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Chemical Control of *Paecillomyces variotii* the Causal Agent of Pistachio Die Back by Using Fungicides

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ABSTRACT: Die-back of pistachio is one of the most important pistachio diseases. The disease caused by various factors such as *Paecillomyces variotii.* In present study *in vitro* antifungal effects of the seven fungicides consists of Copper oxychloride, Rovral TS, Benomyl, Mankozeb, Elite, Tilt and Artyva on mycelial growth of pathogen were investigated. In first experiment, doses of 0, 5 ppm, 10 ppm, 25 ppm and 50 ppm of fungicides were used. In second experiment, optimal doses of fungicides were tested on the branch by two methods. Fungicides Benomyl and Rovral TS showed the most effective against mycelial growth of pathogen. The minimum growth inhibition of pathogen was related to fungicide Elite. In horizontal position of branches, the maximum reduction of branch canker was observed in application of fungicides Benomyl. Fungicides Elite and Artyva were subsequently placed. Eventually, in vertical position of branches fungicides Artyva , Elite, Rovral TS, Mankozeb as well as tilte showed the maximum reduction of branch canker, respectively.

***Keywords*:** Artyva , Benomyl, Copper oxychloride, Elite, Mankozeb, Rovral TS, Tilt

INTRODUCTION

Pistachio is one of the most agricultural products in Iran. This export products is important in different aspects of economic, social, environment. Production values of this unique product is about 10 percent of non-oil revenues. Pistachio is considered as latest cultivable crops. It means that if pistachio orchards were eliminated, no other products could be cultivated. As well as desertification and desert progress will be followed. Hence, efforts to preserve this product will be followed by decentralization desert beside economic benefits. The die-back of pistachio is one of the most important pistachio diseases.

The disease caused by various factors such as nutrition, the drought, sodium adsorption ratio as well as plant pathogenic fungi which the most important of them is *Paecillomyces variotii* (Samih , 2005). *Paecilomyces variotii* is the asexual state of *Byssochlamys spectabilis*, in family *Trichocomaceae* and a member of the Phylum Ascomycota (Houbraken , 2008). A mixture of biological, physical, resistant cultivars, agronomic and chemical strategies were used to control of die back. The application of chemical toxins in agriculture still uses as an effective and efficient method in control of plant disease in spite of environmental infection and negative impacts on beneficial organisms. Little research has been conducted on the effect of fungicides on this disease. The only research is an experiments which has been investigated chemical and biological control of citrus branch wilt (*Nattrassia mangiferae*). The results showed that fungicide tilte had the highest inhibition of radial growth of pathogen (Taheri , 2005).

In present study, *in vitro* antifungal effects of different doses of seven fungicides consists of Copper oxychloride, Rovral TS, Benomyl, Mankozeb, Elite, Tilt and Artyva on mycelial growth of *Paecilomyces variotii* were investigated. Then, optimal doses of fungicides were tested on the branch by two methods of horizontal and vertical position of

branches (as a reliable method for horticultural uses). Eventually, the most effective fungicide for disease control were selected.

MATERIALS AND METHODS

2. 1. Isolation of pathogen

In order to isolation of pathogen, infected branches cut off and transported to the laboratory. 1-2 mm^2 of skin and wood were removed between healthy and infected tissue by scalpel blade. Then, they were sterilized using 0.5% sodium hypochlorite solution for 90 s and rewashed by sterile distilled water and dried. Afterward, they were placed on PDA (potato dextrose agar) culture medium. Petri dishes were incubated for 7-10 days at 28°C in the dark. They were examined daily until colony of pathogen grown on PDA medium.

2. 2. Purification of pathogen

Water agar culture medium (WA) was used to purification of isolates. After three days, germinated spores were isolated from culture medium by sterile needle under a stereomicroscope. Then, they were transferred to the petri dishes containing PDA culture medium. Petri dishes were maintained in a refrigerator at 4 °C when entire surface of them were covered by pathogen colonies.

2. 3. Identification of pathogen

The isolates were identified by macroscopic characteristics, form of sexual, colony color, microscopic characteristics such as length of conidial cell, conidiophore branches, shapes and sizes conidia as well as current keys (Samson, 1974; Brown and smith, 1957; Hoog , 2000).

2. 4. Proof of pathogenicity

In order to proof of *Paecilomyces* isolates pathogenicity, healthy branches (with 20 cm length and 2 mm diameter) were collected from pistachio trees cultivar 'Fandoghi'. Branches were surface sterilized using 70% ethanol. Then, 2-3 cm of skin and wood (depth of 1-1.5 mm) was cut off under sterile conditions and a mycelial disc (5mm) from pathogen was placed in it. To control treatment a disc (5mm) from PDA culture medium was placed in the gap between skin and wood. Inoculated branches were maintained in sterile test tubes with sterile distilled water. Test tubes were closed by sterile cotton and were incubated under laboratory conditions.

2. 5. In vitro chemical control on culture media (petri dish)

In order to identify the best type of fungicides and optimal dose of controllers, *in vitro* effect of seven fungicides consists of Copper oxychloride, Rovral TS, Benomyl, Mankozeb, Elite, Tilt and Artyva on pathogen was studied. Doses of 0, 5 ppm, 10 ppm, 25 ppm and 50 ppm of every fungicides were added to 100 ml PDA culture medium separately and were poured into petri dish. Then, a mycelial disc (5mm) from 3-day old culture margins of *Paecilomyces variotii* was placed on the middle of every petri dishes. To control treatments a disc (5mm) from margins of PDA was placed in the middle of the petri dishes. Petri dishes were incubated at 28°C. After 7 days of the incubation period, radial growth of pathogens was recorded.

2. 6. In vitro chemical control on branches

The optimal doses of fungicides used in the previous experiment were tested on the branch with a length of 30 to 40 cm by following two ways.

2. 6. 1. Horizontal position of branches

The branches of every treatments were immersed in suspension of water and the best doses of every fungicides for 4-6 h. Sterile distilled water was used to control treatments. Then, a mycelial disc (5mm) from 3-day old culture margins of *Paecilomyces variotii* was placed between the skin and wood of every branches. Inoculation sites were covered by sterile cotton and then wrapped with parafilm. Branches were irrigated by sterile syringe containing sterile distilled water. Development of fungus on the branches was measured. In order to ensure accuracy in work, the fungus was re-sampled and cultured on PDA.

2. 6. 2. Vertical position of branches

The branches of every treatments were vertically placed in suspension of water and the best doses of every fungicides for 4-6 h. So that only the bottom of them placed into suspension. Other steps were performed same as previous method.

2. 7. Statistical analysis

The experiments were carried out in a completely random design c with three repeats. The treatments were compared at $p \leq 0.01$ by using Duncan's multiple range test (Little and Hills, 1978).

RESULTS AND DISCUSSION

3.1. Proof of pathogenicity

After 21 days, surface of the inoculated branches were covered by pathogen. Necrosis and nigrescence were observed in infected branches. Whereas, there were no symptoms of skin or wood necrosis in control branches.

3.2. In vitro chemical control on culture media (petri dish)

The results revealed that there were significant difference between fungicides in growth inhibition of pathogen ($P<0.01$). As well as various doses of fungicides and interaction between them and various doses showed significant difference ($P<0.01$). Results of figure 1 showed all fungicides could control mycelial growth of pathogen.
Fungicides Rovral TS and Benomyl revealed the most effective against mycelial growth of pathogen. There were no significant difference between these two fungicides. Increasing the doses of fungicides increased inhibition of pathogen growth significantly. Therefore, there was a direct correlation between the concentrations of fungicides and disease control. The results revealed that fungicide Rovral TS controlled disease 100% in all doses. Fungicide Benomyl performed the same as fungicide Rovral TS in all doses except dose of 5 ppm. Fungicide Mankozeb inhibited mycelial growth of pathogen 100% at doses of 25 ppm and 50 ppm. Fungicide Artyva inhibited 85.35% and 85.71% in same doses, respectively. In spite of fungicides Copper oxychloride and Elite showed similar effects at low doses, effect of Copper oxychloride was more than the Elite at doses of 50 ppm.

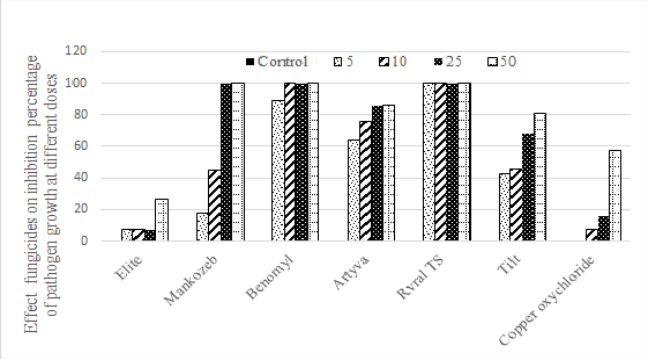

Figure 1. Effect seven fungicides on inhibition percentage of pathogen growth at different doses based on the Duncan's multiple range test

3. 3. In vitro chemical control on branches

The dose of 50 ppm fungicides were used in these experiments.

3. 3. 1. Horizontal position of branches

The results showed that there were significant difference between fungicides in branch canker ($P<0.01$). The maximum reduction of branch canker was observed in application of fungicides Benomyl. Fungicides Elite, Artyva , Rovral TS, Copper oxychloride and Tilt were subsequently placed. Fungicide showed Mankozeb minimum reduction of branch canker (Figure 2).

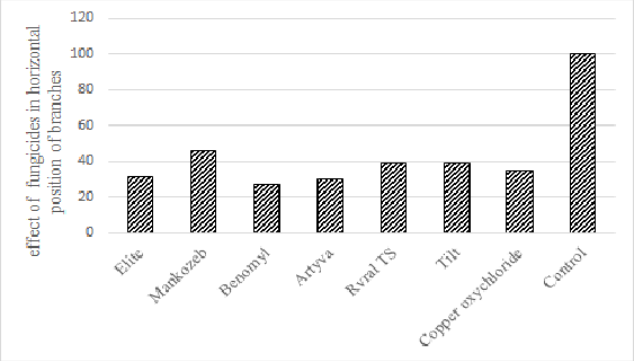

Figure 2. Mean comparison effect of seven fungicides on branch canker in horizontal position of branches based on the Duncan's multiple range test

3. 3. 2. Vertical position of branches

The results revealed that there were significant difference between fungicides in branch canker (P<0.01). Fungicides Artyva , Elite, Rovral TS, Mankozeb as well as tilte showed the maximum reduction of branch canker, respectively. There were no significant difference between these fungicides. Fungicide Benomyl indicated less effect on reduction of branch canker. The minimum reduction of branch canker was observed in fungicide Copper oxychloride (Figure 3).

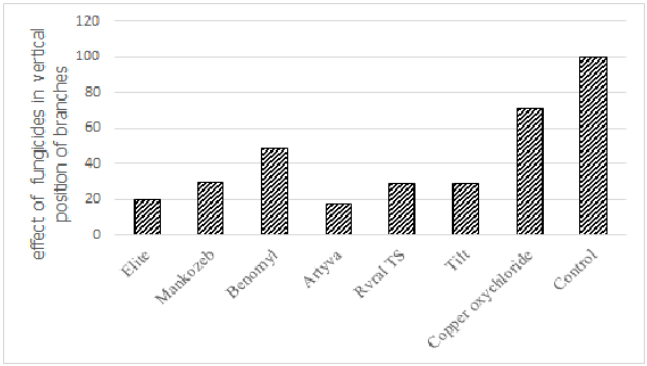

Figure 3. Mean comparison effect of seven fungicides on branch canker in vertical position of branches based on the Duncan's multiple range test

As mentioned it little research has been conducted on the effect of fungicides on this disease. Our results were consisted with results of Taheri et al (2005). Generally, according to the experiments fungicide Rovral TS can be used against the disease immediately after harvest and in month of March. As well as inhibitory effects of this fungicide on primary infection has been shown. After harvest, application of this fungicide is effective on restoration of harvest wounds and also prevent the establishment of fungus. Eventually, it is recommended to investigate effect of fungicides on pathogen under garden conditions, examine the interaction between biotic and abiotic stress in development of disease as well as application fungicides with different management strategies.

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