INSECTICIDAL ACTIVITY OF *EUCALYPTUS* SP. VOLATILE OIL AGAINST BACKSWIMMER INSECT *ANISOPS SARDEA*

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ABSTRACT

The objective of this study was to evaluate a natural bio-insecticide manufacturing from *Eucalyptus* sp. volatile oil. The use of *Eucalyptus* sp. against the Backswimmer insect *Anisops sardea* Herrich-Schaeffer, 1849 predatory of larvae of common carp fish, *Cyprinus carpio* L., in artificial closed ponds in Babylon province represented a new idea in Iraq. The volatile oil of the *Eucalyptus* sp. was extracted by hot water method using the Clevenger, three concentrations of 250000, 450000 and 650000 ppm with benzyl benzoate as a stabilizer were used, which has a boiling point of 324^{O}C (slow evaporation) at field experiment.The results of field and laboratory experiments of the extracted volatile oil in different concentrations, showed that the lower concentration used, the longer exposure time should be, the lowest experimental concentration (250000) ppm was effective for killing half the number of LC50 insects in the field after 30 minutes, with less concentration LC50 killer for laboratory insects 20 minutes after exposure. While the killing rate did not exceed 2.7% at laboratory experiment and 1.5% at field experiment with regard to the effect of the volatile oil on fish larvae was at all times and for different experimental concentrations. A gas chromatography detection of the active compounds that found in the volatile oil of *Eucalyptus* was performed. The results showed that it contained the following compounds: sabinen (0.06%), terpinen (2%), camphenin (3.4%), lemonine (1.08%), myrcine (0.62%), alph-pinene (10%), linalool (0.027%), camphor (6.9%).

Keywords: *Eucalyptus* sp , *A. sardea. Cyprinus carpio*, natural insecticide

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الفعالية القاتلة للحشرات للزيت الطيار لليوكالبتوز .*Eucalyptus* sp ضد حشرة سابحات الظهر *ANISOPS SARDEA*

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المستخلص

تهدف الرسالة الى تقييم مبيد حيوي طبيعي مصنع من الزيت الطيار المستخلص من اوراق اشجار اليوكالبتوز. تعد استخدام الزيوت الطيارة لاوراق اليوكالبتوز ضد سابحلت الظهر ***Anisops sardea*** **Herrich–Schaeffer, 1849**، المفترسة ليرقات اسماك الكارب الشائعة. ***Cyprinus carpio*** **L**، في الأحواض المغلقة الاصطناعية لتربية الاسماك فكرة جديدة في العراق والتي استخدمت في محافظة بابل. استخلص الزيت الطيار من اوراق اليوكالبتوزبواسطة طريقة التقطيربالماء الحار باستخدام **Clevenger** ، تم استخدام ثلاثة تراكيز من مستخلص اليوكالبتوز **250000** و **450000** و **650000** جزء في المليون مع بنزل البنزوات كمثبت، والذي يحتوي على نقطة غليان تبلغ **324** درجة مئوية (ذو تبخر بطيء) في التجربة الميدانية. أظهرت نتائج التجارب الحقلية والمختبرية لمستخلص الزيت الطيار بتراكيز مختلفة، أنه كلما انخفض التركيز المستخدم، كلما طال وقت التعرض، كان أدنى تركيز تجريبي (250000) جزء في المليون فعال في قتل نصف عدد **LC50** الحشرات في الحقل بعد **30** دقيقة ،مع اقل تركيز، قاتل **LC50** للحشرات في المختبر بعد **20** دقيقة من التعرض.في حين أن معدل القتل لم يتجاوز **2.7** ٪ في المختبر و **1.5** ٪ في الحقل فيما يتعلق بتأثير الزيت الطيار على يرقات الأسماك كان في جميع الأوقات وللتراكيز التجريبية المختلفة، تم إجراء فحص كروماتوكرافي الغاز للمركبات الفعالة الموجودة في الزيت الطيار من اليوكالبتوز، اذ أظهرت النتائج أنه يحتوي على المركبات التالية: سابينين (**0.06** ٪)، تربينين (**2** ٪)، كامفينين (**3.4** ٪)، ليمونين (**1.08** ٪)، الميرسين (**0.62** ٪)، ألفا بنين (**10** ٪)، لينالول(**0.027** ٪)، كافور (**6.9** ٪).

الكلمات المفتاحية: زيت لليوكالبتوز، الحشرة المفترسة ***Anisops sardea*** . اسماك الكارب ***Cyprinus carpio*** مبيد طبيعي

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INTRODUCTION

Anisops sardea Herrich-Schaeffer 1849 (Hemiptera: Notonectidae) subfamily Anisopinae (33), is a small-bodied aquatic backswimmer found in high densities in many temporary pools and permanent water bodies throughout India, Which is found in high densities in late-season temporary pools (23, 31), as well as in permanent pools, this species usually live in temporal and permanent fresh, brackish and salt water ponds, lakes and coils with aquatic vegetation (4). Members of Notonectidae (Backswimmers) are very aggressive predators, attacking many pelagic and benthic invertebrates, including their own immature stages, invertebrates that fall onto the water surface (larvae and adults of various insects, mites, small crustaceans, annelids, and mollusks), and small vertebrates (fish larvae and amphibian tadpoles) (13, 19). In general, the genus *Anisops* Spinola, 1837 lives in permanent and brackish freshwater ponds, lakes and springs with aquatic plants; although they are primarily aquatic, they can fly well and thus can be easily transmitted to new habitats or other aquatic environments (9, 26, 31) and being predators can be a good species in the biological control of mosquitoes. Laboratory based predatory experiments have exposed that *A. sardea* have a high predation rate on larval *Culex* mosquitoes (41, 43). The species can be distinguished by a shorter, slender, and laterally slightly depressed body with total size (7.2–8.4 mm) (8, 21, 40). *A. sardea* has a wide distribution range including Africa (tropical region, Algeria, Egypt, Libya, Morocco, Tunisia), Asia (Armenia, Azerbaijan, Georgia, India, Iran, Iraq, Israel, Jordan, Lebanon, Myanmar, Saudi Arabia, Syria, Turkey, Turkmenistan, United Arab Emirates, Yemen) and Europe (Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, France (Corsica), Greek, Italy (incl. Sicily and Sardinia), Malta, Portugal, Spain, Slovakia, Southern Russia) (3, 10,11, 17, 18, 20, 22, 28, 33,34, 39). Plant materials with pesticide properties have traditionally been used around the world without the emergence of resistant species, plant pesticides, when compared with industrial pesticides, are safer for the environment and are generally less expensive and easy to manufacture and use (38, 14), so the need to use essential oils (volatile oils) was an alternative to control many field and house insects pests (35, 36, 37). Volatile oils are aromatic volatile hydrocarbons that give a pungent flavor to the plant and are produced through the secondary metabolic pathways of the plant. Volatile oils have many important functions of the plant, including, protecting the plant tissues from freezing and heat effect, attract or expel insects and use them as a defense against predators and against fungal infections, most essential oils have also been used as flavorings, food additives, perfume ingredients, cosmetics, plastic products and soap. Several studies have shown that the volatile oils, including the volatile oil of *Eucalyptus* sp. have a wide range of effect against insect pests and fungal plant pathogens ranging from insect killer, attractant, repellent, egg laying hindrance and against growth regulator activities (18, 30). The aim of this study was to evaluate the activity of volatile oil of *Eucalyptus* sp. as natural friendly bio-insecticide against *A. sardea* predators of larvae of common carp fish, *Cyprinus carpio* L., which cause losses according to field observations about (70-90) % of the production of these larvae in artificial fish ponds in closed ponds in Babylon province

MATERIALS AND METHODS

1- Preparation of fish larvae for transfer to the artificial pond: A muddy aquarium was selected from artificial fish pond in Babylon province / Al-Musayyib district with an area of 3 * 6 meters for the purpose of performing field experiment with preparing larvae of fish (3-4 days old) for transfer from larval incubators to fish pond in the morning.

2- Preparation of *Eucalyptus* leaves used in the experiment: The leaves of *Eucalyptus* sp. were obtained from trees surrounding the artificial fish pond in Musayyib district, the leaves were collected and cleaned from dust and then dried at room temperature, then crushed as a fine particles and preserved until use, the diagnosis was confirmed by the Herbarium of the College of Science / University of Baghdad.

3 - Preparation and extraction of volatile oil: Volatile oils were extracted using the hot water distillation method by Clevenger (1), and then collects the condensed liquid and

separates the oil from the water using a separation funnel, the extracted oil was stored in special sealed bottles at 4 ° C.

4- Preparation of the extracted volatile oil: The insecticide was prepared by mixing the volatile oil of *Eucalyptus* leaves in different concentrations 250, 450 and 650 ppm of Benzyl benzoate stabilizer, which characterized as boiling temperature of 324 ° C (slow evaporation) that act as a suitable stabilizer for volatile oil when used in the field experiment.

5- Collection and diagnose the insect: *Anisops sardea* was collected and diagnosed by the Iraq Natural History Research Center and Museum / University of Baghdad; the insects were reared using water breeding ponds with a diameter of 50*100 cm.

6- Laboratory control: Insects were collected from breeding ponds and placed in experimental ponds; the insects were divided into six groups and each group into three replicates of 40 insects per repeater with about 350 of breeding fish larvae, Each group was sprayed with 2 ml for each of the above-mentioned concentrations at a height of 30-40 cm with a fine plastic sprayer used to spray cleaning fluids (2) , the experimental ponds were provided with a flowing source of water in order to maintain a constant temperature (15 ± 2)^{o}C throughout the experiment period. Percentage of mortality per insects and breeding fish larvae was calculated after: 5, 10, 15, 20, 25 and 30 minutes after treatment.

7- Field control: The experiment was carried out in the field on 11-7-2018 in a muddy aquarium in the artificial fish pond with an area 3 * 6 meters in Babylon province / Musayyib district, after the experiment pond was sprayed with a volume of 200 ml of concentration 250,000; 450,000 and 650,000 ppm, three replicates of this treatment were taken from the water of the experimental pond. Adults of insect and breeding fish larvae were calculated randomly for each experimental replicates separately,the mortality rate was calculated before spraying and after 10, 15, 20, 25 and 30 minutes after treatment using Abbott's formula:

$$\text{Corrected \%} = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}}\right) * 100$$

Where: n = Insect population, T = treated, Co = control

8- Gas Chromatography: Gas chromatography was used to detect the active compounds in the model; standard compounds were used imported from Supelco Company USA, the prepared standard material was injected at a known concentration of the device and according to the method of work established by the manufacturer to determine the identity of each compound based on the time of retention. The sample was then injected to determine the types of compounds in which it was diagnosed in that sample based on the time of retention and compare the time of retention of standard compounds with the time of retention of peaks that appeared in the model. To determine its concentration, the area under the curve was compared with the area under the curve in the standard compound according to the equation:

Sample Concentration

$$= \frac{Concentration\ of\ standard. \times Area\ sample.}{Area\ of\ standard.} \times \frac{D.F.}{Volume}$$

D.F.= Dilution Factor.

9- Statistical Analysis: The results were analyzed using SPSS (V.25) Probit Regression Analysis to calculate the lethal concentration of half of the experimental number as well as to calculate the frequency and percentages over different time periods and plot the regression curves to extract the correlation coefficient (R2).

RESULTS AND DISCUSSION

In Tables (1-4) two factors were changed concentration of essential oil and exposure time, that the bioactivity of the Eucalyptus essential volatile oil depends upon several factors such as (type and nature of the constituents and their individual concentration. Moreover varies with species, season, location, climate, soil type, age of the leaves, fertility regime, the method used for drying the plant material, and method of oil extraction (6, 9).

Table 1. Effect of volatile oil of *Eucalyptus* sp. In concentrations 450,000 and 650,000 ppm on adults of *Anisops sardeus* in laboratory experiment

Number /		Con.	Number of insects	Observed Responses	Expected Responses	Residual	Probability %
PROBI	1	250.000	40	7	6.686	.314	16.7
T After	2	450.000	40	7	7.640	-.640-	19.1
5 min	3	650.000	40	9	8.674	.326	21.7
PROBI	1	250.000	40	12	12.005	-.005-	30
T After	2	450.000	40	13	12.989	.011	32.5
10 min	3	650.000	40	14	14.005	-.005-	35
PROBI	1	250.000	40	16	16.666	-.666-	41.7
T After	2	450.000	40	21	19.660	1.340	49.2
15 min	3	650.000	40	22	22.667	-.667-	56.7
PROBI	1	250.000	40	26	26.293	-.293-	65.7
T After	2	450.000	40	32	31.448	.552	78.6
20 min	3	650.000	40	35	35.252	-.252-	88.1
PROBI	1	250.000	40	37	36.972	.028	92.4
T After	2	450.000	40	40	39.990	.010	100
25 min	3	650.000	40	40	40.000	.000	100
PROBIT	1	250.000	40	40	40.000	.000	100
After 30	2	450.000	40	40	40.000	.000	100
min	3	650.000	40	40	40.000	.000	100

Table 2. Effect of pilot oil for *Eucalyptus* sp. in concentrations (250,000, 450,000 and 650,000 ppm on breeding carp fish larvae in laboratory experiment

Number /		con	Number of fish larvae	Observed Responses	Expected Responses	Residual	Probability %
PROBIT	1	250.000	650	5	5.081	-.081-	0.8
After 5 min	2	450.000	650	7	6.832	.168	1.1
	3	650.000	650	9	9.087	-.087-	1.4
PROBIT	1	250.000	650	7	6.932	.068	1.1
After 10	2	450.000	650	9	9.140	-.140-	1.4
min	3	650.000	650	12	11.928	.072	1.8
PROBIT	1	250.000	650	10	9.263	.737	1.4
After 15	2	450.000	650	10	11.516	-1.516-	1.8
min	3	650.000	650	15	14.221	.779	2.2
PROBIT	1	250.000	650	10	9.454	.546	1.5
After 20	2	450.000	650	11	12.127	-1.127-	1.9
min	3	650.000	650	16	15.418	.582	2.4
PROBIT	1	250.000	650	12	11.249	.751	1.7
After 25	2	450.000	650	12	13.539	-1.539-	2.1
min	3	650.000	650	17	16.212	.788	2.5
PROBIT	1	250.000	650	12	12.222	-.222-	1.9
After 30	2	450.000	650	15	14.546	.454	2.2
min	3	650.000	650	17	17.232	-.232-	2.7

Table 3. Effect of volatile oil of *Eucalyptus* sp. In concentrations 450,000 and 650,000 ppm on adults of *Anisops sardeus* in field experiment

Number /		Con	Number of insects	Observed Responses	Expected Responses	Residual	Probability %
PROBI	1	250.000	40	5	4.695	.305	11.7
T After	2	450.000	40	5	5.627	-.627-	14.1
5 min	3	650.000	40	7	6.678	.322	16.7
PROBI	1	250.000	40	9	9.009	-.009-	22.5
T After	2	450.000	40	10	9.982	.018	25
10 min	3	650.000	40	11	11.009	-.009-	27.5
PROBI	1	250.000	40	13	14.005	-1.005-	35
T After	2	450.000	40	16	16.960	2.040	42.4
15 min	3	650.000	40	19	20.025	-1.025-	49.1
PROBI	1	250.000	40	15	15.670	-.670-	39.2
T After	2	450.000	40	20	18.648	1.352	46.6
20 min	3	650.000	40	21	21.675	-.675-	54.2
PROBI	1	250.000	40	17	17.831	-.831-	44.6
T After	2	450.000	40	22	20.334	1.666	50.8
25 min	3	650.000	40	22	22.829	-.829-	57.1
PROBI	1	250.000	40	19	19.988	-.988-	≈50
T After	2	450.000	40	25	23.033	1.967	57.6
30 min	3	650.000	40	25	25.969	-.969-	64.9

Table 4. Effect of volatile oil for *Eucalyptus* sp. In concentrations (250,000, 450,000 and 650,000 ppm on breeding carp fish larvae in field experiment

Number /		con	Number of fish larvae	Observed Responses	Expected Responses	Residual	Probability %
PROBI	1	250.000	650	2	1.852	.148	0.3
T After	2	450.000	650	2	2.302	-.302-	0.4
5 min	3	650.000	650	3	2.846	.154	0.4
PROBI	1	250.000	650	3	3.359	-.359-	0.5
T After	2	450.000	650	5	4.264	.736	0.7
10 min	3	650.000	650	5	5.378	-.378-	0.8
PROBI	1	250.000	650	5	5.211	-.211-	0.8
T After	2	450.000	650	7	6.567	.433	0.1
15 min	3	650.000	650	8	8.222	-.222-	1.3
PROBI	1	250.000	650	5	5.721	-.721-	0.9
T After	2	450.000	650	9	7.511	1.489	1.2
20 min	3	650.000	650	9	9.769	-.769-	1.5
PROBI	1	250.000	650	5	5.721	-.721-	0.9
T After	2	450.000	650	9	7.511	1.489	1.2
25 min	3	650.000	650	9	9.769	-.769-	1.5
PROBI	1	250.000	650	5	5.721	-.721-	0.9
T After	2	450.000	650	9	7.511	1.489	1.2
30 min	3	650.000	650	9	9.769	-.769-	1.5

1- Laboratory experimental:

Results in Table 5 shows the concentration of volatile oil extract of Eucalyptus leaves needed to kill half of the total number of *A. sardeus* within time intervals (5,10,15,20,25,30) minutes during laboratory experimental conditions; we note that when exposure periods are short in time, very high concentrations of the extract may be used up to 1,497,696 ppm to kill half of the whole number of *A. sardeus* in laboratory conditions. Therefore, we recommend increasing the exposure period when using appropriate concentrations that do not affect fish larvae. Even if such a high concentration of the extract is adopted, it can be seen that the degree of its effect on fish larvae is low (Table 6, 4).

Table 5. LC50 values of *Eucalyptus* sp. essential oil for (5, 10, 15, 20, 25, 30 min.) exposure on *A.sardea*

Confidence Limits					
	Time (min.)		95% Confidence Limits for con (Ppm)		
			Estimate	Lower Bound	Upper Bound
PROBIT	5	Probability	1,497,696	898.708	3175.387
	10		757,303	632.359	1922.568
	15	50%	472,492	190.127	788.257
	20		237,084	1932.621	64.751
	25		119,280	5923.748	667.179
	30		-	-	-

the concentration of volatile oil extract needed to kill half of the fish larvae within the time periods of exposure (5,10,15,20,25,30) minutes during field experiment conditions (Table 6), as can be seen that in all periods of short or long exposure time, very high concentrations of the extract may reach more than 6,000,000 ppm to kill half of the fish larvae in the experimental conditions.This statistically proves that the use of this prepared Natural pesticide does not pose a threat to the organisms under the water surface. Therefore, even high concentrations of the extract are highly safe in field conditions on fish larvae.

Table 6. LC50 values of *Eucalyptus* sp. essential oil for (5,10,15,20,25,30 min.) exposure on fish larvae.

Confidence Limits					
	Time (min.)		95% Confidence Limits for con (ppm)		
			Estimate	Lower Bound	Upper Bound
PROBIT	5	Probability	4,655,363	3331.122	19943.343
	10		4,573,082	3196.528	19021.098
	15		5,287,805	3086.953	18277.566
	20	50%	4,616,057	3055.758	18061.957
	25		5,813,944	3003.337	17710.930
	30		6,009,829	2967.985	17471.382

2- Field experimental

It is show from Table 7 that suitable concentrations can be used (within the limits of the concentrations used in this experiment 250,000, 450,000 and 650,000. If exposure periods are used within longer periods of time (more than 10 minutes),

Table 7. LC50 values of *Eucalyptus* sp. essential oil for (5, 10, 15, 20, 25, 30 min.) exposure on *A. sardea*

Confidence Limits					
	Time (min.)	Probability	95% Confidence Limits for con (ppm)		
			Estimate	Lower Bound	Upper Bound
PROBIT	5	50%	2,392,027	1177.993	5842.509
	10		2,165,455	871.636	3875.331
	15		648,457	402.641	1577.523
	20		539,270	226.023	1151.729
	25		423,527	38.956	819.601
	30		250,765	678.043	497.683

concentrations from the extract that may be close to 250,000 ppm) may be used to kill half the total number of the aquatic insect *A. sardeus* in the field experimental conditions during a period of exposure (30) minutes to ensure that the fish larvae do not affect, i.e., the greater the exposure period the less concentration of extract used in the field experiment. It is clear from Table 8 (as in the laboratory experiment) that in all periods of short-term or long-term exposure should use very high concentrations of the extract may

reach more than 7,973,526 ppm to kill half of the number of fish larvae in the experimental conditions, which shows the lack of toxicity on fish larvae,the fact that this oil in the spread on the surface of the water has almost no effect on these larvae, and because of the high volatility of the extract, so the use of even high concentrations of the extract is very safe in the field conditions on fish larvae

Table 8. LC50 values of *Eucalyptus* sp. essential oil for (5,10,15,20,25,30 min.) exposure on fish larvae

Confidence Limits					
	Time (min.)	Probability	95% Confidence Limits for con (ppm)		
			Estimate	Lower Bound	Upper Bound
PROBIT	5	50%	7,973,526	3338.869	65245.749
	10		6,363,272	3156.888	61033.428
	15		5,871,187	2980.567	57020.205
	20		4,892,511	2921.042	55659.401
	25		4,892,511	2929.769	55855.719
	30		4,892,511	2936.244	56001.421

The correlation between the concentration of the volatile oil extract of the *Eucalyptus* leaves and the response of both aquatic insects *A. sardeus*, and fish larvae within time periods (5, 10,15,20,25,30) can be estimated by curves in Figures (1-4) where the correlation coefficient value (R2) this relationship, the value of (X) represents one toxic lethal unit, which increases by one unit, the rate of killing increases with different values. From curves in Figure 1 as the concentration of the extract increases by one part per million, the killing rate of *A. sardeus* is increased by ($9.48 * 10^4$) % in exposure period (15) minutes, it is the highest among the rest of the exposure in the laboratory experiment note that the program did not draw this relationship in periods of exposure (25, 30) minutes because the killing rates reached 100%. Curves in Figure 2 show that the greater the concentration of the extract one part per million increase the killing rate of fish larvae by ($5.54 * 10^{4)}$ % in the exposure period (5) minutes, which is the highest among the rest of the exposure periods in the laboratory experiment. While in Figure 3 the higher the concentration of the extract, one part per million, the percentage of killing *A. sardeus* total increases by ($9.78 * 10^4$) % in the exposure period (15) minutes, which is the highest among the rest of the exposure in the field experiment and followed by slightly less ($9.53*10^4$) % in Exposure period (20) minutes. Curves in figure (4) show that the greater the concentration of the extract one part per million increase the killing rate of fish larvae by ($5.54 * 10^{4)}$ % in the last three periods of exposure (20, 25, 30) minutes, which is the highest among the rest of the exposure in the field experiment.

3- Gas Chromatography Analysis:

The result of Gas Chromatography to the *Eucalyptus* sp. essential volatile oil showed presence of (Sabinen 0.06, Terpinen 2.0, Lemonine 1.08, Camphene 3.45, α-pinene 10.0, Myrcine 0.62, Comphore 2.9, Linalool 0.027) % according to the slandered curves While an analysis was performed by Mustafa, (32) on (100g) of dried *Eucalyptus Camaldulensis* leaves showed the presence of compounds (α-pinene 4.68, 4-Terpineol 4.22, Myrtenal 2.46, α-Terpinolene 1.96, α–Terpineol 1.79, Trans-Pinocarveol 1.68, α-Thujene 1.33, Bicyclogirmacrene 0.84, Myrcene 0.65, Terpinene 0.53)%with percentage respectively. The *eucalyptus* spp. oil is a combination of a variety of monoterpenes and sesquiterpenes, and aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones. The pesticidal activity of *eucalyptus* oils has been due to the components such as 1,8-cineole, citronellal, citronellol, citronellylacetate, p-cymene, eucamalol, limonene, linalool, a-pinene, g terpinene, a-terpineol, alloocimene, and aromadendrene (6, 7, 12, 15,24, 25, 28,42).

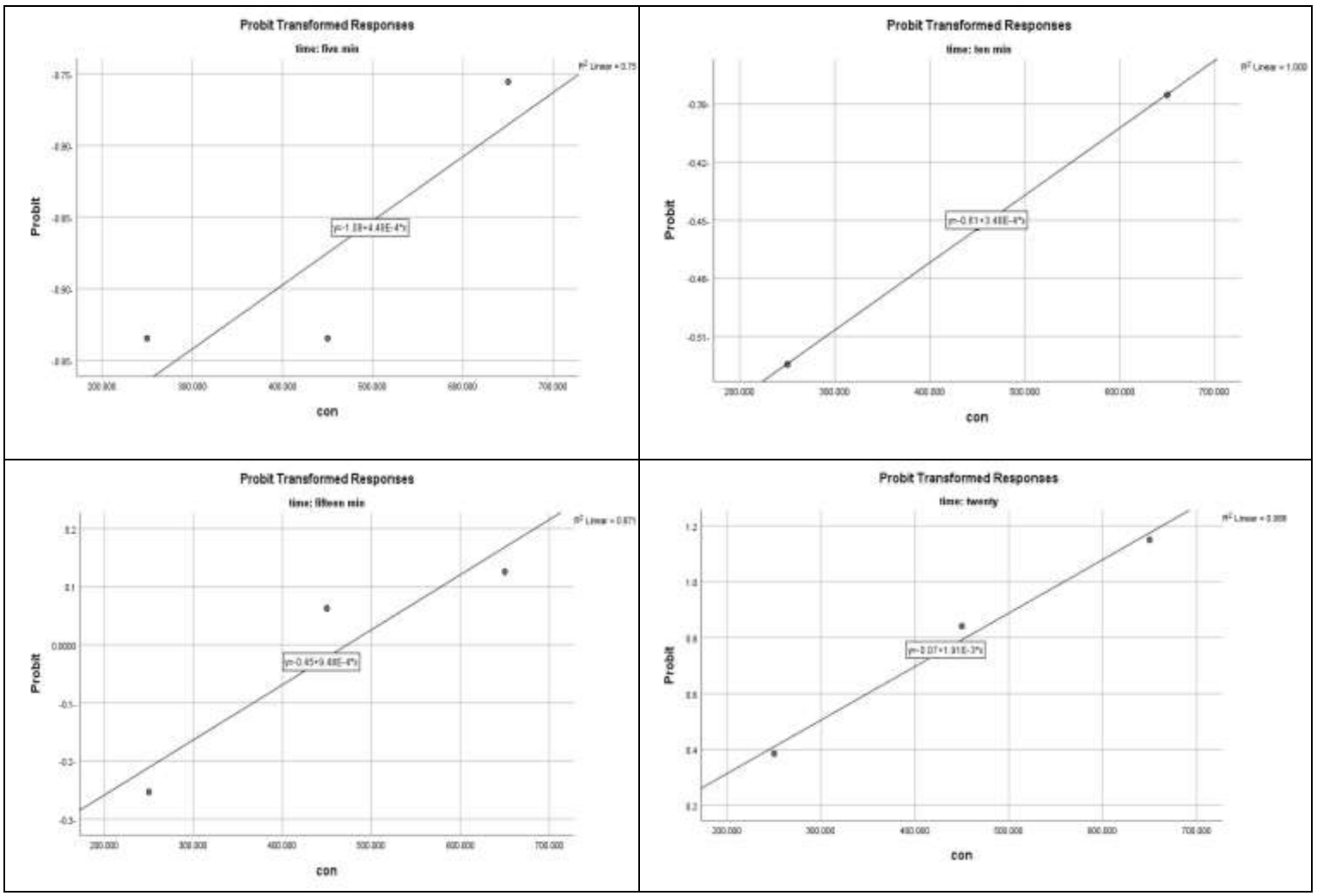

Figure 1. Concentration 5,10,15,20 minute Dose-Response relationship between *Eucalyptus* sp. leaves essential oil and for *A. sardeus* in laboratory experimental

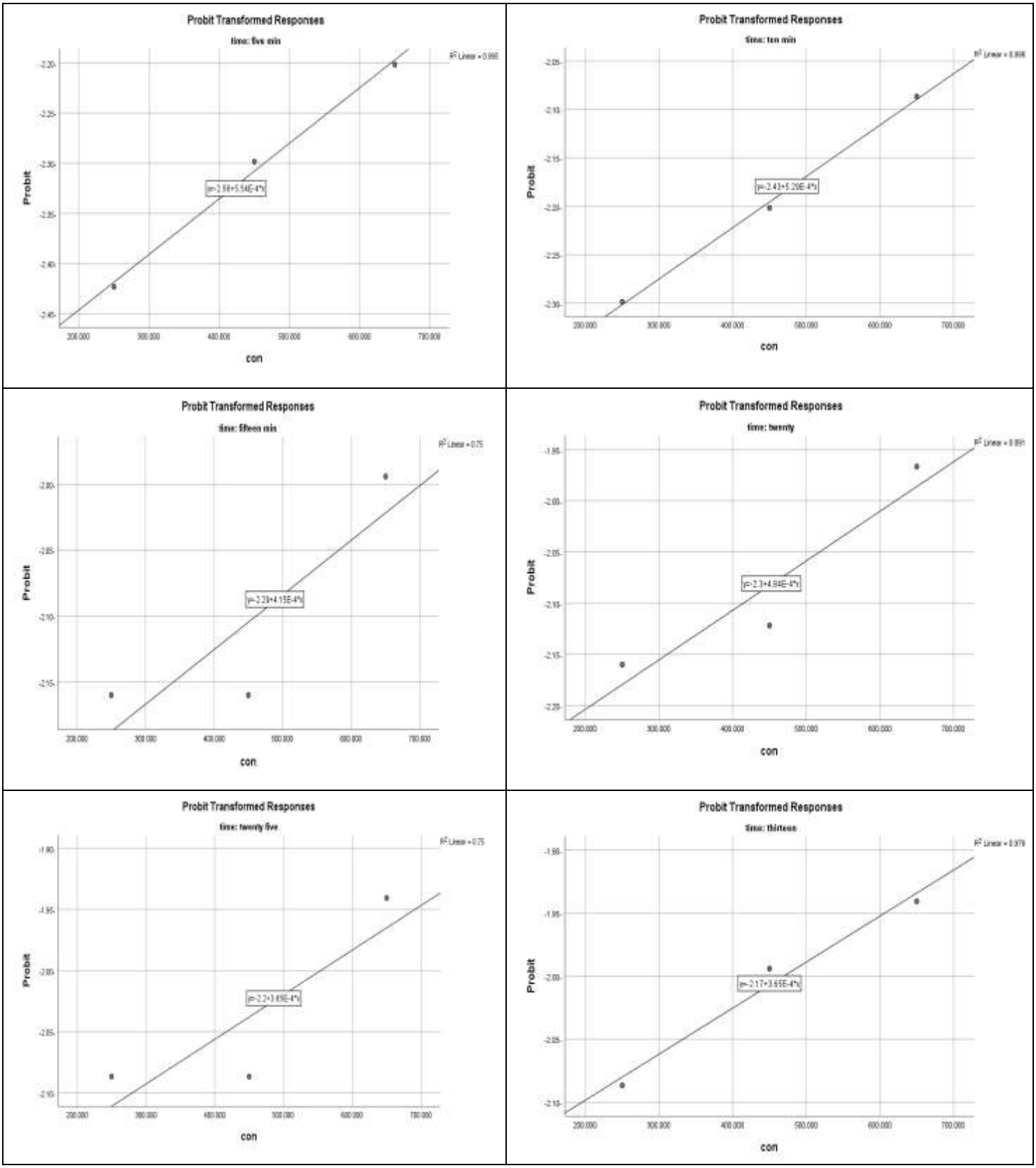

Figure 2. Concentration 5, 10, 15,20,25,30 minute Dose-Response relationship between *Eucalyptus* sp. Leaves essential oil and for fish larvae in laboratory experimental

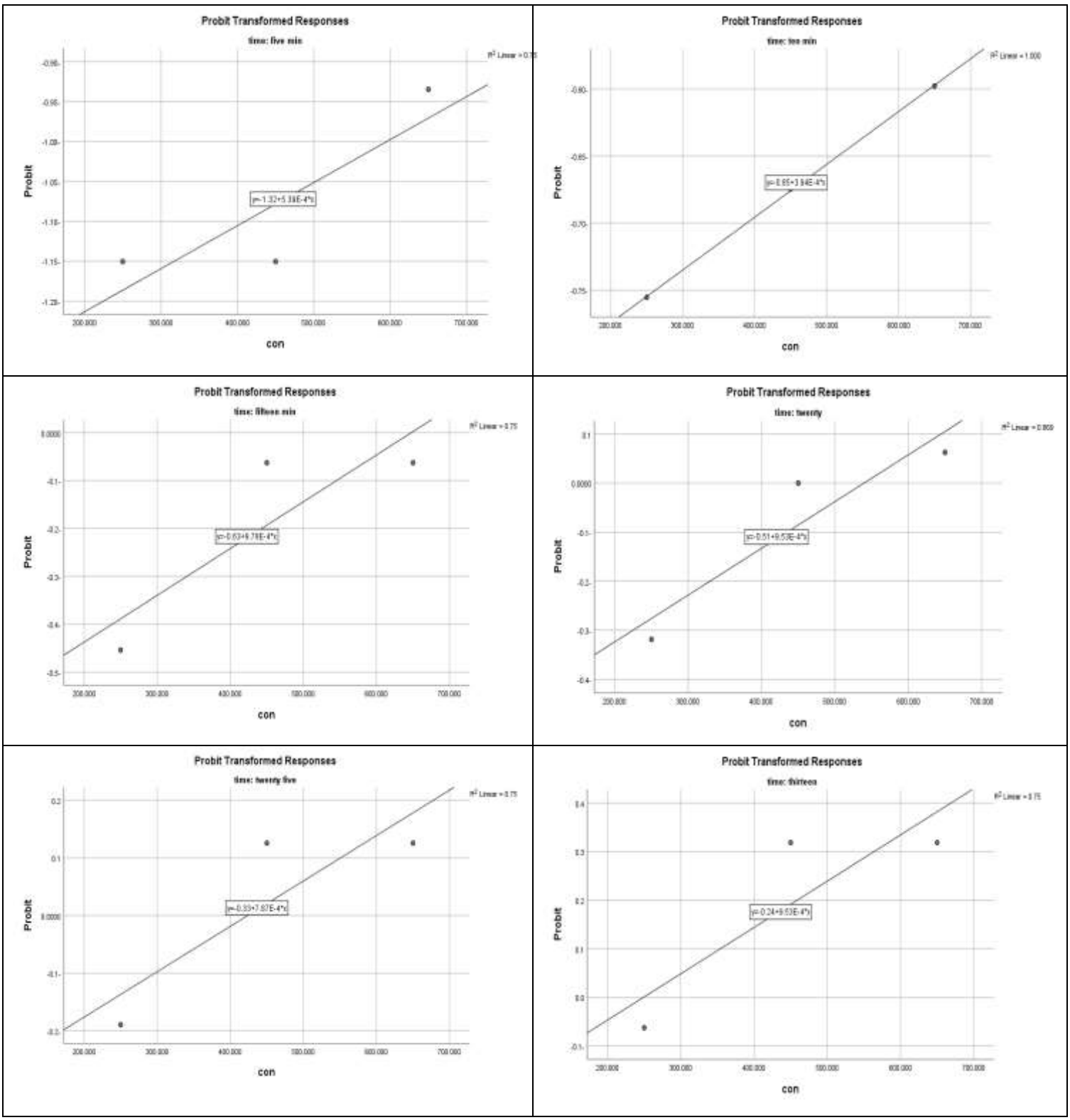

Figure 3. Concentration 5, 10, 15, 20, 25, 30 minute Dose-Response relationship between *Eucalyptus* sp. leaves essential oil and for *A. sardeus* in field experimental

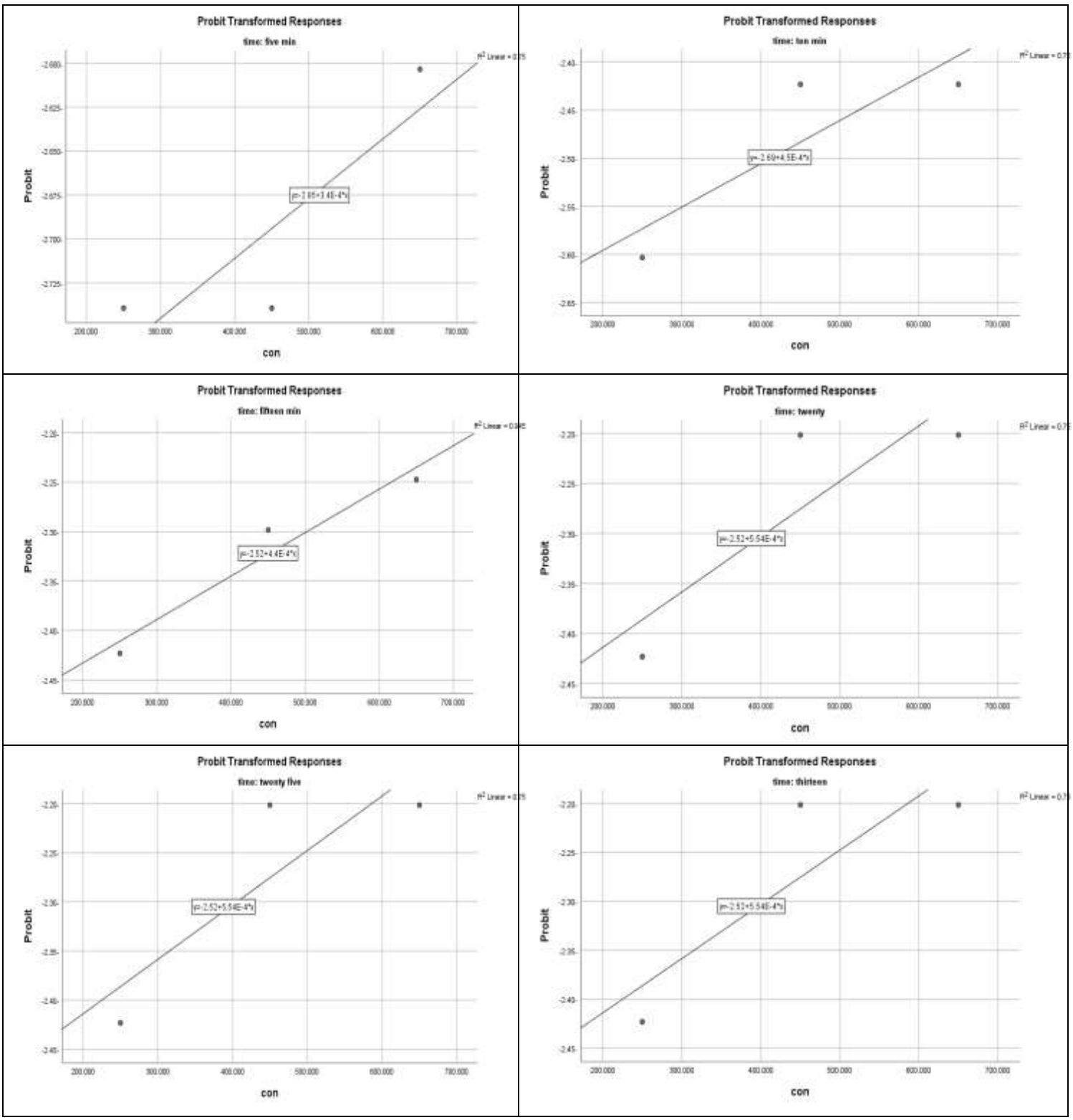

Figure 4. Concentration 5,10,15,20,25,30 minute Dose-Response relationship between *Eucalyptus* sp. leaves essential oil and for fish larvae in Field experimental

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