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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: Protecting sessile oak [*Quercus petraea* (Matt.) Liebl.] seeds from rodents is crucial to ensure successful germination and emergence in activities such as artificial regeneration, afforestation, and seedling production. This study examined the effects of 12 natural or nature-identical substances, believed to have repellent properties, on the germination, emergence, and survival of sessile oak acorns under both laboratory and field conditions. Acorns were coated using a diatomaceous clay-based pellet system, and a Y-maze experiment was conducted to evaluate rodent behaviour. 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 and field trials. Diesel fuel + hair, a conventional repellent, showed poor performance and is not recommended. This study underscores the potential efficacy of natural or nature-identical coatings for protecting seeds from pests in forestry applications.

Keywords: acorn; afforestation; repellent; seed coating; sowing

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Sessile oak (*Quercus petraea*) is widely distributed across Europe and has the largest range among the 17 oak species found in Türkiye (Akkemik 2016; OGM 2020; Girard et al. 2022). One of the factors affecting sowing success in oak is rodent predation on the acorns in nurseries and regeneration areas (Marquis et al. 1976; Quiros, Arce 1998). Some synthetic and natural substances have been reported as effective repellents against rodents such as rats and mice (Sullivan et al. 1988; Vernet-Maury et al. 1992; Crocker et al. 1993; Bouchard et al. 1997; Burwash et al. 1998; Dielenberg, McGregor 2001; Willoughby et al. 2010). In Türkiye, repellents such as diesel fuel, and Thiram (fungicide) have been used for acorn protection (Uğurlu, Çevik 1991; Taşdemir, Karatay 2007; Gülcü 2009).

Seed coating improves moisture and nutrient supply, protects against environmental stress, and may deter pests. It also enhances germination, ensures better soil penetration, and is cost-effective (Günay 1977; Smith et al. 1997; Taylor et al. 1998; Govinden-Soulange, Levantard 2008; Mehrabi, Chaichi 2012; Demir et al. 2017; Başaran, Doğrusöz 2022; Koirala et al. 2023). Although it has been stated that coating seeds with substances that can create a suitable nutrient environment increases germination success in forestry (Boydak, Çalışkan 2014), there is a lack of sufficient research on this subject. Radvanyi (1972) found that film-coated R-55 rodent repellent, containing aluminium powder, endrin, and arasan, effectively protected spruce (*Picea glauca*) seeds from rodents. Similar chemical-based coatings have been tested on *Eucalyptus robusta* and *Ginkgo biloba* (Geary, Millier 2010; Tian et al. 2019). Ostroshenko et al. (2018) applied an organomineral coating (0.3–0.7 mm thick) to Scots pine (*Pinus sylvestris*) and Korean pine (*Pinus koraiensis*) seeds but did not report germination results. Villalobos et al. (2019) tested some deterrents on pedunculate oak (*Quercus robur*) and European beech (*Fagus sylvatica*) seeds against bank voles (*Myodes glareolus*): mink excrement, followed by coconut oil, significantly reduced seed consumption. Hansen et al. (2016) reviewed 54 studies and reported that essential oils and terpenoids are the most effective plant-based repellents, whereas tannins in some oak acorns (*Q. rotundifolia*, *Q. faginea*, *Q. suber*) exhibited limited and inconsistent deterrent effects on *Apodemus sylvaticus*, highlighting the need for more stable natural formulations. No studies specifically focused on sessile oak acorns were found.

In this study, the deterrent effect on rodents and the germination success of sessile oak acorns coated with 12 types of natural or nature-identical active repellent substances were determined.

MATERIAL AND METHODS

Collecting acorns and seed tests. Sessile oak acorns used in this study were collected from the Istanbul Regional Directorate of Forestry (IRFD) fields (Table 1). Acorns were cleaned of foreign materials, floated in water to eliminate non-viable or damaged seeds, and air-dried on clean covers. Before germination, viability was assessed using tetrazolium chloride (1%). Moisture content was determined by the oven-dry method (using 105 °C for 17 ± 1 h). Thousand-seed weight was calculated using eight 100-acorn samples per location (ISTA 1996, 2011).

Essential oils, GC-MS analysis, Y-maze behaviour and phytotoxicity tests. The essential oil plant material (*Carthamus lanatus* L., *Heracleum sphondylium* L., *Foeniculum vulgare*, *Ferulago confusa* Velen.) was collected during the flowering stage from Istanbul, Yalova, and Polonezköy from 96–116 m elevation. Essential oils were extracted by a 4-hour hydrodistillation of 100 g dried aerial plant parts using a Clevenger apparatus (ISOLAB, Germany) and analysed with an Agilent 5977 MSD GC-MS (gas chromatography–mass spectrometry) system (Agilent, USA). Compounds were identified via RRI (rapid repetitive imaging) and spectral libraries.

Y-maze experiment was conducted to test the repellent activity of substances using a custom-built plexiglass setup. Sprague Dawley rats (specific strain of *Rattus norvegicus*) were monitored as they chose between feed with a 10% test substance in ethanol and a control feed with 50% ethanol. Their time spent in each arm (in seconds) was recorded and analysed, and behavioural responses were scored (indifferent – 0, sniff – 1, contact – 2, feeding – 3). Ethics committee permission was obtained for the Y-maze mouse experiment, see Electronic Supplementary Material (ESM) S1.

A phytotoxicity test was conducted on *Lemna minor* to evaluate the effects of active substances and essential oils using a sealed Petri dish setup (Einhellig et al. 1985).

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Table 1. Site characteristics of acorn collection and sowing areas (OGM 2012, 2024; Climate Data 2024; MGM 2024)

Feature	Unit	Forest range					
		IRFD – Binkılıç		IRFD – Kurtkemeri		IRFD – Beykoz	
		collection field	sowing field	collection field	sowing field	collection field	sowing field
Stand types	–	Mc3–Mbc3	FS–L	Mc3–Mbc3	FS	Mc3	FS–Mc3
Location	–	Binkılıç	Binkılıç	Kurtkemeri	Kemerburgaz	M. Şevketpaşa	M. Şevketpaşa
Slope	%	0–20	0–20	0–20	0–20	0–20	0–20
Elevation	m a.s.l.	112–199	165–168	130–160	140	130–160	136
Annual mean precipitation	mm	647.0	647.0	1 129.4	1 129.4	827.0	827.0
Annual mean temperature	°C	12.7	12.7	12.3	12.3	14.1	14.1
Annual mean high temperature	°C	17.1	17.1	17.4	17.4	17.6	17.6
Climate types	–	semi-humid	semi-humid	perhumid	perhumid	humid	humid

IRFD – Istanbul Regional Directorate of Forestry; M – oak; Mbc3 – oak stand, bc development stage (pole-small timber), canopy density 3; FS – forest edge; L – landing

Performing the seed coating process. Diatomaceous soil was chosen for its high porosity, water absorption and repellent preservation, and gradual release (Özbey, Atamer 1987; Madsen et al. 2012). After four demos, the densest mixture, including wood powder, 2% gum arabic, Selvol-205®, a 10% repellent in 50% alcohol, and diatomaceous soil, was selected. The mixture was sieved (0.1 mm) for uniformity, disinfected to prevent fungal contamination with 1.4% Thiram. The wrapped material was dried at room temperature at the end of the process (Günay 1977; İşler 2012). In this study, a semi-automatic stainless-steel candy/drug coating machine was customised as an 'oak acorn coating machine' (OACM) with a mixer and horizontal axis (20–50 rpm, 20–50° incline). Thus, a 'complete film coat' was performed, with diatomaceous clay making up 20% of the seed's weight (Tettero 2012; Patel 2015; ESM S2).

In addition to the control (M0), two conventional mixtures used in Türkiye were tested: M1 = Thiram, alumina powder, and water [15 kg acorn + 800 g Pomarsol Forte (Thiram) + 42 g alumina powder + 1 L water], M12 = diesel fuel (10%) + hair (Genç 1989; Uğurlu, Çevik 1991; Taşdemir, Karatay 2007; Gülcü 2009).

Germination tests in the laboratory and field trials. Germination tests were performed in a seed germination cabinet using sterilised sand-filled glass trays (38.5 × 26.0 × 4.5 cm). The germination experiment included 2 340 acorns (12 coated substances + control) arranged in sand-filled trays with four replicates per group. Acorns were placed horizontally and spaced to prevent contact, following a randomised block design, with 3/4 of each acorn remaining in sand. The germination cabinet was set to 16 h of darkness, 8 h of light, 20 °C ± 0.5 °C, and 75–80% humidity. Germination was monitored for 35 days, and acorns were considered germinated once their roots exhibited geotropism and reached 5 mm in length. Equation (1) below shows how germination percentage (GP) was calculated:

$$GP = \frac{\sum n_i}{N \times 100} \ (\%) \quad (1)$$

where:

n_i – number of germinations on day i;

N – total number of acorns tested (Boydak, Çalışkan 2014).

Acorns were sown at a depth of 5–7 cm (average 6 cm) in pits made with a sowing hoe on gradoni terraces prepared by a 4×4 tractor. They were placed horizontally, in the same direction, with 20 cm spacing. In line with traditional forestry practices in Türkiye, no irrigation was applied at the trial areas. Based on our observations and regional meteorological data, no unusual climatic conditions – including drought – were observed during the 2018 vegetation period. The trial was designed using the 'parcels divided into randomised blocks' method

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Table 2. Field trial design

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

M0 – control; M1 – Pomarsol Forte + alumina 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*; M9 – clay + ferulic acid; M10 – clay + *Heracleum sphondylium*; M11 – clay + benzothiazole; M12 – diesel fuel (10%) + human hair

with four replications and 13 factors. The field trial involved 3 900 coated acorns, with 12 substances and 1 control × 4 replicates × 25 acorns × 3 locations. The acorns were sown in November 2017, and emergence success was evaluated in spring 2018, while seedling survival was assessed at the end of the 2018 vegetation period. Seedling emergence continued until the end of May. Following emergence, standard tending practices, namely weeding and hoeing, were uniformly applied across all three fields from late May to mid-June 2018, when natural rainfall had begun to decline. In addition, acorns were sown along parcel edges for isolation, and emergence (*EP*) and survival (*SP*) rates were calculated per ISTA (1996) standards. Table 2 shows a field trial example from Binkılıç.

Statistical analysis. Variance analyses were performed using one-way analysis of variance (ANOVA) with IBM SPSS Statistics (Version 22, 2013) at a significance level $\alpha = 0.05$. In the phytotoxicity test, differences between the control group and the test substance groups were analysed using the Tukey test. In the Y-maze test, differences between substance and control arms were analysed using the Kolmogorov-Smirnov test, while group differences were assessed with the Dunnett test. Emergence and survival differences across the three locations were classified using the Duncan test.

RESULTS

Tetrazolium (TZ) tests showed that 86% of acorns were viable across all locations. The seed moisture content was sufficient for germination, and the average thousand-seed weight was 1 872 g (Table 3).

According to *L. minor* phytotoxicity test ANOVA test results, significant differences were found between the experimental groups at $\alpha = 0.05$. The differences between the control group and the groups containing test substances are presented in Table 4.

Table 3. Outcomes of seed viability (TZ test), moisture content (W_A), and thousand-seed weight (*TSW*)

Location	Viability (%)	$W_A = \frac{A-E}{A \times 100}$ (%)	*TSW* (g)
Binkılıç	90	42.235	2 200
Kurtkemeri	83	46.883	1 553
Beykoz	86	31.259	1 863
Mean	86	40.125	1 872

TZ – tetrazolium; *A* – initial weight; *E* – dry weight

Rat time spent in Y-maze arms and reaction scores to test substances are shown in Table 5.

Laboratory and field analyses across three locations showed that the 13 active substances (including control) differed significantly ($\alpha = 0.05$) in germination, emergence, and survival, with Duncan test grouping them into homogeneous categories (Table 6).

Table 4. *Lemna minor* phytotoxicity test results

Experimental groups	Inhibition value (%)	*P*
Control	−8.70	–
Pomarsol Forte + alumina powder	−3.31	1.000
Eugenol	100.00	0.000*
Ferulago confusa	27.24	0.403
Foeniculum vulgare	45.20	0.034*
Carthamus lanatus	−14.09	1.000
Ferulic acid	38.02	0.522
Heracleum sphondylium	−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

*Significance level $\alpha = 0.05$ (Tukey test)

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Table 5. Y-maze test results: Arm time and behavioural responses of rats

Experimental groups	*SABS*	*CABS*	*P*-value (BT1)	*SABS*	*CABS*	*P*-value (BT2)	*SAT*	*CAT*	*P*-value (TT1)	*SAT*	*CAT*	*P*-value (TT2)
Feed + Pomarsol forte + alumina powder	0.88	1.63	0.011*	–	–	–	–	–	–	–	–	–
Feed + *Carthamus lanatus*	0.29	1.57	0.013*	–	–	–	–	–	–	–	–	–
Feed + ferulic acid	0.75	1.50	0.026*	–	–	–	–	–	–	–	–	–
Feed + *Ferulago confusa*	–	–	–	0.14	2.00	0.000*	60.02	177.14	0.004*	–	–	–
Feed + *Foeniculum vulgare*	0.50	1.38	0.039*	0.63	2.13	0.002*	–	–	–	–	–	–
Feed + diesel fuel + hair	–	–	–	0.43	1.29	0.001*	–	–	–	–	–	–

*Significance level α = 0.05; *SABS* – substance arm behaviour score; *CABS* – control arm behaviour score; *SAT* – substance arm time (s); *CAT* – control arm time (s); BT – behaviour test; TT – time test

Table 6. Duncan test results of the three locations (Binkılıç, Beykoz, Kurtkemeri, respectively)

Active substance groups	Germination (%)	Homogeneity group	Emergence (%)	Homogeneity group	Survival (%)	Homogeneity group
Pomarsol Forte + alumina powder	87 (91, 86, 81)	a	34 (46, 34, 23)	ab	29 (45, 22, 21)	ab
Ferulic acid	86 (90, 86, 81)	a	34 (51, 37, 14)	ab	31 (51, 29, 14)	ab
Control	86 (90, 85, 81)	a	23 (27, 25, 16)	cd	19 (27, 16, 15)	de
1,8-cineol	85 (91, 86, 76)	a	32 (57, 26, 14)	ab	31 (55, 25, 13)	ab
Foeniculum vulgare	84 (86, 85, 78)	a	35 (56, 35, 13)	ab	31 (53, 28, 13)	ab
Carthamus lanatus	84 (88, 83, 80)	ab	28 (66, 12, 07)	bc	28 (65, 11, 07)	bc
Benzothiazole	83 (88, 85, 75)	ab	26 (47, 19, 13)	bc	25 (47, 16, 13)	cd
Capsaicin	82 (88, 83, 75)	ab	33 (57, 27, 14)	ab	30 (57, 18, 14)	ab
Ferulago confusa	75 (81, 75, 68)	bc	34 (46, 40, 17)	ab	28 (45, 22, 17)	bc
Heracleum sphondylium	73 (93, 76, 48)	c	39 (52, 41, 24)	a	37 (52, 36, 24)	a
Eugenol	73 (80, 75, 63)	c	32 (54, 31, 11)	ab	30 (52, 27, 11)	ab
Camphor	64 (90, 68, 33)	d	39 (56, 36, 26)	a	36 (56, 30, 23)	ab
Diesel fuel + hair	51 (60, 43, 46)	e	16 (27, 12, 10)	d	15 (26, 10, 10)	e

In laboratory tests, Pomarsol Forte + alumina, ferulic acid, 1,8-cineole, and *Foeniculum vulgare* showed the highest germination rates (84–87%), while camphor and diesel fuel + hair had the lowest. Field survival was highest with *Heracleum sphondylium*, camphor, ferulic acid, and *Foeniculum vulgare*, and lowest with diesel fuel + hair and control. *Ferulago confusa*, which exhibited strong repellent activity, also demonstrated optimum germination and survival performance under both laboratory and field conditions. Additionally, both laboratory and field results indicated that the Binkılıç location exhibited higher germination, emergence, and survival performance compared to the other locations.

DISCUSSION

This research used a pellet coating-wrapping technique to retain repellent substances between the acorn and the pellet material, ensuring their long-term effectiveness. Diatomaceous soil was chosen for its high stress resistance, 80–85% porosity, strong water absorption, and 85% SiO_2 content (compatibility with the sand medium) (Özbey, Atamer 1987). Thus, a clay pellet was created that supports air, water, and nutrient circulation, ensuring better germination (Hacıyusufoğlu 2015). The coating met key criteria for effective seed protection. Podlaski et al. (2019) and Demir et al. (2017) found that optimal water potential

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in solid, water-resistant, and organic mineral-based pellets positively affects germination. In this study, the water-resistant and non-dispersible diatomaceous soil coating showed a similar positive impact. Villalobos et al. (2019) found that sand-coated seeds were ineffective in both repelling rodents and supporting germination. This finding supports the choice of diatomaceous soil as the coating material in this study.

This study showed that a 20% clay coating ('complete film coat') (Tettero 2012; Patel 2015) was sufficient to protect sessile oak acorns from environmental stress and enable the controlled release of active substances. Furthermore, a low concentration (10%) of active substances was selected to balance repellency and germination, based on findings by Aydın and Tursun (2010) that higher doses of essential oils can reduce germination.

Agricultural studies have shown that seed coating enhances germination efficiency (Govinden-Soulange, Levantard 2008; Mehrabi, Chaichi 2012; Başaran, Doğrusöz 2022; Koirala et al. 2023), and the laboratory germination results in this research align with previous findings.

Despite the observed phytotoxicity in some substances, the coating process helped reduce their negative effects. *Foeniculum vulgare*, Pomarsol Forte + alumina, ferulic acid, and 1,8-cineole showed high germination rates, while diesel fuel + hair and camphor had the lowest. Camphor's low laboratory germination rate (33%) likely reflects concentration issues or the effects of a closed environment.

Pomarsol Forte + alumina and ferulic acid supported germination and survival but showed weak repellency in Y-maze tests. Further research is needed to validate their deterrent potential.

While the control group's laboratory germination aligned with previous oak studies (Taşdemir, Karatay 2007; Örtel 2011), its field performance was consistently lower, likely due to uncontrolled environmental and wildlife conditions. Similarly, other studies have reported lower field emergence compared to laboratory germination in different oak species (Taşdemir, Karatay 2007; Çalışkan 2014). These findings highlight the role and importance of repellents. While diesel fuel + hair and the control group had the lowest survival rates, *H. sphondylium*, camphor, ferulic acid, and *F. vulgare* showed the highest survival rates. Despite traditional use in Türkiye, diesel fuel + hair showed no repellent effect and negatively impacted germination and survival. Additionally, this study confirmed that the germination success of Pomarsol Forte + alumina is consistent with findings from previous studies (Genç 1989; Uğurlu, Çevik 1991).

The higher germination, emergence, and survival rates observed at the Binkılıç location are consistent with its seed viability (TZ) test results. Variations in the germination performance of the same active substances across different locations are thought to result from differences in seed viability and site characteristics.

In the Y-maze experiment, when the active substance feed-related behaviour of rats was tested, *F. confusa* was found to be the most effective in terms of duration, while *F. confusa* and *F. vulgare* were the most effective based on behaviour scores. Rats avoided feed containing these substances; instead, they consumed the feed in the control arm. It was observed that the animal repellent effect of other active substances remains limited under laboratory conditions.

CONCLUSION

Based on the findings of this research, *F. confusa* demonstrated a strong repellent effect in Y-maze time and behaviour tests and showed optimal performance in laboratory germination, field emergence, and survival. Considering all animal and germination experiments, *F. confusa* was identified as the most effective repellent substance. *F. vulgare*, effective in behavioural tests, ranked second due to its positive impact on germination and survival.

Although seed coating supports moisture retention, stress protection, and pest defence, field emergence rates were lower than laboratory germination. Hansen et al. (2016) found similarly that many substances effective in laboratory settings showed limited success in field conditions. These findings highlight the need for further studies on acorn–animal interactions under varying field conditions for these substances.

Rodent-repellent seed coatings can support afforestation, regeneration, soil conservation, and sapling production, while reducing reliance on toxic bait and traps.

This method can be easily applied to acorns in countries such as Türkiye, which have diatomite reserves and natural plants (*F. confusa*, *F. vulgare*). In forestry, new seed coating or pelletising methods

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can be developed for small-seeded species to support regeneration and seedling production efforts.

Rodent-related challenges cannot be solved by a one-size-fits-all seed coating for oak. In this regard, developing new and functional coating methods and conducting interdisciplinary research are essential. Developing sustainable, eco-friendly new seed technologies can promote the use of natural or nature-identical products to control rodents, birds, and insects in forestry.

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