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Research Article

# Etiology and incidence of pistachio endocarp lesion disorder in pistachio orchards of Kerman province, Iran

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**Abstract:** Pistachio endocarp lesion (PEL) is one of the most important disorders that has spread in the pistachio-growing regions of Rafsanjan and Anar in recent years and has caused great economic losses to farmers. In order to identify PEL symptoms, and investigate the biologic factors involved in the development of this disorder, on May 2016, 90 orchards with different levels of PEL, were randomly selected. The rate of development in each tree was scored in- to four levels. The incidence of this disorder was estimated to be 25.8% in Rafsanjan. The seeds with PEL symptoms were differentiated into six types based on the appearance of the symptoms. Then tissue from infected parts of each type were cultured in the PDA. From among the 18 prepared culture media, fungal growth was obtained only in five cultured plates. Isolated fungi were *Ulocladium* sp. *Penicillium* sp., *Verticillium* sp. and *Fusarium* sp. To prove their pathogenicity, the spore spray technique was used on detached cluster *in vitro*. None of the purified fungi caused symptoms similar to the symptoms of PEL. Isolated strains from the pistachio seeds with PEL symptom often were saprophytic and soil borne; they were abundant in the orchard and could be transferred to the shoots of trees under the influence of various factors, such as: tillage, soil splashing by rain drops, and wind. In this study, the role of these fungal agents on the development of PEL was rejected.

**Keywords:** pistachio endocarp lesion, Kaleh Qouchi, stylar-end lesion, fungal agents, dark spots

## Introduction

Pistachio *Pistacia vera* L. is one of the most important orchard products in Iran. There are currently 450,000 hectares of fertilized and non-fertilized pistachio orchards in Iran. The annual production of pistachio in Iran is more than 250 thousand tons, which is mainly exported abroad. Rafsanjan, with about 80 thousand hectares of pistachio cultivation area, is considered the largest pistachio production center in Iran (FAO., 2018).

Considering the economic importance of pistachio, identifying the factors influencing the growth and production rate of this crop is essential. One of the factors that reduce the quality and quantity of pistachio is the appearance of lesion on this product. Early studies on the etiology of the lesions have reported several factors, such as the environmental stresses and insect attacks (Bolkan *et al.,*1984; Rice *et al.,* 1985; Uyemoto *et al.,* 1986; Bostock *et al.,* 1987).

Pistachio endocarp lesion (PEL) is one of the most important complications that have spread to

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pistachio-growing regions in Iran in recent years. Based on the opinion of the Agricultural Office of Rafsanjan, in some orchards, the damage has been identified to be over 50%.

PEL begins with the appearance of brown and black spots on the pistachio epicarp. The dark spots quickly progress and make the pistachio endocarp Smooth and soft, and finally most of the seeds drop.

For the first time, researchers observed Signs close to this disorder on epicarp in California pistachio orchards and presented it as stylar-end lesion. Their study showed that the attack of an insect called *Leptoglossus clypealis* and the enzymes secreted from the bite of the pest revealed a dark spot on the pistachio shell (Bolkan *et al.,* 1984; Michailides *et al.,* 1987) but the symptoms of this pest did not conform to the symptoms of PEL (Rice *et al.,* 1985)**.** In further research, no evidence of the effect of pathogens on this disorder was observed (Browne and Doster *et al.,* 2002; Zhang, 2004)**.**

In conducted studies in Iran, the symptoms of this disorder were compared with the symptoms of an attack by all types of green shield bugs, such as *Acrostenum* spp.*, Brachynema* spp.*, Lygaeus panderus,* and *Apodiphus amygdali.* No evidence of the effect of these insects on this disorder was observed (Hashemi Rad and Rajabi, 1998; Hashemi Rad, 2005). The impact of some other insects on the appearance of epicarp symptoms of this disorder has been rejected by Browne and Doster *et al.et al.* (2002).

To date, this disorder has been reported in the Ohadi (Fandoghi), Akbari, Poost Khormaei, and Poost Piazi (Biazi) cultivars in the Kerman Province, the Agh Peste cultivar in the Qazvin Province, and the Abbas Ali cultivar in Damghan (Hashemi Rad, 2005). Under the climatic conditions of the Kerman Province, this disorder starts at the beginning of May and continues until mid-June. Since the time of its outbreak, this disorder has been coincident with spring and the occurrence of spring rainfalls. Certain agricultural experts in the region identified the cause of this disorder as fungal agents and while stopping the irrigation of orchards, they used a variety of fungicides to reduce this disorder (Mozafari and Tajabadi Pour, 2005). However, Zhang (2005) and Hashemi Rad (2005) have identified nutritional deficiencies, such as calcium deficiency, as one of the reasons for this disorder. Thus far, no comprehensive research and reliable scientific documentation has been recorded on the factors influencing the occurrence and development of this disorder and the results of studies by researchers (Sajadian and Hokmabadi, 2011; Adibfar *et al.,* 2012) have so far failed to reduce the damage in this region. For this reason, the experts and pistachio farmers of the region have different opinions about the causes of this disorder. Some people suppose involvement of pathogens as a cause of this disorder, and others consider it to be affected by management and environmental factors. Owing to the complexity and ambiguity that exists in this regard as well as the high level of damage caused by this disorder it is necessary to examine the factors affecting this disorder more accurately. So, this study in a set of studies was conducted and aimed to investigate the etiology of fungal pathogens of PEL and determine the percent incidence of this disorder in the pistachio orchards of Rafsanjan and Anar.

## Materials and Methods

### Percent incidence of PEL

To evaluate the percent incidence of PEL, a total of 90 pistachio orchards with the symptoms of PEL were randomly identified and selected in different areas of Rafsanjan (Suburbs of Rafsanjan, Nugh and Koshkoueieh) and Anar on May 2017 (Figure 1). The study area has extended within the geographical range of 55°1'36.159" to 56°6'31.691" E and 30°10'35.78" to 31°5'26.675" N. The mean altitude in the sampled points is 1498 m above sea level.

The orchards were selected based on different levels of appearance of PEL and on the extent of geographical area covered. In each orchard, a tree with different level of PEL symptoms was selected randomly and the important information including, geographical location and altitude, irrigation system, irrigation interval as well as pistachio



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cultivar of each tree was recorded to determine the susceptibility of cultivars to PEL disorder. The incidence of infection of the selected trees was scored in four levels (I) = less than 10%, (II) = 11 - 35%, (III) = 36 - 60%, and (IV) = more than 60%. Then, the percent incidence of this disorder for each area, its districts, and the whole city were obtained from Eq. (1) (Thomas *et al.,* 1987; Alaei *et al.,* 2009; De Backer *et al.,* 2011).

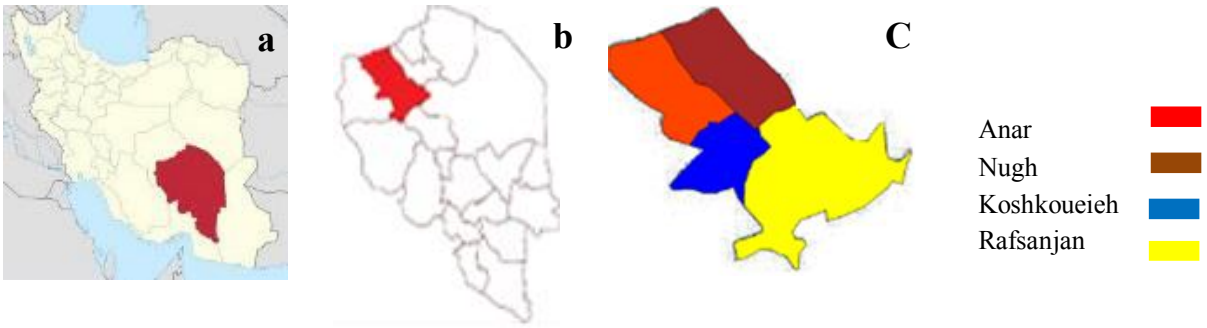


**Figure 1** (a) Geographical location of Kerman province in Iran. (b) Geographical location of study area in Kerman province. (c) Border divisions in the study area.

$$\text{The percent incidence of PEL disorder} = \left[\frac{((1\times n_2)+(2\times n_2)+(3\times n_3)+(4\times n_4))}{4\times(n_2+n_2+n_3+n_4)}\right]\times 100 \qquad \text{(eq. 1)}$$

where 1, 2, 3 and 4 represent the I, II, III, and IV codes of the disorder level, respectively, and $n_1$ = the number of trees with an infection level I, $n_2$ = the number of trees with an infection level II, $n_3$ = the number of trees with an infection level III, and $n_4$ = the number of trees with an infection level IV.

**Sampling**

For the etiological study of the PEL disorder, one pistachio cluster with PEL symptoms was sampled from each of the 90 selected pistachio trees showing the PEL symptoms. The samples were transferred to the lab in special containers on ice. Due to the wide variation of PEL symptoms, the seed samples were grouped according to the morphological characteristics of the symptoms. For this purpose, in each cluster, all the seeds with dark spots on the epicarp were observed by a binocular microscope (BH2, BHTU) at magnification of 10 folds. In terms of the type and cause of the symptoms, they were divided into two groups including (A) Symptoms of pistachio endocarp lesion of known etiology (factors such as environmental conditions and insect bite) and B: Symptoms of pistachio endocarp lesion produced due to unknown factors. Finally, the seeds of Group A were observed on the basis of the differences in the apparent symptoms and based on causal agents were divided into four groups. The seeds of group (B) were also classified into six different types.

**Evaluation of the different types of symptoms in terms of the fungal infection**

To check for etiology of the PEL disorder in terms of fungal agents, three samples from each of the symptoms types of B Group (18 samples in all) were prepared and cultured onto potato dextrose agar (PDA) medium. Small pieces of the symptomatic tissue were cut and disinfected with 0.5% sodium hypochlorite solution for 1 to 2 min. the samples were washed using sterilized distilled water, dried and placed in the culture plates and incubated at 25 °C under dark condition. Meanwhile, in order to assess the possibility of the presence of the pathogenic agents limited to



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the vessel, vessels of the cluster and stem end of the infected pistachio seeds were sampled and cultured. After 72 h, the grown of fungi were microscopically studied. Purification of the fungal colonies was done by single-spore method (Booth, 1971), and for further research, pure fungal colonies were kept in the refrigerator.

**Identification of the isolated strains**

The initial identification of all fungal isolates was made based on morphological characteristics of conidia and conidiophores as well as the colonies. Microscopic slides of each isolate was examined in lactophenol cotton blue mounts by light microscope (BH2, Olympus, Japan). The fungal isolates were identified using macro- and microscopic observations and standard diagnostic mycological keys (Barnett and Hunter, 1972). In this study, the identification of the species was not carried out, but the isolates were compared based on the apparent similarity of the colonies with the species reported in Iran and the probable species was examined.

**Pathogenicity of isolated fungi**

Detached cluster method was used to study and prove the pathogenicity of isolated fungi under *in vitro* conditions (Michailides and Morgan, 1992). Healthy fruit clusters of Kalleh Quchi cultivar with no symptoms were collected from the orchards in May 2017 (Before the development of the shell). Ordinary sterilization was done using 0.5% sodium hypochlorite. Then, they were individually placed in sterile Petri dishes and inoculated by spraying using each fungal spore suspension at concentration of $10^7$ spore $ml^{-1}$. The Petri dishes were placed inside plastic containers at the bottom of which sterilized distilled water was poured in order to have saturated humidity. The containers were stored at a temperature of 25 ± 0.5 °C and then checked every day. Finally, similarity of the observed symptoms was objectively compared with those of the PEL disorder.

## Results

**Percent incidence of PEL**

In order to define the Percent incidence of the PEL disorder in studied area, distribution of the different levels of PEL on trees was calculated in different areas (Table 1). The results showed symptoms of PEL disorder varied from less than 10% to over 60% in different areas. Percent incidence was grouped in 4 levels including low (level I), moderate (level II), high (level III) and very high (level IV). The Percent incidence of the samples with level I in Rafsanjan and Anar was more than 50%, but in Nugh and Koshkoueieh, more than 50% of the samples showed level II. Despite the fact that 7% of the Rafsanjan samples had level IV and this level was not observed in other areas. In Koshkoueieh and Nugh, the Percent incidence of the samples with II and III levels were much higher than in comparison to Rafsanjan. In other words, it could be predicted that many of the orchards of these areas will reach the very high level and this will increase the damages in the areas mentioned more than before. Since the number of samples and PEL levels of the studied trees differ in the target areas, the incidence severity of PEL disorder was also calculated separately for each region and related areas (Table 2). The results showed that the incidence severity of PEL was increased in Anar, Rafsanjan, Koshkoueieh and Nugh, respectively. The rate of PEL occurrence in the studied area was estimated to be 25.8%.

**Table 1** Distribution of different levels of pistachio endocarp lesion (PEL) disorder in studied areas.

| Area | Number of samples | Levels of PEL disorder (%) | | | |
|---|---|---|---|---|---|
| | | Low (level I) | Medium (level II) | High (level III) | Very High (level IV) |
| Rafsanjan | 48 | 58 | 29 | 6 | 7 |
| Koshkoueieh | 16 | 19 | 62 | 19 | 0 |
| Nugh | 17 | 12 | 71 | 17 | 0 |
| Anar | 9 | 55 | 33 | 12 | 0 |
| Total | 90 | 42 | 43 | 11 | 4 |



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**Table 2** Estimation of the percent incidence of the pistachio endocarp lesion (PEL) disorder in studied area.

| Area | Incidence severity (%) | Region | Incidence severity (%) |
|---|---|---|---|
| Rafsanjan | 20.0 | Ahmadabad Razavi | 44 |
| | | Eslamabad | 31 |
| | | Mohamadabad | 12 |
| | | Kosarriz | 20 |
| | | Deh Shafii | 16 |
| | | Musaabad, Roknabad, Moheyabad And Rezaabad | 22 |
| | | Abasabad Amin | 5 |
| Koshkoueieh | 33.3 | Fathabad | 40 |
| | | Eslameyeh | 38 |
| | | Aliabad, Musaabad | 20 |
| Nugh | 37.5 | Hasanabad, Ahadabad, Zanughabad | 38 |
| | | Hossaynabad Kiza, Shamsabad, Hajiabad | 33 |
| Anar | 18.2 | Dehno Amin | 40 |
| | | Shemsh | 6 |

**Evaluation and samples grouping based on different types of symptoms in terms of the causal agent**

According to the observations, the dark spots of the studied seeds were divided into two groups: with known causes (A) and unknown causes (B).

**Group A: Dark Spots with Known Causes**

**(A1)** The dark spot appears on the epicarp of pistachio seed as a result of the impact of hailstones and sand particles during severe seasonal storms (Figure. 2a). The size of these dark spots depends on the growth stage of the seed, and with endocarp hardening, the size of the spots become smaller. (**A2)** Severe necrosis and dark spots are observed only on a part of the seed that is exposed to intense sunlight (Figure. 2b). Sun burn not only damages the seed but also damages the epicarp soft tissue cells, and, sometimes, the endocarp. (**A3)** The blackening of the entire immature seed that is caused by spring frostbite at the beginning of the season. These symptoms usually occur at the initial stage of endocarp formation and with further development of endocarp, do not expand. (**A4**) Brown spots with dark brown margins, which result from the bites of certain pests, such as all the types of green shield bugs and pear lace bugs (Fig. 2c) due to the insertion of bugs' proboscis on the green shell of the seed, the plant sap is released in clear droplets. On the inner surface of seeds, there are also white grids. This stage is called Epicarp Lesion. (Rice *et al.,* 1985).

**Group B: Dark spots with Unknown Causes**

Dark or dry spots that start somewhere on the seed and reach the foot of the seed (Fig. 2d). None of the symptoms in this group are similar to items listed in the Group A, such as insect bite, sun burn. Endocarp of Pistachio seeds are also soft and flexible in the dark parts. The seeds of this group were classified according to the similarity of symptoms into six different types. These six types are presented in Fig. 3.

**The distribution frequency of isolated types in the studied areas**

According to the study of symptoms in 90 cluster of the study areas and the separation of the seeds of these clusters based on the type of damage symptoms, Table 3 was obtained. The most obvious and commonly encountered damage can be related to type 3 (the whole seed drying) and type 1 (the dark spots start from the edge of the seed and do not end). The highest and lowest damage of type 3 was observed in Nugh and Rafsanjan, respectively. In type 1, the highest and lowest damage was observed in Rafsanjan and Anar, respectively.



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The results of the study to identify the most sensitive cultivars to PEL showed that in 90 studied trees (Fig. 4) including cultivars Kaleh Qouchi, Ahmad Aqaie (long), Akbari, Fandoghi, and Ohadi; the most sensitive cultivar that showed the most damage at all levels, was the Kaleh Qouchi cultivar (jumbo).

**Identification and pathogenicity of fungal isolates**

A total of 18 symptomatic pistachio samples from six types of PEL symptoms were selected and cultured onto PDA. Fungal isolates were obtained only from five cultured samples. These fungal genera isolated from a sample of type 1 (dark spots from the head of the seed on one side of the seed which extends up to two-thirds of the seed), two samples of type 2 (dry seeds with gum secretion from one point on the pericarp but with no sign of the insect bite), and two samples of type 3 (dry seeds, without gum secretion). Based on morphological and cultural characteristics, the isolates were identified as *Ulocladium* sp., *Penicillium* sp., *Verticillium* sp**.**, and *Fusarium* sp. (Fig. 5). Comparison of morphology of isolated strains from symptomatic seeds with species reported from Pistachio regions of Iran, Shows that they probably belong to the species *Ulocladium* sp., *Penicillium* sp., *Fusarium* sp. and *Verticillium dahliae***.,** No fungal growth was observed out of vessels of the symptomatic cluster and stem ends of the Pistachio seeds in the culture media; indicating no vessel-fungi involvement with this disorder.


**Figure 2** (a) Dark spots resulting from hailstones on pistachio seeds (epicarp) (Rafsanjan, May 2016). (b) Dark spots caused by sunlight on pistachio seed (epicarp) (Rafsanjan, May 2016). (c) Dark spots caused by bugs attack on pistachio seeds (Rafsanjan, May 2016). (d) A disorder known as PEL (Pistachio Endocarp Lesion) (Rafsanjan, May 2016).



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**Figure 3** Different types of symptoms known as pistachio endocarp lesion (PEL) disorder in studied areas. ($b_1$) dark spots that start from the edge of the seed and sometimes cover up to two-thirds of the seed. ($b_2$) seed drying, so that from one point, gum exudes but there is no sign of insect bites at the exit point of the gum. ($b_3$) full seed drying. ($b_4$) darkening of the seed from the side and drying of the epicarp. ($b_5$) black tip seeds. ($b_6$) darkening of pistachio seed that gradually expands**.**

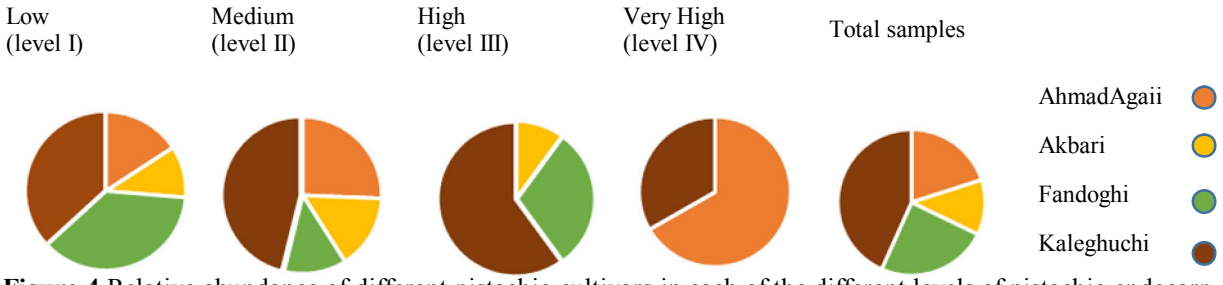


**Figure 4** Relative abundance of different pistachio cultivars in each of the different levels of pistachio endocarp lesion (PEL) disorder.



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Results of the pathogenicity tests on detached healthy pistachio clusters showed that all fungal species produced brown to dark necrotic spots on inoculated pistachio nuts. The severity of the symptoms as well as the development of the spots varied in different isolates but none of them were similar to the endocarp lesion disorder. Only *Penicillium* sp. could create light brown spots on the pistachio pericarp (Fig. 6). Generally, the isolates were capable of causing disease on pistachio seeds, but the symptoms did not correspond to PEL.

**Table 3** Distribution of different types of pistachio endocarp lesion (PEL) disorder (B Group) in the studied area.

| Area | Number of samples | Number of trees with PEL disorder symptoms (% total infection) | | | | | |
|---|---|---|---|---|---|---|---|
| | | B1 | B2 | B3 | B4 | B5 | B6 |
| Rafsanjan | 48 | 31 (64.5) | 23 (47.9) | 35 (72.9) | 6 (12.5) | 12 (25.0) | 3 (6.3) |
| Koshkoueieh | 16 | 7 (43.7) | 9 (18.7) | 14 (87.5) | 2 (14.2) | 1 (6.3) | 1 (6.3) |
| Nugh | 17 | 8 (47.0) | 10 (58.8) | 16 (94.0) | 0 (0.0) | 4 (23.5) | 0 (0.0) |
| Anar | 9 | 0 (0.0) | 4 (44.5) | 7 (77.7) | 0 (0.0) | 3 (33.3) | 0 (0.0) |

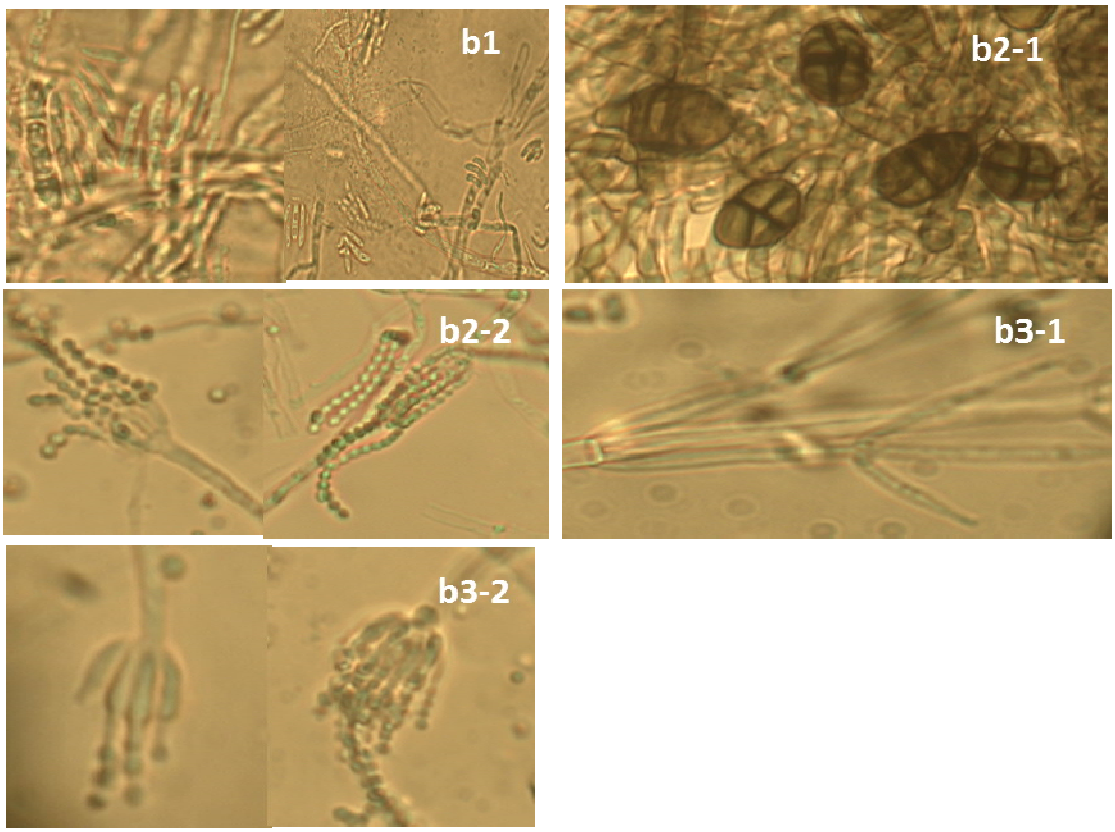


**Figure 5** Information on the extracted species of fungi from pistachio seed with pistachio endocarp lesion (PEL) disorder symptoms ($B_{1=}$ *Fusarium* sp.;$b_{2-1}$ = *Ulocladium* sp.;$b_{2-2}$ = *Penicillium* sp.;$b_{3-1}$ = *Verticillium* sp.;$b_{3-2}$ = *Penicillium* sp.*)*



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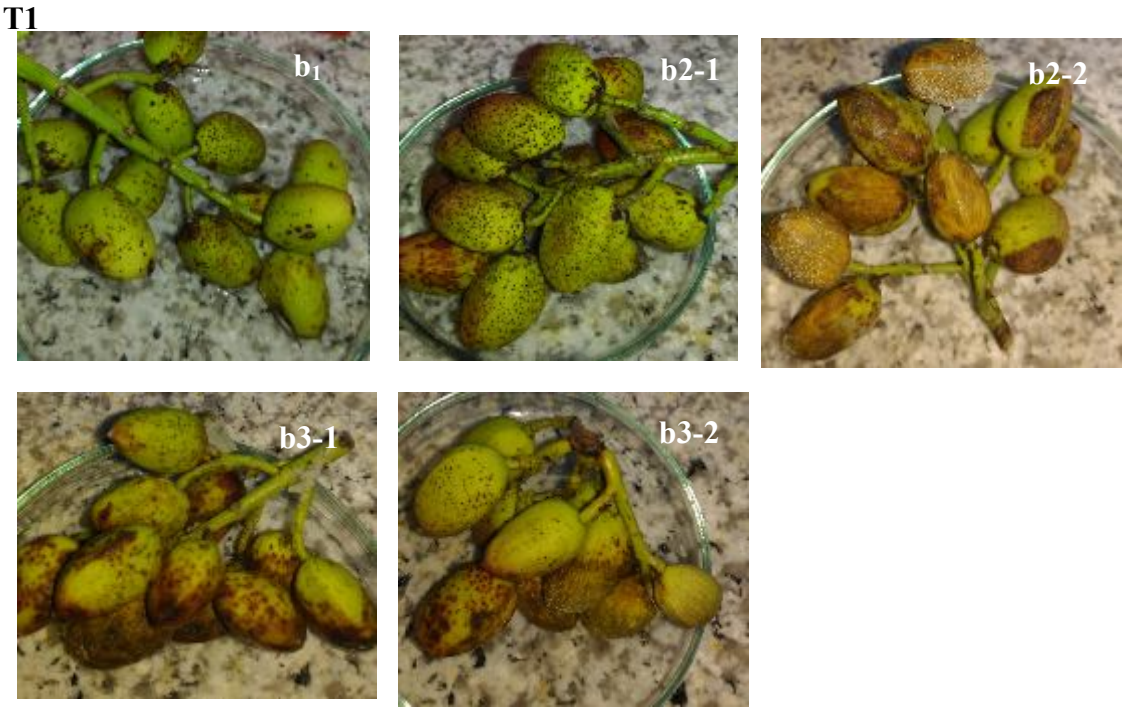


**Figure 6** in vitro Pathogenicity test of fungus strains isolated from pistachio seeds with symptoms of pistachio endocarp lesion (PEL) disorder ($b_1$ = *Fusarium* sp.*;* $b_{2-1}$ = *Ulocladium* sp.*;* $b_{2-2}$ = *Penicillium* sp.*;* $b_{3-1}$ = *Verticillium* sp.*;* $b_{3-2}$ = *Penicillium* sp.).

## Discussion

In this study, different levels of the PEL disorder were observed in different areas. Currently, more than 25% of the studied areas are exposed to different levels of PEL**.** The obtained results reinforce this concern that if the factors affecting this disorder are not considered and removed in the coming years, the level of damage will increase. It is predicted that the spread of PEL disorder is greater in Nugh and Koshkoueieh in comparison to other areas. Although a few trees with an infection level of four were all in Rafsanjan, moderate and high levels of PEL have been frequent in Nugh and Koshkoueieh**.** Since the average salinity of the soil sampled from these two regions was higher than Rafsanjan, so the weakness of the trees in these two regions can be attributed to salinity as a contributing factor to the PEL disorder. On the other hand, the soil texture of Nugh and Koshkoueieh at a depth of 40-80 cm (adjacent to the root) was heavier than in Rafsanjan and the low water permeability under rain and irrigation conditions can retain the soil moisture for longer and the soil moisture content is higher. On average, the elevation of the sampling points in Nugh and Rafsanjan, above sea level, is about 200 m lower than Rafsanjan. These factors might render Nugh and Koshkoueieh more susceptible to this disorder.

In the present study, the Kaleh Quchi cultivar was more susceptible to this disorder than the other cultivars. Kaleh Quchi is one of the most valuable pistachio cultivars, which has attracted growers in recent years owing to high international demand. In addition to the sensitivity to PEL, this cultivar has many physiological disorders, such as aging, severe fallout of seeds, osteoporosis, and seed deformity (Ferguson *et al.,* 2005), that strongly affect its yield. This cultivar is more sensitive to shortages of water and nutrients than other cultivars. Owing to early flowering, the cultivar is more vulnerable to the risk of spring frost loss.



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Pistachio is a hard seed in which the thin pericarp and fleshy epicarp cover the endocarp. In the process of preparing the product for the market, the epicarp is removed and the apparent quality of the endocarp is very important for a marketing (Polito and Pinney, 1999).

Evaluation of various types of endocarp lesions, which affect the quality of this valuable product, showed various forms of the dark spots in the study area, which included the impact of sand and hail, green shield bug and pear lace bug attacks, and spring frostbite and heat stroke**.** In addition, dark brown spots and black spots that begin from the head and side of the seeds, with the destruction of the epicarp and endocarp in the dark part, advance towards the seed base. The epicarp drying, in some cases, happens in such a way that the cell sap leaks out from some points. These symptoms are a part of the shell dark spots, which are known as PEL. Many indigenous experts and farmers in the studied areas believe that these symptoms, especially the dark spots starting from the head and side of nuts that cause softness in the dark part, depend on fungal activity; in the local language, this is called a "Qarchou'' (Means fungi in Persian) and growers used fungicides to prevent its spread. Our etiological studies of PEL disorder in showed that the genera *Ulocladium* sp., *Penicillium* sp., *Fusarium* sp., and *Verticillium* sp., were isolated from the infected seeds with the symptoms of this disorder. Among these four genera, *Ulocladium* sp., *Penicillium* sp., and *Fusarium* sp. are very diverse in terms of distribution and species. They are abundant in the air, dust, plant organs, and active in all the seed orchards and fields in the world (Alexopoulos *et al.,* 1996; Doster and Michailides, 1999; Saremi, 2005). Hence, due to the use of manure and the presence of the plant residues, there are undoubtedly different species of these fungi in the soil; following soil tillage and the splashing of soil particles by rain and wind, these fungi can be transferred into the shoot and new clusters. The *Verticillium* sp. has been also reported in all the regions of the world, including tropical and semi-tropical areas. They cause *Verticillium* wilt in suitable hosts. Since this genus is a group of vessel fungi, it damages the plant by destroying the vessels (Pegg and Brady, 2002). In pistachio, it enters the xylem via the shell infection and transferred to the trunk with the production of the conidia (Hiemstra, 1998). It disrupts the transfer of water and nutrients. The symptoms of pistachio tree infection with this fungus are a gradual deterioration of pistachio trees and the discoloration and dieback of branches. The lack of growth of this fungus in the cultured samples from the vessels leading to the seed and the apparent health of the selected trees at the sampling stage can reject the involvement of this fungus in this disorder. Spore of this fungus could be transferred from the soil to the seeds and shoots of pistachio. Since high temperatures destroy the fungus on the shoot, the beginning of the growing season, which coincides with the development of the PEL disorder, is the best time to observe these species on the shoot.

In examining the confirmation of the pathogenicity of the isolated genera under laboratory conditions, no similarity was observed between the symptoms of the pistachio spots with PEL. Only in the genus *Penicillium* sp., under advanced growth conditions of the fungus, light brown spots were created on the shell of the pistachio seeds. If these spots are observed in the orchard, they can be identified by mistake as a PEL disorder.

Owing to the abundance of the spores of the isolated strains in the soil due to soil amendment with organic matter, manure and presence of weeds in orchards, it is possible to conclude that the increase in moisture content will cause the germination and growth of these strains; if these conditions are due to rain and irrigation early in the growing season (April and May), the symptoms of the growth of these fungi will be observed on pistachio seeds, and increase the severity of the symptoms of the PEL disorder, while these strains cannot be the main cause of PEL but may affect other factors involved in this disorder, including nutritional factors.

The name of the disorder as "PEL" is inaccurate given that many factors, such as



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heat, cold, and insects' impact and bite, can affect it; there is a need to correct the name of this disorder in order to distinguish it from other disorders. In this study, calling a set of symptoms referred to as "PEL" with no sign of heat stroke, frostbite, impact, and bug attack, such as "Pistachio fruit blotch", is suggested. These symptoms are introduced as follows: 1- Dark, black, and flexible pistachio seeds, from the head and side progressively moving towards the foot. 2- Dry green shell of the pistachio in which the soft shell sap drops out but there is no sign of insect injury.

## Conclusions

Contrary to popular belief, PEL is not affected by living organisms such as fungi. However, at the beginning of pistachio endocarp development until it becomes rigid, high moisture content (rainfall and irrigation) causes saprophytic fungus growth on the green shell of pistachio. Since the time of the occurrence of these spots coincides with the creation of the spots caused by this disorder (EL or PEL), fungi are mistakenly thought to be the cause of this disorder. Of course, spraying at this stage will not only be expensive and pollute the environment but also will have no effect on reducing the damage caused by this disorder.

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# سبب شناسی و بررسی درصد بروز عارضه لکه پوست استخوانی در باغات پسته استان کرمان، ایران

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**چکیده:** پدیده لکه پوست استخوانی (PEL) یکی از مهم‌ترین عوارضی است که طی سال‌های اخیر در مناطق پسته‌کاری رفسنجان و انار شیوع پیدا کرده و باعث بروز خسارت‌های اقتصادی زیادی به کشاورزان شده است. به‌منظور معرفی انواع تیرگی‌های پوست استخوانی و شناسایی علائم PEL و بررسی عامل یا عوامل مؤثر در ایجاد این پدیده، در اردیبهشت ۱۳۹۵، ۹۰ باغ با سطوح مختلف گسترش PEL، به‌صورت تصادفی، انتخاب و میزان گسترش پدیده در هر درخت در ۴ سطح تقسیم‌بندی و نمره‌گذاری شد. درصد وقوع این پدیده به‌طور متوسط در سطح شهرستان رفسنجان ۲۵/۸ درصد تخمین زده شد. دانه‌های دارای علائم PEL، در مطالعه حاضر براساس تفاوت در شکل علائم، به ۶ تیپ تفکیک شدند و از قسمت‌های آلوده هر تیپ با سه تکرار در محیط کشت PDA، کشت داده شد. به‌منظور بررسی عوامل پاتوژن وابسته به آوند، از آوندهای منتهی به دانه‌های پسته با علائم PEL و قائده دانه‌ها، نمونه‌گیری و کشت داده شد. از ۱۸ محیط کشت آماده شده، در ۵ محیط کشت، قارچ رشد یافته شامل *Ulocladium* sp.، *Penicillium* sp.، *Verticillium* sp. و *Fusarium* sp. بودند. جهت اثبات بیماری‌زایی آنها، از روش اسپری اسپور بر خوشه‌های بریده، استفاده شد. در این مرحله هیچ‌کدام از قارچ‌های خالص شده در مرحله قبل، علائم مشابه با پدیده لکه پوست استخوانی ایجاد نکرد. گونه‌های جداسازی شده از دانه‌های پسته مبتلا به لکه پوست استخوانی، اغلب ساپروفیت و خاکزی بوده و در باغ به فراوانی وجود دارند. لذا این عوامل تحت تأثیر عوامل مختلف مانند خاک‌ورزی، پاشش خاک توسط ضربه قطرات باران و باد می‌توانند به اندام‌های هوایی گیاه منتقل شوند. در این مطالعه تأثیر عوامل قارچی بر ایجاد پدیده لکه پوست استخوانی بر دانه‌های پسته به اثبات نرسید.

**واژگان کلیدی:** رفسنجان، لکه پوست استخوانی پسته، کله قوچی، stylar-end lesion، عوامل قارچی، لکه‌های تیره



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