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On the Red Squill Powder (*Drimia maritima*) As a Botanical Rodenticide; a Preliminary Study on Wild *Rattus norvegicus* in Ankara Province

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ABSTRACT
Rodents constitute an essential place among pest-vector animals; they also have the potential to cause economic losses and health problems. The Red squill plant, which contains scilliroside, especially in its bulbs, has been known as a rodenticide since the early 20th century. However, little research reveals the Red squill's potential as a rodenticide. For this purpose, we produced the baits, including different amounts of red squill powder with peanut butter, and we applied them on *Rattus norvegicus* in the field in Ankara province and in laboratory conditions. In field studies, it was determined that there was no feed avoidance behavior against the bait containing 25 % or below Red squill powder corresponding to 400 mg kg. In laboratory trials, it was found that if Red squill is not covered with suitable material, such as paraffin wax, it deteriorates rapidly and loses its effectiveness due to contact with air. The food contains between 15 – 25 % dried Red squill powder, corresponding to 400 mg/kg, which was found to cause 50 % and more mortality percentages, and it is suitable to apply as a rodenticide.

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ÖZET
Zararlı vektör hayvanlar arasında önemli bir yer tutan kemirgenler, ekonomik kayıplara ve sağlık sorunlarına da neden olma potansiyeline sahiptir. Özellikle soğanında scilliroside içeren ada soğanı bitkisi, 20. yüzyılın başlarından beri kemirgen öldürücü etkiye sahip olduğu bilinmektedir. Bununla birlikte, ada soğanının bir kemirgen öldürücü olarak potansiyeline ışık tutan çok az araştırma bulunmaktadır. Bu amaçla fıstık ezmesi ile farklı miktarlarda ada soğanı tozu içeren yemler hazırlanarak Ankara ilinde Norveç sıçanı (*Rattus norvegicus*) üzerinde sahada ve laboratuvar koşullarında uygulanmıştır. Saha çalışmalarında % 25 ada soğanı tozu içeren yemlere karşı yemden kaçınma davranışının olmadığı belirlenmiştir. Yapılan laboratuvar denemelerinde ada soğanının parafin mumu gibi uygun bir malzeme ile kaplanmadığı takdirde hızla bozulduğu ve hava ile temasından dolayı etkinliğini kaybettiği görülmüştür. 400 mg/kg veya daha fazlasına tekabül eden %15 – 25 arasında kurutulmuş ada soğanı tozu içeren yemlerin %50 ve daha fazla ölüm oranına neden olduğu ve bitkisel rodentisit olarak kullanılabileceği saptanmıştır.

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INTRODUCTION
Since the beginning of humankind, human beings have considered many animal species harmful and developed different methods for struggling against

them. Rodents are one of the most important groups of animals; they have the potential to cause economic losses and health problems as pest-vector. Grain production is seriously damaged by rodents in 113 countries (Meerburg et al., 2009). Some rodents reside in close proximity to human settlements and are termed synanthropic rodents (ISSG, 2017), whereas others are regarded as agricultural pests. Especially synanthropic rodents are important in the spread of zoonotic diseases such as humans bubonic plague, typhus, Weil's disease, toxoplasmosis, trichinosis, hantaviruses, babesiosis, Lyme disease, tularemia, cutaneous leishmaniosis, etc. (Buckle & Smith, 1994; Atkinson, 2000; Steppan et al., 2005; Gratz, 2006; WHO, 2006; Robins et al., 2008; Khlyap & Warshavsky, 2010; Khlyap et al., 2012; Kosoy et al., 2015; Buckle, 2012; Wilson et al., 2016).

Around 70 rodent species are distributed in Türkiye (Yiğit et al., 1998; Yiğit et al., 2006). There are mainly a few synanthropic rodent species in Türkiye such as *Mus musculus* (House Mouse), *Mus macedonicus* (Balkan house mouse), *Rattus norvegicus* (Norway rat), and *Rattus rattus* (Black rat). Other rodent species belonging to the genus *Microtus* species, known as voles, are the primary pest in agricultural areas in Türkiye. Rat species are originally native to south-eastern Asia (Buckle % Smith 1994; Steppan et al., 2005; Gratz, 2006; Robin et al., 2008; Wilson et al., 2016). According to the criteria given by McNeely et al. (2001), synanthropic rodents are called invasive alien species in Türkiye.

Biocides, which encompass rodenticides, are now categorized under the term pesticides according to Regulation (EU) No 528/2012. The management of rodent pests in agricultural and urban areas is regulated by the Ministry of Agriculture and Forestry and the Ministry of Health, respectively. Rodenticides are generally considered acute and chronic (subacute) effects under two groups. Acute-acting rodenticides cause sudden death, while chronic ones cause death after day four after ingesting the lethal dose (Buckle & Smith, 1994; Buckle et al., 1994; Pelz et al., 1995). Traditionally, the most commonly used acute-acting rodenticide is wheat prepared by zinc phosphide, known as poisonous wheat, and is commonly used in agricultural fields but banned in urban places in Türkiye. The disadvantage of acute rodenticides is that the rodent develops avoidance behavior of poisoned baits and causes secondary poisoning to other animals (Rowe et al., 1970). This poisoning occurs by direct consumption of the bait or via food chains. Especially in agricultural areas, uncontrolled use of acute rodenticides is known to cause the death of non-target animals such as birds and mammals in Türkiye.

Considering the risks created by acute rodenticides, anticoagulant rodenticides have been used, especially in urban areas, after the 1950s. Firstly, the first-generation anticoagulant rodenticides (FGARs) were used in rodent control; after a while, the resistance to FGARs appeared in rodents, and then the second-generation anticoagulant rodenticides (SGARs) was developed, but resistance against SGARs has been detected in European and US rat populations (Buckle & Smith, 1994; Pelz et al., 2005; Pelz & Muller, 2007; Rost et al., 2009; Buckle, 2012; Runge et al., 2013). Nowadays, the researchers are focused on the third-generation anticoagulant production. In such a circumstance, this may lead to increased interest in acute rodenticides in the future.

One of the acute rodenticides is scilliroside (CAS number; 507-60-8 and European Community (EC) number; 208-077-4) and is naturally found in the bulbs of the plant named red squill (*Drimia maritima*) merits as an alternative rodenticide to zinc phosphide, an advantage of this substance is that it stimulates the vomiting reflex in accidental poisoning of human and other mammals. Also, low water solubility reduces the risk of environmental contamination (Munch et al., 1929; Buck, 1936; Crabtree, 1947; Verbiscar et al., 1986; Bozorgi et al., 2017). This substance is not approved as a rodenticide in the EU and US. The acute toxicity category is 2 in EU regulations (Oral dose of LD50 ranges from 50 to 500 mg kg). Scilliroside is known to cause death due to delayed myocardial repolarization and tachycardia. However, further research is required; the precise histopathological effects of these anticoagulant rodenticides on organs and tissues are not well understood. Studies have been primarily limited to LD50 dose investigations and have not extensively covered wild races of the Norway rat and black rat. The toxicity and efficacy of scilliroside were reported for bandicoot rat (*Bandicota bengalensis*) with different LD50 doses for males (0.8 mg kg) and females (0.5 mg kg) (Brooks and Htun, 1980). In another report on scilliroside, Lewis (2004) reported that LD50 of scilliroside is orally 430 mg kg (mg/kg or mg kg $^{-1}$) for laboratory rats. Also, it was stated that the scilliroside may have where acute toxicants are to be used. Red squill powder doses vary from female / male 150 – 825 mg/kg (Munch et al., 1929; Buck, 1936; Crabtree, 1947; Verbiscar et al., 1986; Bozorgi, 2017).

This study gives the results of the analysis of field and lab trials with a botanical rodenticide consisting of powder from the Red squill bulb. The study aims to determine the palatability and effectiveness of botanical rodenticides for rats. In this context, the study involves the evaluation of a plant-based rodenticide with the potential for rodent control, using wild Norway rats captured in Ankara province.

MATERIALS and METHODS

Wild Norwegian Rats

Norway rats (*Rattus norvegicus*) were caught in and around Ankara using Sherman live trap. All samples were captured with the permission of the Ethics Committee decision frame numbered 2020-11-92 and the BAP project numbered 18B0430005 of Ankara University.

Processing of Raw Material of Red Squill

MetaMeta Anatolia Company provided the bulbs of red squill used in this study. The outer covers of the bulbs were peeled, and the bulbs were divided into small pieces. They were dried at 80oC for 48 hours. Dried pieces were ground with a grinder into powder. This powder was kept in containers in the refrigerator (Munch, 1929; Verbiscar et al., 1986).

The Locations and Field Trials

Field trials were conducted in the city center of Ankara and its surrounding towns. Each night, a total of 50 Sherman traps, comprising 25 with poisonous baits and 25 with non-poisonous baits, were used. The poisonous bait was prepared by adding red squill powder to the peanut butter, while the plain bait was peanut butter only. Both baits were placed in carrots, and the carrot was piece set up in the traps. Considering the average weights of Norway rat samples previously caught from Ankara, the weights of the rats varied from 174 to 345 grams, and the average weight was around 260 grams (Yiğit et al., 1998). Thus, 100 mg of red squill powder was added to every bait in the traps during trapping (100 mg of red squill powder remains within reference dose limits of 150 – 825 mg/kg; these values correspond to 25 – 206 mg / 260 g rat).

Captured rats were transferred to the laboratory and taken in individual cages, separating those caught in the poisonous trap and those caught with plain baits. The cages of both groups of rats were given standard rat food (water and rat pellets), so rats were fed ad libitum with solid feed and had free access to water.

Choosing the Dose and Determination of Doses for Palatability

There is ongoing controversy regarding the LD50 doses for *Rattus norvegicus*, and no precise dose has been proposed, particularly for wild Norway rats. The amount of poison used in bait in the references is given both for pure scilliroside form as well as in red squill powder form; the doses of pure scilliroside range from female/male rats 1.1 – 4.3 mg/kg; red squill powder doses vary form female/male 150 – 825 mg/kg (Munch et al., 1929; Buck, 1936; Crabtree, 1947; Verbiscar et al., 1986; Bozorgi, 2017). Fresh and dried red squill powder covered with paraffin wax was used in experiments. 400 mg/kg dose was chosen by the considering reference range of 150 – 825 mg/kg. Thus, the trials started with testing reference doses of 400 mg/kg red quill powder regardless of gender. First, all rats tested were weighed, and the food amount given to the cages was calculated to the corresponding amount of red squill in the total food. For example, a 200 g rat needs 80 mg of red squill powder. So 0.32 gr of bait (75 % Peanut – 25 % red squill powder) for group 3 corresponds to 400 mg/kg red squill powder. These baits calculated per individual were added to the ad libitum feeding, considering that the rats consumed up to 20 g of food per day. In this way, besides determining the efficacy of Red squill powder, the different ratios of the baits used to test the palatability of red squill powder due to its bitter taste;

0 % peanut – 100 % red squill powder
50 % peanut – 50 % red squill powder
75 % peanut – 25 % red squill powder
80 % peanut – 20 % red squill powder,
85 % peanut – 15 % red squill powder,
90 % peanut – 10 % red squill powder,
95 % peanut – 5 % red squill powder,
97.5 % peanut – 2.5 % red squill powder.

Dose modifications were made to determine whether the animals consumed the bait in each experimental group and their consumption rates. Also, the gastric gavage method gave Red squill powder suspended in water to directly test the efficiency of the 400 mg/kg dose.

Laboratory Observations

The rats caught in the plain traps were transferred to a laboratory and placed in separate cages. All animals were kept in constant conditions (temperature and photoperiod, ad libitum feeding) for five days for acclimatization. After that, the poisoned bait was given in different doses in the cages. In this way, the palatability and effectiveness of poisoned baits and the mortality rate were recorded.

Statistical Analysis

Test groups that applied a different amount of red squill powder were analyzed statistically by the Chi-Square test (IBM, 2013; SPSS).

RESULTS AND DISCUSSION

Percentage of Rats Captured in Sherman Traps with Poisonous and Non-Poisonous Baits

The percentage of catching in non-poisonous and poisonous traps (n: 16) is found to be 44 % in poisonous and 56 % in non-poisoned traps (Figure 1). It can be said that there was no substantial difference in the percentage of Norway rats captured in traps with the poison bait, compared to captures with non-

poison baits (Table 1). However, it was found that there was an avoidance of trapping in Norway rats. More than 10 video recordings were taken from the animals wandering around and not entering the traps, at equal numbers of poisonous and non-poisonous traps. In one case, we put four traps for three nights at the same place; the photo camera recorded that Norway rats wandered around the traps on the first and second nights, and two animals were caught on the third night. This indicates that rats are suspicious of traps because they are neophobic and fall into the Sherman traps after acclimation. Adult Norwegian rats caught around Ankara weighed between 210 and 340 grams, with an average weight of 250 grams.

Table 1. Norway rats captured from Ankara province in field trails; measurements (mm), weights (g), and bait types (F: female, M: Male, NP: non-poisonous, P: poisonous).

Çizelge 1. Ankara ilinden tarlalarda yakalanan Norveç sıçanları; ölçüler (mm), ağırlıklar (g) ve yem türleri (K: dişi, E: Erkek, NP: zehirsiz, P: zehirli).

Nr/sex/age *Numara/cinsiyet/Yaş*	Total body length (mm) *Toplam vücut uzunluğu (mm)*	Tail length (mm) *Kuyruk uzunluğu (mm)*	Weight (gram) *Ağırlık (gram)*	Bait type *Yem Çeşidi*
1/F/juvenile *1/D/yavru*	110	60	16	NP
2/M/juvenile *2/E/yavru*	130	70	19	NP
3/F/adult *3/D/yetişkin*	364	175	245	NP
4/F/adult *4/D/yetişkin*	385	185	250	P
5/F/adult *5/D/yetişkin*	401	187	300	NP
6/M/adult *6/E/yetişkin*	415	190	340	NP
7/F/adult *7/D/yetişkin*	425	189	205	P
8/F/adult *8/D/yetişkin*	364	175	145	P
9/F/adult *9/D/yetişkin*	400	190	300	P
10/M/adult *10/E/yetişkin*	390	170	210	NP
11/F/Adult *11/D/yetişkin*	394	192	214	P
12/M/Adult *12/E/yetişkin*	425	195	305	P
13/M/juvenile *13/E/yavru*	275	115	59	NP
14/F/Adult *14/D/yetişkin*	365	175	215	NP
15/F/Adult *15/D/yetişkin*	340	162	225	NP
16/M/Adult *16/E/yetişkin*	340	165	145	P

Laboratory Trials with Red Squill Powder

The palatability test was done with red squill powder and peanut butter without paraffin wax, and it was seen that the baits containing 25 % or less red squill powder were preferred. In addition, it was determined that the effectiveness of the baits prepared without paraffin covering decreased after a while. The effectiveness of 25 % red squill powder was also confirmed by giving it to rats (n: 8) via gastric gavage. Due to the oxidation problem of scilliroside, the baits were prepared in a wax block to cut off their contact with air. Munch (1929) noted that red squill powders stored in screw-top vials in the light for 15 months showed no chance of lethal dose. In this case, red squill powder air contact was cut off. Munch (1929) also suggested that a 10 percent squill biscuit that has been baked was as toxic a year later as the squill content would require. This report is inconsistent with the finding of this study that air contact causes

red squill powder to lose efficacy. Verbiscar et al. (1986) pointed out that the effectiveness of red squill powder would decrease with contact with moisture and air, and it was suspended in agar to be prepared as a product with high uniformity and bioavailability.

After the rats were accustomed to the cages, the trials began with poisonous bait with the bait combination given in Table 2 and the bait form seen in Figure 1. In all bait combinations, while the peanut butter and red squill ratio was changed, the paraffin wax ratio was kept at 60 %. The poisonous baits given to test groups were consumed totally on the same day, likewise for the control groups, indicating that the bait's taste is palatable to rats. The palatability test was also verified with the bait-choice test using poisonous and non-poisonous bait in the same cages and in a laboratory trial for efficacy test performed with 54 rats, 48 rats that were fed poisonous bait containing 400 mg/kg red squill powder in the laboratory died around day four except for six rats. In the pre-mortem phase (1–2 days), any change in daily activity behavior was not monitored after eating the poisoned bait. The behavior of rats exposed to poisoned baits showed signs of sickness such as not responding to stimuli, reduced mobility, avoidance of feeding, and inability to control the body. By the 3rd–4th day, the rat became increasingly immobile, and especially on the fourth day, rats showed slight hind foot convulsion at the time of near death. The convulsion was similarly reported by Crabtree (1947) and Verbiscar et al. (1986). In this duration, adult rats exposed to poisonous bait lost weights between 15 and 98 g averaging 38 g. The weight losses of the control and experimental groups are given in Figure 2. There was no significant change in the weight loss between the control and experimental groups. This suggests that the weight loss is due to laboratory stress in wild Norwegian rats. The rats did not prefer to eat the baits containing 50 % or more red squill powder. 50 % and more deaths were recorded except for the second and third groups (Table 2).

Figure 1. Baits in the paraffin wax
Şekil 1. Parafine karıştırılmış yemler

Table 2: Dose-dependent trials and the efficacy in ad libitum feeding with the bait.
Çizelge 2: Doza bağlı denemeler ve yemle ad libitum beslemedeki etkinlik.

Groups *Gruplar*	Percentage of Red squill powder, peanut butter, and paraffin in the baits *Yemlerdeki ada soğanı tozu, fıstık ezmesi ve parafin yüzdesi*	The total individual number exposed *Maruz kalan toplam birey sayısı*	Total amount of red squill consumption (mg) per individual during trials *Denemeler sırasında birey başına toplam ada soğanı tüketimi miktarı (mg)*	Mortality rate % *Ölüm oranı %*
1	2.5 %, 37.5 %, 60 %	2	250 (3200 mg needs to reach 400 mg kg for 200 g rat)	100
2	5 %, 35 %, 60 %	5	878 (1600 mg needs to reach 400 mg kg for 200 g rat)	40
3	10 %, 30 %, 60 %	14	392 (800 mg needs to reach 400 mg kg for 200 g rat)	21
4	15 %, 25 %, 60 %	2	1772 (530 mg needs to reach 400 mg kg for 200 g rat)	50
5	20 %, 20 %, 60 %	23	1071 (400 mg needs to reach 400 mg kg for 200 g rat)	69
6	25 %, 15 %, 60 %	8	2196 (320 mg needs to reach 400 mg kg for 200 g rat)	63

Inconsistency was observed in the results despite the feed consumption of less than 400 mg/kg in groups 1–3 due to the small number of samples in group 1. Another reason may be that the red squill powder is oxidized or might have different amounts of scilliroside in the red squill onion. It was first reported by Crabtree (1947) that the amount of scilliroside in red squill onions varies. Also, EMEA (1999) reported that Scilliroside varies by 0.01 – 0.53 % in red squill onions. Also, Bozorgi et al. (2017) pointed out two disadvantages of scilliroside, which is one of the miscellaneous bioactive substances in red squill onion: variation in toxic properties and relatively low toxicity.

It is shown that groups 4–6 in which the bait consumption above 400 mg/kg yielded 50 % or more mortality (Table 2). Lewis (2004) reported that LD50 of scilliroside is orally 430 mg/kg for laboratory race of Norway rats. This LD50 dose was found to be almost similar to this study's results, and a 50 % mortality rate was obtained in this study at this dose.

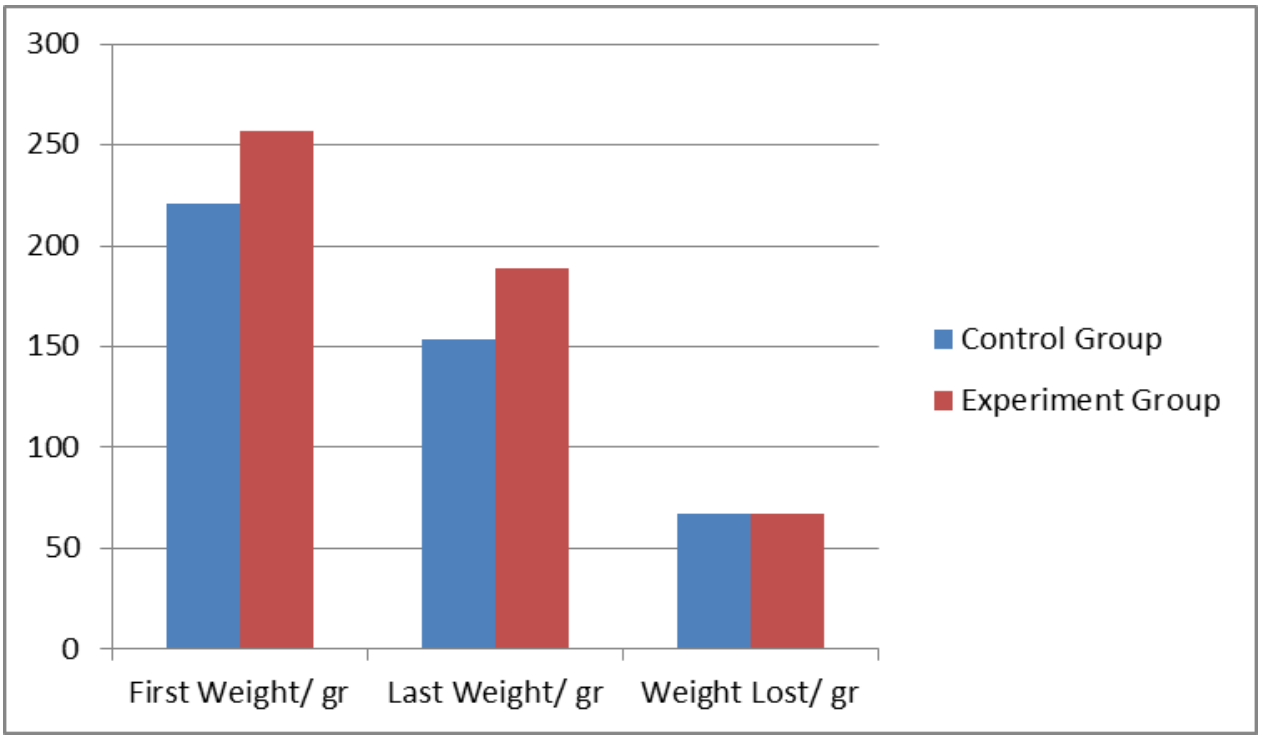

Figure 2. Changes in the weight loss between control and experimental groups
Şekil 2. Kontrol ve deney grupları arasındaki ağırlık kaybındaki değişim

Statistical Analyses

The chi-square test, which was performed considering all groups, was found to be significant ($p < 0.05$). This means that the efficacy of red squill powder varies between groups depending on the dose. However, there was no significant difference ($p > 0.05$) between groups 3–5 with a mortality rate of 50 % and above. This indicates that the doses containing 15 % – 25 % red squill powder can be used as botanical rodenticides.

Post Mortem External and Internal Examination of Rats

In the dissection performed in the post-mortem phase, no abnormal morphology other than weight loss was detected in the external morphology. According to the physical examination of the internal organs, color changes in the form of darkening were detected, especially in the liver and intestines. However, although it is known that death is due to cardiac arrest, scilliroside is thought to affect the liver and intestines intensely. Also, no morphological difference was observed in the heart.

Unexplained deaths due to stress were frequently observed in the wild Norway rats during the trials. The fact that the wild Norway rats usually die in 4 days shows that this product can be considered a rodenticide with a subacute effect.

Oxidation Problem of Red Squill Powder and Seasonally Variable Amount of Scilliroside

As indicated by Verbiscar et al. 1986, laboratory trials of this study have also shown that this botanical rodenticide is easily oxidized and can be used practically in a protective cover such as a wax block. Maddock and Schoof (1970) reported that stabilized scilliroside is highly effective against Norway rats. In addition, Crabtree (1947), EMEA (1999), Verbiscar et

al. (1986), and Bozorgi et al. (2017) reported that the scilliroside content of red squill product varies seasonally, and the active substance (Scilliroside) in dried bulbs varies from 0.01 % to 0.53 % (EMEA, 1999). Due to this, the dose of LD50 in the laboratory trials varied from 400 mg/kg to 7500 mg/kg. This study also identified oxidation issues and efficacy differences observed in various red squill bulbs, aligning with the findings of the researchers mentioned above.

CONCLUSION

The main findings can be summarized below:

- In areas where wild Norway rats are found, the daily trapping success does not exceed 4%, regardless of whether the bait is poisonous or non-poisonous. This proves trap avoidance behavior.

- The laboratory trials also confirmed that red squill powder has a high palatability of up to 25 % in the bait when mixed with peanut butter and paraffin wax.

- Overall mortality was found to average 48 % within four days (in the range of 1–15 days) in all groups tested. For this reason, the red squill powder has a subacute effect on rats as a botanical rodenticide.

- In experiments with bait containing 15–25 % red squill powder, the mortality rate was found to be 50 % and above (25 % corresponds to 400 mg red squill powder/kg).

- It is thought that the differences in the doses consumed and the mortality rate may be due to the difference in the active substance in the red squill bulbs.

- An important advantage of red squill powder is its emetic effect, so it does not harm non-target animals other than rats. However, its bitter taste, the variable amount of active substance in red squill onions, and relatively low toxicity can be considered a disadvantage.

- Trials of this study have shown no difference in the effectiveness of dried or fresh red squill powder when isolated from the air with a suitable material such as paraffin wax.

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Researchers' Contribution Rate Statement Summary

The authors declare that they have contributed to the article as 30 % NY, 25 % EB, 15 % ŞE, 15 % LB, and 15 % DÇ.

Conflict of Interest Statement

The article's authors declare that they do not have any conflict of interest.

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