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Isolation of Pseudomonas Syringe from Hazelnut Trees in West Part of Mazandaran Province

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ABSTRACT:
Symptoms such as yellowing, wilting and vascular discoloration in hazelnut orchards (*Corylus avellanae*) observed in the Dohezar region of Tonekabon. After sampling taken from plants which have mentioned symptoms and culture on nutrient agar containing sucrose, positive levan Pseudomonases were isolated. The isolates produce Green Fluorescent Pigments in King,s B Medium. These isolates didn't grow in Medium containing %5 salt and %1 TTC and unable to create soft rot on the potato. The isolates were negative in terms of oxidase and pectinase but they were positive in terms of make an ice core and catalase. Isolated bacteria showed hypersensitive reaction on tobacco leaves but they unable to hydrolysis gelatin, starch tween 80 and create soft rot on potato. Obtained strains used casein, trehalose, glucose, L – glutamate and aspartate but they unable to use M- tartarate, L-lysine and beta-alanine as a carbon source. Isolates with inoculate suspension of bacteria on 2 years seedlings of hazelnut was Pathogenic and they had ablility to produce Syringomycin in biological assessment on *Bacillus subtilis* and *Geotricum candidum* in PDA Medium. Isolates were identified as a *P.syringe* based on obtained results of physiological and biological tests. This is the first report from *P.syringe* bacteria as causal agent of hazelnut decline in Iran.

Key words: bacteria, canker, hazelnut, *Pseudomonas syringe*

I.INTRODUCTION

Hazelnut (*Corylus avellanae*) is deciduous shrub and a Monoecious of Alder family. Common hazelnut originated from regions such as the south of Europe and Asia Minor and the planted of it was prevalent in Europe from the past. Provinces such as Urmia, Gorgan, Gillan and Mazandaran are the important regions for grow the hazelnut in Iran (*Khoshkhuy et al., 1991*).
Bacterial canker is a very dangerous disease for hazelnut. This disease cause to yellowing, wilting Falling leaves and buds and ultimately the death of infected trees which make an irrecoverable damage for Orchardist in epidemic years. During five years, over 1800 hazelnut trees lost just in the garden located in Rom due to this disease (*Scortichini and Tropiano, 1994*).
Hazelnut Bacterial canker reported from Greek in 1979 (*Psallidas and Panagopoulos, 1979*). During 1995, 1996 and 1997, *P. syringe pv. avallanae* on infected hazelnut trees was isolated and reported from different regions of Italy (*Scortichini and Morone, 1997*).
Disease occurrence and wilter of hazelnut branches due to *Pseudomonas syringe pv. coryli* was reported during 2005 and 2007 from Italy(*Cirvilleri et al., 2007; Scortichini et al., 2005*) and from Germany in 2006 (*Poschenrieder et al., 2006*). Taxonomy of bacterial canker disease of hazelnut studied in 1984 (*Psallidas, 1984*). Reclassification of *pseudomonas syringe pv.avellanae* as *pseudomonas avellanae* (Spec.nov) reported by Psallidas and Vrijer (*Psallidas and Vrijer, 1996*). Molecular analysis performed about *pseudomonas syringe pv. coryli* by lortil *et al* (*Loretil et al.,2008*). Current report was the result of studies about *P. syringe* which its existence reported first in Iran.

II.MATERIAL AND METHODS

Survey and Sampling
In taken Survey from Hazelnut orchards of Tonekabon Township, suspicious trees were observed which have yellow, wilting and decline leaves (fig. 1).

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Fig.1- yellowing of foliage, the leaves wilting quickly due to bacterial canker disease

The main symptoms are the decline and wilting of trees (fig. 2).

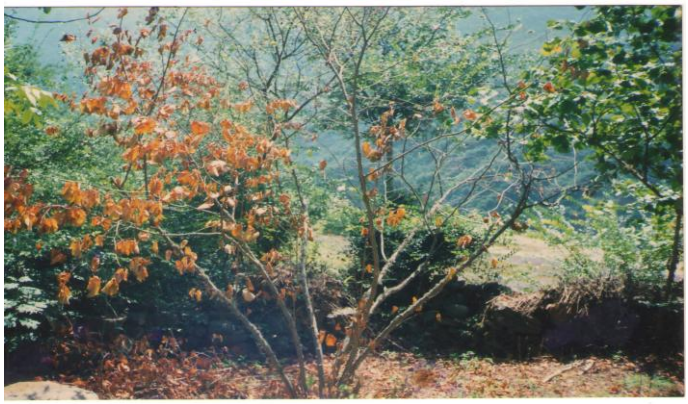

Fig. 2- decline and wilting of hazelnut tree due to bacterial canker

Some gardens have completely dry and aphyllous trees (fig. 3).

Fig. 3- leaves falling and the death of hazelnut tree due to bacterial canker

Samples of infected trees shoot collected from hazelnut gardens and transferred to laboratory with paper pocket.

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Isolation of the bacterial pathogen

Parts of infected sample separated after washing with water and cut to slice in Basin of sterile containing some distilled water. After about 30 minutes kept at laboratory temperature, a drop of the resulting suspension was striped on the medium of the nutrition agar containing half percent of sucrose (NAS, 23 gr nutrition agar + 5 gr sucrose in 1 L water) . 2 days after culture and kept Petri dish in laboratory temperature, separated single colonies was striped to Purification and reproduction on the king. B culture medium (king *et al*., 1954).

Pathogenicity

Hazelnut seeds planted at greenhouse condition in pots containing sterile soil (autoclaved in 24 hours twice) and inoculated with suspension of the bacteria with 10^8 concentration in each ml. by use of handy solution spray in 2 years old. Parts which sprayed with solution wounded by stick sterile pin. Sterile distilled water used for control pots. Inoculated pots placed under plastic cover for 48 hours and kept in greenhouse up to appearance of the symptoms after take the plastic cover.

Biochemical and Physiological Tests

Biochemical and physiological tests was conducted based on prevalent methods (Fahy and Hayward, 1983; Lelliott and Stead, 1987; Schaad *et al.*, 2001) .The use or nonuse of the carbon sources of the isolates was accomplished on the Mineral medium of the Ayer base (Schaad *et al*., 2001). Sugars, organic acids and amino acids added to the base environment with Tyndall method sterile and in 0/2-%1 ultimate concentration. To investigate the hypersensitive reaction of tobacco against bacterial leaf injection method was used as described by klement, 1967. For bacterial suspension (10^8 cells per ml) was injected by syringe below the lower epidermis of leaves of white burley kind of the tobacco. Inoculated plants were kept in a greenhouse with a temperature of 20-27°C.

III.RESULTS AND DISSCUTION

Isolation of the bacterial pathogen

2 days after planting, colonies of the bacteria appeared on the medium of the nutrition agar containing sucrose (NSA) and became spherical –shaped after 3 to 4 days (embowed) with 2-3mm diameter and in pearl white dye. After 24 hours, these colonies produced green fluorescent dye on the medium of the king B. Use or nonuse results of carbon sources than obvious sample (Air-environment without carbon source) evaluated based on growth level comparison and the change of the medium acidity upto three weeks after culturing and maintenance of the Petri dish at 25-28 °c. Strains of this bacterium except difference in urea hydrolysis and 2-ketogluconate have a same reaction production in other biochemical tests. tests with negative reaction in this bacteria are: oxidase, arjenin d-hydrolyze, nitrate, gelatin, starch, tween 80, soft rot, without growth in medium containing %5 salt and % 0/1 TTC and also use M-tartarat L-lizin and beta- alaninas an only carbon source, in other hand, levan production, create freeze core, use casein, 3-haluze glucose, L-glutamat and spartat about positive strains. Hypersensitivity test (HR) was positive for all strains. Two microorganisms such as *Geotricum candidium*, *Bacillus subtilis* used to produce toxin Sirnigumaysin on the PDA medium which tests reaction confirm toxin production with strains. Determine of patovar kind of mentioned bacteria is studying.

Pathogenicity demonstration

Symptoms appeared inoculation in form of seedlings necrosis 2 months after. No symptoms observed in sample plant and inoculated bacteria separated again from infected seedlings.

Bacterial canker observed during 3 successive years in Tonekabon and Isolates were identified as a *P.syringe*. This is the dieback and decline factor of the hazelnut trees (*Corylus avellana*). Main created symptoms including wilting of the budding, trees branches during spring and summer, quickly. Leaves became dry wilted and connected to the branch many long-term. The skin and cambium of the trees changed the color, first roots became necrosis and ultimately cause to plant death.

This study showed *P.syringe* bacteria existence on hazelnut for the first time in Iran. This bacterium seems very dangerous for *C. acvellanae* kind of hazelnut. Whereas, this study is almost as same as studies achieved in Greek and Italy explained that such organism which able to kill plant in an agricultural season, according to symptomatology, this disease is equivalent of element which exist with *P.syringe Patovar avellanae* . By existence obvious symptoms of the bacterial canker on hazelnut trees in Dohezar region of Tonekabon and result of conducted laboratory studies, separated from infected trees due to have individual properties of *Pseudomonas syringe* such as gram- negative, produce fluorescent on KB medium, have colonies with yellow color and change tissue to embowed at the final growth stages of the negative reaction to oxide tests from D-arginine hydrolysis, urease and no power to create crushed potato slides, reduction of nitrate and produce Indole were identified and introduced as *Pseudomonas syringe*.

IV.CONCLUSION

Twig and branch diebacks were frequently observed on the local hazelnut cultivars. Isolation was performed in spring and autumn from symptomatic tissues. Identification of the bacterial pathogen associated with these symptoms was performed by using King's medium B and nutrient sucrose agar. Based on the results obtained from biochemical and Pathogenicity tests we conclude that the twig and branch diebacks observed in the hazelnut orchards of Dohezar surveyed in the present study are caused by *P.syringe*. This is the First report of this pathogen in this area.

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Biography

I have been working as a researcher (Assistant Professor) in Dept. of plant protection research in Natural Resources and Agricultural Research center of Mazandaran. I am holding MSc and PHD degree in plant pathology and environmental science respectively. I have 22 years experience in above field. I have published and presented more than 20 papers in various national and international conferences and journal. I was awarded by head of Iran citrus research Institute and head of Sari Jahad-Agricultural organization and etc. my research area is pesticide residue and plant diseases.