



Detection of Metabolite Content in Local Bitter White Lupin Seeds (*Lupinus albus* L.) and Acaricidal and Insecticidal Effect of its Seed Extract

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ABSTRACT

This study investigated the acaricidal and insecticidal effect of local lupin (*Lupinus albus* L. Fabaceae) seed extract against *Tetranychus urticae* Koch (Acari: Tetranychidae), *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae), *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae) and metabolite content of seeds. In tests for *T. urticae* leaf-disk bioassay was employed. Contrarily, two µL of the *L. albus* extract were topically applied using a micro-applicator on *C. maculatus* and *P. interpunctella*. In the assays of *T. urticae* and *P. interpunctella*, the concentrations of 0.78, 1.56, 3.12, 6.25, 12.5, 25% (w/w) of the plant extracts were used. Furthermore, the concentrations of 0.625, 1.25, 2.50, 5, 10% (w/w) were applied to *C. maculatus*. Mortality data was collected 24, 48 and 72 hours after application. In the results, *L. albus* extract was found to be quite effective to *C. maculatus* adults with LD₅₀ of 7.26, 1.21 and 0.55% after 24, 48 and 72 hours, respectively. Moreover, lupin extract was effective

to *T. urticae* adults with LC₅₀ values of 4.03, 3.15 and 2.73% for the same durations. *L. albus* extract was showed low insecticidal effect against the larvae of *P. interpunctella*.

In seeds metabolite content were detected, which contained 686.99 mg GAE / 100 mg total phenol, 22.06 mg QE / 100 mg total flavonoid, DPPH 26.04 mg TE / 100 g having antioxidant activity. The bitter taste of stem and seeds of the plant is due to their metabolite content which has a toxic effect.

In conclusion, results indicated that lupin seed has a high secondary metabolites content and also its extract had the high toxic effect against *T. urticae* and *C. maculatus*.

Keywords: *Callosobruchus maculatus*, *Tetranychus urticae*, *Plodia interpunctella*; *Lupinus albus* extract, Metabolite content

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1. Introduction

Pests have a negative effect on the growth of culture plants grown in greenhouses and fields, resulting in the loss of stored products. *Tetranychus urticae* Koch (Acarina: Tetranychidae) is a major polyphagous pest that damages many agricultural crops both in the field and in greenhouses.

Callosobruchus maculatus F. (Coleoptera: Chrysomelidae) is a major pest especially for legumes and are able to survive both in the storages and in the field.

Plodia interpunctella (Hubner) (Lepidoptera: Pyralidae) is a major pest for grain and grain products with dried fruits being the main ones, as well as for processed and non-processed products like milk powder, corn flour, wheat flour. Synthetic chemical pesticides have been used widely in pest management. Potential risks posed by synthetic chemical pesticides for mammals, consumer concerns regarding insecticide residues, and increased resistance of pest populations against insecticides, have encouraged researchers to discover new approaches for pest management. The approach of using plants for insecticide production has been brought up by different researchers (Gökçe et al. 2007; Erdoğan et al. 2012; Elma 2014; Ali et al. 2017). Given the destructive nature of synthetic chemical insecticides, it is critical to develop safe and environment friendly resources for better and safer pest management.

White lupin (*Lupinus albus* L.) is a species of the genus *Lupinus* in the family Leguminosae (Duranti et al. 2008). This plant is named termis, acibakla or termiye in Turkey and a local population of bitter white lupin is conventionally cultivated around lakes region.

The lupin plants have a secondary metabolite in its seeds and stem (Oomah et. al. 2006; Reinhard et al. 2006). They can deter a number of herbivores (nematodes, aphids, caterpillars, beetles, locusts, snails) (Gegeer et al. 2007). Deterrent or toxic effects

of alkaloid such as sparteine, lupanine and cytosine against phytophagous insects have been evaluated (Wink 1992; Bermudez-Torres et al. 2009).

In previous studies, *L. albus* seed extract has not been tested against *C. maculatus*, *T. urticae* and *Plodia interpunctella* yet. The aim of this study was to determine of metabolite content of lupin seed and evaluating the insecticidal and acaricidal activities of its extracts against *C. maculatus*, *T. urticae* and *Plodia interpunctella*.

2. Material and Methods

2.1. Plant material

Lupin seeds (local *L. albus* landrace) that had been cultivated in the Deştiğin/Doğanhisar district (37° 59' N, 31° 25' E) of Konya, Turkey, in 2012, were obtained from a local seller. After this transaction, the lupin seeds were desiccated and they were cleaned manually to remove all foreign matter such as dust, dirt, stones and chaff as well as immature, broken seeds.

2.2. Metabolite content in seeds

Three analyzes in this section were performed in 2019 with the procurement of services in the Laboratory of Food Institute of TÜBİTAK-Marmara Research Center.

2.2.1. Total phenolic compound (TPC)

TPC was measured determined by spectrophotometry, using gallic acid as a standard, according the method described by Singleton & Rossi (1965). Briefly, 0.2 mL of the diluted sample extract was transferred in tubes containing 1.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water. After waiting for 10 minutes, 0.8 mL of a sodium carbonate solution (7.5% w/v) was added to the sample. The tubes were then allowed to stand at room temperature for 30 min before absorbance at 743 nm was measured. The TPC was expressed as gallic acid equivalents (GAE) in mg/100 mL of fruit juice. The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 0.2 to 4 mg L⁻¹.

2.2.2. Estimation total flavonoid

Total flavonoid contents of lupin seed (mature) were estimated by using the aluminium nitrate colorimetric method as described by Ashokkumar et al. (2010). Quercetin was used to make the calibration curve.

2.2.3. Total antioxidant activity

The free radical scavenging effects of the methanol extracts were estimated according to the method of Blois (1958) with minor modifications. Antioxidant activity was found by "DPPH free radical scavenging activity method" and the results were given as "Trolox equivalent antioxidant capacity (TEAC)".

2.3. Plant extraction

Plant extracts were prepared as described by Gökçe et al. (2007). The dried grains were ground using a grinder (Retsch SM100) and 50-gram portions were transposed into a glass jar. Then 500 mL methanol (Merck 99.5%) was supplemented. The jar was covered with aluminum foil and were left in room temperature for 6 days. At the end of this period, plant suspension was sieved using a filter paper and the liquid part was extracted and methanol was left to evaporate at low pressure at a temperature of 42 °C using Rotary Evaporator (Heidolph-VAP Precision). Then lupin extract was diluted using purified water and six concentrations (0.78, 1.56, 3.12, 6.25, 12.5, 25% w/w) were prepared for *T. urticae* and *P. interpunctella* and five concentrations (0.625, 1.25, 2.5, 5, 10 % w/w) were prepared for *C. maculatus*.

2.4. Growing the insect cultures

Callosobruchus maculatus and *P. interpunctella* were obtained from the stock culture rearing in the climate cabinet that operates under dark situations at a temperature of 28±1 °C and at 55±5% RH. On the other hand, *T. urticae* red form was reproduced on bean (*Phaseolus vulgaris*) obtained from the stock culture in the laboratory of the Plant Protection Department (the Faculty of Agriculture of Selcuk University) and grown at a temperature of 28±1 °C, and at 65±5% RH, under a 16:8 h (L: D) photoperiod.

2.5. Toxic effect experiment

In the experiment, the newly emerged adults (1 day old) of *C. maculatus* and 3rd- 4th-period larvae of *P. interpunctella* were used. Each concentration of the extract was applied at two µL/insect using a micro-applicator on the dorsal part of *C. maculatus* and on the dorsal abdomen of *P. interpunctella* larvae. Prior to experiment, *C. maculatus* adult and *P. interpunctella* larvae were

immobilized by storing them for 5-10 minutes at a temperature of 2 °C. The adults that completed the dripping application were placed in petri dishes and the petri dishes were held in climate cabinet until the end of the experiments. Experiment was carried out using 3 repetitions in random parcels and for each repetition, 20 adults in each petri dish were used. Only water was used for controls. Mortality data was recorded 24, 48 and 72 hours after the experiment. In the experiments for *T. urticae* Erdoğan et al's (2012) method was adapted. *T. urticae* adults were transferred into bean leaf discs placed in Petri dishes with a layer of wet cotton of 3 cm diameter inside. Leaf discs were dipped for ten seconds into the lupin extract and water (control) and then allowed to dry for 30 min. Then *T. urticae* adults were transferred to leaf disc in the petri dish. Twenty adults were transferred to leaf discs. Each experimental unit (0.78, 1.56, 3.12, 6.25, 12.5, 25% w/w-) consisted of a glass petri dish (10 cm x 2 cm) containing three leaf discs and 60 adults. Insect mortality from Petri dishes assays was appraised after 24, 48 and 72 hours of exposure.

2.6. Data analysis

All mortalities data were corrected for control mortality using “Abbott’s formula” (Abbott 1987).

$$\text{Abbotts formula} = [(A-B)/A] \times 100 \quad (1)$$

Where; A: Live insect in control after treatment (%); B, live insect in treated after treatment (%). Bioassay results were analyzed using Polo-PC probit package program (LeOra Software, 1987), and confidence intervals were determined with LD₅₀/LC₅₀ and LD₉₀/LC₉₀ values.

3. Results and Discussion

3.1. Metabolite content of lupin seeds

The lupin plants have a secondary metabolite in its seeds and stem, which gives it bitterness. These metabolites include alkaloids, phenols, flavonoids, antioxidants etc. According to result of analysis, local lupin seeds contain 686.99 mg GAE / 100 mg total phenol, 22.06 mg QE / 100 mg total flavonoid, DPPH 26.04 mg TE / 100 g antioxidant activity (Table 1). As can be seen here, it is seen that lupin seed has high total phenol content (TPC), flavonoid and antioxidant activity.

Table 1- Antioxidant activity, phenolic and flavonoid content in lupin seeds

Content	mg 100g ⁻¹
Total phenolic	686.99 Gallic acid equivalent
Total flavonoid	22.06 Quercetin equivalent
Total antioxidant capacity	26.04 Trolox equivalent

According to Siger et al. (2012), total phenolic content (TPC) ranged between 4.92 and 7.31 mg g⁻¹ dry material for cvs. Butan (*L. albus*) and Parys (*L. luteus*), respectively. TPC of the boiled legumes varied between 11.8 and 25.9 mg GAE/100 g, simple polyphenols varied from 0.32 to 2.4 mg 100 g⁻¹ (Kalogeropoulos et al. 2010). *L. luteus* of TPC was 14.56 mg GAE/g dry material in 6 day-long germination process provides samples (Buszewski et al. 2019). Karamac et al. (2018) white lupin and wild forms compared the phenolic content and antioxidant capacities who was found 4.36-7.24 mg GAE/g dry matter total phenolic ingredient.

Oomah et al. (2006), eight lupin (*Lupinus angustifolius* L.) genotypes grown at four locations in south central Alberta in 2004 were analysed for phenolic components and antioxidant activity obtained by a photochemiluminescence assay. Phenolic content in cultivars changed between 11.9 and 14.7 mg catechin equivalent, 4.15 and 4.95 mg routine equivalent g⁽⁻¹⁾ lupin for TFC and flavonoid contents, respectively. Lupin cultivars displayed weak antioxidant activity based on water-soluble substances (ACW) of 0.54 to 1.07 mu mole Antioxidant capacities TE/g with lag time varied from 70 to 153 s and an antioxidant index of 6.7 to 14.5 and 1.9 to 3.3 mu mole TEAC/g based on analyses of lipid-soluble substances (ACL). In lupin genotypes, antioxidant activity was not correlated with seeds phenolic contents. Lupin, the reduced alkaloid content induced the sensibility for aphid invasion (Philippi et al. 2016).

According to these results, the local population has more phenols, flavonoids and antioxidants capacity than other genotypes. These results are the first report of the local population.

3.2. Acaricidal effect of lupin seed extract on *Tetranychus urticae* Koch

Lupin seed extract resulted in death at a rate of over 95% in all time of exposure at 25% and 12.5% concentrations. Especially at 25% concentration, 100% death rate was observed for all test periods (Table 2).

Table 2- Mortality percent of lupin seed extract against *Tetranychus urticae* Koch adults at different concentrations and time of exposure

Concentrations (% w/w)	Mortality (% ± SE)		
	Time of exposure (h)		
	24	48	72
25	100±0.00	100±0.00	100±0.00
12.50	96.43±2.92	98.18±1.48	100±0.00
6.25	67.86±4.37	72.73±4.45	75.00±1.57
3.12	49.99±3.86	54.55±3.93	57.69±4.15
1.56	32.14±3.86	36.37±1.48	42.31±8.16
0.78	12.50±1.46	27.28±3.93	28.85±4.15

The LC₅₀ and LC₉₀ values of lupin extract against the *T. urticae* are shown in Table 3. Lupin seed extract was found effective on *T. urticae* adults after 24, 48 and 72 hours with LC₅₀ of 4.03%, 3.15% and 2.73%, respectively.

Table 3- LC₅₀ and LC₉₀ values and fiducial limits of lupin seed extract against *Tetranychus urticae* at different times

Time of exposure (h)	n ^a	Slope±SE	LC ₅₀ (Fiducial limit) ^b	LC ₉₀ (Fiducial limit) ^b	Heterogeneity	χ ²
24	360	0.227±0.029	4.039 (3.13-4.94)	9.676 (8.30-11.79)	0.86	13.72
48	360	0.220±0.031	3.155 (2.12-4.08)	8.981 (7.57-11.25)	0.41	6.48
72	360	0.240±0.033	2.733 (1.59-3.68)	8.076 (6.72-10.37)	0.49	7.77

a: Total number of tested adults; b: 95 % lower and upper fiducial limits

Even though there are several studies on *T. urticae* regarding plant-based compounds prepared using different plants, no study carried out using Lupine seed extract has been observed in the literature. In studies regarding acaricidal effect of extracts made from different plants on *T. urticae*, it was observed that the resulting effect varied depending on the plant species, extract concentration and application period (Tunc & Sahinkaya 1998; Topakcı et al. 2005; Sertkaya et al. 2010; Topuz & Madanlar, 2011; Chen & Dai 2017). In our study, it was observed that the lupine plant extract resulted death rate of 50% at above concentration starting from 3%. Based on these results, it appears that lupine plant extract has a high acaricidal effect on *T. urticae*. The seeds of lupine contain lupinine, sparteine, anagryne and angustifoline as alkaloids; they also contain lupine and vernine as glycosides (Tüzün 2013). One might conclude that the insecticidal effect of the lupine extract may be resulting from these alkaloids and secondary compounds such as glycoside. Previous studies have already demonstrated the nematocidal, molluscicidal and insecticidal effects of lupinine and sparteine (Duke 1992; Bermudez-Torres et al. 2009; Yildiz 2011).

3.3. Insecticidal effect of lupin seed extract on *callosobruchus maculatus* F.

Lupin seed extract resulted a mortality rate of 100% against *C. maculatus* adult at 10% concentration observed after 72 hours. Excluding the lowest application concentration over 90% mortality rate was observed using lupin extract as the data was recorded after 72 hours (Table 4).

Table 4- Mortality percent of lupin seed extract against *Callosobruchus maculatus* F. adults at different concentrations and time of exposure

Concentrations (% w/w)	Mortality (% ± SE)		
	Time of exposure (h)		
	24	48	72
10	68.42±3.75	91.10±2.35	100±0.00
5	43.89±2.25	75.03±2.21	96.89±3.37
2.500	38.63±1.79	76.74±3.01	96.33±2.98
1.250	8.74±0.82	75.03±1.73	88.89±1.89
0.625	8.74±0.95	16.08±0.32	44.44±0.94

Lupine seed extract was the most effective with LD₅₀ values of 0.55 % after 72 hours and 1.21% after 48 hours of treatment (Table 5).

Table 5- LD₅₀ and LD₉₀ values and fiducial limits of lupin seed extract against *Callosobruchus maculatus* at different times

Time of exposure (h)	n ^a	Slope±SE	LC ₅₀ (Fiducial limit) ^b	LC ₉₀ (Fiducial limit) ^b	Heterogeneity	χ ²
24	360	0.166±0.026	7.26 (5.70-9.48)	14.98 (12.00-21.18)	1.22	15.98
48	360	0.151±0.031	1.21 (0.48-1.80)	8.06 (5.38- 20.08)	2.32	30.27
72	360	0.193±0.33	0.55 (0.24-0.81)	4.98 (2.78-22.75)	1.65	21.33

a: Total number of tested adults; b: 95 % lower and upper fiducial limits

Based on these results, it was observed that the lupin extract demonstrated a high level of insecticidal effect on *C. maculatus*. No previous study on the insecticidal effect of lupin seed extract on *C. maculatus* has been found in the literature. Previously some researchers have indicated that certain plant extracts had insecticidal effects on *C. maculatus* (Mahdian & Rahman 2008; Adedire et al. 2011; Cetin & Elma 2017; Louise et al. 2018).

3.4. Insecticidal effect of lupin seed extract on *Plodia interpunctella* (Hubner)

Lupin seed extract resulted in a low toxic effect on *P. interpunctella* larvae in general. Looking at Table 6, it can see that the death rate was observed just 50% after 24 hours at a concentration level of 25% and the highest was recorded 64% after 72 hours. And with other concentrations, the mortality remained below 40% for all exposure times.

Table 6- Mortality percent of lupin seed extract against *Plodia interpunctella* (Hubner) larvae at different concentrations and time of exposure

Concentrations (% w/w)	Mortality (% ± SE)		
	Time of exposure (h)		
	24	48	72
25	50.84±8.42	59.32±7.19	64.40±9.59
12.50	28.80±4.15	42.37±3.66	42.37±1.38
6.25	20.33±7.71	20.33±3.66	20.33±7.71
3.12	6.77±2.77	8.46±4.15	10.16±1.38

The LD₅₀ and LD₉₀ values of lupin extract against the *P. interpunctella* are shown in Table 7. Lupin seed extract showed low toxic effect on *P. interpunctella* adults after 24, 48 and 72 hours with LC₅₀ of 23.08%, 19.45% and 18.36%, respectively.

Table 7- LD₅₀ and LD₉₀ values and fiducial limits of lupin seed extract against *Plodia interpunctella* at different times

Time of exposure (h)	n ^a	Slope±SE	LC ₅₀ (Fiducial limit) ^b	LC ₉₀ (Fiducial limit) ^b	Heterogeneity	χ ²
24	360	0.067±0.010	23.08 (18.14 -33.18)	42.12 (32.36-67.07)	1.94	25.21
48	360	0.076±0.010	19.45 (15.83-25.25)	36.33 (29.28-50.82)	1.66	21.57
72	360	0.079±0.010	18.36 (15.53-22.32)	34.58 (29.03-44.24)	1.10	14.41

a: Total number of tested adults; b: 95 % lower and upper fiducial limits

Bouayad et al. (2013), studies the impact of methanol extracts of ten plants on the growth of the *P. interpunctella* larvae. They indicated that the growth rate was below 2.5% for larvae, which were exposed to *Ajuga iva*, *Rosmarinus officinalis*, *Centaurium erythraea* extracts.

There is the restricted number of studies on the insecticidal activity of lupin extract on pests. Isaev (1939), reported that 0.05% concentration of *Lupinus angustifolius* was sprayed as a spray on the adults of *Nematus ribesii* (gooseberry sawfly) (Tenthredinidae- Hymenoptera) and 100% mortality was observed after 48 hours. In another study, the insecticidal effect of 3 lupin species extracts (*L. montanus*, *L. stipulatus* and *L. aschenbornii*) and the spartein alkaloid obtained from them, was researched on *Spodoptera frugiperda* larvae. As a result, *L. stipulatus* extract was found to be the most toxic against the larvae. And also, the extracts of the other two lupin species as well was found to be at least as effective as sparteine (Bermudez-Torres et al. 2009).

4. Conclusions

Local lupin seed has high phenol, flavonoid and antioxidant activity. Lupin seed extract showed a toxic effect on *Callosobruchus maculatus* and *Tetranychus urticae*. Synthetic pesticides, which are widely used today, are known for causing adverse effects on human beings, the environment, and other creatures. Therefore, especially in the last 15-20 years many studies have been conducted on plants which are known for their biological activities towards pests. From this perspective, further studies that will determine the active ingredients or substances contained in lupin extract that have the insecticidal effect and research the effects of these ingredients on these pests would provide significant findings. It was also concluded that further studies on their acaricidal efficacy under field and greenhouse conditions would provide new findings resulted the potential of lupin seed extract having the potential to be used to manage *Tetranychus urticae* especially sustainable agriculture and integrated pest management.

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