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Identification and control of die-back disease of mulberry trees using a combination of formulations based on ethane peroxic acid in green space of Tehran

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Abstract

Branch die-back disease caused by *Nattrassia mangiferae* is one of the most important fungal diseases of berries. The disease is most commonly seen in urban green space in high humidity and high temperatures during most seasons when trees are exposed to the sun. In this study, the causative fungus was sampled and isolated from mulberry trees on Matin Street in Tehran Municipality, District 17. Proof of pathogenicity of the causative fungus was performed on cut branches of mulberry and biennial seedlings. They were identified based on morphological and molecular characteristics (based on nucleotide sequencing of ITS-rDNA regions amplified with ITS1 and ITS4 primers). The composition formulated on the basis of ethane peroxic acid 20% prepared by Pak Gostar Parand Company against the fungus causing the disease in laboratory conditions and urban green space was studied in a completely randomized design with 4 replications. The results of laboratory studies on the mixing method in PDA culture medium showed that at a concentration of 3000 ppm it was 91.25%. While at concentrations above 4000 ppm, it completely prevented the growth of mycelial fungi. Moreover, the results of comparing the effect of the composition formulated with Berdofix fungicide on the branches of mulberry trees in urban green space showed that there was a statistically significant difference in reducing the symptoms of die-back disease and the composition formulated at a concentration of 5000 ppm compared to Berdofix which inhibited the spread of the disease in the berry trees of the municipality of District 17. Therefore, this combination can be used to control die-back disease in line with environmental goals.

Keywords: berry, die-back disease, formulation, green space

Introduction

The causative fungus *Nattrassia mangiferae* die-back of trees is one of the pathogenic fungi with a wide host range that has widely contaminated on the branches of green space due to various physiological factors and lack of proper nutrition of trees and other stresses. In some cases, this complication affects a large number of trees in the green space every year and the effects of its damage are evident. The causative agent in Tehran forest parks including Chitgar Park, Lavizan Park, eastern forests and green space inside the city are *Nattrassia mangiferae* Dyko & Sutton, coniothyrium sp. and in some cases Cytospora kunzei Sacc. The canker caused by the attack of Botryosphaeria sp in the forests of Bibi Shahrbanoo caused significant damage. It is noteworthy that the disease of coniferous twigs in Tehran and Karaj afforestations was often intensified after irrigation stress and tree weakness (Mirabolfathi, 2001). Keykhosravi et al. (2017) in the sycamore trees of Bustan Salehan located in the municipality of District 17 extensively reported a large volume of infection with paper-skin disease or die-back *Nattrassia mangiferae.*

Aminaei and Ershad (1993), isolated N. mangiferae from oranges, grapefruits, lemons, walnuts, almonds, pistachios, apples, peaches, plums, cherries, junipers, eucalyptus, figs and pomegranates in Kerman province. This fungus can cause severe damage by causing canker, die-back and gum secretion. Alizadeh et al. (2000) reported that the disease of citrus tree branch drought in Safiabad area of Dezful completely destroyed more than 100 hectares of Lisbon lemons in 3 to 5 years after the onset of symptoms and introduced the disease as *N. mangiferae*. It has been stated that this fungus needs a primary wound to cause disease. Najafinia (2006) isolated, identified and reported two species of fungi Bipolaris australiensis and *Nattrassia mangiferae* from citrus.

The pathogenicity of the above fungi was confirmed on cut branches and green space. So far, the fungi in the area have been isolated from oranges, grapefruits, tangerines and lemons. In Jiroft climate, the disease is not active in winter and intensifies in late spring, summer and early autumn. It seems that in suitable temperature conditions, untimely pruning and wounding of the surface layer due to sunlight is necessary to start the contamination. A kind of branch head dryness with gum secretion on Jiroft citrus was similar to the symptoms of *N. mangiferae*, but no pathogens were isolated from them (Najafi Nia Mousa. 2006). So far, the control of this disease in the green space by removing environmental stresses and removing infected branches or by clearing (wire brush) on the complication in the branches and main trunks of trees with one percent Bordofix has controlled the disease, but, since the use of Berdofix in the dressing method does not definitively cure the disease, it is cleared by rain and provides the regrowth of the disease, so in this study, in addition to identifying the causative agent, the control effects of the ethane formulation based on ethane peroxic acid 20% of Pak Gostar Parand Company has been tested in the green space.

Research method

A) Isolation and identification

Observing the symptoms of the disease in the form of paper and black powdery skin in the branches of mulberry trees on Matin Street, located in the second district of Tehran, 17th district, 20 mulberry trees were sampled and each infected branch was sent to the Plant Laboratory of the Research Research Center and green space consultation by washing and surface disinfection of Clorax 10% of the two skin layers of the branches were cultured in PDA medium.

Then, the disease-causing fungus cultured in the incubator was kept at 28 ° C for incubation. By observing the growth of the fungus in the petri dish, grayish-white filaments with spore formation and colony turning black and preparing a microscopic slide were identified as the fungus is the causative agent of *N. mangiferae*. Morphological identification was performed by considering valid identification keys. For further identification, it was performed using ITS4 and ITS5 general primers and after sequencing and BLAST, it was identified in NCBI database (Figure 1).

Figure 1: Right to left, respectively, disease symptoms, disease fungal petri mats, microscopic slides of filaments and spores

Methods of proving pathogenicity in laboratory and greenhouse

Infected parts, especially wounds, were sampled on the main trunk. Proof of pathogenicity of the causative agent was done by infecting seedlings in the greenhouse. In this way, a wound was made on the stem of each seedling and a disk of the growing fungus colony was placed at the wound site on the stem of the seedling. Pathogenicity was confirmed after three weeks with caries at the wound site.

Laboratory method of cut branches: In this study, due to the special conditions of growth and pathogenicity of the fungus, which requires a temperature of 35 ± 1 ° C and a relative humidity of 85 ± 5%, this method was used. In this method, from any berry tree, the subject of the design was prepared from healthy and succulent branches of 8 trees with a diameter of one centimeter and a length of 25 centimeters and the same age. After superficial disinfection of the branches with pure ethyl alcohol, by means of a perforated cork in a suitable place (approximately in the middle of each branch) a disc of skin with a diameter of 5 mm to the surface of the cambium layer is removed, and a young shoot was removed. The growing fungus was placed on it from the culture medium, and the inoculation site was covered with a layer of parafilm and two layers of paper glue. For the control treatment, a fungus-free disc was used. The inoculated branches were placed in plastic containers (containing some water so that the branches do not come into contact with water), and after closing, they were kept in the incubator for two weeks at the 35 ± 1centigrade and relative humidity of 85 ± 5%.

After the test period has elapsed, the skin of the branches in the inoculation area was removed so that the decays in the margins of the wounds are shown.

Draw the edges of the wounds on clear plastic wrap and transfer to white paper. Using a digital planimeter, the area of the wounds was measured and the mean size of the wounds was statistically analyzed.

Method of infecting seedlings in green space: In this method, two-year-old, healthy and lush berry seedlings were used. Drill a hole in the stem of each seedling (about 15 cm from the surface of the pot) with a cork, remove a disc 5 mm in diameter from the bark of the

stem of each seedling to the cambium layer, and place a disc the size of a young clover and stalk. A disease was placed on the culture medium, and the inoculation site was covered with a layer of paraffin and two layers of paper glue. A fungus-free disk was used for the control treatment. Inoculated seedlings were stored in a greenhouse at 35 ± 1 ° C and 85 ± 5% relative humidity for two weeks. After the experimental period was over, the stems of the tested seedlings were cut at a distance of 5 cm from the top and bottom of the inoculation area. After removing the skin in the inoculation site, the rest of the tests and calculations of the caries level were performed according to the method of cut branches.

Laboratory tests of the fungicide formulation of Fermycin Gold

The compound formulated based on ethane peroxic acid 20% from Pak Gostar Parand Company was tested to investigate the fungicidal effect. For this purpose, three-day culture of *N. mangiferae* in culture medium containing different concentrations including 1000, 2000, 3000, 4000, 5000 ppm was added by injection of filtramilipur in sterile PDA culture medium and in four replications compared to the control in a period of one week, the growth inhibitory power of the pathogenic fungus was evaluated in vitro. Yarn growth in culture medium containing formulation concentrations was measured and recorded at 48 hours and 72 hours and after 7 days with a ruler compared to the control.

In another experiment, the combination of the formula with the oil-accompanying substance (Fatty Amine) in the category of non-ionic surfactants of 50% of Pak Gostar Parand Company was tested for better effect.

Additional green space tests

In this experiment, 30 mulberry trees in Matin Street, located in the second district of the 17th district of Tehran, were selected as infected with the disease agent, and with concentrations of 5000 and 10000 ppm and control, an experimental treatment was applied in comparison with the Bordofix composition.

In this experiment, first, by cleaning the fungus causing Nattrassia disease with a wire brush on the infected trunks and branches of mulberry trees in this area, the tested treatments were described as described in Table (1).

Table (1): Treatments and number of mulberry trees tested on Matin Street

Number of trees	Dose	Treatments	N
-	-	control	۱
۵	5 per thousand	Ethane peroxic acid	۲
۵	10 per thousand	Ethane peroxic acid	۳
۵	10 per thousand in net	Broadfix	۴
۵	5 per thousand + 0.5 per thousand	Ethane peroxic acid + phthalamine	۵

۵	10 per thousand + 0.5 per thousand	Ethane peroxic acid + phthalamine	۶

First, the diameter and area of the wound parts for each tree were measured and recorded before the treatment, and, then, after cleaning and application of the treatments, in the interval of 72 hours, one, two and three weeks and two and three months were studied and calculated. The sporogenic power of the causative agent was calculated with the onset of black marks on the wounds. The control power of the experimental doses was calculated.

Findings

The concentration of 5000 ppm had the highest inhibitory power of yarn growth and control of the causative agent of Nattrassia disease in vitro compared to other concentrations.

According to Figure (2), the concentration of 2000 ppm had less than 20% of the inhibitory effect of yarn, but the concentration of 3000 ppm maintained up to 90% of the inhibitory effect of yarn in laboratory conditions. In the second experiment, the concentration of 3000 ppm had the effect of static fungus. But concentrations of 4000 and 5000 ppm have a completely inhibitory effect in a week and prevent the formation of yarn in petri dishes and compared to the control and concentrations of 1000 and 2000 ppm had the highest inhibitory and control effect.

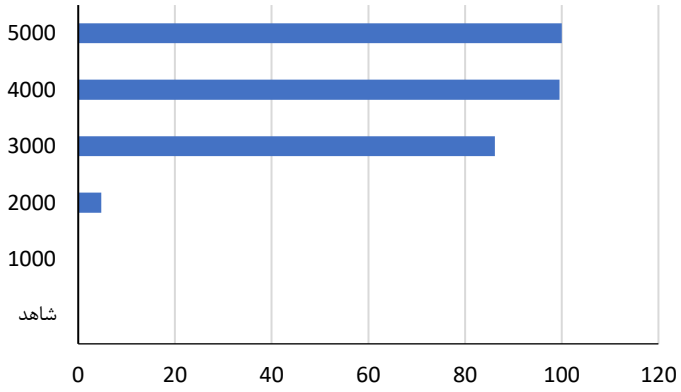

Figure (2): The effect of controlling different concentrations of compound formulated in vitro on pathogens of fungal filaments

Experimental results at the level of green space

Based on the comparison of control effects of different treatments, including a formulation based on ethane peroxic acid 20% and Berdofix and mixing of phthyamine with a compound formulated based on ethane peroxic acid 20% in executive conditions in the green space of Matin street, it was shown that mixing phthyamine with the formulated compound increases the inhibitory effect of fungal growth and brings definite control of the pathogen in infected branches and trunks on the trees of this street after three months and compared to the pure Bordofix compound which was applied as a staining on

the trunk and head of the branches. It has a completely separate function, so that in Berdofix, the growth of the fungus resumes after two months and the effect of Berdofix is removed by rain, but the formulated composition alone with a concentration of 5 per thousand as a foliar application on fungal diseases in trees, it was able to stop the growth of fungi and the concentration of 10 per thousand (one percent) compared to pure Bordofix (one percent) showed 100% control of die-back disease and mulberry tree trunks in this street.

Table 2: Mean of different treatments for mycelium growth after three months on mulberry trees, Matin Shahrdari St., District 17, Tehran

Treatment	Concentration	Fungal mycelium growth rate (mm)
-	-	74.4
Bordofix	10	45
Formaicin gold	5	34.6
Formaicin gold	10	21.2
Formaicin gold+ Fatty Amin	5+ 0.5	18.8
Formaicin gold+ Fatty Amin	10+ 0.5	0

According to Table (2), the compound formulated 10 per thousand in the amount of 21.2% after three months of growth of the disease agent on the trunk and head of the branch. However, the formulated combination of 10 per thousand with half-per-thousand of phthalamine has resulted in definite control if the fungus does not grow by re-sampling the trees.

In the sampling of treated trees and replanting in vitro, the growth of pathogenic fungi in petri dishes with a composition formulated based on 20% ethane peroxyacid followed.

Figure 3: Control image by formulated composition (left) compared to control (right) after three months - Matin St.

Discussion

The fungus is pathogenic on many fruitful and non-fruitful trees, including walnuts, various citrus species, various species of eucalyptus and berries, and there are reports of its pathogenicity on silver cypress in Iran. Rahnama (1998) has isolated and reported

Nattrassia mangiferae from silver cedar in Golestan province [6]. Mirabolfathi (2001,) has also isolated and reported this fungus from silver cypress [1].There is a potential risk for other fruitful and non-productive host pathogen trees in the country.

What can be seen in this case is that, firstly, eucalyptus trees, due to their imported and non-native nature, and secondly, the lack of familiarity with their growth needs such as (soil texture, soil moisture, ambient moisture, nutrients, manner), has caused these trees to fail at their planting site in Iran and for various reasons, including severe pruning or thin skin and sensitivity to radiation and sunlight, wounds on the trunk and its branches appears, sensitizing them to the pathogen of the wound, which is a pathogen of the wound, and spreading the fungus on them. What is important about the infection of eucalyptus trees with this disease [6].

However, at the level of green space, by removing the infected branches and managing the maintenance of the trees along with the nutritional principles, it can be a way to prevent Nattrassia disease.

According to the results obtained in this paper, the use of compounds with new formulations that have good smoothing and control properties can be an effective way to control trees that are prone to disease severity in the green space, as well as the Bordofix compound that is widely used for dressing this complication. The color view on the trunks of the trees is bad in terms of city appearance, and therefore, in addition to definitive control of die-back disease of the trees by the formulated composition, it does not leave any traces of color treatment on the trees. It is one of the important appearance features and the important result of this research is based on the results of the manufacturer's analysis of a compound formulated based on ethane peroxic acid 20%, which has no natural non-toxicity and Karns period and turns into acetic acid and oxygenated water as soon as used. It is completely environmentally friendly.

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