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The Investigation of Resistance to Prunus Bacterial Spot among Some Cultivars of Three *Prunus* Species and Chemical Control of PBS in Golestan Province of Iran

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Abstract: Prunus bacterial spot, caused by *Xanthomonas arboricola* pv. *pruni* continues to be an important issue for stone fruits gardeners in all continents. This disease has been reported from Iran. Yet by sampling from blossoms, leaves and young twigs of peach, nectarine and plum (in Golestan province) we faced with symptoms which were similar to this disease. Isolation of the associated bacteria showed that the casual agent of disease is *X. a*. pv. *pruni* (according to biochemical characteristics). To find a suitable method of controlling this disease regarding the conditions of different regions of this province, some chemical treatments and resistance of cultivars of peach, plum and nectarine were investigated. Our study showed that the incidence of the disease in Shastkola, the most humid region in our investigation, is more than two other regions. Gojeh Saa'di and Bokhara are the most resistant cultivars of plum; however, Santrosa seams to be sensitive to this disease.Early Glu is the most resistant cultivar of peach and Anjiri sounds to not have resistance to PBS. Nectard is the most resistant cultivars of nectarine and in this case Red Glu showed the least resistance. The most effective chemical method to control this disease is three time application of Bordeaux which significantly reduced disease severity.

Key words: *Xanthomonas arboricola* pv. *pruni,* Cultivars, Chemical compounds

INTRODUCTION

Prunus bacterial spot (PBS) disease was first described in 1902 in North America (Bradbury, 1986) and it has been reported from Iran in (Jami *et al.*, 2004). The casual agent of PBS is *Xanthomonas arboricola* pv. *pruni* which naturally infects all cultivated *Prunus* species and hybrids, including apricot ,almond, cherry, plum, peach and nectarine. Furthermore several other *Prunus* species and *Sorbus japonicum* can be infected by artificial inoculation. (Bradbury, 1986).

A warm, moderate season with temperatures of 19-28°C and with light, frequent rains accompanied by fairly heavy winds and heavy dews is most favourable for severe infection (Bradbury, 1986. Du Plessis, 1988. Fahy *et al.,* 1983). The disease tends to appear and spread in the spring, and then makes little progress through the summer, but late infections occur in the autumn. In culture, bacteria have survived ice-box conditions of -2°C to +2°C for 5 months. The disease is not usually found in arid regions. However it is most serious in areas with warm and wet or humid conditions during the growing season (Bradbury, 1986. Du Plessis, 1988. Fahy *et al.,* 1983). Currently, the disease is known to occur in all continents. (Anonymous, 1978. Civerolo *et al*., 1993. CMI, 1987).

The symptoms of the disease are various in different species, including lesions on leaves developing as small, or irregular (0.5 – 1mm diameter) watersoaked spots. Moreover, developing lesion became dark red, purple, brown or black. The dark spots may be surrounded by grey to yellowish tissue. Fruit lesions initially appear as small, circular water soaked spots becoming grey to brown on the surface. As the spots develop, they become light, dark brown, purplish black or black (Civerolo *et al*., 1993). The bacterium causes cankers on twigs, especially on plum and apricot (Anderson HW).

The management of PBS is based on resistant cultivars and or hybrids, integrated cultural practices and treatment with chemicals (Du Plessis, 1983. Du Plessis, 1987. Keil, 1979. Keil and Carrol, 1976. Moffett, 1973). Several *Prunus* species, cultivars and hybrids are relatively resistant to PBS disease (Du Plessis, 1987. Keil and Fogle, 1974. Topp *et al*., 1989.Topp *et al*., 1990). So investigation of resistance among different

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species and cultivars may help to suppress this disease. Chemical sprays of copper-containing compounds (e.g. oxychloride and cupric hydroxide plus lime) and antibiotics can reduce the amount of leaf and fruit infection when applied at the proper time throughout the growing season (Du Plessis, 1983. Du Plessis, 1987. Keil and Carrol, 1976). Thus determining the most efficient compound and time of applying chemical compounds sounds important in controlling this disease.

In Iran it has not yet been investigated on control of this disease but the prevalence of bacterial spot of stone fruits can be clearly seen and has become epidemics in some orchards of Golestan province (which is situated in the north of Iran). In some orchards infection severity of this disease roughly hits 100%. In this survey we have tried to find applicable and suitable method of control and resistant cultivars to suppress the diseases and introduce it to gardeners.

MATERIAL AND METHODS

Isolation of the agent of the disease:

The tissues which had the symptoms of the disease were washed thoroughly in running water and after washing in distilled water some young lesions or the margins of older lesions were selected. Small pieces of given issue were cut out by a sharp sterile scalpel. The pieces were crushed in the sterile distilled water and we streaked the resulted water onto the surface of YDC agar medium by a loop. Petri plates were incubated at 25-27^{oc} for 72hours to form single colonies. To detect the genus, species and pathovar, we applied differential and semi selective media such as NAS, YDC and XPS; furthermore, other biochemical tests were carried out. These tests include using NAS (5%), Gram's reaction, Litmus milk, catalase reaction, starch hydrolysis, Producing acid from different carbohydrates, etc (Bradbury, 1986. Civerolo *et al*., 1993. Schaac *et al*., 2001). LOPAT tests were carried out (Lelliot and Stead, 1987). Hypersensitivity of the isolated bacterium was investigated by infiltration of geranium (*Pelargonium hortorum*) (Rahimian *et al*., 2004).

The investigation of distribution and severity of the disease:

To study the distribution and severity of th disease in Golestan province, in each region (Shastkola and Ali Abad and Gonbad) 48 trees of each orchards were chosen on random and 10 branches from each nectarine were randomly opted to compare number of spots on twigs twice (before and after blooming) and ultimately data were analysed.

The confirmation of Pathogenicity:

To confirm the pathogenicity of the bacterium, distilled water as control treatment and suspension of the bacterium were inoculated artificially to host plants including peach and nectarine (Rahimian *et al*., 2004). After injection of the bacterial suspension under rind of young branches, symptoms of the diseases were scrutinized for one to three months. Also inoculation of diluted suspension of four strains of the bacterium (10^6 CFU /ml) to four young leaves of two year old peaches caused the symptoms to appear.

The reaction of cultivars to the disease:

To study the reaction of different cultivars of stone fruits to this disease, severity of bacterial infection was assessed by counting the number of infected branches. For each cultivar four trees (four to six years old) were applied as our treatments. In this survey resistance of six cultivars of plum including Bokhara, Gojeh Saa'di, Shablon Ripening Early, Santrosa, Ghatreh Tala, and Shablon Ripening Late, five cultivars of peach including Early Glu, Anjiri, Spring Crest, Red Cup and Dexy Red, and ultimately six cultivars of nectarine including Nectard, Red Glu, Sun Keen, Independent, Moghan Gerd and Sun Glu were studied. Investigation on finding resistant cultivars and chemical control method took place by complete randomized block with four replications for 7 treatments (in 5% degree). Results were analysed by Duncan's multiple range test (using SAS software).

The compression of different methods of chemical control of disease:

To compare different methods of controlling this disease we investigated different treatments which are mentioned following:

1. Pruning + Spraying Copper Oxychloride 5per1000 in the first stage (in late February), 2per 1000 in the second stage (one week before blooming) and 2.5 per 1000 in the third stage (one week after blooming)
2. Pruning + Spraying Bordeaux 18% (10 per 1000 in the first stage) and 5per 1000 in the second and the third phases.

3. Pruning+ Spraying Bordeaux (the first and second phases) and Spraying copper Oxychloride (the third stage)
4. Pruning +Spraying Copper Oxychloride (the first and the second stages), and Bordeaux (the third stage)
5. Pruning + Spraying Bordeaux (the first stage), Copper Oxychloride (the second stage) and Bordeaux (the third stage).
6. Pruning + Spraying biological solution (in three stages)
7. The control treatment

Each tree was as one replication and experiments of each region was carried out on 24 trees. Sampling was carried out one month after blooming in three one month intervals. Eventually the data of the experiments were analysed (in 5% degree) by Duncan's multiple range test (using SAS software).

RESULTS AND DISCUSSION

Some of the typical morphological, physiological, and biochemical characteristics of the strains of bacteria which were isolated from peach, nectarine and plum are mentioned in the following table:

Table1: Biochemical characterization tests of the pathogen *Xanthomonas arboricola* pv. *pruni*".

Tests	Results	Tests	Results
Mucoid growth on YDC	+	Yellow to orange colonies	+
Levan formation	+	Gram's Reaction	-
Tolerance onNacl 3%	+	Fermentative	-
Tolerance onNacl 6%	-	Oxidase	-
Litmus milk	Alkaline	Protein Digestion	+
Pathogenicity	+	Growth on 35 ^{c}	+
Starch Hydrolysis	-	Acid from Arabinose	+
Nitrate Reduction	-	Catalase Reaction	+
Hypersensitivity in geranium	+	Lipase activity	±

According to figure number 1, the highest amount of severity of stone fruit trees which were infected by *X. a.* pv. *pruni* was related to Shastkola Region and then Ali Abad region, and the lowest infection was observed in Gonbad region. It was probably due to high relative humidity of Shastkol region (which is situated between Caspian Sea and Golestan Forest) because high relative humidity plays an important role in prevalence of this bacterial disease.

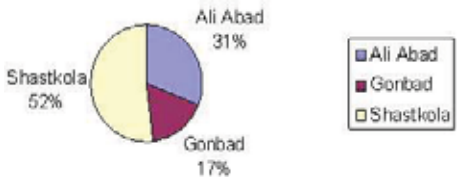


Fig. 1: Infection of Nectarine by PBS in three different regions.

According to graph number 2 the degree of infections of peach, nectarine and plum differs significantly in the 5%level. As different cultivars of peach which were infected averagely 22.5% and were the most sensitive kind of species to this disease (among these three species), next tree was nectarine with 11% infection and plum trees were the most resistant cultivars with just 3.5% incidence of the disease. We can say average infection of different cultivars of plum trees with bacterial spot of stone fruits had a significant difference in the 5% level. Among different cultivars of plum to this disease (the figure number3) Bokhara and Gojeh Saa'di were completely resistant to the disease; however, Shablon Ripening Early, Ghatreh Tala and Shablon Ripening Late were rather sensitive cultivars to this disease. Santrosa was the most sensitive cultivar to bacterial spot of stone fruits. Comparing of sensitivity to the disease among different cultivars of peach (figure 4) (Civerolo and Hatting, 1993). indicates Early Glu and Spring Crest are the most resistant cultivars and then Red Cup, Dexy Red and Anjiri are placed respectively. Thus, Early Glu with just 8%infectoin was the most resistant cultivar; in contrast, Anjiri which was infected roughly 48% was the least resistant cultivar.

Infection degree among different cultivars of nectarine differs significantly (in 5% level) as the least infection was observed in Nectard and Sunkeen cultivars (figure No.5); and Red glu, Sunglu, Moghan Gerd and Independent were the most sensitive cultivars respectively. Also our investigation illustrated Nectard cultivar which was infected about 2% and Sunkeen cultivar with approximately 4% infection were the most resistant cultivars of nectarine, whilst Red Glu cultivar showed the most sensitivity with 25% incidence of the diseases, and Independent, Moghan Gerd and Sunglu were infected something in between 10% and 15%.

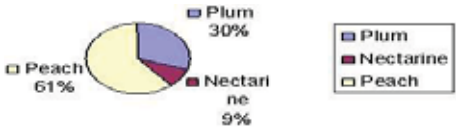


Fig. 2: Infection of different Prunus species by PBS.

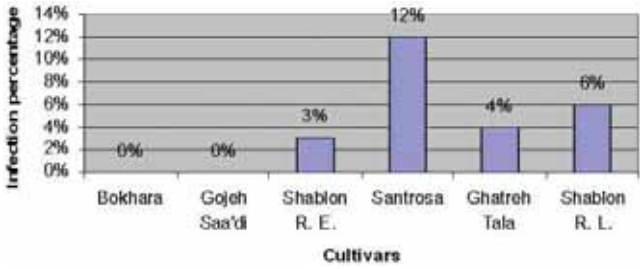
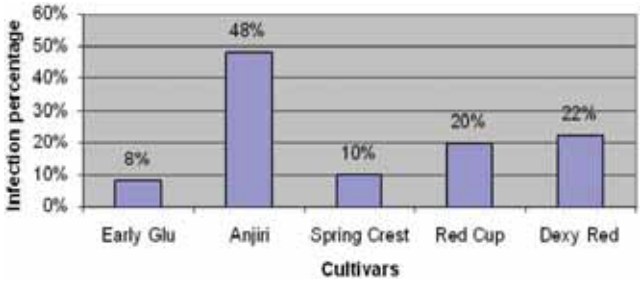


Fig. 3: Infection of different cultivars of Plum by PBS.



Fug. 4: Infection of different cultivars of Peach by PBS.

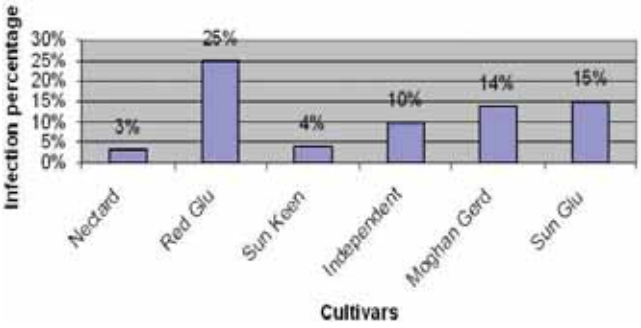


Fig. 5: Infection of different cultivars of Nectarine by PMS.

Different spraying treatments bring out a substantial drop in infection in comparison with the control treatment. comparison of different methods of chemical controls against bacterial canker of stone fruits (the figure number 6) reveals three time spraying by Bordeaux, which reduced the infection to just 5%, was the most effective method of spraying; and that follows by Copper Oxychloride-Bordeaux and Bordeaux spraying, and spraying of Bordeaux, Copper Oxychloride and Bordeaux in comparison with the control treatment which caused roughly 35% infection. The least decrease of infection occurred by three time spraying of Copper Oxychloride, Copper Oxychloride-Copper Oxychloride-Bordeaux spraying and applying the biological control. Thus, according to the result of the experiment the most efficient method of controlling bacterial spot of stone fruits is planting resistant cultivars and also applying Bordeaux after pruning (10 per 1000) in winter and then spraying (5per 1000) before and after glooming. Because of the high sensitivity of cultivars of peach trees it is suggested that planting resistant cultivars of nectarine and plum is better than planting peaches cultivars in Shastkola region. However, planting different cultivars of peach is more suitable for Aliabad and Gonbad regions.

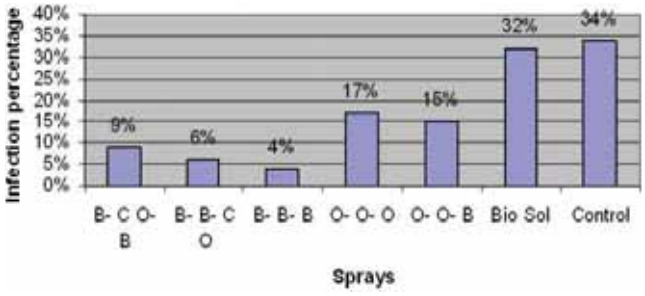


Fig. 6: Impacts of different sprays on incidence of PBS (In Ali Abad region).

Field survey of these regions of Golestan province showed a high incidence of this disease in this province. The pathogen was confirmed as *Xanthomonas arboricola* pv. *pruni*. Moreover our investigation showed that the increasing of relative humidity may result in expanding of the incidence of the diseases. According to the percentage of infection among Plum, Nectarine and Peach, planting of Plum is suggested also the resistance of different cultivars should be paid attention. Furthermore in the old orchards, three time spraying of Bordeaux including winter spraying (after pruning), and in spring (before and after blooming) sounds to be an efficient method to decline the incidence of this disease.

Although these chemical compound could be efficient for controlling this disease, other method of applying these and other chemical compounds should be investigated. In our study the biological solution could not decrease this disease, but under other conditions and applying different biological agents may contribute to suppress the severity of PBS.

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