Journal of Ornamental Plants
Available online on: www.jornamental.iaurasht.ac.ir
ISSN (Print): 2251-6433 ISSN (Online): 2251-6441

Pathogenicity of Fungal Agents Related to the Diseases of Conifers of Cupressaceae in Greenhouse Conditions

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Received: 08 November 2016 Accepted: 13 January 2017
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Abstract

Conifers, a component of beauties of parks and landscapes, are infected with diverse pathogens, especially fungi. In this research, samples were collected from conifers displaying symptoms of disease in parks and landscapes of Rasht in Iran and for the isolation and identification of pathogenic fungi, pieces of infected tissues were placed in PDA and at the next stages in WA, PCA and SNA media and in total, 58 isolates were isolated and identified based on morphological characters. These fungi belonged to *Alternaria franseriae, Alternaria tenuissima, Curvularia pallescens, Fusarium sambucinum* and *Pestalotia* sp. Afterward, pathogenicity test of these fungi was done on *Chamaecyparis lawsoniana, Chamaecyparis lawsoniana 'Minima Aurea', Thuja sp., Juniperus horizontalis, Juniperus chinensis* and *Cupressus arizonica* and to do so, a spore suspension was prepared with the concentration of 5 × 104 spores per ml distilled water and then, the studied plants were inoculated. Final assessments were carried out 10 days later to determine disease intensity. Results showed that *A. franseriae* on *C. lawsoniana, A. tenuissima* on *C. lawsoniana* 'Minima Aurea', C. pallescens on *C. lawsoniana and J. chinensis, F. sambucinum on J. horizontalis* and *Pestalotia sp*. on *C. arizonica* and *C. lawsoniana* 'Minima Aurea', were pathogenic. The most disease severity was caused by *A. franseriae* on *C. lawsoniana* and the least disease severity by *Pestalotia sp*. on *C. lawsoniana* 'Minima Aurea'. Among the studied plants, *C. lawsoniana* and *C. lawsoniana* 'Minima Aurea' showed the greatest sensitivity and the most resistance, respectively. Analysis of variance revealed significant differences in disease intensity of the studied fungi. The occurrence of *A. franseriae, A. tenuissima* and *C. pallescens* on conifers has been reported for the first time from Iran on conifers.

Keywords: Conifers, Fungi, Greenhouse condition, Pathogenicity.

INTRODUCTION

Conifers are economically very important. The wood quality of most coniferous species is optimum with wide application in wood industries (Zare, 2001).

All genera and species of Cupressaceae are evergreen, monoecious or dioecious plants which are seen as trees or shrubs and erect or creeping. Cupressaceae includes Callitroideae and Cupressoideae subfamilies. Callitroideae include 12 genera among which *Chamaecyparis* Spach. is the most important one. It is one of the most important coniferous genera with a special place in horticulture and the cultivation of plants. This genus has 7 species among which *Chamaecyparis lawsoniana* Parl. is the most important one (Brickell, 2008; Zare, 2001; Mozafarian, 2004). *Cupressus* spp. belongs to Cupressoideae with 15-20 known species. The famous species is *Cupressus arizonica* Greene. This beautiful tree is native to Arizona and California and is an important conifer for horticulture, landscapes and park design (Sabeti, 2008). The second genus of this subfamily is *Juniperus* L. which is a big genus including 60 species. The most important species are *J. chinensis* L., *J. commus* L., *J. excelsa* M.B., *J. conferta* Parl. and *J. horizontalis* Moench., whose different forms and varieties have been identified (Mottaghi, 1985; Zare, 2001; Mozafarian, 2004). The third important genus is *Thuja* L. that is evergreen tree that is rarely shrub. These plants grow very narrow, tall and conical. *Thuja* has six species. The most important species are *T. occidentalis* L. and *T. orientalis* L. These species have a lot of varieties which are widely grown in gardens and parks around the world (Zare, 2001; Mozafarian, 2004).

Fungi are important pathogenic agents which affect the quantity and quality of conifers. Various pathogenic fungi on conifers have been reported around the world (Jafarpoor, 1992).

Endophytic fungi were isolated from foliage of four host species of Cupressaceae sampled from 19 sites in Oregon. *Chamaecyparis lawsoniana* and *Thuja plicata* showed high overall rates of infection (30-50%) while *Calocedrus* decurrens and *Juniperus occidentalis* showed lower rates (10-35%). For any particular host, samples from homogeneous stands with a closed canopy showed higher infection rates than those from mixed stands with an open canopy (Petrini and Carroll, 1981).

In a study in Florida, eight famous pathogenic fungi were isolated from the roots of *Pinus clausa* in which *P. cinnamomi* was the most common pathogenic agent (Barnard *et al.*, 1985). Seedling blight of longleaf pine caused by *Rhizoctonia solani* in Florida nurseries was studied by English *et al*. (1986). The first symptoms appeared as water-soaking and chlorosis in roots. These spots were yellow and then, brown in upper parts of the roots and became darker in terminal buds and taproots. Finally, the infected parts were rotten (English *et al.*, 1986).

Phytophthora cinnamomi is a soil-borne fungus affecting over 900 plant species. Foresters in southern US were well familiar with *P. cinnamomi* because of conifers stubborn disease, especially short-leaf pines (Barnard, 1988).

In a study about needle cast disease, the needle-like leaves of the plants host were separated and fell. The causal agents of the disease were species of *Lophodermium, Elytroderma, Scirrhia, Hypodermella, Bifusella, Adelopus, Rhabdocline* and other related genera. The general symptoms included light green to yellow spots on the leaves turning finally into red or brown (Jafarpoor, 1992).

In a study in Italy, *Fusarium* sp., *Rhizoctonia* sp. and *Pythium* sp. were isolated from *Pinus nigra* (Annesi *et al.*, 1992). Macrophoma die-back disease was first reported in Nowshahr on *Pinus eldarica.* The causal agent was *Diplodia* sp. In addition to saprophytic life on the wood of fallen pines, this fungus attacked weak pines as wound parasite and killed its branches and twigs (Ershad, 1977; Browne, 1968). Pines (Pinus spp.) in Florida were exposed to many root-infecting fungi in 1985 (Barnard *et al.*, 1985; Barnard, 1999).

Barnard and Meeker (1995) found that one of the root-borne fungi was ascomycetes from the genus *Ophiostoma* (anamorph: *Leptographium*). These fungi were mostly accompanied with one or some bark-eating insects or wood-borer insects (Barnard and Meeker, 1995).

In a study, it was found that root rot caused by *Heterobasidium annosum* was an important and fatal disease infecting the conifers in northern moderate regions of the world (Barnard, 1999).

Menkis *et al*. (2006) introduced *Rhizoctonia* sp. and *Pythium* sp. as the causal agents damping off and root rot in conifers seedlings.

In the past years, different fungi like *Pestolotiopsis funerea, Rosellinia necatrix, Tramates radiciperda, Collybia xylophila* and *Phytophthora cryptogea*, were reported as the causal agents of diseases in *Chamaecyparis* spp. in Iran (Ghodskhah and Karim, 2015).

In a study, *Pestolotiopsis funerea* was isolated from *Cupressus sempervirens* var. *horizentalis* and pathogenicity of this fungus has been done on vase and nurseries seedlings. The results showed infection severity on Altappeh area was 4.17% and distribution of diameter and high of infected trees showed same age curve (Borhani *et al.*, 2004).

In a study in Iran, the causes of the death of coniferous seedlings in Lakan nurseries were studied (Herfehdoust *et al.,* 2009). According to the results of pathogenicity tests, *Rhizoctonia solani, Pythium* sp., *F. oxysporum, F. semitectum, F. solani* and *Fusarium* sp. were the pathogenic agents (Herfehdoust *et al.*, 2009).

In another research in Iran, about the identification of soil-borne pathogenic fungi in coniferous forest plantations in Fars province, *Fusarium solani, F. sambucinum, F. avenaceum, F. graminearum, F. subglutinans, F. crookwellens, Rhizoctonia solani* and *Pythium okanoganens* were isolated and identified (Zarghani *et al.*, 2010).

In a study, endophytic *Alternaria alternata, Alternaria pellucida*, and *Alternaria tangelonis* were recovered from healthy *Cupressaceous* trees. Biodiversity and bioactivity of recovered endophytic *Alternaria* species were a matter of biogeography and host identity. *In vitro* assays also indicated that endophytic *Alternaria* species significantly inhibited the growth of cypress fungal phytopathogens (Soltani and Hosseyni Moghaddam, 2014).

Like all other plants in the world, conifers are exposed to infection by many pathogenic fungi resulting in growth loss, yellowing and finally, the death of the trees. The main objective of the present study was to evaluate the pathogenicity of fungal agents related to the diseases of conifers of Cupressaceae in greenhouse conditions in Rasht city of Guilan province.

MATERIALS AND METHODS

Sample collection

The sample were collected from infected leaves of such conifers as *Chamaecyparis lawsoniana*, *Chamaecyparis lawsoniana* 'Minima Aurea', *Thuja sp., Juniperus horizontalis, Juniperus chinensis* and *Cupressus arizonica* that had showed the symptoms of the disease. The infection samples were placed in plastic bags separately and were sent to laboratory for the isolation of pathogenic agents (Herfehdoust *et al.*, 2009).

Culture, isolation, purification, storage and identification of fungal isolates

The pieces were isolated from the space between infected tissues and healthy tissues of leaves. Then, they were disinfected with sodium hypochlorite 0.5% and were cleansed with distilled water. Following hydration, the pieces were cultured in various media including PDA, WA PCA and SNA for the isolation and growth of fungal colony, purification and single-spore and for morphological identification of fungal isolates, respectively. Fungal isolates were stored by sterile filter paper technique (Safari Motlagh *et al.*, 2005). After the isolation and purification of fungi, their macroscopic properties and then microscopic properties were examined. The fungi were identified by their morphological traits including shape and color of colony and conidia, growth form of mycelium, singularity or collectiveness, dimensions and color of conidiophores, conidia length and width, and number of septa of conidia. Different keys of fungi identification were used for this purpose (Simmons, 2007; Ellis, 1971; Lesli and Summerell, 2006; Sivansesan, 1987; Nag Raj, 1993).

Pathogenicity tests

Pathogenicity tests were conducted on the basis of Koch's postulates in greenhouse environment at 25°C and >90% relative humidity in seven treatments and three replications. First, a sample of garden soil was autoclaved and was transferred to disinfected plastic pots under sterile hood. Then, one-year-old seedlings of conifers were planted in pots. Two groups of pots, each including three pots, were considered for the study, one treatment and one control. Then, pathogenicity tests were conducted. First, the seedlings of both control and treatment pots were sprayed with distilled water under sterile hood. Then, the suspension for inoculation was prepared. To prepare spore suspension, the fungi (seven fungi and from each 3 isolates) were cultured in four petri dishes containing WA medium and were placed in incubator at 26°C for three weeks. In all studies, suspension concentration was measured by hemocytometer. The fungal spore suspension concentration used for inoculation was 5 × 104 spores per ml distilled water. To increase adsorption, Tween® 20 with the ratio of 1% was applied. The final assessment was done 10 days later. After a certain time when the symptoms appeared, they examined and the isolated pathogens were compared with the initial pathogens. The basis of the evaluation was the visual observations of the symptoms. The symptoms were described and graded as follows: 1. Health leaves with no symptoms; 2. Leaves with small, undeveloped spots; 3. Leaves with moderate, developed spots; 4. Leaves with complete blight (Horsfall and Barratt, 1945). Finally, disease intensity was calculated by the following equation on the basis of the number of spots on leaves (Safari Motlagh, 2010):

$$\text{Disease intensity}=(N_1\times 1)+(N_2\times 2)+\quad+(N_t\times t)/(N_1+N_2+\quad+N_t)$$

where, N was the number of leaves at different grades.

It should be noted that due to lack of some plants during the opening rhythm of growth (one-year-old seedlings), pathogenicity tests were carried out on some plants not all plants.

Data analysis

The study was based on a CRD with seven treatments and three replications. The means were compared by Tukey method. The data were analyzed by SAS Statistical Software Package, and the graphs were drawn by MS-Excel Software Package.

RESULTS AND DISCUSSION

In total, 58 fungal isolates were isolated from the collected samples and were classified in five groups according to morphological evaluations: *Alternaria franseriae* E.G. Simmons (8 isolates), *Alternaria tenuissima* (Kunze) Wiltshire (10 isolates), *Curvularia pallescens* (Boedijn) (16 isolates), *Fusarium sambucinum* Fuckel (8 isolates), and *Pestalotia* sp. DE Not. (16 isolates).

In the present study, the fungal isolates were inoculated regardless of the host from which

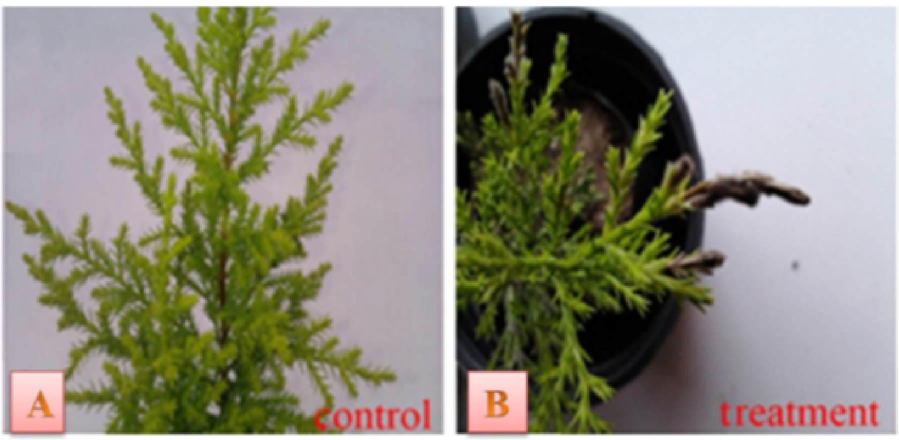

Fig. 1. A) C. lawsoniana (control), B) Symptoms of infection of *A. franseriae* on *C. lawsoniana.*

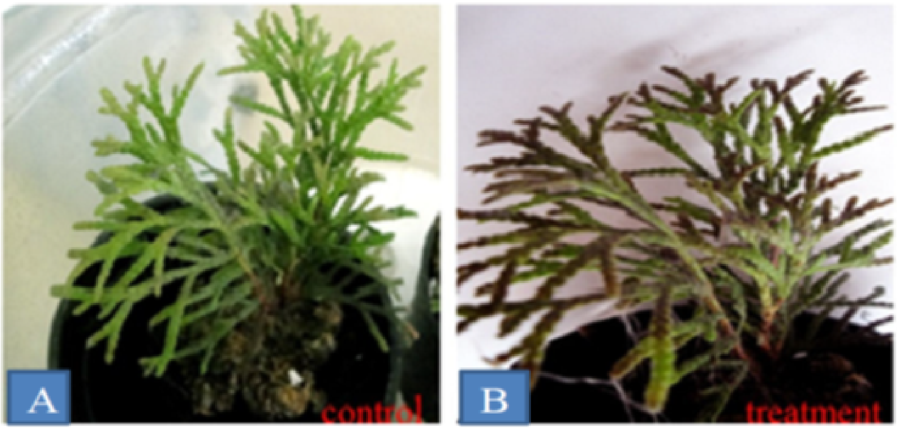

Fig. 2. A) *Thuja* sp. (control), B) Symptoms of infection of *A. tenuissima* on *Thuja* sp.

they were isolated because of the absence of plants required at the initial growth stages (seedling). The followings were observed about the disease trends and symptoms in each fungal isolate on different hosts.

Pathogenicity test of *A. franseriae* was carried out on one-year-old seedlings of *Chamaecyparis lawsoniana.* The first symptoms appeared as small yellow spots on the tip of upper leaves four days after inoculation. On days 5 and 6, the number of these spots increased on upper leaves and their color turned darker. Also, small white spots appeared on lower leaves. On days 7 and 8, the yellow spots at tip of the upper leaves developed and their color turned to brown. As well, small spots appeared on middle leaves. On day 9, the number of spots increased. Finally on day 10, the spots joined each other and they became black covering half of leaf area and creating necrotic state (Fig. 1).

Pathogenicity test of *A. tenuissima* was carried out on one-year-old seedlings of *Thuja* sp. The first symptoms were visible as light green or yellow spots on upper leaves four days after inoculation. On days 5 and 6, the spots started to develop from the tip of the leaves towards petioles. On days 7 and 8, the number of spots on upper leaves increased and some small yellow spots were observed on middle leaves. On day 9, the yellow spots darkened and finally on day 10, the spots on leaves became black and necrotic state was observed in leaves (Fig. 2).

Pathogenicity test of *C. pallescens* was carried out on one-year-old seedlings of *C. lawsoniana* and *Juniperus chinensis*. In *C. lawsoniana*, the first symptoms appeared as small yellow spots at the tip of the upper leaves four days after inoculation. On days 5 and 6, small yellow spots appeared at the tip of the middle leaves. On days 7, 8 and 9, the yellow spots at the tip of the leaves developed downward and became black. Finally on day 10, some leaves entered necrotic state

Fig. 3. A) *C. lawsoniana* (control), B) Symptoms of infection of *C. pallescens* on *C. lawsoniana, C J. chinensis* (control), D) Symptoms of infection of *C. pallescens* on *J. chinensis*.

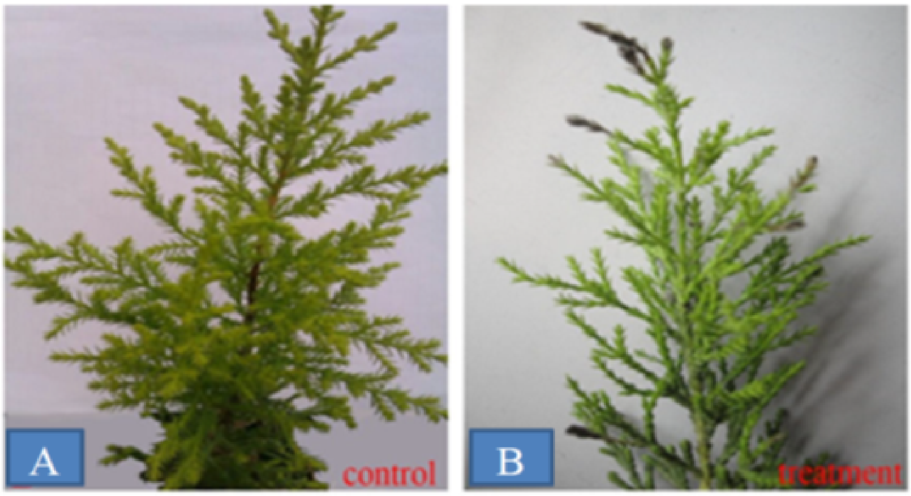

Fig. 4. A) *C. lawsoniana* (control), B) Symptoms of infection of *F. sambucinum* on *C. lawsoniana.*

(Figs.). In *J. chinensis*, the first symptoms were visible as small brown spots on some middle and lower leaves five days after inoculation. Finally on day 10, leaves showed necrotic state (Fig. 3).

Pathogenicity test of *F. sambucinum* was carried out on one-year-old seedlings of *C. lawsoniana.* The first symptoms were small white spots on petioles of upper leaves appeared four days after inoculation. On days 5 and 6, the spots developed towards the tip of the leaves. On days 7 and 8, the color of the spots changed to gray. On day 9, the spots developed and covered whole leaf area. Finally on day 10, leaves exhibited necrotic state (Fig. 4).

Pathogenicity test of *Pestalotia* sp. was conducted on one-year-old seedlings of *C. lawsoniana* and *C. lawsoniana* 'Minima Aurea'. In *C. lawsoniana*, the first symptoms appeared as white spots at the tip of upper leaves four days after inoculation. On day 5, the spots developed from the tip towards petiole. On days 6 and 7, the number of spots increased. On day 8, the spots became yellow and brown and spore-containing acervuli which were black in color appeared on spots. On day 9, the spots darkened and the number of black acervuli on spots increased. Finally on day 10, some leaves showed necrotic state. In *C. lawsoniana* 'Minima Aurea', the first symptoms appeared as small yellow spots at the tip of the leaves five days after inoculation. On days 6 and 7, the spots developed. On day 8, spore-containing acervuli appeared on spots. On day 9, the spots became brown. Finally on day 10, leaves showed necrotic state (Fig. 5).

Analysis of variance revealed significant differences in disease intensity of the studied fungi

Fig. 5. A) *C. lawsoniana* (control), B) Symptoms of infection of *Pestalotia* sp. on *C. lawsoniana*, C) *C. lawsoniana* 'Minima Aurea' (control), D) Symptoms of infection of *Pestalotia* sp. on *C. lawsoniana* 'Minima Aurea'.

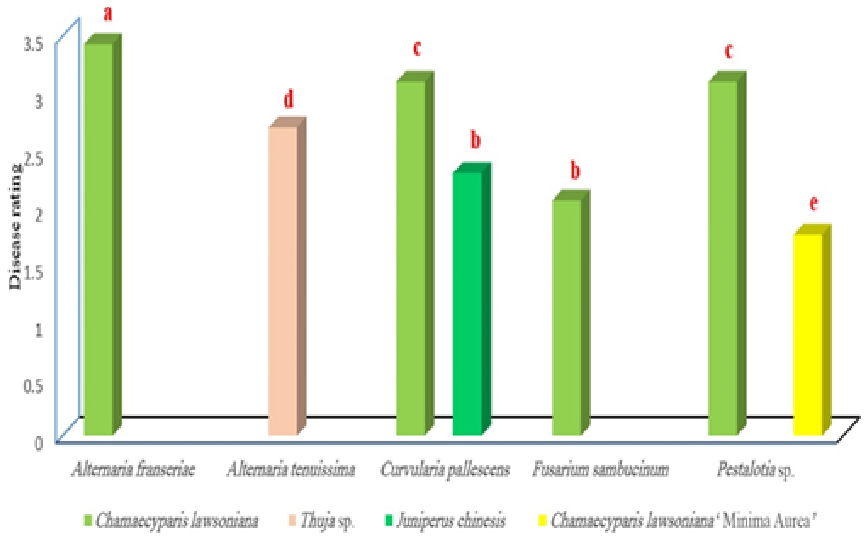

Fig. 6. Comparison of means of disease rating (intensity).
Treatments having at least one similar letter do not show a significant difference at P<0.05.

on different hosts at the 5% probability level (Table 1). According to means comparison for the intensity of disease created by the studied fungi on hosts as compared to control, it can be concluded that all isolated fungi were pathogenic.

Means comparison for different treatments in terms of disease intensity showed that *A. franseriae* caused more severe disease on *C. lawsoniana* followed by *Pestalotia* sp. and *C. pallescens* on *C. lawsoniana, A. tenuissima* on *Thuja* sp., *C. pallescens* on *J. chinensis* and *F. sambucinum* on *C. lawsoniana* and *Pestalotia* sp. on *C. lawsoniana* 'Minima Aurea' showed the lowest disease intensity (Fig. 6).

Means comparison for disease intensity in *C. lawsoniana* and *C. lawsoniana* 'Minima Aurea' as affected by *Pestalotia* sp. showed that the disease caused by *Pestalotia* sp. on *C. lawsoniana* was more intense and for the disease intensity of *C. lawsoniana* and *J. chinesis* as affected by *C. pallescens* showed that *C. pallescens* caused more intense disease in *C. lawsoniana.*

According to means comparison for the intensity of disease caused by *A. franseriae, C. pallescens, Pestalotia* sp. and *F. sambucinum* in the same host, i.e. *C. lawsoniana*, it can concluded that *A. franseriae* showed the most disease severity and then *Pestalotia* sp., *C. pallescens* and *F. sambucinum* had the most disease severity, respectively.

By comparing the average of disease intensity caused by *A. franseriae* on *C. lawsoniana, Pestalotia* sp. on *C. lawsoniana* 'Minima Aurea', *A. tenuissima* on *Thuja* sp. and *C. pallescens* on *J. chinensis* revealed that *A. franseriae* resulted more intense disease on *C. lawsoniana* than other fungi and the next intense diseases were caused by *A. tenuissima* on *Thuja* sp. and *C. pallescens* on *J. chinesis*. The least disease intensity was caused by *Pestalotia* sp. on *C. lawsoniana* 'Minima Aurea'.

In the present study, *C. pallescens* and *Pestalotia* sp. had the most frequency (27.5 %). A. franseriae was isolated from *C. lawsoniana* and its pathogenicity test was carried out on *C. law-*

Table 1. Analysis of variance of disease intensity.

S. o.V	**df**	**Squares of means**
Treatment	6	1.16**
Error	14	0.149
CV (%)		14.58

** Significance at the 5% probability level *soniana.* Observations and results proved its pathogenicity and it was found out that its disease intensity was higher on *C. lawsoniana*, so that it created necrotic state on most leaves and twigs. *A. tenuissima* was isolated from *C. lawsoniana* 'Minima Aurea' and its pathogenicity test was conducted on *Thuja* sp. Observations and results proved its pathogenicity on the studied plants. *C. pallescens* was isolated from *C. lawsoniana* and *J. chinensis* and its pathogenicity test was conducted on *C. lawsoniana* and *J. chinensis*. According to observations and results, its pathogenicity was proved on the studied plants. It was revealed that its disease intensity was higher on *C. lawsoniana* than on *J. chinensis* which can be related to the different in the responses of these two species to the disease agent.

Fusarium sambucinum was isolated from *J. horizontalis.* Its pathogenicity test was conducted on *C. lawsoniana* and its pathogenicity was proved on the studied plants. *Pestalotia* sp. was isolated from *C. arizonica* and *C. lawsoniana* 'Minima Aurea'. Its pathogenicity test was conducted on *C. lawsoniana* and *C. lawsoniana* 'Minima Aurea'. The observations proved its pathogenicity on the studied plants. It was revealed that the intensity of disease caused by *Pestalotia* sp. was more on *C. lawsoniana* than on *C. lawsoniana* 'Minima Aurea'. Since both studied plants belonged to the same species but the varieties were different and however, there were significant differences in disease intensity, it can be related to their different genetic structure and their different responses to the disease.

Results revealed that *A. franseriae* on *C. lawsoniana, A. tenuissima* on *C. lawsoniana* 'Minima Aurea', *C. pallescens* on *C. lawsoniana* and *J. chinensis, F. sambucinum* on *J. horizontalis* and *Pestalotia* sp. on *C. arizonica* and *C. lawsoniana* 'Minima Aurea' were pathogenic.

Results of pathogenicity tests and analysis of variance led to the conclusion that the disease intensity caused by *A. franseriae* and *C. pallescens* on *C. lawsoniana* was higher than those caused by other fungi in the studied plants.

Based on results, the most disease severity by *A. franseriae* on *C. lawsoniana* and the least one by *Pestalotia* sp. on *C. lawsoniana* 'Minima Aurea' were created.

In this research, among the studied plants including *C. lawsoniana, C. lawsoniana* 'Minima Aurea', *Thuja* sp., *J. chinensis, J. horizontalis* and *C. arizonica; C. lawsoniana* had the most sensitivity to the disease and *C. lawsoniana* 'Minima Aurea' was the most resistant.

In 1949, Russian Research Institute reported that the wilting and death of pine seedlings by parasite and non-parasite agents was of the most important diseases that killed pine seedling in 30 Russian nurseries (Ankudinov, 1950). *Fusarium* spp. was reported as the main cause of pine seedling death and then, species of *Alternaria* were identified which was consistent with our finding that species of Alternaria and Fusarium were the causal agent of disease in conifers.

In a study on seeds, seedlings and barks of pine trees in pine nurseries of Southern Georgia, Huang and Kuhlman (1990) identified 41 species of 23 fungal genera, out of which *Alternaria alternata, Fusarium moniliforme* and *Penicillum expansum* caused the death of the seedlings. According to the results of Huang and Kuhlman (1990) and the present study, it can be concluded that a lot of conifers in parks and forests of the world are annually attacked by pathogenic fungi resulting in their loss. Different species of Fusarium and Alternaria are among these fungi.

Denitto and Kliejunas (1991) isolated Phytophthora lateralis from *Taxus brevifolia* and *Chamaecyparis lawsoniana.* Orlikowski and Szkuta (2003) took numerous samples from the leaves and branches of *Picea omorika* and *Thuja* occidentalis in two nurseries in Poland where symptoms of sudden yellowing and browning of twigs had been observed. They identified *Phytophthora citricola* and at the next stages, *Botrytis cinerea, Fusarium avenceum* and *Pestalotia* sp. as pathogens.

In another study, *Phytophthora lateralis* was isolated from the dried and died tips of *Chamaecyparis lawsoniana* in eight forest, protected and tourist gardens in England, Scotland and Northern Ireland (Green *et al.,* 2012).

Zakeri *et al.* (1995) collected numerous samples from the roots and crowns of conifer seedlings including *Cupressus arizonica, Cupressus sempervirens* and *Pinus eldarica* in nurseries of Fars province with symptoms of chlorosis, dwarfing, necrosis, leaf shedding, root and crown rot and seedling death. *Alternaria* sp. and *Penicillum* spp. from the rotten seeds were isolated. One species of *Fusarium* was isolated from the rotten seeds of *Pinus eldarica* and their pathogenicity was proved which was consistent with findings of the present study.

In Fars province, Mansouri (1998) identified *Fusarium equiseti* in pine trees. The symptoms were as the drying of leaves from the tip downward as well as the stagnation of the growth of some trees. The disease symptoms were visible on inoculated twigs four days later, whilst the symptoms in the present study appeared at most after three days. It can be related to the studied organ which was twig in Mansouri's study and leaf, a more sensitive organ, in the present study.

Kavianpey *et al.* (2000) isolated the fungi accompanying seedling root rot and death in forest tree nurseries of Khuzestan and studied their morphology and pathogenicity characters. *Fusarium solani* as the most prevailing species with 89% frequency and high distribution in the nurseries of Khuzestan which was pathogenic on *Cupressus sempervirens, Pinus eldarica* and *Cupressus arizonica.*

In a study in nurseries of Mazandaran province, Borhani and Mousazadeh (2004) showed that *Pestalotiopsis funerea* causes disease on *Cupressus sempervirens, Cupressus arizonica, Sequoia* sp. and *Cedrus* sp. with almost similar symptoms. In a study in Fars province, Zarghani *et al.* (2010) identified *Fusarium* spp., *Rhizoctonia* spp. and *Pythium okanoganens* on conifers displaying such symptoms as growth stunting, yellowing and declining. In the case of the species of *Fusarium*, despite replicated inoculation under drought stress and greenhouse conditions, most *Fusarium* isolates were not pathogenic on the seedlings of *Picea pungens* and *Cupressus sempervirens*. Some isolates of *F. solani* resulted in root and crown rot, yellowing and dwarfing of pine seedlings. In addition, pine seedlings inoculated with *F. sambucinum* showed root and crown rot 2 weeks later which was inconsistent with findings of the present study about appearance time of symptoms.

As can be seen, in most studies some species of *Fusarium* has introduced as the main cause of diseases in conifers which is in agreement with our findings. Furthermore, it was observed that among studied conifers, *C. lawsoniana* was the most sensitive to pathogenic fungi which is consistent with the previous findings that it does not exhibit high resistance in most climatic conditions (Brickell, 2008; Zare, 2001; Mozafarian, 2004).

CONCLUSIONS

Overall, it was found out that diverse fungal groups occur on conifers whose identification and in vitro or greenhouse pathogenic studies can be useful in finding approaches for the management of conifers diseases. It should be noted that with a review of studies in Iran and other countries no reports were found about the occurrence of *Alternaria franseriae, Alternaria tenuissima* and *Curvularia pallescens* on conifers and that these fungi are reported for the first time from Iran.

ACKNOWLEDGMENTS

This experiment was supported by the Islamic Azad University, Rasht Branch, Iran.

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How to cite this article:
Safari Motlagh, M., and Ramezani Rad, F. 2017. Pathogenicity of fungal agents related to the diseases of conifers of Cupressaceae in greenhouse conditions . *Journal of Ornamental Plants, 7(3), 177-187.*
URL: http://jornamental.iaurasht.ac.ir/article_532997_a5d79b7567af0735d35760baf3a91811.pdf