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Pathogenicity of Alternaria Species Isolated from *Chamaecyparis lawsonia In Vitro*

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Abstract

Chamaecyparis lawsonia, which brings beauty in parks and green space, is attacked by a variety of pathogenic agents specially fungi. In this study, some *Chamaecyparis lawsonia* available in parks that had disease symptoms were sampled. After sample collection, in order to isolate fungal pathogens, cuts of the infected plant tissues were placed on PDA (potato dextrose agar) and later were put on WA (water agar) for identifying. Then test of pathogenicity of these isolates on *Chamaecyparis lawsonia* was done inside the box with plastic stopper. To do this, cuts of colonies of 4 day fungi obtained on PDA medium were put on leaves. The final evaluation was performed after 10 days. This experiment was performed in a completely randomized design with 3 replications and 7 treatments. The results showed that isolated fungi are in 2 groups as following: *Alternaria franseriae* and *Alternaria tenuissima*. According to the results, both species were pathogenic on host. Totally, A. franseriae showed more disease severity compared to *A. tenuissima* on *C. lawsonia.*

Keywords: *Alternaria* spp., *Chamaecyparis lawsonia*, Fungi, Pathogenicity.

INTRODUCTION

Chamaecyparis lawsonia is one of important conifers in gardening, planting and growing of ornamental plants and due to its branches form has many fans among designers and gardeners (Zare, 2002). Fungi are the most important factors that affect the quantity and quality conifers (Jafarpour, 1994). Agents of disease of shed leaves of conifers (Needle cast) are species of *Lophodermium, Scirriha, Hypodermella, Adelopus, Rhabdocline* and other related genus that removed leaves from host plant and fall. At first, symptoms of this disease appear as bright green or yellow spots on needle leaves that finally turn to brown or red (Jafarpour, 1994).

Pine dieback disease (*Macrophoma* die-back) was collected and reported for the first time in 1970 from Noshahr from *Pinus eldarica*. The agent of this disease was *Diplodia* sp. (Ershad, 1978). According to the research done, various pathogenic fungi have been reported on conifers around the world. In 1985, in Florida, *Pinus* spp. were exposed many root infecting fungi (Barnard *et al.,* 1985). One of fungi resident in root is *Ophiostoma* sp. (Barnard and Meeker, 1995).

Root rot of *Annosum* that is caused by *Heterobasidium annosum* is one the most important and devastating diseases that affect the conifers of world in northern temperate regions. About 200 forest species including several conifers are host of *H. annosum*; the most common genus are *Abies, Juniperus, Larix* and *Pinus* (Barnard, 1999). Among the research carried out in Iran, identifying pathogenic soilborne fungi in hand planting forest of conifers in Fars province can be referred (Zarghani *et al.*, 2010). The field observations of different hand planting forests in Fars province's geographic area, number of fungi were identified, which only two isolates from *Fusarium. Sambucinum, Rhizoctonia. solani* and *Pythium okanoganens* were pathogenic. *P. okanoganens* had relatively high pathogenicity, while other fungi had relatively mild pathogenicity (Zarghani *et al.,* 2010). Also, the causes of death in conifer seedlings in nursery of Lakan was studied (Herfehdoost *et al.*, 2009). Damping off is one of the common diseases of forest plant nurseries that imposes a lot of damage to plant nurseries. In another study, infected seedlings were studied after being collected and transported to the laboratory, and then pathogens obtained after doing pathogenicity tests were identified as *Rhizoctonia solani, Pythium* sp., *Fusarium. oxysporum, F. semitectum, F. solani* and *Fusarium* sp. (Herfehdoost *et al.*, 2009).

Chamaecyparis lawsonia, are used a lot in greenery and landscape design of urban parks due to its beauty value, variety of colors and their ever greenness in the family of conifers, (Zare, 2002). Therefore, objectives of this study were to study of pathogenicity of Alternaria species isolated from *C. lawsonia* that damages it at different stages of its growth.

MATERIALS AND METHODS

Sample collection

Samples were taken from different infected parts of plants with diseases symptoms (Safari Motlagh, 2000). Infected samples were placed individually in plastic bags and transported to the laboratory immediately for isolation of pathogens and laboratory operations were performed on them.

Identification of fungi

Identification of fungi was done using morphological characteristics as shape of colony, color of colony, mycelial growth mode, conidiophore's being single or group, conidiophore's size and color, conidia's length and width, the number of conidia's septa, and so on. For this purpose, keys of identifying fungi such as Simmons, 2007; Ellis, 1971; Leslie and Summerell, 2006; Nag Raj, 1993 were used.

Pathogenicity test

Pathogenicity tests were performed in plastic box with stopper in dimensions of 5 × 15 × 24 cm. For inoculating on leaves, a piece of dimensions 3 × 2 mm from 4-day cultured fungus on culture medium PDA, was put on leaves. Then, boxes were placed in incubator at 26°C for 10

days. Ten days after inoculation, leaf appearance symptoms were assessed (Kamran and Bani-Hashemi, 1995; Yousefi and Hagian Shahri, 2009).

The measurement was based on visual observations of symptoms. Description of symptoms and grading was done as follows: 1= the leaves were healthy and asymptomatic, 2= creation of small and undeveloped spots on leaves, 3= creating medium and developed spots on leaves, 4= complete blight (Safari Motlagh, 2011). Finally, severity of disease in each treatment was calculated based on the number of spots on the leaves according to this formula:

$$\text{Disease rating} = \frac{(N_1 \times 1)+(N_2 \times 2)+\cdots(Nt \times t)}{(N_1 + N_2 + \cdots N_t)}$$

RESULTS AND DISCUSSION

A total of 50 isolated sampled fungi were obtained and after morphological evaluation, two fungal groups were identified as follows:

First group: ***Alternaria franseriae*** **E.G. Simmons**

Colonies were gray to white velvet. Conidiophores were simple, with a single conidiogenous cell and were proliferate in curve mode that had a terminal helium in each short development. Conidia were oval and elongated oval with a round tip, in some cases without the tip, approximately oval or elliptical in golden brown, smooth and in dimensions 12-8 × 40-30 µm, and had 5-8 septa and sometimes longitudinal (Fig. 1). Characteristics of this group of isolates were consistent with *Alternaria franseriae* (Simmons, 2007).

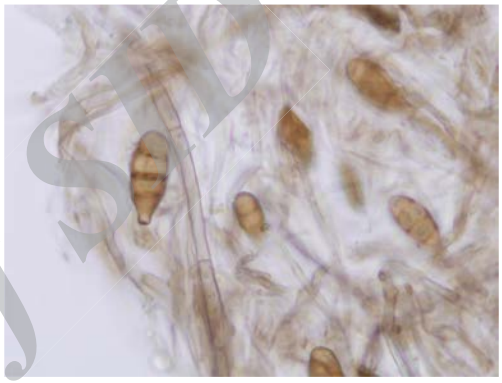

Fig. 1. Conidia and conidiophores of *Alternaria franseriae* (×1200).

Second group: ***Alternaria tenussima*** **(Kunze) Wiltshire**

Colonies were blackish brown with fast growth. Conidiophores were simple or branched individually or in simple or branched groups, straight or curved groups, almost cylindrical, septate, light yellow or light brown, smooth, up to 115 µm in length and 4.6 µm in thickness (Fig. 2). Conidia were present individually or in short chains, straight or curved, bent spindle or oval shape that gradually narrows towards the tip (Fig. 2).Characteristics of this group of isolates were consistent with *Alternaria tenussima* (Ellis, 1971)

Fig. 2. Conidia and conidiophores of Alternaria tenussima (×1200).

In pathogenicity test, by comparing the averages of disease rating caused by the fungi under study in *Chamaecyparis lawsoniana*, compared with control, it can be concluded that both isolated fungi species were pathogenic (Figs. 3 and 4).

In a study, *Phytophthora lateralis* was isolated from head dried, died and infected parts of *Chamaecyparis lawsoniana* in eight forest areas, protected areas and tourist cottages in England, Scotland and Northern Ireland (Green *et al*., 2013). In this study, *P. lateralis* was isolated from young seedlings of *C. lawsoniana* and *Thuja occidentalis* that have disease symptoms. This was the first report of presence of this fungus on this plant (Green *et al.*, 2013). In 1949 in Russia was reported that seedling damping off, wilting and dying of pine trees are the most important diseases which caused pine seedlings to be damaged in 30 Russian nurseries (Ankudinov, 1950). After investigations carried out on seedlings, the most important cause of pine seedlings death was reported

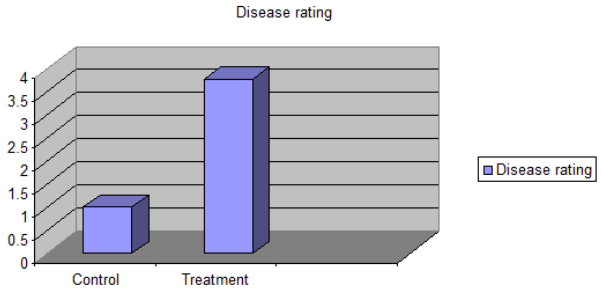

Fig. 3. Diagram of the comparison of *A. franseriae* mean disease rating in treatment and control.

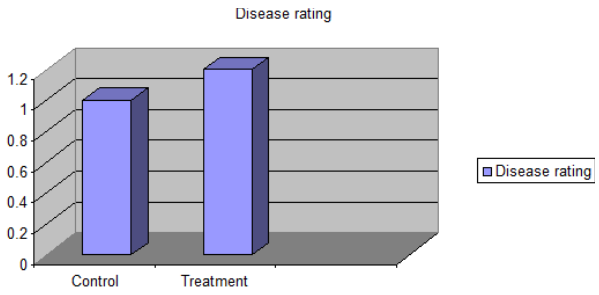

Fig. 4. Diagram of the comparison of *A. tenuissima* mean disease rating in treatment and control.

as *Fusarium* spp. and then some species of *Alternaria* were identified (Ankudinov, 1950). In a study on seeds, seedlings and nursery pine bark of pine trees in south Georgia, Huang and Kuhlman (1990) identified 41 species of 23 fungi genus and 12 species of fungi identified from pine seedlings seed were selected to demonstrate the pathogenicity. Among them, *Alternaria alternata, Fusarium moniliforme* var. *moniliforme,* and *Penicillum expansum* caused seedling damping off.

According to the results obtained in this study it can be concluded that each year many conifers in the parks and forests around the world are attacked by a variety of pathogenic fungi causing their destruction.

According to analysis of variance, there was a significant difference in the disease severity of tested fungi level at probability 5%; and was observed that severity of *Alternaria franseriae* disease was more of *Alternaria tenuissima*.

In Australia, was reported leaf blight of grey mangrove tree caused by *Alternaria alternata* (Chandrashekar and Ball, 1980). In this study, severe blight caused by *Alternaria alternata*, affected leaves, flowers and cuttings of grey mangrove trees in the southern coast of Australia.

Asdaghi *et al*. (2014) investigated diseased trees of *Populus euphratica* in Khuzestan province and leaves with symptoms were collected and transported to the laboratory. Results of pathogenicity test was creating spots on health leaves similar to spots on the leaves of infected trees. By doing these experiments was diagnosed pathogen *Alternaria alternata*. This was the first report of *A. alternata* on *Populus euphratica* in Iran. Kamalakannan *et al*. (2008) investigated disease of leave spot of *Aloe vera* in the state of Tamil Nadu, India with symptoms including oval shaped circles with dark brown necrotic spots, mostly located at the tips of the leaves. *Alternaria alternata* was reported as pathogen. This was the first report of leaf spot disease caused by *Alternaria alternata* in *Aloe vera* in India. In another study, blight symptom was observed in Incarvillea emodi cultivated in India that was caused by *Alternaria* sp. Symptoms of this disease were observed on leaves, flowers and twigs that caused loss of flowers in cool months. This was the first report of *Alternaria* sp. from *I. emodi* around the world (Shanmugan, 2011).

Table 1. Mean disease severity of fungi tested on *Chamaecyparis lawsoniana*.

Fungi	host	Mean disease severing
Alternaria franseriae	*Chamaecyparis lawsoniana*	3.76
Alternaria tenuissima	*C. lawsoniana*	1.23

Table 2. Variance analysis of disease rating in pathogenicity test.

SOV	df	Squares Mean
Treatment	6	6.63**
Error	14	0.058
C.V. (%)		9.46

In the summers of 2007-2008 when the temperature was increased in an unusual way, *Alternaria alternata* caused decay of cluster of grapes in Slovakia (Kakalikova *et al*., 2009). This was the first report of this disease in Slovakia. Soleimani and Esmailzadeh (2007) investigated diseased leaves of apple trees in the northeastern Iran. Symptoms of this disease include black and dark brown spots on the leaves of the apple tree. *Alternaria mali* was isolated from diseased leaves. This is the first report of *Alternaria mali* which causes leaf spot disease on apple trees in Iran.

CONCLUSION

According to the results obtained in this research and previous studies, *Alternaria* spp. were pathogenic on different plants and therefore, further studies in particular greenhouse studies need to be done.

ACKNOWLEDGMENTS

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بیماری‌زایی گونه‌های آلترناریای جدا سازی شده از *Chamaecyparis lawsonia* در شرایط *In Vitro*

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چکیده

گیاه سوزنی برگ *Chamaecyparis lawsonia* که زیبایی را در سطح پارک‌ها و فضای سبز به ارمغان می‌آورد بوسیله‌ی عوامل بیماری‌زای گوناگون بویژه قارچ‌ها مورد حمله قرار می‌گیرد. در این تحقیق از تعدادی *Chamaecyparis lawsonia* موجود در سطح پارک‌ها که دارای علائم بیماری بودند نمونه‌برداری انجام شد. پس از جمع‌آوری نمونه‌ها، به منظور جداسازی پاتوژن‌های قارچی، قطعاتی از بافت‌های آلوده‌ی گیاهی روی محیط کشت PDA قرار گرفت و در مراحل بعدی به منظور شناسایی روی محیط کشت WA قرار گرفتند. سپس آزمایش بیماری‌زایی این جدایه‌ها روی *Chamaecyparis lawsonia* در داخل جعبه‌های پلاستیکی درپوش‌دار انجام گرفت. برای این‌کار قطعاتی از کلنی‌های ۴ روزه‌ی قارچ‌های بدست آمده روی محیط کشت PDA بر روی برگ‌ها قرار داده شدند. ارزیابی نهایی پس از ۱۰ روز انجام گرفت. این آزمایش در قالب یک طرح کاملا تصادفی با ۳ تکرار و ۷ تیمار انجام شد. نتایج نشان داد که قارچ‌های جدا شده در ۲ گروه قرار می‌گیرند که عبارتند از: *Alternaria* franseriae و *Alternaria tenuissima*. بر طبق نتایج هر دوگونه‌ی آلترناریا بر روی میزبان بیماری‌زا بودند. بر اساس جدول تجزیه واریانس اختلاف معنی‌داری در شدت بیماری قارچ‌های مورد مطالعه در میزبان مشاهده گردید. بدین معنی که *Alternaria franseriae* شدت بیماری بیشتری را نسبت به *Alternaria tenuissima* بر روی شبه سروالاوسون نشان داد.

کلید واژگان: *Alternaria* spp.، بیماری‌زایی، شبه سروالاوسون، قارچ‌ها.