Journal of Nuts

Journal homepage: ijnrs.damghan.iau.ir

ORIGINAL ARTICLE

Investigation on the Efficacy of some Fungicides in Controlling Alternaria Late Blight of Pistachio

Hossein Khabbaz-Jolfaee[1], Mehdi Mohammadi-Moghadam[*2], Gholamreza Baradaran[3], Mojtaba Ghalandar[4]

[1]*Department of Plant Pathology, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran*

[2]*Crop and Horticultural Sciences Research Department, Agricultural and Natural Resources Research and Education Center of Semnan Province (Shahrood), AREEO, Shahrood, Iran*

[3]*Department of Plant Protection, Kerman Agricultural and Natural Resources Research and Education Center, AREEO, Kerman, Iran*

[4]*Department of Plant Protection, Markazi Agricultural and Natural Resources Research and Education Center, AREEO, Araak, Iran*

K E Y W O R D S

Captan;
Chemical control;
Mishocap;
Nordox;
Pistachio;
Pyradim

A B S T R A C T

The efficacy of Pyradim® DF with pyraclostrobin+dimetomorf active ingredient was evaluated in comparison with fungicides Captan, Nordox, Mishocap in the control of pistachio Alternaria late blight. The experiment was carried out in Markazi (Zarandieh), Kerman and Semnan in a Randomized Complete Block Design (RCBD) with 8 treatments and 4 replications. Treatments were applied at two stages (before bud swelling and after the petals fall and fruit set). The data were analyzed using SAS software and mean comparison of disease severity and disease incidence percentages were done by Duncan's multiple range test (P =5%). The results showed that Pyradim with dosage of 1 and 0.75 per thousand has a good efficacy in disease control. So that the efficiency of dosage 1g L^{-1} in reducing the severity of the disease compared to the control without spraying was about 88% in Zarandieh and about 77% in Kerman and Semnan. The efficacy of Pyradim at the dose of 0.75g L^{-1} in Zarandieh, Kerman and Semnan was about 76%, 72% and 74%, respectively, but the dose of 0.5g L^{-1} Pyradim was not acceptable and in the three studied areas was between 52 to 55%. Captan fungicide also had acceptable efficacy in controlling pistachio Alternaria late blight disease, so that its efficiency was more than 70% in Kerman and Semnan and 68% in Zarandieh. In contrast, Nordox and Mishocap fungicides did not have good efficiency. Therefore, 0.75g L^{-1} pyradim is recommended for controlling pistachio Alternaria late blight disease.

[*]**Corresponding author**: Email address: mm.moghadam52@gmail.com
Received: 6 September 2022; Received in revised form: 5 January 2023; Accepted: 9 April 2023
DOI: 10.22034/jon.2023.1966444.1193

Introduction

Among the countries of the world, only Iran, USA, Turkey, Syria, China, Greece, Afghanistan and Italy cultivate pistachios economically. The largest producers of this product in the world are Iran, the United States and Turkey, respectively (FAO, 2011; Norozi *et al*., 2019; Sharifkhah *et al*., 2020; Nazoori *et al*., 2022b). According to Food and Agriculture Organization (FAO), Iran is the main center of diversity and has the largest cultivation area of pistachio in the world. This country was the first producer and exporter of this product in the world in 2014, with the production of 440,000 tons of pistachios (Shamshiri and Hasani, 2015; Sharifkhah *et al*., 2020; Behzadi Rad *et al.*, 2021; Nazoori *et al*., 2022b). Pistachio as a strategic and commercially valuable product has a special place among Iranian agricultural products and has a major role in the country's non-oil exports (Eslami *et al*., 2019; Hosseini *et al*., 2022; Nazoori *et al*., 2022a).

Various diseases affect pistachio production in the world and of course in Iran, one of which is Alternaria blight. This disease is one of the most important pistachio diseases in the world (Ozkilinc and Sevinc, 2016; Ozkilinc *et al.*, 2017). The causal agent of the disease is *Alternaria* spp., the main species of which is *Alternaria alternata* (Fr.) Keissl. Of course A. tenuissima (Kunze) Wiltshire and A. arborescens E.G. Simmons have also been mentioned as disease causal agent (Khabaaz jolfaee, *et al*., 2020, Ozkilinc and Sener, 2017). This disease was first reported in Iran by Aminaei and Ershad in 1989 from Kerman. In 2010, Mirabolfathi and Afzali reported the disease from the central regions of the country and Khorasan, and shown the disease to be pathogenic (Khabaaz jolfaee, *et al*., 2020). This disease is still present in pistachio orchards in Semnan (Damghan), Kerman and Markazi provinces and is damaging in favorable environmental conditions. However, it does not cause much damage. The disease begins with the appearance of necrotic spots on the leaf surface that gradually join together and cause premature leaf fall (Karaoglanidis, *et al.,* 2011). Based on the results of a study on the efficacy of several fungicides in controlling pistachio blight in the United States, copper hydroxide and nordox (copper oxide) fungicides significantly reduced Alternaria symptoms on fruits and were introduced as effective fungicides to control the disease (Michailides and Morgan, 1993). In 1995, the fungicide Benomyl was also introduced for this purpose (Michailides *et al.*, 1995). Another study in 1999 confirmed the efficacy of azoxystrobin in controlling the disease (Michailides *et al*., 1999). Two separate studies in 1997 and 2002 confirmed the high efficacy of the fungicides azoxystrobin, iprodione and tebuconazole in controlling pistachio blight (Pryor and Michailides, 2002). In another study, the use of buscalid + pyraclostrobin (pristine), azoxystrobin, pyraclostrobin, trifloxystrobin, and cyprodinil and fludioxonil, especially as periodically (alternatively) in the resistance avoidance (prevention) management program was introduced to control the disease (Allemann, 2007). In the world, various fungicides including different types of fungicide DMI group (inhibitor of sterols demethylation), benzimidazoles, fungicides of QOI group (external quinone inhibitor) such as azoxystrobin, trifloxystrobin and piracloestrobin, fungicides of SDHIs group (succinate dehydrogenase inhibitors) such as buscalid, carboxin, flutolanil and tyflosamide (Avenot and Michailides, 2007) have been recorded

and introduced to control Alternaria pistachio blight disease. Because Alternaria species infect flower buds and cause the disease later during fruit growth, the best time to spray in order disease control on the fruit is before flower buds formation until just before harvest (Doster and Michailides, 1999; Brazauskiené and Petraitiené, 2004; Meena*et al.*, 2004; Ferguson *et al*., 2005). In the present study, the efficacy of several fungicides was evaluated in order to control Alternarialate blight of pistachio.

Materials and Methods

The experiment was conducted in Markazi (Zarandieh), Kerman and Semnan (Damghan) provinces. In the mentioned provinces, a garden with a history of Alternaria late blight of pistachio was selected from the cultivars of Kaleghoochi, Fandoghi and Ohadi, Shahpasand, respectively, with similar trees in terms of age and growth conditions. The experiment was conducted in a randomized complete block design with 8 treatments and 4 replications as follows. Experimental treatments included Pyradim (piraclosterobin + dimethomorph DF) with doses of 0.5, 0.75 and one per thousand, Flint® (WG 50%) 0.2 per thousand, Captan (Captan WP50%) with a dose of 3 per thousand, Nordox ® (Copper oxide WG38%) at a dose of one per thousand and Mishokap® (Copper oxychloride WP35%) at a dose of 3 per thousand and controls (without any operation and by water-spraying). In this study, each replication included two trees about 15 to 20 years old. Among the tested trees, one untreated tree was considered to avoid the effect of treatments on each other. The treatments were applied by The treatments were applied using a motorized lance equipped sprayer in two times, the first time, before the bud opening and the second time, after the petals fall and fruit formation. During harvest, the treated trees were examined and samples were taken from the clusters to evaluate the percentage of disease incidence and severity.

Percentage of disease incidence

From the four main directions and the center of the trees in each plot, 10 clusters were randomly picked and transported to the laboratory in separate bags. In the laboratory, the samples were counted based on the number of pistachio fruits with symptoms of pistachio Alternaria late blight disease and without disease symptoms in each cluster, and according to the following formula, the percentage of the disease was calculated in them (Allemann, 2007).

$$PDI = (nd/N) \times 100$$

In this formula, PDI: percentage of disease incidence, nd: the number of samples with disease symptoms, and N: the total number of samples counted.

Percentage of disease severity

To determine the percentage of disease severity, 10 clusters were randomly picked from the four main directions and the center of the trees in each plot. The symptoms on each cluster were classified based on the percentage of the number of infected fruits in each cluster to the total number of fruits in a cluster from 0 to 4 (Can *et al*., 2014) as follow:

Grade zero: no symptoms (all the fruits of a cluster are healthy), 1: 1 to 15% of fruits' surface of a bunch have blight symptoms, 2: 16 to 30% of fruits' surface of a bunch have blight symptoms, 3: 31 to 70% of fruits' surface of a bunch have blight symptoms, and 4: 71 to 100% of fruits' surface of a bunch have blight symptoms (Can *et al*., 2014).

Then, using the following formula, the percentage of disease severity was calculated.

$$PDS = [\Sigma (n_i \times v_i) / V \times N] \times 100$$

In this formula, PDS: percentage of disease severity, n_i: the number of samples with the same degree of contamination, v_i: disease degree of each sample, N: total number of samples for each replicate, and V: maximum degree of contamination.

Analysis of variance and comparison of means

The results of calculating the percentage of disease incidence and the percentage of late blight disease severity for each plot were analyzed in the SAS statistical program and considering the significance of the difference between the treatments, the mean of the treatments was compared with Duncan's multiple range tests at the five percent probability level.

Results

Due to the fact that the experimental cultivars were located in different provinces and the present significant differences by Bartlett's test; therefore, the data of each of the provinces was analyzed, separately.

Markazi Province

The analysis of variance of data in Central Province showed that the effect of the treatments on reducing the disease severity and the occurrence of the disease in comparison with the control was significant (Table 1). The lowest percentage of disease severity (2.50 and 5.00) and disease occurrence (3.50 and 6.00) were belonged to the treatments. Pyradim with a ratio of 0.75and $1gL^{-1}$ had the highest significant effects to reduce disease severity and occurrence followed by other fungicides (higher than those control). The value of disease severities of Captan, Nordox, Pyradim and Mishocap were 6.75, 8.50, 9.50 and 11.25, and occurrence of the disease 8, 9.25, 13 and 11.50, respectively (Table 2)

Kerman province

Analysis of variance of the data belonged to the Kerman province have shown significant differences between fungicides as well as with control on reducing disease severity and the occurrence of the disease (Table 1). The lowest rate of disease severity by 6.25, 7.50 and 7.75 and disease incidence by 5.75, 6.25 and 6.50 were related to Pyradim 1 g L^{-1}, Pyradim 0.75 g L^{-1} and captain, respectively. The highest rate of disease severity (16.25 and 16.00) and disease incidence (15.37 and 14.62) occurred in trees treated with Mishucap and Nordox, respectively (Table 2).

Semnan province

In Semnan province, the analysis of variance of the data obtained from the evaluation of the leaves of the treated trees showed that the treatments had a significant effect on reducing disease severity and occurrence of disease in comparison with the controls (Table 1). The lowest rate of disease severity (6.50, 7.25 and 7.50) and disease occurrence (7.75, 8.25 and 8.50), respectively, related to Pyradim 1 per thousand, Pyradim 0.75 per thousand and Captan treatments with no significant differences. Other fungicides, including Pyradim, Mishucap, and Nordox, respectively, with disease severity by 13.12, 14.00, and 15.00, and disease incidence of 12.75, 14.37, and 14.37, were found in

the next statistical group and higher than the controls (Table 2).

Table 1. Variance Analysis of disease severity percent and disease incidence percent on fruit in Markazi, Semnan and Kerman provinces

S.O.V.	D.F.	Mean squares					
		Markazi		Samnan		Kerman	
		Disease incidence (%)	Disease severity (%)	Disease incidence (%)	Disease severity (%)	Disease incidence (%)	Disease severity (%)
Replicate	3	22.79^{ns}	2.86^{ns}	18.44^{ns}	9.36^{ns}	83.22^{ns}	83.22^{ns}
Treatment	7	407.625^{**}	222.17^{**}	207.97^{**}	309.69^{**}	1217.24^{**}	1217.24^{**}
Error	21	11.29	4.05	2.03	1.06	87.30	87.30
C.V.		12.2%	14.1%	9.7%	6.91%	24.19%	24.19%

** Significant at 1% level; ns: not significant

Table 2. Mean comparison of disease severity percent and disease incidence percent on fruit in Markazi, Semnan and Kerman provinces

Treatment	Makazi		Samnan		Kerman	
	Mean of disease incidence (%)*	Mean of disease severity (%)*	Mean of disease incidence (%)*	Mean of disease severity (%)*	Mean of disease incidence (%)*	Mean of disease severity (%)*
Pyradim® DF 1 g L^{-1}	3.50a	2.50a	6.75c	6.50d	5.75c	6.15d
Pyradim® DF 0.75 g L^{-1}	6.00ab	5.00ab	8.25c	7.25d	6.25c	7.50d
Captan WP50% 3 g L^{-1}	8.00abc	6.75bc	8.50c	7.50d	6.50c	7.75d
Nordox® WG38%1 ml l^{-1}	9.25bc	8.50cd	14.37b	15.00b	14.62b	16.00b
Mishocap WP35% 3 g L^{-1}	11.50c	11.25d	14.37b	14.00c	15.37b	16.25b
Pyradim® DF 0. 5 g L^{-1}	13.00c	9.50cd	12.75b	13.12bc	12.00b	12.82c
Control (without any spraying)	26.25d	21.25e	25.00a	27.62a	21.00a	27.12a
Control (water spraying)	32.00e	23.25e	26.25a	28.25a	25.25a	29.25a

* The means of each column followed by common letters are not significantly different (Duncan's multiple range test α= 5%).

Discussion

Overall, new Pyramid fungicide with doses of 1 and 0.75 per thousand had the highest efficacy controlling alternaria blight in pistachio reducing disease severity by 88% in Zarandieh and 77% in Kerman and Semnan provinces. The efficiency of Pyradim 0.75g L^{-1} ranged 76%, 72% and 74% in Zarandieh, Kerman and Semnan, respectively. Pyradim treatments with a dose of 0.75 and 1 g L^{-1} were found to be effective to control disease with no significant differences. The efficiency of 0.5 g L^{-1} pyramids in the three investigated provinces was between 52 and 55%. The active ingredient of Pyradim fungicide is pyraclostrobin + dimethomorph. Piraclostrobin belongs to QoI fungicides (external inhibitors of quinones) and its site of action is complex III: cytochrome bc1 (ubiquinol oxidase) in Qo (cyt b gene) and prevents respiration. These groups of fungicides such as azoxystrobin, trifloxystrobin and piraclostrobin have been introduced to control pistachio alternaria blight disease (Avenot and Michailides, 2007).

Dimethomorph inhibits the biosynthesis of cell membrane phospholipids.

Captan also had an acceptable efficiency in controlling pistachio alternaria blight disease, so that it was more than 70% effective in Kerman and Semnan and 68% in Zarandiyeh. On the other hand, Nordox and Mishucap fungicides were not effective and except for Nordox, which was 60% effective in Zarandieh, in other areas, the effectiveness of these fungicides was less than 50%. Although some studies during 1990 to 1992 in the United States showed that copper hydroxide and Nordox (copper oxide) fungicides significantly reduced Alternaria spots on fruits and were introduced as effective fungicides (Michailides and Morgan, 1993), but in the current study, copper fungicides Nordox and Mishucap were not effective in controlling disease. The possible reasons for the difference in results could be mentioned the pathogenic species or race in the present study as well as time or number of fungicide applications, the variety and the age of trees.

Acknowledgements

Hereby, thank you for the sincere cooperation of the Agricultural and Natural Resources Research and Education Center of Kerman, Markazi and Semnan Province in conducting this research.

Conflict of interests

All the authors declare that there is no conflict of interest in the study.

References

Allemann A (2007) A dissertation submitted in partial fulfilment of the requirements for the degree of Master of Science in Agriculture. University of the Free State press. 116pp.

Anonymous (1998) General Directorate of Statistics and Information of the Agriculture Ministry.272pp.

Anonymous (2016) Statistics of the Jihad Agriculture Ministry.

Anwar W, Haider MS, Aslam M, Shahbaz M, Nawaz Khan S, Bibi A (2015) Assessment of Antifungal Potentials of some Aqueous Plant Extracts and Fungicides Against AlternariaAlternata. Journal of Agricalture Research. 53,75-82.

Avenot HF, Michailides TJ (2007) Resistance to Boscalid Fungicide in *Alternaria alternata* Isolates from Pistachio in California. Plant Disease. 91, 1345- 1350.

Behzadi Rad P, Roozban MR, Karimi S, Ghahremani R, Vahdati K (2021) Osmolyte accumulation and sodium compartmentation has a key role in salinity tolerance of pistachios rootstocks. Agriculture. 11(8), 708.

Brazauskiené I, Petraitiené E (2004) Effects of fungicide application timing on the incidence and severity of *Alternaria* blight (*Alternaria brassicae*) and on the productivity of spring oilseed rape (*Brassica napus* L. ssp. *Oleiferaannua* Metzg.). Agronomy Research. 2,121-133.

Can C, Konukoglu F, Ozgun E, Kusek M, Ozkilinc H, Aktan A, Citak M, Sarpkaya K (2014) *Alternaria* Blight Infections of Pistachio Fruits in the Southeastern Anatolia Region of Turkey. Acta Horticulturae. 1028, 171-178.

Doster MA, Michailides TJ (1999) Relationship between shell discoloration of pistachio

nuts and incidence of fungal decay and insect infestation. Plant Disease. 83,259-264.

Eslami M, Nasibi F, Manouchehri Kalantari K, Khezri M, Oloumi H (2019) Effect of exogenous application of larginine and sodium nitroprusside on fruit abscission and physiological disorders of pistachio (*Pistacia Vera* L.) scions. International Journal of Horticultural Science and Technology. 6, 51-62.

Evans N, Michailides TJ, Morgan DP (1997) Environmental and the progress of *Alternaria* light blight development in pistachio. In: California Pistachio Industry. Annual Report, California Pistachio Commission, Fresno, CA. 103 pp.

Ferguson L, Polito V, Kallsen C (2005) Pistachio Production Manual. http:// fruitsndnuts .udavis.edu/crop/pistachio. 14/05/2006.

FAO (2011) Production Year Book. Vol. 58.

Hosseini N, Rezanejad F, ZamaniBahramabadi E (2022) Effects of soil texture, irrigation intervals, and cultivar on some nut qualities and different types of fruit blankness in pistachio (*Pistacia vera* L.). International Journal of Horticultural Science and Technology. 9, 41-53.

Khabbaz Jolfaee H, Bakhtiari MH, Mirabolfathy M (2020) The management of pistachio *Armillaria* rot. Iranian Research Institute of Plant Protection. Registration No. 57770

Karaoglanidis GS, Luo Y, Michailide TJ (2011) Competitive Ability and Fitness of *Alternaria alternata* Isolates Resistant to QoI Fungicides. Plant Disease. 95, 178-182.

Meena PD, Meena RL, Chattopadhyay C, Kumar A (2004) Identification of critical stage for disease development and biocontrol of *Alternaria* blight if Indian Mustard (*Brassica juncea*). Journal of Phytopathology. 152, 204-209.

Michailides TJ, Morgan DP (1993) Control of *Alternaria* late blight of Pistachio using multiple application of organic and other fungicides. Proceedings of the 67th Annual Western Orchard Pest & Disease management Conference. 67, 5-6.

Michailides TJ, Morgan DP, Doster MA (1995) Diseases of Pistachio in California and their significance. Acta Horticulturae. 419, 337-343.

Michailides TJ, Morgan DP, Felts H (1999) Chemical control of *Alternaria* blight of California pistachio in 1998. In: California Pistachio Industry Annual Report Crop Year.

Nazoori F, ZamaniBahramabadi E, Mirdehghan H (2022) Effect of sulfur pesticide on the quality of fresh pistachios in cold storage. International Journal of Horticultural Science and Technology. 9, 453-462.

Nazoori F, ZamaniBahramabadi E, Mirdehghan H, Yousefi M (2022) Preharvest application of sulfur as pesticide on fresh hull and kernel of pistachio (*Pistacia vera* L.). International Journal of Horticultural Science and Technology. 9, 117- 129.

Norozi MM, Valizadeh Kaji B, Karimi R, Nikoogoftar Sedghi MA (2019) Effects of foliar application of potassium and zinc on pistachio (*Pistacia vera* L.) fruit yield. International Journal of Horticultural Science and Technology. 6, 113-123.

Ozkilinc H, Sarpkaya K, Kurt S, Can C, Polatbilek H, Yasar A, Sevinc U, Uysal A, Konukoglu F (2017) Pathogenicity, Morpho-Species and Mating Types of *Alternaria* spp. causing *Alternaria* blight in *Pistacia* spp. in Turkey, Phytoparasitica. https:// doi. org/10 .1007/s12600-017-0624-8.

Ozkilinc H, Sener K (2017) Screening Fungicide Resistance of *Alternaria* Pathogens Causing *Alternaria* Blight of Pistachio in Turkey. YYU Journal Agricalture Science. 27, 543-549.

Ozkilinc H, Sevinc U (2016) Molecular phylogenetic approaches to defining *Alternaria* species associated with *Alternaria* blight of pistachio and its wild relatives. 6th Plant Protection Congress with International Participation. Turkey. pp. 487.

Pryor BM, Michailides TJ (2002) Morphological, pathogenic, and molecular characterization of *Alternaria* isolates associated with *Alternaria* late blight of pistachio. Phytopathology. 92, 406-416.

Shamshiri MH, Hasani MR (2015) Synergistic accumulative effects between exogenous salicylic acid and arbuscular mycorrhizal fungus in pistachio (*Pistacia Vera* cv. Abareqi) seedlings under drought stress. International Journal of Horticultural Science and Technology. 2, 151-160.

Sharifkhah M, Bakhshi D, Pourghayoumi MR, Abdi S, Hokmabadi H (2020) Effect of pollination time on yield and antioxidant properties of some pistachio cultivars. International Journal of Horticultural Science and Technology. 7, 51-58.