EFFICACY OF CONIFEROUS PLANT EXTRACTS [*Pinus nigra* Arnold AND *Picea pungens* Engelm.] AGAINST SOME STORED PEST SPECIES

YEŞILAYER, A.* – KUŞAKOĞLU, E. O.

Department of Plant Protection, Agricultural Faculty, Tokat Gaziosmanpasa University, Tokat 60216, Türkiye
(http://orcid.org/009-008-0025-1316 (Kuşakoğlu, E. O.))

**Corresponding author*
e-mail: ayesilayer@gmail.com; https://orcid.org/0000-0001-7174-9324

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Abstract. The utilization of pesticides is a prevalent in the context of stored pest management. However, as an alternative due to the deleterious effects of pesticides, the use of plant extracts in pest control is becoming increasingly prevalent. In this study, the insecticidal effects of four different concentrations of plant extracts obtained from blue spruce (*Picea pungens* Engelm.) and black pine (*Pinus nigra* Arnold) were analyzed for *Sitophilus oryzae* L., *Sitophilus granarius* L. (Coleoptera: Curculionidae), and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). The application of *S. oryzae* in black pine at the lowest concentration resulted in a mortality rate of 57.56%, while *S. granarius* exhibited a mortality rate of 52.06% by day 15. In blue spruce, the respective mortality rates were 64.42% and 66.03%. The insecticidal effect of black pine extract on mealworms was observed to be lower at the lowest concentration than at the other three concentrations. In this trial, black pine was observed to be more effective than blue spruce in both plant extracts against mealworm, rice weevil, and wheat weevil.
Keywords: *Sitophilus oryzae, Sitophilus granarius, Tenebrio molitor, plant extract, needle plants*

Introduction

Wheat and corn are the primary crops utilized in the production of cereal products, which are subsequently consumed by humans. Furthermore, their use as animal feed in the food industry is of significant economic benefit to producers and provides raw materials to factories (Güneş and Turmuş, 2020). According to data from the Turkish Statistical Institute (TUIK), cereal production in Türkiye experienced an 8.1% increase in 2020 compared to the previous year (Anonymous, 2023), reaching approximately 37.2 million tons. Furthermore, according to FAOSTAT (2024), total cereal production was recorded at approximately 42.2 million tons. The quality of stored grains is influenced by a number of factors, with storage pests representing a significant concern. While pests can cause damage to crops in the field, the majority of contamination occurs during storage (Cotton and Wilbur, 1974; Kabir et al., 2012). Global losses of stored products resulting from the activities of insects or other organisms of biological origin are estimated to range from 10 to 40% (Shani, 2000; Raja et al., 2001; Güçlü et al., 2020). The following pests are responsible for economic damage in warehouses: *Sitophilus oryzae* (rice weevil), *Sitophilus granarius* (wheat weevil), and *Tenebrio molitor* (mealworms) (Longstaff, 1981; Obrepalska-StepLowska et al., 2008; Plarre, 2010; Mebarkia et al., 2010).

Sitophilus oryzae is one of the most geographically widespread pests of cereals, with a host range that includes rice, wheat, barley, rye, and oats across the globe. The pests are highly resistant to chemical control, which is widely employed (Hagstrum and

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Subramanyam, 1995). *S. granarius is a* species of grain-infesting weevil that has a wide dietary range, although wheat and barley are among the most commonly consumed food sources by their hosts (Schwartz and Burkholder, 1991; Buchelos and Athanassiou, 1999; Magan et al., 2003). *T. molitor* is a pervasive species in temperate regions worldwide. It thrives in cereal crops, cereal residues, and animal material such as meat, feathers, and insect remains (Hill, 2003; Robinson, 2005).

Efforts are underway to develop effective pest control strategies for stored product insect species. However, the extensive use of chemical agents, synthetic insecticides, and fumigants in pest management has resulted in significant challenges, most notably the emergence of insecticide resistance among target insect populations (Zettler and Cuperus, 1990; Ribeiro et al., 1997; Lorini et al., 2007). The utilization of chemical insecticides has been demonstrated to have adverse effects on beneficial insects, including the poisoning of humans and animals, the contamination of water, air, and soil, the presence of food residues, the development of pest resistance, and other detrimental consequences (Regnault-Roger et al., 2004). The detrimental impact of pesticides on the environment has led to a growing demand for environmentally friendly alternatives. In recent years, numerous commonly utilized pesticides have been prohibited. Consequently, research on alternative agricultural control methods that can be used in lieu of chemical control has proliferated. The investigation of the utilization of diverse forms of naturally occurring plant materials for the management of pests represents a significant area of emphasis within this context (Akman et al., 2004; Yeşilayer, 2018).

Plant-derived pesticides are used in a variety of ways to control insects, including as repellents, ovicidal agents, and toxicants. These pesticides are generally considered environmentally friendly, non-toxic to non-target organisms, and not designed to induce resistance. However, they are not enduring in nature and may have other unintended consequences (Liu et al., 2000; Sadek, 2003). A significant number of plant species that are perceived as potential alternatives to chemical pesticides have been demonstrated to pest-control properties, and are consequently being evaluated for their suitability for incorporation into pest management strategies (Singh, 2000; Sahayaraj and Raju, 2003; Kathuria and Kaushik, 2004).

The objective of this study was to evaluate the insecticidal efficacy of extracts from coniferous plants against significant storage pests, with the aim of applying the findings to develop a sustainable pest control strategy.

Material and method

The *Sitophilus granarius* and *S. oryzae* specimens were sourced from the stock culture maintained in the Entomology Laboratory of the Department of Plant Protection at Gaziosmanpaşa University in Tokat. *Tenebrio molitor* was placed in plastic containers (40 x 25 x 15) with a 70:30 ratio of bran to wheat flour, as is standard practice for stock cultures. Larvae, pupae, and adults were reared separately in containers of 2-5 liters at 21°C under room conditions. The black pine and blue spruce plants used in the experiment were obtained from the Tokat Gaziosmanpaşa University campus between June and August of 2021-2022. They were subsequently subjected to a drying and grinding process, resulting in a fine powder. The ground plant was weighed and placed in 5 L plastic containers. Methanol was added to achieve complete coverage of the plant material and the containers were then transferred to an orbital shaker set at 200 rpm for a period of 48 hours. The sample was then filtered with filter paper into sterile glass

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containers, poured into petri dishes to remove methanol, and left in an incubator set at 40 ºC for 48 h to remove the solvent (Muhammed et al., 2002).

Toxic effects

In the experiment, we used filtter papers placed in 10 cm Petri dishes and different concentrations were prepared for each bioassay 5%, 10%, 15%, 20%, 20%, 30%, 40% w/v of plant extracts in 2 ml spray bottles and used them as a preliminary experiment on 3-5 day old adult insects (male and female mixed). After waiting for about 30 to 40 seconds for the extract to evaporate, adding 10 adults of each of the starved *S. oryzae* and *S. granarius* and 5 adult *T. molitor* to each Petri dish. The crushed grain was adding than the in to dried filterpapers. The adult mortalities were assesed at 24, 48, 78, and 96 hours (Riba et al., 2003). The experiments were conducted with four replicates and two replications at 26±1°C under controlled laboratory conditions and It was a randomized block, encompassing all tested doses as well as a control. Each experiment was repeated twice, and the control group was treated with methanol.

Statical analysis

The effect was calculated according to the Abbott formula (Abbott, 1925). According to this formula, corrected Percent Mortality Rate= [(A-B) / A] (A: Number of live individuals in the control, B: Number of live individuals in the treatment dose) was corrected with the mortality occurring in the control. The insecticidal effects were evaluated using an analysis of variance (ANOVA), incorporating dose and extract as between-subjects factors, while time was considered a within-subjects factor. Post-hoc comparisons of the means were conducted using Tukey's test, and the results were represented using letter-based annotations. All statistical analyses were performed utilizing the Minitab 17 statistical software package.

Results

In this study, the insecticidal activity of plant extracts obtained from two different conifers, black pine (*P. nigra*) and blue spruce (*P. pungens*), was investigated. In the application, 4 different concentrations obtained from each plant were tested against the storage pests *S. oryzae*, *S granarius* and *T. molitor*, and the data obtained were regularly recorded according to plant and pest. As demonstrated in *Table 1*. In the study examining the insecticidal activity of black pine extract against grain weevils, the maximum concentration resulted in an 95.01% mortality rate by the third day. By the fifteenth day, mortality rates ranged from the lowest to the highest concentration as follows: 52.06%, 64.12%, 69.17% and 100%, respectively. At the lowest concentration, the first mortality was recorded on the fifth day, followed by incremental increases at 10% and 20% concentrations ($F=27.16$, $df=4$, $p < 0.001$).

In 4 different treatments of black pine extract against rice weevil, mortality rates at 20% on day 3 was %09.40. There was no mortality adult the control, whereas by day 15 all treatments with plant extracts had an increase in mortality compared to the control ($F=9.55$, $df=3$, $p < 0.001$, *Table 2*).

The result of the black pine treatment ($F=11.55$, $df=3$, $p < 0.001$, *Table 3*), showed that most of the treatment revealed significantly ($p<0.05$) higher mortality at 15 days of

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exposure when compared to the control. The experimental data indicated a statistically significant variation in the survival and mortality when applied to all treatments.

Table 1. *Efficacy of Pinus nigra extract against Sitophilus granarius*

% Mortality (mean±SE)*							
Treatment %	**3.d**	**5.d**	**7.d**	**9.d**	**11.d**	**13.d**	**15. d**
10	00± 00B	00.21± 00.75C	00.62± 01.00C	03.98± 01.77C	16.51± 00.29C	32.82± 00.19C	52.06± 00.39C
20	00± 00B	02.03± 01.25B	14.94± 01.09B	25.52± 00.36B	34.58± 00.63B	44.63± 01.22B	64.12± 01.07B
30	00± 00B	02.03± 01.25BC	23.21± 00.58B	33.84± 01.15B	46.59± 01.53B	53.17± 01.48B	69.17± 02.63AB
40	95.18± 01.37A	100± 00A	100± 00.00A	100± 00.00A	100± 00.00A	100± 00.00A	100± 00.00A
Control	00± 00C	00± 00D	00± 00D	00± 00D	00± 00D	0.29± 00.61D	01.70± 01.50D

*There is a statistical difference between different letters following the same column (Tukey test, P<0.05)

Table 2. *Efficacy of Pinus nigra extract against Sitophilus oryzae*

% Mortality (mean±SE)*							
Treatment %	**3.d**	**5.d**	**7.d**	**9.d**	**11.d**	**13.d**	**15. d**
5	00± 00B	16.05± 00.61BC	17.10± 00.58C	19.92± 00.75C	42.59± 00.87C	53.21± 01.57C	57.56± 01.47C
10	00± 00B	35.88± 01.29C	43.01± 0.92B	54.27± 01.0B	59.01± 00.99B	67.71± 01.92B	69.71± 01.80B
15	01.08± 01.71B	36.2 ± 01.21AB	47.38± 01.37B	57.36± 01.17B	62.72± 01.01B	68.23± 01.29B	73.04± 00.91B
20	09.40± 01.21A	54.15± 00.67A	77.10± 00.81A	79.45± 01.01A	87.55± 01.77A	92.73± 02.17A	99.38± 01.00A
Control	00± 00B	00± 00D	00± 00D	00± 00D	00± 00D	02.18± 02.67D	02.18± 02.67D

*There is a statistical difference between different letters following the same column (Tukey test, P<0.05)

Table 3. *Efficacy of Pinus nigra extract against Tenebrio molitor*

% Mortality (mean±SE)*						
Treatment (%)	**3.d**	**5.d**	**7.d**	**9.d**	**11.d**	**13.d**
10	0.00± 0.00B	29.85± 02.34BC	47.99± 00.38BC	75.17± 04.10BC	95.75± 02.61B	99.78± 00.75B
20	0.00± 0.00B	30.42± 04.80B	37.46± 00.86B	78.72± 02.88BC	99.53± 01.63A	100± 00.00A
30	0.00± 0.00B	38.75± 02.46B	49.39± 04.42B	88.59± 03.29B	100± 00.00A	100± 00.00A
40	100± 0.00A	100± 00.00A	100± 00.00A	100± 00.00A	100± 00.00A	100± 00.00A
Control	00.00± 0.00C	00.00± 00.00C	00.00± 00.00C	00.00± 00.00C	00.00± 00.00C	00.00± 00.00C

*There is a statistical difference between different letters following the same column (Tukey test, P<0.05)

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In the study, the application of *P. pungens* extract resulted in mortality rates between 3-15 days at 10% concentrations of 00.41%, 05.05%, 12.97%, 31.64%, 47.46% and 63.03%, respectively. Notably, no mortality was observed in the first days at 20% and 30% concentrations, while, mortality was recorded after the 5th day. A mortality rate of over 50% was observed after day 11 at 30% concentration. Mortality rates were statistically significant compared to the control (*Table 4*) (F=32.61, df=4, p < 0.001).

Table 4. *Different doses of Picea pungens ectracts were evaluated for their insecticidal efficacy against Sitophilus granarius*

	% Mortality (mean±SE)*						
Treatment (%)	**3.d**	**5.d**	**7.d**	**9. d**	**11.d**	**13.d**	**15. d**
10	00± 00B	00.41± 00.65C	05.05± 02.23C	12.97± 01.06C	31.64± 00.36C	47.46± 03.19C	63.03± 03.51C
20	00± 00B	01.22± 01.16B	09.63± 01.02B	15.37± 02.81C	35.25± 02.69BC	48.99± 0.45B	70.67± 0.61B
30	00± 00B	04.73± 02.88B	21.25± 01.37AB	37.38± 01.02B	58.32± 03.79B	65.50± 03.02AB	85.92± 03.12AB
40	83.37± 2.42A	100± 0.00A	100.± 0.00A	100± 0.00A	100± 00.00A	100± 0.00A	100± 0.00A
Control	00± 00B	00± 00D	00± 00D	00± 00D	00± 00D	00.29± 00.61D	01.70± 01.50D

*There is a statistical difference between different letters following the same column (Tukey test, P<0.05)

In the study of insecticidal activity of spruce extract against rice weevil, no mortality was observed in the first two days at all concentrations. From the third day to the end of the experiment, the mortality rates at the maximum concentration were 39.38%, 50.56%, 58.58%, 72.79%, 81.72% and 89.44% in 15 days. In addition, *P. pungens* extracts was found to be the superior in insecticidal action on *S oryzae* with highest mortality 89.44% at 20 percent concentration and it was statistically different from all other concentration in the treatments. The test insects were monitored daily for the first 3 days and every other day after the 3rd day (F=11.61, df=3, p < 0.001, *Table 5*).

Table 5. *Different doses of Picea pungens ectracts were evaluated for their insecticidal efficacy against Sitophilus oryzae*

	% Mortality (mean±SE)*						
Treatment (%)	**3.d**	**5.d**	**7.d**	**9. d**	**11.d**	**13.d**	**15. d**
5	00± 00C	14.30± 001.8C	29.25± 01.72C	41.73± 01.75C	56.37± 00.97B	62.43± 00.68C	66.42± 00.63C
10	00± 00C	17.38± 002.65B	32.02± 01.21B	43.73± 01.41C	59.56± 01.67B	69.65± 10.92B	74.35± 02.53B
15	0.10± 00.36 B	27.01± 000.83BC	46.90± 00.41AB	60.38± 00.54B	62.35± 00.44AB	72.53± 01.57AB	81.00± 02.10AB
20	07.74± 01.45A	39.38± 001.37A	50.56± 02.96A	58.58± 20.52A	72.79± 01.60A	81.72± 02.73A	89.44± 02.12A
Control	00±00C	00± 00D	00± 00D	00± 00D	00± 00C	00± 00D	05.09± 02.38D

*There is a statistical difference between different letters following the same column (Tukey test, P<0.05)

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The insecticidal efficacy of blue spruce extract against mealworms other than rice weevil and wheat weevil exhibited significant disparities when compared to the control group. Furthermore, a direct correlation was observed between the doses utilized and the subsequent days, with an increasing mortality rate over time (F=11.61, df=4, $p < 0.001$, *Table 6*).

Table 6. *Different doses of Picea pungens ectracts were evaluated for their insecticidal efficacy against Tenebrio molitor*

	% Mortality (mean±SE)*					
Treatment (%)	**3**	**5**	**7**	**9**	**11**	**13**
10	00± 00B	0.86± 01.33C	06.31± 02.62C	53.82± 00.70BC	92.42± 01.99A	98.08± 01.74B
20	00± 00B	06.31± 02.62BC	43.79± 01.33BC	80.33± 01.52AB	99.2± 02.70AB	100± 00.00A
30	00± 00B	31.16± 02.01AB	50.40± 01.12B	82.22± 02.49AB	100± 00.00A	100± 00.00A
40	93.88± 04.00A	97.42± 02.45A	99.14± 01.33A	100± 00.00A	100± 00.00A	100± 00.00A
Control	00± 00B	00± 00D	00± 00D	00± 00D	00.59± 01.25C	03.95± 03.34C

*There is a statistical difference between different letters following the same column (Tukey test, P<0.05)

Discussion and conclusion

In the present study, the insecticidal effects of the extracts obtained from blue spruce and black pine plants, which can be used as an alternative to chemicals, against three different warehouse pests (rice weevil, wheat weevil, and mealworm) were evaluated. In the experiment with both plants, the mortality rates from low concentration to the highest concentration were examined, and the insecticidal effects of the plants were determined. The results revealed that black pine demonstrated significantly higher insecticidal efficacy against *T. molitor* compared to blue spruce, achieving a mortality rate of 99.78% at the lowest concentration by the conclusion of the 13th day. In contrast, when *Sitophilus* species were examined, blue spruce exhibited a greater mortality rate than black pine. However, based on the obtained data, black pine extract showed greater insecticidal activity against all tested insect species when compared to blue spruce when evaluated comprehensively. The hierarchical effectiveness of black pine and blue spruce extracts was determined as follows: rice weevil, mealworm beetle and wheat weevil. In applications of *P. nigra* and *P pungens* extracts, the mortality rate at the lowest concentration on the 15th day was recorded as 52.06% and 63.03%, respectively, for *S. granarius*. Similarly, for *S. oryzae*, the mortality rates at the same concentration were 57.56% and 66.42%, respectively. Similarly, Karakoç et al. (2009) observed the insecticidal effect of Anatolian sage plant in the treatment of storage pests and concluded that the stem extract had 87% effect and flower extract had 69% effect on weevils (*Sitophilus* spp). No prior studies were identified regarding the application of conifer extracts to the test insects examined in this research. However, similar studies investigating these insect species in relation to other plant extracts were included for comparative analysis.

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In the present experiment, the earliest mortality observed in mealworms was 100% on the 9th day in the blue spruce extract at a 40% dose, and on the 3rd day in the black pine extract. In mealworms, 100% mortality was observed on the 5th day with black pine and blue spruce extracts. In the case of rice weevils, black pine extract and blue spruce extracts resulted in 100% mortality on the 19th day at a dose of 20%. Duyar and Karakaş (2019) observed an insecticidal effect of 6-10 in thyme, parsley, and mint methanol extracts on wheat weevils in their study, while the strongest effect was observed in thyme and the weakest effect was observed in parsley. At a 40% concentration in the present study, the insecticidal effect recorded at the end of the third day was 93.88% and 100% against *T. molitor* for *P. pungens* and *P. nigra*, respectively. Similarly, mortality rates of 83.37% and 95.18% were observed for *S. granarius*. Also reported that, Kanik and Karakoç (2020) among the extracts derived from various plant species, the hexane extract of the chasir plant exhibited the highest toxicity after three days, with a 74% mortality rate against wheat weevils (*S. granarius*). Additionally, the ethyl acetate extract of sneezeweed demonstrated the highest recorded mortality for *T. molitor*, reaching 42%. In the current study, *P. pungens* and *P. nigra* extract showed results against weevils (*S. oryzae* and *S. granarius*) at all the tested concentrations after different exposure times. The results of the present study are consistent with those reported in previous research (Alkan and Gökçe, 2012; Yaman and Şimşek, 2019) who reported that among different plant extracts were proved most effective against rice weevil and wheat weevil.

Chemical control remains one of the most extensively utilized methods for managing stored pests. However, due to its associated adverse effects, there has been a growing interest in exploring alternative pest management strategies. Research has demonstrated that plant-based solutions can play a significant role in insect control (Şener et al., 1998; Erler, 2004; Ertürk, 2006).

In this context, the present study aimed to assess the efficacy of coniferous plant extracts in pest management. The results indicate that these extracts exhibit substantial insecticidal activity against the three examined storage pest species. These results indicate that plant-based pest control methods represent a promising alternative to conventional approaches for grain protection. Farmers can implement this strategy without exclusive dependence on fillers or commercial formulations, ensuring safe application without compromising the integrity of stored food products or leaving harmful residues. However, further research is necessary to determine the actual mode of action and the precise insecticidal components. Field-level experiments are required to determine the effect in stores.

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