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Determination of Mycoflora in Almond Plantations Under Drought Conditions in Southeastern Anatolia Project Region, Turkey

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Abstract: In this study, we aimed to determine the fungi that are among the pathogens leading to diseases in almond orchards in the region. In various organs of totally 8720 almond trees in 23 districts within these three provinces between 2002-2003, the rates of disease symptoms caused by a single fungal pathogen were found to be 98.70% for Monilia (*Monilinia laxa*), 96.11% for Red leaf blotch (*Polystigma ochraceum*), 95.93% for Shot hole (*Stigmata carpophila*), 6.11% for Leaf curl (*Taphrina deformans*) and 3.08% for Wood rots (*Fomes fomentarius*). Of the disease symptoms caused by more than one fungal pathogen, the rates of leaf blight, leaf fruit and branch canker were determined to be 88.22, 69.02 and 33.05%, respectively. In the isolations performed *in vitro* conditions in fruit samples with symptom, *Aspergillus* sp. was found to have the highest value as 26.34%, followed by *Penicillium* sp. as 19.02%, *Alternaria* sp. as 11.55%, *Rhizopus* sp. as 8.46%, *Cladosporium* sp. as 7.54%, *Stemphylium* sp. as 7.05%, *Coryneum beijerinckii* as 5.93%, *Macrophoma* sp. as 3.21% and *Epicoccum* sp. as 2.08%. In leaves, *Alternaria* sp. was isolated at the rate of 28.04%, *Cladosporium* sp. as 17.88%, *Bipolaris* sp. as 14.72%, *Helminthosporium* sp. as 12.16%, *C. beijerinckii* as 10.57%, *Chaetomium* sp. as 1.26% and other fungi as 17.10%. In the isolations performed in samples exhibiting wilt, *Thielaviopsis* sp. was determined as 42.16%, *Fusarium* sp. as 27.00%, *Rhizoctonia* sp. as 9.25% and other fungi as 21.32%. In the isolations in tissues with canker, *Stemphylium* sp. was found to be 16.25%, *Ulocladium* sp. 14.74%, *Manila laxa* 7.25%, *C. beijerinckii* 6.47%, *Pseudobotrytis* sp. 6.17%, *Pyrenochaeta* sp. 3.50% and other fungi at the rate of 19.97%.

Key words: Almond, fungi, diseases, *Fomes fomentarius*, *Polystigma ochraceum*

INTRODUCTION

The Southeastern Anatolia Project (SAP) area located between the subtropical and terrestrial climate zone is within the homeland of almond (*Prunus dulcis* (Mill.) D.A. Webb). Which is consumed both as green fresh fruit and kernel. The provinces Diyarbakır, Elazığ and Mardin located in this region are found moderately suitable in respect of the dormancy period of the almond and these three provinces meet 13% of Turkey's almond production. One of the most important problems of the almond is the loss of yield due to the diseases. Fungi are the most important within these biotic and abiotic factors.

It has earlier been reported that *Monilinia laxa* (Aderh. and Ruhland) Honey and *M. fructicola* (G. Winter) Honey, among the fungal pathogens of this disease, has caused blight in almond, flower and shoots. *Stigmata carpophila* (Lév.) M.B. Ellis, which is widely seen in apricot has formed disease in the almond (Ogawa and English, 1991) and again,

Polystigma ochraceum (Wahlenb.) Sacc, causing blight in almond leaves, has been seen in Lebanon and Israel (Saad and Masannat, 1997). *Taphrina deformans* (Berk.) Tul., making the leaf curl of the peach has been isolated in almond leaves (Syrop, 1975).

It was reported that *Verticillium dahliae* Kleb., one of the fungal pathogens of the disease having a wide host, caused wilt in the almonds in Italy (Luisi *et al.*, 1994) and that *Phytophthora citricola* Sawada and *P. cactorum* (Lebert and Cohn) J.Schröt., leading to canker and drying, were isolated in the roots of almond trees in California (Browne and Viveros, 1999).

It was also reported that the anthracnose was seen in the almonds in Israel (Shabi and Katam, 1983) and that the pathogen causing this disease in California was *Colletotrichum acutatum* J.H. Simmonds (Adaskaveg and Hartin, 1997).

There was determined that the widest fungal pathogens in the leaf and fruits of the almond were *Penicillium*, *Aspergillus* and *Cladosporium*,

other fungi were stated as *Alternaria*, *Botryosphaeria*, *Botrytis*, *Coniothyrium*, *Curvularia*, *Epicoccum*, *Fusarium*, *Mucor*, *Paecilomyces*, *Phoma*, *Rhizopus*, *Stemphylium*, *Trichoderma* and *Ulocladium* (Teviotdale and Hendricks, 1994).

No study has been conducted in the above mentioned provinces for determining fungal pathogens of these diseases. Through this study, the fungal disease pathogens will be determined and the substructure for future Integrated Pest Management (IPM) will be formed.

MATERIALS AND METHODS

Survey area: The study was carried out in totally 23 districts of three provinces, including Diyarbakır, Mardin and Elazığ within the Southeastern Anatolia Project (SAP), in 2002-2003. Of totally 491974 almond tree populations in these three provinces, 8720 were observed. No irrigation or fertilizing program was applied in these almond plantations.

Survey time: The surveys were performed between 15.03.2002-20.10.2003 in different periods from the blooming of almonds to the harvest and at 15 days intervals. In the examination, the flower, shoot, branch, leaf, fruit as well as almond tree roots in drying state were dealt with.

Sampling method: The number of the trees included to the survey was determined through classified sampling method in accordance with Snedecor (1956).

With the known disease pathogens stated in the record tables of the survey, the unidentified samples were examined in the laboratory.

Assessment of samples: The samples with symptoms were taken to the laboratory in an ice box and with nylon package. For the isolation, initially, the samples that were divided into small parts in 5-7 mm length, with sound tissue and disease were washed well under the tap water and then the surface was sterilized for 2-5 min with 1% hypochlorite. The sterilized plant parts were washed with sterile water twice and dried with a sterile drying paper. Of these dried parts, 4 were inoculated to each *Petri* containing sterile nutrition media (PDA, Sugar agar and Alcohol agar). One part from each fungus colonies developed at the end of 3-10 days incubation period was taken and transferred to inclined agar tubes purely. The obtained isolates were grouped initially according to their macroscopic and then microscopic characteristics and their identification were made (Funder, 1968; Barnett and Hunter, 1987).

As a result of the study, the penetration and disease rates of the fungi were calculated (Bora and Karaca, 1970).

RESULTS AND DISCUSSION

Land survey: According to the disease symptoms, whose pathogens were already known in the provinces where the study was conducted, *Monilinia laxa*, *Stigmina carpophila* and *Polystigma ochraceum* diseases were seen in all orchards (Fig. 1), while *Taphrina deformans* in 82.85% of the orchards surveyed. According to the average of these three provinces, the rate of infected tree in the surveyed orchards was determined in turn starting from the highest to the lowest value; *Monilinia laxa* 98.70%, *Polystigma ochraceum* 96.11%, *Stigmina carpophila* 95.93% and *Taphrina deformans* 6.11%. In the previous studies, it has been stated that these pathogenic fungi cause disease in hard-shelled fruits (Ogawa and English, 1991; Adaskaveg and Ogawa, 1992; Saad and Masannat, 1997).

Similar disease symptoms seen in the various parts of the almond tree due to more than one pathogenic fungus: Fruit blight, leaf blight and branch canker have occurred



Fig. 1: Symptom of Red leaf blotch caused by *Polystigma ochraceum* in leaves of almond



Fig. 2: Fruiting body of tinder polypore that causes decaying of wood by *Fomes fomentarius* on trunk of almond

Table 1: The prevalence of diseases and infected trees, determined during the surveys performed in almond orchards of Diyarbakır, Elazığ and Mardin. Survey performed provinces Diyarbakır Elazığ Mardin Averages

| Determined diseases | Diyarbakır | | Elazığ | | Mardin | | Averages | |
|---------------------|------------|--------|--------|--------|--------|--------|----------|--------|
| | PD (%) | IT (%) | PD (%) | IT (%) | PD (%) | IT (%) | PD (%) | IT (%) |
| Monilia | 100.00 | 98.76 | 100.00 | 99.52 | 100.00 | 97.32 | 100.00 | 98.70 |
| Shot hole | 100.00 | 97.80 | 100.00 | 93.68 | 100.00 | 97.45 | 100.00 | 95.93 |
| Red leaf blotch | 100.00 | 97.57 | 100.00 | 95.86 | 100.00 | 94.89 | 100.00 | 96.11 |
| Leaf curl | 7.08 | 4.11 | 81.27 | 9.44 | 80.21 | 3.02 | 82.85 | 6.11 |
| Leaf fruit | 100.00 | 77.38 | 100.00 | 66.53 | 100.00 | 63.74 | 100.00 | 69.02 |
| Wilt | 55.42 | 1.92 | 67.30 | 2.98 | 59.90 | 1.91 | 60.87 | 2.38 |
| Leaf blight | 100.00 | 89.54 | 100.00 | 91.49 | 100.00 | 81.53 | 100.00 | 88.22 |
| Branch canker | 100.00 | 35.19 | 100.00 | 34.37 | 100.00 | 28.55 | 100.00 | 33.05 |
| Wood rots | 39.17 | 1.42 | 80.47 | 5.04 | 59.90 | 1.79 | 59.85 | 3.08 |

Table 2: The rates of fungi obtained as a result of the isolation performed in the samples taken from the almond orchards of Diyarbakır, Elazığ and Mardin

| Diseased plant part | Obtained fungi | Rates of fungi (%) | | | | | | | |
|---------------------------------|-----------------------------|--------------------|----------|------------|----------|------------|----------|--------------|----------|
| | | Diyarbakır | | Elazığ | | Mardin | | Averages | |
| | | No. sample | Rate (%) | No. sample | Rate (%) | No. sample | Rate (%) | Total sample | Rate (%) |
| Spot, blight and decay in fruit | <i>Alternaria</i> sp. | 15.00 | 12.40 | 14.00 | 11.29 | 10.00 | 10.64 | 39 | 11.55 |
| | <i>Aspergillus</i> sp. | 30.00 | 24.79 | 35.00 | 28.23 | 24.00 | 25.53 | 89 | 26.34 |
| | <i>Cladosporium</i> sp. | 10.00 | 8.26 | 8.00 | 6.45 | 9.00 | 9.57 | 27 | 7.54 |
| | <i>Stigmina carpophila</i> | 7.00 | 5.79 | 8.00 | 6.45 | 5.00 | 5.32 | 20 | 5.93 |
| | <i>Epicoccum</i> sp. | 3.00 | 2.48 | 1.00 | 0.81 | 2.00 | 2.13 | 6 | 2.08 |
| | <i>Macrophoma</i> sp. | 5.00 | 4.13 | 3.00 | 2.42 | 1.00 | 1.06 | 9 | 3.21 |
| | <i>Penicillium</i> sp. | 22.00 | 18.18 | 26.00 | 20.97 | 16.00 | 17.02 | 64 | 19.02 |
| | <i>Rhizopus</i> sp. | 8.00 | 6.61 | 12.00 | 9.68 | 8.00 | 8.51 | 28 | 8.46 |
| | <i>Stemphylium</i> sp. | 9.00 | 7.44 | 5.00 | 4.03 | 8.00 | 8.51 | 22 | 7.05 |
| | Other Fungi | 12.00 | 9.92 | 12.00 | 9.68 | 11.00 | 11.70 | 33 | 11.02 |
| Blight on branch | <i>Coniothyrium</i> sp. | 3.00 | 9.37 | 3.00 | 3.12 | | | | |
| | <i>Phoma</i> sp. | 9.00 | 32.14 | 10.00 | 31.25 | 7.00 | 29.16 | 26 | 30.99 |
| | <i>Stemphylium</i> sp. | 8.00 | 28.57 | 11.00 | 34.38 | 9.00 | 37.50 | 28 | 33.79 |
| | Other Funguses | 11.00 | 39.28 | 8.00 | 25.00 | 8.00 | 33.33 | 27 | 33.25 |
| Spot and blight on leaf | <i>Alternaria</i> sp. | 20.00 | 25.31 | 24.00 | 30.37 | 21.00 | 28.00 | 65 | 28.04 |
| | <i>Bipolaris</i> sp. | 12.00 | 15.18 | 10.00 | 12.65 | 12.00 | 16.00 | 34 | 14.72 |
| | <i>Chaetomium</i> sp. | 3.00 | 3.79 | 3.00 | 1.26 | | | | |
| | <i>Cladosporium</i> sp. | 16.00 | 20.25 | 14.00 | 17.72 | 11.00 | 14.66 | 41 | 17.88 |
| | <i>Stigmina carpophila</i> | 7.00 | 8.86 | 10.00 | 12.65 | 7.00 | 9.33 | 24 | 10.57 |
| | <i>Helminthosporium</i> sp. | 11.00 | 13.90 | 9.00 | 11.39 | 8.00 | 10.66 | 28 | 12.16 |
| | Other Fungi | 10.00 | 12.65 | 12.00 | 15.18 | 16.00 | 21.33 | 38 | 17.10 |
| Wilt | <i>Fusarium</i> sp. | 7.00 | 28.00 | 8.00 | 24.24 | 7.00 | 29.16 | 22 | 27.00 |
| | <i>Rhizoctonia</i> sp. | 3.00 | 12.00 | 2.00 | 6.06 | 2.00 | 8.33 | 7 | 9.25 |
| | <i>Thielaviopsis</i> sp. | 11.00 | 44.00 | 16.00 | 48.48 | 9.00 | 37.50 | 36 | 42.16 |
| | Other fungi | 4.00 | 16.00 | 7.00 | 21.21 | 6.00 | 25.00 | 17 | 21.32 |
| | | | | | | | | | |
| Branch with canker | <i>Stigmina carpophila</i> | 3.00 | 7.14 | 4.00 | 6.67 | 3.00 | 5.56 | 10 | 6.47 |
| | <i>Monilinia laxa</i> | 2.00 | 4.76 | 5.00 | 8.33 | 4.00 | 7.41 | 11 | 7.25 |
| | <i>Phoma</i> sp. | 12.00 | 28.57 | 15.00 | 25.00 | 15.00 | 27.78 | 42 | 26.41 |
| | <i>Pseudobotrytis</i> sp. | 4.00 | 9.52 | 3.00 | 5.00 | 3.00 | 5.56 | 10 | 6.17 |
| | <i>Pyrenochaeta 2</i> | 4.76 | 5.00 | 5.00 | 7.00 | 3.50 | | | |
| | <i>Stemphylium</i> sp. | 5.00 | 11.90 | 8.00 | 13.33 | 11.00 | 20.37 | 24 | 16.25 |
| | <i>Ulocladium</i> sp. | 6.00 | 14.29 | 9.00 | 15.00 | 8.00 | 14.81 | 23 | 14.74 |
| | Other Fungi | 8.00 | 19.05 | 13.00 | 21.67 | 10.00 | 18.52 | 31 | 19.97 |
| | <i>Monilinia laxa</i> | 9.00 | 90.00 | 11.00 | 78.57 | 7.00 | 77.77 | 27 | 82.17 |
| Monilia | Other Fungi | 1.00 | 10.00 | 3.00 | 21.42 | 2.00 | 22.22 | 6 | 19.78 |

in all selected orchards and wilt has been determined in 60.87% of these orchards. At the almond trees in these orchards, leaf blight has been observed most with 88.22% value and this has been followed by fruit blight with 69.02%. The branch canker has been determined in 33.05% of the trees (Table 1).

Fomes fomentarius (L.) J. Kickx f. causing the wood decay has been found in 59.85% of the surveyed orchards

and it has been determined in only 3.08% of the trees in these orchards (Table 1 and Fig. 2). In the previous study, it has been stated that this fungus is wide in the forest trees in Turkey (Gezer, 2000).

Isolation of fungi diseases: As a result of the isolations performed in the samples with symptoms; it was determined that *Monilia* disease was caused by

Monilinia laxa, hull rot by *Stigmata carpophila*, Red leaf blotch by *Polystigma ochraceum* and Leaf Curl by *Taphrina deformans*. In previous studies, it was reported that these fungal pathogens caused similar diseases in almond trees (Ogawa and English, 1991; Adaskaveg and Ogawa, 1992; Saad and Masannat, 1997).

The result of the isolations performed in the diseased samples, in which more than one pathogenic fungi form a similar symptom, is shown in Table 2. In the Table 2, *Aspergillus* sp. has been determined as the highest value in 26.34% of the diseased fruit samples, followed by *Penicillium* sp. with 19.02%, *Alternaria* sp. with 11.55%, *Rhizopus* sp. with 8.46%, *Cladosporium* sp. with 7.54%, *Stemphylium* sp. with 7.05%, *Stigmata carpophila* with 5.93%, *Macrophoma* sp. with 3.21% and *Epicoccum* sp. with 2.08%. The rate of the other fungi that could not be diagnosed is 11.02%. These findings indicate that these fungi are also present in the almond trees according to a similar study carried out in California (Teviotdale and Hendricks, 1994).

In the isolations made in the diseased branch samples, *Stemphylium* sp. with 33.79%, *Phoma* sp. with 30.99% and *Coniothyrium* sp. with 3.12% were determined only in the samples brought from the Elazığ province. The rate of the other fungi that could not be diagnosed is 33.25% (Table 2).

In the isolations performed in the *Alternaria* sp. diseased leaf samples, the highest value was obtained at rate of 28.04%, followed by *Cladosporium* sp. with 17.88%, *Bipolaris* sp. with 14.72%, *Helminthosporium* sp. with 12.16%, *Stigmata carpophila* with 10.57% in order and *Chaetomium* sp. with 1.26% rate was determined only in the samples brought from the Diyarbakır province. The rate of the other fungi that could not be diagnosed is 17.10% (Table 2).

In the isolations performed in the samples taken from the almond trees that show wilt, *Thielaviopsis* sp. has been found with 42.16%, *Fusarium* sp. with 27.00% and *Rhizoctonia* sp. with 9.25%. The rate of the other fungi that could not be diagnosed is 21.32% (Table 2).

In the isolations performed in the almond branch samples in which canker formation occurred, *Phoma* sp. is the highest by 26.41%, followed by *Stemphylium* sp. by 16.25%, *Ulocladium* sp. by 14.74%, *Monilinia laxa* by 7.25%, *Stigmata carpophila* by 6.47%, *Pseudobotrytis* sp. by 6.17% and *Pyrenochaeta* by 3.50%. The rate of the other fungi that could not be diagnosed is 19.97% (Table 2).

In the isolations performed in mummified fruit samples, *Monilinia laxa* was found as 82.17% and the rate of the other fungi is 19.78% (Table 2).

The intensity and ranking of the pathogenic fungi, which we found in the isolations performed in branch, leaf and other diseased tissue samples, seem to be compatible with those reported in a similar study performed previously (Teviotdale and Hendricks, 1994).

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