International Journal of Nuts and Related Sciences 3(1):23-28, 2012

Distribution of Pistachio Die-Back in Khorasan-Razavi Province and Application of Some Fungicides for the Disease Control

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Abstract: The die-back of pistachio is one of the most important diseases affecting pistachio trees reducing yield production. In commercial fields, sampling was done from nine different pistachio production areas in Mahvelat, Khorasan Razavi province during 2008-2010. In each orchard, samples were taken from tree branches with canker during spring and summer seasons. Fungal species were identified based on the micro and macro-morphological features, reverse and surface characters of colonies. The ability of the isolates to produce disease was assessed on two year-old pistachio branches. The effect of Mancozeb, Benomyl, Metalaxyl, Rovral TS, Bordeaux mixture and Propiconazole was evaluated on radial mycelia growth of *P. variotii*, *N. mangiferae* and *Stemphylium* spp.. In most orchards, the samples were positive for fungal species and in only a few orchards no fungal isolates could be detected. Based on micro and macro- morphological features; *P. variotii*, *N. mangiferae*, and *Stemphylium* spp. were identified in most sampling areas with different frequencies. In pathogenicity tests, most isolates of *N. mangiferae* and *Stemphylium* spp. were able to establish disease, while about 43 % of *P. variotii* isolates produced die-back on inoculated branches. The results of fungicides produce different levels of control on mycelia growth of fungal species which was varied according to the kind of fungicide and fungal species. Overall, the differences between fungal isolates, biotic and a biotic factors to produce disease as well as contradictory results were obtained by different researchers as described in literature.

Keywords: Pistachio die-back, Pathogenicity, *P. variotii*, *N. mangiferae* and *Stemphylium* spp

INTRODUCTION

Die-back of pistachio is one of the most important diseases affecting the pistachio trees throughout of Iran, economically. The disease will affect different parts of tree, such as leave canopy, branch and trunk which destroy the tree after some years. Several fungal species have been reported to cause die-back of pistachio trees. On the other hand, various biotic and abiotic factors may produce similar symptoms of pistachio die-back or affect the tree which lead to infection by different fungal species.

Toxicity or deficiency of different soil elements such as Na, K, B, Zn, Ca and Cl may cause die-back of pistachio. For example, the toxicity of Na and deficiency of K reported to be as the main factors caused pistachio dieback in the orchards [6 & 8]. The damage can be varied based on tree species/cultivar, orchard management, fungal genera and their ecological and biological requirements as well as the weather conditions [13].

Several fungal species have been isolated from canker of infected trees in Iran; however most of them were not able to produce disease in different pathogenicity tests. Aminaee (1993) reported that the *Paecilomyces variotii* as causal agent of pistachio die-back in Kerman province. Later

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researchers reported several other fungal species such as *Cytospora* sp., *Coniothyrium* sp., *Nattrassia mangiferae*, *Fusarium* sp., *Fusarium equiseti, Alternaria* sp. and *Ulocladium* sp. [3]. They also mentioned among these fungal species only *P. variotii, Cytospora* sp. and *N. mangiferae* were able to produce disease. However, there are contradictory results regarding the ability of the species to produce disease [7]. This indicates the main reason for the pistachio die-back is poor orchard management which caused damage to tree; therefore, there will be possibility for fungal infection. Improving orchard management could reduce fungal infection and disease development on the trees, significantly. For example, [12] mentioned *P. variotii* as biotic factor to produce disease on pistachio tree and application of potassium, calcium and zinc could reduce disease development up to 63 % caused by this species. [15] reported that propiconazole could reduce mycelia growth of *N. mangiferae* under in-vivo conditions. Different species of *Botryosphaeria,* such as *B. dothidea*, *B. ribis* and *B. obtuse*, of *Eutypa lata*; *Xanthomonas* isolated from twig, branches and cluster of pistachio trees in various pistachio producing regions throughout the world [9, 10 ,13&14].

Little information is available on the causal agent of die-back of pistachio trees in Khorsan Razavi province, their pathogenicity and application fungicide to manage the disease.

MATERIALS AND METHODS

Sampling

In commercial fields, sampling was done from nine different pistachio production areas in Mahvelat, Khorasan Razavi province during 2008-2010. Overall, 180 orchards with pistachio die-back were selected. In each orchard, samples were taken from tree branches with canker during spring and summer seasons.

Isolation

Isolation of fungal species was carried out after cutting infected tissue into small pieces. The pieces were sterilized in 0.5 % sodium hypochlorite (NaOCl) for 1min and then washed in sterilized distilled water for three times. After drying, five pieces were placed on petri-dishes including PDA (potato dextrose agar; Merck, Germany) contain 250 mg tetracycline (AppliChem, Germany). The plates were kept at 25 ºC for 7 days in darkness and examined daily for fungal growth. The fungal colony was purified using single hyphal tip method as describe. The fungal colony was sub-culture on thin water agar. After 12-24 hours a single hyphal tip was selected and cut with agar block to establish pure culture using dissecting microscope at 10-30X. The agar blocks were transferred on PDA plates as well as slant PDA for maintenance.

Identification

Identification of fungal species was done based on the micro and macro- morphological features, reverse and surface characters of colonies.

Pathogenicity test

The two year-old pistachio branches, *Pistacia vera*, were inoculated with 7, 8 and 2 isolates of *P. variotii*, *N. mangiferae* and *Stemphylium* spp., respectively, collected from Mahvelat orchards as described by Afeck [1]. The ability of fungal isolates to produce disease was evaluated by assessing the length of lesions on branches 7 days after inoculation as well as re-isolation.

Effect of fungicides on mycelia growth

The experiments were conducted using three isolates of *P. variotii*, six isolates of *N. mangiferae* and two isolates of *Stemphylium* spp. collected from pistachio orchards in mahvelat regions. The effect of Mancozeb (WP 80%), Benomyl (WP 50%), Metalaxyl (Ridomil, G5%), Rovral TS (effective substance was Carbendazim 17.5% and Iprodione 35%), Bordeaux mixture (copper (II)

sulfate-slaked lime-water; 3-3-100) and Propiconazole (EC 25%), on mycelia growth were assessed under in-vitro conditions. A 100 ppm concentration of fungicides were prepared in molten PDA (50 ºC) and 20 ml aliquots were poured into petri dishes. Then, small discs from 3 days old of fungal colony of fungi were cultured in the center of each petri dish to test the inhibition activity of each fungicide. Fungal growth was measured after 5 days of incubation at 30 °C. For the control, medium mixed with sterilized distilled water.

Statistical analysis

The average values of mycelial growth were separately determined for each replication. The data were analysed using Proc GLM procedures (SAS Release Version 9.0, SAS Institute, Inc., Cary, NC). Mean comparisons were made using Duncan's new multiple range test at 5 % probability. When it was necessary data were log-transferred prior to analysis.

RESULTS AND DISCUSSIONS

The symptoms of pistachio die-back were observed in different pistachio orchards affecting by various biotic and a biotic parameters., *Paecilomyces variotii*, *Nattrassia mangiferae*, and *Stemphylium* spp. were isolated in the majority of pistachio orchards (about 90%) (table 1). This is in agreement with reports from Aminaee and Ershad (1987 & 1993); Alizadeh *et al*., (2005) and Mozaffari *et al*., (2005). Several other species of fungi and bacteria have been isolated from pistachio trees with die-back (Rumbos, 1986, Corraza *et al*., 1990, Chitzanidis *et al*., 1995, Swart and Botes, 1995, Facelli *et al*., 2005).

P. variotii was isolated from main limb and branches but isolation of *N. mangiferae* was high in main limb, branches and trunk. These data showed that the *N. mangiferae* can infect all tisuues of the piatchio trees (table1). In contrast, Stemphylium was only isolated from branches. It seems *Stemphylium* has lower pathogenicity on pistachio trees in Mahvelat. Time of isolation showed no difference between the isolates. Late spring or early summer is the best time for isolation of the fungi in pistachio trees.

It seems that several biotic and a biotic parameters such as deficiency or toxicity of different elements, other fungal pathogens, root knot nematode, unsuitable of irrigation, texture and structure soil, pistachio pests can predispose the trees to die-back. These findings are agreement with Alaei (1997)

In pathogenicity tests, 85 and 69% isolates of *N. mangiferae* and *Stemphylium* spp. respectively, produced symptoms of the disease, while about 43 % of *P. variotii* isolates showed die-back symptoms on inoculated branches. These data indicates that isolated fungi from infected trees are different in pathogenicity. So, the fungi especially *P. variotii* can not infect the pistachio trees in all cases. This is also in agreement with the results of Alaei *et al.,* (1997) on pistachio. Heidarian (1995) also reported the *N. mangiferae* isolates are highly aggressive on citrus.

Table 1. Comparison time, regions and infected plant tissue were used for isolation fungal species during 2008-2010

Date	Fungal species	Plant tissue	Region
April or December	*Paecilomyces variotii*	Main limb and branch	Feyz abad
April or December	*Paecilomyces variotii*		Janat abad
April or December	*Paecilomyces variotii*		Shams abad
April or December	*Paecilomyces variotii*		Mehneh
April or December	*Paecilomyces variotii*		Fath abad
April or December	*Paecilomyces variotii*		Abdol abad
April or December	*Paecilomyces variotii*		Hasan abad
April or December	*Nattrassia mangiferae*	Main limb, trunk and branch	Feyz abad
April or December	*Nattrassia mangiferae*		Janat abad
April or December	*Nattrassia mangiferae*		Fath abad
April or December	*Nattrassia mangiferae*		Shams abad
April or December	*Nattrassia mangiferae*		Doogh abad
April or December	*Nattrassia mangiferae*		Abdol abad
April or December	*Nattrassia mangiferae*		Hasan abad
April or December	*Nattrassia mangiferae*		Mehneh
April or December	*Stemphylium* spp.	Branch	Hasan abad
April or December	*Stemphylium* spp.		Abdol abad
April or December	*Stemphylium* spp.		Feyz abad
April or December	*Stemphylium* spp.		Fath abad
April or December	*Stemphylium* spp.		Mehneh

The results of fungicides produced different levels of control on mycelia growth of fungal species which was varied according to the kind of fungicide and fungal species (table 2). Overall, propiconazole, rovral T-S and bordeax mixture inhibit the mycelia growth of different fungal species, completely. The presence of ridomil in culture media had no effects on mycelia growth of *N. mangiferae* and *Stemphylium* spp. isolates compared to control, while the mycelia growth of *P. variotii* decreased, significantly. There was no mycelia growth for the isolates of *P. variotii* and *Stemphylium* spp. when Mancoze added to media, while different isolates of *N. mangiferae* were able to growth although reduce in significant levels. Susceptibility of fungal species to fungicides used in this study reduced in the *P. variotii*, *Stemphylium* spp., *N. mangiferae* and isolates, respectively. On the other hand, *N. mangiferae* isolates were the most aggressive ones in pistachio dieback.

Table 2. Effect of fungicidal activity on radial mycelia growth of *P. variotii*, *N. mangiferae* and *Stemphylium* spp.

	Fungal isolates										
	P. variotii			*N. mangiferae*						*Stemphylium* spp.	
Fungicides	Pae1	Pae2	Pae7	Nat2	Nat3	Nat4	Nat5	Nat6	Nat8	Stem1	Stem 2
Mancozeb	0.0D	0.0D	0.0D	16.0D	15.1D	15.0D	15.8D	15.1D	15.9D	0.0 C	0.0C
Ridomil	36.5C	35.7C	35.7C	48.3A	42.1A	42.9A	44.4A	44.8A	45.9A	13.1A	13.3 A

Benomyl	40.1B	41.4B	42.1B	34.3C	33.8C	33.3C	33.9C	34.0C	34.4C	7.8 B	7.5 B
Propiconazole	0.0 D	0.0 D	0.0 D	0.0E	0.0E	0.0 E	0.0 E	0.0 E	0.0 E	0.0 C	0.0 C
Brodeax	0.0 D	0.0 D	0.0 D	0.0 E	0.0 E	0.0 E	0.0 E	0.0 E	0.0 E	0.0 C	0.0 C
Rovral T-S	0.0 D	0.0 D	0.0 D	0.0 E	0.0 E	0.0 E	0.0 E	0.0 E	0.0 E	0.0 C	0.0 C
Control	42.5A	44A	43.7A	47.5A	43 A	44.6A	43 A	45.0A	46.0A	13.5A	13.0 A

Numbers with similar letter do not differ significantly at 5% level according to Duncan's Multiple Range Test (DMRT)

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