *M. azedarach* extracts may be an effective larvicidal agent which could be used to control population *Ae. aegypti*.

Results in Tables (1 & 2), show that the leaves alcoholic extract of *R. stricta* gave low effective (8-36%) against the 4th larval instars of *Ae. aegypti* with different concentrations (200-1000 ppm). Larval mortality was 8% at 200 ppm increased to 36% at 1000 ppm after 24 h. According the mode of action of *R. stricta* tested plant extract did not appear to give high percentage of mortality against larval stages, although in most cases a clearly delayed inhibition of adult emergence was noted as follow. The survival pupae percentage that produced from treated with different concentrations (200-1000 ppm) of alcoholic extract of *R. stricta* indicated that increased significantly of pupal survival due to decreasing the concentration of the extract. The survival pupae percentage reached 92, 89, 81, 78 and 64 as results of treated with 200, 400, 600, 800 and 100 ppm,

respectively (Table, 1). The obtained data indicated that the adult hatched percent ranged between 7-86 with different concentrations which ranged between 200-1000 ppm, also the adult hatched percent increased with decreased the plant extract concentrations (Table, 1). Inhibition of adult emergency percent reached to 93 when using 1000 ppm, while reached 14 with 200 ppm. The obtained results are harmony with those obtained by El Hag *et al*. (1999) found that extracts of *A. indica, Rhazya stricta* and *Syzygium aromaticum* influence larval development by reducing pupation and inhibiting adult emergence. They also observed that there was no further development of the first instar to the second instar larvae of *Cx. pipiens* after being subjected to a 400.0 ppm methanol extract of *R. stricta*.

Also, three plant extracts; *A. herba alba*, *M. chamomilla* and *J. curcas* were tested with different concentrations; 300-1200, 100-300 and 500-1500 ppm, respectively comparing with Diflubenzuron (0.0001-0.002 ppm) against the 4th larval instars of *Ae. aegypti*,. The percentage mortality of the 4th instar larvae were ranged between 15-78, 16-69 and 9-73, respectively according the concentrations, while in case Diflubenzuron gave 3-30 percent morality. It is evident that all concentration of extract showed ranged between low and moderate larvicidal effects. The obtained results are agreed with those obtained by Chanthakan *et al*. (2012) compared the effects of the purified toxin with crude protein extracts from seed kernels of *Jatropha curcas* and *Ricinus communis*. The larvae of *Cx. quinquefasciatus* were more susceptible to the toxin and both extracts than the larvae of *Ae. aegypti*. After 24 hours of exposure, the extract showed larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* with (LC_{50}) values of 3.89 mg/ml and 0.0575 mg/ml, respectively. The results indicated that the crude protein extract and Jc-SCRIP were more toxic to the third instar larvae of *Cx. quinquefasciatus* than that of *Ae. aegypti*.

The survival pupae percentage of the three tested plant extracts indicated that increased significantly of pupal survival due to decreasing the concentrations. The survival pupae percentage ranged between 22-85, 31-84 and 27-91 for *A. herba alba*, *M. chamomilla* and *J. curcas*, respectively, whereas in case Diflubenzuron gave 70-97percent (Table 1). Due to the site effect of each plant extract and Diflubenzuron did not appear to give high percentage mortality against larval stages, but cumulative effects to pupae and adults, as shown that the inhibition of adult emergency ranged between 22-95, 30-90 and 25-94 due to the action of the three plant extracts, successfully while reached to 19.35-91.40 due to effects of Diflubenzuron (Table 1).

The required values, i.e. IC_{50} and IC_{90} are presented in Tables 1 and Figure 1. Data given summarized the susceptibility of field strains of the 4th instar larvae of *Ae. aegypti* to the tested plant extract and Diflubenzuron. The results clearly showed that *A. herba alba, M. chamomilla, J. curcas*, and Diflubenzuron gave IC_{50} 542.5483, 753.2824, 142.1649 and 0.0003 ppm, while LC_{90s} were 1214.099, 1445.174, 325.8848 and 0.0016 ppm, respectively against field strain. The slope of regression line on field stain of the 4th larval stages of *Ae. aegypti* population when using *A. herba alba, M. chamomilla, J. curcas*, and Diflubenzuron were 3.6636, 4.5292, 3.5573 and 1.7108, successfully. Also results indicated that the tabulated X^2 $(Chi)^2$ were 7.3041, 4.1193, 1.10144 and 1.7661, while calculated X^2 $(Chi)^2$ were 7.8 in four treatments respectively. Therefore, it can be inferred from the present study that the previous plant extracts may be an effective larvicidal agent which could be used to control populations of *Ae. aegypti*. Ikram and Farman (2013) evaluated the larvicidal activity of methanol extracts of roots, stem and leaves of *Artemisia vulgaris* against *Culex quinquefasciatus*. The LC_{50} value for roots extract was 9141.0 ppm, stem extract 2224.2 ppm and leaves extract 803.2 ppm. The findings of the present study presented the methanol extract of the leaves of *A. vulgaris* as a good source of preparations for pest control especially mosquito control. Also, Zhu and Tian (2013) found the total, 56 compounds extracted from *Artemisia gilvescens* corresponding to 98.20 % of the total oil were identified and the major compounds identified were camphor (13.49 %), eucalyptol (12.13 %), terpine-4-ol (9.65 %), germacrene D (8.62 %), caryophyllene oxide (4.65 %), and caryophyllene (4.29 %). Essential oil induced 8, 46, 80, 85, 94, and 100 % larval mortality at the concentrations of 25, 50, 75, 100, 125, and 150 mg/l and the LC_{50} and LC_{90} values were 49.95 and 97.36 mg/l, respectively. Among the

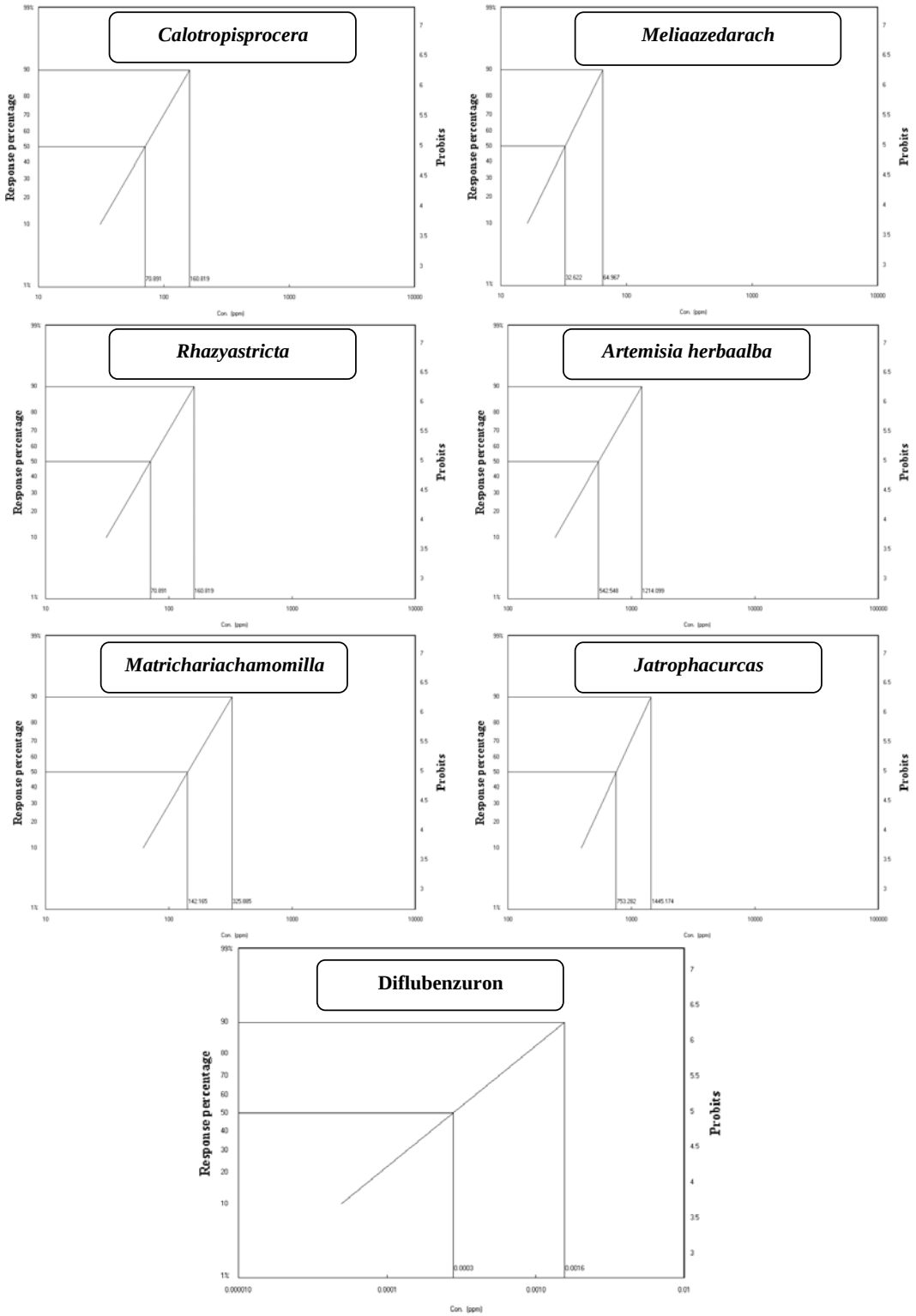

Fig. 1: Regression lines for different juvenile hormone analogues on the 4th larval stage of *Aedes aegypti* (L.)

six compounds, the most potent larvicidal compound were caryophyllene oxide, germacrene D, Terpine-4-ol, eucalyptol and caryophyllene.

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