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Original article

Population development of the *Anthonomus amygdali* Hustache (Coleoptera: Curculionidae) in almond orchards in Gaziantep, Kahramanmaraş and Adıyaman provinces

Gaziantep, Kahramanmaraş ve Adıyaman illerinde bulunan badem bahçelerinde *Anthonomus amygdali* Hustache'nin popülasyon gelişimi

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ABSTRACT

This study was carried out between 2016 and 2017 to determine the population development of *Anthonomus amygdali* Hustache, which is a pest in almond orchards in Gaziantep (Şahinbey), Kahramanmaraş (Pazarcık) and Adıyaman (Besni) provinces. The study used the beating method in almond orchards, where the population was followed. It was determined that *A. amygdali* started its flights between April and May in the region where the study was conducted and continued until the end of June. While the pest reached its highest level, with 52 units/100 knocks in Besni district on 20 May 2016, the next year, its population reached its maximum level with 44 units/100 knocks, in Besni district on 01 June 2017. Thus, the highest pest population was Besni district of Adıyaman province. The pest has also been found to be an early pest in almond orchards, feeding on the buds and flowers of almonds.

INTRODUCTION

Almond (*Prunus dulcis* Miller) is from the Rosaceae family and is native to Central and Western Asia and Southwestern Asia. It was first grown in Iran, Türkiye, Syria, and Palestine and was taken from there to Spain, Italy, North Africa, Greece, and then to North America (Küden et al. 2014).

All regions of our country are suitable for almond cultivation except the coastal areas of the Black Sea region and high plateaus. Almond cultivation is mainly carried out in the Aegean, Mediterranean, and South-eastern Anatolia regions of the country.

There are many factors affecting yield and quality in almond production areas. These factors begin with pest and disease control and cultural practices such as fertilization, irrigation, and pruning. Especially pests and diseases are the most important factors that cause a loss in almond production. Due to our country's continuous enhancement of almond

orchards in recent years, plant protection problems are expected to increase.

Many insect species cause yield losses in almond orchards. The adult or pre-adult stages of these insects cause damage to the fruit directly, as well as causing severe yield losses by damaging different parts of the tree and drying the trees from the root. There have been many studies on almond pests and diseases in the World (Adaskaveg et al. 1998, Barnet 1965, Dicenta et al. 2003, Ivanov et al. 1962, Russo et al. 1993, Talhouk 1977, Vasileva 1974). In our country, some studies showed that harmful insect species reduce yield and product quality in almond orchards (Bolu et al. 2005a, Bolu and Özgen 2007, Bolu and Özgen 2010, Ekici and Günaydın 1969, Maçan 1986, Nizamlıoğlu 1961).

Anthonomus species belonging to the Curculionidae family are among the insects that cause yield losses in orchards. Tolga and Yoldaş (2020) identified *A. amygdali* and *A. pyri* species that cause damage to almond orchards in their study in Datça, Fethiye, and Seydikemer, Akhisar, and Kula districts. Bolu and Özgen (2007) obtained *A. amygdali*, *A. bituberculatus*, *A. brevipennis* and *A. variabilis* species in their study on the determination of *Anthonomus* species that cause damage in the almond orchards of Diyarbakır, Elazığ and Mardin provinces. *A. amygdali* has an important place among these harmful insect species. Lodos et al. (1978) reported that this species is an important almond pest and that the larvae of the pest develop in the flower buds of almond trees, but these flower buds do not open. In addition, it has been determined that *A. amygdali* causes severe yield losses in almond orchards in the South-eastern Anatolia Region (Bolu et al. 2005b). Önuçar and Zümreoğlu (1985) determined that *A. amygdali* caused 20-32% infestation in almond orchards in Datça on *Anthonomus* spp., which damages fruit trees in the Aegean region.

In the region where the study was conducted, almond orchards are increasing. However, detailed studies have not been conducted on the insects that cause damage to almond orchards in this region. Herein, the population of *A. amygdali* in almond orchards in Gaziantep, Kahramanmaraş and Adıyaman provinces was tried to be determined. Thus, the critical periods that must be combated with the pest have been determined. It aims to contribute to the farmers' success in the control of *A. amygdali*.

MATERIALS AND METHODS

Material

The study material consisted of adult almond trees, *A. amygdali* adults, a Steiner funnel, a suction bottle and a stick with a rubber tube.

Method

This study was conducted in Gaziantep (Şehitkamil), Kahramanmaraş (Pazarcık) and Adıyaman (Besni) provinces between 2016 and 2017 to determine the population development of *A. amygdali*, one of the important almond harmful insect species. Population monitoring was realized in one orchard in each district. Pest population monitoring was carried out once a week between March and November of each year in two almond orchards in each of the indicated provinces. Samples were collected from orchards that were not treated with chemicals. In the study, the method used by Bolu and Özgen (2007) before was used.

The beating method was used in the study to monitor the pest population. Four branches of 25 trees selected randomly from the orchard were knocked three times with a stick with a rubber tube on end, causing the adult stages of the motile pest to fall on the Steiner funnel, and the falling insects were collected with a suction bottle. After the collected insect samples were killed with the help of a killing bottle, they were brought to the laboratory and counted.

RESULTS AND DISCUSSION

In the almond orchards where the study was carried out, it was observed that *A. amygdali*, belonging to the Curculionidae family, which is seen as one of the important pest species, is an early-period pest of almond trees and causes intense flower shedding in the almond orchards where it is located. Lodos et al. (1978) reported that this species is an important almond pest and that the larvae of the pest develop in the flower buds of almond trees and these flower buds do not open.

Population change of Anthonomus amygdali in Gaziantep/ Sahinbey district

In Şahinbey district, the first adults of *A. amygdali* were sighted in early May 2016, and adults continued to be encountered until the end of June. The first adults were sighted on 2 May 2016, and the highest population was recorded on May 23, with 20 adults/100 knocks. It was noted that the average air temperatures when the pest started its flights were between 14-16 °C (Figure 1).

Bolu and Özgen (2007) determined the highest population density of the pest as 28 adults/100 knocks on May 29, 2003, in Gezin district of Elazig province.

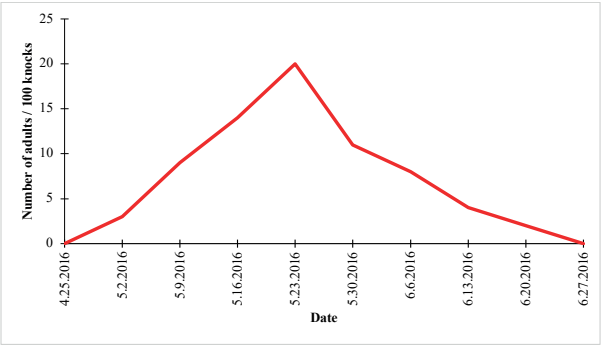

Figure 1. Population change of *Anthonomus amygdali* in Şahinbey in 2016

The first adults of *A. amygdali* started to appear on May 15 2017, and adults continued to be encountered until June 26 2017, the highest population ratio was recorded on May 29, 2017 with 17 adults/100 knocks (Figure 2).

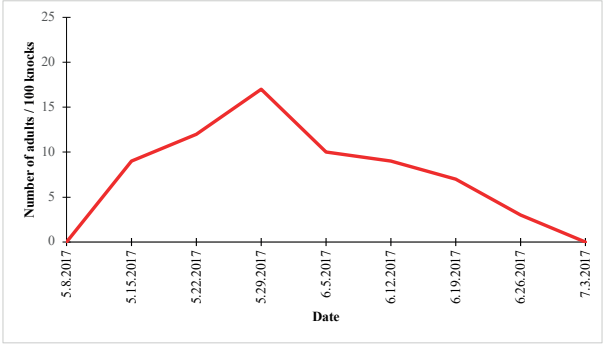

Figure 2. Population change of *Anthonomus amygdali* in Şahinbey in 2017

During the period when the adult emergence begins, the maximum air temperatures are 22-24 °C and the minimum air temperatures vary between 15-17 °C. Bolu and Özgen (2007) stated that the pest appeared in Çermik district of Diyarbakır province in 2004 from the end of April and beginning of May and continued to fly until the end of June.

Population change of Anthonomus amygdali in Kahramanmaraş/Pazarcık district

The development of the population of *A. amygdali* in Pazarcık district in 2016 is shown in Figure 3. According to this, the first adults were encountered on May 3, 2016. The highest population value of the pest was recorded on 24 May 2016 with 30 adults/100 knocks, and the last adults were seen in mid-June (Figure 3). Bolu and Özgen (2007) reported in their study conducted in Akbağ and Ömerli districts in Mardin province that the adults of the pest were still found until the end of June.

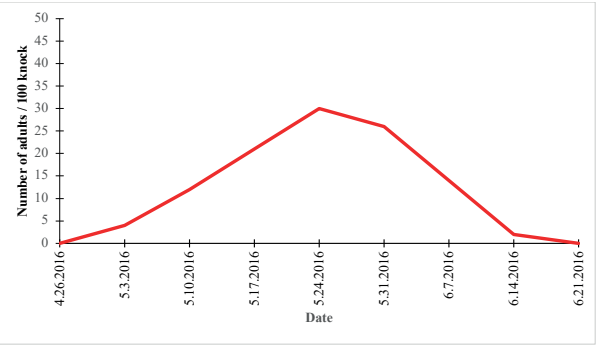

Figure 3. Population change of *Anthonomus amygdali* in Pazarcık district in 2016

The first adults of *A. amygdali* were sighted in Pazarcık on 10 May 2017, and adults continued to be encountered until the end of June. The highest population ratio was recorded on 24 May, 2017 with 17 adults/100 knocks (Figure 4).

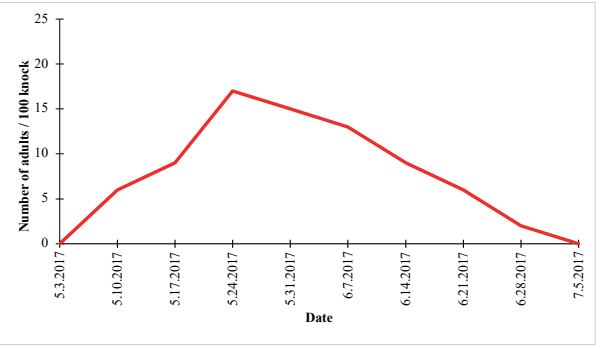

Figure 4. Population change of *Anthonomus amygdali* in Pazarcık district in 2017

The two-year population changes were parallel to each other, and the first adult flights started later in 2017 compared to the previous year due to the climate difference. In addition, it was noticed that the general population of the pest decreased slightly in 2017.

Population change of Anthonomus amygdali in Adıyaman/ Besni district

Figure 5 shows the population change of *A. amygdali* in Besni district in 2016. It was determined that the first adult flights of the pest started in almond orchards at the end of April, and the maximum population value was determined as 52 adults/100 knocks on May 20, 2016 (Figure 5). It was determined that the average air temperatures ranged between 16-20 °C on the dates when the pest started to fly. Adult flights of the pest continued until the end of June. This study shows parallelism with the study mentioned above.

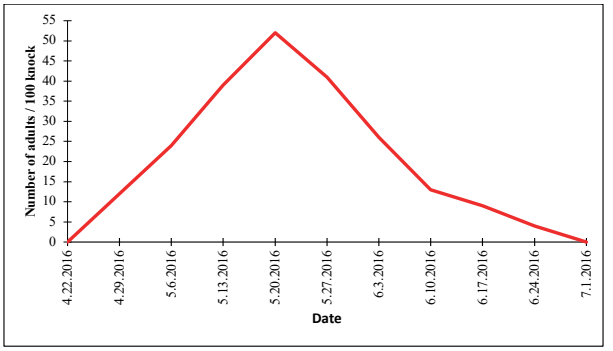

Figure 5. Population change of *Anthonomus amygdali* in Besni district in 2016

The first adult flights of *A. amygdali* in 2017 in Besni district started on May 4 and continued until May 22. Flights of adults continued for about two months in Besni district, and the population reached its maximum value at the end of May with 44 individuals/100 knocks (Figure 6). Among all the regions where the study was conducted, it was observed that the densest population of the pest was in Besni district.

Besides, it has been determined that adults are withdrawn under tree bark, soil and crevices in the soil at the end of June and the first week of July.

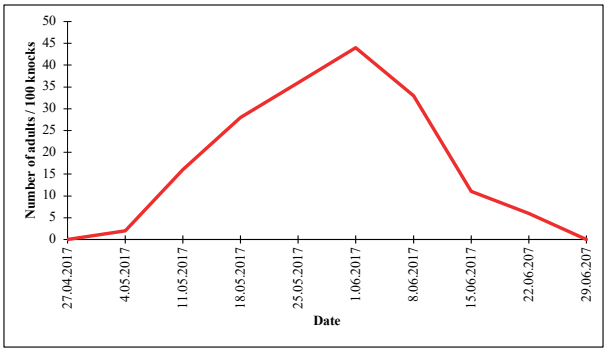

Figure 6. Population change of *Anthonomus amygdali* in Besni district in 2017

It has been observed that the pest is a high-potential pest in the regions where the study was conducted. In general, it was determined that the flight of the pest started between April and May and continued until the end of June. It has been revealed that the region where *A. amygdali* is most concentrated in almond orchards in Adıyaman/Besni district; less density was found in Şahinbey and Pazarcık districts, and parallelism was observed between the development of the populations. In the study conducted by Bolu and Özgen (2007) in almond orchards in Diyarbakır, Elazığ and Mardin provinces, they reported that the highest population of *A. amygdali* was in Diyarbakır and flight of the pest started between April and May and continued until the end of June. The results obtained from the two studies are similar to each other.

It has been determined that the hatched larvae feed on the flower petals, ovary and anther part of the male organ in almond trees. In addition, it was observed in the study that the damaged flowers did not open, the opened flowers did not produce fruit, and the adults retreated under the tree bark, soil and crevices in the soil between the end of June and the first week of July. Tolga and Yoldaş (2020) reported that in the study conducted in Muğla and Manisa provinces, it was determined that the pest feeds on ovaries, stalks, anthers and male organs. In the study, it was determined that the pest overwintered in its adult stage. Monaco (1967) reported that adults also feed on fresh leaves, sprouts and shoots and adults spend the winter under tree barks, stones, and leaf debris or in cracks and cracks in the soil.

Although there are not many studies on the pest in question in our country, it is necessary to carry out studies to determine the economic damage threshold of the pest. To control the pest, the producers must conduct the necessary surveys by the end of April. By shaking the branches of the trees from the time the buds burst until the flower buds appear fallen adults and damaged flowers can be collected and destroyed in gardens where the pest is present. Moreover, during pruning, branches with damaged buds should be cut and removed. It is of great importance to carry out studies on the biological and biotechnical control of the pest in the future.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Bu çalışma Gaziantep (Şahinbey), Kahramanmaraş (Pazarcık) ve Adıyaman (Besni) illerinde badem bahçelerinde zararlı durumunda bulunan *Anthonomus amygdali* Hustache'nın popülasyon gelişimini belirlemek amacıyla 2016-2017 yılları arasında yürütülmüştür. Çalışmada popülasyon takibinin yapıldığı badem bahçelerinde darbe yöntemi kullanılmıştır. Çalışmanın yürütüldüğü bölgede, *A. amygdali*'nin uçuşlarına nisan-mayıs ayları arasında başladığı ve haziran ayı sonuna kadar devam ettiği saptanmıştır. Zararlı Besni ilçesinde 20 Mayıs 2016 tarihinde 52 adet/100 darbe ile en yüksek seviyesine ulaşırken bir sonraki yıl ise yine Besni ilçesinde 01 Haziran 2017 tarihinde 44 adet/100 darbe ile popülasyonu maksimum seviyesine ulaşmıştır. Böylece

zararlının popülasyonunun en yüksek olduğu ilçe Adıyaman ilinin Besni ilçesi olmuştur. Ayrıca zararlının badem bahçelerinde erken dönem zararlısı olduğu ve bademde tomurcuk ve çiçek içinde beslendiği tespit edilmiştir.

Anahtar kelimeler: *Anthonomus amygdali*, badem popülasyon gelişimi, Curculionidae

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Original article

An updated data on bionomics of walnut weevil, *Alcidodes porrectirostris* Marshal (Coleoptera: Curculionidae) under laboratory conditions and its extent of damage in *Juglans regia* (L.) of the Kashmir valley, India

Ceviz kurdu, *Alcidodes porrectirostris* Marshal (Coleoptera: Curculionidae)'nin laboratuvar koşulları altında biyonomisi ve Hindistan'ın Keşmir vadisinde *Juglans regia*'daki (L.) zarar derecesi hakkında güncellenmiş veri

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ABSTRACT

Walnut is one of the most important fruits, which has been defined as a "Superfood" in recent years. It is infected by a wide array of insect pests. Among these pests, the walnut weevil is a destructive one. Its adults feed on flowers and buds while its larvae feed inside the fruits and are extremely destructive in causing premature dropping. We report two generations of walnut weevil in a year and only a single larva per fruit. Adults emerge from the soil in April and feed on walnut leaf buds, petioles of leaves, and floral buds. Adult females lay 1-2 eggs on fruits, mainly in May and early June, hatch in 4.9±0.74 (SD) days and 4.5±0.97 days in the first and second generations. They develop through three instars and the total developmental time lies in the range of 46-55 days (49.1±2.51). The adults of the second generation undergo overwintering to avoid harsh environmental conditions. Comprehension of biology, life cycles, and the nature of damage aids in keeping track of specific insect pests at the proper time.

INTRODUCTION

English walnut, *Juglans regia* L. (Juglandaceae: Fagales) is an important and widely distributed cultivated nut species acclaimed for its nutritious kernels and timber (Bayazit et al. 2007). It has evolved in ancient Persia (Mahmoodi et al. 2019). It is one of the important hard-shelled fruit species with a widespread area in the world and is the most significant species traded among many species due to its excellent fruit characteristics (Guney et al. 2021, Kafkas et al. 2020). On a global scale, walnut ranks first in nut production after cashews and almonds (Anonymous 2019a) with India holding the

seventh rank in its production globally. It is assessed least concerned species according to the International Union for Conservation of Nature (IUCN) Red List of threatened species (Rivers and Allen 2017) and is included in the group of priority plants by FAO (Raja et al. 2017) and is a lucrative crop that is in high demand globally (Hassankhah et al. 2017). It possesses a wide range of medicinal properties in almost every part like bark, kernel, fruit, leaves, green husk, and flower, and contains phenolic compounds with high antioxidant activity (Jahanban-Esfahlan et al. 2019). It is found between altitudes of 1200 to 2100 meters from Kashmir to Bhutan, Khasia, S. Tibet, and Nepal (Anonymous 2019b). However, several insect pests infest walnut orchards as well as nurseries globally. They are attacked by leaf defoliators that harm leaves, twigs, and branches leading to nut drops (Abbas et al. 2015). Beetles can be considered pests and a few of them cause considerable direct and indirect loss damage (Kailash et al. 2015, Paunikar 2015). Certain grubs of insect pests dig extensive tunnels into the roots and stems feeding in the interior tissue as they progress upwards (Khan et al. 2013). Some of the most serious pests that damage walnuts around the world are the European red mite (*Panonychus ulmi* Koch), Codling moth (*Cydia pomonella* L.), Walnut aphid (*Chromaphis juglandicola* Kalt.) Walnut scale (*Quadraspidiotus juglansregiae* Comstock) and San Jose scale (*Quadraspidiotus perniciosus* Comstock) (Ohlendorf and O'Neill 2009). Asian walnut moth, *Garella musculana* Erschoff is also a serious pest of *J. regia* (Bostanci et al. 2021, EPPO 2019, Khan et al. 2023) and *J. nigra* (Bostanci et al. 2019). In the plethora of insect pests affecting the walnut trees in Jammu and Kashmir (J&K), Walnut weevil, *Alcidiodes porrectirostris* Marshal (Coleoptera: Curculionidae) is one of the most destructive pests causing about 36.7% fruit damage (Mir and Wani 2005) and in some situations up to 90% output loss (Caliskan et al. 2020). The walnut weevil is a medium-sized pest with characteristics of the Mecysolobini tribe of the weevil family Curculionidae (Bhagat 2017). It was reported as a new insect pest on walnuts in the Kashmir division of J&K in 1990 (Gaffar and Bhat 1990). Coleoptera insects have a large array of adaptability to environmental conditions and have a cosmopolitan distribution (Kritika and Jaimala 2017). They are holometabolous with separate phases of development: egg, 1 to n instar larvae, pupa, and adult (Zhang and Zhang 2008). The majority of super family curculionids of order Coleoptera attack fruits, seeds, and leaves of cereals and grains (Bhatti et al. 2018). The adults and grubs of *A. porrectirostris* are damaging in nature with the former feeding on exposed aerial parts of the tree (petioles, leaves, and branches) and the latter feeding inside the fruit and destroying the kernels.

Walnuts are one of the major cash crops and a significant component of Kashmir's economy. *A. porrectirostris* infestation can lead to severe yield and quality loss to walnuts. In addition, the pest is monophagous and attacks *J. regia,* which can be a challenge to manage and control in outbreak years. It can cause heavy economic losses in years with higher infestation levels and has developed resistance to a variety of insecticides. Understanding the life cycle of insects and the type of crop affected can go a long way in helping with the production of agriculture to translate conclusively the stage that causes the kernel damage and devise future management plans for this destructive insect pest. Keeping in view the economic importance of walnut in Jammu and Kashmir the present work was therefore, undertaken to elucidate the complete biological aspect of *A. porrectirostris* and the nature and mode of extent of damage it causes to walnut trees.

MATERIALS AND METHODS

Study area

The field surveys were conducted across the Kashmir Valley, a part of the North western Himalayas. The Kashmir valley lies between the Great Himalaya and Pir Panjal region that includes all ten districts of Union Territory (UT), J&K to record infestation to common walnut trees by the coleopteran insect pest *A. porrectirostris*. The Kashmir Valley lies between latitude 33° and 35°N, and longitude 73° and 76°E. At all places, walnut trees suffering from pest attacks were selected randomly and shoots/fruits/branches were examined for infestation. The study was conducted from 2017-2019 and the location maps of the sampling sites are represented in Figure 1.

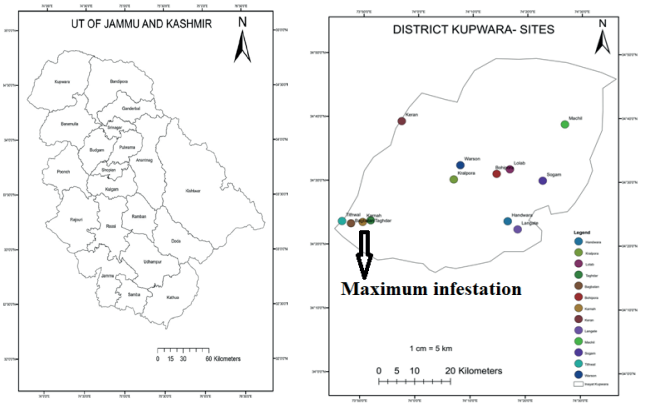

Figure 1. Map of UT of J&K and Sampling sites in district Kupwara

Laboratory rearing (stock culture)

A new progeny was obtained from male and female adults of *A. porrectirostris* collected from the walnut fields by hand picking and placed in (12×12×12) cm glass containers, with whole, unshelled, unprocessed walnuts, and leaves as food and substrate. About sixty adult specimens were collected and observed daily for pre-mating, mating, pre-oviposition,

and oviposition behaviour and duration until new breeding was obtained. The eggs laid were transferred cautiously and larvae were removed carefully with a fine hairbrush and placed in another glass container of the same dimensions along with the same food for their development. Pupae were separated and put into different containers until they reached the adult stage. Each glass container was covered with meshed cages (Olivero-Verbel et al. 2013).

Life cycle estimation of Alcidiodes porrectirostris in the laboratory

Twenty pairs of recently emerged adults were randomly selected to characterize their life cycles. These specimens were placed in glass jar containers covered with a plastic mesh, allowing them to copulate. Ten eggs were collected and individually placed in petri dishes of dimensions (10×1) cm and each one was designated as a replicate. Each egg was observed daily until its hatching to witness the hatching period. Emerge larvae were examined every day; recording growth and survival for each instar (Leon et al. 2005). The modification from one instar to the next was manifested by the existence of the exuvia. After attaining the pupal stage, every specimen was examined daily, and its development was recorded up to the emergence of the adult. The length of each instar and subsequent pupae were measured and the mean was calculated with standard error and standard deviation in MS Excel 2010. Identification of the specimens was done using the taxonomic literature of Kumar et al. (2020).

Statistical analysis

Biological parameters, such as duration, and size of each developmental stage were analyzed using descriptive statistics with the calculation of means and SD.

RESULTS

Holometaboly is the most prevalent life cycle type in beetles, in which individuals hatch from eggs as larvae, develop through several instars, pupate, and eventually emerge as adults (Bouchard et al. 2009). The life cycle of *A. porrectirostris* was characterized in this study, and each stage of development is described below.

Eggs

During the act of copulation, the male weevil initially taps the body of the female with its snout and after getting a green signal, it then mounts on the top of the body of the female weevil. The copulation lasts for more than one hour and takes place during the daytime before noon. The female lays its eggs on the developing fruits. Using its mandibles, it makes a pit on the rind of the fruit measuring about 1 to 2 mm in diameter. These were observed laying 1-2 (1.4±0.52) eggs singly in one pit usually on the opposite end of the fruit (stalk end of the fruit) and near the node of the walnut twig. The eggs are spindle-shaped and snowy white in colour. It measures 0.2-1.0 mm in length and 0.6 mm in breadth (Figure 2). The eggs hatch between 4-7 days (5.4±1.07) in the first generation whereas it ranges between 3-6 days (4.8±1.13) in the second generation.

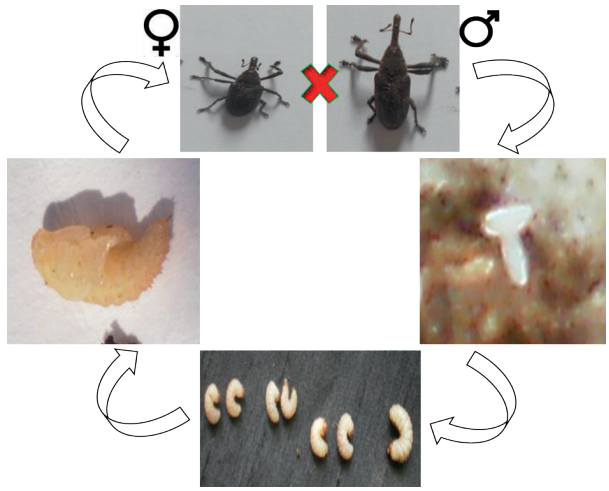

Figure 2. Life cycle of *Alcidiodes porrectirostris* Marshal (Coleoptera: Curculionidae) on common walnut (*Juglans regia*) in Kashmir Valley

Larval instars and duration

The three larval instars were reported in the pest development (Figure 3a-c). The complete larval development takes place inside the immature walnut fruit until its complete development to adulthood.

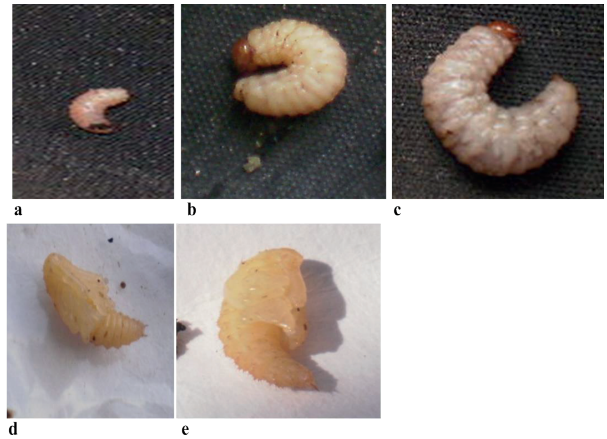

Figure 3. Larval instars of *Alcidiodes porrectirostris* Marshal (Coleoptera: Curculionidae) and pupa (a-c). First, second and third instar, (d-e). Pupa formation

First Instar-The colour of the first instar is creamy white. It measures 2-3 mm in length and 1-1.2 mm in breadth.

Second Instar- It intensifies in growth and measures 4-6 mm in length and 1.2-1.5 mm in breadth. It is bulky and dark creamy white in colour. The segmentation is visible.

Third Instar- It is a mature bulky, creamy white larva that intensifies in growth, and because of its large size, it occupies a major portion of immature walnut fruits. It measures 10-18 mm in length and 2.5-3 mm in breadth. It is the voracious feeder of immature walnut meat.

The total larval duration was observed between 32-44 (37.3±3.89) days in the study area. The first instar stage (1st generation) lasts between 10-14 days (11.9±1.29) in May and the 2nd generation lasts between 10-16 days (12.9±1.91) in August. The second instar stage (1st generation) lasts between 8-12 days (10.44±1.33) in May and (the 2nd generation) lasts between 10-16 days (12.6±1.84) in August. The third instar stage (1st generation) lasts between 12-20 days (14.6±2.71) in June and (the 2nd generation) lasts between 11-19 days (13.6±2.55) in September.

Pupa

The pupa is naked and develops inside the infested fruit in the midst of black decayed nutmeat in 1st generation whereas in 2nd generation it develops inside the rind of the walnut (Figure 3d-e). It ranges in size between 10-11 (10.5±0.53) mm in length and 5-6 (5.4±0.52) mm in breadth and is creamy white in colour. The curved rows of fine bristles are present on all the segments. There were also two spines at the extremity of the abdomen. A thick rostrum lies beneath the head and is directed backward. The antennae, whipcords, and legs are just below and along the sides of the snout. The pupal period of 1st generation ranges between 10-15 (11.8±1.93) days and the pupal period of 2nd generation ranges between 13-18 (14.6±1.51) days. During the pupal phase, the minimum and maximum temperature was recorded between 7.5 °C to 35 °C (21.25 °C), and the minimum and maximum humidity was recorded between 42-96% (69%). The pupation of 1st generation takes place inside the fruit whereas that of 2nd generation takes place inside the rind of the fruit as in case of 2nd generation the delicate first instar larva is not able to bore through the solid shell of the walnut fruit. To tide over the cold temperature of the study area, the second-generation adult hibernates inside the soil and becomes active in April (next) when favourable atmospheric condition sets in. The total life cycle duration of *A. porrectirostris* from egg laying to adult emergence lies in the range of 46-55 (49.1±2.51) and is presented in a tabular form (Table 1).

Adults

The adult weevil excavates a small oval shaped emergence hole with the help of its strong snout and makes its way out into surrounding from its pupal chamber (Figure 4). The adult weevils after emergence usually feed on normal walnut leaf buds, leaf petioles, floral buds, and fruits. The male ranges in size between 10-11 mm in length and 3-4 mm in breadth whereas the female ranges in size between 12-13 mm in length and 3.5-4 mm in breadth. The head and rostrum are smooth but the surface of thorax and elytra are granulated. Its apical end is wider than middle. Eyes are large, oval, and black in colour (Figure 5).

Figure 4. Intra fruit development of *Alcidiodes porrectirostris* Marshal (Coleoptera: Curculionidae) and its emergence through emergence hole of walnut fruit, (d). Leaf damage

Table 1. Time line of *Alcidodes porrectirostris* Marshal (Coleoptera: Curculionidae)

Stage	Timeline	Duration	Average	Temperature (°C)			Rel. Humidity (%)		Average (%)
				Min.	Max	Avg	Min	Max	
Incubation	April-May	04-07	5.5	3	29.5	16.5	30	96	63
1st instar	May	10-14	11.9±1.29	3	35	19	31	96	63.7
2nd instar	May	8-12	10.44 ±1.33						
3rd instar	June	12-20	14.6±2.71						
Total larval period	April-June	32-45	37.8±5.31						
Pupation	June -July	10-15	11.8±1.93	7.5	35	21.25	42	96	69
Total Duration	April-July	46–55	49.1±2.51						

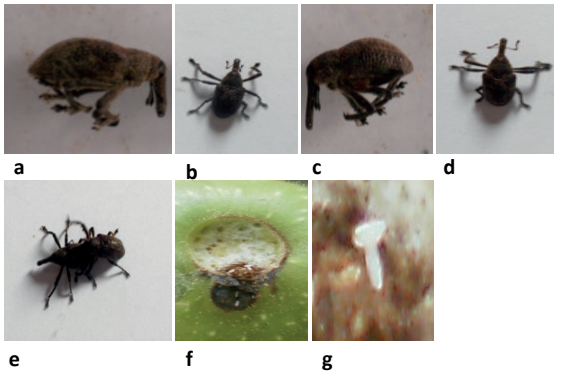

Figure 5. General habitus of *Alcidiodes porrectirostris* Marshal (Coleoptera: Curculionidae) and their eggs (a–b). Lateral and dorsal view of male adult, (c-d). Lateral and dorsal view of female adult, e. Mating, f-g. Site of oviposition with eggs

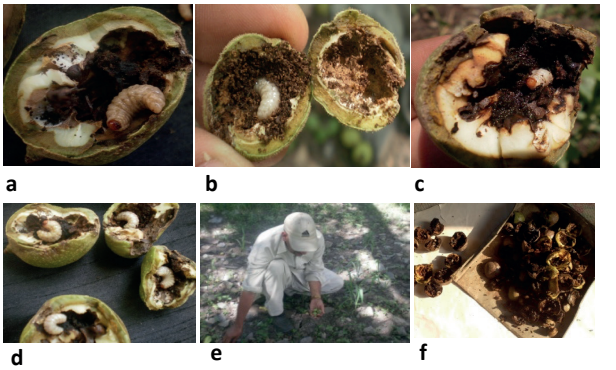

Figure 6. Walnut damage by the larvae of *Alcidiodes porrectirostris* Marshal (Coleoptera: *Curculonidae*) (a-c). Black rotten mass by the action of larva inside the fruit, (d) Presence of a single larva in each nut, (e-f). Collection of walnuts for yield estimation

Life cycle of Alcidiodes porrectirostris

Egg laying, larval development (3 instars), pupation and emergence of the adult in 1st generation take place from April up to July whereas in 2nd generation the egg laying, larval development (3 instars), pupation, and emergence of the adults take place July up to September. To tide over the harsh conditions of the study area the adults of 2nd generation undergo overwintering from October up to April (next) till the suitable climatic factors set in. The 2nd generation of the same pest ranges between 226-265 days (245.5 days) from July up to April (next) including the overwintering period of 180-190 days (90 days) of the adult from October up to April (next).

Mode and extent of damage

A. porrectirostris has been recorded in all the walnut growing regions throughout the world including in J&K. In the Kashmir division, the pest has been found to attack the walnut fruits in the Karnah region of Kupwara district where its percentage of damage lies above 35%. The pest prefers the superior thin shelled to less valuable thick-shelled types of walnut fruit. It is found at a height of 6000-7000 above mean sea levels, causing serious damage to walnut fruit. The attack is more often so serious that no tree produces any normal fruit. The adult weevils were found to feed on leaf buds, petioles of leaves, female floral buds, and male fruit. The larva bores inside walnut fruits, converts the kernels into rotten black powder (Figure 6a-c), and causes the fruits to drop before their maturity. The larva damages the inner side of walnut till its shell hardens. The adults of 2nd generation after their emergence from infested walnuts were observed to cause notch type damage in the rind of the normal fruit.

DISCUSSION

Walnut weevil, *A. porrectirostris* has brought considerable damage to *Juglans regia* across Jammu and Kashmir thereby causing economic loss to the farmers. Hence, it was imperative to study the life cycle of this weevil in walnut growing areas of the Kashmir region to translate conclusively the stage that causes the kernel damage. During the investigations, it became apparent that *A. porrectirostris* has two generations per year (bivoltine), starting from the last week of April or the first week of May when egg production begins. These findings conform with the results of Hussain and Khan (1949) who also reported two generations of the pest in the Northern Himalayas but against the study conducted by Guroo et al. (2021) and Shah et al. (2012) who reported only one generation in Jammu region of J&K and Manoor valley (Kaghan) of Pakistan respectively. The differences in life cycle duration can be due to altitude and temperature variations (Inward et al. 2012). Besides, in our study, the number of larvae found in the infected fruit was against the studies of Mir and Wani (2007) who reported 3-4 larvae in a single fruit while Guroo et al. (2021) reported two larvae in a single fruit and Hussain and Khan (1949) reported eleven larvae in a single infected nut. The total number of larvae reported per nut was found to be independent of nut size and number of punctures, as previously documented in pecan weevil infestations (Harris 1976). The pupation in *A. porrectirostris* takes place within the seed and second generation adult emerges through the emergence hole of the dry nut and hibernates within the soil. The harsh and below freezing temperature may be the reason for second generation adults to hibernate inside the soil. Besides the adult diapauses in coleopteran, occur in 90% of beetle species (Danks 1987) that belong to

families Coccinellidae and Curculionidae. Pupation inside the soil has been reported in the larvae of other weevils such as *Curculio caryae* (Boethel and Eikenbary 1979) and *Conotrachelus juglandis* (Corneil and Wilson 1979). After overwintering the adult weevils lay white creamy eggs and hatch directly into the nuts and feed on liquid endosperm which results in early fruit dropping from the second week of May with larva found pupating either in fallen fruit or inside the soil. Usually, the larvae after emerging from the dropped nuts enter into the ground and pupate there (Stebbing 1902) and in those situations where two generations per year are found, the larvae of 1st generation pupate inside the fruit whereas, that of 2nd generation inside the rind of the fruit (Hussain and Khan 1949). Overall, these findings suggest that either pupa formation can occur in a variety of places, due to the hardness of the soil forming a physical barrier that prevents the larvae from entering it (Schraer et al. 1998) or mature larvae may not be able to leave their shells, forcing them to pupate inside the fallen fruit.

Pupation inside the soil has been reported in the larvae of other weevils such as pecan weevil (Boethel and Eikenbary 1979, Harp and Van Cleave 1976) and Butternut curculio (Corneil and Wilson 1979), etc. The emergence of adults from pupae started in the last week of June. These adults continued feeding on walnut leaves till the end of September before overwintering from early October to the third week of April next year. However, it must be noted that the time of adult emergence from the soil cannot be precisely so as reported above, this is because the weevil emergence from the soil depends upon various factors which can pre or postpone the emergence of the walnut weevils. For example, an increase in soil moisture due to rainfall can lead to the early emergence of weevils (Hinrichs and Thompson 1955, Tedders 1974) while as prevalence of drought conditions and hardness can delay the emergence of weevils from soil (Alverson et al. 1984, Harris and Ring 1980). Hence, it can be abstracted that the weevil emergence from soil can vary depending upon the prevailing weather conditions at that time. Furthermore, it can be established that the proper management of adults through the application of insecticides, when necessary, depends greatly on the regular monitoring of weevil emergence from the soil (Harris 1983). Otherwise, applied chemicals will result in waste.

The walnut weevil is a very serious insect pest of Persian walnut. These damaging insect pests begin attacking the kernels in the developing nuts while the nuts are still on the canopy, decreasing the yield until not harvested. The affected fruits also show dark brown spots, which are dried resinous excretions that render a large portion of the crop unmarketable. The feeding of weevil during the watery stage typically causes the fruit to abort and fall to the ground (Boethel and Eikenbary 1979, Calcote 1975). Females make shallow punctures with their beaks in the shucks of immature nuts and deposit 1-2 eggs in each nut. Similarly, females of *Curculio dieckmanni* (Coleoptera: Curculionidae) lays eggs on developing hazelnuts and the larva feeds on the kernels upon hatching (Zhang et al. 2021). Both phases (adult and larval form) are destructive in nature. Mid-season adult feeding on nut-lets causes premature nut drop and grub damage to kernel occurs after shell hardening. It overwinters as adults in the soil and emerges in spring when the environmental conditions become feasible. The complicated nature of the life cycle, behaviours, and habitat of weevils make some insecticides difficult to spray as the larva and pupa occur inside nuts and soil (Ree et al. 2011) thereby leaving the opportunities to control the weevil limited to adults only.

CONCLUSIONS

Juglans regia is a significant nut fruit crop with more than 90% production in the UT of J&K. It is a precious crop in terms of its nutritional value, wood products, and rural development. However, several insect pests have been attacking this crop. Our visual observations demonstrate that *Alcidodes porrectirostris* has caused more harm in the Kupwara district than rest of the studied locations. Hence, the required management techniques must be employed at proper moment to halt further damage to other walnut growing areas of the Kashmir Valley. The crucial components in effectively managing walnut weevils involve monitoring walnut phenology, initial walnut weevil emergence from the soil, and activity of adults in the canopy. Monitoring data should be integrated with details on walnut cultivars, price estimates, and treatment costs to aid in making management decisions. Nuts become vulnerable to oviposition with the onset of spring and various traps can detect the emergence of walnut weevil and thereby prevent economic loss. To reduce losses, an integrated pest management (IPM) strategy uses resistant varieties, scouting and economic thresholds, pheromone traps and biological insecticides, and synthetic pesticides to minimize losses. The reliance on chemicals for insect pest management, combined with their careless and imprudent application, has had several negative repercussions on these crops, including environmental contamination, ecological imbalance, pesticide resistance, pest revival, secondary pest outbreaks, etc. The several biological control agents that could be used as a crucial tool in IPM include predators, parasites, pathogenic microorganisms, and competitors (Khan et al. 2009). Future research is indispensable on the biology, ecology, and management of the weevils, especially

A. porrectirostris which is expected to pose economic concerns to J&K, India, and other nations.

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Author's Contributions

Maximum contribution by Sajad Ahmad by 65% and 35% equally by Inayat Ullah Lone, Deen Mohd Bhat, and Mohd Feroz.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Ceviz, son yıllarda "Süper gıda" olarak tanımlanan en önemli meyvelerden biridir. Çok çeşitli böcek zararlıları tarafından enfekte edilir. Bu zararlılardan birisi de ceviz kurdudur. Erginleri çiçeklerle ve tomurcuklarla beslenirken, larvaları meyvelerin içinden beslenir ve erken döküme neden olarak son derece zarar verir. Ceviz kurdunun yılda iki döl verdiği ve meyve başına sadece tek bir larva bulunduğu belirlenmiştir. Erginler, nisan ayında topraktan çıkar ve ceviz yaprağının tomurcukları, yaprak sapları ve çiçek tomurcukları ile beslenir. Ergin dişiler özellikle mayıs ve haziran ayı başında meyvelere 1-2 yumurta bırakır, birinci dölde 4.9±0.74 (SD) günde, ikinci dölde ise 4.5±0.97 günde yumurtadan çıkarlar. Üç dönem boyunca gelişirler ve toplam gelişim süresi 46-55 gün (49.1±2.51) aralığındadır. İkinci dölün erginleri, sert çevre koşullarından kaçınmak için kışlamaya girerek kışı geçirirler. Biyolojisinin, hayat çemberi ve hasar durumunun anlaşılması, belirli böcek zararlılarının uygun zamanda takip edilmesine yardımcı olacaktır.

Anahtar kelimeler: fındık, ceviz kurdu, yaşam döngüsü, yetiştirme, zararın boyutu

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Original article

Evaluation of some cotton genotypes for resistance to *Verticillium dahliae Kleb.* under field conditions

Bazı pamuk hatlarının tarla koşullarında *Verticillium dahliae Kleb.* solgunluğuna duyarlılıklarının belirlenmesi

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ABSTRACT

Verticillium wilt caused by the soil-borne pathogen is one of the most significant diseases affecting the yield of cotton and is almost incurable with chemical agents. For this reason, it will be inevitable to cultivate resistant varieties. In this direction, this study was carried out to determine the response of cotton varieties and inbred lines obtained from cotton breeding projects of the GAP International Agricultural Research and Training Center (GAPUTAEM) in Diyarbakır, Türkiye to Verticillium wilt disease. The trial was conducted in a randomized complete block design with four replications during the 2016 and 2017 cotton growing seasons. In the study, 12 advanced inbred lines (8, 20, 30, 34, 38, 57, 58, 63, 64, 8/4, 5/7, and 8/1) and 3 control varieties [Stoneville 468, Carmen (tolerant control), and Çukurova-1518 (sensitive-control)] were tested for the response to Verticillium wilt under naturally infected field conditions. Disease severity was determined in the leaf at 5-10% and 50-60% of the boll opening stages and in the stem section after harvest. Additionally, some yield parameters and fiber quality properties were investigated in the study. The results indicated that there were significant differences among genotypes for most of the investigated characteristics.It was determined that with regard to foliar disease index (FDI) as mentioned boll opening stages and disease index of stem cross-section (SDI) the most tolerant genotype was inbred line 38, while the most sensitive variety was Çukurova-1518. As a result of their low disease index value and high yield capacity, the inbred lines 30, 57, 38, and 20 were recommended.

INTRODUCTION

The cotton grown in hot climate conditions is a one-year plant whose effective root depth is generally accepted as 90 cm with not much soil selectivity but needs more water (Aydogdu et al. 2018). It is a significant agricultural crop that meets the crude material demands of many industry branches. The rise in the standard of living in developing

societies leads to an increase in fashion brand awareness, and therefore the cotton and textile sectors are gaining importance day by day. Cotton is mostly produced and processed in developing countries, whereas the highest per capita consumption of cotton occurs in developed countries due to the significant resource of cellulose in natural fibers. Türkiye has desirable ecological farming conditions and cotton production practices that have lasted for centuries. With its direct and indirect employment effects, cotton is considered a source of income for a lot of people in Türkiye, and is grown intensively in the Southeastern Anatolia Region, the Aegean Region, Adana, and Antalya regions, especially with the determinant climatic factors. Among the biotic stresses, plant disease is a vital limiting factor that disrupts plant production. Over 40 diseases induced by nematodes bacteria, fungi, and viruses have been identified in cotton. In particular, fungi are responsible for approximately two-thirds of infectious plant diseases (Carris et al. 2012). *Verticillium dahliae* is the leading cause of Verticillium wilt and its resting body microsclerotia can survive for up to 14 years in the absence of a host or under adverse conditions (Short et al. 2015). Verticillium wilt is most important in temperate regions, occurs less frequently in the subtropics, and is rare in the tropical areas of the world (Inderbitzin and Subbarao 2014). In recent years, the disease has become increasingly serious due to climatic variation, long-term monoculture, and frequent introduction of new cotton varieties in different countries and regions in the world (Ranga et al. 2020). The control of disease is difficult due to its being a soil-borne pathogen and impractical and expensive soil sterilization practices (Bicici and Kurt 1998). In 1914, Verticillium wilt was first reported in Virginia and afterward spread to many cotton-growing areas worldwide. Enormous losses occur every year in many cotton-producing areas of the world, which restrict certain factors for cotton production. Contaminated plants usually exhibit symptoms of marginal necrosis or chlorosis in their leaves, discoloration of the stem vascular bundles, decreased photosynthesis, and increased respiration, which result in a significant reduction of the plant's biomass and a heavy loss of yield (Hampton et al. 1990). The disease can be controlled with the use of tolerant plants and traditional practices. Nevertheless, no genetic resources of resistance prevent contamination of the vascular system, and neither of the recent upland cotton cultivars is resistant to *V. dahliae*. Hence, determining the susceptibility of cotton cultivars and cultivar candidates bred in Türkiye and brought from abroad is essential. In Türkiye, Verticillium wilt was first detected by Iyriboz in Manisa Kırkağaç in 1941, but the *Verticillium dahliae* Kleb. was reported by Karaca et al. (1971). Then, the disease was reported to be spread in the Aegean and Mediterranean regions by Esentepe (1979). Verticillium wilt also causes a significant reduction in yield in the Southeastern Anatolia Region. The frequency of occurrence of Verticillium wilt disease was 16.27%, and the prevalence rate was 79.28% in some districts of the region (Siirt, Mardin, Batman, Diyarbakır, Adıyaman and Şanlıurfa). By conducting numerous trials in those regions, it has been observed that this disease's widespread rate was 86% (Sağır et al. 1991). Plants infected at early stages are severely stunted. At first glance, Verticillium wilt migrates from the root to the tissue, nestles in the xylem, and causes occlusion of the stem veins. Chlorosis, necrosis and vascular discoloration on leaves and stems are considered the first signs of disease, and then wilting appears. Pathogens prevent the transport of water and other mineral substances from the roots to the leaves and tissues. From the bottom leaves, this disease stimulates wilting, drying, a reduced photosynthesis rate, and reduced yield and quality of fiber parameters. Significantly, infected plants shed all their leaves and most of their young bolls. The need for improving significant strategies against cotton wilt has emerged. Thus, the use of resistant varieties derived from genetic resources has been considered the most practical and effective way of managing the disease (Baran et al. 2022).

Marani and Yaacobi (1976) observed that appropriate scanning for wilt resistance in Israel appears practical by examining foliar symptoms throughout the second part of the bloom period when the area is uniformly infected by the fungus under convenient temperatures. Bolek et al. (2005) determined that by using four *Verticillium dahliae* isolates (V44, V76, TS-2 and PH) in scanning four cotton cultivars (Acala 44, Pima S-7, M-315 and Acala Prema), Pima S-7 and Acala Prema executed the tolerance reactions, while Acala 44 was considered the most sensitive cultivar. This study revealed that the number of uninfected leaves and total shoot weight were considered the best signs of resistance. Erdogan et al. (2006), evaluated cultivars' tolerance to the disease and concluded that Carmen's yield and fiber properties made it a good choice in contaminated fields. As a consequence of the increased population, natural fibers and cotton are becoming more important and demandable. This experiment was conducted to determine the most tolerant inbred lines that were developed by GAPUTAEM's breeding programs as well as contribute to future cotton breeding programs.

MATERIALS AND METHODS

The trial was conducted in GAPUTAEM's experimentel field during the 2016-2017 years in Diyarbakır, Türkiye. In the study, 12 inbred lines (8, 20, 30, 34, 38, 57, 58, 63, 64, 8/4, 5/7 and 8/1) and three control varieties were planted in the

infected field with Verticillium wilt. Stoneville-468 (tolerant), Carmen (tolerant) and Çukurova-1518 (susceptible) were tested as control varieties in the trial. The study was designed as a randomized complete block design with four replications. Each plot comprises four rows of 12 m in length, 70 cm interrow row spacing, and 15-20 cm above row spacing.

Soil characteristics of the experimental area

The experimental site was flat and devoid of organic substances, and had no salinity issues. Depending on the abundance of clay minerals, the soil profile was expanded and swollen during winter, and deep cracks were formed 80-90 cm from the top level of soil in summer (Avşar and Karademir 2022). Soil specimens received from the 0-30 cm soil stratum of the experimental area were analyzed in the GAPUTAEM soil analysis laboratory (Table 1).

Table 1. Soil properties of the research area

Texture	Clay-Loam (C-L)
EC (dS m^{-1})	1.27
pH	8.10
$CaC0_3$ (%)	11.46
P_2O_5 (kg ha^{-1})	3.21
K_2O (kg ha^{-1})	243
Organic Matter (%)	0.98
Bulk Density (g/cm^3)	1.19

Meteorological data for the experimental area

The average temperature, average maximum temperature, precipitation amount, and average relative humidity taken from the meteorological service in Diyarbakır are presented in Table 2. The maximum temperature values of June, July and August 2016 and 2017 were higher than long-term mean values, while the precipitation amounts of April 2017 and May, June and September 2016 were long-term mean values. The relative humidity of July, August, September and October in both years of the trials was below the mean values for long years. In Diyarbakır province, long-term climatic data indicated that there was 210 mm of total precipitation and a 21.56 °C mean temperature. The highest average maximum temperature was 40.7 °C in July 2017, and the highest average rainfall was 68.5 mm in April 2017. The disease severity index was measured using data on foliar and vascular symptoms. Leaf and stem sections of 50 consecutive plants were examined for disease in each plot.

Determination of foliar disease severity index in leaves

Wilt disease in the leaves, was evaluated when plants reached approximately 5-10% and 50-60% boll opening time, and to calculate the foliar disease severity index (FDI), a 0-4 scale discovered by Bejarano-Alcazar et al. (1995) is used (Table 3). FDI was measured with the index formula given below (Karman 1971).

$$FDI= \frac{(0)(a)+(1)(b)+(2)(c)+(3)(d)+(4)(e)}{N} \quad (1)$$

n= (a+b+c+d+e)

a,b,c,d,e: Number of plants included in each scale value

n: Total of the plants

0,1,2,3,4: Scale data

In the "0, 1, 2, 3, and 4" scale data of the leaf disease severity index, "a, b, c, d, and e" symbolized the number of plants included in each scale value, and "N" indicates the total number of plants. As the data goes towards 0, the leaf becomes more tolerant to the disease. However, when the trend goes

Table 2. Monthly climate data during the growth period of cotton in 2016-2017 and long-term averages in Diyarbakır*

Months	Avg. temp. (°C)			Avg. max.temp. (˚C)			Precipitation (mm)			Avg. relative humidity (%)		
	2016	2017	Long term avg.	2016	2017	Long term avg.	2016	2017	Long term avg.	2016	2017	Long term avg.
April	15.7	12.8	13.8	28.8	19.5	20.2	29	98.8	68.7	56.2	68.5	63
May	19.9	18.8	19.2	27.5	26.3	26.5	41.4	30.6	42.8	51.9	57.6	56
June	26.8	26.9	26.3	34.7	35	33.5	18.4	2.6	8	32	30	31
July	31.6	32.3	31.1	39.2	40.7	38.3	0	0	0.7	23	19.4	27
August	31.9	31.1	30.4	40.5	39.9	38.2	0	0	0.4	22.7	22.8	28
September	24.2	26.8	24.9	31.9	36.4	33.2	5.2	0	3.9	29.9	22.3	32
October	18.8	17.2	17.3	26.7	24.8	25.3	13.6	22	31.7	36.9	39.2	48
November	8.2	10	9.5	16.4	16.3	16.2	52	21.2	53.8	54	67.5	55

*Source: Turkish State Meteorological Service, Diyarbakırasu

toward 4, it means the sensitivity to disease is increasing (Karman 1971).

Table 3. Scale values of wilt disease in leaves

Disease Scale Value (0-4)	Disease Symptoms
0	No symptoms on the plant (plants are healthy)
1	On the leaves of the plant, there are symptoms at the beginning stage, very little yellowing and unclear symptoms (1-33%)
2	Yellowing of the leaves, interveinal necrosis and leaf fall (34-66%)
3	Local necrosis between the leaf veins of the plant, defoliation and shriveling of all parts of the plant (i.e. going towards death) (67-97%)
4	Dying and death plant (98-100%)

Determination of severity disease index in cross stem section

Cotton plants were cut at a height of 5 cm from the soil level at an angle of 45 degrees from the root collar. By examining the discoloration of the wood tissue of the cut plants, a 0-3 scale that was discovered by Buchenauer and Erwin (1976) for stem cross-sectioning was used (Table 4).

Disease index of stem cross-section (SDI) was measured with index formula given below;

$$SDI = \frac{(0)(a)+(1)(b)+(2)(c)+(3)(d)}{N} \quad (2)$$

n= (a+b+c+d)

a,b,c,d: Sum of plants included in each scale value

n: Sum of the plants

0,1,2,3: Scale data

The measured grades "0, 1, 2, 3" represent the scale data in accordance with the stem section disease severity index; "a, b, c, d" represent the number of plants comprised in each scale value; and "N" stands for the total number of plants processed. As the stem section disease severity index data goes toward 0, the stem section indicates that plants are getting more resistant to the disease. Nevertheless, if the data is directed towards 3, that means plants are getting more sensitive to the disease (Karman 1971). Statistical analyses were conducted using JMP 5.0.1 statistical software with the LSD (0.05) test.

Table 4. Scale values of wilt disease on stem section

Disease Scale Value (0-3)	Disease Symptoms
0	No browning (discoloration) in wood (xylem) tissue
1	The browning and black spots (discoloration) 1-33% in the wood (xylem) tissue of the plant
2	The 34-67% of browning and black spots (discoloration) in the wood (xylem) tissue of the plant
3	Browning and darkening 68-100% (discoloration) in the plant wood (xylem) tissue

RESULTS AND DISCUSSION

Based on variance analysis outcomes of cotton genotypes, there were statistically considerable differences at the $P<0.01$ level between genotypes with regard to FDI at the boll opening stage of 5-10%, 50-60%, and SDI (Table 5). Two different defense systems, called resistance and tolerance, ensure a host's survival against infectious diseases. Resistance is based on the ability of the host to kill pathogens while tolerance is defined as a plant's ability to sustain yield in the presence of disease (Newton 2016). The mean values of observed traits for years, genotypes and year*genotypes interactions were presented in Table 6. Values of disease severity based on FDI at the boll opening stage of 5-10% were observed to be prominent between cotton genotypes ($P\leq0.01$) (Table 6). Additionally, FDI at the boll opening stage of 5-10% grouped into "e" (0.61-0.65) were the most tolerant genotypes to wilt disease (*Verticillium dahlia* Kleb.). In this study regarding FDI at the boll opening stage of 5-10%, inbred lines 20, 38, 57 and 58 had lower values in comparison with Carmen, although Carmen is presented as a tolerant variety to Verticillium wilt globally (Stathakos et al. 2006). Compared to other genotypes, Çukurova-1518 was the most susceptible variety with a 0.98 value. These results were in parallel with Erdoğan et al. (2015), who reported that the highest disease intensity value was observed in Çukurova 1518 (2.53) in accordance with the severity of the disease. As seen in Table 6, data analysis indicated that the differences among years in FDI at the boll opening stage of 5-10% were statistically significant. As years compared, lower FDI at the boll opening stage of 5-10% was obtained in 2016 with a 0.68 value, while a higher value was observed in 2017 with a value of 0.76. A similar study carried out in Diyarbakır stated that Carmen, Golda, and Teks were tolerant while Stonville 453, Sayar 314 and Maraş 92 cultivars

were susceptible varieties based on the disease severity in leaf (Karademir et al. 2012). Genotypes and year*genotypes interactions for FDI at 50-60% boll opening stage were significant, while the differences between years were insignificant. Genotypes for FDI at the boll opening stage of 50-60%, ranged from 0.71 for an inbred line called 58 to the value of 1.15 for the Çukurova-1518 variety. According to Baran (2022), the leaf-disease severity varied between 0.12-3.09 at 50-60% at the boll opening period, whilst stem cross-section values ranged between 0.36-2.30 and a positive correlation was found between the indices. Our results contribute to the outcomes of Erdoğan (2009) and Korkmaz (2005) who recorded that cotton genotypes had different susceptibilities to Verticillium wilt disease, even though Stoneville-468 is considered one of the most tolerant cotton varieties in worldwide. According to this study, taking into account FDI at the boll opening stage of 5-10%, inbred lines 8, 20, 38, 58 and Carmen variety were depicted with lower values compared to Stoneville-468 which is known to be to Verticillium pathogens (Sağır et al. 2021).

Table 5. Variance analysis of mean squares

Variance Sources	DF	FDI at boll opening stage of 5–10%	FDI at boll opening stage of 50–60%	SDI
0	1	52.2796**	0.9588	3514.715**
1	14	9.6375**	4.1137**	21.0122**
2	14	2.354**	1.8893*	9.0306**
3	84	0.007958	0.023901	0.016347
	119	2.2211592	4.207997	13.86106

** P<0.01, * P<0.05, DF: Degrees of Freedom, FDI: Foliar Disease Index, SDI: Severity Disease Index of stem cross-section

Table 6. Mean values of FDI at boll opening stage of 5–10%, FDI at boll opening stage of 50–60%, and SDI

Genotypes	FDI at boll opening stage of 5-10%			FDI at boll opening stage of 50-60%			SDI		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
8	0.78 cd	0.72 c-e	0.75 b-d	0.75 ef	0.82 d-f	0.79 cd	1.4 f-h	1.72 cd	1.56 de
20	0.53 g	0.7 c-f	0.61 e	0.7 fg	0.82 d-f	0.76 cd	1..25 h-k	1.75 b-d	1.5 ef
30	0.63 e-g	0.72 c-e	0.67 c-e	0.98 b-d	0.82 d-f	0.90 bc	1.6 d-e	1.72 c-d	1.66 cd
Stoneville-468	0.73 c-e	0.75 c-e	0.74 b-d	0.73 e-g	0.9 c-f	0.81 cd	1.1 k-m	1.87 a-c	1.49 ef
34	0.73 c-e	0.8 c	0.76 b	0.9 c-f	0.89 c-f	0.89 bc	0.95 m	1.52 e-f	1.24 ı
38	0.58 fg	0.72 c-e	0.65 e	0.75 ef	0.77 d-f	0.76 cd	0.95 m	1.75 b-d	1.35 g-ı
57	0.5 g	0.77 cd	0.64 e	0.93 c-e	0.87 d-f	0.9 bc	1.65 de	1.9 a-c	1.78 bc
58	0.58 fg	0.67 d-f	0.62 e	0.53 g	0.9 c-f	0.71 d	1.35 f-ı	1.41 f-h	1.38 f-h
CARMEN	0.63e-g	0.71 c-e	0.67 de	0.75 ef	0.81 d-f	0.78 cd	1 lm	1.61 de	1.31 hı
63	0.75 c-e	0.77 cd	0.76 bc	0.85 d-f	0.87 d-f	0.86 cd	1.2 l-k	1.85 a-c	1.53 e
64	0.8 c	0.82 c	0.81 b	0.85 d-f	0.9 c-f	0.88 bc	0.95 m	1.75 b-d	1.35 g-ı
8/4	0.53 g	0.77 cd	0.65 e	0.75 ef	0.92 c-e	0.84 cd	1.75 b-d	1.92 ab	1.84 ab
Çukurova-1518	0.95 ab	1.2 a	0.98 a	1.2 a	1.1 a-c	1.15 a	1.9 a-c	2 a	1.95 a
5/7	0.8 c	0.75 c-e	0.78 b	0.88 d-f	0.82 d-f	0.85 cd	1.15 j-l	1.72 cd	1.44 e-g
8/1	0.83 bc	0.82 c	0.82 b	1.15 ab	0.9 c-f	1.03 ab	1.3 g-j	1.47 e-g	1.39 f-h
Mean	0.68 B	0.76 A		0.84	0.87		1.30 B	1.73 A	
CV(%)		12.27			17.64			7.94	
$LSD_{(0.05)}$									
Year		0.026**			N.S.			0.017 **	
Genotypes		0.08**			0.15 **			0.12 **	
Year*Genotypes		0.12**			0.21 **			0.17 **	

*and** significant at the 0.05 and 0.01 probability level respectively, N.S: Non Significant, CV: Coefficient of Variation

Table 7. Mean values of seed cotton yield, fiber yield and ginning percentage (%)

Genotypes	Seed cotton yield (kg ha^{-1})			Fiber Yield (kg ha^{-1})			Ginning Percentage (%)		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
8	4384 bc	1590 mn	2987 d-f	1869a-d	674 no	1271 e-f	42.6a-f	42.4 c-g	42.5 b-d
20	4310 bc	2229 kl	3270 b-e	1875 a-d	932 lm	1403 b-e	43.5 ab	41.8 f-ı	42.6 a-c
30	4880 a	2295 kl	3588 ab	2050 a	996 l	1523 ab	42 e-ı	43.4 a-c	42.7 ab
Stoneville-468	3823 de	2479 jk	3151 de	1656 e-g	1019 l	1341 d-f	43.5 ab	41.1 h-j	42.3 b-e
34	4114 cd	2461 jk	3288 b-d	1712 d-f	1073 kl	1392 b-e	41.6 f-ı	43.6 a	42.6 a-c
38	4690 ab	2339 k	3515 a-c	1952 a-c	1006 l	1478 b-c	41.6 f-ı	43 a-e	42.3 b-e
57	4414 a-c	2636 l-k	3525 a-c	1787 b-e	1081 kl	1434 b-d	40.5 j	41 ıj	40.7 f
58	3580 ef	2375 k	2978 d-f	1522 f-h	1024 l	1273 ef	42.5 b-g	43.1 a-d	42.8 ab
CARMEN	3484 e-g	1861 lm	2673 gf	1493 g-ı	763 mn	1129 gh	42.9 a-e	41 ıj	41.9 c-e
63	3750 d-f	1427 mn	2589 g	1541 f-h	605 no	1073 h	41.1 h-j	42.4 c-g	41.7 e
64	3600 ef	2288 kl	2944 ef	1496 g-h	963 l	1230 f-g	41.6 g-ı	42.1 d-h	41.8 de
8/4	4120 cd	2345 k	3233 c-e	1772 c-e	973 l	1372 c-e	43 a-e	41.5 g-j	42.2 b-e
Çukurova-1518	2874 h-j	1285 n	2080 h	1251 j-k	554 o	902 ı	43.5 ab	43.1 a-d	43.3 a
5/7	4640 ab	3018 g-ı	3829 a	1972 ab	1283 j	1627 a	42.5 b-g	42.5 b-g	42.5 b-d
8/1	3320 f-h	3102 g-ı	3211 c-e	1378 hj	1315 ıj	1347 d-f	41.5 g-j	42.4 c-g	41.9 c-e
Mean	3998 A	2248 B		1869 A	951 B		42.6	42.2	
CV(%)		10.71			10.2			1.7	
LSD (0.05)									
Year		12.41 **			42.79 **			N.S.	
Genotypes		33.12 **			134.02 **			0.72 **	
Year*Genotypes		46.86 **			189.54 **			1.01 **	

As indicated in Table 6, the differences among years, genotypes, and year*genotypes interactions were significant with regard to SDI value. When years were compared in terms of SDI, the value of 2017 (1.73) was higher than the value of 2016 (1.30). Çukurova 1518 variety (1.95) and 8/4 (1.84) inbred lines were detected as the most susceptible genotypes, while inbred line 34 with 1.24 SDI value was reported as the most tolerant genotype. These results were parallel with the study conducted by Erdoğan et al. (2015), who indicated the sensitivity of 13 cotton varieties improved by breeding against Verticillium wilt. It was reported that the minimum disease severity was noticed in Carmen, and the maximum disease severity value was defined in Çukurova 1518. Similarly, it was informed that the differences among cotton varieties were statistically significant with regard to the SDI values (Göre et al. 2017, Yaşar 2022). Even though Carmen is considered one of the most tolerant cotton varieties worldwide (Wheeler and Woodward 2016), in this study regarding SDI, inbred line 34 was shown lower value compared to the Carmen variety.

As seen from Table 7, there were statistically important differences between genotypes for seed cotton yield. The mean seed cotton yield values ranged from 2080 kg ha-1 (Çukurova - 1518) to 3829 kg ha-1 (5/7 inbred lines). The maximum value was obtained from the number inbred line 30, with a 4880 kg ha-1 and 4690 kg ha-1 value from number 38 in 2006, respectively. The data stated that some vulnerable genotypes had high yield values. The reason for this situation might be linked to the late onset of the disease. Higher seed cotton yield (3998 kg ha-1) was attained in the trial's first year (2016). It was estimated that the differences detected between the years of the experiment may be due to climatic or cultural alterations. As seen in Table 7, data analysis indicated that differences between years, genotypes and year*genotype interactions for fiber cotton yield were significant. Since the Çukurova-1518 variety is considered susceptible to Verticillium disease, high values were attained from FDI (5-10% and 50-60% boll opening stage) and SDI values, and also low yield values were seen in this variety. Lower cotton fiber yield was obtained in the trial's second year (2017). In 2017, higher precipitation amounts compared to average precipitation in long years, resulting in a delay in the sowing date, led to a decreased yield. Significant differences were received in terms of the ginning percentage of genotypes and year*genotypes and variety interactions at $p<0.01$ probability level, while differences between years

Table 8. Mean values of 100 seed weight and first flowering date

Genotypes	100 Seed Weight (g)			First flowering date (day)		
	2016	2017	Mean	2016	2017	Mean
8	11.4 c-e	12 b	11.7 b c	69	70	69
20	10.2 h-ı	11 d-f	10.6 e f	69	69	69
30	11.6 b c	11.5 b-d	11.6 b c	69	69	69
Stoneville-468	10.2 h-ı	10.5 f-g	10.4 f	68	69	68
34	11.6 bc	12 b	11.8 b	68	68	68
38	11.3 c-e	11.5 b-d	11.4 c	69	69	69
57	13.3 a	12 b	12.7 a	69	69	69
58	10.9 e f	11 d-f	11 d	69	69	69
CARMEN	10.2 h-ı	11 d-f	10.6 e f	68	69	69
63	10.9 e-f	12 b	11.5 b c	69	70	69
64	11.4 c-e	11.5 b-d	11.5 b c	69	70	69
8/4	10.6 f g	11 d-f	10.8 d e	69	69	69
Çukurova-1518	10.8 f	10 h-ı	10.4 f	69	70	69
5/7	10.5 f g	11 d-f	10.8 d e	68	69	69
8/1	9.9 ı	11 d-f	10.5 e f	68	69	68
Mean	11,02	11,27		68,5	69,1	
CV(%)		3,23			1,64	
LSD (0.05)						
Year		N.S.				
Genotypes		0.35 **				
Year*Genotypes		0.49 **				

Table 9. Mean values of fiber fineness, fiber length, fiber strength, fiber uniformity index, short fiber index and spinning consistency index

Genotypes	Fineness (mic)	Fiber Length (mm)	Fiber Strength (g/tex)	Fiber Uniformity Index (%)	Short Fiber Index (%)	Spinning Consistency Index (SCI)
8	3.42 c-e	30.2	32.6	84.32	7.26	150.5 b-d
20	3.47 cd	29.2	28.3	84.16	7.87	135.5 e
30	3.22 ef	29.2	32.4	84.7	7.38	159.5 ab
Stoneville-468	3.48 cd	29	28.9	84.42	7.72	149.5 b-d
34	3.75 ab	29.4	31.6	85.15	6.41	149.5 b-d
38	3.53 bc	29.5	32.6	85.76	6.43	159.5 ab
57	3.86 a	29.9	32.3	84.51	7.48	142 de
58	3.15 f	29.3	31.5	83.98	7.05	164.5 a
CARMEN	3.26 d-f	29.4	30.6	83.63	6.78	153.5 a-c
63	3.45 c-e	30	31.6	84.45	7.01	157 ab
64	3.22 ef	29.7	29.9	84.6	7.58	154 a-c
8/4	3.45 c-e	29.7	31.7	84.02	7.62	150.5 b-d
Çukurova-1518	3.28 d-f	29	29.8	83.66	6.78	151.5 b-d
5/7	3.61 bc	29.9	32.3	84.51	7.48	144.5 c-e
8/1	3.56 bc	29.6	30.5	83.86	7.45	137.5 e
Mean	3.45	29.5	30.98	84.51	7.19	150.6
CV(%)	4.63	2.1	7.3	1.21	10.15	5.19
LSD (0.05)	0.23**	N.S.	N.S.	N.S.	N.S.	11.13**

Table 10. Correlations coefficient among the investigated characteristics

FDI at boll opening stage of 50–60%	FDI at boll opening stage of 5–10%	0.5416	<.0001	
SDI	FDI at boll opening stage of 5–10%	0.2966	0.0010	
SDI	FDI at boll opening stage of 50–60%	0.2779	0.0021	
First flowering date	FDI at boll opening stage of 5–10%	0.0670	0.4674	
First flowering date	FDI at boll opening stage of 50–60%	0.0808	0.3803	
First flowering date	SDI	0.2694	0.0029	
Seed cotton yield	FDI at boll opening stage of 5–10%	-0.4012	<.0001	
Seed cotton yield	FDI at boll opening stage of 50–60%	-0.1574	0.0860	
Seed cotton yield	SDI	-0.5973	<.0001	
Seed cotton yield	First flowering date	-0.2549	0.0050	
Ginning Percentage	FDI at boll opening stage of 5–10%	0.0986	0.2839	
Ginning Percentage	FDI atboll opening stage of 50–60%	-0.0719	0.4351	
Ginning Percentage	SDI	-0.0368	0.6900	
Ginning Percentage	First flowering date	0.0066	0.9429	
Ginning Percentage	Seed cotton yield	-0.1160	0.2071	
100 seed weight	FDI at boll opening stage of 5–10%	-0.2011	0.0276	
100 seed weight	FDI at boll opening stage of 50–60%	-0.0520	0.5728	
100 seed weight	SDI	0.1249	0.1740	
100 seed weight	First flowering date	0.1550	0.0910	
100 seed weight	Seed cotton yield	-0.0108	0.9068	
100 seed weight	Ginning Percentage	-0.3022	0.0008	

were found to be insignificant. Çukurova-1518 variety (43.3%) and 20 (42.6%), 30 (42.7%), 34 (42.6%) and 58 (42.8%) inbred lines had higher ginning percentages and were classified in the same group.

The mean values regarding 100 seed weight (g) and first flowering date (day) were given in Table 8. As indicated in the table, there were significant differences between genotypes in accordance with 100 cotton seed weights. Among the genotypes, the maximum 100 seed weight value was obtained from inbred line 57, while the lowest was obtained from the Stoneville-468 and Çukurova-1518 varieties. The differences between genotypes relating to the first flowering date were stated as insignificant.

Verticillium wilt may reduce the deposition and reorganization of cellulose molecules in cotton fiber. This could affect fiber yield and fiber properties, including micronaire, fiber maturity, short fiber content, and immature fiber content, as these are all related to cellulose deposition and reorganization in cotton fiber development (Ayele et al. 2020).

Fiber fineness (micronaire), fiber length (mm), fiber strength (g tex-1), fiber uniformity index, short fiber index, and spinning consistency index values were reported in Table 9. The genotypes were significantly different at P<0.01 level in terms of fiber fineness (micronaire), which related to maturity and spinning consistency index values. Genotypes with lower values for FDI at 5-10% boll opening stage had

coarse fibers, as indicated in Table 6 and Table 8. This may be due to genotype-environment interactions as well as sowing dates and different cultural implementations. Regarding fiber fineness observed from Table 9, the values ranged from 3.15 mic. (inbred line 58) to 3.86 mic. (inbred line 57). Green and Culp (1990) reported that environmental variability, especially the differences in weather conditions, could influence the fiber quality parameters of cotton genotypes.

The differences among genotypes with respect to fiber length, fiber strength, fiber uniformity index, and short fiber index were indicated as insignificant (Table 9). Taking the spinning consistency index (SCI) into account, inbred line 58 had the highest value (164.5) and inbred line 20 had the lowest value (134.5).

Based on Table 10, FDI at the boll opening stage of 50-60% was positively and significantly correlated with FDI at the boll opening stage of 5-10%; positively correlated with SDI and first flowering date, and negatively correlated with seed cotton yield, ginning percentage and 100-seed weight. According to the research conducted by Baran and Temiz (2021), a positive correlation (r = 0.5616) was reported between the severity index for leaf disease at the boll opening period of 50–60% and the stem section. Likewise, Khaskheli (2013) informed us that positive and high correlation values (r = 0.966) between stem section and leaf disease severity index were found in all genotypes. In this research, SDI had a positive correlation with FDI at the boll opening stage of 5-10%, while the first flowering date had an important and negative correlation between SDI and seed cotton yield. The one hundred-seed weight of cotton was negatively correlated with seed cotton yield, ginning percentage, FDI at the boll opening stage of 5-10%, and FDI at the boll opening stage of 50-60%. On the other hand, a positive correlation was noticed between SDI and first flowering time (Table 10). In parallel to the study, similar research was performed to define the responses of several cotton varieties of different origins to wilt disease. According to outcomes from the trial carried out by Akışcan and Tok (2019), a highly positive and significant correlation (r= 0.972) between the disease severity indices data determined from the leaf and stem sections of the different cotton genotypes has been observed.

CONCLUSION

Verticillium dahliae Kleb. is an important fungal pathogen in cotton as in many plants. The disease limits the cotton yield at a great rate in Türkiye and worldwide, which does not have any economical chemical control way. In cotton breeding programs, developing resistant varieties is crucial to combat Verticillium wilt. The results showed that most of the properties examined in the study differed significantly among cotton genotypes (P<0.01). In light of these observations, 5/7, 30, 57, 38, and 20 inbred lines demonstrated tolerance in terms of three different periods of disease, and due to their high cotton yield performance, they could be recommended for infected areas. These genotypes can be used in breeding studies after testing in different locations, and the achieved results could be a guide for the forthcoming trials on the response against Verticillium wilt.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Toprak kaynaklı patojenlerin neden olduğu Verticillium solgunluğu kimyasal mücadelesi olmayan ve pamuk verimini etkileyen en önemli hastalıklardan biridir. Bu nedenle dayanıklı çeşitlerin geliştirilmesi kaçınılmazdır. Bu çalışma ile GAP Uluslararası Tarımsal Araştırma ve Eğitim Merkezi Müdürlüğü (GAPUTAEM) tarafından ileri aşamalara getirilmiş verim ve kalitesi yüksek genotiplerin, Verticillium solgunluk hastalığına karşı toleransının belirlenmesi amaçlanmıştır. Bu amaçla, GAPUTAEM tarafından geliştirilmiş 12 adet ileri hat (8, 20, 30, 34, 38, 57, 58, 63, 64, 8/4, 5/7 ve 8/1) ve 3 adet kontrol çeşiti [Stoneville-468, Carmen (tolerant-kontrol), Çukurova-1518 (duyarlı-kontrol)] projede kullanılmak üzere seçilerek, hastalıkla doğal olarak bulaşık tarla koşullarında 2016 ve 2017 yıllarında test edilmiştir. Hastalık şiddeti yaprakta %5-10 ve %50-60 koza açma dönemlerinde ve hasattan sonra gövde kesitinde belirlenmiştir. Araştırmada bazı verim ve lif kalite parametreleri incelenmiştir. İncelenen birçok özellik arasında önemli düzeyde farklılıkların olduğu, belirtilen dönemlerde ve gövde kesiti hastalık okuma değerleri bakımından en tolerant genotipin 38 numaralı hat olduğu tespit edilmiştir. Çalışma sonuçları değerlendirildiğinde 5/7, 30, 57, 38 ve 20 numaralı hatlar düşük hastalık indeks değerleri ve yüksek verim kapasiteleri nedeniyle önerilebilir bulunmuştur.

Anahtar kelimeler: pamuk, *Verticillium dahliae*, genotip, hastalık şiddeti

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Original article

Outbreak conditions of Sunn pest, *Eurygaster maura* L., 1758 (Hemiptera: Scutelleridae): Model of Central Anatolia (Türkiye)

Süne, *Eurygaster maura* L., 1758 (Hemiptera: Scutelleridae)'nın salgın koşulları: Orta Anadolu (Türkiye) örneği

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ABSTRACT

In this study, outbreak conditions of Sunn pest, *Eurygaster maura* L., 1758 (Hemiptera: Scutelleridae), the main agricultural pest of wheat, *Triticum aestivum* L., 1753 (Poaceae: Poales) which is the most important crop of the Central Anatolia (Türkiye) in terms of cultivation area and production was investigated. For this purpose, population density of adult sunn pests in its overwintering site and wheat cultivation area were evaluated for Central Anatolian provinces for the years between 1988 and 2021. It is observed that population density of sunn pest reached the level that requires control in 1988 for the first time. After, there were three infestation periods up to 2021 and each of them lasted for 4 years. First of them occurred between 1993 and 1996. It is observed that when average density of overwintered adult sunn pests exceeds 30 per square meter, there can be infestation in all overwintering sites of Central Anatolia. Also, there is a correlation between this density and the intensity of the infestation. Throughout the infestation periods, average temperature was determined as 20 °C during the reproduction and development period, and it was recorded over 19 °C during the season when temperature changes and relocations were high in overwintering site.

INTRODUCTION

The control against harmful organisms which cause yield losses, primarily against organisms that cause disease, pests and weeds, takes also an important place, in addition to the techniques used to increase agricultural production. Worldwide, 36.50% field loss occurs in all agricultural products, of which 10.2% is caused by insects (Agrios 2005).

Sunn pest is one of the major pests causing yield loss in wheat in Central Anatolia, as well as in the entire country of Türkiye (Özkan et al. 1999, Özkan and Babaroğlu 2015). Nymphs and adults of sunn pest, which gives one offspring per year, result in a large-scale loss of both germination power and bread and pasta quality of the grains, due to the suction of cereals found in various phenological periods. Without control in the area during years when the pest

density is high, losses up to 100% in quantity and quality are likely to occur (Özkan and Babaroğlu 2015).

The life cycle of the sunn pest is divided into active and passive periods. Sunn pest spends a passive period of about 8-9 months in mountains called overwintering sites. This period is divided into two parts: aestivation period covering the period from July to October-November following the harvest and hibernation period covering the period from October-November to March-April of the following year. Sunn pests that passed the winter begin to migrate from overwintering sites towards the plains as spring comes and the temperature rises. With the start of migrations to the plains, the passive period ends and the active period begins. Overwintered adults that have come to the plains feed and mate for 1.5-2 months and lay eggs. At the end of this period adults die. Depending on the climatic conditions, the eggs hatch within 2-3 weeks and nymphs emerge. Undergoing 5 periods in about 1 month, nymphs become the new generation adults (NGAs). The NGAs, feeding voraciously and storing the necessary energy, are drawn to the overwintering site with the beginning of harvest season.

In Central Anatolia, sunn pest control is mainly based on pesticides, which are applied to control pest nymphs. Before deciding on chemical control by carrying out surveys, chemical control is carried out on areas of higher density with above economic damage threshold.

Overwintering site surveys are made in order to estimate the density of the adult sunn pest population in overwintering sites and, consequently, the severity of the infestation. From the overwintering site surveys done twice throughout the same life cycle, the first overwintering site survey is done in autumn, generally in November, after sunn pests are drawn to overwintering sites and the replacement on overwintering sites are completed. The second overwintering area site survey is done in spring, generally in April, after sunn pest have passed winter just before descending to the cereal fields. These surveys are carried out in the same areas of the overwintering sites which can represent the province studied each year. Counts in overwintering sites are made in the south and north directions of the lower, middle and high points in terms of height. The surveys are made to determine the density of overwintered adults in wheat fields. The studies start with the completion of the descent of the overwintered adults.

MATERIALS AND METHODS

In this study, Central Anatolian provinces such as Afyon, Ankara, Aksaray, Burdur, Eskisehir, Isparta, Karaman, Kırıkkale, Kırsehir, Kayseri, Konya, Nigde, Nevsehir and Yozgat, located in the middle of the Anatolian peninsula where wheat ranks first among the grown agricultural products, were evaluated. The spatial distiribution of average temperature taken into account for the selection of provinces.

In this study, the overwintering sites and fields surveys carried out between 1988 and 2021 for control of the sunn pest in the Central Anatolian provinces by the Ministry of Agriculture and Forest, Directorates of Provincial staffs were evaluated. Evaluations were made regarding average values based on years.

The results of the field surveys were evaluated through categorizing them into four groups:

1. Economically non-damaging density (0.1-0.4 number m^2 overwintered adults),
2. Most likely economically non-damaging density except in hot and dry years when wheat is weak and sunn pest shows strong growth (0.5-0.7 number m^2 overwintered adult),
3. Density that will cause potential damaging (0.8-1.0 number m^2 overwintered adults),
4. Density that absolutely will cause damage (1 < number m^2 overwintered adults).

Temperature data used in this study were obtained from the General Directorate of Meteorology.

Binary logistic regression analysis were used to determine the time periods (months) in which population density was affected, and regression analysis was used to determine the relationship between spring overwintering site density and overwintered adult density in wheat cultivated areas. Analyses were done using SPSS 21 statistical software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Size and rate of infected area

Sunn pest, whose presence has been known in Central Anatolia since 1942 (Alkan 1942), became a problem for the first time in wheat cultivation areas in Ankara, Burdur and Isparta in 1988 when its population density reached levels that required control, and each year showed different levels of prevalence up to day (Table 1).

While in 1988, when the control was done for the first time, the amount of infected area was 100 thousand hectares, in 1993 it exceeded to 500 thousand hectares, and overall covered over 1 million hectares until 2000. The infected area, which increased to over 1.5 million hectares after 2000, is observed as average 2 million hectares today (Table 1). When the infected area ratios that are analyzed 3% of the

Table 1. Wheat cultivation areas in Central Anatolia between 1988 - 2020, sunn pest infected area, infected area rate (%), chemical control applied area and the loss rate (%)

Year	Cultivation area (ha)	Infected area (ha)	Infected area rate (%)	Chemical control applied area (ha)	Damage rate (%)
1988	3343116	101127	3.02	21995	
1989	3341234	243956	7.30	34526	6.25
1990	3317275	308703	9.31	1735	
1991	3415174	238530	6.98	18600	
1992	3473904	176589	5.08	19469	9.40
1993	3491215	573789	16.44	105952	14.93
1994	3468171	1425856	41.11	348069	4.89
1995	3286248	1570220	47.78	302247	3.41
1996	3145633	697191	22.16	92372	4.03
1997	2973325	1047521	35.23	72090	2.06
1998	2990267	1421156	47.53	5927	1.61
1999	3037004	828880	27.29	39099	2.33
2000	3061838	1431627	46.76	79031	2.55
2001	3057128	1642377	53.72	322155	2.33
2002	2987854	1529502	51.19	327036	2.50
2003	2940039	1772085	60.27	231851	2.00
2004	2981195	2075106	69.61	257723	1.07
2005	2945496	2588019	87.86	34812	0.89
2006	2735501	1983377	72.51	10702	1.74
2007	2711509	1864203	68.75	202563	2.70
2008	2704102	2182163	80.70	355287	2.45
2009	2582224	2201426	85.25	303301	1.91
2010	2651908	2262449	85.31	323779	1.98
2011	2728293	2249055	82.43	132833	1.13
2012	2507629	1972068	78.64	267153	1.29
2013	2651446	2050688	77.34	252793	0.86
2014	2714808	1989955	73.30	194407	0.93
2015	2699054	1970702	73.01	71128	0.64
2016	2711865	1821149	67.15	54544	0.53
2017	3048814	1896109	62.19	125118	0.74
2018	2907239	1911167	65.74	175256	1.12
2019	2711263	2052502	75.70	321278	1.75
2020	2596009	1954534	75.29	87391	0.75
2021	2071717	1814212	87.57	59173	0.53

cultivation areas were infected with this pest in 1988, while the infection rate has increased to double digits (16.44%) since 1993. The increase in the rate of infection continued and reached the highest level in 2005 with a value of 87.86%, covering a large part of the cultivation area, and today its prevalence is around 70% as seen in Table 1.

The reasons of population growth

Changes in climate, especially in temperature, together with favorable nutrient abundance and weakness of beneficial fauna in wheat ecosystem are the most important factors of sunn pest population size growth in Central Anatolia. The high susceptibility of insect populations to changes in abiotic conditions, such as the temperature that affects insect life and development, have been reported by Kansu (2005) and Schowalter (2011). Kansu (2005) states that the suitability of one or two factors in a mixed ecological habitat does not manifest itself on the population, however; it is observed that the suitability of one or two of the factors on the few populations living in a simpler ecological habitat, such as wheat fields with a large cultivation area, can have rapid effects and an extraordinary increase in population in a short time. Furthermore, it was this numerical rise that cause to the infestations.

During the overwintering sites surveys carried out in since 1955 within the framework of control carried out against wheat sting bug, *Aelia rostrata* Boh., 1852 (Hemiptera: Pentatomidae), one of the important pests of wheat in Central Anatolia, an increase of overwintering sunn pest population was observed for the first time in 1988.

Among the most important factors of the infestations of the sunn pest is the rise in average temperatures up to more than 20 ºC since the beginning of 1990s in June, which is the reproduction and development period of pests, in provinces where the study was conducted between 1955 and 2016 (Figure 1). It has been reported by different researchers that the most favorable temperature for development of the egg, nymph and NGA of the sunn pest is 20-24 ºC and the probability of an outbreak is high when temperatures during May-June are above 20 ºC for 2 consecutive years (Doronina and Makarova 1973, Fedotow and Botchowara 1955, Racz 1975, Zwölfer 1942).

As a result of global warming, an increase in temperature was observed in Türkiye as well as globally. Türkeş (2012) reports that warming tendencies are getting stronger and are manifested during summer and autumn seasons. He has also explained that the observed warming tendency has been accelerating since 1980s and a significant leap has been turned into an important hot period in the last 20 years. The transition to a period in which warmer conditions prevail over long-term averages took place in the mid-1980s in some stations and in the early 1990s in others. The studies conducted in Türkiye has revealed that the temperature change trend in all months, especially in summer, was positive, that the temperatures had been increasing particularly since the 1990s and that these temperature variations were compatible with global temperature changes (Bahadır 2011, ÇŞB 2013, Demir et al. 2008, Şensoy et al. 2007).

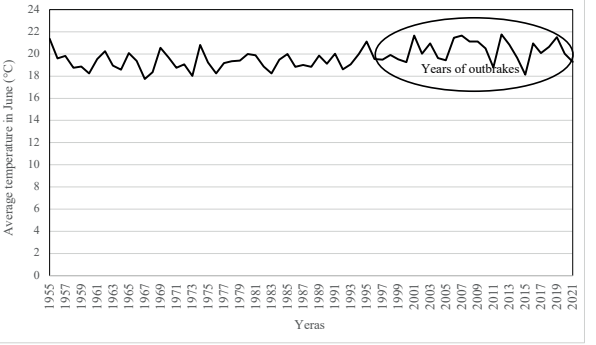

Figure 1. Average temperatures in June in Central Anatolia between years 1955-2021

Sunn pest being actually a pasture insect became harmful after pastures were converted to cereals agriculture. As in all of Türkiye, with the help of mechanization since the 1950 in Central Anatolia, expansion of cultivation areas of wheat and barley as sunn pest hosts, also application of agricultural techniques such as usage of fertilizers and irrigation for yield incensement, turning the host into a more suitable food source for the pest happens to be one of the most important causes of sunn pest infestations together with the appropriate climatic conditions. Indeed, it has been reported by many researchers that insect population growth depends on the food usability, especially the abundance of suitable food sources.

Schowalter and Lowman (1999) report that many species do not attract attention due to the fact that their population fluctuates without causing economic damaging. However, under changing environmental conditions, some of these species cause infestations, especially the changing of the natural environment by people, the expansion of sensitive dense monoculture farming areas and the procedures for rapid development of commercial agricultural products cause widespread infestations of the insects.

Kareiva (1983) reports that insect outbreaks are more frequent and intense in product systems where natural food sources are relatively unlimited, in natural monocultures or where disturbance provides suitable environments.

Boyce (1984) reports that insect with the " K " strategy like sunn pest are rarely harmful, but they become harmful

with the increasement of suitable areas as a result of human activities and it is quite difficult and expensive to control such pests once they spread.

It is reported by different researchers that monoculture wheat cultivation along with suitable climatic conditions play important role in sunn pest infestations in large areas by providing unlimited suitable food for them (Doronina and Makarova 1973, Fedotow and Botchowara 1955, Lazarov et al. 1969, Nizamlıoğlu 1955, Yüksel 1968, Zwölfer 1942).

In Central Anatolia, which has unsuitable climate conditions for beneficial organisms and a wheat ecosystem, the life and activities of beneficial organisms especially egg parasitoids, which limit the population growth of the sunn pest are adversely affected due to the incorrect and / or widespread use of insecticides that are used to control sunn pest as well (Babaroğlu and Uğur 2009, 2011). In Central Anatolia particularly in local and narrow areas, only 1-2% of sunn pest population in infected areas, which vary over years, can be suppressed by egg parasitoids and these areas can be excluded from spraying due to parasitization.

Distribution of population densities

During the 34-year period of examination it has been determined that a large part of the adult density in the infected area was 0.1-0.4 overwintered adults per m^2, which is the economically non-damaging density (Table 2). Data from fields surveys, put forward that especially during the period from 2005 to today, approximately half of the total wheat cultivation areas contain overwintered sunn pest adults at this density. After 2000 the density of 0.5-0.7 adults per m2, that is most likely economically non-damaging, had exceeded to over 300 thousand hectares, except for the hot and dry years when wheat growth is weak and sunn pest growth is strong, and this amount of infection continues today. The adult density reached its peak in 2010 with an area of approximately 700 thousand hectares, which is 26.17% of the wheat cultivation area.

In this research three periods encountered in which the density of 0.8 ≤ overwintered adults per m^2, which is considered as potentially economically damaging, exceed 100 thousand hectares. The first of these periods, which is considered as outbreak period, was between 1993 and 1996 covering a 4 year period. In the second outbreak period, which also lasted for 4 years, between 2001 and 2004, the area with density of 0.8 ≤ overwintered adult per m^2 has exceeded 200 thousand hectares. The third 4 years epidemic with lower severity than the first two outbreak occurred between 2008 and 2011 (Table 2). Furthermore, the sunn pest population reached a density that is not described as an epidemic period in 2018-2019, but it did inflict economic damage. As it can be seen from these results, the population of sunn pest showed a cyclical fluctuation in Central Anatolia. The population responses to environmental conditions can be identified by synthesizing the cycles in the population fluctuation of the sunn pest, which are powerful tools that can be used to predict future outbreaks.

It has been reported by different researchers that there are fluctuations from region to region and from year to year in the population density of the sunn pest. Lazarov et al. (1969) report that *Eurygaster integriceps* Put., 1881 (Hemiptera: Scutelleridae) was first detected in Bulgaria in 1952 and the high population density seen in 1954 was seen again, 10 years later, in 1963. Doronina and Makarova (1973) reports that according to the population density of sunn pest and the severity of the damage, there are 3 types of populations; the first type population with a consistently high pest density and high loss causing rate, the second type population with periodically high density and high loss causing rate and the third type of the population with periodic high density and moderate loss causing rate. When the Central Anatolian population is evaluated according to density and loss rates (Tables 1 and 2), it appears as a population with high density and moderate loss causing rate appearing periodically. Doronina and Makarova (1973) report that there is 2-3 years decline in populations with consistently high pest density, and 5-6 years decline in populations with a high periodic density during 10-year periods.

Racz (1975) reports that sunn pest species causes periodic infestations in local areas in Hungary. According to the results of the study he conducted between 1966 and 1974, there was an increase in the population only between 1968 and 1969. The rate of damage was 10.65% and 7.41% between 1968 and 1969 respectively and it has not exceeded the acceptable limit of damage during all other years of the study. Mustatea and Lonescu (1977) reported that sunn pest population density in Romania varies from region to region and from year to year. Stamenkovic (1992) reported that they determined the highest population density of the sunn pest in the overwintering site in Yugoslavia in 1964 and between 1968 and 1970 during surveys done from 1964 to 1991 and that they applied control to the pest in the widest areas in these years. Popov et al. (1996) emphasized that the sunn pest is common in 22 regions of Romania, however, it is always present in high concentrations in the northern and eastern regions, with varying concentrations from year to year and emphasized that there are significant increases in population density in years with generally favorable climatic conditions. Radjabi (1994) and Javahery (1996) underlined that in their study where they investigate the causes of the infestation of the sunn pest between 1960 and 1992 in Iran, they concluded that the climate conditions, especially

Table 2. Overwintered adult population densities in wheat cultivation areas between 1988 and 2020 period in Central Anatolia

Year	Overwintered adult density (number m-²)									
	0.1-0.4		0.5-0.7		0.8-1.0		0.8 <		1.0 <	
	Infected		Infected		Infected		Infected		Infected	
	area (ha)	area rate (%)	area (ha)	area rate (%)	area (ha)	area rate (%)	area (ha)	area rate (%)	area (ha)	area rate (%)
1988	73522	2.20	2825	0.08	8400	0.25	24780	24.50	16380	16.20
1989	158507	4.74	39146	1.17	34657	1.04	46302	18.98	11645	4.77
1990	293014	8.83	12725	0.38	1647	0.05	2965	0.96	1318	0.43
1991	223255	6.54	12585	0.37	1708	0.05	2690	1.13	9820	0.41
1992	146375	4.21	19493	0.56	4769	0.14	10721	6.07	5952	3.37
1993	406834	11.65	62890	1.80	83306	2.39	104066	18.14	20760	3.62
1994	804016	23.18	138838	4.00	273803	7.89	483002	33.87	209199	14.67
1995	860301	26.18	209283	6.37	494685	15.05	500637	31.88	5952	0.38
1996	869317	26.18	162657	5.17	144487	4.59	165216	23.70	20729	2.97
1997	903840	30.40	96117	3.23	46614	1.57	47564	4.54	950	0.09
1998	1155952	38.66	179389	6.00	55930	1.87	85815	6.04	29885	2.10
1999	590956	19.46	184821	6.09	53103	1.75	53103	6.41	0	0.00
2000	1217336	39.76	165667	5.41	46795	1.53	48625	3.40	1830	0.13
2001	821351	26.87	379489	12.41	316110	10.34	441537	26.88	125427	7.64
2002	1095986	36.68	225149	7.54	140585	4.71	208368	13.62	67783	4.43
2003	1244183	42.32	309827	10.54	153384	5.22	218076	12.31	64692	3.65
2004	1258394	42.21	469052	15.73	264246	8.86	347659	16.75	83413	4.02
2005	2200177	74.70	324117	11.00	59075	2.01	63725	2.46	4650	0.18
2006	1731626	63.30	219158	8.01	32593	1.19	32593	1.64	0	0.00
2007	1647995	60.78	188754	6.96	26605	0.98	27455	1.47	850	0.05
2008	1722741	63.71	357385	13.22	96227	3.56	102036	4.68	5809	0.27
2009	1561919	60.49	534567	20.70	94899	3.68	104939	4.77	10040	0.46
2010	1412196	53.25	693966	26.17	130984	4.94	156286	6.91	25302	1.12
2011	1608673	58.96	544290	19.95	95929	3.52	96092	4.27	163	0.01
2012	1546683	61.68	399672	15.94	25043	1.00	25712	1.30	669	0.03
2013	1613933	60.87	410860	15.50	25895	0.98	25895	1.26	0	0.00
2014	1568488	57.78	377128	13.89	44339	1.63	44339	2.23	0	0.00
2015	1536245	56.92	412427	15.28	21390	0.79	22030	1.12	640	0.03
2016	1442125	53.18	344614	12.71	34410	1.27	34410	1.89	0	0.00
2017	1592021	51.99	296429	9.68	7660	0.25	7660	0.25	0	0.00
2018	1376897	37.46	426771	11.61	78939	2.15	107439	2.92	28560	1.49
2019	1229664	45.03	531413	19.46	239982	8.79	291426	10.67	51443	2.51
2020	1448583	55.80	448565	17.28	57386	2.21	57386	2.21	0	0.00
2021	1611806	77.80	172178	8.31	1080	0.05	1530	0.07	450	0.03

Table 3. Overwintered adult population densities in Central Anatolia provinces overwintering site between 1988 and 2021

	Overwintered adult density (number m-^{2})	
	Spring overwintering site surveys	Autumn overwintering site surveys
1988	18.81	71.37
1989	50.27	17.73
1990	14.32	17.85
1991	16.19	26.29
1992	23.68	54.65
1993	51.65	66.31
1994	61.24	65.14
1995	60.11	62.63
1996	47.58	32.48
1997	26.47	33.52
1998	23.04	24.66
1999	21.71	46.17
2000	35.63	63.98
2001	49.60	62.15
2002	59.81	67.10
2003	52.70	61.13
2004	50.28	33.30
2005	22.88	19.64
2006	18.52	26.26
2007	25.87	38.40
2008	36.45	32.17
2009	27.94	39.13
2010	35.19	34.99
2011	28.38	24.65
2012	22.01	18.70
2013	18.31	22.84
2014	21.73	18.84
2015	17.56	14.03
2016	13.06	12.03
2017	7.96	12.22
2018	9.996	44.18
2019	41.44	34.07
2020	30.24	17.34
2021	12.61	10.95

temperature and precipitation, played the main role in the population dynamics of the sunn pest and that there was an epidemic every 6-8 years.

Relationship between overwintering site and field population densities

The control against sunn pest is based on a series of surveys aimed at determining the population density based on the life cycle of the sunn pest (Özkan and Babaroğlu 2015). When the population densities of overwintering sites are investigated for over a 32-years period, from surveys (spring) carried out from 1990 to 2021 to determine the adult sunn pest population density in the overwintering site in order to estimate the severity of the infestation; it can be seen that, although it varies in years, there is a continuous population of sunn pest in different densities in the overwintering sites (Table 3). As in the wheat cultivation areas, in the overwintering site, the population of the sunn pest shows cyclical oscillations, and the density increases approximately every 3-5 years. According to the results of the spring overwintering site surveys, it can be seen that there is an average density of 50 and above adults in m^{2} during the 1st and 2nd outbreak periods and an average of 30 and adults in m^{2} during the 3rd epidemic period. This intensity, which marks the beginning of the infestation cycle above, is called the outbreak threshold. Despite the importance of this threshold in outbreaks, few studies have been conducted on the effect of this threshold on pest species outbreaks. During this period, the population spreads to other suitable habitat areas with migrations.

It is observed that the severity of the infestations in cultivation areas changes according to the density of adults in overwintering sites above outbreak threshold (Figure 2). As the density of the adults in the overwintering sites increases, the sites with densities of 0.8 ≤ overwintered adults per m^{2}, which can be economically potentially damaging in cultivation areas, expand in parallel (r = 0.860; p = 0.000) (Table 4). Likewise, areas with 1 and above overwintered adults per m2 are expanded (r = 0.648; p = 0.000).

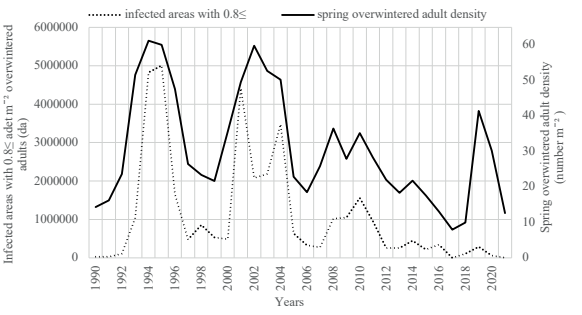

Figure 2. Wheat cultivation areas in Central Anatolia with density of 0.8 ≤ overwintered adults number m^{-2}. and spring overwintered adult density

Table 4. Relation between spring overwintering site density and wheat cultivation area in Central Anatolia

Variables		Adult density in wheat fields (number m-²)				
		1 <	0.8 ≤	0.8-1	0.5-7.0	0.5-7.0
Spring overwintering site density	Correlation coefficient	0.648*	0.860*	0.782*	-0.048	-0.275
	Significance level	0.00	0.00	0.00	0.798	0.135
	N	32	32	32	32	32

*Correlation is important at the 0.05 level

It has been reported by different researchers that there is a close relationship between population density in overwintering site and population density in wheat fields, furthermore it is cited that as the population density in the overwintering site increases, the control area expands accordingly. As a result of the work done in Bulgaria by Lazarov et al. (1969), it is reported that if 8-10 adults per m^2 are detected in the autumn surveys of overwintering site, the pest intensity is expected to be high in the consequent year.

Mustatea and Ionescu (1977) stated that, as a result of their study in Romania between 1969 and 1974, they concluded that when the density of the population in overwintering site does not exceed 3-4 overwintering adults per m^2, it is expected to be no issues in terms of control.

Jovaniç and Stamenkoviç (1978) emphasize that, in their study where they examine the population dynamics in Yugoslavia for 20 years (1958-1977) concluded that, the infestation can be expected if there are 30 or more adults per m^2 in the overwintering site and this threshold should be accepted as the tolerance limit.

Adıgüzel (1981) reported that as a result of the studies carried out in the Southeastern Anatolia Region it is proven that, an infestation can be expected if there is an average of 25-30 individuals per plant in 25 overwintering sites. Moreover, parallel to the increase in the population density in the overwintering site, the area of control will expand, and the severity of the epidemic may also increase. Şimşek et al. (1989) stated that infestations can be expected in the same region when the density of the sunn pest exceeds 20 individuals per plant in the overwintering site, and the area of control expands as the pest density increases. Karaca et al. (2009) stated that there is a 49.5% positive correlation between the adult density detected in the overwintering site of the Southeastern Anatolia region and the areas of chemical control applied in cereal cultivation areas.

As mentioned above, a part of the life cycle of the sunn pest proceeds in the overwintering site (passive period) and a part of it proceeds in the wheat fields (active period) in the plain. As a result of the analyses it was revealed that the population size in the active period varies depending on the density of the sunn pest in spring overwintering site. The density in the passive period, that is, the density of sunn pest in the overwintering site varies depending on the new generation adult density in the active period. In other words, the density of the sunn pest, which descends to the plains as the weather gets warmer in the spring, varies depending on the autumn overwintering sunn pest density that migrated from the plains to the overwintering site a year ago. As can it be seen here, the size of population of new generation adults in the field, together with the climatic conditions in the winter, affects the size of the infestations that will occur 1 year later in the same area.

Forecasting of outbreaks

When results of overwintering site surveys conducted in autumn of previous year to determine the severity of the infestation are analyzed, it is seen that the intensity was high during years of infestation. As the proportion of the wheat cultivation areas that have 0.8 ≤ overwintered adults per m^2 increases, the density of the adults in the overwintering site increases. We already mentioned that the most important reasons for this increase in the population are the suitable nutrient abundance and the favorable climatic conditions, especially the temperature. As a result of analyzes conducted to determine the relationship between the average temperatures of the 14 provinces between 1990 and 2016 and the population size in these years; it was determined that the average temperatures of especially June and September are important in the growth of the population (Table 5, Figure 3).

Presence of physiologically strong individuals within the population plays an important role in the increase of population size. In June, which is the breeding and development period of the sunn pest, if the temperature is about 20 °C and above, favorable conditions are created for feeding and numerically significant population growth occurs. Also, this is a time period during which it is fed for the purpose of storing the nutrients it will spend during its passive period (aestivation + hibernation). During

this period, it can store more nutrients, as temperatures above 20 °C will create favorable conditions for feeding. Consumption of the vast majority of the stored nutrients by the sunn pest occurs during estivation. During this period, especially in September when temperatures are highly variable, disproportionately high nutrient consumption occurs. During these two months, when high temperatures in June and high temperature variations in September prevails, breaking of the outbreak occurs.

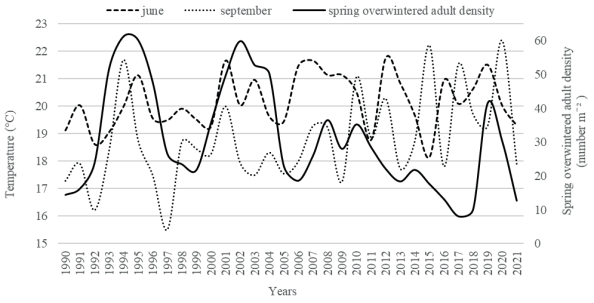

Figure 3. The periods when there was an infestation of the sunn pest in Central Anatolia and the most favorable conditions for the infestation

Zwölfer (1942) reported that the sunn pest is affected by climatic conditions and the 1st -3rd nymphal periods are very sensitive. In this period, which coincides with May-June interval, the average monthly temperature of 20-22 °C and 10-20 mm of precipitation generate the most favorable climatic conditions, and if these conditions persist for two consecutive years, sunn pest outbreaks can occur.

Fedotow and Botchowara (1955) states that during the development of eggs, nymphs and new generation adults, nutritional and climatic conditions are vital and the optimum development temperature is 20-24 °C. It is emphasized that because of the delay in the development of eggs, nymphs and new generation adults under unfavorable conditions, the compatibility between sunn pest's biology and phenology of wheat is disrupted, causing malnutrition especially in the new generation adults. Under constant humid conditions during the estivation period, high temperatures also provide a disadvantage in terms of survival.

Ouchatinkaia (1955) reported that changes in the physiological conditions of the pests occur at the beginning of diapause until temperatures reach close to the required level and after diapause until the beginning of the flight of

Table 5. The relationship between the average monthly temperatures between 1990 and 2021 and population size in Central Anatolian

Descriptive variables	B	Std. Error	Wald	df	significance level	Exp (B)
January	.124	.272	.208	1	.648	1.132
February	.140	.346	.163	1	.687	1.150
March	.084	.542	.024	1	.876	1.088
April	-.685	.449	2.323	1	.127	.504
May	-.834	.729	1.310	1	.252	.434
June	2.056	.975	4.444	1	.035	7.816
July	-1.716	.982	3.056	1	.080	.180
August	-.545	.530	1.056	1	.304	.580
September	1.435	.641	5.008	1	.025	4.200
October	-.849	.571	2.211	1	.137	.428
November	-.232	.385	.365	1	.546	.793
December	.488	.348	1.966	1	.161	1.629
Constant	15.743	19.991	.620	1	.431	6873338.736
Number of observations	32					
Log-Likelihood value	23.197					
Cox & Snell R^2	0.465					
Nagelkerke R^2	0.628					
Correct classification percentage						
Outbreak will happen	76.9					
Outbreak will not happen	94.7					
Overall Percentage	87.50					
Hosmer and Lemeshow Test	X^2=11.156; df=8; P=0.193					

*P < 0.10 significance level

the overwintered adults to the fields. During these periods, unfavorable climatic conditions cause the activity of changes in the pest body to increase and the food to decrease rapidly. However, it is stated that during the hibernation period as the pest remains immobile during diapause it keeps the exchange rate of stored nutrients at low levels and to ensure that it remains resistant to unfavorable conditions.

As a result of his studies in Southeastern Anatolia, Yüksel (1968) reports that if the average temperatures in May are 17.5 ºC or above, and the total monthly precipitation is 30-35 mm or less, and if these conditions persist for two consecutive years, the sunn pest outbreak can happen.

Doronina and Makarova (1973) state that they achieved similar results with Ouchatinkaia (1955) as they report that the pest is not only dependent on the amount of nutrients accumulated during the feeding, but also on the usage of them during other periods of life cycle, and that temperature is the factor affecting the accumulation of nutrients intensively during the feeding period of the new generation adults. It is reported that the pest intensity drops significantly if the temperature is below or around 20 ºC during nymph development. It is pointed out that in such years, nymphs develop slowly and erratically, and some of the sunn pest nymphs could not mature until the harvest and individuals who are retreated to overwintering sites are weak in terms of survival. Again, it is stated that also the temperatures during estivation period affect the population size. However, the relationship between the temperature during the hibernation period and the population size is insignificant.

Gospodinov (1973) indicated that in Russia, when the temperatures are 14-15 ºC in May, 18 ºC in June and 20 ºC in July, an increase in outbreak level in *E. integriceps* densities is observed.

Racz (1975) reported that as a result of his study in Hungary shows, if the average temperatures are above 21 ºC for 2 consecutive years in May and June, the probability of *E. integriceps* outbreak is high. The researcher states that low temperatures and high precipitation in June create unfavorable conditions for an *E. integriceps* infestation.

CONCLUSIONS

Changes in density, which is one of the basic elements of population dynamics, are the most important data in explaining the characteristics of the population. In this respect, revealing the insect density and the factors affecting it helps to explain the population dynamics. In the evaluation made in this study, the relation of the growth of the population size of the sunn pest with respect to the temperatures and the overwintered adult density was determined. Apart from these, it is a known fact that food and the effect of natural enemies play a role in the related issue. It is presumed that in the case of temperatures being around 20 ºC in June, approximately over 19ºC in September, and low temperature variations in September, and overwintered adult density of 30 ≤ pests per m^2 in the overwintering sites of 14 provinces of Central Anatolia, the probability of an outbreak is high.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Bu çalışmada Orta Anadolu'da (Türkiye) yetiştiriciliği yapılan tarım ürünleri içerisinde ekim alanı ve üretim bakımından ilk sırada yer alan buğday, *Triticum aestivum* L., 1753 (Poaceae: Poales)'ın ana zararlısı Süne, *Eurygaster maura* L., 1758 (Hemiptera: Scutelleridae)'nin salgın koşulları incelenmiştir. Bu amaçla, Orta Anadolu'ya ait illerin 1988-2021 yıllarını kapsayan periyottaki kışlak ve buğday ekim alanlarındaki ergin süne popülasyon yoğunlukları değerlendirilmiştir. Orta Anadolu'da mücadeleyi gerektiren popülasyon yoğunluğu ile ilk kez 1988 yılında dikkati çeken Süne, 2021 yılına kadar, birincisi 1993-1996 yıllarında olmak üzere 4'er yıl süren 3 salgın dönemi gerçekleştirmiştir. Orta Anadolu'ya ait tüm kışlaklarda ortalama kışlamış ergin yoğunluğunun m2'de 30 adedin üzerine çıkması durumunda buğday ekim alanlarında salgının oluşabileceği ve kışlak ergin yoğunluğuna bağlı olarak salgının şiddetinin de değişebileceği belirlenmiştir. Salgınların oluştuğu dönemlerde kışlaktaki ergin yoğunluğu ile birlikte üreme ve gelişme periyodunda ortalama sıcaklığın 20 ºC, kışlakta sıcaklık değişimlerinin ve yer değiştirmelerin yüksek olduğu dönemde ise 19 ºC'nin üzerinde seyrettiği belirlenmiştir.

Anahtar kelimeler: iklim değişikliği, tahmin, sıcaklık, buğday

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Original article

Lygus species (Hemiptera: Miridae), their distribution, and population dynamics in cotton production areas in Diyarbakır province, Türkiye

Diyarbakır ili pamuk alanlarındaki *Lygus* (Hemiptera: Miridae) türleri, yayılış alanları ve popülasyon gelişimi

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ABSTRACT

Many insect pests infest cotton (*Gossypium hirsutum* L.) crop, resulting in significant economic losses. The tarnished plant bugs (*Lygus* species) greatly damage the cotton crop during all growth stages. This study determined Lygus species infesting cotton in Diyarbakır province, their distribution, and population dynamics during 2020 and 2021. *Lygus* species samples were collected from 244 fields in 7 districts of the province by using either D-vac or sweep net based on the phenological period of cotton. Population dynamics of the species were monitored weekly by using sweep net and D-vac from two fields situated in the Sur (Gencan village) and Çınar (Şükürlü village) districts of the province during both years. Two species including *Lygus gemellatus* (Herrich-Schäffer) and *L. pratensis* (Linnaeus) were identified during the study. *Lygus gemellatus* was the most common and abundant species observed in 91.2% and 74.7% of the surveyed fields during 2020 and 2021, respectively. The species were recorded at the end of May (before flowering) during both years, and their populations increased afterward. The peak population of both species was observed during the boll maturation period. The D-vac trapped a statistically higher number of *Lygus* bugs than the sweep net at Gencan ($p < 0.05$), while both methods trapped statistically similar numbers of bugs at Şükürlü. It is concluded that the population density of both species can change from year to year depending on biotic and abiotic factors. It is suggested that survey studies must be conducted at the start of a vegetative period of cotton to monitor these species.

INTRODUCTION

Cotton (*Gossypium hirsitum* L.) is used in a variety of industries, principally the textile industry. It is also utilized as a raw material for the paper and cellulose industries, as well as in the furniture and healthcare sectors in Türkiye worldwide (Egbuta et al. 2017). Because of its variety of uses, it is a significant product from the perspective of strategy.

China (25%), USA (20%), India (16%), Pakistan (9%), Brazil (5%), Türkiye (4%), and Uzbekistan (4%) are the seven producers of cotton worldwide (Deguine et al. 2008). Türkiye is also rated sixth with 2.250.000 tons of cotton produced (TUIK 2021). In Türkiye, the Southeastern Anatolia Region produces 39.6% of cotton. Şanlurfa province leads the way in terms of production in this area with 892.906 tons, followed by Diyarbakır province in second with 309.229 tons (TUIK 2021).

Cotton, like many other crops, is susceptible to damage from a wide range of biotic and abiotic conditions that reduce production and quality. Particularly influential are pests and diseases (Kranthi et al. 2002, Matthews 1989, Özgür et al. 2019, ZMTT 2008). The important pests reported in cotton cultivation are *Helicoverpa armigera* (Hübner 1908) (cotton bollworm), and *Earias insulana* Boisd. (spiny bollworm), *Pectinophora gossypiella* (Saunders, 1844) (pink bollworm), *Spodoptera littoralis* (Boisduval, 1833) (cotton leafworm), *Tetranychus urticae* Koch, 1836 (two-spotted spider mite), *Lygus* spp. (tarnished plant bugs), *Thrips tabaci* Lindeman, 1889 (thrips), *Bemicia tabaci* Genn. (whitefly), *Empoasca decipiens* Paoli, 1930, *Asymmetrasca decedens* (Paoli, 1932) (leafhoppers) and *Aphis gossypii* Glover (cotton aphid) (ZMTT 2008). One of the notable challenges encountered in cotton production is the presence of *Lygus* spp., a pest species belonging to the Miridae family (Mccoll et al. 2011, Özgür et al. 2019, Ugine 2012, Wood et al. 2016, 2017, ZMTT 2008). The members of the Miridae insect family are called the tarnished plant bug (*Lygus* spp.) (George et al. 2021, Gore et al. 2012, Scales and Furr 1968). These insects are regarded as a pest in agriculture because it consumes a wide range of plants, including crops, and can seriously harm crops in both their nymph and adult stages (Layton 2000, Mccoll et al. 2011, Özgür et al. 2019, Wheeler 2001, ZMMT 2008).

Tarnished plant bugs are classified as piercing-sucking insects, as they employ their elongated mouthparts to puncture plant tissues and withdraw sap. These organisms consume a variety of plant components, such as buds, flowers, and developing fruits. The act of feeding by certain organisms can result in various detrimental effects on plant tissues, including distortion, discoloration, and abortion. These consequences ultimately contribute to a decrease in both crop yield and lint quality of cotton (Musser et al. 2009, Ugine 2012, Wood et al. 2016, 2017, ZMTT 2008).

Some researches have been conducted on Miridade family in Türkiye (Ateş 2018, Çerçi et al. 2021, Demirel 2009, Efil and İlkan 2003, Fent 2011, Kıyak and Ersoy 2022, Önder et al. 2006, Özgür et al. 2019, Tepecik and Dursun 2020, Yazıcı and Yıldırım 2018). However, there is not sufficient research on the cotton fields of Diyarbakır province. The research conducted in Şanlıurfa province has exclusively identified *Creontiades pallidus* Rambur as the only species of interest. It has been reported that this particular species is responsible for inducing substantial economic losses in cotton production (Efil and İlkan 2003). The objective of this study was to acquire noteworthy findings about the harmful species within the Miridae family, including their prevalence, abundance, seasonal population dynamics, and their association with the phenology of cotton plants in the cotton cultivation regions of Diyarbakır province. The objective was to enhance comprehension of these pests and offer valuable perspectives for their efficient control.

MATERIALS AND METHODS

The studies were conducted in Kayapınar, Çınar, Sur, Bismil, Bağlar, Yenişehir, and Ergani districts of Diyarbakır province, where cotton production is mostly produced (Figure 1). It has been observed that the most common cotton varieties are 455May, DP499, Golden West Esperia, Orion, and Lazer in these districts' cotton-growing areas.

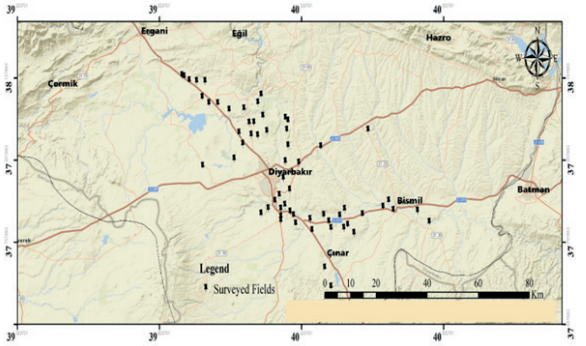

Figure 1. Distribution of the surveyed fields in Diyarbakır province to determine the infestation of *Lygus* species

Determination of Miridae species in cotton fields of Diyarbakır province

The research was conducted in Bağlar, Bismil, Çınar, Ergani, Kayapınar, Sur and Yenişehir the districts of Diyarbakır province in the years 2020 and 2021 (Table 1). For the survey studies, a modified "Husqvarna 132HBV" type vacuum insect collector called D-vac and a standard sweep-net was used to collect insects during the vegetative growth stages of cotton. The collected insects were placed in transparent plastic bags of 5 liters and transported to the laboratory. After being placed in a killing bottle containing ethyl acetate, adult individuals of Lygus species were separated under a stereo binocular microscope. The identification of the species belonging to the Miridae family was conducted by Prof. Dr. İnanç ÖZGEN (Fırat University, Faculty of Bioengineering, Department of Biomolecular Engineering, Elazığ, Türkiye).

Table 1. The distribution of surveyed cotton fields to determine Lygus species in Diyarbakır province

Surveyed Districts	Surveyed the number of cotton fields	
	2020	2021
Kayapınar	6	6
Ergani	6	6
Bağlar	4	4
Bismil	18	18
Çınar	28	28
Yenişehir	32	32
Sur	28	28
Total	122	122

The population dynamics of Lygus species in cotton fields

Population dynamics studies were carried out in the Sur and Çınar districts throughout 2020-2021. The research encompassed a total of four different fields, with two fields being investigated in each district. Sweep-net was employed to monitor the population dynamics of *Lygus* species in 2021. A total of 50 sweep nets were taken along the cotton rows on each occasion to collect adult individuals (ZMTT 2008). Sweep net and D-vac sampling techniques were used in the second-year study to evaluate their performance in obtaining Lygus species through the sampling technique. During the sampling procedure utilizing the D-vac method, Lygus species were acquired by employing the vacuum technique to extract them from the cotton plants. This process was conducted for 60 seconds at each of the six different points within the field, resulting in an overall sampling time of 6 minutes (Mutlu et al. 2008). The collected samples were transported to the laboratory and placed in a freezer to kill insects. Following this, the aforementioned insect samples were carefully transferred into appropriate plastic containers, and subsequently, the Lygus species were meticulously isolated and quantified.

Relationships between cotton phenology and damage caused by Lygus species

The research was carried out in 2022, focusing on the phenology of the cotton plant across three distinct stages: basic development, flowering, and maturation periods. The data collection involved employing a direct visual counting method. Sampling was conducted on 100 cotton plants, and the plants were randomly selected from various locations within the field. The sampling was carried out once a week, starting from the 3-5 leaf stage of cotton until the boll maturation stage. The direct counting method was employed for data collection. During the assessment, a comprehensive examination was conducted on the bolls of every cotton plant. The objective was to identify and record the black spots resulting from the feeding activities of Lygus species within the bolls. The percentage of damaged bolls was determined by comparing the number of damaged bolls to the total number of bolls counted.

RESULTS

Determination of Miridae species in cotton fields of Diyarbakır province

During two years, surveys were conducted in the cotton fields of the study area, resulting in the determination of two species belonging to the Miridae family: *Lygus gemellatus* (Herrich-Schäffer, 1835) and *L. pratensis* (Linnaeus) were recorded (Figure 2). Table 2 presents the data about the specific locations where the species under consideration were observed.

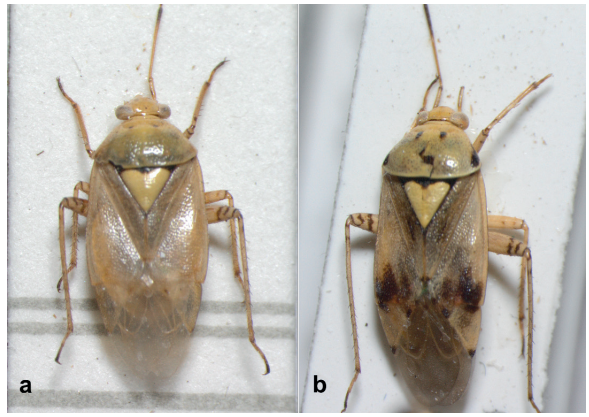

Figure 2. The tarnished plant bugs, *Lygus gemellatus* (a), *Lygus pratensis* (b) identified in the study

Table 2. *Lygus* species observed in cotton fields in Diyarbakır province

	Lygus gemellatus		*Lygus pratensis*	
Districts	2020	2021	2020	2021
Bağlar	-	-	-	-
Bismil	9	4	1	-
Çınar	23	22	5	7
Ergani	-	-	-	-
Kayapınar	-	-	-	-
Sur	85	125	6	38
Yenişehir	39	17	4	10
Total	166	168	16	57

Lygus species were not identified in the cotton fields of the Bağlar, Ergani, and Kayapınar districts in either of the two years (Table 2). The data acquired showed that *L. gemellatus* was the most common and abundant species in the cotton

production areas, accounting for 91% in 2021 and 75% in 2022. Besides, *L. gemellatus* was found to be most prevalent among the surveyed districts in the cotton fields of the Sur district.

The population dynamics of Lygus species in cotton fields

The data regarding the population dynamics of *Lygus* spp. in Diyarbakır province for the years 2020-2021 are presented in Figure 3 and Figure 4.The research started on May 22, 2020, in the cotton fields located in the Gencan and Şükürlü locations. The initial observation of *Lygus* species occurred in the Gencan of Sur district during the middle of June, before the onset of cotton flowering. On June 26, 2020, a total of 10 individuals were captured per sweep-net. On July 24, 2020, there was an observed increase in the count of individuals/ sweep-net, specifically during the cotton flowering period. The quantification of adult/sweep-net during the cotton maturation phase yielded a count of 8 on August 7, 2020, and a count of 4 on September 18, 2020. Following this point, a decline in the population of pests was observed. The presence of pests was detected in the village of Şükürlü, located in the Çınar district, during the initial week of July. This occurrence coincided with the flowering phase of the cotton crop, where a count of 3 adults per sweep-net was recorded. Following a particular period, there were variations observed in the population dynamics. Specifically, during the maturation phase on August 21, 2020, the population reached its peak count of 8 adults per sweep-net. In both study sites (Figure 3), it was observed that the population of Lygus spp. demonstrated a notable increase, particularly during the squaring and boll developmental stages of cotton.

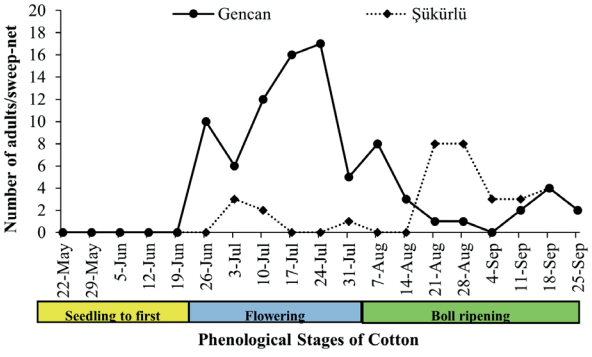

Figure 3. The population dynamics of *Lygus* spp. in cotton planted in Diyarbakır province during 2020

The study's second year started on May 22, 2021, at the Gencan and Şükürlü sites. *Lygus* species were observed in Gencan during the initial week of June. The population in the specified location was recorded as 2 adults per sweep-net on June 19, 2021, 5 adults per sweep-net on July 17, 2021, and 7 adults per sweep-net on August 7, 2021. The population had its lowest level during the period of cotton maturation, which typically occurs in late August. In the Şükürlü field, Lygus species were observed during the early week of June. The pest population observed in the present field showed a notable decrease in comparison to the population recorded in the Gencan field. In the Şükürlü region, the presence of one adult per sweep net was recorded on two separate occasions: August 7, 2021, and August 21, 2021 (Figure 4).

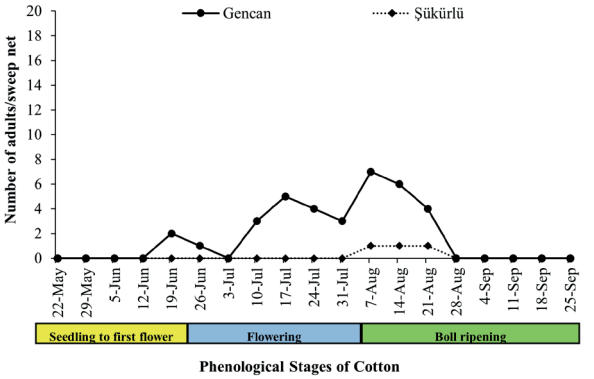

Figure 4. The population dynamics of *Lygus* spp. in cotton planted in Diyarbakır province during 2021

A comparative analysis of two distinct sampling methods was conducted on the cotton field situated in the Gencan location during the year 2021. Adult *Lygus* individuals were initially observed during the third week of May, coinciding with the vegetative growth stage of cotton, as illustrated in Figure 5. On May 29, a sampling procedure utilizing the D-vac method resulted in the acquisition of 57 adult individuals. Conversely, the sweep-net method resulted in the capture of 17 adult *Lygus* specimens. The population showed a decline in both methods; however, a subsequent increase was observed during the flowering and squaring stages of cotton. Notably, the D-vac method resulted in a total of 15 adult individuals. During the period of maturation, the population declined to its minimum level using both methods (Figure 5).

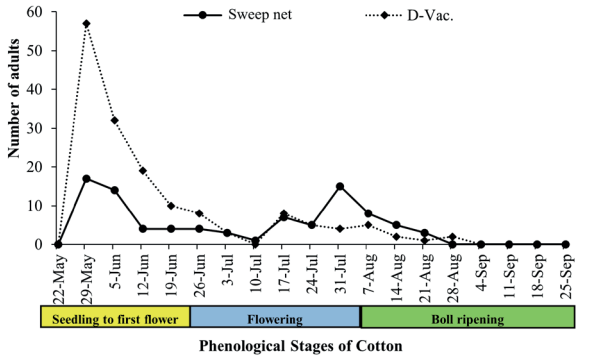

Figure 5. Population dynamics of *Lygus* species determined by Sweep-net and D-vac methods in Gencan during 2021

In another sampling conducted in the cotton field of Şükürlü, the first adult *Lygus* was determined in the last week of May (during the vegetative growth stage of cotton) (Figure 6). The same number of adult *Lygus* (7 individuals) were collected using both sampling techniques. The population in this field fluctuated during the blossoming and squaring seasons, although more adult *Lygus* were collected using the D-vac method than with the sweep-net technique. The population in both ways reached its lowest point in the maturation period.

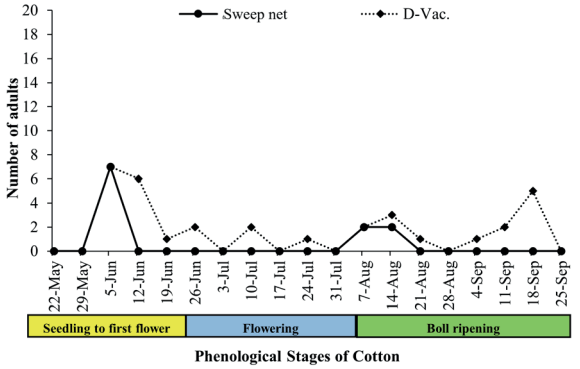

Figure 6. Population dynamics of *Lygus* species determined by Sweep-net and D-vac methods in Şükürlü during 2021

In the Gencan location field, the sweep-net method resulted in a capture rate of 36.5% (average 4.7 adult), whereas the D-vac the method resulted in a capture rate of 63.5% (average 8.2 adults). There was a significant capture in terms of D-vac sampling (t (19)= 3.865, p= 0.001) compared to the sweep net in the Gencan field. Similarly, in the Şükürlü location, the proportion of individuals captured using the sweep-net method was found to be 25.0% (average 0.58 adult), whereas the D-vac the method provided a high capture rate of 75.0% (average of 1.73 adults) (Figure 7). However, despite there was no statistically significant difference between the two methods (t (19)= 1.505, p= 0.150) at the Şükürlü location, relatively more *Lygus* spp. were obtained with D-vac.

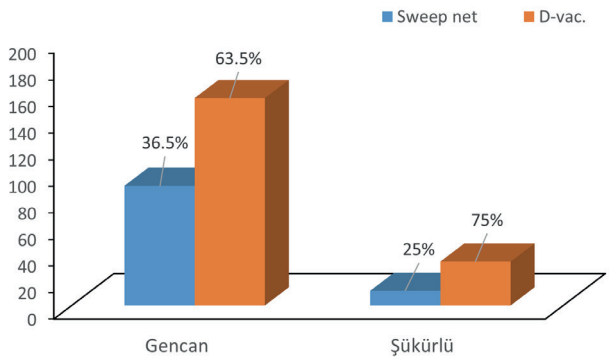

Figure 7. The relative proportion of Lygus species trapped by sweep net and D-vac sampling methods in Diyarbakır province during 2021

Relationships between cotton phenology and damage caused by Lygus species

Observations were carried out in the Gencan and Şükürlü districts during the period that extended from the final week of May to the initial week of October in both 2020 and 2021. The objective of these observations was to determine the relationship between *Lygus* species and cotton phenology, specifically about the extent of damage caused.

Damage to newly grown cotton bolls began in both districts during the early week of July, coinciding with the generative stage. The Gencan location exhibited the greatest number of damaged bolls during the middle of September (September 18, 2020), with a total of 180 infested bolls (Figure 8). Likewise, the Şükürlü location showed the greatest number of damaged bolls, precisely 84 infested bolls, on September 11, 2020 (Figure 8).

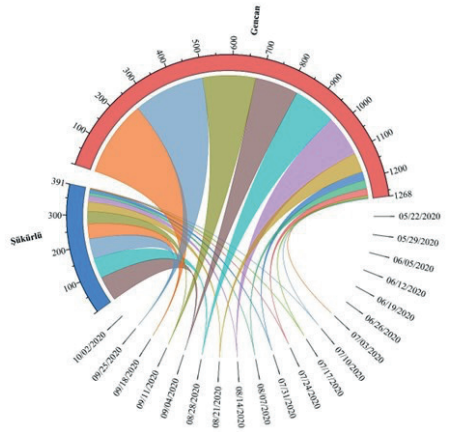

Figure 8. The number of damaged bolls due to *Lygus* spp. in Gencan and Şükürlü during 2020

In the second year of the study, which was the year 2021, the first boll damage was observed starting from the last week of June, which coincides with the beginning of the flowering period. After this period, an increase in the number of infested bolls was noticed, and it was determined that the highest number of damaged bolls occurred at the end of August and the beginning of September (during the maturation period). The highest boll damage was recorded in the Gencan field during the first week of September (September 4, 2021), with 378 infested bolls, while in the Şükürlü location, 410 infested bolls were identified at the end of August (August 28, 2021). The number of damaged bolls in the cotton plant was determined for both years (Figure 9). Consequently, it was determined that *Lygus* species cause damage to cotton plants during the squaring and boll-forming stages, with the most significant damage observed during the boll stage.

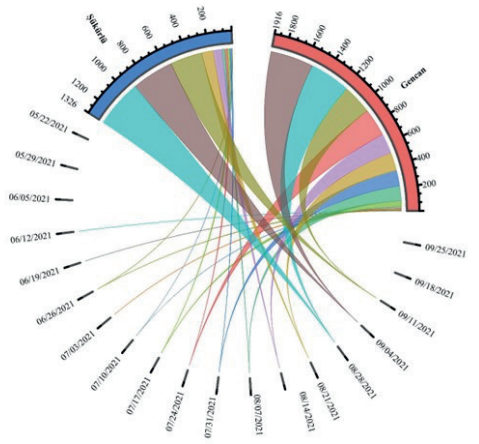

Figure 9. The number of damaged bolls due to *Lygus* spp. in Gencan and Şükürlü during 2021

Figure 8 displays the data about the quantity of bolls affected by *Lygus* species in four distinct cotton fields in Diyarbakır. In the first year of the study (2020), 20.000 bolls from 100 cotton plants in the field at Gencan were examined. Out of these, 1.268 were determined to be damaged and recorded as infested by *Lygus* species, while the remaining 18.732 were thought to be undamaged. As a result, 6.3% of the Gencan field's bolls were infested compared to those that were not affected. Similarly, in the field at Şükürlü, 100 plants totaled 15.500 bolls, of which 15.110 were found to be undamaged and 390 to be infested. 2.5% of the bolls in this field were determined to be damaged.

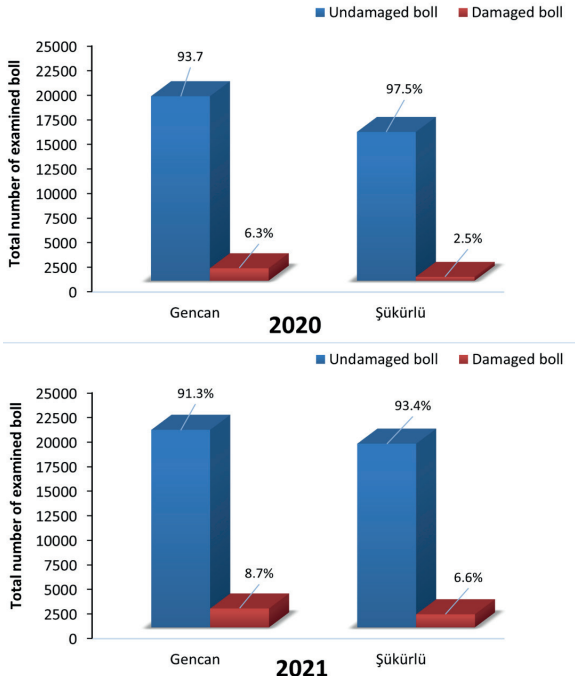

Figure 10. The number of damaged and undamaged bolls, and damage ratios caused by *Lygus* spp. in Gencan and Şükürlü locations

During the second year of the study, in the year 2021, a comprehensive examination of 22.000 bolls was conducted in the field at Gencan. Out of this total, 1.916 bolls were recorded as infested, while the remaining 20.084 bolls were identified as undamaged. Hence, the proportion of infested bolls to undamaged bolls in the Gencan was calculated to be 8.7%. In the Şükürlü cotton field, where the research was conducted, a comprehensive count of 20.000 bolls was conducted. Out of these, 18.674 bolls were found to be undamaged, while 1.326 bolls were identified as infested. The study determined that the proportion of bolls damaged by *Lygus* species in Şükürlü Village was 6.6% (Figure 10).

DISCUSSION

Over two years of surveys conducted in cotton cultivation areas in Diyarbakır province, two *Lygus species, L. gemellatus and L. pratensis*, were determined. Several studies have been conducted on tarnished plant bugs, including those found on cotton and other field crops In Turkiye (Arı 1999, Demirel 2009, Önder et al. 2006, Özgür et al. 2019, Yıldırım 2020). In these studies including cotton and other field crops *L. rugulipennis, L. lineolaris, L. borealis, L. elisus, L. hesperus, L. italicus, L. kalmi, A. linealatus, L. lucorum, C. pallidus, E. gemellatus, E. pratensis* and *A. lucorum* were determined. Besides, Wood et al. (2016, 2017) stated that *L. lineolaris* is a significant pest in cotton fields in the central United States.

According to several studies focusing on the cotton plant, it has been observed that the species C. pallidus is found in cotton fields located in Şanlıurfa province. Conversely, in the cotton cultivation areas of Hatay's Amik plain and Aydın province, the species *L. gemellatus, L. pratensis* and *C. pallidus* have been identified (Ateş 2018, Demirel 2022, Efil and İlkan 2003). In addition to *C. pallidus*, the species *L. italicus* has also been identified in the cotton fields located in Adana province. The findings of this study indicate that the species observed are consistent with those previously identified in various regions of Türkiye, thereby providing support for the assertion that *L. gemellatus* and *L. pratensis* are the most prevalent and abundant species in cotton fields. According to a study conducted by Efil and İlkan (2003), C. pallidus has been observed as a prevalent species in the cotton cultivation areas of Şanlıurfa province. In the present study, despite the carrying out of surveys over two years, the identification of the species *C. pallidus* could not be identified in Diyarbakır province.

In contrast to the findings of the present study, previous research conducted in Aydın province has reported a notably low occurrence of *E. gemellatus* and *E. pratensis,* with *C. pallidus* identified as the most prevalent and abundant species (Ateş 2018). It is widely acknowledged

that species within the Miridae family, specifically *Lygus* bugs, are subject to significant effects from abiotic factors, resulting in fluctuations in their population densities across different years and seasons. The population dynamics of these insects are affected by abiotic factors, including temperature, humidity, precipitation, irrigation, and other environmental conditions (Adams et al. 2013, Asiimwe et al. 2014, Harper et al. 1993, Lu et al. 2014, Schotzko and O'Keeffe 1986). In this research, *C. pallidus* was not determined despite the sampling of 244 cotton fields over two consecutive years. Hence, it can be concluded that *C. pallidus* does not exhibit any population within the ecological conditions of Diyarbakır. Therefore, it is recommended that comprehensive investigations be undertaken to investigate the occurrence of the aforementioned *Lygus* species in cotton fields within the Şanlıurfa province.

Both harmful *Lygus* species were observed before the flowering period of cotton, and after this period, their population density began to increase. During the squaring and boll formation period (July and August), it reached its peak. In the cotton fields of Aydın province, it has been determined that Miridae species pass from clover to cotton areas starting from the formation of cotton bolls and the highest population density on cotton occurs in August and September when the bolls are present (Ateş 2018). It has been also recorded that *C. pallidus* is observed during the flowering period of cotton and its density significantly increases during the boll formation period in Şanlıurfa province (Efil and Bayram 2009). This study's findings are supported by the fact that in the cotton fields of the Çukurova region, both *C. pallidus* and *L. italicus* show an increase in density from mid-July and reach their peak levels starting from August. Consequently, it can be stated that mirid species, in general, start increasing their population densities in cotton fields from the generative period onward and are considered harmful. The study also revealed that *L. gemellatus* demonstrates a high population density during the cotton boll period, whereas *L. pratensis* exhibits its highest population density during the boll maturation period.

The efficacy of sweep-net and D-vac methods in capturing adult *Lygus* individuals were evaluated, particularly during phenological periods characterized by the highest numerical intensity of harmful density. The D-vac method provided greater numbers of individuals in studies conducted to assess the population densities of both *Lygus* species. The population density of the pest in the Sur district was determined to be 36.5% through the utilization of the sweep-net method, whereas the D-vac method yielded a population density of 63.5%. In the Çınar district, it was observed that the rates obtained through the sweep-net method were 25%, while the rates obtained through the D-vac method was 75%. Based on the data acquired from both sampling locations, it has been concluded that the D-vac method exhibits higher effectiveness in capturing *Lygus* species in comparison to the sweep-net method. Nevertheless, an investigation carried out in Aydın province revealed that the sweep-net method provided the most favorable outcomes, exhibiting the highest capture rate among the various methods employed (Ateş 2018). According to Schotzko and O'Keefe (1986), they obtained approximately the same number of individuals while sampling *Lygus* hesperus Knight species from lentils using the D-vac and sweep net methods. However, they obtained a lower number of *L. hesperus* nymphs with the sweep net method. Harper et al (1993) reported that differences between sweep net and D-vac insect population estimates varied over sampling dates and years and were dependent on the insect species, their developmental stages, and abiotic factors. Buffington and Redak (1998) found a significantly higher number of Miridae species when employing the D-vac sampling method. Besides, Parajule et al. (2006) stated that there was no significant difference in catch efficiency between vacuum and sweep net methods for cotton mirid species, *Pseudatomoscelis seriatus* (Reuter). Although this study resulted in a higher number of *Lygus* species using the D-vac sampling method when compared to the sweep net, the accuracy of insect estimates obtained through the use of sweep net and D-vac methods is dependent upon various factors, including the specific insect species under consideration, their developmental stage, their spatial distribution within the canopy, the particular crop being sampled, and the influence of abiotic factors (Harper et al. 1993). Hence, taking into account the aforementioned factors, it is believed that both methods can be utilized in an integrated way to achieve precise population estimations.

In the present investigation, an examination was conducted to establish a correlation between the detrimental effects caused by *Lygus* spp. species and the phenological stages of cotton. The findings of this study revealed that these species cause damage specifically during the squares and boll periods, an effect that can be attributed to the population and increase of these pests within the cotton ecosystem. Nevertheless, it has been established that the period during which they cause the most severe damage is during the phase of cotton boll formation. Similarly, Özgür et al. (2019) reported that when the Miridae population is high, there is an increase in the number of bolls with discoloration and the number of bolls being shed onto the ground. They also observed an increase in the damage rates caused by *C. pallidus* on cotton bolls. Besides, Ateş (2018) stated similar findings to those obtained in our study, indicating that in

Aydın province, *Lygus* spp. start causing damage to newly forming small cotton bolls and increase their population significantly after this period.

According to the findings of damage assessment studies conducted in 2020, it was observed that the average percentage of small black spots on bolls in Gencan Village of Sur district was 6.3%, whereas, in Şükürlü Village of Çınar district, the rate of these spots was recorded as 2.5%. In the year 2021, the rate of pest-related damage in Gencan Village was recorded to affect approximately 8.7% of the bolls. Similarly, in Şükürlü Village, the damage caused by pests accounted for approximately 6.6% of the bolls. This damage was primarily attributed to the feeding activities of pests, which involve piercing and sucking. During the second year of the study, although the *Lygus* population was lower than the previous year, there was a proportional rise in the number of discolored bolls. The observed decrease in population size during the second year is thought to be attributable to the negative consequences of insecticides applied by cotton cultivators to control various cotton pests on the population density of *Lygus* species. This observation supported by Musser et al (2009) the management of tarnished plant bugs in cotton fields primarily involved the use of insecticides targeted at other pests during the flowering stage, therefore, economic damage from tarnished plant bugs during flowering was relatively uncommon.

According to the findings of Özgür et al. (2019), it was observed that even in the presence of low mirid populations, cotton crops in the Çukurova region experienced substantial damage. The study conducted in Harran Ovası revealed a negative correlation between the population of *C. pallidus* and the timing of cotton field planting. Specifically, early-planted cotton fields exhibited a lower population of *C. pallidus,* whereas cotton fields planted at later dates demonstrated a higher population of this species. The increased presence of squares and young bolls in early-planted cotton was identified as the contributing factor to the more favorable conditions that facilitated the population growth of *C. pallidus* (Efil and İlkan 2003). Furthermore, it has been determined that the initial damage caused by *Lygus* species occurred in early June, while the most significant occurrence of boll damage occurred in August. In this study, an investigation was conducted to establish a correlation between *Lygus* spp. species and cotton phenology. The findings of this study revealed that *Lygus* spp. species cause damage to cotton plants specifically during the boll formation and boll period. Furthermore, the highest degree of damage was once again observed during the period of cotton boll formation and boll development.

It is widely known that *Lygus* species population densities fluctuate year to year in response to plant types, biotic and abiotic environmental conditions as well and agronomic practices (George et al. 2021, Oeller et al. 2021, Özgür et al. 2019). The number and distribution of *Lygus* bugs may fluctuate over time as a result of the influence of several factors on population dynamics. The population dynamics of *Lygus* bugs in different environments can be greatly affected by both biotic factors, such as the availability of host plants and the presence of natural enemies, and abiotic factors, like temperature, humidity, and rainfall. For successful pest management techniques and long-term agricultural sustainability, an awareness of these aspects is crucial. Additionally, it was found that populations of *Lygus* spp. in Diyarbakır exceeded the economic threshold during both years of the study. Therefore, cotton producers need to be vigilant against plant bug species in cotton production areas from the flowering period onward. As the population of pests increases, the damage rate during the flowering, boll formation, and boll period may rise (Efil and İlkan 2003). Thus, appropriate cultural measures should be taken against *Lygus* species. Moreover, if chemical control is necessary, it is essential to choose pesticides with minimal impact on beneficial insects. This ensures the preservation of ecosystem balance and effective control of pests while maintaining the contribution of natural enemies, making it crucial for sustainable agricultural practices.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Pamuk üretimi boyunca birçok zararlı etmen ortaya çıkmakta ve önemli ürün kayıplarına yol açmaktadır. Bunlar içerisinde yer alan *Lygus* spp. (Bitki tahtakuruları) pamuğun generatif döneminden başlayarak meydana getirdikleri zarar ile önemli ürün kayıplarına yol açabilmektedir. Bu çalışma Diyarbakır ili pamuk üretim alanlarındaki *Lygus* spp. türleri, yayılış alanları ve pamuktaki popülasyon gelişimini belirlemek amacıyla 2020 ve 2021 yıllarında yürütülmüştür. *Lygus* türlerini belirlemek için Diyarbakır ilinde 7 ilçeden toplam 244 adet tarladan örneklemeler pamuğun fenolojik dönemine göre atrap ve böcek toplama aleti (D-vac) kullanılarak

yapılmıştır. Popülasyon değişimi çalışmaları ise iki farklı örnekleme yöntemiyle (atrap ve D-vac) ile her iki yıl Sur ve Çınar ilçelerinde iki farklı tarlada haftalık olarak gerçekleştirilmiştir. Çalışmada *Lygus gemellatus* (Herrich-Schäffer) and *Lygus pratensis* (Linnaeus) olmak üzere toplam iki bitki tahtakurusu türü saptanmıştır. Bu iki tür arasında *Lygus gemellatus* en yoğun ve yaygın tür olduğu, 2020 yılında %91.2, 2021 yılında ise %74.7 oranında bulunduğu belirlenmiştir. Zararlıların çalışma yapılan her iki yılda, mayıs ayı sonunda (çiçeklenme döneminden önce) pamuk alanlarında görüldüğü belirlenmiştir. Bu dönemden sonra popülasyonun arttığı görülmüş ve özellikle *L. gemellatus* popülasyonunun pamuğun taraklanma (koza oluşturma) döneminde en yüksek seviyeye ulaştığı tespit edilmiştir. Buna karşın *L. pratensis* popülasyonu ise koza (olgunlaşma) döneminde en üst düzeye çıkmıştır. D-vac Gencan'da atrap metoduna göre istatistiksel olarak daha fazla sayıda tahtakurusu yakalarken ($p < 0,05$), Şükürlü'de her iki yöntem de istatistiksel olarak benzer sayıda tahtakurusu yakalamıştır. Biyotik ve abiyotik faktörlere bağlı olarak her iki zararlının popülasyon yoğunluğunun yıldan yıla değişebildiği sonucuna varılarak, pamuğun generatif döneminden itibaren bu zararlılara karşı yapılacak sürveylerle dikkatli olunması gerektiği önerilmektedir.

Anahtar kelimeler: sürvey, zararlı böcekler, zarar, popülasyon, bitki tahtakuruları

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Original article

Fumigant and contact toxicity of *Ruta chalepensis* l. (Rutaceae) essential oil against five coleopteran stored product pests and its effects on cholinesterases

Ruta chalepensis L. (Rutaceae) uçucu yağının beş coleopteran depo ürün zararlısına karşı fumigant ve kontak toksisitesi ile kolinesterazlar üzerine etkileri

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ABSTRACT

The essential oil composition of aerial parts of *Ruta chalepensis* L. was analyzed with GC-MS. Seventy-nine compounds were detected representing 85.93 ± 1.08% (n = 3) of the essential oil. The major components of the essential oil were 2-undecanone 21.52 ± 0.21%, 2-nonanone 18.31 ± 0.27%, and 2-nonyl acetate 13.22%. The highest insecticidal contact toxicity of the oil was observed against *Rhyzopertha dominica* F. with 0.018 µl/insect LD50 and 0.039 µl/insect LD90 after 24h. Essential oil also produced considerably low 0.50 and 0.59 µl/insect LD50 values after 24h against *Sitophilus oryzae* L. and *Sitophilus granarius* L. respectively. The lowest contact toxicity was observed against Tribolium castaneum Herbst. and *Tribolium confusum* Jacquelin du Val. 0.138 and 0.078 µl/insect LD50 after 24h respectively. The highest fumigant toxicity was observed against *S. granarius* for the application concentration of 10 µl, 10% oil/acetone (v:v) in a 10 ml chamber which afforded 100.00 ± 0.00% mortality after 48h. The essential oil also produced high fumigant toxicity against *S. oryzae*, *T. castaneum* and *R. dominica* which were 95.47 ± 3.41%, 93.30 ± 5.54%, and 85.47 ± 3.41% mortality at 20 µl application concentration of the oil solution after 48h. The *R. chalepensis* essential oil produced low acetylcholinesterase enzyme 5.29 ± 1.20% (n=3) inhibition and mediocre butyrylcholinesterase enzyme inhibition 42.6 ± 0.71% (n=3). According to the insecticidal activity assays performed, the essential oil *R. chalepensis* seems to be a promising source that could yield natural compounds that could be employed in stored product pest management.

INTRODUCTION

The appropriate storage of agricultural products is very important to reduce product losses. Product loss during storage is caused by many factors, including pests such as insects, rodents, and microorganisms as well as the conditions during storage such as humidity, temperature, etc. (Bouajaj et al. 2014, Chebli et al. 2004, Eisen 2013, Shah et al. 2015, Shaaya et al. 1997, Yazdgerdian et al. 2015). The most significant damage among the factors listed that affect the product is caused by stored product insects. The primary (*S. granarius, S. oryzae, R. dominica*, etc.) and secondary (*T. confusum, T. castaneum, O. surinamensis,* etc.) stored product insect species cause serious damage to the product during the storage (Atay et al. 2023, Polatoğlu and Karakoç 2016, Prakash and Rao 1996, Rajendran and Sriranjini 2008). Unless they are controlled, they can produce serious losses in the product (Çam et al. 2012, Hernandez-Lambraño et al. 2015, Kamanula et al. 2010). Product losses can be between 40% and 100%, particularly if modern storage techniques are not used (Campbell and Sinha 1976, Shaaya et al. 1997, Yıldırım et al. 2001). Currently, synthetic chemical compounds with contact and fumigant activity—often consisting of organic phosphorus—are frequently utilized in large-scale pest control of stored products against insects (Alkan et al. 2023, Çam et al. 2012, Evlice and Alkan 2023, Martin et al. 2015, Udo 2005). Natural pest control methods are becoming more popular as a result of increased environmental and health concerns, as well as new laws implemented to address these issues, such as the European Pesticide Regulation (EC) No. 1107/2009 (Alkan and Atay 2023, Atay and Alkan 2023, Isman 2006, 2008, Villaverde et al. 2014).

Ruta species of Rutaceae is only represented by *Ruta chalepensis* L. in Cyprus (Viney 1994). *Ruta* species are plants native to the Mediterranean and they found many uses in traditional medicine (Pollio et al 2008). These medicinal uses include uses for dropsy, inflamed eyes, colics, spasms, diarrhoea, insect/scorpion/spider bites, snake bites, poisoning, headache, migraine, epilepsy, hysteria, internal parasites, catarrh, common cold, cough, toothache (Lardos 2006). Until now from the *Ruta* species, many different types of secondary metabolites including alkaloids, coumarins, flavonoids, triterpenes, sterols, saponins, and tannins were isolated (Chen et al. 2001, Hnatyszyn et al. 1974). The furocoumarins (psoralens) are specifically observed in the species of Rutaceae, Umbelliferae, Moraceae, Fabaceae, and Apiaceae. Psoralens cause phytophotodermatitis in humans (Pathak et al. 1962). *Ruta* species is also known for its quinolone alkaloids. The essential oils of the *Ruta* species were also investigated comprehensively. However, most of the reports only indicate a few identified substances in the composition of the essential oil except for a couple of comprehensive reports that identify many substances in the composition of the oil (Ulubelen et al. 1986).

The *Ruta* essential oils are characterized with alcohols (2-nonanol), esters (nonyl acetate, 2-methyloctyl acetate), ketones (2-undecanone, 2-dodecanone, 2-decanone, 2-nonanone) of saturated fatty acids (Abbad et al. 2014, Akkari et al. 2015, Rustaiyan et al. 2002, Tounsi et al. 2011, Tzakou and Couladis 2001). Usually, the essential oils of *Ruta* species contain geijerene (2-Isopropenyl-1-methyl-1-vinyl-3-cyclohexane) and moskachan (psoralen) derivatives in minor quantities (Joulain et al. 1991, Stashenko et al. 1995, Stashenko et al. 2000, Yaacob et al. 1989). The *Ruta* essential oils are reported to have herbicidal, molluscicidal, nematicidal, antimicrobial, antibacterial, antifungal, anti-inflammatory, antipyretic, emmenagogue and antihelmintic effects (Bouabidi et al. 2015, Günaydin and Savcı 2005, Haddouchi et al. 2013, Hmamouchi et al. 2000, Iauk et al. 2004, Ntalli et al. 2011,). Additionally, the essential oils of *Ruta* species are known for their considerable insecticidal activity against a broad spectrum of insect species. *R. chalepensis* has already been reported to have larvicidal, repellent and biting deterrent activity against vectors of many diseases namely *Aedes albopictus Skuse*, *Aedes aegypti* (L.), *Culex pipiens pallens Coquillett, Anopheles quadrimaculatus* Say (Diptera: Culicidae) (Ali et al. 2013, Conti et al. 2013, Kim et al. 2002, Perez Lopéz et al. 2015). Furthermore, the essential oils of *Ruta* species were tested against agricultural product insects namely *Orgyia trigotephras* Boisd. (Lepidoptera), *Tribolium confusum* Jacquelin du Val, *T. castaneum* (Herbst) (Coleoptera) and found active against these pests (Abbad et al. 2014, Akkari et al. 2015, Majdoub et al. 2014). The extracts of *Ruta* species were reported to have aphicidal, insecticidal, and nematicidal activity against *Alphitobius diaperinus* Panzer, *Trogoderma granarium* Everts (Coleoptera), *Aphis punicae* Passerini, *Bemisia tabaci* Gennadius (Hemiptera), *Hypsipyla grandella* Zeller (Lepidoptera), *Locusta migratoria* L. (Orthoptera), *Meloidogyne arenaria* Chitwood, *M. hapla* Chitwood, *M. javanica* (Treub) Chitwood, *M. incognita* (Kofoid and White) Chitwood (Tylenchida), *Pediculus humanus* L. (Phthiraptera), *Psammotermes hybostoma* Desneux (Blattodea), *Xiphinema index* Thorne and Allen (Dorylaimida) species (Abdellaoui et al. 2014, Al-mazr'awi and Ateyyat 2009, Alshehry et al. 2014, Jorge et al. 2009, Madkour et al. 2012, Mancebo et al. 2001, Marcomini et al. 2009, Moawad and Al-Barty 2011, Sasanelli 1992, Sasanelli and D'Addabba 1993, Soto Monterrosa et al. 2007). Natural substances isolated from *Ruta* species namely quinoline, quinoline-4-carbaldehyde, quinoline-3-carbaldehyde, and quinolone carboxylic acid derivatives were reported to have fumigant and contact toxicity against *S. oryzae* (Jeon et al. 2013). The 3-(2″,2″-dimethylbutenyl)-

3′-hydroxyl-dihydrofuropsoralen and rutamine isolated from *R. chalepensis* were reported to have larvicidal activity against *Spodoptera littoralis* (Emam et al. 2009). The active essential oil component of *R. graveolens* 2-isopropyl-5-methylphenol and its derivatives namely 5-isopropyl-2-methylphenol, 4-isopropyl-3-methylphenol, 2-methylphenol, 3-methylphenol, 4-methylphenol, 2-isopropylphenol, 3-isopropylphenol and 4-isopropylphenol were tested against *S. oryzae, S. zeamais* and *Lasioderma serricorne* (Fabricius) with fumigant toxicity assay; highest activity was observed for 3-isopropylephenol (Jeon et al. 2015). *R. chalepensis* essential oils were also reported to have antifungal activity against the fungal species that produce damage to agricultural products such as species from *Alternaria, Aspergillus, Botrytis, Fusarium* and *Phytophthora* genus (Bouabidi et al. 2015, Bouajaj et al. 2014, Chebli et al. 2004, Haddouchi et al. 2013). Additionally, *R. chalepensis* essential oil was reported to have seed germination inhibition against *Triticum durum* Desf. and *Phalaris canariensis* L. (Bouajaj et al. 2014, Mejri et al. 2012).

Inhibition of the cholinergic system enzymes is reported to be one of the possible insecticidal action mechanisms of essential oils (Rattan 2010). Essential oil components with inhibitory properties against AChE were reported in the literature (López and Pascual-Villalobos 2010, Miyazawa et al. 1997, Miyazawa and Yamafuji 2005). Previously, AChE inhibitory properties of methanol and water extracts of *Ruta graveolens* L. were reported (Adsersen et al. 2006). Another report shows that hexane extract of the same species inhibits AChE and BChE enzymes better than its methanol extract (Wszelaki et al. 2010). This report also indicates that non-polar substances which include components of the essential oil present in the plant could be responsible for the previously reported insecticidal activity.

A considerable number of reports regarding the insecticidal activity of the *Ruta* species as well as a lack of any reports on the chemical composition of *Ruta chalepensis* from Cyprus presented a high potential for discovering new insecticidal lead compounds from this genus which prompted us to do present research. Herein, it is aimed to determine insecticidal, AChE, and BChE inhibitory activity and essential oil composition of *R. chalepensis* as a part of our phytochemical and insecticidal activity screening study of Cypriot plants.

MATERIALS AND METHODS

Plant materials

Plant samples were collected on 25 May 2015 from St. Hilarion - Kyrenia. The voucher specimen has been placed at the Herbarium of the Near East University of Cyprus' Institute of Environmental Sciences (Voucher number. 6890). Dr. Salih Gücel from the Institute of Environmental Sciences, Near East University, Nicosia, identified the plant materials.

Isolation of the essential oils

Using a Clevenger-style device, aerial parts (100 g) of the air-dried plant were hydro distilled for 3 hours to obtain essential oils with yields of 1.27% (v/w). After being dried over anhydrous Na2SO4, oil was kept in an amber vial at -20 °C until the day it was tested.

Gas chromatography-mass spectrometry analysis

With Agilent 7890B GC and 5977B EI MSD equipment, the GC-MS analysis was carried out. Samples of essential oils were diluted by 1/10 (v/v) in n-hexane. Temperatures were set at 250 ˚C for the MS transfer line and injector. The 50:1 split ratio was chosen. Both GC/MS studies employed an Innowax FSC column (60 m x 0.25 mm, 0.25 m film thickness) and helium as the carrier gas (1 ml/min). The temperature of the oven was set at 60 °C for 10 minutes before being increased to 220 °C at a rate of 4 °C/min. After being held at 220 °C for 10 minutes, the temperature was increased to 240 °C at a rate of 1 °C/min. At 70 eV, mass spectra covering the m/z range of 35 to 425 were captured. The integration of the peaks in MS chromatograms was used to determine the relative percentage quantities of the separated substances. The components of essential oils were identified. The commercial Wiley 8th Ed./NIST 05 Mass Spectra Library, the Adams Essential Oil Mass Spectral Library, and the Pallisade 600K Complete Mass Spectral Library were used for computer matching throughout the mass spectra comparison process. The findings of the analysis were presented as the mean standard deviation after being performed in triplicate. Figure 1 shows the GC-MS chromatogram for the study of *Ruta chalepensis* aerial parts oil.

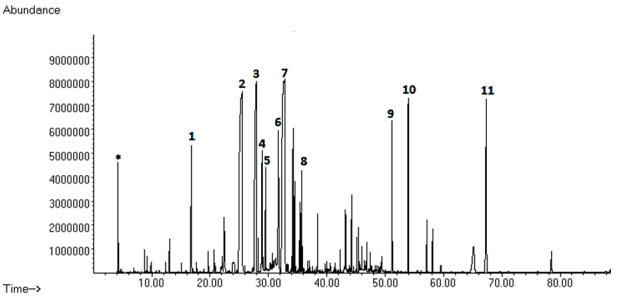

Figure 1. GC/MS Chromatogram of *Ruta chalepensis* aerial parts oil. Compounds: *. Solvent peak (n-hexane); 1. Limonene; 2. 2-Nonanone; 3. 2-Nonyl acetate; 4. 2-Decanone; 5. 2-Nonanol; 6. Pregeijerene; 7. 2-Undecanone; 8. 2-Dodecanone; 9. Moskachan A; 10. Moskachan B; 11. Moskachan D

Stored product insect cultures

Sitophilus oryzae, Sitophilus granarius, Rhyzopertha dominica, Tribolium confusum and *Tribolium castaneum* were collected from the infested stored products in Türkiye. Insect cultures were prepared at Çankırı Karatekin University, Yapraklı Vocational School, in the laboratory of the Animal and Plant Production Department according to the previous methods (Karakoç et al. 2006, Pimentel et al. 2008).

Insecticidal activity assays

Insecticidal contact toxicity assay

To create a 10% (v/v) stock solution, *Ruta chalepensis* essential oil was diluted with acetone. The dorsal side of the thorax of the insects was exposed to a stock solution of oil sample (1 µl) using a 50 µl Hamilton syringe (Gokce et al. 2010). The same volume of acetone was used in the empty controls. Following the sample or blank application, the insects were transferred to 60 mm glass petri plates containing 5 g of either whole wheat (for *S. granarius* and *S. oryzae*) or broken wheat (for *T. castaneum, T. confusum* and *R. dominica*) (n = 10 for each bug species and treatments). For 72h the insects were raised at 50 ± 10% relative humidity and 27 ± 2 °C. At 24, 48, and 72 hours, the samples and controls were observed, and the number of dead insects was noted. Each sample and blank treatment were made at least three times.

Insecticidal fumigant toxicity assay

The fumigant toxicity of *Ruta chalepensis* essential oil against the mentioned insects was determined according to a previous protocol (Çam et al. 2012). At the 48th hour, samples and controls were checked, and the number of dead insects was noted. Each sample and blank treatment were made at least three times.

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity

The previously mentioned technique was used to assess the *Ruta chalepensis* essential oil's inhibitory effect on AChE and BChE (Ellman et al. 1961). AChE (Electric Eel) or BChE was added to the reaction mixture at a concentration of 0.0325 U/ml to initiate reactions. The average and standard deviation of three parallel tests were used to display the experiment's results.

Statistical analysis

In order to calculate the arcsin values, the activity findings from the insecticidal activity assays were converted into % death values (Zar 1996). ANOVA was performed on the arcsin values before Tukey's multiple comparison tests with a $P<0.05$ significance threshold. The software package Minitab Release 14 (McKenzie and Goldman 2005) was used for all of the statistical analyses.

RESULTS

The essential oil composition of aerial parts of *Ruta chalepensis* was given in Table 1. Seventy-nine compounds were detected representing 85.93 ± 1.08 % (n = 3) of *R. chalepensis* aerial parts oil. The essential oil was dominated by the derivatives of n-alkanes. The major components of the essential oil were 2-undecanone 21.52 ± 0.21%, 2-nonanone 18.31 ± 0.27%, and 2-nonyl acetate 13.22%.

However, there were differences in the relative percent amount of the major components of the essential oil as well as the composition of the secondary components of the oil. The essential oil investigated in the present study afforded moskachan derivatives as the components that have a relative percentage in the range of 2-5% very similar to a previous study (Joulain et al. 1991) unlike the other studies.

The insecticidal contact toxicity of the *Ruta chalepensis* oil against *S. granarius, S. oryzae, T. castaneum, T. confusum* and *R. dominica* was given in Table 2. *R. chalepensis* essential oil was active against all the insects with different LD50 and LD90 values. The highest activity of the oil was observed against *R. dominica* with 0.018 µl/insect LD50 and 0.039 µl/insect LD90 after 24h. The highest activity was observed after 24h of application against all insect species except for *R. dominica*. The oil showed the highest activity after 24h against *R. dominica* and there was no change after 48h and 72h of applications. *S. oryzae* and *S. granarius* were also affected considerably with 0.50 and 0.59 µl/insect LD50 after 24h respectively. However, this activity increased with longer exposure times and produced lower LD50 values. The lowest contact toxicity was observed against *T. castaneum* and *T. confusum* at 0.138 and 0.078 µl/insect LD50 after 24h respectively. The contact toxicity of the essential oil slightly increased at longer exposure periods for *T. confusum* and *T. castaneum*.

The fumigant toxicity of the *Ruta chalepensis* oil against *S. granarius, S. oryzae, T. castaneum, T. confusum* and *R. dominica* was given in Table 3. The highest fumigant toxicity was observed against *S. granarius* for the application concentration of 10 µl, 10% oil/acetone (v:v) in a 10 ml chamber which afforded 100.00 ± 0.00% mortality. The toxicity of the oil produced by the oil was decreased to 98.86 ± 3.41% mortality at 5 µl and to 0.00 ± 0.00% mortality at 2.5 µl application of the oil solution for *S. granarius*. The essential oil also produced high fumigant toxicity against *S. oryzae, T. castaneum* and *R. dominica* which were 95.47 ± 3.41%, 93.30 ± 5.54% and 85.47 ± 3.41% mortality at 20 µl

Table 1. The essential oil composition of aerial parts of *Ruta chalepensis*

No	RRI[1]	RRI Lit.[2]	Compound	I[3]	II	III	Ave[4]	SD[5]	IM[6]	Literature
1	1023	1032	α-Pinene	0.24	0.21	0.22	0.22	0.02	RI[7], MS[8]	Başer et al. 2000a)
2	1027	1176	α-Thujene	0.02	0.02	0.02	0.02	0.00	RI, MS	(Tabanca et al. 2011)
3	1033	1035	2-Methyl-3-buten-2-ol	0.18	0.17	0.17	0.17	0.01	RI, MS	(Başer et al. 2000b)
4	1038	1042	Isopropyl butyrate	0.01	0.01	0.01	0.01	0.00	RI, MS	(Narain et al. 2007)
5	1051	1061	Isopropyl-2-methyl butyrate	0.13	0.12	0.13	0.13	0.01	RI, MS	(Başer et al. 2002a)
6	1069	1079	Ethyl isovalerate	0.01	0.01	0.01	0.01	0.00	RI, MS	(Kamatou et al. 2008)
7	1075	1083	Butanoic acid, 3-methyl-1methylethyl ester	0.02	0.02	0.02	0.02	0.00	RI, MS	(Kaya et al. 2003)
8	1087	1093	Hexanal	0.03	0.03	0.03	0.03	0.00	RI, MS	(Başer et al. 2000a)
9	1112	1118	β-Pinene	0.13	0.12	0.12	0.12	0.01	RI, MS	(Başer et al. 2000a)
10	1126	1132	Sabinene	0.4	0.38	0.38	0.39	0.01	RI, MS	(Başer et al. 2000a)
11	1168	1174	Myrcene	0.11	0.11	0.11	0.11	0.00	RI, MS	(Başer et al. 2001)
12	1184	1188	α-Terpinene	0.03	0.03	0.03	0.03	0.00	RI, MS	(Başer et al. 2000a)
13	1186	1192	2-Heptanone	0.03	0.03	0.03	0.03	0.00	RI, MS	(Başer et al. 2004)
14	1205	1203	Limonene	2.49	2.34	2.4	2.41	0.08	RI, MS	(Başer et al. 2000a)
15	1210	1213	1,8-Cineole	0.05	0.05	0.05	0.05	0.00	RI, MS	(Başer et al. 2001)
16	1215	1213	4-Methoxy-2-methyl-2-mercaptobutane	0.01	0.01	0.01	0.01	0.00	RI, MS	(Kumazawa and Masuda 1999).
17	1224	1232	(E)-2-Hexanal	0.11	0.1	0.1	0.10	0.01	RI, MS	(Kırımer et al. 2000)
18	1238	1244	2-Pentylfuran	0.03	0.03	0.03	0.03	0.00	RI, MS	(Başer et al. 2000a)
19	1251	1255	γ-Terpinene	0.06	0.06	0.06	0.06	0.00	RI, MS	(Başer et al. 2000a)
20	1258	1265	(E)-β-Ocimene	0.01	0.01	0.01	0.01	0.00	RI, MS	(Başer et al. 2002b)
21	1277	1278	m-Cymene	0.06	0.01	0.01	0.03	0.03	RI, MS	(Kılıç et al. 2010)
22	1290	1295	2-Octanone	0.3	0.29	0.29	0.29	0.01	RI, MS	(Başer et al. 2006)
23	1294	1296	Octanal	0.14	0.14	0.14	0.14	0.00	RI, MS	(Başer et al. 2001)
24	1318		Geijerene derivative I	0.08	0.08	0.08	0.08	0.00	MS	
25	1332	1338	Geijerene	1.64	1.53	1.72	1.63	0.10	RI, MS	(Tabanca et al. 2007)
26	1344	1348	6-Methyl-5-hepten-2-one	0.01	0.01	0.01	0.01	0.00	RI, MS	(Başer et al. 2000a)
27	1387	1391	(Z)-3-Hexen-1-ol	0.04	0.04	0.03	0.04	0.01	RI, MS	(Kırımer et al. 2000)

28	1410	1398	2-Nonanone	18.58	18.04	18.32	18.31	0.27	RI, MS	(Başer et al. 2006)
29	1423	1426	2-Octanol	0.08	0.08	0.08	0.08	0.00	RI, MS	(Van Vuuren et al. 2006)
30	1479	1470	2-Nonyl acetate	13.41	13.04	13.21	13.22	0.19	RI, MS	(Van Vuuren et al. 2007)
31	1486	1483	Acetic acid octyl ester	0.31	0.31	0.31	0.31	0.00	RI, MS	(Başer et al. 2000c)
32	1501	1493	Isogeijerene c	0.03	0.03	0.03	0.03	0.00	RI, MS	(Tabanca et al. 2007)
33	1505	1491	2-Decanone	2.3	2.21	2.23	2.25	0.05	RI, MS	(Bendimerad et al. 2005)
34	1509	1506	Decanal	0.23	0.24	0.23	0.23	0.01	RI, MS	(Başer et al. 2001)
35	1516	1516	Geyrene	0.06	0.06	0.06	0.06	0.00	RI, MS	(Das 2015)
36	1524	1521	2-Nonanol	1.98	1.93	1.93	1.95	0.03	RI, MS	(Kıvçak et al. 2004)
37	1528		Geijerene derivative II	0.14	0.14	0.14	0.14	0.00	MS	
38	1535		Geijerene derivative III	0.04	0.04	0.04	0.04	0.00	MS	
39	1556		Geijerene derivative IV	0.13	0.14	0.13	0.13	0.01	MS	
40	1577	1572	Pregeijerene B	0.92	0.9	0.88	0.90	0.02	RI, MS	
41	1591	1594	Pregeijerene	3.68	3.61	3.5	3.60	0.09	RI, MS	(Tabanca et al. 2005)
42	1628	1604	2-Undecanone	21.73	21.32	21.51	21.52	0.21	RI, MS	(Kırımer et al. 2000)
43	1635	1651	6-Methyl-1-octanol	0.07	0.07	0.07	0.07	0.00	RI, MS	(Wen et al. 2014)
44	1665	1664	1-Nonanol	0.23	0.23	0.23	0.23	0.00	RI, MS	(Başer et al. 2001)
45	1682	1674	2-Dodecanone	1.19	1.15	1.15	1.16	0.02	RI, MS	(Kawakami and Kobayashi 1991)
46	1688	1687	Decyl acetate	0.07	0.07	0.07	0.07	0.00	RI, MS	(Tabanca et al. 2006)
47	1694	1694	p-Vinylanisole	0.01	0.01	0.01	0.01	0.00	RI, MS	Başer et al. 2002b)
48	1698	1676	1,8-Menthadien-4-ol	0.03	0.03	0.02	0.03	0.01	RI, MS	(Kollmanns-berger et al. 1992)
49	1707	1706	α-Terpineol	0.3	0.29	0.29	0.29	0.01	RI, MS	(Başer et al. 2001)
50	1715	1766	1-Decanol	0.73	0.71	0.71	0.72	0.01	RI, MS	(Kırımer et al. 2000)
51	1719	1719	1-Heptadecene	0.02	0.01	0.01	0.01	0.01	RI, MS	(Kamariah et al. 1999)
52	1724	1722	2-Undecanol	1.36	1.35	1.35	1.35	0.01	RI, MS	(Kırımer et al. 2000)
53	1735	1728	(Z,E)-α-Farnesene	0.05	0.05	0.05	0.05	0.00	RI, MS	(Özek et al. 2010)
54	1758	1758	(E,E)-α-Farnesene (= α-farnesene)	0.12	0.12	0.12	0.12	0.00	RI, MS	(Demirci et al. 2006)
55	1766	1766	1-Decanol	0.04	0.04	0.04	0.04	0.00	RI, MS	(Kırımer et al. 2000)
56	1770		2-Methyl-undecanal	0.11	0.11	0.11	0.11	0.00	MS	

57	1797	1800	Methyl salicylate	0.02	0.02	0.02	0.02	0.00	RI, MS	(Kırımer et al. 2000)
58	1819	1815	2-Tridecanone	0.62	0.67	0.6	0.63	0.04	RI, MS	(Demirci et al. 2006)
59	1837	1835	β-Damascenone	0.01	0.01	0.01	0.01	0.00	RI, MS	(Başer et al. 2000a)
60	1845	1845	trans-Carveol	0.01	0.01	0.01	0.01	0.00	RI, MS	(Başer et al. 2000a)
61	1901	1904	Ethyl dihydrocinnamate	0.1	0.1	0.1	0.10	0.00	RI, MS	(Polatoğlu et al. 2010)
62	1955	1958	(E)-β-Ionone	0.04	0.04	0.04	0.04	0.00	RI, MS	(Demirci et al. 2006)
63	2026	2029	Methyl eugenol	0.04	0.05	0.04	0.04	0.01	RI, MS	(Başer et al. 2001)
64	2093	2099	Hedycaryol	0.4	0.4	0.39	0.40	0.01	RI, MS	(Joichi et al. 2005)
65	2101	2181	Anthranilic acid	0.17	0.17	0.15	0.16	0.01	RI, MS	(Pino et al. 2009)
66	2134	2131	Hexahydrofarnesyl acetone	0.06	0.05	0.07	0.06	0.01	RI, MS	(Başer et al. 2000a)
67	2186	2185	γ-Eudesmol	0.07	0.07	0.06	0.07	0.01	RI, MS	(Başer et al. 2001)
68	2197	2192	τ-Cadinol	0.02	0.02	0.02	0.02	0.00	RI, MS	(Tümen et al.1998)
69	2241	2250	α-Eudesmol	0.06	0.06	0.06	0.06	0.00	RI, MS	(Başer et al. 2001)
70	2251	2257	β-Eudesmol	0.09	0.09	0.08	0.09	0.01	RI, MS	(Demirci et al. 2006)
71	2342		Moskachan A	1.95	1.93	1.91	1.93	0.02	MS	
72	2383	2181	1-Nonadecanol	0.03	0.03	0.03	0.03	0.00	RI, MS	
73	2400	2400	Tetracosane	0.02	0.02	0.02	0.02	0.00	RI, MS, Ac[9]	
74	2449		Moskachan B	2.77	2.75	2.72	2.75	0.03	MS	
75	2504	2500	Pentacosane	0.04	0.04	0.04	0.04	0.00	RI, MS, Ac	
76	2551		4-(3,4-Methylenedioxy-phenyl)-2-butanone	0.77	0.77	0.77	0.77	0.00	MS	
77	2617	2622	Phytol	0.11	0.11	0.11	0.11	0.00	RI, MS	(Kırımer et al. 2000)
78	2700	2700	Heptacosane	0.03	0.02	0.01	0.02	0.01	RI, MS, Ac	
79	2793		Moskachan D	5.38	5.33	5.3	5.34	0.04	MS	
			Monoterpenes	10.27	9.82	9.94	10.01	0.23		
			Oxygenated Monoterpenes	0.44	0.43	0.42	0.43	0.01		
			Sesquiterpenes	0.17	0.17	0.17	0.17	0.00		
			Oxygenated Sesquiterpenes	10.80	10.70	10.61	10.70	0.10		
			Diterpenes	0.11	0.11	0.11	0.11	0.00		
			Others	65.34	63.82	64.36	64.51	0.77		
			Total	87.13	85.05	85.61	85.93	1.08		

[1]Relative retention index; [2]Relative retention index of the compound given in the literature that uses similar methodology; [3]The GC-MS analyses were performed in triplicate and results were given as relative percent amounts calculated from the chromatograms; [4]Average relative percent amounts obtained from the analyses; [5]Standard deviation of the relative percent amounts; [6]Identification method used for the compound; [7]RI: Relative retention index match of the compound with the literature that uses same/similar chromatographic conditions; [8]MS: Mass spectra match of the compound; [9]Ac: Relative retention index and mass spectra similarity of the compound with the authentic compound (co-injection).

Table 2. The contact toxicity of the *Ruta chalepensis* oil given for 24, 48, and 72h periods as LD50 and LD90

Insect Species	24 h				48 h				72 h			
	LD_{50} (µL/insect) (Fiducial limits)	LD90 (µL/insect) (Fiducial limits)	Slope±SE	X^2	LD_{50} (µL/insect) (Fiducial limits)	LD90 (µL/insect) (Fiducial limits)	Slope±SE	X^2	LD_{50} (µL/insect) (Fiducial limits)	LD90 (µL/insect) (Fiducial limits)	Slope±SE	X^2
S. granarius	0.059 (0.052-0.067)	0.107 (0.089-0.141)	5.02±0.77	7.98	0.052 (0.045-0.059)	0.096 (0.081-0.125)	4.76±0.66	11.37	0.052 (0.045-0.059)	0.096 (0.081-0.125)	4.76±0.66	11.37
S. oryzae	0.050 (0.040-0.061)	0.090 (0.072-0.138)	4.97±0.69	22.94	0.046 (0.038-0.053)	0.083 (0.069-0.110)	4.92±0.67	13.94	0.040 (0.034-0.045)	0.071 (0.061-0.088)	5.06±0.68	7.93
T. castaneum	0.138 (0.118-0.162)	0.327 (0.259-0.480)	3.42±0.49	11.65	0.116 (0.092-0.146)	0.316 (0.231-0.559)	2.95±0.39	22.57	0.111 (0.089-0.137)	0.311 (0.230-0.522)	2.86±0.38	19.43
T. confusum	0.078 (0.060-0.099)	0.340 (0.238-0.628)	2.01±0.31	11.90	0.078 (0.060-0.099)	0.340 (0.238-0.628)	2.01±0.31	11.90	0.073 (0.055-0.093)	0.324 (0.227± 0.597)	2.011±0.31	12.48
R. dominica	0.018 (0.011-0.023)	0.039 (0.029-0.066)	3.83±0.59	11.63	0.018 (0.014-0.022)	0.038 (0.029-0.062)	3.92±0.60	11.31	0.018 (0.014-0.022)	0.038 (0.029-0.062)	3.92±0.60	11.31

application concentration of the oil solution in acetone. The fumigant toxicity of the oil was concentration-dependent. According to the results presented in Table 3, the most susceptible insect species was *S. granarius* to the essential oil of *R. chalapensis*. The essential oil did not produce any fumigant toxicity against *T. confusum*.

The contact toxicity study of the essential oil provided a different activity profile than the fumigant toxicity study. The highest contact toxicity was observed for *R. dominica* whereas the highest fumigant toxicity was observed for *S. granarius*. This fact states that active substances were less active in the vapor phase for *R. dominica*. In contact toxicity, this fact could be explained by different cuticle penetration amounts of active substances in different insects.

The AChE and BChE inhibitory activity of the oil was determined at 20 µl application volume of 10 mg/ml oil solution in methanol in the assay mixture. The oil produced 5.29 ± 1.20% inhibition of the AChE and 42.6 ± 0.71% inhibition of BChE. The oil produced very low AChE inhibition and mediocre BChE inhibition, therefore observed insecticidal activity could be related to another mechanism than the action on the cholinergic system.

DISCUSSION

Previously insecticidal activity of 2-undecanone was reported against *Keiferia lycopersicella* (Walsingham) and *Spodoptera exigua* (Hübner) of Lepidoptera (Lin et al. 1987). Additionally, the biting deterrence and larvicidal activity of *R. chalepensis* essential oil, 2-undeacanone, 2-nonanone and 2-nonyl acetate against *Aedes aegypti* L. and *Anopheles quadrimaculatus* Say. were documented in detail (Ali et al. 2013). The insecticidal activity observed for *R. chalepensis* essential oil could be related to the major components namely 2-undeacanone, 2-nonanone, and 2-nonyl acetate.

The essential oil of *Ruta chalepensis* from Cyprus has very high fumigant insecticidal activity against *S. granaries* and

Table 3. The fumigant toxicity of the *Ruta chalepensis* oil given for 48 h period as % mortality

Test insects	Mortality±SD (%)				
	Doses (µl (10% (v/v) oil/acetone)[1]				
	Control[2]	20	10	5	2.5
Sitophilus granarius	0.00±0.00	100.00±0.00	100.00±0.00	98.86±3.41	0.00±0.00
Sitophilus oryzae	0.00±0.00	95.47±3.41	56.69±0.34	25.00±5.54	N.A.
Tribolium castaneum	0.00±0.00	93.30±5.54	80.69±1.66	4.53±3.41	N.A.
Tribolium confusum	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	N.A.
Rhyzopertha dominica	0.00±0.00	85.47±3.41	53.35±1.35	6.70±5.54	N.A.

[1]The volume (µl) of essential oil solution (essential oil is diluted with acetone 10% (v/v)) applied in the fumigant insecticidal activity assay (chamber size is 10 ml). [2]Control (blank) is essential oil-free acetone.

S. oryzae, Ryzopherta dominica and *Tribolium castaneum*. The oil also produced considerably high insecticidal activity against *Ryzopherta dominica, S. granaries,* and *S. oryzae*. The major components of the *Ruta chalepensis* oil namely 2-undeacanone, 2-nonanone, and 2-nonyl acetate were previously reported to have insecticidal and repellent activity against other insect species (Ali et al. 2013, Lin et al. 1987). Consequently, these substances are thought to be the cause of the insecticidal effects seen in the current study on the species under investigation. However, the insecticidal activity of 2-undeacanone, 2-nonanone, and 2-nonyl acetate should be investigated in detail against *S. granarius, S. oryzae, T. castaneum, T. confusum* and *R. dominica* to prove their activity. Additionally, the *R. chalepensis* essential oils from different locations were reported to have seed germination inhibition against *Triticum durum* Desf. and *Phalaris canariensis* L. which contains 2-undeacanone and 2-nonanone major compounds (Bouajaj et al. 2014, Mejri et al. 2012). The inhibitory activity of 2-undecanone and 2-nonanone on germination of radish was reported to be 38% and 30% respectively which could be related to low inhibition values (De Feo et al. 2002). The essential oil of *R. chalepensis* appears to be a promising source from which natural compounds can be extracted that can be used in the pest control of stored product.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Ruta chalepensis L.'nin toprak üstü aksamından elde edilen uçucu yağ kompozisyonu GC-MS ile analiz edildi. Uçucu yağın %85.93 ± 1.08 (n = 3)'i temsil eden yetmiş dokuz bileşik tespit edildi. Uçucu yağın ana bileşenleri olarak %21.52 ± 0.21 oranında 2-undekanon, %18.31 ± 0.27 oranında 2-nonanon ve %13.22 oranında da 2-nonil asetat belirlendi. Uçucu yağı en yüksek insektisidal kontak toksisitesi 24 saat sonra 0.018 µl/böcek LD50 ve 0.039 µl/ böcek LD90 değerleri ile *Rhyzopertha dominica* F. üzerinde gözlemlenmiştir. Uçucu yağ, ayrıca *Sitophilus oryzae* L. ve *Sitophillus granarius* L. karşısında 24 saat sonra sırasıyla 0.50 ve 0.59 µl/ böcek değerleriyle oldukça düşük denebilecek LD50 değerlerini gösterdi. En düşük kontak toksisite 24 saat sonra *Tribolium castaneum* Herbst. ve *Triboliom confusum* Jacquelin du Val.'e karşı sırasıyla 0.138 ve 0.078 µl/böcek LD50 değerleri şeklinde gözlemlendi. *S. granarius*'a karşı en yüksek fumigant toksisite 48 saat sonra %100 ölüm oranını sağlayan ve 10 ml'lik haznede uygulanan 10 µl, %10 yağ/aseton (v:v) konsantrasyonunda görüldü. Uçucu yağ ayrıca *S. oryzae, T. castaneum* ve *R. dominica*'ya karşı yüksek fumigant toksisiteyi de ortaya çıkardı. 48 saat sonra 20 µl yağ çözeltisi uygulama konsantrasyonunda ölüm oranı sırasıyla %95.47 ± 3.41, %93.30 ± 5.54 ve %85.47 ± 3.41 idi. *R. chalepensis* uçucu yağı ile %5.29 ± 1.20'lik düşük bir asetilkolinesteraz enzim inhibisyonu ile %42.6 ± 0.71'lik orta derecede bir bütirilkolinesteraz enzim inhibisyonu elde edildi. Yapılan insektisidal aktivite denemelerine göre, *R. chalepensis* uçucu yağının, depo ürün zararlılarına karşı mücadelede kullanılabilecek doğal bileşiklerin elde edilmesi adına umut verici bir kaynak olduğu düşünülmektedir.

Anahtar Kelimeler: *Ruta chalepensis*, uçucu yağ analizi, depo ürün zararlıları, asetlkolinesteraz, bütirilkolinesteraz, enzim inhibisyonu

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Original article

qPCR analysis of *Puccinia* spp. in turfgrass areas in Türkiye

Türkiye'deki çim alanlarında *Puccinia* spp.nin qPCR analizi

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ABSTRACT

In recent years, with the increase in parks, gardens, recreation areas, picnic areas and refuges with urbanization, the interest in turfgrass areas that beautify the aesthetic appearance of these areas has also increased. In these areas, rust diseases are also becoming increasingly common and causing problems. Rust diseases are an important pathogen group that needs to be monitored due to their ability to create new races and their airborne transmission. In this study, surveys were conducted in turfgrass areas in 8 provinces of Türkiye and 110 turfgrass leaf samples showing rust symptoms were collected. As a result of DNA isolation and qPCR analysis from pustules on the leaf surface, 37 *Puccinia coronata*, 32 *P. graminis*, 18 *P. striiformis* and 23 *Puccinia* spp. have been detected. It was determined that the most common rust species that causes disease in turfgrass areas in Türkiye is *P. coronata*. This species was followed by *P. graminis* and *P. striiformis*, respectively. While *P. striiformis* was mostly isolated from Kayseri and Istanbul provinces, *P. coronata* and *P. graminis* were mostly isolated from İstanbul.

INTRODUCTION

Puccinia genus is a group of fungi that includes airborne and obligate pathogens species that cause problems in turfgrass areas. The agents that cause rust diseases in turfgrass damage the leaves due to the pustules they form on turfgrass leaves, seriously deteriorate the quality of the turfgrass and can cause great economic losses. The increase in rust diseases is closely related to temperature and humidity. When the disease first begins, yellow-orange urediospore pustules appear on the leaves, which causes necrosis on the leaves in later periods. In time, the pustules spreads on the leaves and absorbs nutrients in plant cells, destroys chlorophyll and prevents the plant from performing photosynthesis and respiration, which negatively affects the physiology and general health of the plant. All turfgrass varieties are sensitive to the disease (Smiley et al. 2005), and it has been determined that it can be seen in all regions of Türkiye when suitable conditions for disease development occur.

There are currently a total of 39 different species of rust fungi (*Puccinia, Physopella* and *Uromyces*) known to be pathogenic to turfgrasses (Smiley et al. 2005). But *Puccinia coronata* Corda (crown rust), *Puccinia graminis* Pers (stem rust),

Puccinia striiformis Westend (stripe rust), *Puccinia recondita* Roberge ex Desmaz. and *Puccinia brachypodii* G. Otth (leaf rusts) are considered the most prevalent rust pathogens of turfgrass (Karakkat et al. 2018, Smiley et al. 2005).

While intermediate hosts, spore structures on intermediate and main hosts, their biology of rust diseases other than turfgrass plants have been examined in more detail, less information is available on turfgrass rusts than on rust diseases in other cereals. To develop different methods of control against a pathogen, such as resistant varieties, all morphological forms and the biology of the agent must be well known. Since intermediate hosts come into play in the biology of rusts, it is difficult to follow them. Additionally, since there are so many different *Puccinia* species, it is difficult to distinguish their spore differences visually and the risk of making mistakes is high. Before the development of molecular techniques, studies such as effective control strategies and the development of resistant varieties in turfgrass rust diseases were difficult due to the insufficient information available about these factors. Given the enormous economic and aesthetic problems associated with turfgrass rust diseases and the confusion in accurately identifying and classifying these pathogens, improving turfgrass breeding programs and expanding knowledge about the turfgrass rust system is an important priority. Evaluating these fungi at the molecular level, developing an accurate identification procedure, and implementing an effective inoculation protocol to maintain pathogen cultures will improve the ability to study turfgrass rust fungi.

It is reported that if rust diseases are not controlled, they negatively affect turfgrass seed production and this causes significant losses in the seed production industry. Studies report that rust diseases reduce seed yield by 25% - 98% (Beirn et al. 2011, Pfender 2009). Since rusts are diseases that can spread very quickly with the wind and constantly create new races, it has become important to monitor them regularly and develop fast and accurate diagnostic methods take new precautions and develop strategies. In addition, since the sensitivity of different fungi to fungicides varies, correct identification of the fungus is essential for effective control of the disease. Classical diagnosis of *Puccinia* species is usually made based on the shape, size and color of their spores under the microscope (Zhang et al. 2022). However, the fastest and most accurate diagnosis is important in distinguishing the species. Molecular analysis methods are considered the most reliable and fastest method for rust fungi (Stackhouse et al. 2020). Quantitative real-time polymerase chain reaction (qPCR) is a rapid, accurate, and highly sensitive molecular detection method widely used to quantify pathogenic fungi, viruses and bacteria, and fungi from a wide variety of hosts (Mirmajlessi et al. 2015, Stackhouse et al. 2020). Several PCR-based molecular assays have been developed for turfgrass pathogens, including SYBR green probes to identify *Ophiosphaerella agrostis* in *Agrostis stolonifera* (creeping bentgrass), a set of real-time qPCR assays for the detection of Puccinia species causing turfgrass rust diseases, and a TaqMan assay to detect *Magnaporthiopsis* poae in Kentucky bluegrass (Beirn et al. 2011, Kaminski et al. 2005, Zhao et al. 2012). This study is the first comprehensive rust survey and detection study conducted on turfgrass areas in Türkiye.

MATERIALS AND METHODS

Survey

In the survey studies in 2015, leaf samples with rust symptoms were collected from parks and gardens, recreation areas, and refuges in İstanbul, Ankara, Kayseri, Bursa, Antalya, İzmir, Aydın and Muğla provinces. Depending on the size of the controlled land, in the surveys made to represent that area, 5 samples up to 5000 m2, 10 samples between 5000-10000 m2, 15 samples up to 10000 m2-15000 m2, 20 samples from areas larger than 15000 m2 were collected (Aktaş 2001). The collected leaves were placed in ice boxes and brought to the laboratory for molecular identification.

Real-time PCR analysis

For molecular identification, urediniospores were carefully scraped on turfgrass leaves and transferred into 1.5 ml microtubes, DNAs were extracted using DNeasy Plant Mini Kit (Qiagen, USA). Real-timePCR studies were carried out using different specific primers and probes (Table 1) (Beirn et al. 2011) with genomic DNA obtained from plants with rust symptoms and rust types were identified. Roche LightCycler 480 II Real time-PCR device was used in the study and the raw data were analyzed with the "Absolute Quantification/Second Derivative Maximum" method in Lightcycler 1.5 software and the results were obtained. In addition, the raw data were examined with the "Fit point" method and the effects of Background, log phase and plateau phase on CP (Crossing Point) values were evaluated.

In real-timePCR studies, the Roche Lightcycler® Probes Master ready mix was used, taking into account the optimum values specified in the kit manual. In a preliminary study, the suitability of the device for "ramp rate (°C/s)" and waiting times at relevant temperatures were tested for a total of 10-20 ng template DNA and 20 µl reaction volume. In addition, the optimum binding temperature was 60 oC and the two-step (denaturation and binding/extension) replication step was found to be more efficient and accordingly; PCR reaction for each sample; 10 µl 2x Roche Probe Master Mix, 2 µl primer/probe mix (containing 10 mM primer 1, 10 mM primer 2 and 10 mM probe), 3.0 µl molecular biological purity water, 5 µl template DNA (10-20 ng) It was carried out in a total reaction volume of 20 µl. Real-time PCR conditions are; initial denaturation 95 °C 10 min, denaturation 95 °C 15 sec, binding/extension 60 °C 60

Table 1. Polymerase chain reaction primers and hydrolysis probes used in this study

Primers and Probes	
FrITS2Cr (Puccinia coronata forward ITS2);	3'-TTTGTGGATGTTGAGTGTTGC-5'
RrITS2Cr (P. coronata reverse ITS2);	3'-TCCCACCTGATTTGAGGTCT-5'
FrITS1Pu (P. graminis and P. striiformis forward ITS1);	3'-CCTGCGGAAGGATCATTATT-5'
RrITS1Pu (P. graminis and P. striiformis reverse ITS1);	3'-TTTGGTTACATTCATTTAAACTTGTG-5'
PuSTM-ITS1 (Puccinia graminis probe);	3'-Cy3-TTAGAGTGCACTTTATTGTGGCTCAACTCTCT-BHQ1-5'
PuSTR-ITS1 (Puccinia striiformis probe);	3'-FAMCGTAACTTCTTTATTGAATGTTGCATTACCCTCCC-BHQ1-5'
PuCR-ITS2 (Puccinia coronata probe);	3'-FAM-TACTTGCCATCTTTTGAAAGGAGGGA-BHQ1-5'

sec, cooling 40 °C 5 min.

RESULTS AND DISCUSSION

As a result of the survey studies, 110 leaf samples showing rust symptoms were taken from grass areas in 8 provinces. In field observations, it was observed that the rust disease started as spots ranging from light green to yellow on the leaves, leaf sheaths and stems, and advanced symptoms turned into regular or scattered yellow, orange or brick red pustules, depending on the type of rust (Figure 1).

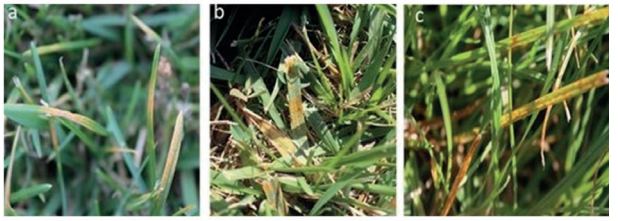

Figure 1. Symptoms of *Puccinia striiformis* (a), *Puccinia graminis* (b) and *Puccinia coronata* (c) on turfgrass leaves in the survey areas

Puccinia spp. were identified by real-time PCR studies using different specific primers and probes. As a result of the identification studies, 37 *P. coronata*, 32 *P. graminis* and 18 *P. striiformis* species were identified. No results were obtained from 23 samples with the primers and probes used. It was concluded that these 23 rust species belong to species other than the three species. Rust species were seen in all regions, but *P. striiformis* was more common in Kayseri and İstanbul provinces (Table 2). *P. coronata* and *P. graminis* were mostly isolated from İstanbul.

As a result of the real-time PCR study, the most common rust species that cause disease in turfgrass areas in Türkiye was determined to be *Puccinia coronata*. This species was followed by *P. graminis* and *P. striiformis*, respectively. The frequency of *P. coronata, P. graminis* and *P. striiformis* isolation was 33.63%, 29.09% and 16.36%, respectively. It was concluded that 23 rust species belonged to the other rust species that were not included in the three species, as the primers and probes used for the three species did not give the desired curves (Figure 2). Among the *Puccinia* spp. in turfgrass areas, only these three species can be identified molecularly (Stackhouse et al. 2020). Studies should be carried out to diagnose other species of rust using molecular methods, and effective primers and probes need to be developed. In this regard, a study was conducted in the USA, 66 rust-infected turfgrass samples were collected from different regions of the USA and these collected isolates were evaluated by qPCR study using primers and probes developed from the ITS-5.8 rDNA region. Analysis results showed that 67% of the samples were P. coronata, 28% were *P. graminis* and 5% were *P. striiformis* (Beirn et al. 2011). In this study, the most isolated species was P. coronata and all three rust species were detected in all provinces of Türkiye. *P. graminis* and *P. striiformis* are also common rust species in wheat production areas in Türkiye (Aktaş 2001).

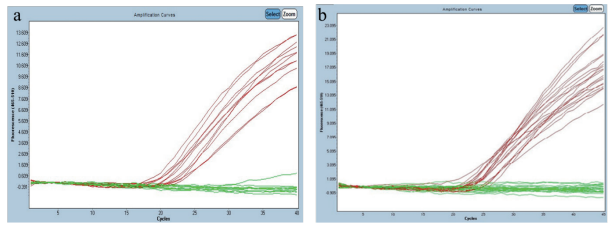

Figure 2. Amplification curves of some samples obtained in real-time PCR with *Puccinia graminis* (a) and *Puccinia striiformis* (b) specific primers and probes (red curves refer to positive samples, green curves refer to negative samples and the template belongs to the control without DNA

Most turfgrass varieties used in the turfgrass areas in Türkiye are sensitive to rust diseases (Smiley et al. 2005), and it has been determined that rust diseases can be seen in all regions of Türkiye when suitable conditions for disease development occur. Rust fungi, located in the Pucciniales order of the Basidiomycetes class, constitute the largest group of obligate pathogenic fungi. It is estimated that there are more than 7000 species of rust in the world, and only 3/4 of them have been fully identified (Hawksworth et al.

Table 2. The number of samples taken from the provinces, determined rust species, numbers and percent occurrence rates of rust species

Provinces	Number of leaf samples with rust symptoms taken	*P. coronata*	*P. graminis*	*P. striiformis*	Other rust species
İstanbul	26	10	7	4	5
Ankara	19	8	5	1	5
Bursa	18	6	6	2	4
Kayseri	18	5	4	7	2
İzmir	10	4	3	1	2
Aydın	7	2	3	1	1
Muğla	6	1	2	1	2
Antalya	6	1	2	1	2
	110	37	32	18	23
Percent occurrence rates (%)		33.63	29.09	16.36	20.90

1995). The largest genus, *Puccinia,* contains approximately 4000 species, nearly 650 of which are pathogenic to various plants within the Poaceae family (Abbasi 1996). There are currently a total of 39 different species of rust fungi (belonging to *Puccinia, Physopella* and *Uromyces* genus) known to be pathogenic to turfgrass but 10 of these commonly cause infection in turfgrasses (Smiley et al. 2005). In another study, 56 *Puccinia* species and 14 *Uromyces* species were associated with the invasion of the Poaceae family, which includes grass and non-grass hosts (Afshan 2008). Considering the complications of morphology-based rust taxonomy, the use of molecular sequence data in conjunction with morphology has become a powerful tool for the study of these fungi at the species level (Aime et al. 2006, Beirn et al. 2011, Maier et al. 2007, Van der Merwe et al. 2007). In addition, there are many different races of rust fungi and the disease severity of these races on different turfgrass varieties varies. Some turfgrass varieties may become susceptible to some types of rust because they can create new strains over time that can also infect resistant varieties (Avasthi et al. 2023). For this reason, the detection and monitoring of rust species in turfgrass areas is important.

In a study conducted in the Midwestern United States, rust species in turfgrass areas were monitored and determined via real-time PCR between 2013 and 2015. In the study, the most isolated species from Kentucky bluegrass was *P. graminis* with a rate of 69%. This was followed by *P. coronata* with a rate of 17%. Both species of rust were detected in 13% of the samples taken. In the same study, the population of 2 rust species was monitored in the region for 3 years. Both year and location effects were observed in population distribution. Additionally, variability was observed in pathogen-host relationships in the following years. It has been revealed that rust species differ between locations in the same year, and data taken from the same location for several years also show differences in terms of rust diseases distribution and density. For this reason, the density and distribution of rust species in turfgrass areas should be monitored at regular intervals. Watkins and Gaussoin (1992) stated that the rust disease caused by *Puccinia graminis* in turgrasses is commonly seen in *Poa pratensis* (fescue grass), *Lolium perenne* (English ryegrass), *Festuca arundinaceae* (reedy fescue) and *Zoysia japonica* (Japanese ryegrass). They also stated that cool climate turfgrass plants (*P. pratensis, L. perenne, F. arundinaceae*) are damaged more severely in the summer (Smiley et al. 2005). There is currently no licensed commercial fungicide for conrol against rust diseases in turfgrass areas in Türkiye. In the future, studies should be carried out to control rust diseases in turfgrass.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Son yıllarda şehirleşmeyle birlikte park, bahçe, rekreasyon alanları, piknik alanları ve refüjlerin artmasıyla birlikte bu alanların estetik görünümünü güzelleştiren çim alanlarına ilgi de artmıştır. Bu alanlarda pas hastalıkları da giderek yaygınlaşmakta ve sorun oluşturmaktadır. Pas hastalıkları, yeni ırk oluşturma kapasiteleri ve hava yoluyla taşınmaları sebebiyle takip edilmesi gereken

önemli bir patojen grubudur. Bu çalışmada, Türkiye'nin 8 ilindeki çim alanlarında sürveyler düzenlenerek, 110 adet pas belirtisi gösteren çim yaprak örneği toplanmıştır. Yaprak yüzeyindeki püstüllerden DNA izolasyonu ve qPCR analizi sonucunda 37 adet *Puccinia coronata*, 32 adet *P. graminis,* 18 adet *P. striiformis* ve 23 adet *Puccinia* spp. tespit edilmiştir. Türkiye'de çim alanlarında hastalığa neden olan en yaygın pas türünün *P. coronata* olduğu saptanmıştır. Bu türü sırasıyla *P. graminis* ve *P. striiformis* takip etmiştir. *P. striiformis* en fazla Kayseri ve İstanbul ilinden izole edilirken, *P. coronata* ve *P. graminis* en fazla İstanbul ilinden izole edilmiştir.

Anahtar kelimeler: çim, pas, real-time PCR, Türkiye

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