Using eco-friendly green insecticides for storages pest management: Prangos ferulacea (essential oil and powder) against Callosobruchus maculatus (Col. : Chrysomelidae)

Zeinab Hosseini
Yasuj University of Medical Sciences
Gholamhossein Shahraki
vahabsh@yahoo.com
Yasuj University of Medical Sciences
Amin Sedaratian-Jahromi
Yasouj University

Article

Keywords: Prangos ferulacea, Callosobruchus maculatus, aluminum phosphide, essential oil, insecticide

Posted Date: November 26th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7967791/v1

Additional Declarations: No competing interests reported.

Abstract

To promote the use of eco-friendly green pesticides as storage pests, this study was designed to evaluate the insecticidal effects of *Prangos ferulacea* Lindl. essential oil and powder on *Callosobruchus maculatus* F in vitro.

The LC_{50} value of the essential oil was 16.28 µL/L, and the LD_{50} value of the powder was equal to 1.6 g/kg food after 72 h. The LT_{50} value of the essential oil was 36.623 h for stability and analysis of the probit slope (1.596 ± 0.291), and the logarithm of time and mortality rate were significant (95% CI). The LT_{50} value was determined to be 38.72 h for the powder and 50.85 h for the aluminum phosphide tablets; thus, the essential oil had a greater effect on the powder formulation as well as the aluminum phosphide. A comparison of the durability of the treatments revealed that during 24 h, the essential oil with a mortality rate of 80.55 ± 6.10% was significantly superior to the aluminum phosphide tablets (40.50 ± 7.43% at 99% CI).

The results of this study revealed that *P. ferulacea* essential oil has acceptable respiratory toxicity to the examined storage pest.

Introduction

Legumes are attacked by various insect mite pests during storage, and 50% of the beans are sometimes destroyed by pests during the 3–4 month storage period. The cowpea seed weevil (*Callosobruchus maculatus* F. (Col. : Chrysomelidae: Bruchinae) is one of the most important storage pests that damages legumes such as beans, peas, mung beans (*Vigna radiata* (L.)), lentils, etc. (Shakarami et al., 2004).

In some countries, pesticides such as aluminum phosphide are used to preserve legumes from insects. Rice pellets (aluminum phosphide) are highly toxic substances and dangerous combinations of phosphides that are used as pesticides, insecticides, and fumigants in the agricultural industry to preserve grains, cereals and rice(Daglish et al., 2018). Annually, more than 300,000 deaths from pesticide poisoning occur worldwide, most of which are caused by aluminum phosphide poisoning (Gunnell & Eddleston, 2003). According to a forensic medicine report from Iran, mortality due to aluminum phosphide poisoning is increasing, and the number of people who die because of aluminum phosphide poisoning has increased from 214 cases in 2008 to 598, 636, 825 and 919 cases in 2012, 2014, 2018 and 2019, respectively (Organization, 2020; Soltani nejad, 2019).

Aluminum phosphide poisoning is one of the deadliest poisonings, and no solution has yet been found to save patients' lives. The most severe form of acute poisoning with this substance is caused by eating half a 3-gram tablet. This toxic substance is easily absorbed by the gastrointestinal tract and produces dangerous phosphine gas after being dissolved in body fluids and stomach moisture. The resulting phosphine disrupts the cytochrome oxidase enzyme and causes cell death in various organs, including the heart, liver and lungs, in less than 6 h after ingestion (Shadnia et al., 2006).

In general, it has been proven that some plants have compounds leading to fast insect death in addition to repellent effects, antifeeding and inhabitation of oviposition. Among the plants with extraordinary toxic potential for storage pests, those with essential oil and medicinal and nutritional properties and rapid degradation in nature, in addition to being low risk to humans and other mammals, are known (Karahroudi et al., 2010; Negahban & Moharamipour, 2007). Since plant compounds affect the physiology and behavior of pests, the use of these compounds and their products has attracted much attention for controlling pests, particularly storage pests. However, some of these compounds are terpenoids found in plant essential oils and seem to be safe alternatives to chemical pesticides (Taghizadeh & Moharramipour, 2011).

Among these plants, *Prangos ferulacea* (L.) (Apiaceae) is notable. The Prangos genus has approximately 30 species, of which 15 are in Iran and only 5 are native to Iran (Sajjadi & Mehregan, 2003). In addition to Iran, other species of this genus are distributed in Turkey, the Balkans, Italy, Syria, Kazakhstan and the Caucasus (Coşkun et al., 2004; Rechinger, 1982). *Prangos ferulacea* is distributed in the provinces of East Azerbaijan, West Azerbaijan, Kurdistan, Kermanshah, Hamedan, Semnan, and the southern regions of the Alborz, Isfahan, Lorestan, Ilam, Kohgiluyeh and BoyerAhmad, Kerman and the mountains of Fars Province in Iran (Zargari, 1997). *Prangos ferulacea* is a long, tall and fragrant plant belonging to the Umbelliferae family that grows in many mountainous areas of Iran and is used as one of the main plants supplying winter fodder for livestock (H Amiri, 2007; Hamzeh Amiri, 2007; Kafash-Farkhad et al., 2013; Mozafari et al., 2007). *Prangos ferulacea* is a moisture-friendly plant that needs plenty of moisture, cold and frost to complete its life cycle and is often found in areas that are cold and snowy. Its growth rate in clay soils is better than that in other soils. These climatic needs are met at relatively high altitudes and slope directions (Hasani & Shahmoradi, 2007; Rechinger, 1982; Zargari, 1997). terpenoid compounds, especially monoterpenoids, can be used as low-risk compounds for humans and the environment and as alternatives to synthetic chemical compounds in the control of storage pests (H Amiri, 2007; Kafash-Farkhad et al., 2013; Mobarakian et al., 2016). The identification of compounds found in *P. ferulacea* essential oils in all three phases (before flowering, flowering, fruiting) has shown that the main components of the essential oils of this plant in all three phases are monoterpene compounds, especially alpha- and beta-pinene, such that these two compounds constitute more than 65% of the essential oils (Hamzeh Amiri, 2007). The insecticidal effects of *P. ferulacea* on the flour moth *Ephestia kuehniella* (Ercan et al., 2013), its antimicrobial effects (Hamzeh Amiri, 2007; Badalamenti et al., 2022) and its antioxidant activity (Badalamenti et al., 2022; Bruno et al., 2021) have already been studied.

Thus, in the present research, the insecticidal effects of *P. ferulacea* essential oils and powders on *C. maculatus* were analyzed as alternatives to aluminum phosphide tablets, which have been used for many years as fumigants to protect stored grains from insects in some Middle East countries.

Materials and methods

Insect rearing

The storage pest *C. maculatus* was prepared from the laboratory of the Department of Plant Protection, Faculty of Agriculture, Yasouj University. To rear and reproduce the insects, 100 male and female insects (50:50 ratio) were transferred to 250 g of mung bean (*Vigna radiata*) in a container (approximately 3 liters) and kept at 25±2 °C in darkness. To eliminate possible contamination, first, the *V. radiata* used in the present study were kept at -18 °C for 72 h and then used in the experiments.

After one day, the adult insects were removed via an electric aspirator from the breeding containers and transferred to new containers containing healthy, noncontaminated *V. radiata*. The *V. radiata* seeds containing one-day-old weevil larvae were subsequently kept at ambient temperature in darkness until a new generation of insects emerged. Notably, one- to three-day-old adult insects (*C. maculatus*) were used for the bioassay experiments.

Collection of P. ferulacea

At the end of May 2018, the aerial parts of *P. ferulacea* were collected from mountains located in Ab-Nahr, Yasuj (lat: 30.68183, Lon: 51.68183), and dried under appropriate shading and ventilation conditions. *P. ferulacea* samples were purchased from a local farmer with permission. No wild plants were collected, and all procedures complied with national and international guidelines (IUCN and CITES). The dried plants collected for the experiments weighed approximately 20 kg. The collected plant species (*P. ferulacea*) was approved by Dr. Azizolah Jafari, Assistant Professor of Plant Systematics, Yasouj University. A voucher specimen of *P. ferulacea* (voucher no. HYU 350–1750) has been deposited in the Herbarium of Yasouj University (HYU), Iran.

Preparing essential oils

To prepare the essential oil, the aerial parts of *P. ferulacea* were fully pulverized by an electric shredder (Pars Khazar, Iran) in the shortest time before essential oil extraction. To extract the essential oil, 100 g of plant powder with 1200 ml of distilled water was poured into a Clevenger essential oil extractor (Pyrex Fan, 2000 cc, Iran). The extraction of essential oil after boiling the suspension of dried plant powder was continued for 3 h at 100 °C. The essential oils enriched with anhydrous sodium sulfate were dehydrated and stored in 2 ml microtubes with an aluminum coating in a refrigerator (4 °C).

Preparing powder

Prior to each experiment, an amount of *P. ferulacea* that had previously been dried under appropriate conditions was powdered with an electric grinder and sieved through 70 mesh.

Aluminum phosphide tablet

Aluminum phosphide tablets were purchased from pesticide distribution centers in 10-tube packaging sealed by necessary licenses.

Testing conditions

The experiments were performed at a temperature of 25 ± 2 °C, a relative humidity of 75 ± 5% and darkness in a laboratory incubator (Rad Teb, Iran).

Evaluation of the respiratory toxicity of essential oils to C. maculatus adults

The bioassay was performed in dark McCarthy containers (70 ml). Given that the probit model is used in data analysis, basic tests were conducted to find a range of concentrations associated with 20–80% mortality in all the experiments. The different concentrations studied included 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.55, 0.6, 0.65, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, and 8.8 µl. By performing primary assays, low concentrations (20% mortality) and high concentrations (80% mortality) of essential oils were determined, and appropriate concentrations ranging from 20–80% mortality were selected. The selected concentrations are as follows: 0.50, 0.60, 0.70, 0.90, 1.00, 2.00, 2.50, 3.00, 3.50 and 4.50 µl (equivalent to 7.14, 8.57, 10, 12.85, 14.28, 28.57, 35.71, 42.85, 50 and 64.28 µl/l air, respectively).

The essential oil used at the aforementioned concentrations was poured with a sampler (Clever, Germany) on pieces of filter paper (Watman, UK) that had previously been cut to the size of a lid, and then the filter was inserted into the lid of the container used in the experiments. Then, 10 adult insects aged 1–3 days were transferred to each container. To prevent the insects from contacting the filter paper and eliminate the effect of contact toxicity, the containers were covered with fine mesh nets that the studied insects could not pass through, and then the lid containing the filter paper impregnated with essential oil was tightly closed. To prevent the evaporation of essential oil from the container, they were properly closed with parafilm tape (American National Can Co.). The number of dead insects in the control and treatment containers was counted after 24, 48, and 72 h of essential oil extraction. At the end of this period, the insects whose appendix could not be moved after brush stimulation were considered dead. The experiments were performed in at least 5 replicates, with the control at a temperature of 25±2 °C, a relative humidity of 75±5% and darkness. In the control group, the same steps were performed, but the essential oil was not used on the filter paper pieces.

Evaluating the toxicity of P. ferulacea powder to C. maculatus adults

To evaluate the toxicity of *P. ferulacea* powder on cowpea seed weevils, experiments were performed in McCarthy containers. To find the right weight, a series of basic experiments were performed. Basic tests were performed to find a range of concentrations with a mortality range of 20–80%. The different weights studied included 0.001, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.1, 0.3, 0.5, 0.8, 1, 1.3, 1.5, 1.8, 2, 2.3, 2.5, and 3 g of *P. ferulacea* powder for 10 g of food, as measured by digital scales (AND, Japan), with an accuracy of 0.001 g. The powder was mixed with *V. radiata*. Low concentrations (20% mortality) and high concentrations (80% mortality) were used. The selected concentrations included 0.001, 0.005, 0.01, 0.02, 0.03, 0.04, 0.08, 0.3, 0.5 and 1.00 g of *P. ferulacea* powder for every 10 g of food. In the next step, 10 adult insects (1–3 days old) were transferred to each container. After 24, 48, 72 and 96 h, the number of dead insects in each container was recorded. The experiments at this stage were performed with at least 5 replications. *P. ferulacea* powder was not used in the control group.

Evaluating the toxicity of aluminum phosphide tablets to C. maculatus

In this step, the experiments were performed in 3.5 L containers. To determine the appropriate concentration, several series of basic tests were performed. As in the previous stage, basic tests were performed to determine the range of concentrations that cause mortality of 20–80%. The different concentrations included 0.001, 0.0001, 0.0002, 0.0003, 0.0004, 0.0005, 0.0006, 0.0007, 0.0008 and 0.0009 g of aluminum phosphide tablet. By performing basic experiments, low and high concentrations of tablets were determined, and appropriate concentrations with 20–80% mortality were selected. The selected concentrations, including 0.0002, 0.0003, 0.0004, 0.0005, 0.0006, 0.0007, 0.0008, and 0.0009 g (equivalent to 0.00005, 0.00008, 0.00011, 0.00014, 0.00017, 0.00020, 0.00022, and 0.00025 g/l air), were poured into Petri dishes with two or three small holes attached to the lids of the container. A set of 10 insects was placed in another Petri dish inside the container, and then, the lid was tightly closed. The lids of the glass containers were opened 24, 48 and 72 h after the start of the experiment, and the number of dead insects per concentration was recorded. The experiments of this stage were performed in at least 5 replications. The conditions of the experimental environment were similar to those of the previous steps. Notably, no aluminum phosphide tablets were used in the control group.

Evaluating the durability of the essential oils and powders of P. ferulacea and aluminum phosphide tablets

To evaluate the durability of the essential oil, the LC_{50} values of the respiratory toxicity of the essential oil, powder and aluminum phosphide tablets were determined via SPSS. This index for essential oil, powder and aluminum phosphide tablets was 1.14 µl per 70 ml of air (16.28 µl/l air), 0.015 g per 10 g of food (1.6 g/kg food), and 0.0006 g per 3.5 liter of air (0.00017 g/l air). To investigate the durability of *P. ferulacea* essential oil, 1.14 µl of essential oil was poured by a sampler on filter paper that was previously cut to the size of a container lid, and then the filter paper inside the lid of the containers was

used in the experiments. As in the previous stages, the containers were covered by fine mesh nets that the studied insects could not pass through, and then, the lid was tightly closed. To prevent the essential oil from escaping, the lids were properly closed with parafilm tape (American National Can Co.). Over a period of one, 24, one, two, three or four weeks after essential oil extraction, 10 1–3-day-old adult insects were transferred to the test containers, and 24 h later, the mortality rates of the studied insects were recorded. The experiments were conducted in 5 replications under the previously mentioned conditions.

To evaluate durability, 0.015 g of *P. ferulacea* powder was mixed with 10 g of beans, and over a period of one month, 24 h, and one, two, three and four weeks after the powder was added to the containers, 10 adult insects aged 1–3 days were left in the test containers, and mortality was recorded 24 h after each period.

The method used to evaluate the durability of the studied tablets was the same as that used for *P. ferulacea* powder. In this stage, 0.0006 g of aluminum phosphide tablet was measured and then poured into Petri dishes with two or three small holes attached to the lids of containers. Over a period of one month, at 24 h, one-, two-, three- and four-week intervals after the tablets were added to the environment, 10 adult cowpea seed weevils aged 1--3 days were placed in another Petri dish in containers, and the lid was then tightly closed. Twenty-four hours later, the mortality rate was recorded.

Data analysis

A probit model was used to evaluate the lethality of the essential oils and powders of *P. ferulacea* and the aluminum phosphide tablets. In the analysis of this stage, SPSS (Ver.18) was used. Analysis of variance was used to evaluate the durability of the plant and chemical materials used. If a significant difference was observed between the averages at this stage, Tukey's test was used to categorize the averages ($P<0.05$).

Results

Toxicity of P. ferulacea essential oil to C. maculatus after 24, 48 and 72 h

The results of the experiments revealed that the mortality rate of adult insects increased with increasing essential oil concentration and duration of extraction. The highest mortality at the highest concentration of essential oil (64.28 µl/l air) and over a period of 48 h was approximately 100%, and the lowest mortality rate at the lowest concentration (7.14 µl/l air) and over a period of 24 h was 20%. Therefore, it can be concluded that with increasing concentration and time, the respiratory toxicity of the abovementioned plant essential oils increased (Table 1).

Table 1. Toxicity of Prangos ferulacea essential oil to Callosobruchus maculatus

% Mortality by time intervals			Concentrations
72 h	48 h	24 h	(µl/l air)
62.00±13.16	45.00±22.36	20.00±0.00	7.14
67.40±17.14	53.59±18.67	30.00±14.14	8.57
69.09±9.03	58.80±2.88	43.94±6.94	10
70.00±0.00	59.09±13.91	52.50±5.00	12.85
73.87±15.12	61.25±13.56	54.43±13.43	14.28
75.33±10.87	70.66±16.39	66.30±4.84	28.57
76.74±16.17	72.39±10.08	68.20±8.06	35.71
80.00±10.00	77.50±17.07	72.50±17.07	42.58
80.00±10.00	78.57±22.67	76.00±16.73	50
100.00±0.00	100.00±0.00	80.00±5.77	64.28

In this study, a lethality of 20–80% was not obtained at the concentrations selected for the LC_{50} analysis after 72 h; thus, the lethal effects were reported only at 24 and 48 h (Table 1). The LC_{50} values and the results of the probit analysis of the bioassay data for *P. ferulacea* essential oils are shown in Table 2. The logarithm of the concentration and mortality probit of *C. maculatus* caused by *P. ferulacea* essential oil after 24 and 48 h revealed that the mortality of the insects tended to increase, and with probit analysis, the LC_{50} values of the essential oil were 16.28 and 12.88 µl/l air after 24 and 48 h, respectively, indicating that with increasing time, the mortality rate of *C. maculatus* increased. According to the results, the insecticidal power increased over time, which was due to the decrease in the LC_{50} values over time. Figures 1 and 2 show that the mortality rate is different at various concentrations and that the applied concentrations are directly related to the mortality rate, which means that with increasing concentration, the mortality rate of *C. maculatus increases*.

Table 2. LC_{50} values determined via probit analysis of the bioassay data of *Prangos ferulacea* essential oil on *Callosobruchus maculatus* after 24 and 48 h

Efficacy time (h)	LC_{50} (µL/L)	Slope(a)±SE	Intercept(b)±SE	Chi-square (χ^2)	*P*
24	16.28 (13.85-18.84)*	1.672±0.184	-0.095±0.059	32.66	0.996
48	12.88 (8.01-16.55)*	1.197±0.259	0.054±0.75	39.729	0.655

*Low and high 95% CIs are shown in parentheses **SE=standard error

Determination of the lethality rate of P. ferulacea powder to C. maculatus after 24, 48, 72 and 96 h

In this study, given that the mortality rate of *C. maculatus* caused by powder was less than 100%, 4 time intervals of 24--96 hours were used to evaluate the lethality rate of *P. ferulacea* powder on *C. maculatus*.

The results of the experiments revealed that the mortality rate of *C. maculatus* increased with increasing powder concentration, and the highest mortality rate was observed at the highest concentration. The highest mortality rate at the highest concentration of powder was 78% for 1.00 g per 10 g of food after 96 h, and the lowest mortality rate was 9.54% at the lowest concentration, 0.001 g per 10 g of food after 24 h. Therefore, it can be concluded that with increasing concentration and time, the toxicity of the aforementioned plant powder increased (Table 3).

Table 3. Toxicity of *Prangos ferulacea* powder to *Callosobruchus maculatus* after 24, 48, 72 and 96 h at different concentrations

% Mortality by time intervals				Concentrations
96 h	72 h	48 h	24 h	(g per 10 g food)
62.00±10.97	33.33±5.77	27.95±17.71	9.54±11.91	0.001
64.80±8.67	91/4±16/39	29.54±13.82	13.62±7.52	0.005
65.00±3.74	46.83±7.75	33.66±14.73	20.70±9.73	0.01
65.30±5.13	50.40±8.76	35.60±15.53	22.22±14.52	0.02
65.60±6.07	52.33±4.04	40.75±6.99	26.00±6.00	0.03
66.00±5.87	56.42±4.75	42.00±12.02	30.42±10.06	0.04
68.60±13.22	61.33±2.30	43.00±10.00	32.00±5.17	0.08
70.60±6.84	69.00±10.14	44.00±11.43	33.50±10.75	0.3
72.00±4.47	70.00±0.00	46.00±11.43	33.00±4.91	0.5
78.00±13.03	75.75±5.05	47.75±14.55	33.33±5.77	1.00

In this study, according to the results shown in Table 3, at 24, 48 and 96 hours, a lethality of 20–80% was not obtained at the concentrations selected for the LC_{50} analysis, so the lethal effects were reported only at 72 hours. The LD_{50} values and the results of the probit analysis of the bioassay data of *P. ferulacea* powder on *C. maculatus* are given in Table 4.

Table 4. The LD_{50} values were computed via probit analysis of the bioassay data of *Prangos ferulacea* powder on *Callosobruchus maculatus* after 72 h.

Efficacy time (h)	LD_{50} (g/kg)	Slope(a)±SE	Intercept(b)±SE	Chi-square(χ^2)	*P*
72	1.6 (0.6-3.3)*	0.366±0.074	0.660±0.128	6.936	1.000

*Low and high 95% CIs are shown in parentheses **SE=standard error

The logarithm of the concentration and mortality probability of *C. maculatus* caused by *P. ferulacea* powder after 72 h revealed that the mortality of the insects tended to increase, and after probit analysis, the LD_{50} value of the powder was 1.6 g/kg food after 72 h, which indicates that, with increasing time, the mortality rate of *C. maculatus* increased. According to the results, the insecticidal power increased over time, which was due to declining LD_{50} values over time. Figure 3 shows that with increasing concentration, the percentage of mortality in *C. maculatus* increased. In this study, significant mortality was observed in adults only at high concentrations of *P. ferulacea* powder.

Toxicity of aluminum phosphide to C. maculatus after 24, 48 and 72 hours

The results of the experiments revealed that the mortality rate of *C. maculatus* increased with increasing aluminum phosphide concentration and duration; thus, the highest mortality rate was observed at the highest dose, and the mortality rate reached 70--100% at 72 h. The highest mortality rate was 100% at the highest aluminum phosphide concentration, i.e., 0.00025 g/l air after 72 h, and the lowest mortality rate was 15.28% at the lowest concentration, i.e., 0.00005 g/l air after 24 h. Therefore, with increasing concentration and time, the toxicity of aluminum phosphide increased (Table 5).

Table 5. Toxicity of aluminum phosphide to *Callosobruchus maculatus* after 24, 48 and 72 h

% Mortality by time interval			Concentration
72 h	48 h	24 h	(g/L air)
46.66±11.54	33.33±5.77	15.28±5.47	0.00005
65.00±14.14	34.00±5.47	18.89±11.14	0.00008
71.66±20.20	40.13±12.47	23.33±5.77	0.00011
90.00±0.00	46.66±15.27	36.67±11.55	0.00014
92.00±5.77	57.57±28.93	45.45±31.01	0.00017
93.33±8.16	68.33±7.63	55.00±12.90	0.00020
100.00±0.00	90.00±0.00	62.00±16.43	0.00022
100.00±0.00	92.00±13.03	70.00±17.32	0.00025

In this study, according to the results shown in Table 5, a lethality of 20–80% was not obtained at the concentrations selected for LC_{50} analysis after 72 h; therefore, the lethal effects were reported at 24 and 48 h. The LC_{50} values and the results of the probit analysis of the aluminum phosphide bioassay data of *P. ferulacea* on *C. maculatus* are given in Table 6. The logarithm of the concentration and mortality probability of *C. maculatus* caused by aluminum phosphide after 48 and 72 h revealed that the mortality of the insects increased, and with probit analysis (Figures 4 and 5), the LC_{50} values of aluminum phosphide were 0.00017 and 0.00011 g/l air after 24 and 48 h, respectively, indicating that with increasing time, the mortality rate of *C. maculatus* increased. According to the results, the insecticidal power increased over time, which was due to declining LC_{50} values over time.

Table 6. The LD_{50} values were computed via probit analysis of bioassay data of aluminum phosphide in *Callosobruchus maculatus* after 24 and 48 h.

Efficacy time (h)	LC_{50} (g/L air)	Slope(a)±SE	Intercept(b) ±SE	Chi-square (χ^2)	*P*
24	0.00017 (0.00017-0.00022)*	2.558±0.366	8.182±1.212	27.238	0.660
48	0.00011 (0.00011-0.00014)*	2.435±0.439	8.226±1.474	20.147	0.633

*Low and high 95% CIs are shown in parentheses **SE=standard error

Determination of the LT_{50} value of P. ferulacea essential oil for C. maculatus

To determine the LT_{50} value of *P. ferulacea* essential oil to determine its stability, the LC_{20} values of the essential oils were equal to 5.11 µl/l air. The experiments were performed at various periods of 24, 48, 72, 96 and 120 h with the control treatment, which is shown in Figure 6 as a logarithm of time and mortality rate. With probit analysis, the LT_{50} calculated for the effect of essential oil was 36.623 h, the significance of which is shown in Table 7. In other words, essential oils caused 50% mortality in adult insects after 36.623 h.

Table 7. The computed LT_{50} of *Prangos ferulacea* essential oil for *Callosobruchus maculatus*

LT_{50}(h)	Slope(a)±SE	Intercept(b) ±SE	Chi-square (χ^2)	*P*
36.623 (27.24-44.45)*	1.596±0.291	-2.496±0.504	0.170	0.919

*Low and high 95% CIs are shown in parentheses **SE=standard error

Determination of the LT_{50} value of *Prangos ferulacea powder* for *Callosobruchus maculatus*

To determine the LT_{50} value of *P. ferulacea* powder, the LD_{50} value of the powder used was 1.6 g/kg food. The experiments were performed at various concentrations, which are shown as logarithms of concentration and mortality in Figure 7. The calculated LT_{50} for the effect of essential oil by probit analysis was 38.72 h, the significance of which is shown in Table 8. In other words, *P. ferulacea* powder caused 50% mortality in insects after 38.72 h.

Table 8. The computed LT_{50} of *Prangos ferulacea* powder against *Callosobruchus maculatus*

LT_{50}(h)	Slope(a)±SE	Intercept(b)±SE	Chi-square (χ^2)	*P*
38.72 (31.89-44.83)	2.096±254	-3.329±0.452	0.577	0.902

*Low and high 95% CIs are shown in parentheses **SE=standard error

Determination of the LT_{50} values of aluminum phosphide tablets on C. maculatus

To determine the LT_{50} value of aluminum phosphide, the LC_{20} value of aluminum phosphide tablets was used, which was 0.00008 g/l air. The experiment was evaluated at different times (24, 48, 72, 96 and 120 hours) with the control treatment, and the logarithms of the concentrations and mortality rates are shown in Figure 8. The calculated LT_{50} for the effect of aluminum phosphide by probit data analysis was determined to be 50.85 h, the significance of which is shown in Table 9. In other words, aluminum phosphide could cause a mortality rate of 50% in adult insects after 50.85 hours.

Table 9. The computed LT_{50} of aluminum phosphide on *Callosobruchus maculatus*

LT_{50}(h)	Slope(a)±SE	Intercept(b) ±SE	Chi-square (χ^2)	*P*
50.85 (44.069-58.188)	2.314±0.313	-3.949±0.547	0.674	0.714

*Low and high 95% CIs are shown in parentheses **SE=standard error

Comparison of the durability of P. ferulacea essential oil and powder with aluminum phosphide in adult C. maculatus

To compare the durability of *P. ferulacea* essential oil and powder with aluminum phosphide tablets, the LC_{50} values (or LD_{50} values for powder) used were 0.00017 g/L air for aluminum phosphide, 16.28 µl/L air for essential oil and 1.6 g/kg food for powder. The experiments were performed at different times, namely, 24 h and one, two, three and four weeks after exposure, with respect to the control group.

The results of the analysis of variance revealed that there was a significant difference in the mortality rate of *C. maculatus* among the different durations of essential oil (P = 0.000, $d\,f$ = 4, F=15.016) and *P. ferulacea* powder (P = 0.004, df = 4, F= 4.382) use. There was no significant difference in the *mortality rate of C. maculatus among the different durations of aluminum phosphide use* (P = 0.056, df = 4, F = 2.77) (Table 10). According to Table 10, the results of the analysis of variance of *P. ferulacea* essential oil also revealed that there was a significant difference between 24 hours of exposure at all testing times (CI = 95%, P <0.05) and between the first week and fourth week (P=0.014).

The results also revealed a significant difference in the mortality rate of *C. maculatus* between 24 hours of contact and one week after contact (CI= 95%, P = 0.013) and the second week (CI = 95%, P = 0.020). Furthermore, there was a significant difference among the effects of essential oils, powders and aluminum phosphides on the mortality rate of the tested insects during 24 hours of exposure to 95% C. I (P = 0.000), and Tukey's test revealed that there was a significant difference only between the essential oils and the tablets (P = 0.047).

Table 10. Durability of aluminum phosphide tablets, *Prangos ferulacea* essential oils and powders on adult *Callosobruchus maculatus* according to the efficacy time

Samples*	% Mortality (Mean±SE)** by times after exposure					*P*
	24 h	1 week	2 weeks	3 weeks	4 weeks	
Essential oil	80.55±6.10	38.54±5.38	12.00±2.91	9.50±3.02	3.00±1.53	0.000
Powder	10.00±2.98	31.00±6.74	30.00±2.98	24.00±4.99	15.00±3.07	0.004
AP Tablet	40.50±7.43	17.82±5.89	14.00±4.00	6.00±2.45	4.00±2.45	0.060

* P_{value} between 3 samples is 0.000 (99% CI, ANOVA) ** SE=Standard error *** AP= aluminum phosphide

According to the results and as shown in Figure 9, at 24 h of exposure, the essential oil, aluminum phosphide and powder had the highest insecticides, and the *P. ferulacea* essential oil, accounting for 80.55%, had a greater mortality rate than the aluminum phosphide tablets, accounting for 40.50%. Over one week, the respiratory effects of the essential oils and tablets still resulted in significant mortality rates of 38.54% and 17.82%, respectively, with a mild decreasing trend for the next week, and after one

month, the effects of the essential oils and tablets were minimized by 3.00% and 4.00%, respectively, in terms of mortality. Although this decreasing trend was statistically significant until the third week for essential oil, the mortality rate did not significantly differ for aluminum phosphide after the first week. However, a fully different trend was observed for *P. ferulacea* powder, which can be attributed to its nature compared with that of the essential oils and tablets. Hence, the lowest insecticide effect was observed after 24 hours, and the highest insecticide effect was observed in the first week. These effects lasted until almost the second week, and in the end, the insecticide effects decreased over time. However, it showed greater durability than did the essential oils and tablets and remained at a higher level than the other two oils did at the fourth week, with an average mortality of 15%.

Discussion

The results of this study revealed that *P. ferulacea* essential oil had appropriate respiratory toxicity to *C. maculatus*, which has been reported by other researchers in terms of the respiratory toxicity of similar plant essential oils to insects (Ercan et al., 2013; Hamzavi et al., 2014; Owolabi et al., 2014; Roozbahani et al., 2014; Taghizadeh & Moharamipour, 2010; Taghizadeh & Moharramipour, 2011). In the present study, the toxicity of *P. ferulacea* essential oil to *C. maculatus* was significantly greater than that of aluminum phosphide, especially in the first 24 h of contact (more than 80.5% mortality versus 40.5% caused by aluminum phosphide). Additionally, essential oil has a faster effect than aluminum phosphide (LT_{50} = 36.62 h for essential oil and LT50 = 50.85 h for aluminum phosphide). The durability chart also shows that *P. ferulacea* essential oils compete well with aluminum phosphide tablets. Taghizadeh and Moharramipour (Taghizadeh & Moharamipour, 2010) also confirmed that *Prangos acaulis* (Apiaceae) has a favorable effect on *C. maculatus* (LC50 = 1.31 µl/L). Many researchers have reported the resistance of storage pests to aluminum phosphide and its decreasing effect (Lee et al., 2004; Rajendran & Sriranjini, 2008; Whitacre & Ware, 2004).

According to the results, with increasing concentrations and durations of essential oil extraction, the mortality rate of adult insects increased so that *P. ferulacea* essential oil caused 100% mortality at a concentration of 64.28% (the highest concentration tested) over 48 h, and as shown in previous reports, several researchers have reported the proportions of the concentration and duration of essential oil extraction with respect to the severity of storage pest losses (Ercan et al., 2013; Hamzavi et al., 2014; Roozbahani et al., 2014; Taghizadeh & Moharamipour, 2010).

In this study, *P. ferulacea* essential oil with an LC_{50} of 16.28 µl/L air was less toxic to *C. maculatus* after 24 h of essential oil extraction than aluminum phosphide tablets with an LC_{50} of 0.00017 g/L air. In other studies, *P. acaulis* essential oil with an LC_{50} of 1.31 µl/L air and *Thymus persicus (Lamiaceae)* with an LC_{50} of 2.39 µl/L air were toxic to *C. maculatus* (Taghizadeh & Moharamipour, 2010). In another study, *P. ferulacea* essential oil with an LC_{50} of 0.57 µl/L air showed desirable toxicity to another storage pest (*E. kuehniella*) ((Ercan et al., 2013). The pennyroyal essential oils with an LC_{50} of 0.35 µl/L air, the rosemary essential oils with an LC_{50} of 0.42 µl/L air, the case essential oils with an LC_{50} of 0.53 µl/L air, and the

mugwort essential oils with an LC_{50} of 0.99 µl/L air had respiratory toxicity effects on *Oryzaephilus surinamensis* (L.) (Col. : Silvanidae) over 24 h in research conducted by Roozbehani and others (Roozbahani et al., 2014).

In this study, *P. ferulacea* powder can be a moderate and desirable option in terms of durability (between essential oils and aluminum phosphide) as a contact insecticide when the processing time does not matter (especially after the first week of exposure). In Africa, the leaves of essential oil-containing plants are traditionally placed on stored seeds for preservation from pest damage(Golob & Webley, 1980). Plant powders and essential oils are classified by the International Environment Agency as bioactive compounds from third-generation insecticides. These compounds are less harmful than conventional pesticides are and are very effective in integrated pest management (IPM) when produced and released properly. Features such as low toxicity to mammals, rapid decomposition, low molecular weight, volatility, and low durability have caused them to receive a great deal of attention today (Pérez et al., 2010; Rosell et al., 2008). In particular, they are very effective in competing with formerly popular pesticides such as aluminum phosphide for simplicity and a wide range of use. Another dimension is the cost of *P. ferulacea* (powder/essential oil), although its widespread use in agriculture is due to the lower price of aluminum phosphide; however, owing to its numerous therapeutic (pain relief, inflammation and diabetes), antibacterial, viral and fungal applications, it can be given to customers with mass production and at a low and competitive price with aluminum phosphide (Kafash-Farkhad et al., 2013).

Conclusion

It can be concluded that *P. ferulacea* essential oil has acceptable respiratory toxicity to the examined storage pest (*C. maculatus*) and could be used as an alternative to aluminum phosphide tablets. Furthermore, *P. ferulacea powder* after the addition of essential oil is also useful for the management of storage pests.

Suggestions

Although P. ferulacea has been shown to have high lethality but low durability, these studies were performed under in vitro conditions and in an environment outside the storage product mass. Moreover, many factors can affect natural storage conditions, including the type of storage product, volume and weight of the product, temperature and humidity of the storage mass used to absorb essential oil, repellent effects and durability; moreover, the unpleasant smell of *P. ferulacea* may have an unfavorable effect on the taste of storage products. Therefore, the above factors should be considered in future research. On the other hand, given the poor dispersing potential of essential oil compounds, novel technologies must be used to apply these compounds to prepare suitable formulations. Furthermore, a Gc/Ms analysis revealed that these essential oils could be considered promising candidates for pharmaceutical and nutraceutical preparations; however, flower essential oils are harmful at low and wide-spectrum concentrations (Badalamenti et al., 2022), and therefore, the toxicity safety analysis of food consumption was performed.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest.

Funding

This study was financially supported by Yasuj University of Medical Sciences, Yasuj, Iran.

Author Contribution

ASJ and ZH contributed to performing the experiments. GH was a major contributor in writing the manuscript. SH and ASJ, supervised the study and contributed to the critical revision of the manuscript. All the authors read and approved the final manuscript.

Acknowledgement

This article is derived from a research project approved by Yasuj University of Medical Sciences (ethics code IR.YUMS.REC.1396.62). This article is also resulted from collaboration of the Department of Entomology, Faculty of Agriculture, Yasuj University. We would like to appreciate the sincere cooperation of Vice President for Research and Botany Department of Yasuj University of Medical Sciences.

Data Availability

Data is provided within the manuscript or supplementary information files

References

1. **Amiri, H. (2007)**. Chemical Composition and Antibacterial Activity of Essential Oil of Prangos ferulacea (L.) Lindl. *Journal of Medicinal Plants*, *1*(21), 36-41.
2. **Amiri, H. (2007)**. Essential oil variation of Prangos ferulacea Lindl. in different stage of plant growth. *Iranian Journal of Medicinal and Aromatic Plants*, *1*(23), 121-127.
3. **Badalamenti, N., Maresca, V., Di Napoli, M., Bruno, M., Basile, A., & Zanfardino, A. (2022)**. Chemical Composition and Biological Activities of Prangos ferulacea Essential Oils. *Molecules*, *27*(21). https://doi.org/10.3390/molecules27217430
4. **Bruno, M., Ilardi, V., Lupidi, G., Quassinti, L., Bramucci, M., Fiorini, D., Venditti, A., & Maggi, F. (2021)**. Composition and biological activities of the essential oil from a Sicilian accession of Prangos

ferulacea (L.) Lindl. *Nat Prod Res*, *35*(5), 733-743. https://doi.org/10.1080/14786419.2019.1598996

5. **Coşkun, B., Gülşen, N., & Umucalılar, H. (2004)**. The nutritive value of Prangos ferulacea. *Grass and Forage Science*, *59*(1), 15-19.
6. **Daglish, G. J., Nayak, M. K., Arthur, F. H., & Athanassiou, C. G. (2018)**. Insect pest management in stored grain. In *Recent advances in stored product protection* (pp. 45-63). Springer.
7. **Ercan, F., Bas, H., Koc, M., Pandir, D., & Öztemiz, S. (2013).** Insecticidal activity of essential oil of Prangos ferulacea (Umbelliferae) against Ephestia kuehniella (Lepidoptera: Pyralidae) and Trichogramma embryophagum (Hymenoptera: Trichogrammatidae). *Turkish Journal of Agriculture and Forestry*, *37*(6), 719-725.
8. **Golob, P., & Webley, D. (1980)**. *The use of plants and minerals as traditional protectants of stored products*. [Record #7 is using a reference type undefined in this output style.]
9. **Hamzavi, F., Moharramipour, S., & Talebi, A. (2014).** Repellent activity and persistence of essential oils from Eucalyptus camaldulensis Dehnh. and Callistemon viminalis (Gaertn.) G. Don on some species of stored-product beetles. *Iranian Journal of Medicinal and Aromatic Plants*, *30*(2), 332-341.
10. **Hasani, J., & Shahmoradi, A. (2007)**. Autecology of Prangos ferulacea in kurdistan province.
11. **Kafash-Farkhad, N., Asadi-Samani, M., & Khaledifar, B. (2013)**. A review on secondary metabolites and pharmacological effects of Prangos ferulacea (L.) Lindl. *Journal of Shahrekord University of Medical Sciences*, *15*(3), 98-108.
12. **Karahroudi, Z., Seifi, F., & Rahbarpour, A. (2010)**. Study on the fumigant insecticide effect of essential oil of five medicinal plants on three stored product pests. *Plant Protection Journal*, *2*(3), 197-207.
13. **Lee, B.-H., Annis, P. C., & Choi, W.-S. (2004)**. Fumigant toxicity of essential oils from the Myrtaceae family and 1,8-cineole against 3 major stored-grain insects. *Journal of Stored Products Research*, *40*(5), 553-564.
14. **Mobarakian, M., Zamzni, A. A., Karimzadeh, J., Moeeny Naghadeh, N., & Emami, M. (2016)**. Insecticidal Effects of Crude Extracts of Six Officinal Plants on the Cowpea Weevil, Callosobruchus maculates F. (Col.: Bruchidae). *29*(4), 499-510.
15. **Mozafari, S., Sadeghi, S. E., Mohamadi, M., & Ali, B. (2007)**. Study some biological aspects and infestation rate of Pachymerus acaciae (Col.: Bruchidae) in rangelands of Kohgiouyeh and Boyer-Ahmad Province. *Iranian Journal of Forest and Range Protection Research*, *50*(1).
16. **Negahban, M., & Moharamipour, S. (2007)**. Efficiency of Artemisia sieberi Besser and Artemisia scoparia waldst et kit essential oils on biological activity of Callosobruchus maculatus F.(Col.: Bruchidae).
17. **Organization, I. L. M. (2020)**. *Increasing 11% mortality rate by rice tablet*. https://www.lmo.ir/news
18. **Owolabi, M. S., Padilla-Camberos, E., Ogundajo, A. L., Ogunwande, I. A., Flamini, G., Yusuff, O. K., Allen, K., Flores-Fernandez, K. I., & Flores-Fernandez, J. M. (2014)**. Insecticidal activity and chemical composition of the Morinda lucida essential oil against pulse beetle Callosobruchus maculatus. *The Scientific World Journal*, *2014*.

19. **Pérez, S., Ramos-López, M., Zavala-Sánchez, M., & Cárdenas-Ortega, N. (2010)**. Activity of essential oils as a biorational alternative to control coleopteran insects in stored grains. *Journal of Medicinal Plants Research*, *4*(25), 2827-2835.
20. **Rajendran, S., & Sriranjini, V. (2008)**. Plant products as fumigants for stored-product insect control. *Journal of Stored Products Research*, *44*(2), 126-135.
21. **Rechinger, K. (1982)**. Flora Iranica Graz-Austeria, Akademische Druck-U. In *Verlagsantalt* (pp. 439-440).
22. **Roozbahani, Z., Kocheili, F., Shakarami, J., & Mosadegh, M. (2014).** Fumigant toxicity of essential oils from four plant species on adult Oryzaephilus sueinamensis(L.) (Coleoptera: Silvanidae). *Plant Protection (Scientific Journal OF Agriculture(36*(4), 1-9.
23. **Rosell, G., Quero, C., Coll, J., & Guerrero, A. (2008)**. Biorational insecticides in pest management. *Journal of Pesticide Science*, *33*(2), 103-121.
24. **Sajjadi, S. E., & Mehregan, I. (2003).** Chemical composition of the essential oil of Prangos asperula Boiss. subsp. haussknechtii (Boiss.) Herrnst. et Heyn fruits. *Daru*, *11*(2), 79-81.
25. **Shadnia, S., Soltaninezhad, K., Ghaemi, M., & Abdollahi, M. (2006)**. REVIEW ON.
26. **Shakarami, J., Kamali, K., Moharramipour, S., & Meshkatassadat, M. (2004).** Effects of three plant essential oils on biological activity of Callosobruchus maculatus F.(Coleoptera: Bruchidae).
27. [Record #8 is using a reference type undefined in this output style.]
28. **Taghizadeh, S. A., & Moharamipour, S. (2010)**. Fumigant toxicity of essential oil from Thymus persicus (Lamiaceae) and Prangos acaulis (Apiaceae) against Callosobruchus maculatus (Coleoptera: Bruchidae). *Plant Protection (SCIENTIFIC JOURNAL OF AGRICULTURE(33*(1), 55-68.
29. **Taghizadeh, S. A., & Moharramipour, S. (2011)**. Oviposition deterrence and persistence of essential oils from Thymus persicus (Roniger ex Reach f.) compared to Prangos acaulis (dc.) Bornm against Callosobruchus maculatus F. in laboratory.
30. **Whitacre, D., & Ware, G. (2004)**. The pesticide book. *Ohio: Meister Media Worldwide*, 3-13.
31. **Zargari, A. (1997)**. Iranian medicinal plants. *Tehran: Tehran University Publications*.

Figures

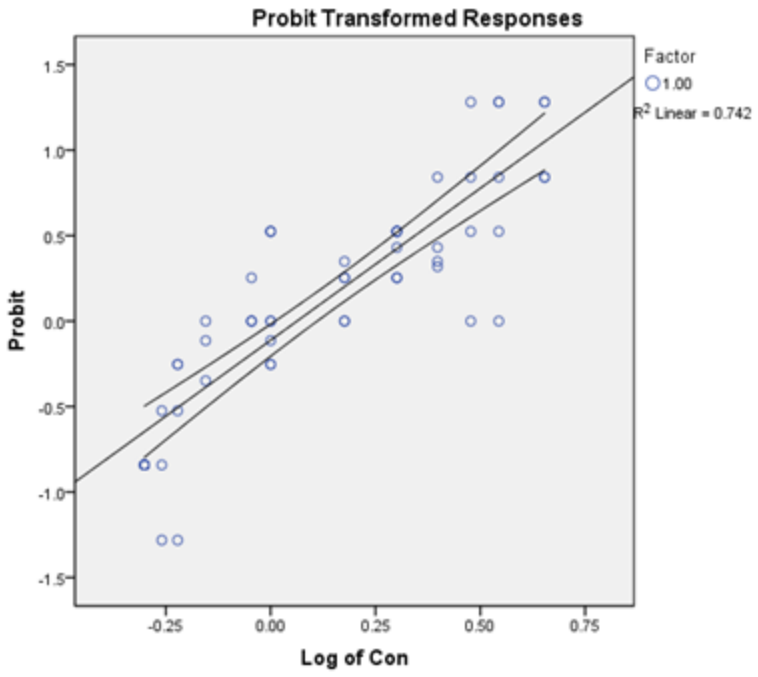

Figure 1

The logarithm of the concentration and mortality probability of *Callosobruchus maculatus* caused by *Prangos ferulacea* essential oil after 24 h

Figure 2

The logarithm of the concentration and mortality probability of *Callosobruchus maculatus* caused by *Prangos ferulacea* essential oil after 48 h

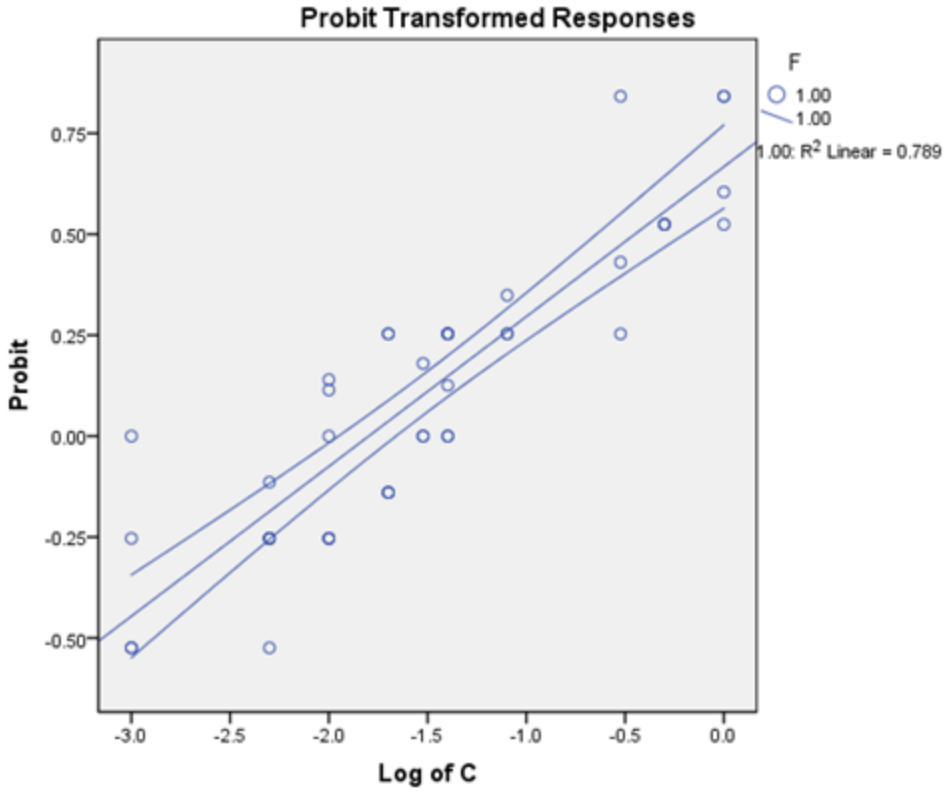

Figure 3

The logarithm of the concentration and mortality probability of *Callosobruchus maculatus* caused by *P. ferulacea* powder after 72 h

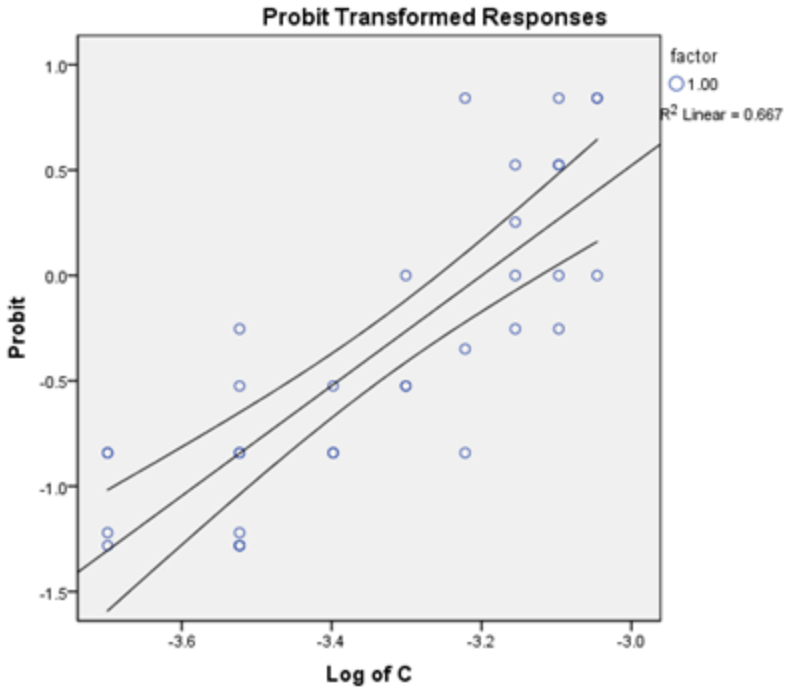

Figure 4

The logarithm of the concentration and mortality probability of *Callosobruchus maculatus* caused by aluminum phosphide after 24 h

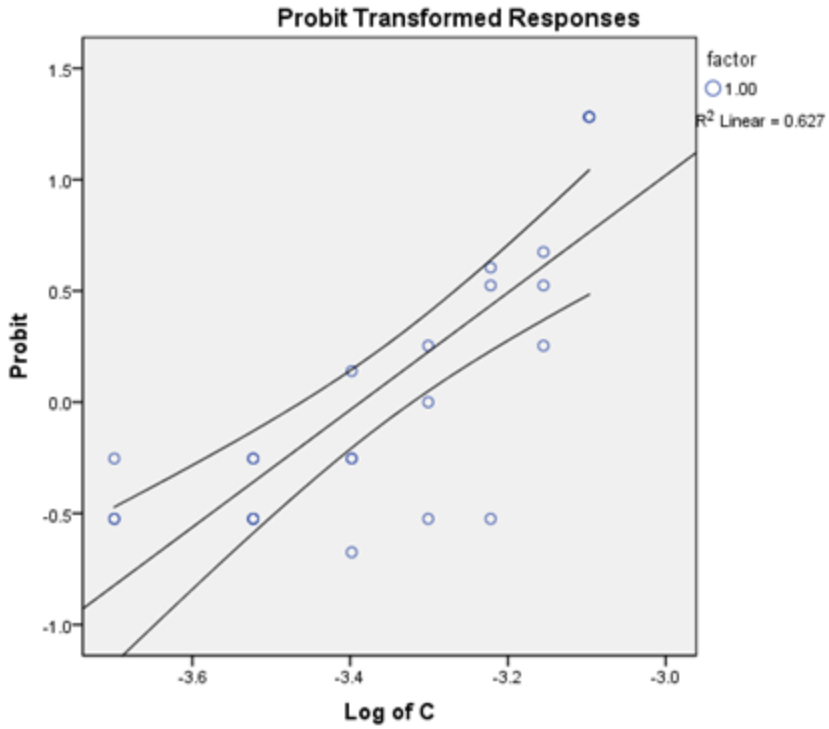

Figure 5

The logarithm of the concentration and mortality probability of *Callosobruchus maculatus* caused by aluminum phosphide after 48 h

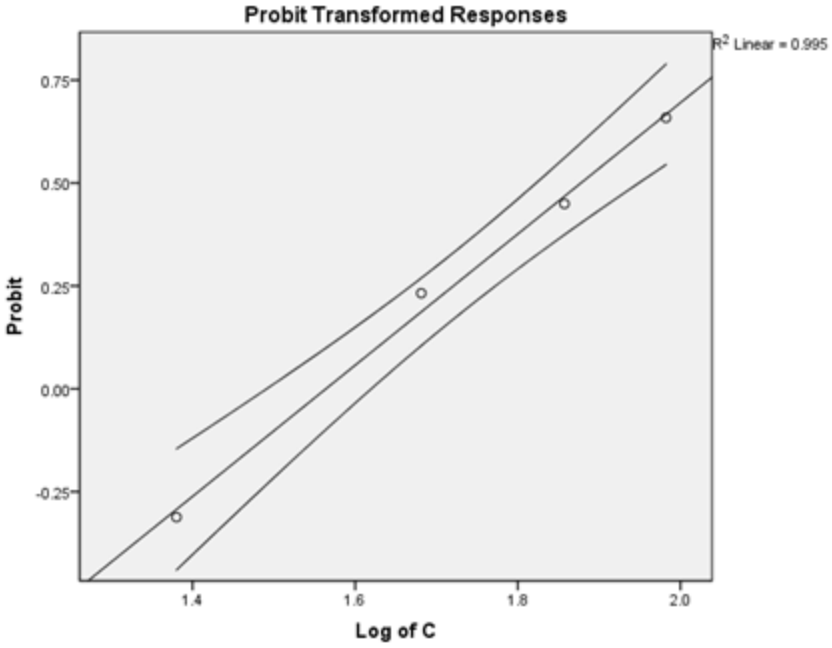

Figure 6

The logarithm of the concentration and mortality probability of *Callosobruchus maculatus* caused by *Prangos ferulacea* essential oil

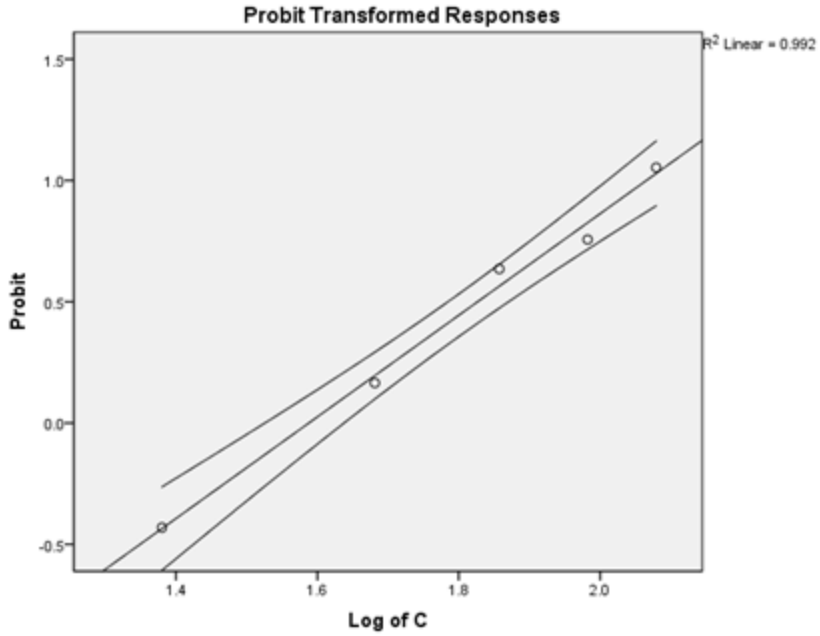

Figure 7

The logarithm of the concentration and mortality probability of *Callosobruchus maculatus* caused by *Prangos ferulacea* powder

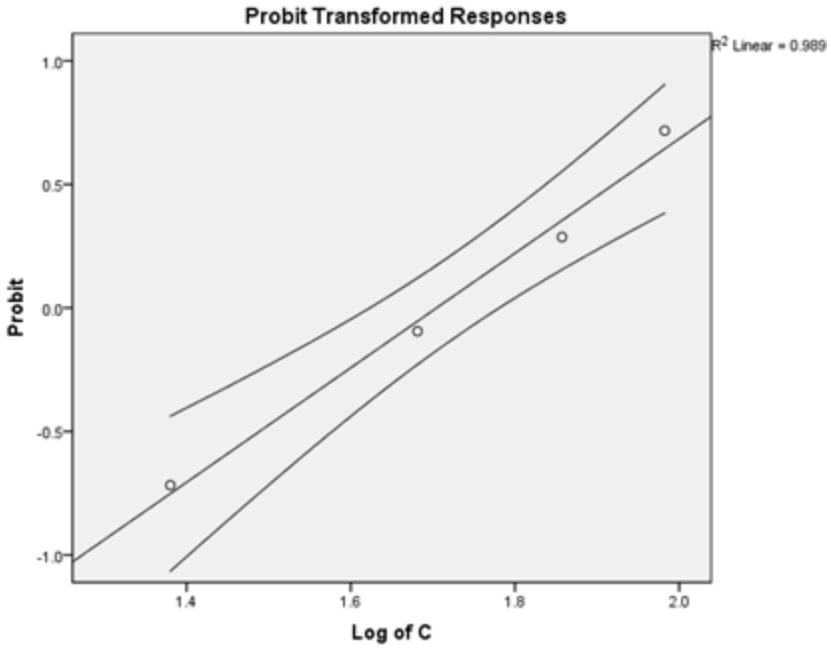

Figure 8

The logarithm of the concentration and mortality probability of *Callosobruchus maculatus* F caused by aluminum phosphide

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Analysis.zip