Evaluation of fruits cultivar and different harvest times of Damghan pistachio to early split complications and contamination to the *Aspergillus flavus* fungus

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**Abstract:** Study the effect of fruit cultivar (intact, early split, irregular crack) on (Akbari, Abbasali, Khanjari) cultivars in Damghan city to *Aspergillus Flavus* fungus and effect of harvest time in early split pistachio in 1389, sampling from orchards has been performed. Samples after grinding by dilute cultivating in a specific AFPA medium bottles in a completely randomized model were three times recultivated. Colonies of *A. flavus* after 3 to 7 days, were isolated, identified and counted. The results of Duncan grouping were analyzed by SAS software. Results of that study of contamination of samples to *A. Flavus* fungal showed. The contamination of the samples to the fungus *A. Flavus* differ from each other, there is less contamination in the Khanjari cultivar and more contamination in Akbari cultivar. With delay in the harvest time, percentage of intact fruits and without front skin cracking decrease. The lowest percentage of early split (0 first) was at first harvesting time (Shahrivar 10) in Akbari cultivar and the highest percentage of early split (Shahrivar 20) in the second harvest time in the Akbari cultivar.

**Keywords:** *Aspergillus flavus*, cultivar, early split, harvesting times

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**INTRODUCTION**

Pistachio fruit is sensitive to contamination by Fungi such as *Aspergillus flavus* and *Aspergillus parasiticus* that produce *Aflatoxins*. Fruit skin cracking reduce the fruit quality that during harvest and after harvest will reduce fruit skin quality and affect its market friendliness [3].

Fruit cracking may occur when the fruit is still attached to the tree. Fruit cracking in stone fruit, seeded fruit, grapes and vegetables has been seen. Skin cracking and surface cracks of fruit may be the result of a number of factors including water stress, insect, spraying.

Early split pistachios, are abnormal Pistachio nuts that both green skin and bony skin, simultaneously crack at the split place. In other words, cracks created on the Green skin, exactly coincide with the split of bony skin and so the kernel of pistachio is visible. In this cultivar of cracks because the edible part of fruit (the kernel) is directly exposed to the attack of spores and fungi, is one of the most important factors in *Aflatoxin* production in orchards [1].

These Pistachios are abnormal because the hard skin split occurs before maturity. (Prior to separation of the, soft skin from, bony skin), so in this case the soft skin is attached to the bony skin and does not have the necessary flexibility. By premature Splitting of hard skin the, soft skin also crack from the hard skin split spot. This condition is called early split [2].

When the harvest time for Pistachio nuts draws near, *Aflatoxin* generator fungus population density increase within orchards and it appears to be a

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The Aspergillus flavus fungus group and Aspergillus niger population increases by approaching harvest time due to the increased abundance of cracked poured Pistachio nuts on the orchard floor and contamination increase by contact with the fungus mentioned above. We can also mention more time exposer to the spores in the atmosphere of Pistachio nuts orchards. Timely and Early harvesting reduce Pistachio nuts fruit contamination and also reduce contamination of new Pistachio nuts [8].

Fruit growth of Pistachio nuts in orchards is associated with soft skin and bony skin. When the fruit matures the soft skin separate from the Hard skin. And cracking of Hard skin is important for Fruits market friendliness [6].

The purpose of this study is assessment the effect of fruit cultivar (early split, irregular cracks and intact) and different times of harvesting phenomenon on early split of Pistachio nuts on (Akbari, Khanjari and Abbasali), cultivar.

MATERIALS AND METHODS

Sampling: In order to study the extent of contamination of Pistachio nuts of Semnan province to A. flavus group of fungus aflatoxin different parts of Damghan city Pistachio nuts cultivation of fresh pistachios in 1389 were sampled.

From late Mordad, coincide with the product maturation, we select the orchards and collect samples of three varieties of Pistachio nuts (Akbari, Abbasaly Khanjari). of 1.5 kg from each cultivar, in sampling with interval of 10 days during the three phases, at each phase the percentage of nut side pistachios (intact, early split and irregular cracks) are calculated and after separation of this Pistachio nuts from each other we separate the green skin and sun-dried them.

Since the distribution of toxic and contamination dispersion in different parts of the kernel of pistachio is different and estimating the extent of fungal A. flavus infection and aflatoxin requires achieving a quite uniform and homogeneous sample, pistachio samples are grinded. Grinded samples by dilute cultivating method cultured on surface environment of Aspergillus flavus parasiticas Agar (AFPA) medium. (Completely randomized design with three replications). Fungi of this group on the AFPA specific medium produce Conidieles of typical yellow to olive green.

From each cultivar the amount of 10 gr kernel of pistachio in diluted method was added to 90ml peptone water 1% and then diluted to 10^-1, 0.01 ML of above sample were cultured on surface of AFPA medium petri dish. Petri dishes were kept at 26 °C.

After 3 to 7 days of cultivation, colonies of A. flavus were counted and separated, the contamination in various samples were compared. Obtained Data using SAS software analyzed and compare with Duncan test was performed at 5% level.

RESULTS AND DISCUSSION

Results of A. flavus colony counts is shown in Table 1. As can be seen in Table the results from these A. flavaus colonies suggest that The fungal contamination of the samples of kernel of pistachio is different for various Pistachios. As such in some cases there was no fungal contamination of A. flavus and some samples showed high contamination of this fungus. (Table 1). Evaluating the result
(Duncan's multiple range tests) is indicated the difference between that average numbers of fungal colonies different samples of *Pistachio nuts* in 1% statistical level is of significance. (Table 2) The results of these investigations show the delay in the time of harvest reduces percentage of intact, and un-cracked front skin fruits. This difference between the times of first (Persian date Shahrivar 10), and second (Persian date Shahrivar 20) and third (Persian date Shahrivar 30) is quite significant.

Effects of harvest time on the percentage of the early split product showed the lowest percentage of early split is zero in the first harvest (Persian date Shahrivar 10) in Akbari cultivar, and most early split of Pistachio nuts 7.5% in the second harvest (Persian date Shahrivar 20) in Akbari cultivar.

<table>
<thead>
<tr>
<th>No. almond sample</th>
<th>The average number fungal colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5.7×10^4</td>
</tr>
<tr>
<td>3</td>
<td>2.1×10^2</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>1.33×10^5</td>
</tr>
<tr>
<td>6</td>
<td>6.3×10^4</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0.333×10^2</td>
</tr>
<tr>
<td>10</td>
<td>0.066×10^5</td>
</tr>
<tr>
<td>11</td>
<td>1.6×10^2</td>
</tr>
<tr>
<td>12</td>
<td>2.3×10^2</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>1×10^5</td>
</tr>
</tbody>
</table>
Percentage difference of early split between the second and third times with first time means the early harvesting can be effective in reducing contamination of pistachio *A. flavus* fungi. Assessment of different harvest time effects showed that the delay in harvesting increases the fungi *Aspergillus flavus* contamination of the product. (Figure 2)

Fig 1: Means of *A. flavus* fungi in different pistachio cultivars
The results showed that the delay in harvesting reduces the percentage of intact fruit (without cracking the green skin). Intact skin of fruits until harvest is important because as long as the front skin is intact, it acts as a deep barrier against penetration of external agents such as insects, fungal spores, and dust, and also helps moisture loss of the fruit.

In this study, the delay in harvest increases the fruit early split. Shtatzky and colleagues (1997) reported that the early split is the main cause of fungal contamination and aflatoxin production.

The results of this study showed that the highest fungus *Aspergillus flavus* contamination occurs in the last harvest, and least contamination in the first harvest. In other words, harvest at the first weeks after physiological maturity of product, reduced product contamination to fungus *Aspergillus flavus*. Also, the delay in the harvest increases tarnished pistachio.

Evaluation of the effects of different harvest times showed that the contamination in the first harvest has a significant difference with the second and third times of harvest. The test results are consistent with the results of Taj Abadi Pur (1383) study about the effects of harvest time.

Results of research on Kerman Ohadi Pistachio show that the best time of harvest for highest weight dry product occurs in the first week of Mehr and maximum percentage of early split fruit occurs in the third week of Mehr. Most productive fruits occur at the fourth week of Mehr and least productive fruits occur at second week of Shahriwar [4].

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