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Biological Control of *Rhizoctonia solani,* the Causal Agent of Rice Sheath Blight by Antagonistics Bacteria in Greenhouse and Field Conditions

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Abstract: *Pseudomonas fluorescens* isolates that inhibited growth of *Rhizoctonia sola*ni Kühn, the rice sheath blight pathogen were collected the rhizosplane and surrounding soil of healthy and rice sheath blight disease in farming of the Guilan province, Iran. Two hundred eighty eight isolates tested and among them only antagonistic ability of 8 isolates were demonstrated by using the dual culture method. According to the results of biochemical and morphological trials all isolates were identified as *P. fluorescens* biovar 3. By determining the effects of volatile metabolites, secretion of extracellular and antibiotics of these isolates inhibited mycelial growth of *R. solani in vitro.* All *P. fluorescens* isolates produced siderophore on King' s medium B, inhibited the mycelial growth of the *R. solani.* Antagonistics isolates reduced the germination and cause the lysis of sclerotia of *R. solani.* In greenhouse conditions antagonistic isolates were used by seed coating, soil drenching and foliar spray. Statistical analysis of data indicated that there existed significant differences between seed, soil and plant treatments. All of the isolates in seed coating are more effective. In the field conditions foliar spray of isolate B_{41} mixed with benlate were applied. The disease intensity in B_{41} isolate for seed coating, soil drenching and seed coating + foliar spray were 10.5, 11.75 and 18.75%, respectively, while the control plants showed 52% disease intensity. These results suggest that the *P. fluorescens* isolates studied have an excellent potential to be used as biocontrol agents of *R. solani* in rice at the field conditions.

Key words: Sheath blight, *Rhizoctonia solani*, *Pseudomonas fluorescens,* biological control

INTRODUCTION

Sheath blight caused by *Rhizoctonia solani* Kühn is one of the most widespread diseases of rice (*Oryza sativa* L.) and cause serious yield losses under favorable environmental conditions[1]. *Rhizoctonia solani* has a very wide host range and strong of source resistance in rice against this disease are not available. All cultivars of rice are susceptible, but the degree of susceptibility are vary[2]. Yield loss ranging from 25 to 50% has been reported[3]. The sclerotia of the fungus survive in soil and are disseminated by irrigation water[4]. The disease is initially soil-born, subsequent spread is foliar. Seed treatment[5], soil application[6] and foliar spray with systemic fungicides and antibiotics[7] have given effective control of the disease. However, these treatments are expensive and add pesticide to the environment. In recent years, fluorescent pseudomonads have drawn attention worldwide because production of secondary metabolites such as siderophore, antibiotics, volatile compounds, HCN, enzymes and phytohormones[8]. Fungi from *Trichoderma* genus are among the biological control agents of *R. solani*[9,10] also bacteria belonging to *Pseudomonas* and *Bacillus* genus have been also used[11]. The ideal biocontrol agent for the management of foliar infection by a soil-borne pathogen may be the one that can survive in both rhizosphere and phyllosphere. Among the various biocontrol agent, fluorescent pseudomonads are known to survive both in rhizosphere[12] and phyllosphere[13]. Florescent pseudomonads are known to induce disease resistance against foliar disease[14]. Bacterial suspensions of florescent pseudomonads have been used for control of rice sheath blight by Mew and Rosales[15]. Certains of fluorescent pseudomonad isolates have antagonistic activity based on producing antibiotic wherase for other isolates such as *Pseudomonas putida* WCS358 based on competition for iron[16]. According to present and future regulations on the use of chemical fungicides such as Hinosan, benlate and Rovral and considering that treatments must prevent environmental pollution, we have considered the use of biocontrol agents to control *R. solani* that effect rice plants. The selection of bioantagonistic microorganisms, other to take into account the direct effect on pathogen

development, must consider conditions where the bioantagonist should be develop, I. e. salinity that pH of soil and different temperature, among other[17]. The objectives of the present research, isolation of florescent pseudomonads from rhizosphere and phyllosphere of infected rice that could control *R. solani in vitro* and *in vivo* and their characterization in term of antagonistic mechanisms used to control the pathogen and conditions for growth similar to those present in the field.

MATERIALS AND METHODS

Isolation of *Rhizoctonia solani*: Rice sheath blight disease was collected in 2001 from infected farming in Rasht, Lahijan, Foman, Talesh and Astara of Guilan Province, Iran. For isolation of *R. solani*, some small pieces of infected sheath leaf, were washed and surface sterilized with 5% sodium hypochlorite for 10 min. The infected tissues were cultured on acidified Potato Dextrose Agar (PDA). The plates were incubated at room temperature (26±2°C) for a week. The growing colonies of fungi were transferred to new plates for purification and identification.

Preparation of inoculum *R. solani* and pathogenicity test
Three hundred gram of barly seed sterile were put in Erlenmeyer, then in each erlen five 4 mm mycelial disc from a 4 days old culture of *R. solani* on PDA was placed in the erlens and they were incubated at room temperature (26±2°C) for 3 week. The colonies of fungi were developed and produced many sclerotia[31]. For pathogenicity test, approximately 0.2 g of inoculum (mycelium and sclerotia) placed inside the leaf sheath with a few drop of sterile water invariably induced single dicreate uniform size lesion, irrespective of type of inoculum used (i.e., sclerotium or mycelium)[18].

Isolation of fluorescent pseudomonads isolates and identification
Rhizosphere colonizing fluorescent pseudomonads were isolated in 2001, from fresh roots of rice paddies in Rasht, Lahijan, Foman, Talesh and Astara in Guilan province, Iran. After vigorous shaking of excised roots to remove all but tightly adhering soil, root segment, (1 g) were shaked in 100 mL of sterile distilled water for 25 min. Fluorescent pseudomonads were isolated on King's medium B (KB). According to the methodology of Schaad[18] antagonistics isolates of bacteria were identified by biochemical, physiological and biological tests.

Antagonism
Efficacy of *P. fluorescens* isolates to inhibit *R. solani in vitro*: *Rhizoctonia solani* isolated from a diseased leaf sheath blight of the rice cultivar Kazar, was shown to be highly virulent isolates in a subsequent pathogenicity test. Efficacy of the *P. fluorescens* isolates in inhibiting growth of *R. solani* was tested by streaking each bacterial isolate on one side of a petri dish containing potato dextrose agar and nutrient agar (PDA+ NA) medium[19]. One 5 mm mycelial disc from a 4 days old culture of *R. solani* on PDA+ NA was placed at the opposite side of the petri dish and experiments were independently repeated four times. Growth of fungus was inhibited when it grew toward the bacterial colony and the inhibition zone was measured from the edge of mycelium to the bacterial colony edge. The bacterial isolates that inhibited *R. solani* were identified by specific tests for *P. fluorescens*[20].

Production of volatile antibiotic: Two hundred fifty micro liter of a antagonistic bacterial suspension (10^8 CFU mL^{-1}) were placed at the petri dish containing KB and a 5 mm disk of a four days old pure culture of *R. solani* was placed at the center of another petri dish containing PDA. Both half plates were placed face to face preventing any physical contact between the pathogen and the bacterial suspension and were sealed to isolate the inside atmosphere and the prevent loss volatiles formed. Plates were incubated at 26°C for 6 days and the growth of the pathogen was measured and compared to controls developed in the absence of the bioantagonist (mocked inoculation with 6 mm disk of PDA). Each experiment considering a single bacterial isolate was run in triplicate and was repeated at least three times[21]. Results are expressed as means of % inhibition of the growth of *R. solani* in the presence and absence of any bacterial isolate. Percent inhibition was calculated using the fallowing formula[21]:

% inhibition = (1- (fungal growth / Control growth)) x 100

Secretion of extracellular: These test were performed in 250 mL Erlenmeyer flasks containing 100 mL of sterile nutrient broth (NB). One milliliter bacterial suspension isolates (10^8 CFU mL^{-1}) were added to the flasks containing NB. The flasks were then incubated at 26°C for 6 days on a rotary shaker at 175 rpm at room temperature (26±2°C). Bacterial cells were pelleted by centrifugation at 5000 g for 12 min. The supernatants were sterilized with 0.22 μm filtrate. 5, 15 and 25 % (v/v) of culture filtrate were mixed with PDA and a 5 mm disk of a four days old pure culture of *R. solani* was placed at the center of petri dish. The experiments were independently repeated four times.

Production of diffusible antibiotic: This effect was tested according to Montealegro *et al.*[17] PDA plates, covered with a cellophane membrane, were inoculated in the center

with 250 μL of a bioantagonistic bacterial suspension (10^8 CFU mL^{-1}). After incubation for 48 h at 26°C, the membrane with the grown bacterial isolate was removed and the plate was inoculated in the middle with a 6 mm disk of a pure culture of *R. solani* plates were future incubated at 26°C for 5 days and the growth of the pathogen was measured. Control were run with mocked inoculated PDA containing plates on the cellophane membrane (replacing the bacterial suspension by sterile distilled water) and future incubated with *R. solani*. Each experiment considering a single bacterial isolate was run in triplicates and was repeated at least three times.

Effect of Fe^{+3} on antagonism level: This effect was tested according to Pumarino[22], using $FeCl_3$ x $6H_20$ at 5, 50 and 100 μMol concentrations added to the KB medium. For all tests, the experimental unit was one petri dish.

Bacterial ability for root colonization: Ability of bacteria to colonize roots was established according to Misaghi[2]. The pre-germinated rice seeds were inoculated with bioantagonistic bacterial suspension (10^8 CFU mL^{-1}) containing 1% gum After incubation for 1 h at 26°C, rice seeds were placed in the tube containing 2/3 sand sterile and the tubes were incubated for 21 days at 26°C, for evaluation, radicale apex were isolated and crushed in stomacher plastic containing 1 mL distill water sterile and the bacterial suspension obtained was diluted. Fifty microliter of these dilutions were seeded on petri dish containing KB and incubated for 48 h at 26°C and cfu were counted. The experimental unit was three replications for each antagonistic isolate.

Production of protease: This effect was tested according to Maurhofer *et al.*[42]. Efficacy of the *P. fluorescens* isolates in production of protease was tested by streaking each bacterial isolate on Skim Milk Agar medium (SMA) on the petri dish. Each experiment considering a single bacterial isolate was run in triplicates and was repeated at least four times. The bacterial isolates that produced protease were identified by a halo zone arounding of bacterial colony and were measured[22].

Effect of antagonistic isolates on germination of sclerotia of *R. solani in vitro*: Sclerotia of *R. solani* were produced, their surface sterilized with 2.5% sodium hypochlorite and dipped in the Erlenmeyer flasks containing 50 mL of KMB and 1 mL suspension antagonistics isolates (10^8 CFU mL^{-1}) were inoculated. The bacterial cells suspensions in KMB medium were placed for 24 h on a rotary shaker at 175 rpm. at room temperature (26±2°C) and 5 sclerotia were inoculated in each bacterial isolate and was placed for 24 h on a rotary shaker at 175 rpm. at room temperature. After incubation for 3 days at 26°C, the germination of the sclerotia pathogen was measured. Control were run with inoculated replacing isolates antagonistics suspension by sterile distilled water. Each experiment considering a single bacterial isolate was run in triplicates and was repeated at least three times.

Effect of antagonistic isolates on lysis of sclerotia of *R. solani in vitro*: Two hundred μL of a bioantagonistic bacterial suspension (10^8 CFU mL^{-1}) were placed at the petri dish containing KB. After incubation for 72 h at 26°C, sclerotia of *R. solani* were produced, their surface sterilized with 2.5% sodium hypochlorite and were placed for 6 weeks at 26°C, the lysis of the sclerotia pathogen was measured. Each experiment considering a single bacterial isolate was run in triplicates and was repeated at least three times. Control were run with inoculated replacing isolates antagonistics suspension by sterile distilled water.

Effect of antagonistic isolates on germination of sclerotia of *R. solani* in soil: This effect was tested according to Knudsen and Eschen[23], 5 kg of soil were sterilized and placed at the pot (15 cm in diameter), sclerotia of *R. solani* were produced, their surface sterilized with 2.5% sodium hypochlorite and soil were drenched with 50 mL of a bioantagonistic bacterial suspension (10^8 CFU mL^{-1}), the pots were placed for 6 weeks at 26°C. After incubation for 3 days at 26°C, the germination of the sclerotia pathogen was measured. Each experiment considering a single bacterial isolate was run in triplicates and was repeated at least three times. Control were run with inoculated replacing isolates antagonistics suspensions by sterile distilled water.

Effect of benlate fungicide on *R. solani* and antagonistics isolates *in vitro*: This effect was tested according to Horsfall[24]. The development of antagonistic bacteria and *R. solani* were tested under Benlate concentrations of 1, 3, 5, 10, 100 and 1000 ppm at room temperature (26±2°C). For *R. solani*, 2 mL of each Benlate concentrations which mixed with PDA at 50°C and a 5 mm disk of a four days old pure culture of *R. solani* was placed at the center of petri dish containing PDA. After incubation for 5 days at 26°C and the growth of the pathogen was measured. Control were run with mocked inoculated PDA on the PDA medium with (replacing fungicide concentrations by sterile distilled water). For antagonistics isolates, the 5 mm disk of wathman paper was dipped in the benlate concentrations for 2 min and was placed at the petri dish containing KB. After incubation for 48 h at 26°C the inhibition zone was measured. Four replication per concentration were maintained. In greenhouse conditions,

1000 ppm concentration of benlate was used for seed treatment, soil drenching and foliar spray.

Results are expressed as means of % inhibition of growth of *R. solani* and zone inhibition of antagonistics bacteria.

Greenhouse and field

Efficacy of *P. fluorescens* isolates to control *R. solani* in the greenhouse and field: For evaluate greenhouse and field works were used cultivar Khazar that very sensible to sheath blight. The fluorescent pseudomonad isolates that inhibited *R. solani in vitro* were tested for their efficacy to control sheath blight in the greenhouse.

Preparation of bacterial inocula: Cells of antagonistic bacteria for use in greenhouse and field experiments were grown in King's Medium B broth (KMB) to late exponential phase at 27°C with shaking at 175 rpm. Cells were harvest by centrifugation (5000 rpm/min, 10°C, 15 min), washed twice and resuspended in 0.5% sterile NaCl solution (for greenhouse) or tap water (for field experiments). The bacterial suspension was adjusted turbidimetrically to about 10^8 colony forming units (CFU mL^{-1}) for each experiment. The bacterial cells suspensions in KMB medium were used for seed coating, soil drenching and foliar spray.

Efficacy of seed coating, soil drenching and foliar spray with *P. fluorescens* isolates and fungicide: In greenhouse conditions rice seeds (Cv. Khazar) were surface sterilized in 0.6% sodium hypochlorite for 10 min and then air dried in a fume hood and the seeds were soaked in the antagonistic bacterial suspensions (10^8 CFU mL^{-1}) in 1% methyl cellulose for 24 h. Inoculum of *R. solani* was added to soil in pot and infested soil was covered with plastic film and incubated 48 h at room temperature then five grams of seeds was sown in each pot (25 cm in diameter). Separately, at the same time, soil in pot were drenched with suspensions of the antagonistic bacteria isolates and benlate at a total concentration of 10^8 CFU mL^{-1} and 1000 ppm, respectively. Pots were maintained in the greenhouse under 25±3C and relative humidity 95% conditions. Four-weeks-old rice plants were spray inoculated with antagonistic bacteria suspensions (10^8 CFU mL^{-1}). Control treatments were inoculated with sterile distilled water and seedling with sheath blight disease symptoms were recorded 4 weeks after planting. Plants were arranged in a randomized completed block design with five replications. Sheath blight intensity was assessed 120 days after *R. solani* inoculation.

Field experiments were performed in 2003 at the University of Guilan Field Station. The experimental plot consisted of a Randomized Complete Block Design with three replications. Each replication consisted of about 30 plants. The experimental plot was surrounded by a buffer zone of approximately 10 m of fallow soil. Field rice was upland and seedling rice were planted. Seed coating and foliar spray with antagonistic bacteria suspensions (10^8 CFU mL^{-1}) and bacteria suspensions (10^8 CFU mL^{-1}) add benlate were used similar to greenhouse conditions. Rice plants were sampled 120 days after planting[25].

Evaluated of disease intensity of *R. solani*: Disease intensity was scored using the formula described by Sharma *et al.*[26] where the Highest Relative Lesion Height (HRLH) was equal to the highest lesion height/the highest plant heighx100. This index is based on the highest point reached by a sheath blight lesion relative to the highest point of the plant and is adapted from the relative lesion height method described by Ahn *et al.*[27]. The disease intensity was scored for each tiller in the 5 plants at 120 days after sowing (90 days after seedling) in the stage of earing and five replications were maintained for *P. fluorescens* isolate. The pots were arranged in factorial design. The trial were repeated at least twice with similar results.

Statistical analysis: The data obtained were subjected to analysis of variance and the means separated by using Duncun's Multiple Range Test[28] and ANOVA. Tests were used to establish significant differences. Values in percentages were analyzed statistically after carrying out angular transformation.

RESULTS

Pathogenicity test of *R. solani* on rice: After two weeks rice seedling (Cv. Khazar) showed that sheath blight symptoms. Initial symptoms develop as lesions on sheaths of lower leaves near the water line. These lesions develop below the leaf collar as oval-to-elliptical, green-gray, water-soaked spots about 0.75 cm in wide and 2 to 6 cm in long. *R. solani* AG-1 IA was isolated on rice sheath blight showed that the high disease intensity. There are no significant difference between of 4-day-old of mycelium and 6-day-old of sclerotia inoculums.

Isolation of bioantagonistics bacteria: Two hundred eighty eight bacterial isolates, were initially collected from the rhizoplan and rhizosphere of rice sheath blight disease in farming of different areas of the Guilan province-Iran. Among them, 8 isolates were found to inhibit growth of *R. solani in vitro*. All of them were identified as *Pseudomonas fluorescens* biovar 3 according to the methodology of Schaad[18].

Efficacy of antibiosis of *P. fluorescens* isolates to inhibit *R. solani in vitro*

Dual culture: No physical contact was observed between any of the antagonistics bacteria tested and *R. solani*; moreover, an inhibitory halo was observed suggesting the presence of fungistatic metabolites secreted by the bacteria. On the other hand, a change in mycelial color was observed closed to the colony end of *R. solani*, being this one of a darker brown than the one observed at the center of colony. Microscopy observation of this zone, allowed to detect cytoplasmic leakage that could be observed up to the hyphal septum, resulting in deformation and sliming of their apex up to 1/7 of its original size. Similar results were obtained by Montealegro *et al.*[17]. *P. flourescens* B_{41} and B_{22} with zone inhibition of 45 and 39 mm were the most inhibited of *R. solani*.

Volatile antibiotics: All antagonistics isolates were showed there are significant difference with control (p=0.05). *P. fluorescens* B_{41} were the antagonistic bacteria isolate that showed the best inhibitory effect on the growth of *R. solani.* The % inhibition of *P. flourescens* B_{41} at 72 h culture of antagonistic isolate was 72.7, while although all bacteria showed inhibitory effect on *R. solani* grow (Table 1).

Diffusible antibiotics: Results similar to those obtained when the effect of volatile antibiotics, were obtained when the effect of diffusible antibiotic was tested (Table 1). Isolate B_{41} and B_{22} with % inhibition of 85.8 and 78.5, respectively were the most inhibited of *R. solani,* while isolate B_4 with % inhibition 65.2 was the less inhibited by diffusible antibiotics on the growth of *R. solan* (Table 1).

Secretion of extra cellular: All antagonistics isolates were seen there are significant different between isolates and concentration of juices (p= 0.05). *P. fluorescens* B_{41} % inhibition 87.6 (25% v/v) was the most inhibited of *R. solani* (Table 1).

Production of protease: All eight antagonistics isolates were able to produce protease on SMA medium. Among isolates, *P. flourescens* B_{41} was found most effective which had 23 mm of halo zone arounding bacterial colony. It consider that all of the eight isolates were able to secrete of the enzymes involved in biocontrol and that all had the ability to control *R. solani* through secretion of diffusible and volatile metabolites, it may be concluded that they use these two latter mechanisms of biocontrol as opposite to some fungal biocontrol microorganisms that also use fungal cell wall hydrolyzing enzymes within their biocontrol mechanisms[29].

Table 1: Effect of antibiosis of *Pseudomonas fluorescens* isolates in inhibition of mycelia growth of *Rhizoctonia solani in vitro*

	Pseudomonas fluorescens isolates							
Antibiosis (Inhibition (%))	B_4	B_6	B_{17}	B_{18}	B_{22}	B_{24}	B_{41}	B_{42}
Daul culture	43.5c	44.6c	51.3b	50.6b	56.3b	52.2a	57.6a	51.5b
Volatile antibiotics simultaneously	55.2c	54.4c	55.7c	54.8c	61.3b	54.3c	66.3a	56.3c
Volatile antibiotics 72 h	59.4c	55.9c	59.3c	59.2c	65.2b	58.5c	72.7a	56.6c
Antibiotics	65.2c	67.2c	64.1c	66.9c	78.5b	68.7c	85.8a	65.7c
Secretion of extra-cellular (25% v/v)	76.7c	73.7c	74.3c	74.7c	80.9b	75.2c	87.6a	73.5c
Sclerotia lysis	44.7c	43.7c	45.5c	44.3c	55.3b	45.3c	66.7a	46.5c
Germination of sclerotia (KB)	41.7c	42.7c	44.7c	45.7c	52.7b	43.3c	69.3a	44.3c
Germination of sclerotia (soil)	43.7c	42.3c	43.3c	43.3c	53.7c	43.3c	68.5a	45.6c

Table 2: Effect of different methods of treatment by *Pseudomonas fluorescens* isolates and benlate in control of rice sheath blight in greenhouse conditions

	Disease intensity (%)		
Antagonistics isolates and benlate	Methods of application		
	Seed coating	Soil drenching	Foliar spray
B_4	16.5b	17.25b	26.50b
B_6	15.5b	18.75b	26.25b
B_{17}	14.0c	15.00c	22.75c
B_{18}	14.5c	16.00c	23.25c
B_{22}	10.5d	10.50d	20.00d
B_{24}	13.0c	14.75c	23.00c
B_{41}	10.5d	11.75d	18.75d
B_{42}	13.5c	14.50c	21.25c
$B_{41}+B_{22}$	9.0e	11.25e	16.50e
Benlate	9.0e	10.00e	15.50e
Benlate+$B_{41}+B_{22}$	7.0f	8.00f	13.00f
Control	65.0a	65.00a	65.00a

Table 3: Effect of different methods of treatment by *Pseudomonas fluorescens* isolates and benlate in control of rice sheath blight in field conditions

	Disease intensity (%)		
Antagonistics isolates and benlate	Methods of application		
	Seed coating	Foliar spray	Seed coating+Foliar spray
B_{41}	28d	32d	22d
B_{22}	29d	30d	23d
$B_{41}+B_{22}$	23c	26c	17c
Benlate	21c	25c	16c
Benlate+B_{41}	16b	18b	12b
Benlate+B_{22}	17b	19b	13b
Benlate+$B_{41}+B_{22}$	11a	13a	9a
Control	52e	52e	52e

Means followed by a common letter in a columne are not significantly different according to Duncan's multiple range test (p=0.05)

Siderophore: The eight antagonistic bacteria showed similar behavior on *R. solani* growth at any of the Fe^{+3} concentration tested. Among them, *P. flourescens* B_{41} was found most effective which had 45% inhibition of growth *R. solani* at 5 µMol concentrations of $FeCl_3$.

Lysis and inhibition of germination of sclerotia: All antagonistics isolates were seen there are significant difference (p=0.05). *P. fluorescens* B_{41}, sclerotia lysis, inhibition of germination in KB and soil with 66.7, 69.3 and 68.5%, respectively were the most inhibited sclerotia of *R. solani* (Table 1).

Colonization of rice roots by antagonistic bacteria: During the 4 weeks of growth in sterile soil in the tube, there are significant difference were seen between the numbers of bacteria (CFU g^{-1} of roots)on rice roots for all isolates. The counts of each isolate were 5.4-5.5 log CFU g^{-1} of roots 1 day after inoculation and they were increased at the level after 45 days 3-5 x 10^8 CFU g^{-1} of roots. During further growth in non- sterile soil at pots in the greenhouse, they were still at the same level 8.15-8.55 log CFU g^{-1} of roots 120 days after inoculation.

Greenhouse and field conditions: In greenhouse conditions, statistical analysis of data on the Highest Relative Lesion Height (HRLH) cause by *R. solani*, indicated that there existed significant differences between seed coating, soil drenching and spray foliar. All of the isolates in seed coating are more effective. Among these eight isolates, *P. flourescens* B_{41} was the most effective for control of rice sheath blight. The disease intensity in B_{41} isolate for seed coating, soil drenching and seed coating + foliar spray were 10.5, 11.75 and 18.75%, respectively, while the control plants showed 65% disease intensity (Table 2). The results of used the mixture of isolates (B_{41} and B_{22}) showed that there existed significant differences between application isolates antagonistics with were used these isolates alone. Maximum control were obtained when B_{41} and B_{22} suspensions were added benlate in treatment seed coating (Table 2).

In the field conditions, *P. flourescens* B_{41} and B_{22} isolates were used. The disease intensity in B_{41} isolate for seed coating, soil drenching and seed coating + foliar spray were 28, 32 and 22%, respectively, while the control plants showed 52% disease intensity (Table 3). During the 120 days of growth rice in the field, there are no significant difference were seen between the B_{41} and B_{22} on disease intensity for all treatments, while when the mixture of these isolates were applied, there are significant difference were seen between application of isolates antagonistics with were used these isolates alone, also there are no significant difference between application of mixture these isolates and benlate in all treatments. Maximum control in field with disease intensity 9 was obtained when B_{41} and B_{22} suspensions were added benlate in treatment seed coating + foliar spray (Table 3).

DISCUSSION

In recent years, fluorescent pseudomonads have drawn attention worldwide because production of secondary metabolites such as siderophore, antibiotics volatile compounds HCN, enzymes and phytohormones.

Although isolates of *P. fluorescens* could be obtained from the rhizosphere of different rice paddy, antagonistic potential of these isolates appears to vary a great deal[30,51]. The ability of *P. fluorescens* isolates to serve as biocontrol agent of sheath blight is described here. The results of dual culture studies showed that *P. fluorescens* isolates were inhibited the growth of *R. solani* on plates. Members of the genus *Pseudomonas* spp. are well known antagonistic fungi[31]. They are known to produced volatile compounds such as hydrogen cyanide[30].

Iron is a fundamental element for resprition of several aerobic and facultative microorganisms and therefore, its availability in soil is essential[32]. On the other hand, siderophores are low weight compounds with high affinity for Fe^{+3}[33], which are produced under limiting concentration of iron. These compounds are able to transport this element inside the cell for metabolic functions[34] and microorganism which are able to produce sidrophores show competitive advantage as compared to those that do not produce them. From this pint of view, the competence for iron increases in conditions where this element is limiting, but this condition is reverted when iron is added to the culture medium[35]. The results of this study were similar to results of Montealegro[32]. If it is considered that iron available in soil fluctuate 1.8 and 27 ppm, it may be concluded that this element is not limiting for the antagonistic activity of these bacteria on *R. solani*.

Root exudates are believed to determined which microorganisms colonized roots in the rhizosphere[36]. The use of bactrise to exert an appropriate biological control of *R. solani* and of other soil borne fungi relies on their ability to colonize roots efficiently, otherwise, their biocontrol character would be non-sense. The ability to colonize rice roots is variable between rhizobacteria, being this characteristic a reflection for their ability to compte for ecological niches in the rhizosphere[37].

P. fluorescens isolates effectively controlled rice sheath blight when it was applied to seed or soil or foliar spray. These results showed that *P. fluorescens* isolates were more effective as a seed coating compared to foliar spray and soil drenching with bacterial suspension. Most biocontrol trials have dealt with use of *P. fluorescens* B_{41} against rice sheath blight. In greenhouse and field conditions, combination of B_{41} and B_{22} were comparable to benlate application.

P. fluorescens effectively controlled rice sheath blight when it was applied to seed coating. The bacteria appeared to move epiphytically from seed to roots, stems and leaves. It has been shown in other studies that fluorescent pseudomonads could be isolated from aerial parts of plants grown from seeds treated with the bacteria[18,38,39]. Fluorescent pseudomonads have also been detected in substomatal cavities of leaves[40,41]. The epiphytic bacteria could have controlled *R. solani*. Induction of disease resistance against foliar diseases by soil inoculum of fluorescent pseudomonads has also been widely reported[42-45]. Control of diseases with fluorescent pseudomonads applied to the foliage has been reported[15,19,40,46-49]. Survival of *Pseudomonas fluorescens* in the phyllosphere[46] may explain the effectiveness of foliar sprays. Foliar spray with under field conditions, primary inoculum of *R. solani* is from soil and irrigation water[4]. A combined application of bacteria suspension isolates were added with benlate for seed coating+foliar spray was the most effective method for control of rice disease in the field. Possibly both rhizosphere and phyllosphere population of *P. fluorescens* helped to control disease. Both direct inhibition of the pathogen and systemically induced resistance in the rice plants could be involved in control[50]. The results of field trial in the current study indicate the potential usefulness of seed coating with *P. fluorescens* isolates suspension when added benlate in controlling sheath blight of rice.

From all these results it may be concluded that the biocontrol effect of antagonistic bacteria isolated from rhizosplane of healthy and rice sheath blight disease against *R. solani* are adequate for their use at the rice field in different areas of Iran. The low disease intensity of sheath blight of rice with B_{41} isolate suggests that the antibiosis metabolites are conductive for rapid inhibition agent, *R. solani*. The multiple activity may be useful under natural conditions in which the same crop suffer from disease other than sheath blight.

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