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SURVEY AND POPULATION DENSITY EVALUATION OF OLIVE TREES MITES WITH APPLICATION OF SOME INTEGRATED CONTROL OF MITES PESTS

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ABSTRACT

Numerous mite species frequently affect olive trees worldwide; phytophagous mites induce damage to leaves, buds, and fruits. This research work was conducted as an exploratory assessment of the mite fauna of olive trees, its population prevalence, and the composition of control strategies by comparing the efficacy of predation of *Amblyseius swirskii* with acaricide Danisaraba 20% SC (active constituent cyflumetofen) for control of pest mites on olive trees in the open field. Results showed that 12 species from 11 genera belonging to 6 families were identified. Six species were identified as a new record for Aljouf region, Saudi Arabia. Eriophyid mites were the most prevalent phytophagous species associated with olive aerial parts. The temperature and relative humidity in various months significantly influence mites' activity and population density. At an average temperature of 20 to 40 ˚C and 12 to 30% rh, the population density of mites began to rise at the beginning of March and peaked in June. Comparison of predation of *A. swirskii* with acaricide Danisaraba 20% SC in field revealed that the enumeration of different stages of pest mites started declining gradually. The Eriophyid population recorded the lowest number on the 48th day after the second release of *A. swirskii* at a predator/prey ratio of 1:7. While population steadily increased on the 16th day after the second launch of the acaricide Danisaraba. *A. swirskii* has proved to be a promising candidate for biological control of olive trees mites compared with acaricide Danisaraba 20% SC in an open field.

Keywords: Olive mite, survey, *Amblyseius swirskii*, acaricide, integrated control.

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INTRODUCTION

One of the earliest known plant tree species is the olive, *Olea europaea* L. (Oleaceae) which is found across the Mediterranean area from Portugal to the Levant as well as in the Far East, Arabian Peninsula, and South Asia (Terral *et al.*, 2004). In Aljouf region of Saudi Arabia, there are more than thirteen million olive trees (Hemida *et al.*, 2014). The olive tree has a high concentration of essential nutrients as well as active ingredients with medicinal importance (Parvaiz *et al.*, 2013). They also added the olive fruit has a substantial concentration of hydrophilic, lipophilic, and phenolic compounds in the fresh pulp weight, which have been shown to have anticarcinogenic, antioxidant, antibacterial, laxative, antihypertensive, anti-inflammatory and antiplatelet properties. The health advantages of olive oil are ascribed mainly to the antioxidant ability of the phenolic compounds, secoiridoids, hydroxytyrosol, tyrosol, phenolic acid, and flavones (Dini *et al.*, 2020).

The polyphagous spider mite *Tetranychus urticae* Koch has been recorded on olive trees in Greece (Hatzinikolis, 1982). It is a global mite that may severely harm numerous horticultural crops grown in open fields and greenhouses; it feeds on around 1100 host plant species, with over 150 economically important species (Maleknia *et al.*, 2016; Pavela, 2017; Sousa *et al.*, 2019). Olive-infesting eriophyid, tenuipalpid, and tetranychid mites have been reported on olives in Saudi Arabia, Tunisia and Turkey (Al-Atawi and Halawa, 2011; Chatti *et al.*, 2017; Kamuran, 2020). Tzanakakis (2003) indicated that 12 of the 30 mite species on olives belonged to the family Eriophyidae. Among these mites, Hatzinikolis (1973) reported that species like *Aceria oleae* significantly inhibited the growth of olive trees and damaged olive flowers and young fruits in olive plantations.

The eriophyid mites are one of the most commercially significant phytophagous mites groups (Lindquist and Amrine Jr, 1996). They cause direct harm to their hosts and frequently transfer plant viruses, although no evidence of microbe transmission on olive trees has been found (Paliwal, 1980; Duso *et al.*, 2010). Furthermore, they have a significant invasive ability because of their capability to disperse unnoticed through windborne or human-mediated dispersion (Michalska *et al.*, 2010; Navia *et al.*, 2010).

Tenuipalpidae family, widely known as flat mites or false spider mites, is distributed all around the world, and there are 891 valid species in 34 genera; all tenuipalpid species are phytophagous, meaning they feed on the epidermal cells of stems, foliage and fruits directly, causing losses in plants (Mesa *et al.*, 2009). They also added that genera *Brevipalpus* & *Tenuipalpus* include the vast majority of species harming cultivated plants.

Phytophagous mites are serious pests agricultural ecosystems, controlled by synthetic pesticides, but pesticides adversely affect natural enemies and lead to environmental pollution, human health problems, harmful side effects, and risk of pest resistance (Fathipour and Maleknia, 2016). Mites from the family Phytoseiidae are diverse predators who live in a wide variety of plants, frequently associated with phytophagous mites and insects, reducing pest infestations significantly; Their importance in biological and integrated control has been shown in recent years (Tixier, 2018; Jorge *et al.*, 2021). After its release onto the market in 2005, *A. swirskii* had proved to have greatest effective biological control in protected agriculture (Calvo *et al.*, 2015). It is a generalist predatory mite with a great prey consumption capability that can reproduce and feed on *T. urticae*, insects, pollen, plant exudates, and honeydew (McMurtry *et al.*, 2013). It is recently the most extensively utilized as augmentative biological control agent (Knapp *et al.*, 2018).

Due to the lack of specific information regarding olive tree mites in Aljouf region of Saudi Arabia, the purpose of this study was to conduct an exploratory evaluation of mite species and population density on olive trees (*Olea europaea* L.), as well as the application of some biological and chemical control methods with a comparison between them in the study area.

MATERIALS AND METHODS

Study location: Aljouf region is located in the north of Saudi Arabia between longitudes 37˚ to 42 ˚E & latitudes 29˚ to 32 ˚N (Fig. 1). This area which is important for agriculture; is famous for its olive tree plantations. According to geographical parameters, the districts chosen for the research are homogenous from four governorates, as shown in the following Table (1) in Aljouf region.

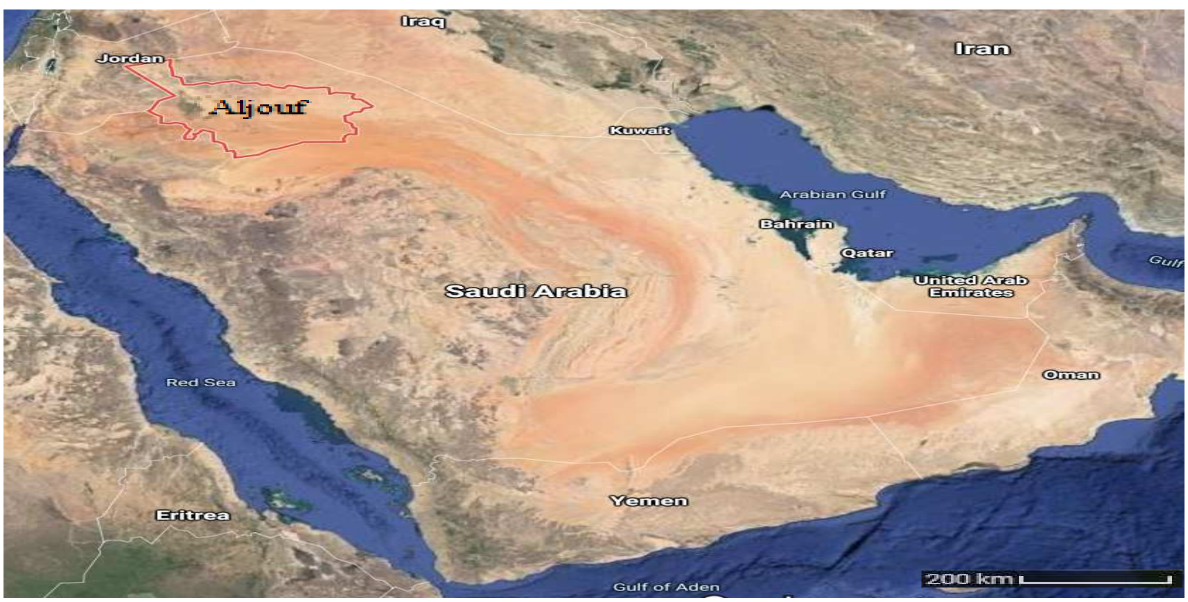


Fig. 1. Map of the Saudi Arabia in which the region of this study is specified, in the red margin (Aljouf).

Table 1. Governorate location and sea level of Aljouf region.

Governorate code	Governorate	Above sea level	Location
A	Sakaka	560 m	29.97 °N 40.21°E
B	Doumat Aljandal	580 m	29.81°N 39.86°E
C	Tubarjal	550 m	30.50 °N 38.22°E
D	Al Qurayyat	505 m	31.33 °N 37.34°E

Climate: The climate in Aljouf area in the summer is hot and dry, while in the winter cold and rainy. In January, the average low temperature is 9.76 ˚C, while in August, the average low temperature is 32.96 ˚C. Rainfall is sporadic with an annual average of 55 mm which occurs from October to May of each year (meteorological station in Sakaka).

Plant samples collection: The samples were taken from olive trees orchards of four governorates in Aljouf region (Sakaka, Doumat Aljandal, Tubarjal & Al Qurayyat) monthly during December 2020 to November 2021. From each district, an orchard was selected, ten trees located at different points from the four cardinal directions and middles in orchards were sampled, 50 cm long shoots containing leaves, buds, flowers or fruits were collected from each selected tree.

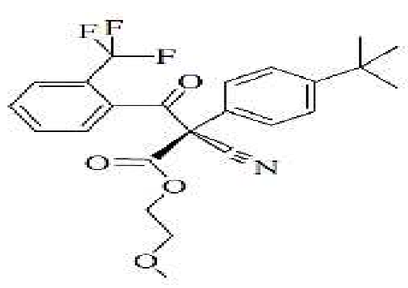
Mite identification: Method of De Lillo *et al.* (2001) was followed for extraction of Eriophyid mite species live freely on plant surfaces such as leaves, buds and fruits. Specimens collected were prepared and slide-mounted as stated by Keifer (1975), then dried for one week at 40 °C (Zhang, 2003). A compound microscope with phase contrast and a x100 objective was used to examine slide-mounted specimens. Morphological nomenclature as stated by Lindquist *et al.* (1996), and systematic classification applied by Amrine Jr *et al.* (2003) were used for identification of mites.

All other mite samples were sorted and mounted in Hoyer's medium placed on glass slides in accordance with method of Krantz (1978) under the prior microscope and recognized at the species level using suitable taxonomic references, according to Baker (1965) for Tydeidae, Chant (1965) for Phytoseiidae, Keifer (1982) & Zaher (1986) for Eriophyidae, Stigmaeidae, Tenuipalpidae, and Meyer (1987) for Tetranychidae.

Pest mites control: To study the effect of different types of control agents, two treatments were carried out on olive trees, using the predatory mites and the acaricide:

- Predatory mites *Amblyseius swirskii* (Athias-Henriot) (Acari: Mesostigmata: Phytoseiidae).
- Acaricide Danisaraba 20% SC is a commercial formulation of cyflumetofen, at the recommended application rates (40 ml 100 L^{-1}) having spray able material as 2 L/tree.

Molecular formula cyflumetofen: $C_{24}H_{24}F_3NO_4$
Structural formula cyflumetofen:



S-cyflumetofen

The experimental results were compared with a control group. Two years old field-grown trees were experimental units that were all infested during the previous season. Sprayings and releasing started when the population density of pest mites exceeded 8 individuals/leaf. Mite numbers were assessed prior to treatment by collecting (10 leaves/tree) from 10 trees/ treatment. Every 8 days, samples (100 leaves) were taken for each treatment.

Cochran formula (Cochran, 1977) was used to calculate total number of pest mites/experimental trees.

$$n = \frac{p(1-p)z^2}{e^2} \rightarrow (1)$$

n = sample size
p = the population proportion (p=0.5=50%)
e = acceptable sampling error (e=0.05)
z = z value at reliability level or significance level.
- Reliability level 95% or significance level 0.05; z = 1.96 (Chaokromthong and Sintao, 2021)

The required population size of the predatory mite for each experiment based on the number of prey extracted from formula (1) was calculated according to formula (2).

$$\text{Predator released number} = \frac{\text{Total number of pest mites/experimental trees}}{\text{Proposed predator/prey ratio}} \rightarrow (2)$$

- Proposed predator/prey ratio = 1/7.

Two sprayings of acaricide and two manual releases of predatory mites, the first on the 1st of March 2022 and the second on the 18th of April 2022, were carried out in Sakaka governorate.

Data analysis: Numerical data were analyzed using SPSS program version 26 (SPSS Inc. Chicago, IL, USA). The independent-samples' *t*-test was applied to compare the means of mite populations between every two groups. The *p*-value < 0.05 was statistically significant. Microsoft Excel program were applied for graphs designation.

RESULTS

In the current study, eleven species from ten genera and five families were recognized. The mites were extracted from olive trees (leaves, buds & fruits) in four governorates in Aljouf region [Sakaka (A), Doumat Aljandal (B), Tubarjal (C) and Al Qurayyat (D)]. According to the current data, mite species are classified into two orders and two suborders; Order Acariformes was represented by suborder Actinedida, which was represented by the families Eriophyidae (three genera, three species); Tenuipalpidae (one genus, two species); Stigmaeidae (one species) and Tydeidae (two genera, two species). Order Parasitiformes was represented by a single suborder Gamasida, that included just one family, Phytoseiidae (three species in three genera). Six species were identified as a new record for Aljouf region Table 2.

Table 2. Taxonomical categories of mite species obtained from olive trees orchards from four governorates (A, B, C, & D) in Aljouf region, Saudi Arabia.

Family and species	Trophic group	Record on plant: Leaves	Buds	Fruits	Locality	Remarks
Order: Acariformes						
Suborder: Actinedida						
1. Family: Tetranychidae Donnadieu						
Tetranychus urticae Koch	I	+	-	+	A, B	*
2. Family: Eriophyidae Nalepa						
Aceria oleae (Nepela) ●	I	+	-	+	A, B, C	***
Oxycenus maxwelli (Keifer)	I	+	+	+	A, B, C, D	***
Tegolophus hassani Keifer●	I	+	-	+	A, B, C	**
3. Family: Tenuipalpidae Berlese						
Brevipalpus phoenicis (Geijskes)	I	+	-	+	A, D	**
Brevipalpus olearius Sayed●	I	+	-	+	A, B, C	**
4. Family: Stigmaeidae Oudemans						
Agistemus exsertus Gonzalez●	II	+	-	-	A, B	*
5. Family: Tydeidae Kramer						
Brachytydeus formosus (Cooreman) ●	III	+	-	-	A, B	**
Tydeus californicus (Banks)	III	+	-	-	A, B, C	**
Order: Parasitiformes						
Suborder: Gamasida						
6. Family: Phytoseiidae Berlese						
Amblyseius swirskii (Athias-Henriot)	II	+	-	-	A, B, C, D	*
Typhlodromus athiasae Porath & Swirski●	II	+	-	-	A, B	*
Euseius scutalis (Athias-Henriot)	II	+	-	-	A, B, D	*

● = new record in Aljouf region, Saudi Arabia.
I = Phytophagous II = Predaceous III = Miscellaneous
(*, ** & ***) = (<4, 4-8 & >8) numbers of mites respectively/leaf, bud or fruit

Fig. 2 shows the percentage of mite species obtained from olive trees orchards in the study areas. In Fig. 3, mite species were categorized according to their feeding habits into three categories: six species belong to phytophagous mites and presented 50%, four species belong to predacious mites and presented 33.33%, while two species belong to miscellaneous mites and representing 16.67% of the total amount of collected mite species. It was observed that the phytophagous mites had the highest number found in four governorates followed by the predaceous then miscellaneous species in Sakaka and Doumat Aljandal. While number of predaceous was similar to miscellaneous in Tubarjal and Al Qurayyat.

Relation of phytophagous mites to olive trees host: The olive mites were present on leaves, flowers and fruits, according to our findings in Aljouf area. Phytophagous mites prefer younger leaves and are the most commonly seen on the undersides of leaves. The mites feed on the growing points on the leaves, causing deformations that obstruct plant development. The growth of the trees become delayed. Leaves were disfigured and lost their stellate hairs, especially at the underside, creating green spots on a silvery background. At high population densities, the fruits were disfigured.

Family: Tetranychidae Donnadieu
***Tetranychus urticae* Koch**

One species on olive trees represented the family Tetranychidae: *T. urticae*, the results showed that it was recorded with few numbers (< 4 mites/leaf). The mites often were found on the leaves at flush growth and its economic impact seems negligible.

Family: Eriophyidae Nalepa
1- Species: *Aceria oleae* (Nalepa, 1900)
Olive gall mite

In this study it was observed that both leaf surfaces were affected by *A. oleae* with distinctive alterations in leaf form and color, in the form of development of yellow and white spots on the upper surface, which correlated to hypertrophy and swellings on the undersurface.

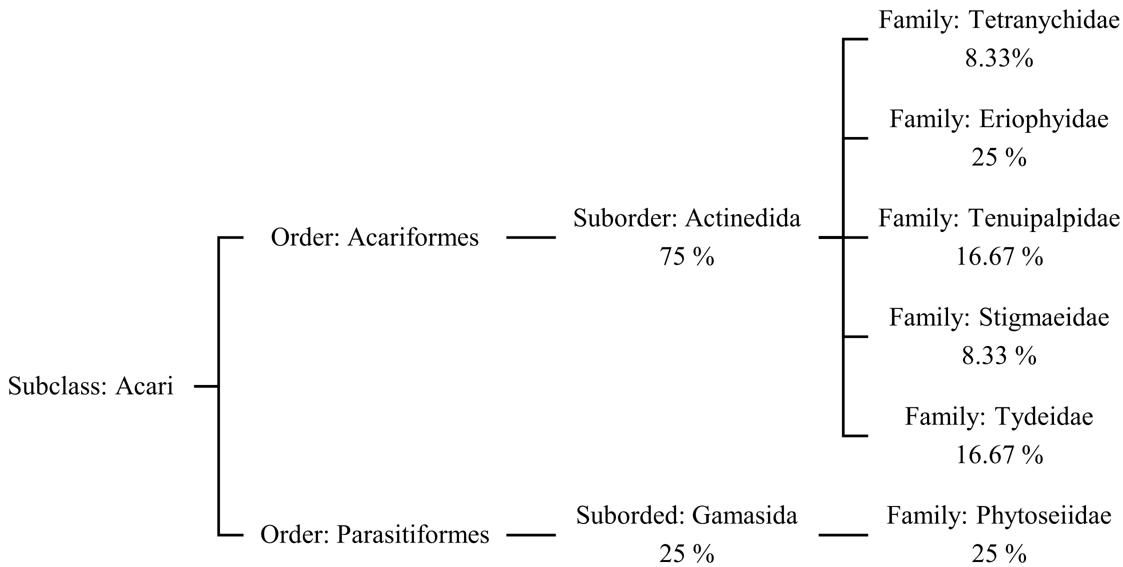


Fig. 2. Percentage of mite species on olive trees in Aljouf region of Saudi Arabia, according to taxonomic Subclass, Order, Suborder, & Family (Krantz, 1978).

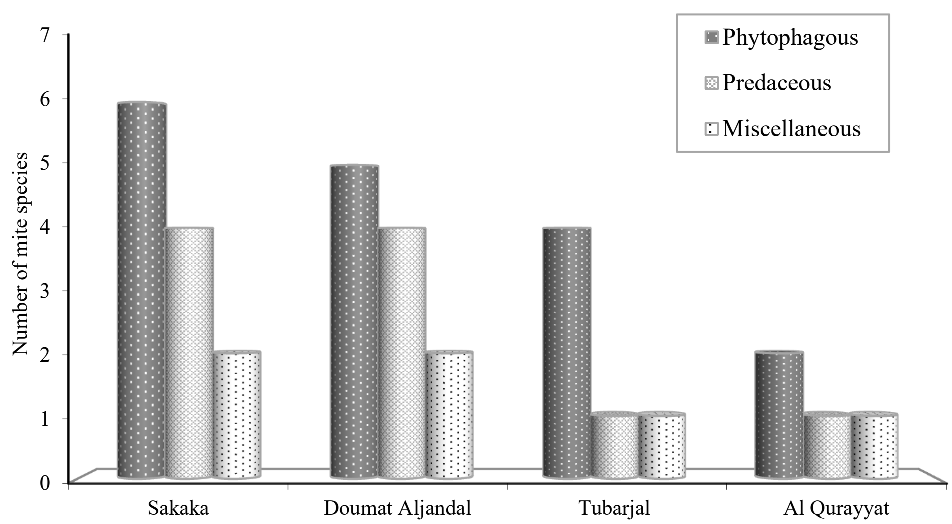


Fig. 3. Number of mite species associated with olive trees according to feeding habits and occurrence recorded in Aljouf region,

Notes: *Aceria oleae* a new record for Aljouf region, Saudi Arabia.

2- Species: *Oxycenus maxwelli* Keifer, 1939

Olive bud mite

This species was recorded on the upper surface of olive leaves as well as new growth, with the presence of distortions on the affected parts of the plant includes inflorescence abscission, sickle-shaped leaves, vegetative buds damage in the spring, flower buds discoloration, blossom blasting and decreased shoot growth.

Notes: *Oxycenus maxwelli* previously first recorded in Sakaka governorate, Saudi Arabia by (Elmoghazy, 2016)

3- Species: *Tegolophus hassani* Keifer, 1959

Olive rust mite.

In the current survey, the species *T. hassani* have been recorded on the top surface of the olive leaves and blossoms causing deformation in the leaves and fruits of olives and causes structures resembling rust-colored appearance, especially in fruits. Twists and distortion of foliage and fruits are caused by severe infestations.

Notes: *Tegolophus hassani* a new record for Aljouf region, Saudi Arabia.

Family: Tenuipalpidae Berlese

False spider mites appear dorsoventrally flat and move slowly. In the current study, two mites species in genus *Brevipalpus* have been found in orchards of olive trees in Aljouf *B. olearius* & *B. phoenicis*. Small white spots on the leaves are the most common symptom, which can lead to desiccation and leaf drop in severe infestations.

Notes: *Brevipalpus olearius* a new record for Aljouf region, Saudi Arabia.

Family: Stigmaeidae Oudemans

One species on olive leaves represented the family Stigmaeidae: *Agistemus exsertus* was recorded with few numbers in areas (Sakaka & Doumat Aljandal).

Notes: *Agistemus exsertus* a new record for Aljouf region, Saudi Arabia

Family: Tydeidae Kramer

Two species were detected in moderate numbers (4 - 8 mites/leaf) on olive leaves: *Brachytydeus formosus* (Cooreman) was found in regions, Sakaka & Doumat Aljandal, while *Tydeus californicus* (Banks) was found in areas (Sakaka, Doumat Aljandal & Tubarjal).

Notes: *Brachytydeus formosus* a new record for Aljouf region, Saudi Arabia.

Family: Phytoseiidae Berlese

On olive trees aerial parts, three species of the Phytoseiidae family were detected: In all locations, *Amblyseius swirskii* (Athias-Henriot) was detected in few numbers. *Typhlodromus athiasae* Porath & Swirski was found in few numbers (< 4 mites/leaf) in locations (Sakaka & Doumat Aljandal). *Euseius scutalis* (Athias-Henriot) was found in few numbers in regions (Sakaka, Doumat Aljandal & Tubarjal).

Notes: *Typhlodromus athiasae* a new record for Aljouf region, Saudi Arabia.

Population density of mite

The information in Fig. 4 describes the population density of moving mites in study regions. Studies on population density revealed that temperature and relative humidity in different months according to regions play an important role in governing activity and the population of mites. The population density of mites started to increase at the beginning of March, and the number reached the highest peak in June at an average temperature between 20 to 40 ºC and relative humidity of 12 to 30%. The following are the results of the independent samples T test comparing between regions: in Sakaka, and (Doumat Aljandal, Tubarjal, & Al Qurayyat) was (F= 0.002, DF= 7.990, P= 0.426), (F= 0.341, DF= 7.118, P < 0.05), and (F= 1.905, DF= 5.343, P < 0.01) respectively. Also, on Doumat Aljandal, and (Tubarjal, & Al Qurayyat) was (F= 0.309, DF= 7.255, P = 0.063), and (F= 1.954, DF=5.435, P < 0.05) respectively. On Tubarjal, & Al Qurayyat was (F= 1.525, DF= 6.553, P= 0.180), then started to decrease with the beginning of September to reach the lowest number in December at an average temperature of 30 to 8 ºC and relative humidity 70 to 55 %: In Sakaka, and (Doumat Aljandal, Tubarjal, & Al Qurayyat) was (F= 0.042, DF= 11.969, P= 0.785), (F= 1.749, DF= 10.962, P = 0.313), and (F= 2.213, DF= 10.732, P =0.161) respectively. Also, on Doumat Aljandal, and (Tubarjal, & Al Qurayyat) was (F= 0.663, DF= 10.670, P = 0.495), and (F= 0.905, DF=10.431, P =0.282) respectively. On Tubarjal, & Al Qurayyat was (F= 0.035, DF= 11.981, P= 0.609).

Pest mites control

Results in Fig. 5 show the mean number of moving phytophagous mites after the release of the predatory mites *Amblyseius swirskii* and spraying of acaricide Danisaraba 20% SC on olive trees on the 1st of March when the mites number exceeded 8 individual/leaf during the treatment. From the obtained results, it was generally noticed that the predatory mite and acaricide reduced the population density of mites on olive trees.

In the predatory mite treatment, the pest mite started declining gradually. It reached 5 individuals/leaf on the 48th days after the first launch, and it reached its lowest population on the 48th day after the second launch, 3.2 individuals/leaf. While in the treatment of the acaricide, the decrease started after the first spray, and then the number increased until it reached its highest number on the 48th day, 4.6 individuals/leaf after the first spray. Then it decreased to its lowest number on the 8th day, 0.6 individuals/leaf after the second spraying, then increased gradually until the end of the experiment.

The independent-samples T test comparing the effects of the different treatments yielded the following results: *A. swirskii* & control was (F= 0.758, DF= 20.778, P < 0.0001), Danisaraba 20% SC and control was (F= 0.102, DF= 20.831, P < 0.0001), and *A. swirskii* & Danisaraba 20% SC was (F= 1.006, DF= 18.249, P < 0.01).

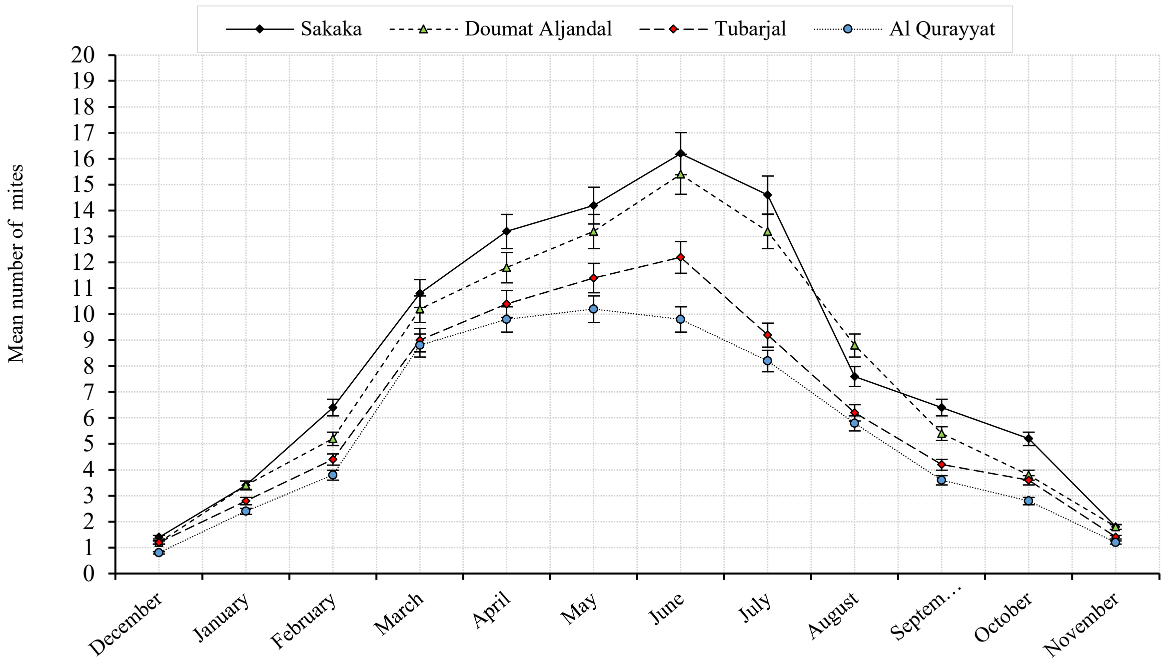


Fig. 4. Population density of mean number mites (moving stages)/ leaf on olive trees orchards from four governorates in Aljouf region, Saudi Arabia.

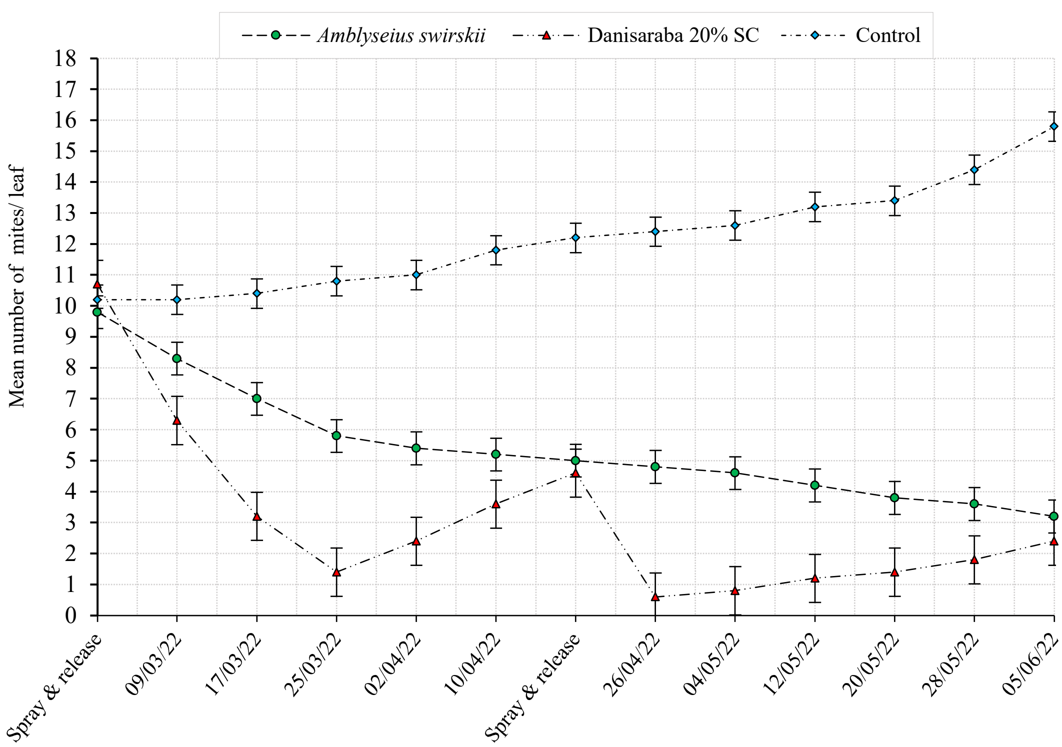


Fig. 5. Mean number density of moving phytophagous mites/ leaf on olive trees under field conditions affected by releasing predatory mites and spraying acaricide at Sakaka governorates, Saudi Arabia during March to June 2022 season.

DISCUSSION

The findings of the present work are consistent with earlier research. In the Saudi Arabia and Mediterranean region *Aceria oleae* and *Oxycenus maxwelli* infest olive trees, and it is particularly harmful to young olive trees (Elhadi and Birger, 1997; Al-Atawi and Halawa, 2011; Elmoghazy, 2016). It causes leaf and fruit deformation and a significant reduction in the quality & quantity of olives and oils extracted. The pest in Tunisia is prevalent in olive-growing areas, achieving extremely high population densities; It is frequently seen in association with *O. maxwelli* (Chatti *et al.*, 2017).

The findings in our research regarding *O. maxwelli* were in agreement with previous studies (Russo, 1972; Keifer, 1975), which reported that these mites usually feed on the top surfaces of olive leaves, but when numbers are large, they infest the lower surfaces; stems, fresh buds and foliage are preferable, heavy *O. maxwelli* infestations may result in the early fall of olive flowers, as well as spotting and leaf deformation.

In several studies, it was reported that *Tegolophus hassani* sucks the cell content of olive leaves, causing deformation on the leaves, shedding and cause yellow spots on the top surface of the leaves, increasing plant water loss due to crusting on the undersurface of the leaves with causing rust in the fruit; It has also been noted that in severe infections, the pest causes curling and deformation in leaves, deformity in fruit and loss of quality and quantity in fruits (Jeppson *et al.*, 1975; Shahini *et al.*, 2009).

Family Tenuipalpidae is widely distributed throughout the world and includes numerous economically significant pests polyphagous species (Castro *et al.*, 2020). *Brevipalpus* species had been recognized as vectors of rhabdoviruses, which cause diseases as passion fruit green spot virus, reducing plant productivity and lifetime (Mesa *et al.*, 2009; Vacante, 2010). Also, it had been recognized as vectors of *Citrus leprosis* complex, associated with two unrelated taxa of viruses distributed in South, Central, and North America which cause diseases as passion fruit green spot virus, These viruses cause local lesion infections in all known hosts (Roy et al., 2015). *Brevipalpus* spp. attacks stem, leaves, inflorescences and fruits of olive trees in Tunisia (Chatti *et al.*, 2017).

The Stigmaeidae family is comprised of more than 32 genera in plant areas worldwide. *Agistemus* is the 3rd richest genus of Stigmaeidae, with approximately 87 species; moreover, this genus is frequently present on the plants aerial parts (Stathakis *et al.*, 2014; Rehman *et al.*, 2018). *Agistemus* species are considered important predators on phytophagous mites, which are considered as potential predators against of the mite families Tenuipalpidae, Tetranychidae, Tarsonemidae & Eriophyidae (Johann *et al.*, 2013; Silva *et al.*, 2020).

Tydeidae species are widely distributed worldwide. They are frequently linked with plants and can be found in moss, litter, straw, soil, or humus, fungus, bird nests, and stored food items; some are predaceous on small plant pests, including phytophagous mites, while others feed on fungi (Elmoghazy, 2002; Walter and Proctor, 2013).

The findings of the present work revealed that the enumeration of different stages of pest mites started declining gradually until it reached its lowest population 48 days after the second launch of *A. swirskii* at a prey/predator ratio of 7/1. Regarding acaricide Danisaraba, the decrease started after the first spray of the acaricide, and then the number increased until it reached its highest value after 48 days; this was in agreement with earlier research of Grasswitz (2012) who reported that the trial with tree infestation levels in the untreated controls with acaricides declined over the experimental period due to the activities of a predatory mite. Still, eriophyid populations in most treatments with acaricides started to increase 5 weeks after the first applications. Many commercialized phytoseiid predators are sold to control Tetranychidae (Gerson and Weintraub, 2007). However, it is known that the generalist Phytoseiidae (McMurtry and Croft, 1997) feed on not only spider mites but also prove to be key predators of eriophyid mites. Momen and Abdel-Khalek (2008) studied the *Typhlodromips swirskii* to evaluate their development and reproductive potential when fed on mobile stages of *Aculops lycopersici*, this type of prey proved to be acceptable food for it.

Conclusions: In the current research, 12 species from 11 genera belonging to 6 families were recorded. Six species were identified as a new record in Aljouf region, Saudi Arabia. Additionally, the current research showed that among phytophagous mite species linked to olive aerial parts, eriophyid mite species were more common. This study explored the effect of predatory mite *Amblyseius swirskii* compared with acaricide Danisaraba 20% SC to enhance pest control. *A. swirskii* is expected to be one of the keys to achieve successful biological control of phytophagous mites in olive trees in areas with high pest mites. Hence, the biological control system for olive trees can be simplified, and its robustness greatly enhanced by using this highly efficient predatory mite.

Conflict of Interest: No potential conflict of interest was reported by the authors.

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