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Development of sessile oak (*Quercus petraea* (Matt.) Liebl.) seed coating material against rodents and evaluation of its performance on seed germination and emergence

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Abstract

Protection of sessile oak (*Quercus petraea* (Matt.) Liebl.) seeds, which are sown for various purposes such as artificial regeneration, afforestation, rehabilitation, seedling production, and seed supplementation in natural regeneration, from rodents is one of the factors affecting germination and emergence success. In this study, the effects of 12 natural or natural-identical substances (*Foeniculum vulgare*, *Carthamus lanatus* L., *Ferulago confusa* Velen, *Heracleum sphandylum* L. essential oils, Ferulic acid, 1,8-cineol, Benzothiazole, Capsaicin, Eugenol, Camphor, Pomarsol Forte+alumina powder, Diesel fuel+hair) of different origins—thought to have a repellent effect—on the germination, emergence and survival characteristics of oak acorns were tested under laboratory and field conditions. The repellent effects of the substances on rodents were tested in the laboratory using the "Y-maze" test. Additionally, a new oak acorn coating equipment and coating method (using diatomaceous clay) was developed and applied to coat sessile oak acorns with these substances. As a result of the research, among the tested substances, *Ferulago confusa* and *Foeniculum vulgare* were recommended as rodent repellents due to their success in laboratory germination, field emergence and survival. It was determined that the germination, emergence and survival percentages of the conventionally applied "Diesel fuel+hair" treatment were lower than the Control and other substances and it did not have a repellent effect on harmfuls.

Keywords: Acorn, coating, afforestation, rodents, germination.

Introduction

Sessile oak (*Quercus petraea* (Matt.) Liebl.) has the most extensive distribution area among the 17 oak species in Türkiye, accounting for 29.42% of the total forested area (OGM 2020), according to Akkemik (2016) and Kantarcı (1974). The acorns of sessile oak, ranging from 1 to 2 cm in diameter and 2 to 3 cm in length, are initially green and turn brown upon maturation within one year (Yaltırık 1984). These acorns are shed from October to November (Ürgeç 1998a). In Türkiye, oak acorns are utilized in natural regeneration-seed supplementation (Saatçioğlu 1969), sapling production, afforestation through planting or sowing (Yaltırık 1984), soil conservation and rehabilitation works (OGM 2005; 2022).

To facilitate the easy germination of oak acorns and the formation of seedlings and saplings, the soil should ideally be loose, uncovered, and fertile (Saatçioğlu 1979; Atay 1990; Odabaşı et al. 2004). It has been reported that deep soil tillage (60 cm) and "sowing in holes on terraces" are successful practices in oak afforestation areas where machinery is used (Uğurlu and Çevik 1991).

One of the factors affecting planting success in oak is rodents that eats the acorns in nurseries and regeneration areas (Marquis et al. 1976; Stanturf et al. 1988; Quiros and Arce 1998; Gültekin 2010a). Madsen and Magnus (2005) emphasized that protection methods should be developed for rodents in oak sowings. Genç (1989) suggested that the acorns should be treated to protect them from bird and underground rodent damage.

In nurseries and regeneration areas in Türkiye, substances such as diesel fuel, fungicide (thiram) and insecticide (endrin, chlorpyrifos-ethyl) are often used as "repellents" to protect the acorns against rodents (Genç 1989; Uğurlu and Çevik 1991; Ürgeç 1998a, Smith et al. 1997; Özer and Bul 1998; Taşdemir and Karatay 2007; Gülcü 2009). However, due to the toxic effects and persistence of these substances, some restrictions and bans have been imposed on their use (Daniel et al. 1979; Smith et al. 1997; TOB 2015).

Willoughby et al. (2010) reported that capsaicin derived from pepper showed great potential as a repellent on wood mice (*Apodemus sylvaticus* L.) and gray squirrels (*Sciurus carolinensis* G.). In another study, it was stated that the odorous substances found in the urine of predators of animals such as mice and squirrels (foxes, wild cats, other members of the feline family) have a defensive, anxiety-inducing, and repellent effect on these animals (Sullivan et al. 1988; Burwash et al. 1998; Dielenberg and McGregor 2001). Additionally, it has been reported that some substances, whether produced synthetically or found in various natural sources, have repellent effects on rats and mice (Bouchard et al. 1997; Crocker et al. 1993; Vernet-Maury et al. 1984; Vernet-Maury et al. 1992).

Seed coating (seed ball, clay ball, seed packs) is a practice that dates back to ancient Egyptian civilization and is also found in Turkish traditions. In Anatolia, shepherds would place seeds of pear, mahaleb, thuja, juniper, pine and oleaster seeds in balls with clay outside and humus on the inside and leave them in the fields (Anonymous 2013).

In the area allocated for conventional rice production in Japan, a mixture of 5 parts red clay and 1 part seeds by volume was used to form seed balls with diameters ranging from 10 to 80 mm (Wikipedia 2024). Commercially, seed coating was developed in America during World War II in the 1940s and later spread to Europe (Leep et al. 2012; Günay 1977). In Türkiye, although seed coating practices are commercially applied to sugar beet and lettuce seeds (Günay 1977; Johnson 1997; Hacıyusufoğlu 2016), they are not yet common for other species.

Seed coating offers numerous benefits, including moisture and nutrient supply, the ability to transport chemicals when needed, protection from environmental stress (such as moisture, wind, sun, and soil acidity), and defense against birds and rodents. Additionally, seed coating provides effective penetration, has a positive effect on germination, and is economical (Günay 1977; Taylor and Harman 1990; Taylor et al. 1998; Taylor 2003; Khiabani and Çelen 2014; Gökçöl and Duman 2014; Demir et al. 2017; Javed et al. 2022; Chirag et al. 2023; CSC 2024). Various pesticides (herbicides, insecticides), bacteria, and fungicides can be added to seeds to ensure resistance to pathogenic effects in humid environments and to protect against other diseases and pests (Leep et al. 2012; Hacıyusufoğlu 2016). It has been stated that the quality and germination power of the seeds coated with fungicide or insecticide is preserved (Sola et al. 2010; Dökülen and Yılmaz 2018).

The logic of coating the seed with pellets is to make the seed, which is light in density, heavier, uniform, and as spherical as possible and to apply the process to each seed in the ongoing coating process (Kubik 2024; Kayaalp 2014). The coating process varies depending on the number of seeds to be coated, seed sizes and the thickness of the coating. The materials to be used in coating should be determined because of preliminary trials (Günay 1977; İşler 2012). Most researchers use vermiculite, bentonite, garden soil, sand, peat, perlite, wood sawdust, compost, activated charcoal powder, etc. substances and their different mixtures as coating materials (Günay 1977; Hacıyusufoğlu 2016). In addition, sugar water, glucose, sucrose solutions, gelatin, starch, and gum Arabic are the most commonly used substances as adhesives to ensure that the seed and coating material adhere to each other (Güven 1967; İzgü 1974; Günay 1977).

Soulange and Levantard (2008) found that coating increased the germination percentage of tomato seeds (*Lycopersicon esculentum* Mill.) but prolonged the germination period. Koirala et al. (2023) discovered that pelleting increased the germination of pennycress plant (*Thlaspi arvense* L.) seeds by 4-30% after 3 months of storage. Mehrabi and Chaichi (2011) stated that coating methods (organic, hydrogel and mineral) affects the germination speeds of sainfoin seeds (*Onobrychis viciifolia* Scop.). Başaran and Doğrusöz (2022) primed alfalfa (*Medicago sativa* L.) seeds with 50 and 100 mM salt solutions then coated them to half of the seed weight with clay (20%), ash (20%), and lime (20%). They found that seeds coated with these materials were superior to normal or commercially coated seeds in their study.

According to Podlaski et al. (2019) in coated sugar beet seeds, the abrasion and water resistance of the pellet, as well as the physicochemical properties of the pellet, are effective on the seed's optimum water capacity and germination.

It has been stated in forestry planting that in barren lands where planting is difficult, coating the seeds with substances that can create a suitable nutritional environment increases germination success (Boydak and Çalışkan 2014). Radvanyi (1972) developed the film-coated "R-55 Rodent Repellent" using chemical substances such as aluminum powder, endrin and arasan, which are conventionally used to protect spruce (*Picea glauca* (Moench) Voss) seeds from rodents. He then compared the performance of the R-55 substance through animal testing and germination conditions, and found that this substance was significantly effective. In addition, although seed coating with different chemical substances has been tried in Eucalyptus (*Eucalyptus robusta* Sm.)

and Ginkgo (*Ginkgo biloba* L.) species (Geary and Millier 2010; Fang et al. 2019), only two research studies have focused on natural or nature-identical substances:

Ostroshenko et al. (2018) pelleted Scots pine (*Pinus sylvestris* L.) and Korean pine (*Pinus koraiensis* Siebold et Zucc.) seeds with an organomineral mixture to a thickness of 0.3-0.7 mm, but did not provide detailed information about their germination characteristics. Villalobos et al. (2019) experimented with four different substances to repel seeds of pedunculata oak (*Quercus robur* L.) and European beech (*Fagus sylvatica* L.) from bank vole mice: Chilli/coconut fat, Citronella/rapeseed oil, Mink (*Mustela vison*) excrement and Sand-coating. It was found that mink excrement and coconut oil significantly reduced the touching and consumption of seeds by mice compared to the other groups, and mink excrement was recommended.

This study was conducted to test new methods for the protection and germination of sessile oak acorns sown in nurseries and regeneration areas using herbal extracts with natural or nature-identical like repellent effects against rodents. Sessile oak acorns, which belong to the recalcitrant (cannot be dried) seed group (Karrfalt 2008) and contain 40-45% water when collected (Saatçioğlu 1971), start to lose viability if their moisture content falls below 40% (Poulsen 1992; Ürgenç 1998b, Doody and Reilly 2008). Örtel (2011), referring to Krah1-Urban (1959), states that the minimum value for germination in oaks is 22%. Therefore, protecting the moisture content of oak acorns through seed coating methods is important for increasing germination rates.

In the study, the deterrence from rodents, germination success of oak acorns coated with 12 types of active substances were determined.

Material and methods

Material

Seed collection and trial locations

Sessile oak acorns used in the research were collected from in the "c" or "d" development-age stands of the Istanbul Regional Directorate of Forestry: Bahçeköy-Belgrad-Kurtkemerî, Çatalca-Binkılıç, and Kanlıca-Beykoz Forests.



Fig 1 Tohum toplanan lokasyonların konumu (OGM 2019)

After the laboratory studies, sowings were on established trial patterns made on 3 forest soil areas close to these stands (Fig 1; Table 1).

Table 1 Features of acorn collected and sowed areas (OGM 2012; 2019, CD 2019, MGM 2016)

Forest range							
		Binkılıç		Kurtkemer/Kemerburgaz		Beykoz	
Feature	Unit	Acorn collected field	Sowing field	Acorn collected field	Sowing field	Acorn collected field	Sowing field
Stand types (M:Oak)	-	Mc3-Mbc3	(FS)-L	Mc3-Mbc3	FS	Mc3	(FS)-Mc3
Location	-	Binkılıç	Forest edge-Landing	Kurtkemer	Kemerburgaz	M.Şevketpaşa	M.Şevketpaşa
Slope	%	0-20	0-20	0-20	0-20	0-20	0-20
Altitude	m	112-199	165-168	130-160	140	130-160	136
Annual precipitation	mean mm	647	647	1129.4	1129.4	827	827
Annual mean temperature	°C	12.Tem	12.Tem	12.Mar	12.Mar	14.Oca	14.Oca
Annual mean high temperature	°C	17.Oca	17.Oca	17.Nis	17.Nis	17.Haz	17.Haz
Climate types	-	Semi humid	Semi humid	Perhumid	Perhumid	Humid	Humid

Plant material

The plant material used in the research, was collected from the areas of Istanbul, Yalova and Polonezköy. Plant materials were collected at the flowering stage. Collection locations and dates were given in Table 2.

Table 2 Plant material from which essential oils are obtained, locations where they are collected

Plant Name	Family	Location	Altitude (m)	Collecting Date
<i>Carthamus lanatus</i> L.	Asteraceae	Polonezköy	96	21.07.2016
<i>Heracleum sphondylium</i> L. subsp. <i>ternatum</i>	Apiaceae	Yalova	113	26.06.2017
<i>Foeniculum vulgare</i>	Apiaceae	Istanbul	118	07.06.2017
<i>Ferulago confusa</i>	Apiaceae	Istanbul	116	07.06.2017

Coating material

Acorns were coated using diatomaceous soil and wood powder as filling material, 2% gum Arabic in water as binding agent. Pest repellent active substances were impregnated on the mid-layers of coating (essential oil and other pure substances) at 10% (v/v) concentration in 50% (v/v) ethanol.

Acorn coating equipment

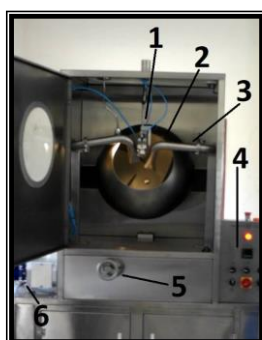


Fig.2 Horizontal axis semi-automatic Acorn Coating Equipment (1-Liquid Sprayer, 2-Rotary acorn coating drum, 3- Air blower, 4-Control panel, 5- Coating drum adjustment unit, 6- High pressure pump)

In previous studies, rotary drums (Madsen et al. 2010; 2012; İşler 2012; Sesvanderhave 2024) were used to mix the seeds and coating materials. In this study, semi-automatic stainless steel candy coating/drug coating equipment was used to coat the acorns. The equipment was customized as an "oak acorn coating machine" (OACM) with mixer feature and horizontal axis. The machine can coat all seeds with equal thickness (Figure 2). Number of revolutions and angle of the drum can be adjusted on the machine. In order to coat the seeds, equipment was adjusted to rotate at an inclination range of 20°-50° and a speed of 20-50 revolutions per minute (rpm). The number of revolutions was kept low to prevent damage to the coat material on the acorns and to ensure a uniform coating.

Method

Acorn selection methodology

Acorns were collected from trees 75–100 meters apart from each other (Arslan et al. 2013). Then, they were brought to a well-ventilated, enclosed area where they were mixed in small polypropylene sacks. After separating the acorns from foreign substances, they were placed in water-filled buckets to float wormy, rotten, or low-germination ability seeds (Ergun 2009). The acorns removed from the water were spread on large, clean covers and mixed several times a day until the coating process began.

Tetrazolium test (TZ)

After the acorn material was prepared, acorn viability was observed before germination by testing 4 samples of 100 acorns from each location with 1% "tetrazolium chloride" under laboratory conditions (ISTA 2011). Based on the staining status of the tissues, the acorns were qualitatively evaluated as "living," "weak but living," "weak, dead but unstained," and "dead-unstained" (ISTA 2011; Anonymous 2014).

Determination of moisture content

Before conducting germination experiments, the acorns were stored in an environment with 83-87% humidity. During this period, the seed moisture content was monitored using the oven dry method. Samples of 20 grams each (three samples per location) were taken following the guidelines of ISTA (1996). After recording the initial weights of the acorn samples, they were placed in a drying cabinet at 105 °C for 17±1 hours. The samples were then re-weighed to determine the moisture content. Additionally, the moisture content results were verified using a Precisa XM 50 seed moisture analyzer. The environmental humidity was measured with an HTC hygrometer.

Determination of thousand seed weights (TSW)

Randomly, 8 samples of 100 acorns each were selected from each location and weighed with a precision scale sensitive to 0.001 grams. Their average weight (X) was calculated. Then, it was tested whether the sampling represented the seed weight by calculating the standard deviation (S) and coefficient of variation (r). The averages were multiplied by 10 to find the thousand seed weight (10*X) (ISTA 1996).

Obtaining essential oils

Essential oils were obtained by hydrodistillation (4 hours) of the air dried, aerial parts (100 g) of the plant material by Clevenger Apparatus. The obtained essential oil was dried over Na₂SO₄. The yield of the essential oils were given as v/w% (volume/mass) formula. Essential oil content was analyzed with Agilent 5977 MSD GC-MS system.

Gas chromatography, mass spectrometry Analysis

Gas chromatography mass spectrometry tests were performed on "Agilent 7890B GC and 5977B EI MSD" system. Essential oil samples were diluted 1/10 (v/v) with *n*-hexane. Injection and mass spectrometer transfer line temperatures were set at 250°C. The injection split ratio was set to 50:1. Innowax FSC capillary chromatographic column (60 m x 0.25 mm, 0.25 µm film thickness) was used in the analyses. High purity helium gas was used as the mobile inert phase at a flow rate of 1 mL/min. The temperature program was used in the analysis. The temperature program started at 60°C, and maintained for 10 minutes, then 220°C was reached at a rate of 4°C/minute, the temperature was maintained constant at 220°C for 10 minutes and it was increased to 240°C at a rate of 1°C/min. The mass spectrum was measured with an energy of 70 eV and mass range of m/z

35 to 425. "Relative percentage of compounds separated by chromatography" were determined from the integration of chromatograms obtained from the mass spectrometer detector. In the identification of essential oil components, mass spectra comparison and comparison of RRI (relative retention index calculated according to the retention times of the *n*-alkane series) of the substances with literature values were used. Mass spectra matches were done by comparing the observed mass spectra with mass spectra of known substances from commercial Wiley 8th Ed./NIST 05 Mass Spectrum Library, and Adams Essential Oil Mass Spectrum Library.

Y Maze behavior test

In order to test the repellent activity of the test substances "Y maze" experimental setup was employed. A specially designed Y maze made of transparent plexiglass material was constructed. The selection arms ended with a 10 cm feed compartment and in the back wall a small computer fan blowing air, passing from the perforate wall and feed towards the entrance arm. The entrance arm is 45 cm long and it opened two opposite arms where feed is located. The set up was designed in a way where animals can move forward easily (100 cm). In the experiment, feed containing only the a 10% test substance in ethanol was placed in one arm, and feed containing blank (50% alcohol) was placed in the other arm (control arm). In order to determine the repellent effect of substances, the behavior of Sprague Dawley rats were monitored in the experimental setup. The experiments were video-recorded before and after the substance was administered.

Using the recorded videos of the experiment was analysed the time spent in the Y maze arms by the animals was measured. Test days were repeated in order to eliminate the learning factor of the animals On the "repeat day", the places of the petri dishes containing the test substance containing feed with repellent odor in the Y maze arms were changed (Test day 1 and Test day 2). In addition the behavioural patterns of the animals interacting with the feed was evaluated by scoring (Indifferent-0, Sniff-1, Contact-2, Feeding-3) according to their behavior towards the feed with the test substance and with the blank feed. Ethics committee permission was obtained for the Y maze mouse experiment (Supplement 1) and the experiments were recorded with video.

Phytotoxicity test

Duckweed (*Lemna minor*) plant was used as a model to determine the phytotoxic activities of substances and oils (Einhellig et al. 1985). *Lemna minor* with three fronds ($n=10$) were placed on a petric dish which contained growth medium (10 mL) and the test substance at 50% concentration in ethanol was impregnated on a 1 x 1 cm filter paper which was attached to the lid. These prepared solutions were applied in the middle of a 10 mL petri dish in a volume of 50 μ L. The lids of the petri dish were colosed and sides of the petri dish were sealed with parafilm, than they were placed in a growth cabinet and incubated at 30 C° and $56 \pm 10\%$ relative humidity with 12h day/night cycle for seven days. The growth inhibition of *Lemna minor* was evaluated by counting the generated/surviving fronds. A blank control test which used the same conditions except the test sample (blank ethanol) were also tested. Each experiment was repeated 4 times, and results is given as growth inhibition mean value $\pm$ standard deviation. Growth inhibition percentage was calculated according to comparison of the sample to the blank control sample using the following formula.

Growth inhibition = (Number of leaves in the petri that does not contain the sample–Number of leaves in the sample) / Number of leaves in the petri that does not contain the sample x 100.

Performing the seed coating process

In this study, "pellet coating" was applied to oak acorns. Diatomaceous clay, the only mineral of biological origin (Çetin and Taş 2012), was used as the main pelletizing material. Diatomaceous clay was preferred for several reasons: its high surface area due to its porous structure and sharp microscopic edges (Madsen et al. 2012), which can contribute to the germination and survival chances of acorns. Its ability to absorb repellent substances. Its capacity to create a carrier layer that can release these substances slowly with time.

To determine the mixture for the research, four demos were conducted using varying amounts of diatomaceous clay, wood powder, and adhesive binder. The dispersion properties of the coatings were tested by assessing their dissolution capacity in both water and running water. The mixture with the highest density was selected. This mixture included powder filling material (wood powder), 2% gum Arabic (dissolved in water), adhesive binder (Selvol-205®), 10% active substance in 50% alcohol (repellent agent), and diatomaceous soil. During the coating process, water was sprayed onto the seeds in the rotating drum of the OACM. The coating process was standardized by using the same amounts, order, and proportions for each repellent agent. The mixture was passed through a 0.1 mm sieve to ensure homogeneity. As a result, the acorns were coated with a

mixture of diatomaceous clay, wood powder, adhesive, and repellent agent. Each mixture was disinfected against fungal pathogens with 1.4% thiram. After coating, the seeds were removed from the drum and dried, forming a hard pellet coating on the seeds (Günay 1977; İşler 2012). At the end of each process, the inside of the coating machine was cleaned with 70% alcohol before starting a new process.

Layers of covering material were applied to the seeds through a "wrapping operation." Initially, the acorns were coated without the repellent. The active substance was added by spraying it on before the final wrapping layer. This method ensured that the active substance did not come into direct contact with the acorn, minimizing any negative effects on germination. Adding an extra layer over the active substance allowed for its controlled release. As described by Tetters (2012) and Patel (2015), the "complete film coat" process was performed to cover the entire seed uniformly, with a diatomaceous clay layer making up 20% of the seed's weight. Then the oak acorns were let to dry at room temperature, allowing the pellet to harden and making them ready for planting. The coated sessile oak acorns are shown in Fig. 3 and Supplement 2.



Fig. 3 Sessile oak acorns coated by oak acorn coating machine

Apart from "control and clay coating" processes; a mixture conventionally used in Türkiye (15 kg seeds + 800 g Pomarsol Forte (thiram) + 42 g alumina powder + 1 L water) was mixed until it reached the consistency of yoghurt and then coated onto the acorns (Genç 1989; Uğurlu and Çevik, 1991; Gülcü 2009) and tested. In another process, acorns were coated with another conventional mixture (100 L water + 10 L diesel fuel) (Taşdemir and Karatay 2007; Gülcü 2009) and supplemented with hair, and tested.

Testing germination "inhibition"

Germination tests in the laboratory

Germination tests were conducted on glass trays measuring 38.5x26.0x4.5 cm within a seed germination cabinet. The trays were filled with sifted sand, which was sterilized in a drying oven at 160 °C for 120 minutes (Pekbay 2005). The quantities of sand and distilled water added to the trays were recorded. Each tray was also thoroughly wiped and disinfected with a 5% sodium hypochlorite (NaOCl) solution before use. Since the diatomic material coating the seeds contained 89.9% SiO₂, ensuring compatibility with the sand used as the germination medium.

The acorns were placed horizontally in the germination trays, aligned in the same direction, and spaced apart to prevent contact. Acorns coated with 12 different substances were arranged in the sand trays in four replicates, following a randomized block design, with ¾ of each acorn remaining exposed. The germination cabinet was set to provide 16 hours of darkness, 8 hours of light, a temperature of 20±0.5 °C, and 75-80% ambient humidity. Glass grilles were inserted between the trays to ensure even air circulation. The germination experiment involved 2340 coated acorns, including 12 substances and one Control group, with 4 replicates, 15 acorns per replicate, and 3 locations. The trays were observed daily for 35 days, and acorns were considered germinated if their roots showed geotropism and reached a length of 5 mm.

Germination percentages (GP) obtained at the end of the germination period were calculated separately for each substance. The germination percentage was calculated as a proportional expression of the germinated acorns relative to the total number of acorns within a certain period, starting from the beginning of the germination test (Boydak and Çalışkan 2014):

$$GP (\%) = \sum ni / N \times 100$$

GP: Germination percentage

ni: Number of germinations on day i

N: Total number of acorns tested

Determination of emergence and survival rates in the field

A sowing depth of 5-7 cm (average 6 cm) was applied in the pits opened with a sowing hoe on the gradoni terraces prepared with a 4x4 tractor in the field. The acorns were sown horizontally, facing the same direction, 20 cm apart (Genç 1989; Uğurlu and Çevik 1991; Özer and Bul 1998; Taşdemir and Karatay 2007; Willoughby et al. 2004; Boydak and Çalışkan 2014).

The percentage of emergence in the spring and the survival rates after a period of vegetation of the acorns were examined. The acorns were sown outside the vegetation season (Pamay 1969; Genç 1989; Uğurlu and Çevik 1991; Boydak and Çalışkan 2014) in November in the field. Weeding and hoeing operations were carried out on the saplings until the second week of June 2018.

The trial design was established using the "parcels divided into randomized blocks" method with four replications and 13 factors. The field trial was carried out with 3900 coated acorns, with "12 substances and 1 control x 4 replicates x 25 acorns x 3 locations." The acorns were sown on the edges of the parcels for isolation. Emergence percentages (EP) and survival percentages (SP) were calculated as the rate of acorns that emerged or survived within a certain period relative to the total number of acorns. Fig. 4 illustrates an example of the field trial at the Binkılıç location. Fig. 5 provides examples from both laboratory and field studies.

Block 1												
M10	M9	M0	M3	M11	M2	M8	M6	M7	M1	M12	M4	M5
Block 2												
M9	M6	M7	M8	M10	M2	M3	M4	M5	M11	M0	M12	M1
Block 3												
M7	M9	M4	M0	M10	M11	M8	M12	M6	M3	M2	M1	M5
Block 4												
M4	M8	M3	M7	M5	M11	M2	M10	M6	M12	M9	M1	M0

Fig. 4 Trial pattern for field work

These coating types are as follows;

- M0: Control.
- M1: Pomarsol Forte+alumine powder+water.
- M2: Clay+Capsaicin.
- M3: Clay+Eugenol.
- M4: Clay+*Ferulago confusa*.
- M5: Clay+1,8-cineol.
- M6: Clay+Camphor.
- M7: Clay+*Foeniculum vulgare*.
- M8: Clay+*Carthamus lanatus* L.
- M9: Clay+Ferulic acid.
- M10: Clay+*Heracleum sphondylium* L.
- M11: Clay+Benzothiazole.
- M12: Diesel fuel10 % + Human hair.



Fig. 5 Images from laboratory and field germination tests

Tests such as Tetrazolium, thousand seed weight, moisture content, emergence and survival in the field were performed according to (ISTA, 1996) norms. Under ISTA rules, the number of acorns tested in the field was taken as 3 locations x 4 replicates x 13 substance x 25 acorns (total 3900 acorns). In the laboratory tests, due

to the limited conditions in the air conditioning cabin, the number of seeds tested was taken as 3 locations x 4 replicates x 13 substance x 15 acorns (total 2340 acorns). In many similar studies on the applying "priming" to seeds before sowing, different numbers of seeds had to be tested in laboratories for various reasons. (Aydın and Tursun 2010; Marquis et al. 1976; Knot and Sochorec 2012; Shah et al. 2012; Çalışkan 2014).

Statistical analysis

One-way ANOVA was used in the variance analysis in the study. Differences regarding seed germination, emergence, and seedling survival averages were grouped using the post hoc Duncan test. For the *Lemna minor* phytotoxicity test, "Tukey advanced statistical method" was used. In the Y maze test, the difference between the arms containing the substance and the arms containing the Control within the same experimental group was evaluated by the "Kolmogorov-Smirnov test." Additionally, the differences between the groups were evaluated with the advanced statistical method by Dunnett by calculating the total time spent by the animals in the arms and the total feeding time for all tested active substances on the test days. The statistical results were analyzed at the $P < 0.05$ significance level.

Results

Tetrazolium test (TZ)

In the TZ test, acorn viability was determined according to locations (Table 3). Due to the tests performed for all 3 locations, 86% of the acorns were qualitatively evaluated as "living".

Table 3 Tetrazolium test of collected acorns

Location	Living %	Non-living %
Binkılıç	90	10
Kurtkemer	83	17
Beykoz	86	14
Result	86	14

Moisture content

The mean moisture content of oak acorns before sowing, according to locations, is shown in Table 4.

Table 4 Moisture content (WA%) test of collected acorns

Location	A=Initial Weight (g)	E=Weight after drying (g)	%WA= A-E/A*100
Binkılıç	20.24	11.69	42.235
Kurtkemer	20.296	10.776	46.883
Beykoz	20.516	14.103	31.259
Mean	20.35	12.189	40.125

According to these results, it was understood that the seed moisture content was sufficient for the collected sessile oak acorns to germinate.

Thousand seed weight (TSW)

Due to the measurements, the average thousand seed weight of the acorns in the three locations was calculated as 1872 g (Table 5).

Table 5 Thousand seed weight of locations

Location	Standard deviation (S)	Coefficient of variation ($r = (S/X) \cdot 100$)	TSW (g) (10.X)
Binkılıç	8.08	3.6	2200
Kurtkemer	5.75	3.7	1553
Beykoz	7.02	3.7	1863
Mean	-	-	1872

There are different opinions about the effect of seed size and weight on germination characteristics. (Chauan and Riana 1980; Shepard et al. 1989; Toon et al. 1990; Edwards and Kassaby 1996; Chaisurisri et al. 1994; Davidson et al. 1996; Indira et al. 2000; Khera et al. 2004; Tilki and Alptekin 2005; Moles and Westoby 2006; Ögüt 2006; Delgado et al. 2009; Örtel 2011; Çalışkan, 2014; Aksu and Tilki 2015). Therefore "seed diameter and weight" were not used in this research, and only information about the "thousand seed weight" was provided.

Essential oils, GC-MS analysis, phytotoxicity and Y Maze test

The yields of the essential oils and the main components of the essential oils are presented in Table 6.

Table 6 Essential oil yields and their main components

No	Plant name	Family	Collected location	Essential oil yield (v/w)	Main substance	Composition
1	<i>Carthamus lanatus</i> L.	Asteraceae	Polonezköy	0.03%	allo-Himachalol	12.58%
2	<i>Heracleum sphondylium</i> L.	Apiaceae	Yalova	0.28%	Octylacetate	59.85%
3	<i>Foeniculum vulgare</i>	Apiaceae	Istanbul	2.20%	(E)-Anethole	60.86%
4	<i>Ferulago confusa</i>	Apiaceae	Istanbul	1.61%	Elemicin	50.25%

In the phytotoxicity test, significant differences were observed between the experimental groups at $P < 0.05$ (Table 7). The differences between the Control group and the groups containing test substances were analyzed using the Tukey test (Table 8).

Table 7 *Lemna minor* Phytotoxicity Test ANOVA test results

	Mean squares	df	Mean	F	P
Between groups	79669.435	12	6639.120	16.064	0
Within groups	16118.726	39	413.301		
Total	95788.161	51			

* Different at $p < 0.05$ significance level

Table 8 *Lemna minor* Phytotoxicity test results

Experimental groups	Inhibition value (%)	P
Control	-8.70	-
P.Forte+alumine powder	-3.31	1
Eugenol	100.00	0.000***
<i>Ferulago confusa</i>	27.24	0.403
<i>Foeniculum vulgare</i>	45.20	0.034*
<i>Carthamus lanatus</i> L.	-14.09	1
Ferulic acid	38.02	0.522
<i>Heracleum sphondylium</i> L.	-7.80	0.995
Benzothiazole	92.81	0.000***
Diesel fuel+hair	49.69	0.012*
Camphor	100.00	0.000*
1,8-cineol	99.10	0.000*
Capsaicin	31.73	0.237

*** = Significant P value of 0.001%, * = Significant 0.05% P value, TUKEY test.

In animal experiments, the time spent by rats in the arms of the Y tube and the scores of their reactions to the applied test substances were measured and presented in Table 9.

Table 9 Y Maze test, time and behavioral responses of animals

Experimental groups	SABS	CABS	P value (BT1)	SABS	CABS	P value (BT2)	SAT	CAT	P value (TT1)	SAT	CAT	P value (TT2)
Feed + P. forte+alumine powder	0.88	1,63	0,011*									
Feed + <i>Carthamus lanatus</i> L.	0.29	1,57	0,013*									
Feed + Ferulic acid	0.75	1,50	0,026*									
Feed + <i>Ferulago confusa</i>				0,14	2	0,000*	60,02	177,14	0,004*			
Feed + <i>Foeniculum vulgare</i>	0.5	1,38	0,039*	0,63	2,13	0,002*						
Feed + Diesel fuel-hair				0,43	1,29	0,001*						
SABS: Substance arm behavior score CABS: Control arm behavior score SAT: Substance arm time CAT: Control arm time BT: Behavior test *Different at p<0.05 significance level TT: Time test												

Testing germination “inhibition”

Germination test in the laboratory

According to the variance analysis results for laboratory germination, it was revealed that the 12 different active substances applied in 3 locations differed from each other in terms of germination percentage at each location at the p<0.05 significance level (Table 10).

Table 10 Laboratory germination Analysis of Variance ANOVA results

Trial fields		Mean squares	df	F	P
Binkılıç	Between groups	10.895	12		
	Within groups	96.100	767	7.246	0.000*
	Total	106.995	779		
Kurtkemer	Between groups	24.515	12		
	Within groups	134.300	767	11.67	0.000*
	Total	158.815	779		
Beykoz	Between groups	4.415	12		
	Within groups	127.400	767	2.215	0.010*
	Total	131.815	779		

* Different at p<0.05 significance level

The active substances with differences in germination percentage at each location were ranked according to their germination rates using the Duncan test. Accordingly, the substances divided into three homogeneous groups (a, b, c) in Binkılıç and Beykoz locations and four homogeneous groups (a, b, c, d) in the Kurtkemer location (Table 11).

Table 11 Laboratory germination Duncan Test results

Active substance groups	Binkılıç GP (%) Homogeneity group	Kurtkemer GP (%) Homogeneity group	Beykoz GP (%) Homogeneity group
<i>Heracleum sphondylium</i> L.	93.3a	48.3d	76.7ab
1,8-cineol	91.7a	86.7a	76.7ab
P. Forte+alumine powder	91.7a	86.7a	81.7ab
Camphor	90.0ab	33.3d	68.3bc
Control	90.0ab	85.0ab	81.7ab
Capsaicin	88.3ab	75.0abc	83.3ab
Ferulic acid	88.3ab	90.0a	81.7ab
<i>Foeniculum vulgare</i>	85.0ab	78.3abc	86.7a
<i>Carthamus lanatus</i> L.	83.3ab	80.0abc	88.3a
<i>Ferulago confusa</i>	81.7ab	68.3bc	75.0abc
Eugenol	80.0ab	63.3c	75.0abc
Benzothiazole	75.0b	88.3a	85.0ab
Diesel fuel+hair	46.7c	46.7d	60.0c

The highest germination rates under laboratory conditions were determined to be "*Heracleum sphondylium* L." in Binkılıç (93.3%), "Ferulic acid" in Kurtkemer (90.0%), and "*Carthamus lanatus* L." in Beykoz (88.3%). The lowest germination rates were observed for "Diesel fuel+hair" (46.7%) in Binkılıç, "Camphor" (33.3%) in Kurtkemer, and "Diesel fuel+hair" (60.0%) in Beykoz.

Findings of emergence and surviving in the field

According to the results of the field emergence variance analysis (Table 12), it was detected that 12 different substances applied in 3 locations, differed from each other at the $p < 0.05$ significance level in terms of the percentage of emergence level in each location.

Table 12 Acorn emergence Analysis of Variance ANOVA results (Field)

Trial fields		Mean squares	df	F	P
Binkılıç	Between groups	15.411	12	5.340	0.000*
	Within groups	309.540	1287		
	Total	324.951	1299		
Kurtkemer	Between groups	13.509	12	5.839	0.000*
	Within groups	248.130	1287		
	Total	261.639	1299		
Beykoz	Between groups	4.792	12	2.954	0.000*
	Within groups	173.980	1287		
	Total	178.772	1299		

* Different at $p < 0.05$ significance level

The active substances were sorted according to their emergence rates using the Duncan test. Based on this test, the substances were divided into three homogeneous groups (a, b, c) in the Binkılıç and Beykoz locations, and five homogeneous groups (a, b, c, d, e) in the Kurtkemer location (Table 13).

Table 13 Acorn emergence Duncan Test results (Field)

Active substance groups	Binkılıç EP (%) Homogeneity group	Kurtkemer EP (%) Homogeneity group	Beykoz EP (%) Homogeneity group
<i>Carthamus lanatus</i> L.	66.0a	12.0e	07.0c
1,8-cineol	57.0ab	14.0de	26.0a
Capsaicin	57.0ab	27.0abc	14.0bc
<i>Foeniculum vulgare</i>	56.0ab	35.0ab	13.0bc
Camphor	56.0ab	36.0ab	26.0a
Eugenol	54.0ab	31.0abc	11.0c
<i>Heracleum sphondylium</i> L.	52.0ab	41.0a	24.0ab
Ferulic acid	51.0ab	37.0ab	14.0bc
Benzothiazole	47.0b	19.0cde	13.0bc
<i>Ferulago confusa</i>	46.0b	40.0a	17.0abc
P. Forte+alumine powder	46.0b	34.0ab	23.0ab
Control	27.0c	25.0cde	16.0abc
Diesel fuel+hair	26.0c	12.0e	10.0c

The highest emergence rates under field conditions were determined to be *Carthamus lanatus* L. (66.0%) in Binkılıç, *Heracleum sphondylium* L. (41.0%) in Kurtkemer and Camphor (26.0%) in Beykoz. The lowest germination rates were observed for Diesel fuel+hair (26.0%) in Binkılıç, Diesel fuel+hair (12.0%) in Kurtkemer, and *Carthamus lanatus* L. (7.0%) in Beykoz.

According to the results of the field survival variance analysis (Table 14), it was detected that 12 different substances applied in 3 locations, differed from each other at the $p < 0.05$ significance level in terms of the percentage of survival level in each location.

Table 14 Survival Analysis of Variance ANOVA results (Field)

Trial fields		Mean squares	df	F	P
Binkılıç	Between groups	14.892	12	5.155	0.000*
	Within groups	309.83	1287		
	Total	324.772	1299		
Kurtkemer	Between groups	7.988	12	4.080	0.000*
	Within groups	209.990	1287		
	Total	217.978	1299		
Beykoz	Between groups	4.000	12	2.513	0.000*
	Within groups	170.720	1287		
	Total	174.720	1299		

* Different at p<0.05 significance level

The active substances whose survival percentages differed in each location were sorted according to their survival rates using the Duncan test. Based on this test, the substances were divided into three homogeneous groups (a, b, c) in Binkılıç and Beykoz locations, and four homogeneous groups (a, b, c, d) in the Kurtkemer location (Table 15).

Table 15 Survival Duncan Test results (Field)

Active substance groups	Binkılıç SP (%) Homogeneity groups	Kurtkemer SP (%) Homogeneity groups	Beykoz SP (%) Homogeneity groups
<i>Carthamus lanatus</i> L.	65.0a	11.0d	07.0c
Capsaicin	57.0ab	18.0bcd	14.0abc
Camphor	56.0ab	30.0ab	23.0a
1,8-cineol	55.0ab	13.0d	25.0a
<i>Foeniculum vulgare</i>	53.0ab	28.0abc	13.0abc
<i>Heracleum sphondylium</i> L.	52.0ab	36.0a	24.0a
Eugenol	52.0ab	27.0abc	11.0bc
Ferulic acid	51.0ab	29.0abc	14.0abc
Benzothiazole	47.0b	16.0cd	13.0abc
<i>Ferulago confusa</i>	45.0b	22.0bcd	17.0abc
P. Forte+alumine powder	45.0b	21.0bcd	22.0ab
Control	27.0c	16.0cd	15.0abc
Diesel fuel+hair	26.0c	10.0d	10.0c

The highest survival rates under field conditions were detected in *Carthamus lanatus* L. (65.0%) in Binkılıç, *Heracleum sphondylium* L. (36.0%) in Kurtkemer and 1,8-cineol (25.0%) in Beykoz. The lowest germination rates were observed for Diesel fuel+hair (26.0%) in Binkılıç, Diesel fuel+hair (10.0%) in Kurtkemer, and *Carthamus lanatus* L. (7.0%) in Beykoz. The differences between the means for laboratory germination, field emergence, and survival, calculated for each location, are shown collectively in Table 16.

Table 16 Comparison of laboratory germination, field emergence and survival rates between locations

Active substance groups	Locations	Laboratory GP (%)/Homogeneity group	Field emergence EP (%)/Homogeneity group	Field survival SP (%)/Homogeneity group
Control	Binkılıç	90.0a	27.0a	27.0a
	Kurtkemerli	85.0a	25.0a	16.0b
	Beykoz	81.0a	16.0a	15.0b
Pomarsol Forte+alumine powder	Binkılıç	91.0a	46.0a	45.0a
	Kurtkemerli	86.0a	34.0ab	22.0b
	Beykoz	0.81a	23.0c	21.0b
Capsaicin	Binkılıç	88.0a	57.0a	57.0a
	Beykoz	83.0a	27.0b	18.0b
	Kurtkemerli	75.0a	14.0c	14.0b
Eugenol	Binkılıç	80.0a	54.0a	52.0a
	Beykoz	75.0a	31.0b	27.0b
	Kurtkemerli	63.0a	11.0c	11.0c
<i>Ferulago confusa</i>	Binkılıç	81.0a	46.0a	45.0a
	Beykoz	75.0a	40.0a	22.0b
	Kurtkemerli	68.0a	17.0b	17.0b
1,8-cineol	Binkılıç	91.0a	57.0a	55.0a
	Kurtkemerli	86.0ab	26.0b	25.0b
	Beykoz	76.0b	14.0b	13.0c
Camphor	Binkılıç	90.0a	56.0a	56.0a
	Beykoz	68.0b	36.0b	30.0b
	Kurtkemerli	33.0c	26.0b	23.0b
<i>Foeniculum vulgare</i>	Beykoz	86.0a	56.0a	53.0a
	Binkılıç	85.0a	35.0b	28.0b
	Kurtkemerli	78.0a	13.0c	13.0c
<i>Carthamus lanatus</i> L.	Beykoz	88.0a	66.0a	65.0a
	Binkılıç	83.0a	12.0b	11.0b
	Kurtkemerli	80.0a	07.0b	07.0b
Ferulic acid	Kurtkemerli	90.0a	51.0a	51.0a
	Binkılıç	86.0a	37.0b	29.0b
	Beykoz	81.0a	14.0c	14.0c
<i>Heracleum sphondylium</i> L.	Binkılıç	93.0a	52.0a	52.0a
	Beykoz	76.0b	41.0a	36.0b
	Kurtkemerli	48.0c	24.0b	24.0b
Benzothiazole	Kurtkemerli	88.0a	47.0a	47.0a
	Beykoz	85.0a	19.0b	16.0b
	Binkılıç	75.0a	13.0b	13.0b
Diesel fuel+hair	Beykoz	60.0a	27.0a	26.0a
	Kurtkemerli	46.0a	12.0b	10.0b
	Binkılıç	46.0a	10.0b	10.0b

Averages of three locations

To examine the combined effects of the 12 tested active substances, three different areas were evaluated with three replicates each. Thus, the average effect of each substance group on emergence and survival percentages in the field was determined. According to the variance analysis results from the three locations, it was revealed that the applied substances differed significantly from each other in terms of the germination percentage at the $p < 0.05$ significance level (Table 17).

Table 17 Laboratory germination Analysis of Variance ANOVA results of three locations

	Mean squares	df	F	P
Between groups	24.287	12		
Within groups	379.044	2327	12.425	0.000*
Total	403.332	2339		

* Different at $p < 0.05$ significance level

Using the Duncan test, the active substances were sorted according to their germination percentages and divided into a total of five homogeneous groups (Table 18).

Table 18 Laboratory germination Duncan Test results of three locations

Active substance groups	Germination percentages (%)	Homogeneity group
P. Forte+alumine powder	87	a
Ferulic acid	86	a
Control	86	a
1,8 -cineol	85	a
<i>Foeniculum vulgare</i>	84	a
<i>Carthamus lanatus</i> L.	84	ab
Benzothiazole	83	ab
Capsaicin	82	ab
<i>Ferulago confusa</i>	75	bc
<i>Heracleum sphondylium</i> L.	73	c
Eugenol	73	c
Camphor	74	d
Diesel fuel+hair	51	e

According to the variance analysis results for the three locations, it was revealed that the applied substances differed from each other in terms of emergence percentage at the $p < 0.05$ significance level (Table 19).

Table 19 Acorn emergence Analysis of Variance ANOVA results of three locations (Field)

	Mean squares	df	F	P
Between group	823.070	3887		
Within group	14.888	12	5.859	0.000*
Total	837.984	3899		

* Different at $p < 0.05$ significance level

With the Duncan test, the active substances were sorted according to their emergence percentages and divided into a total of four homogeneous groups (Table 20).

Table20 Emergence Duncan Test results of three locations (Field)

Active substance groups	Emergence percentage (%)	Homogeneity group
Camphor	39	a
<i>Heracleum sphondylium</i> L.	39	a
<i>Foeniculum vulgare</i>	35	ab
<i>Ferulago confusa</i>	34	ab
P. Forte+alumine powder	34	ab
Ferulic acid	34	ab
Capsaicin	33	ab
1,8-cineol	32	ab
Eugenol	32	ab
<i>Carthamus lanatus</i> L.	28	bc
Benzothiazole	26	bc
Control	23	cd
Diesel fuel+hair	16	d

According to the variance analysis results for the three locations, it was revealed that the applied substances differed from each other in terms of survival percentage at the end of the vegetation period at the $p < 0.05$ significance level (Table 21).

Table 21 Survival Analysis of Variance ANOVA results of three locations

	Mean squares	df	F	P
Between groups	783.627	3887		
Within groups	13.0260	12	5.384	0.000*
Total	796.652	3899		

* Different at $p < 0.05$ significance level

Using the Duncan test, the active substances were sorted according to their survival percentages and divided into a total of five homogeneous groups (Table 22).

Table 22. Survive Duncan Test results of three locations (Field)

Active substance groups	Survival percentage (%)	Homogeneity groups
<i>Heracleum sphondylium</i> L.	37	a
Camphor	36	ab
Ferulic acid	31	ab
<i>Foeniculum vulgare</i>	31	ab
1,8- cineol	31	ab
Eugenol	30	ab
Capsaicin	30	ab
P.Forte+alumine powder	29	ab
<i>Ferulago confusa</i>	28	bc
<i>Carthamus lanatus</i> L.	28	c
Benzothiazole	25	cd
Control	19	de
Diesel fuel+hair	15	e

Discussion

Villalobos et al. (2019) experimented with four different substances (coconut oil, citronella oil, mink excrement, sand coated seed) to repel bank voles from seeds of pedunculate oak and European beech in research areas. They

detected that mink excrement and coconut oil reduced the touching and consumption of seeds by mice. The sand-coated seeds, which were less effective in moisture and nutrient transport compared to diatomaceous structures, did not succeed in repelling rodents or promoting germination. These findings support the preference for diatomaceous soil in this study. According to Villalobos et al. (2019), more research and field studies are needed for conclusive recommendations.

The primary reason for using pellet coating-wrapping (Madsen et al. 2010; 2012; Geary and Millier 2010; Sesvanderhave 2024; Ostroshenko et al. 2018) in this research is to ensure that the repellent substances remain between the seed and the pellet powder, maintaining their effectiveness long time. Additionally, diatomaceous soil was used its high environmental stress resistance, 80-85% porosity, exceptional water absorption capacity, 30-60% moisture content in reserve, large specific surface area, and at least 85% SiO₂ content (Özbey and Atamer 1987). This created a clay ball that facilitated forming agglomerates and allowed for air, water, and nutrient circulation, thus creating favorable germination conditions. According to Hacıyusufoğlu (2015), chosen coating materials should ensure that the pelletizing agent covers the seed, homogeneously. The coating must protect the seed from damage during storage, transport, and sowing, support germination with sufficient moisture, and not impede the plant's growth. The experimental layer developed in this study is believed to meet these criteria.

In this research, a minimum clay thickness of 20% (Complate Film Coat) (Tettero 2012; Patel 2015), covering the entire acorn was found to be sufficient. This method was chosen because the primary goal was to protect the oak acorn from environmental stress and to ensure the controlled release of the active substance. This thickness can be adjusted for new oak acorn coating trials and various purposes in the future. Aydın and Tursun (2010) observed that, in studies with different essential oils and weed seeds, germination rates decreased as the dose increased. Therefore, in this study, a low dose of the active substance (10% active substance in 50% alcohol) was used in the coating mixture. Thus, the repellent odor was conserved without affecting germination.

In the phytotoxicity tests, it was observed that Eugenol, Benzothiazole, Camphor, 1,8-cineole, *Foeniculum vulgare*, and Diesel fuel+hair groups had a phytotoxic effect on *Lemna minor*. However, it was found that the germination of coated acorns with some of these substances was successful (for example, 78.3-86.7% for *Foeniculum vulgare*). This shows that the coating method can mitigate the negative effects of the repellent substances on oak acorn germination, except for Camphor.

The laboratory test results for Camphor in the Kurtkemerli location show a low germination value of 33.3%. This outcome is thought to result from either the negative effect of the active substance in closed conditions or an inability to adjust its concentration in the coating material. Despite showing the second-lowest germination rate in laboratory tests, the Camphor group exhibited the highest germination rate in field studies. This discrepancy can be attributed to Camphor's negative effect on germination in enclosed environments (such as germination cabins), which diminished in open field conditions. In the germination cabin tests, acorns were sown close to each other and exposed more intensely to the substance, as the evaporated substance was maintained a limited area. In studies conducted in the field, this substance was diffused outside and shown its repellent effect. A similar interpretation can be expressed for *Heracleum sphondylium* L. oil. Apart from these two substances, *Foeniculum vulgare*, *Ferulago confusa*, Pomarsol Forte+alumine powder, Ferulic acid, Capsaicin, 1,8-cineole, and Eugenol formed the second most homogeneous group in terms of the highest emergence rate in the field. In the laboratory germination tests, Pomarsol Forte+alumine powder (87.0%), Ferulic acid (86.0%), Control (86.0%), 1,8-cineole (85.0%), and *Foeniculum vulgare* (84.0%) demonstrated higher germination rates than the others. Diesel fuel+hair was identified as the group with the lowest statistical rate, with a germination rate of 51.0%. In laboratory tests, Camphor exhibited the second lowest germination value at 74.0%.

The highest survival rate among the substances tested in field studies was observed with *Heracleum sphondylium* L. oil. This was followed by *Foeniculum vulgare*, Camphor, Pomarsol Forte + Alumina powder, Ferulic acid, Capsaicin, 1,8-cineole, and Eugenol. When comparing locations, higher germination, emergence, and survival rates were recorded at the Binkılıç site in both laboratory and field studies. These results align with the acorn viability (TZ) and thousand seed weight test findings of the Binkılıç location. In field tests, the Diesel fuel + hair group had the lowest emergence rate (16%) and survival rate (15%) across the three locations. The Control group, with no treatment, had the next lowest rates, with 19.0% emergence and 23.0% survival.

For a long time in Türkiye, especially in oak sowings for afforestation, acorns have been treated with 10% diesel fuel to protect against rodents such as mice, rabbits, moles, and squirrels (Taşdemir and Karatay 2007; Gülcü 2009). However, this research reveals that the use of Diesel fuel+hair does not provide protection,

on the contrary, negatively impacts the germination and survival rates of the acorns. In fact, this treatment may give effect more harm from rodents. Ceasing this practice alone could improve the success of oak afforestation efforts. A mixture of Thiram (Pomarsol Forte), alumine powder, and water has been used in forest nurseries to protect against both fungal infections and rodents (Genç 1989; Ugurlu and Çevik 1991; Gülcü 2009). In this study, the germination success of Pomarsol Forte+alumine powder in laboratory and field conditions was consistent with other studies (Sola et al. 2010; Dökülen and Yılmaz 2018). While effective for germination, emergence, and survival, this substance was found to lack repellent properties in the Y-maze test. Thus, further research is needed to enhance its repellent effectiveness. Similar investigations should also be conducted for Ferulic acid.

According to laboratory germination results, the findings from all three locations in the Control group are similar to those from previous studies on different oak species (Taşdemir and Karatay 2007; Örtel 2011). The lower emergence and survival rates in the field Control group compared to laboratory conditions are thought to be caused by exposure to various rodents, birds, pigs, and other factors and absence of repellent in the coating process. Similarly, Taşdemir and Karatay (2007) found lower emergence rates in field studies compared to laboratory germination rates for Persian Oak (*Quercus brantii* Lindl.) and Hairy Oak (*Quercus cerris* L.). Although the three locations are suitable for oak sowing, differences in germination performance for each substance are thought to be due to variations in micro-topography, climate, and fauna.

In the Y maze experimental setup, when the active substance feed-related behavior of rats was tested, the most effective group was found to be the *Ferulago confusa* group in terms of duration; and the *Ferulago confusa* and *Foeniculum vulgare* groups were found to be the most effective group in terms of behavior score. *Ferulago confusa* was evaluated as having the highest subject repellent effect due to it had the shortest subject visitation time in the Y-maze test. Additionally, in the scoring studies, it was observed that the animals in the *Ferulago confusa* and *Foeniculum vulgare* groups did not eat the feed with these substances and ate the feed in the control arm. It was observed that the animal repellent effect of other active substances remains limited under laboratory conditions. According to these data, it was revealed that germination and animal experiments results should be evaluated together.

Conclusions and Recommendations

Since there is no repellent/protective substance in the field-control group, it is an expected situation that seed emergence and survival rates will be low due to biotic and abiotic factors. It is known that seed coating has moisture supply, protection from "environmental stress and harmfuls" (Günay 1977). It is thought that the low emergence and survival results seen in the control group in field studies are due to the lack of coating and repellent substances in this group. Since field emergence rates are lower compared to laboratory germinations, with new research in the field (under controlled conditions) monitoring the effects of acorn-animal behavior will provide us with more concrete information.

In this research, we tested whether germination, emergence, and survival rates in the laboratory and field varied according to the active substances and whether these substances had a repellent effect on rodents. *Ferulago confusa* active substance was identified as having a repellent effect based on the Y maze-time and behavior tests. When all animal and germination experiments were evaluated together, *Ferulago confusa* showed optimal performance in terms of laboratory germination, field emergence, and survival. *Foeniculum vulgare*, which was also found to be effective in behavioral tests, was evaluated as the second repellent active substance due to its effectiveness in germination and survival tests.

Gültekin (2010b) revealed in a study on 8 oak species in the Lakes Region of Anatolia animals rapidly consumed over 99% of fallen acorns within the first week. Therefore, coating sessile oak acorns with rodent repellents will significantly contribute to achieving targets of afforestation, soil conservation, regeneration, and sapling production studies. Additionally, this method offers further benefits, such as avoiding the cutting of branches that shelter harmful, eliminating the use of toxic feed, and removing the need for traps.

In this research, the coating made with diatomaceous soil did not hinder radicle emergence or the intake of air and water by the acorns. This finding highlights the importance of selecting appropriate coating techniques, materials, and their ratios based on the seed type. Therefore, before starting the coating, it is essential to define the coating's purpose, the shape of the seed, the materials and supplements to be used, the wrapping operation, the number and method of layers. It's important to carefully plan out this "process". Demir et al. (2017) demonstrated that the coated sugar beet (*Beta vulgaris* Saccharifera L.) varieties at different soil-water capacities variety exhibited better germination results at low field water capacity. It is thought that a similar effect can be achieved in oak plantings in areas with low soil-water capacities, with coated seeds potentially

germinating better due to the advantages provided by the structure of the diatomaceous soil. According to Podlaski et al. (2019) optimum water potential in pellets composed of solid, water-resistant, and organic minerals positively affected germination. In this study, the diatomaceous soil coating material, tested for water resistance and composed of organic minerals, showed a similarly positive effect on germination.

Almost any type of seed can be coated (Hacıyusufoğlu and Güner 2015; CSO 2024), but there is a need the well-determined purpose, practical, cheap and robust methods and models. According to Kayaalp (2014), seed properties, pelletizing materials, and pelletizing technology are critical in creating seed pellets. Therefore, the physical and biological properties of forest tree seeds should be examined, and pelletizing processes should be developed separately for each genus.

Seed coating producers often keep their methods, pelleting materials, and R&D studies confidential for commercial reasons. However, in forestry, which is primarily conducted by public entities, seed coating techniques could be disseminate without such commercial constraints. There is no produced equipment for acorn coating. Taking inspiration from medicine tablet coating machines, a new coating equipment and coating method was developed for large-seeded species such as oak and chestnut in this study.

There aren't many studies on seed coating in forestry, except for a few chemical substance experiments on *Picea glauca*, *Eucalyptus* and *Ginkgo biloba* seeds. (Radványi 1972; Geary and Millier 2010; Ostroshenko et al. 2018; Fang et al. 2019). In this research, the effectiveness of germination in oak seed coating, which was tried for the first time in the world, found similar to the results of many other studies (Koirala et al. 2023; Soulangue and Levantard 2008, Mehrabi and Chaichi 2011, Villalobos et al. 2019, Başaran and Doğrusöz 2022).

In agriculture, especially for expensive and small diameter seeds (e.g., a sugar beet, lettuce), seed coating with pellets is done since single grain sowing is not suitable. Similarly, in forestry, in the future, seed coating, pellet making, and a machine sowing with different small-seeded species will be possible on sloped fields or in nurseries.

As in agriculture (Johnson 1997; Khiabani and Çelen 2014; Gökçöl and Duman 2014) increasing the germination and emergence rates possible by coating seeds with substances such as potassium nitrate (KNO₃) and gibberellic acid (GA₃) in forest nurseries or fields.

According to Çetin and Taş (2012), Türkiye's diatomite reserves amount to 125 million tons, featuring very abundant and high-quality deposits. Additionally, plant materials such as *Foeniculum vulgare* and *Ferulago confusa*, from which repellent active substances are derived, are species that naturally occur in Türkiye (Anonymous 2024) and can be easily obtained.

The challenges that seedlings face during their development phase cannot solve with a one-size-fits-all approach, relying solely on a few simple seed coatings. To achieve significant progress, it is essential to investigate the interactions among different seed coatings, develop new methodologies, and conduct interdisciplinary studies involving chemistry, machinery, soil science, and microbiology (Chirag et al. 2023). According to Hacıyusufoğlu (2016), there are very few researchers and professionals in Türkiye with adequate knowledge and experience in seed coating. Given that seed coating technologies in forestry are a relatively new field, it is important to give graduate courses or graduation assignments on this topic in forestry faculties, similar to some agricultural faculties (Duman 2018). This approach will allow for a more detailed examination of the subject.

It is thought that with the development of such sustainable, environmentally compatible methods and seed technologies, natural or nature-identical products can be produced and disseminated to fight rodents, birds, and insects in forestry.

Supplementary information: The online version contains supplementary material available at <https://doi.org/...> (Ethics Committee Application Permission, a video of oak acorncoating was added)

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