RESEARCH ARTICLE

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The insecticidal effects of different plant extracts on confused flour beetle, *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae)

Yunus GURAL[1] • **Erhan KARAMAN**[2] • **Gozde BAYDOGAN**[2] • **Inanc OZGEN**[2]

[1] Department of Statistic, Facultyof Science, Fırat University, Elazığ, Turkiye
[2] Department of Bioengineering, Faculty of Engineering, FıratUniversity, Elazığ, Turkiye

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Correspondence: Inanc OZGEN
E-mail: inancozgen@gmail.com

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Abstract
This study was carried out to determine the insecticidal effects of different plant extracts on confused flour beetle, *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae) and time-varying LD50 and LD90 values. LD50 and LD90 of the formulations were determined by the probit analysis method. The studies were conducted under controlled conditions in a climate cabin and air conditioning room at 25 ± 2°C and 60% humidity 2019 to 2020 in Fırat University Bioengineering Department in Turkey. In this study, Grainpro AITC-ALPHA (Monoterpen, Allyisothiocyanate,Cocoate methyl esters), Grainpro AITC-GAMMA (Gamma terpene, Allyisothiocyanate, Cocoate methyl esters-Eugenol), Grainpro GAMMA-EUG (Gamma terpinene, Eugenol, Cocoate methyl esters), and Grainpro AITC-EUC (Eucalyptus oil, Allyisothiocyanate, Cocoate methyl esters) plant formulations were used in doses (In all doses, different µL drug doses prepared in 1 ml were mixed in 1 liter of water and applied by obtaining 0.2 µL, 0.4 µL, 0.6 µL, 0.8 µL, 1 µL doses for each formulation). The two upper and two lower doses of the most effective drug obtained were then tested and their effectiveness was determined. It was determined that the insecticidal efficacy of the pesticides increased as the pesticide dose increased and two lower and two upper doses of the most effective dose of 1 µL were subjected to efficacy trials. Among these formulations, 1 µL and 1.10 µL doses of Gainpro AITC-EUC formulation were the most effective on the pest.
Keywords: Insecticidal effect, LD50, LD90, Plant extracts, *Tribolium confusum*

INTRODUCTION

The adults and larvae of confused flour beetle *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae) are a secondary pest and commonly found in houses, supermarket warehouses and granaries, mills and flour warehouses (Ebelling, 1992). This species does not directly damage whole grains, but feeds on grains damaged by other store pests. Furthermore, *T. confusum* provides a source of cracked kernels and powdered food for them from mechanical harvest damage when trying to consume other cereal crops. Gases released from the thorax of adults produce odor in products. This leads to the product getting moldy, the color of the flour turning grayish and its quality decreasing (Aitken, 1975; Hill, 1990). This pest causes significant losses in cereal crops in our country and the world (Trematerra et al., 2011). In the control of this pest, both chemical and organic preparations have been used from time to time in various studies.

In some studies conducted in our country: Determination of the fungicidal effect of essential oils of 32 different plants against all biological stages of *T. confusum* was carried out. Again, the effects of essential oils obtained from garlic and onion

and some active substances such as diallyl sulfide, diallyl disulfide and dipropyl disulfide and their mixtures against the pest were studied. Determination of the fumigant effect of the main compounds (allyl isothiocyanate and allyl disulfide) of *Rosmarinus officianalis* (Rosemary) L. (Lamiales: Lamicaea) and *Laurus nobilis* L. (Laurales: Lauracea) essential oils together with carbon dioxide (CO2) and nitrogen (N2) is considered among some important studies conducted in recent years (Erler 2005; Isikber et al, 2006; Karci, 2006; Gozek, 2007).

In previous studies, it was found that 0.1, 1 and 5 ppm doses of spinetoram insecticide had a fumigant effect on *T. confusum*, *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae) and *Rhyzopertha dominica* Fabricius, 1792 (Coleoptera: Bostrichidae) feeding on wheat. In the same study, essential oils extracted from *Pagostemon heyneaus* Benth. (Lamiales: Lamiacea), *Ocimum basilicum* L. (Lamiales: Lamiaceae) were found to have contact effect on *T. confusum* (Shaaya et al., 1997). Essential oils were extracted from the leaves of *Curcuma longa* L. (Zingiberales: Zingiberacea) plant known as Indian saffron and its contact and insecticidal effects were studied against *R. dominica, S. oryzae* and *Tribolium casteneum* Herbst, 1797 (Coleoptera: Tenebrionidae) (Tripathi et al., 2002).

The use of plant-based insecticides has come to the forefront, especially due to the degradation problems and toxic effects of chemical pesticides. The use of plant-based insecticides has come to the forefront, especially due to the degradation problems and toxic effects of chemical pesticides. Especially the formation of resistance to chemicals also affects the success of the pest management (Tiryaki et al., 2010).

The main objective of this study was to develop alternative control methods to chemical control in terms of control of stored product pests and to determine the lethal doses of new generation plant-based insecticides. These insecticides are Grainpro AITC-Alpha, Grainpro AITC-Gamma, Grainpro Gamma-Eug and Grainpro AITC-Euc herbal insecticides developed by Biohaust company. These insecticides were developed by the aforementioned company for the control of storage and warehouse pests. The results of this study will be used both in the licensing studies of insecticides and in the control of warehouse and warehouse pests.

MATERIALS AND METHODS

Material

The studies were conducted under controlled conditions climate cabin and air conditioning room (25 ± 2°C and 60% humidity) to between 2019 to 2020 years in Bioengineering Department in Turkey. *Tribolium confusum* adults, flour-bran mixtures, Grainpro AITC-Alpha, Grainpro AITC-Gamma, Grainpro Gamma-Eug and Grainpro AITC-Euc pesticides contests are given below were the main materials of the study (Table 1, Figure 1). All formulations are developed by the company Biohaust. No excipients were used in the formulations. The formulations are designed as liquid formulations.

Table 1. Plant formulations and their ingredients used in the determination of LD50 and LD90 values on *Tribolium confusum*.

Pesticide Used	Ingredients
Grainpro AITC-Alpha	Monoterpen, Allyisothiocyanate, Cocoate methyl esters
Grainpro AITC-Gamma	Gamma terpene, Allyisothiocyanate, Cocoate methyl esters-Eugenol
Grainpro-Gamma-Eug	Gamma terpinene, Eugenol, Cocoate methy esters
Grainpro AITC-Euc	Eucalyptus oil, Allyisothiocyanate, Cocoate methyl esters

Figure 1. The test pesticides used in the study: "Grainpro AITC-Alpha, Grainpro AITC-Gamma, Grainpro Gamma-Eug and Grainpro AITC-Euc".

Method

Insect Rearing and Applications

The pest was placed on flour and bran in 15x30x5 cm container at a temperature of 25±1°C in laboratory conditions and kept until it reached the adult stage. The study was initiated with larvae hatched from the eggs of the new generation of adults produced under laboratory conditions. First of all, the broken grains that the pest would feed on were treated with pesticide for 10 seconds by dipping method.The grains were then dried on an absorbing paper (Figure 2). The dried wheat was taken into 10x5 cm tubes with 5 repetitions for each dose. Ten adult *T. confusum* pests were placed on 15 grams of cracked wheat (Figure 2) LD 50 and lD90 mortality rates of herbal insecticides (Biohaust Company, 789 Teviot Road, JimboombaQld 4280, Australia) were determined on the tested material.

For all botanical insecticides, 1 ml mixture was obtained by using 100 µL formulation + 900 µL pure water. Thus, 1 ml of mixture containing 100 µL pesticide at a concentration

of 0.1 was obtained. This mixture was diluted in 100 ml of water and a mixture with a concentration of 0.001 was obtained. For each pesticide, doses of 0.2 µL, 0.4 µL, 0.6 µL, 0.8 µL and 1 µL were used. To provide these doses, insecticidal activities were analyzed by taking 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml from the mixture, respectively. Studies were performed in 5 cuvettes for each dose. Adult individuals were examined at 24-hour intervals and the results were noted.

The Determination of The LD50 and LD90 values and Statistic Analyses

The LD_{50} and LD_{90} values of the dose causing the highest mortality rate of each pesticide were determined. Descriptive statistics of the data set were expressed as mean±standard deviation. Differences between means were compared with one-way ANOVA followed by Tukey's post-hoc test. The values of lethal doses (LD_{50} and LD_{90}) were determined for certain times using probit analysis. All statistical analyses were carried out using the IBM SPSS software version 21.0 statistical program. The doses determined for LD50 and LD90 were determined by us according to the experimental composition by taking the recommended doses that the company wants to be applied to warehouse pests and/or the precedent doses of similar plant-based drugs used against this pest as an example.

Figure 2. Cracked wheat to which the formulations were applied.

RESULTS AND DISCUSSION

The insecticidal effects of Gainpro plant extracts prepared at different concentrations on insects are shown in Table 2. According to the ANOVA test, the Gainpro AITC-Alpha viability ratio showed a significant difference according to doses ($p<0.05$) (Table 2). When the lethal effects of the application doses were compared according to the Tukey test, the 1 mL dose (23.61±14.48) was significantly more effective than the 0.2 mL (40.33±7.76) and 0.4 mL (33.48±11.28) doses, while the 0.8 mL (24.46±13.16) and 0.6 mL (27.76±15.96) doses were significantly more effective than the 0.2 mL dose (40.33±7.76) ($p<0.05$). The LD_{50} and LD_{90} values and time-dependent insecticidal effect of Gainpro AITC-Alpha plant extract on *T. confusum* are given in Tables 3&4 and Figures 3&4.

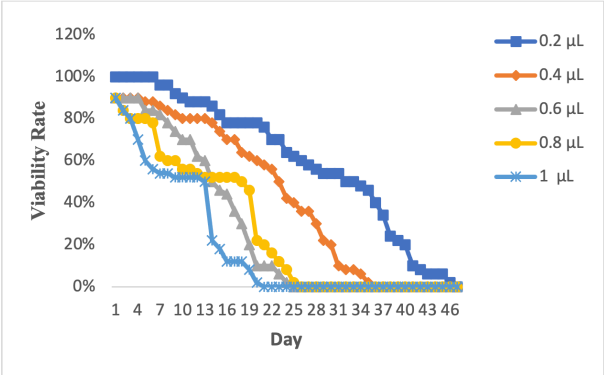

Figure 3. Effect of different doses of Gainpro AITC-Alpha formulation on *Tribolium confusum*.

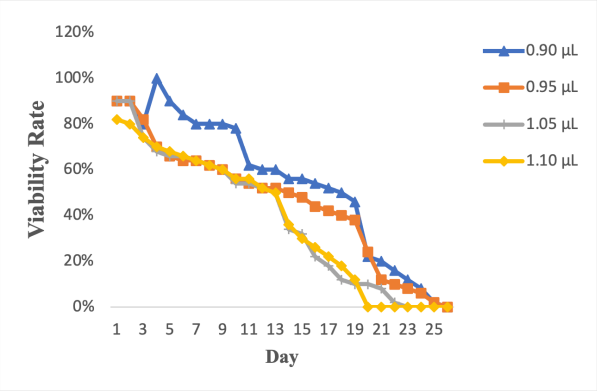

Figure 4. Effect of different doses of Gainpro AITC-Alpha formulation on *Tribolium confusum*.

The viability rate of Gainpro AITC-EUC insecticide showed a significant difference according to the doses ($p<0.05$) (Table 2). When the lethality effects of the application doses were compared according to the Tukey test, 1 mL (18.55±13.28), and 0.8 mL (18.65±13.01) doses were found to be statistically significantly more effective than the 0.2 ml dose (27.20±9.23) (Table 2). The LD_{50} and LD_{90} values and time-dependent insecticidal effect of Gainpro AITC-EUC plant extract on *T. confusum* are given in Tables 5&6 and Figures 5&6.

Table 2. The percentage mortality of Gainpro extracts on *Tribolium confusum*.

	0.2 mL		0.4 mL		0.6 mL		0.8 mL		1 mL		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p value
Grainpro AITC-Alpha	40.33a	7.76	33.48ab	11.28	27.76bc	15.96	26.46bc	13.16	23.61c	14.48	**0.000***
Grainpro AITC-Gamma	27.20a	9.23	26.26ab	10.83	22.53ab	12.93	18.65b	13.01	18.55b	13.28	**0.015***
Grainpro-Gamma-Eug	39.03a	4.59	38.03a	5.97	32.36ab	10.42	26.56bc	11.54	21.68c	12.71	**0.000***
Grainpro AITC-Euc	23.96	6.98	21.20	9.45	22.83	8.25	21.06	9.58	20.32	11.62	0.565

* $p<0.05$

Table 3. LD_{50} and LD_{90} values of Gainpro AITC-Alpha (Doses 0.2, 0.4, 0.6, 0.8 and 1).

Day	Lethal Concentration Doses	
	LD_{50} (95% confidence limit)	**LD_{90} (95% confidence limit)**
2	3.342 [a] (1.663–102.81)	19.236 [a] (4.828–21837.296)
3	2.346 [a] (1.424–13.43)	10.240 [a] (3.706–423.769)
4	1.668 [a] (1.203–3.939)	5.390 [a] (2.715–36.942)
5	1.360 [a] (1.059–2.357)	4.047 [a] (2.341–15.203)
6	1.242 [a] (1.003–1.923)	3.403 [a] (2.119–9.992)
7	1.146 [a] (0.921–1.730)	3.821 [a] (2.294–11.490)
8	1.101 [a] (0.888–1.636)	3.787 [a] (2.274–11.319)
9	1.115 [a] (0.869–1.819)	4.910 [a] (2.624–20.724)
10	1.059 [a] (0.823–1.733)	5.176 [a] (2.677–24.411)
11	1.088 [a] (0.828–1.915)	6.033 [a] (2.897–37.245)
12	0.990 [a] (0.772–1.598)	5.245 [a] (2.667–26.369)
13	0.924 [a] (0.736–1.385)	4.512 [a] (2.448–18.083)
14	0.652 [a] (0.559–0.781)	2.119 [a] (1.519–3.834)
15	0.599 [a] (0.246–3.807)	2.129 [a] (1.013–27859.61)
16	0.545 [a] (–)	2.033 [a] (–)
17	0.521 [a] (–)	1.898 [a] (–)
18	0.481 [a] (–)	1.758 [a] (–)
19	0.435 [a] (–)	1.402 [a] (–)
20	0.372 [a] (0.063–0.620)	0.868 [a] (0.542–33.541)
21	0.358 [a] (0.047–0.600)	0.823 [a] (0.513–40.640)
22	0.331 [a] (0.042–0.542)	0.793 [a] (0.494–26.616)
23	0.313 [a] (0.081–0.480)	0.707 [a] (0.463–4.866)
24	0.276 [a] (0.058–0.422)	0.619 [a] (0.406–3.874)
25	0.265 [a] (0.043–0.411)	0.538 [a] (0.352–5.338)
26	0.254 [a] (0.036–0.394)	0.501 [a] (0.329–6.291)
27	0.249 [a] (0.016–0.395)	0.500 [a] (0.322–14.476)
28	0.237 [a] (0.058–0.348)	0.473 [a] (0.323–2.267)
29	0.223 [a] (0.184–0.256)	0.436 [a] (0.379–0.530)
30	0.221 [a] (0.183–0.254)	0.426 [a] (0.371–0.519)
–: not calculated. a: µg/ml		

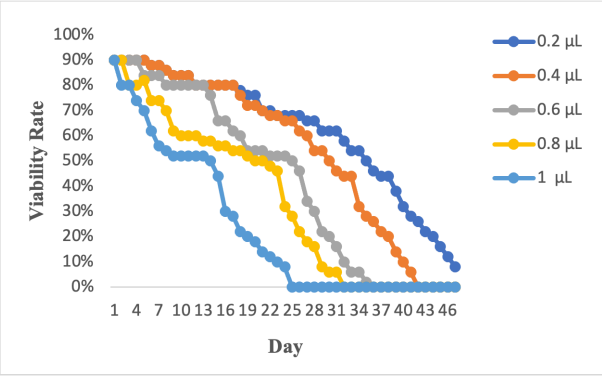

Figure 5. Effect of different doses of Grainpro AITC-EUC formulation on *Tribolium confusum*.

In the case of Gainpro AITC-Gamma insecticide, the viability rate differed significantly according to the doses ($p<0.05$) (Table 2). When the lethality effects of the application doses were compared according to the Tukey test, the 1 mL dose (21.68±12.71) was found to be statistically significantly more effective than the 0.2 mL (39.03±4.59), 0.4 mL (38.03±5.97) and 0.6 mL (32.36±10.42) doses, and the 0.8 mL (26.56±11.54) dose was found to be significantly more effective than the 0.2 mL (39.03±4.59) and 0.4 mL (38.03±5.97) doses (Table 2). The LD_{50} and LD_{90} values and time-dependent insecticidal effect of Gainpro AITC-Gamma plant extract on *T. confusum* are given in Tables 7&8 and Figures 7&8.

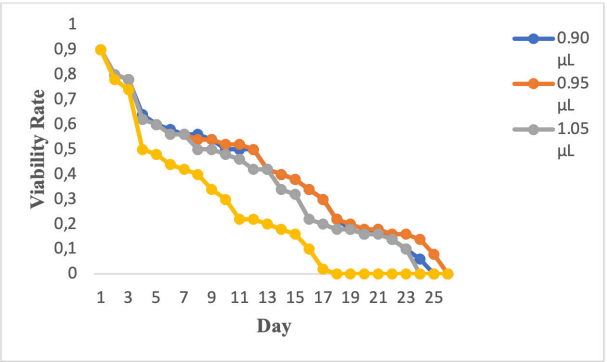

Figure 6. Effect of different doses of Grainpro AITC-EUC formulation on *Tribolium confusum*.

Table 4. LD_{50} and LD_{90} values of Gainpro AITC-Alpha (Doses 0.90 0.95 1.05 1.1).

Day	Lethal Concentration Doses	
	LD_{50} (95% confidence limit)	**LD_{90} (95% confidence limit)**
1	2.122 [a] (–)	4.804 [a] (–)
2	1.843 [a] (–)	3.617 [a] (–)
3	1.766 [a] (–)	4.635 [a] (–)
4	1.185 [a] (–)	1.569 [a] (–)
5	1.241 [a] (–)	1.961 [a] (–)
6	1.293 [a] (–)	2.423 [a] (–)
7	1.287 [a] (–)	2.574 [a] (–)
8	1.231 [a] (–)	2.304 [a] (–)
9	1.188 [a] (–)	2.105 [a] (–)
10	1.118 [a] (1.035–3.468)	1.872 [a] (1.383–970.380)
11	1.294 [a] (–)	9.937 [a] (–)
12	1.127 [a] (–)	5.371 [a] (–)
13	1.066 [a] (–)	3.346 [a] (–)
14	0.945 [a] (0.760–1.005)	1.476 [a] (1.235–5.755)
15	0.936 [a] (0.821–0.985)	1.344 [a] (1.187–2.287)
16	0.915 [a] (0.818–0.958)	1.237 [a] (1.136–1.619)
17	0.906 [a] (0.820–0.947)	1.186 [a] (1.107–1.428)
18	0.899 [a] (0.827–0.936)	1.132 [a] (1.075–1.276)
19	0.891 [a] (0.826–0.926)	1.092 [a] (1.047–1.191)
20	0.815 [a] (0.664–0.871)	1.010 [a] (0.972–1.091)
21	0.780 [a] (0.555–0.850)	0.973 [a] (0.928–1.039)
22	0.780 [a] (0.533–0.848)	0.937 [a] (0.880–0.984)
23	0.678 [a] (0.000–0.809)	0.894 [a] (0.013–0.957)
24	0.539 [a] (–)	0.833 [a] (–)
25	0.552 [a] (–)	0.740 [a] (–)
–: not calculated. a: µg/ml		

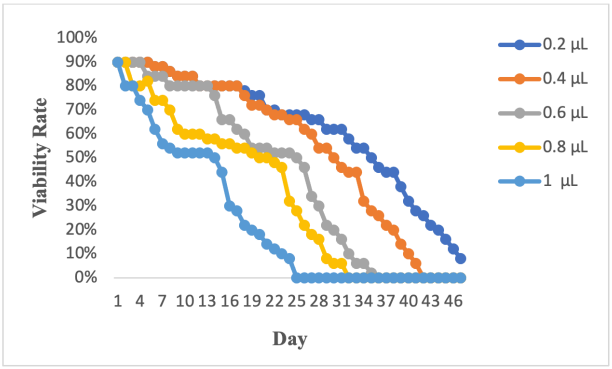

Figure 7. Effect of different doses of Gainpro AITC-Gamma formulation on *Tribolium confusum*.

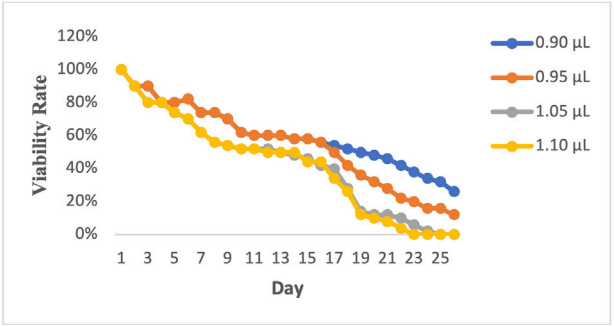

Figure 8. Effect of different doses of Gainpro AITC-Gamma formulation on *Tribolium confusum*.

The viability rate of Gainpro Gamma-EUG did not differ significantly between doses ($p>0.05$) (Table 2). The LD_{50} and LD_{90} values and time-dependent insecticidal effect of Gainpro Gamma-EUG plant extract on *T. confusum* are given in Tables 9&10 and Figures 9&10.

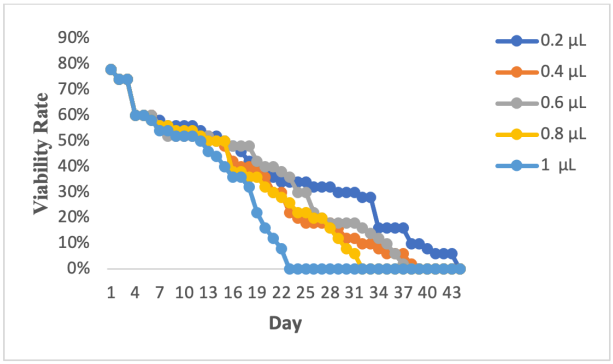

Figure 9. Effect of different doses of Grainpro Gamma EUG formulation on *Tribolium confusum*.

Figures 3-10 show that the viability rate decreased as the dose increased. At 1 ml dose, the viability of insects decreased significantly after the 13th day. Similarly, at a dose of 0.8 ml, the viability of the insects decreased drastically after day 19 (Figure 3).

Table 5. LD_{50} and LD_{90} values of Grainpro AITC-EUC (Doses 0.2 0.4 0.6 0.8 1).

Day	Lethal Concentration Doses	
	LD_{50} (95% confidence limit)	**LD_{90} (95% confidence limit)**
1-4	–	–
5	2.653 [a] (–)	12.355 [a] (–)
6	1.927 [a] (–)	117.193 [a] (–)
7	1.621 [a] (–)	113.752 [a] (–)
8	1.501 [a] (–)	140.394 [a] (–)
9	1.136 [a] (0.686–40.846)	46.602 [a] (–)
10	0.936 [a] (0.604–6.791)	29.807 [a] (–)
11	0.841 [a] (0.532–8.121)	35.051 [a] (–)
12	0.712 [a] (0.390–195.75)	56.391 [a] (–)
13	0.498 [a] (0.306–0.763)	8.906 [a] (2.879–1102.948)
14	0.450 [a] (–)	5.870 [a] (–)
15	0.403 [a] (0.263–0.532)	3.720 [a] (1.866–25.825)
16	0.378 [a] (–)	2.300 [a] (–)
17	0.342 [a] (–)	1.619 [a] (–)
18	0.316 [a] (–)	1.303 [a] (–)
19	0.293 [a] (–)	1.131 [a] (–)
20	0.274 [a] (–)	1.089 [a] (–)
21	0.226 [a] (–)	0.998 [a] (–)
22	0.197 [a] (–)	0.939 [a] (–)
23	0.178 [a] (0.093–0.245)	0.840 [a] (0.644–1.377)
24	0.158 [a] (0.078–0.222)	0.703 [a] (0.551–1.075)
25	0.145 [a] (0.067–0.209)	0.648 [a] (0.509–0.971)
26	0.141 [a] (0.067–0.202)	0.579 [a] (0.460–0.827)
27	0.131 [a] (0.058–0.190)	0.527 [a] (0.418–0.738)
28	0.088 [a] (0.018–0.154)	0.472 [a] (0.351–0.702)
29	0.082 [a] (0.015–0.147)	0.427 [a] (0.311–0.619)
30	0.085 [a] (0.018–0.149)	0.411 [a] (0.328–0.646)
–: not calculated. a: µg/ml		

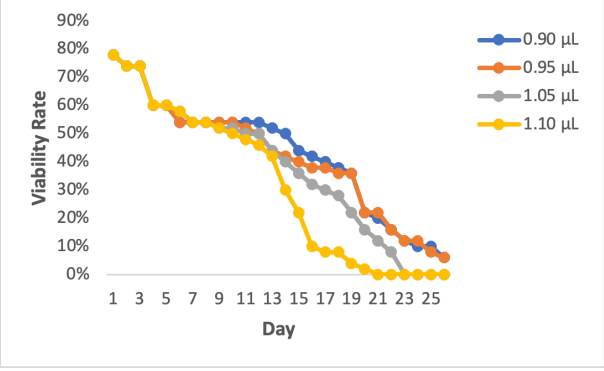

Figure 10. Effect of different doses of Grainpro Gamma EUG formulation on *Tribolium confusum*.

A concentration of 1 µg/ml of Gainpro AITC-Alpha extract showed a very strong insecticidal effect and by day 21 there were no living insects and all individuals were dead (Figure 3). The LD_{50} value of the plant formulation observed on day 21 was 0.358 µg/ml and the LD_{90} value was 0.823 µg/ml (Table 3). The 1.10 µg/ml concentration of Gainpro AITC-Alpha extract showed a very strong insecticidal effect and no live insects were observed on the 20th day of the application (Figure 4). The LD_{50} value of the plant formulation observed on day 20 was 0.815 µg/ml and the LD_{90} value was 1.010 µg/ml (Table 4).

A concentration of 1 µg/ml of Gainpro AITC-EUC extract showed a very strong insecticidal effect and no live insects were observed on day 19 (Figure 5). The LD_{50} value of the plant observed on the 19th day was 0.293 µg/ml and the LD_{90} value was 1.131 µg/ml (Table 5). A concentration of 1.10 µg/ml of Gainpro AITC-EUC extract showed a very strong insecticidal effect and no live insects were observed on day 18 (Figure 6). The LD_{50} value of the plant formulation observed on day 18 was 0.778 µg/ml and the LD_{90} value was 1.044 µg/ml (Table 6).

A concentration of 1 µg/ml of Gainpro AITC-Gamma extract showed a very strong insecticidal effect and no live insects were observed on day 25 (Figure 7). The LD_{50} value of the plant formulation observed on day 25 was 0.426 µg/ml and the LD_{90} value was 1.359 µg/ml (Table 7). The 1.10 µg/ml concentration of Gainpro AITC-Gamma extract showed a very strong insecticidal effect and no live insects were observed on day 23 (Figure 8). The LD_{50} value of the plant formulation observed on day 23 was 0.872 µg/ml and the LD_{90} value was 0.997 µg/ml (Table 8).

Table 6. LD_{50} and LD_{90} values of Grainpro AITC-EUC (Doses 0.90 0.95 1.05 1.1).

Day	Lethal Concentration Doses	
	LD_{50} (95% confidence limit)	**LD_{90} (95% confidence limit)**
1-2	–	–
3	4.198 [a] (–)	50.718 [a] (–)
4	1.182 [a] (–)	2.907 [a] (–)
5	1.156 [a] (–)	3.374 [a] (–)
6	1.062 [a] (–)	2.672 [a] (–)
7	1.043 [a] (–)	2.643 [a] (–)
8	0.997 [a] (–)	2.037 [a] (–)
9	0.974 [a] (–)	1.749 [a] (–)
10	0.941 [a] (–)	1.684 [a] (–)
11	0.937 [a] (0.787–0.991)	1.404 [a] (1.209–3.291)
12	0.928 [a] (0.782–0.980)	1.367 [a] (1.193–2.724)
13	0.857 [a] (0.03–0.941)	1.486 [a] (–)
14	0.847 [a] (0.383–0.925)	1.345 [a] (1.161–6.192)
15	0.834 [a] (0.379–0.914)	1.306 [a] (1.143–4.613)
16	0.831 [a] (0.611–0.898)	1.155 [a] (1.073–1.549)
17	0.834 [a] (–)	1.078 [a] (–)
18	0.778 [a] (–)	1.044 [a] (–)
19	0.709 [a] (–)	1.039 [a] (–)
20	0.687 [a] (–)	1.017 [a] (–)
21	0.734 [a] (–)	1.023 [a] (–)
22	0.665 [a] (–)	0.993 [a] (–)
23	0.616 [a] (–)	0.955 [a] (–)
24	0.669 [a] (–)	0.885 [a] (–)
25	0.466 [a] (–)	0.731 [a] (–)

–: not calculated. a: µg/ml

Table 7. LD_{50} and LD_{90} values of Gainpro AITC-Gamma (Doses 0.2 0.4 0.6 0.8 1).

Day	Lethal Concentration Doses	
	LD_{50} (95% confidence limit)	**LD_{90} (95% confidence limit)**
1-2	–	–
3	16.597 [a] (–)	–
4	6.635 [a] (2.036–3843.36)	145.497 [a] (–)
5	4.447 [a] (1.750–3310.741)	70.368 [a] (–)
6	2.424 [a] (1.331–24.327)	24.379 [a] (5.776–9001.231)
7	1.903 [a] (1.189–7.907)	14.346 [a] (4.592–600.628)
8	1.699 [a] (1.091–6.308)	13.837 [a] (4.463–556.365)
9	1.444 [a] (0.979–4.129)	11.390 [a] (4.031–274.216)
10	1.387 [a] (0.957–3.645)	10.445 [a] (3.864–199.409)
11	1.387 [a] (0.957–3.645)	10.445 [a] (3.864–199.409)
12	1.569 [a] (0.979–8.153)	19.698 [a] (5.033–4436.794)
13	1.492 [a] (0.954–6.512)	17.486 [a] (4.779–2330.720)
14	1.331 [a] (0.893–4.282)	13.740 [a] (4.274–757.059)
15	0.999 [a] (0.748–1.859)	7.309 [a] (3.123–74.317)
16	0.808 [a] (–)	3.999 [a] (–)
17	0.752 [a] (–)	3.433 [a] (–)
18	0.684 [a] (–)	3.118 [a] (–)
19	0.616 [a] (–)	2.908 [a] (–)
20	0.597 [a] (–)	2.631 [a] (–)
21	0.553 [a] (–)	2.850 [a] (–)
22	0.523 [a] (–)	2.522 [a] (–)
23	0.502 [a] (–)	2.409 [a] (–)
24	0.453 [a] (–)	1.753 [a] (–)
25	0.426 [a] (–)	1.359 [a] (–)
26	0.398 [a] (–)	1.198 [a] (–)
27	0.361 [a] (0.002–0.662)	1.034 [a] (–)
28	0.340 [a] (0.069–0.537)	0.953 [a] (0.590–20.663)
29	0.311 [a] (0.037–0.498)	0.806 [a] (0.502–23.218)
30	0.300 [a] (0.072–0.456)	0.754 [a] (0.491–5.580)

–: not calculated. a: µg/ml

Table 8. LD_{50} and LD_{90} values of Gainpro AITC-Gamma (Doses 0.90 0.95 1.05 1.1).

Day	Lethal Concentration Doses	
	LD_{50} (95% confidence limit)	**LD_{90} (95% confidence limit)**
1-2	–	–
3	1.486 [a] (–)	2.407 [a] (–)
4	–	–
5	1.863 [a] (–)	5.489 [a] (–)
6	1.355 [a] (–)	2.342 [a] (–)
7	1.260 [a] (–)	2.375 [a] (–)
8	1.140 [a] (1.058–1.812)	1.761 [a] (1.371–12.397)
9	1.127 [a] (1.042–2.956)	1.867 [a] (1.386–259.598)
10	1.120 [a] (–)	2.592 [a] (–)
11	1.129 [a] (–)	3.243 [a] (–)
12	1.100 [a] (–)	2.726 [a] (–)
13	1.084 [a] (–)	2.525 [a] (–)
14	1.066 [a] (–)	2.836 [a] (–)
15	1.016 [a] (–)	1.924 [a] (–)
16	0.990 [a] (–)	1.937 [a] (–)
17	0.943 [a] (0.621–1.012)	1.561 [a] (1.258–35.221)
18	0.905 [a] (0.753–0.956)	1.297 [a] (1.159–2.111)
19	0.897 [a] (0.835–0.931)	1.103 [a] (1.055–1.207)
20	0.888 [a] (0.824–0.923)	1.083 [a] (1.040–1.174)
21	0.879 [a] (0.810–0.915)	1.070 [a] (1.030–1.156)
22	0.868 [a] (0.799–0.903)	1.036 [a] (1.002–1.102)
23	0.872 [a] (0.819–0.900)	0.997 [a] (0.971–1.043)
24	0.869 [a] (0.816–0.895)	0.973 [a] (0.950–1.014)
25	0.874 [a] (0.822–0.897)	0.960 [a] (0.940–1.003)
–: not calculated. a: µg/ml		

Table 9. LD_{50} and LD_{90} values of Grainpro Gamma EUG (Doses 0.2 0.4 0.6 0.8 1).

Day	Lethal Concentration Doses	
	LD_{50} (95% confidence limit)	**LD_{90} (95% confidence limit)**
1-8	–	–
9	2.950 [a] (–)	–
10	2.950 [a] (–)	–
11	2.950 [a] (–)	–
12	1.420 [a] (–)	–
13	0.597 [a] (–)	–
14	0.417 [a] (–)	–
15	0.273 [a] (–)	–
16	0.164 [a] (–)	–
17	0.099 [a] (–)	–
18	0.062 [a] (–)	–
19	0.089 [a] (–)	39.457 [a] (–)
20	0.099 [a] (–)	9.918 [a] (–)
21	0.079 [a] (–)	6.732 [a] (–)
22	0.085 [a] (–)	3.959 [a] (–)
23	0.099 [a] (–)	1.736 [a] (–)
24	0.104 [a] (–)	1.241 [a] (–)
25	0.099 [a] (–)	1.223 [a] (–)
26	0.093 [a] (–)	0.968 [a] (–)
27	0.093 [a] (–)	0.924 [a] (–)
28	0.103 [a] (0.020–0.181)	0.782 [a] (0.565–1.632)
29	0.098 [a] (0.019–0.172)	0.687 [a] (0.505–1.287)
30	0.101 [a] (0.025–0.170)	0.589 [a] (0.444–0.952)

–: not calculated. a: µg/ml

Table 10. LD_{50} and LD_{90} values of Grainpro Gamma EUG (Doses 0.90 0.95 1.05 1.1).

Day	Lethal Concentration Doses	
	LD_{50} (95% confidence limit)	**LD_{90} (95% confidence limit)**
1-8	–	–
9	1.281 [a] (–)	91.031 [a] (–)
10	1.131 [a] (–)	14.908 [a] (–)
11	1.033 [a] (–)	6.441 [a] (–)
12	0.997 [a] (–)	4.941 [a] (–)
13	0.892 [a] (–)	3.139 [a] (–)
14	0.891 [a] (–)	1.607 [a] (–)
15	0.861 [a] (–)	1.412 [a] (–)
16	0.875 [a] (0.730–0.927)	1.194 [a] (1.103–1.572)
17	0.871 [a] (0.737–0.922)	1.169 [a] (1.089–1.464)
18	0.858 [a] (0.701–0.914)	1.162 [a] (1.082–1.478)
19	0.865 [a] (0.761–0.910)	1.101 [a] (1.047–1.247)
20	0.769 [a] (0.444–0.855)	1.049 [a] (0.994–1.266)
21	0.789 [a] (0.569–0.859)	1.014 [a] (0.970–1.119)
22	0.763 [a] (0.472–0.844)	0.976 [a] (0.924–1.054)
23	0.766 [a] (0.405–0.842)	0.920 [a] (0.825–0.965)
24	0.684 [a] (0.000–0.811)	0.903 [a] (0.222–0.964)
25	0.665 [a] (–)	0.878 [a] (–)
–: not calculated. a: µg/ml		

The 1 µg/ml concentration of Gainpro AITC-Gamma-EUG extract showed a very strong insecticidal effect and no live insects were observed on day 23 (Figure 9). The LD_{50} value of the plant formulation observed on day 23 was 0.099 µg/ml and the LD_{90} value was 1.736 µg/ml (Table 9). The 1.10 µg/ml concentration of Gainpro AITC-Gamma-EUG plant formulation showed a very strong insecticidal effect and no live insects were observed on day 21 (Figure 10). The LD_{50} value of the plant formulation observed on day 21 was 0.789 µg/ml and the LD_{90} value was 1.014 µg/ml (Table 10).

In terms of study results, the effectiveness of these extracts used against this pest or similar pests has not been determined in previous studies. In this study, the LD_{50} and LD_{90} values of these formulations were determined. However, different results were obtained in studies with some conventional and plant extracts against this pest and similar pests. Yiğit et al. (2021) determined the efficacy, LD_{90}, and LT_{90} values of three different doses (0.1%; 0.5% and 1.0%) of thyme essential oil (*Thymbra spicata var. spicata, Origanum majarona* and *O. saccatum*). In the study, it was stated that the LD_{90} values of three different thyme oils in 0.1% dose were 8.36, 4.81, 8.99, and 6.82, 1.90, 4.33 in 1% dose, respectively. Also, almost 100% death occurred on the 4th day of *O. majarona*. In our study, however, the 1.10 µg/ml dose of Gainpro AITC-Gamma-EUG showed the highest effect, but 100% mortality occurred at the end of the 21st day. This difference is thought to be due to the fact that the dose used in our study was approximately 99% lower than the study on thyme. In our next study, it is thought that this effect will be achieved in a very short time with a dose increase.

In our study, % lethal dose values and viability rates of the extracts were determined depending on time. In addition, the LD_{50} value of 1.10 µg/ml dose of Gainpro AITC-gamma-EUG was found to be 1.281 on the 9th day, while this value was found to be 0.10 on the 4th day in *Origanum majorana*, which is the most effective among thyme. This difference is due to the dose difference, as we mentioned above.

Mahmoud & Sabbour (2020) studied the insecticidal effects of four different natural essential oils on *T. confusum* and *T. castaneum*. They determined that coriander and caraway oils were highly effective against both pests, and that 2% dose in nano-formulations caused 70%-85% mortality on the 7th day of application. Gokturk et al. (2020) determined the effects of essential oils of *Ocimum basilicum L*., *Rosmarinus officinalis L*. and *Artemisia dracunculus* on *T. confusum*. At the end of 96 hours, it was determined that *O. basilicum* had 98.3%, *R. officinalis* 98.3%, *A. dracunculus* 93.3% lethal effects. The dose of plant oils used in the study was 20 µL, which is about 20 times the highest dose used in our study. In addition, while spraying method and petri dish application were used for pests in this study, a larger application plastic container was used compared to the food supply and petri dish in our study. In this study, the LD_{50} and LD_{90} values determined in our study were not studied.

Memon et al. (2020) studied the effects of 2%, 1.5%, 1%, and 0.5% doses of *Mentha longifolia* against *T. confusum*. It was determined that the 2% dose reduced the population the most and caused the least weight loss after feeding on wheat grain. In addition, it was determined that the 2% dose caused 96.70% mortality within 7 days. However, the dose rate in this study was investigated higher than in our study and no lethal dose study was performed.

Işıkber et al. (2019) studied the effects of mustard oil alone and with modified atmosphere applications against *Tribolium confusum*. They stated that the mustard essential oil and the 92% CO_2 concentration were more toxic to the larvae, pupae and adults of the pest. They determined that 10 µl/l mustard oil caused nearly 100% mortality in the pest. It was determined that LC_{50} value was 2.25 and LC_{90} value was 3.67 at the 24th hour of application of mustard oil against pest adults. In our study, the LD_{50} value of 1.10 µg/ml dose of Gainpro AITC-Gamma-EUG as the most effective dose was found to be 1.281 on the 9th day. This difference is thought to be due to the dose difference of the plant extract used.

As a result, it was observed that the insecticidal effectiveness of the plant extracts used in this study increased in parallel with the increase in dose, and the LD_{50} and LD_{90} values decreased depending on time due to the low concentration and dose. Diversification of the compounds with new studies including dose increase depending on the perspective of stored pests and organic pest control will provide an effective approach in the fight against warehouse pests. Among these formulations, 1 µL and 1.10 µL doses of Gainpro AITC-EUC formulation were the most effective on the pest.

COMPLIANCE WITH ETHICAL STANDARDS

This research article complies with research and publishing ethics.

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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