American-Eurasian J. Agric. & Environ. Sci., 10 (1): 84-88, 2011
ISSN 1818-6769

The Role of *Pseudomonas syringae* as a Bio Control of *Echinocloa*

[1]*Abbas Akhavan Sepahy,* [2]*Mohaddeseh Larypoor and* [1]*Neda Jafarian*

[1]Department of Microbiology, Islamic Azad University, North Branch, Tehran, Iran
[2]Department ofMedical and Veterinary Mycology, Faculty of Veterinary Sciences,
Islamic Azad University, Science and Research Branch, Tehran, Iran

Abstract: *Pseudomonas syringae* is a Gram-negative, rod, aerobic and phytopathogenic bacteria. This study has described the effect of *P. syringae* on the *Echinocloa cruss-galli* as a weed, the main weed on rice fields. For this reason the microbial suspension of *Echinocloa cruss-galli* was applied in different concentration on seeds, stems, leaves and roots of weed. When this experiment was repeated, the microbial suspension had not any effect on the seeds, stems and leaves of weed. Whereas after addition of this suspension to sterile soil and cultivation of the plants in this soil, weeds have dried after 48-72 hours. The experiment was repeated on rice and this suspension had not any effect on rice.

Key words: Pseudomonas syingae · Echinocloa cruss-galli · Weed

INTRODUCTION

Pseudomonas genus is a complex consisting of gram-negative, aerobic and non fermentative bacilli which live in soil and water. These bacteria spread widely in their natural habitat and have a prominent role in the degradation of organic matter. Several species of the subgroup of this genus are pathogenic to animals and plants [1, 2].

Pseudomonas syringae is a kind of gram-negative, aerobic and short rod bacterium, with the optimum growth temperature of about 24°C. These bacteria are known as the cause of pathogenesis in plant [3].

Weeds are kinds of plants which have been adapted to man-made habitat and are generally considered as harmless wild plants in their geographical origin. Weeds have spread unwontedly through the activities of human beings and domestic animals, so that they have became native undesirably. Most of weeds are herbaceous, but some shrubs and trees such as Prosopis, Acacia, Dew berry (rubus) and Cactuses like *Opuntia spp.*, are extremely dangerous weeds in some places [1].

Echinocloa cruss-galli is an annual plant from cereal family, which is one of the most problematic weeds in the word tropical and temperate areas. This kind of weed is a 4 carbon plant, thus has a rapid growth and tillering. It germinates in the seed plot sooner than rice and during the direct planting of rice and generates many new leaves, tarsuses and adventitious roots during the first three weeks and finally dominates the rice habitat.

This kind of weed consumes a considerable amount of soil nutrients and competes with the major product for water and sunlight. It also produces a lot of seeds which spread widely and are effective in the resettlement of the weed. Echinocloa cruss-galli is similar to rice in the seed plot and it is difficult to distinguish it as it requires experience and carefulness. The leaves of this kind of weed are pale green in color and its major leaf vein is paler in color than the major leaf vein of rice and is whiter, so called. There are two blades in the joint between leaves and sheath, named stipule and ligula, whereas the mentioned weed does not have one or both of the blades [4-6].

Biological control is defined as applying a pathogenic agent or an insect in order to decrease the loss due to a kind of noxious agent. This method is especially appropriate for places such as greenhouses and large artificial areas. The goal of biological control is not the extirpation of noxious agents which cause damage to plants, but is to reduce their population to a level that cause the least detriment to the farmer or the environment. In this method, in fact, the noxious agents are controlled via the environmental interaction among the living creatures. In the natural environment, where human being puts the least interference to ecosystem, noxious agents and plant disease always exist, but if the direct

Corresponding Author: Mohaddeseh Larypoor, Department ofMedical and Veterinary Mycology, Faculty of Veterinary Sciences, Islamic Azad University, Science and Research Branch, Tehran, Iran. E-mail: mlarypoor@yahoo.com.

interference of human being does not exist, their population is always in equilibrium and in a normal level.

Biological control of weeds is one of the methods for controlling them, which is performed through exposing weeds to their natural enemies. Many of the weeds present in our surrounding environment, have been the native of other regions and transferred to other areas by seed, growth organ, wind, water and insect. Unfortunately, natural enemies of weeds are not transferred to new regions with them and therefore weeds are able to grow easily and expose the native and desirable plant life to danger.

Biological control suggests methods to set natural balance between the weeds and their surrounding environment and this process is done through introducing the insect and disease which attack the noxious plants.

One method is applying a natural enemy, such as herbicides, which is in direct contact with the weed, like plant pathogenic agents. This method is mostly used on farms and to control cereal weeds [7- 9].

The goal of this study is to perform this process and to study the effect of Pseudomonas syringae on Echinocloa cruss-galli weed and whether these bacteria could be used to control and destroy the mentioned weed; considering the fact that these bacteria have been isolated from rice field soil and if they have an appropriate effect on weed (destroying it), then the use of chemical poisons against this kind of weed could be reduced and spread of the weed on rice fields and the reduction of rice quality are prevented.

MATERIALS AND METHODS

Sampling: Samples of rice field soil and weed and seeds rice were collected from Khoozestan (Ramhormoz, Baghmalek), Gilan and Esfahan (Falavarjan, Mobarakeh, Lenjan) provinces.

Isolation and Characterizing the Bacterium: Pour plate technique was applied in order to isolation Pseudomonas syringae bacteria from the collected soil samples. In this process the dilution series was prepared from the soil samples and 1 ml of the existing dilutions was added to the sterile plate, then the sterile specific culture medium of Pseudomonas syringae which had been cooled for about 45-50°C was added to the sterile plates containing 1 ml of the sample.

The cultivated plates were incubated for 24 hrs at 25-28°C and in aerobic conditions. After this period, If a yellow colonies appeared on the plate surface, then lams were prepared from these colonies. Finally, the necessary biochemical tests were conducted in order to characterize the bacterium and the bacterium was purified [10, 11].

For more characterizing bacterium, molecular technique and PCR were used. Then the bacterium genome was sequenced and comparisoned with the standard bacterium.

Preparing Microbial Suspension: In this stage, the bacterium was inoculated in to Nutrient Broth (NB) culture medium and then was aerated in an incubator equipped with a shaker for 6 days. After this period, the obtained microbial suspension was centrifuged and the top liquid was isolated and selected and in order to repeat the experiment, the microbial suspension was used at different rotations of the centrifuge [12].

Planting the Seeds (Of Weed and Rice): In order to plant the seeds of weed and rice, the seeds were first kept in the fridge for a week and then were placed in a plate containing a filter paper. For doing this process, the seeds were first kept in 70% alcohol and then were dealcoholized with sterile distilled water and after that the seeds were kept in 0.4% Javelle water and rinsed with sterile distilled water, again and finally placed in the sterile plate.

Inoculating the Microbial Suspension on to the Seeds (Of Weed and Rice): In order to inoculate the microbial suspension onto the weed and rice seeds, the microbial suspension prepared at different rotations of the centrifuge, was used. Whit this aim, in the seed planting stage (we used of 5 seeds in each plate), after rinsing the seeds with Javelle water and distilled water, they were kept in the microbial suspension prepared at different centrifuge rotations, various time and different amounts (for example 1 ml of one centrifuged microbial suspension for one second; and the same amount whit different centrifuge rotation and various time, the same centrifuge rotation whit different a month and various time and the same time whit different centrifuge rotation and different amount) and then rinsed with sterile distilled water, added to the sterile plate and were kept at the conditions similar to the seed planting [10, 11].

Inoculating the Microbial Suspension onto the Plant Leaves and Stems (Weed and Rice): In this stage, after that the planted seeds germinated and their leaves and stems appeared, the microbial suspension prepared at

different centrifuge rotations, various times and with different amounts (for example 1 ml of one centrifuged microbial suspension for one second; and the same amount whit different centrifuge rotation and various time, the same centrifuge rotation whit different a month and various time and the same time whit different centrifuge rotation and different amount), was applied to the leaves and stems. For doing this process, a series of leaves and stems, after making injuries on them (with a sterile needle) and a series of the samples, without making injuries, were kept under the microbial treatment. Then the treatment leaves and stems were planted in sterile soils and kept in greenhouse conditions [2, 12].

Inoculating the Microbial Suspension onto the Plant Roots (Weed and Rice): In order to perform this stage, the soil was first sterilized and autoclaved, then the microbial suspension (prepared at different centrifuge rotations and various amounts for example 1 ml of one centrifuged microbial suspension for one second; and the same amount whit different centrifuge rotation and various time, the same centrifuge rotation whit different a month and various time and the same time whit different centrifuge rotation and different amount) was added to the sterilized soils in the plant pots and mixed thoroughly with the soils.

After that, the plants (weed and rice) which had grown to the height about 15-20 centimeter, were cultivated in the plant pots and kept in greenhouse conditions.Each of the above experiments was performed several times and each time on several samples.

Preparing Blank Samples from Weed and Rice: In order to prepare blank samples from weed and rice, the seeds of them were first planted in Petri dishes and transferred, after germinating, to plant pots containing the sterile soil, which no microbial suspension had been added to them [7, 9, 10].

All the above stage were also performed, using standard bacteria (Pseudomonas syringae) and the obtained results were then compared.

RESULTS

Isolating and Characterizing the Bacterium: In this stage, lames were prepared from the yellow colonies made in the specific culture medium and the gram-negative bacilli were observed. The results obtained from doing biochemical tests and the characterization of the purified samples, are as the following Table 1:

Table 1: The results obtained from biochemical tests

Oxidase	+	OF	+	Casein hydrolysis	+
Catalase	+	MR	-	Citrate	-
INM	+	VP	-	Proline hydrolysis	-
Starch hydrolysis	-	SIM	-		

After sequencing of bacterium, a difference sequence of isolated bacterium in some nucleotide than the standard bacterium was observed and this bacterium is similar to standard bacterium with 98 percent similarity.

The Genome of Standard Bacterium:
GCAACCGCTCGACACTGGTCACCAGCCAGATGCCC
GGTGGACAAATGGACAAATGGCATGCGCTAATCG
GCGATCCCACCTTGGGCGACGCGATCCTCGACCGGC
TGGTGCACAACGCTTATCGGATCGAACTGAAGGGC
GATCGAACTGAAGGGCGATCGAGTCGATGCCGCAG
ACGCGCAACGAAAAATTGACGACGGCAGGGACTT
CAGACTAATGCGAACCTGCGTCGCTGCTGCGCTCCG
ACTGCCTGTCCGGATGATCGTGGAACGGGTGTCCG
GATGTTGGTGGGCTGAGTGTCCAGATGGCGTGGAAT
CCGCACACCATGAACGGAACTGCAAAAGCAAGG
TAGCCCTCCTGCGTCCAGGTTGACCGGTAATAGGG
TCGGCTGATTCGTTCGCTCGTTGCATGGGCACTTTA
CTTTTGCGTGCTGTTAAATGTGTTCGTTATGGTTTG
GGTCATGGCCAGTGAGCGGGTTCGCACTCAATGGT
ATCGGCGGTGAGTTTTGCTCTGTATCAGGTGGCAAG
GCCCCGATCGCCAGTGCGGATTTCTCTHACCAHTC
HCCCAHCGGTCGTTCGCTCGTTGCATGGGCACTTAC
TTTTGCCTGCTGTTAAATGTGTTCGCTTGGTTTGGG
TCATGGCCAGATGAGCGGGTTCGCACTCAATGGTA
TCGGCGGTGATTTTGCTCTGTATCAGGTGGCAAGGC
CCCGATCGCCAGTGCGGATTTCTCTGACCAGTCGCC
CAGCGGTTGGGCCGGTAGTCTTGTTCTGCCCTATCT
GTTCTGCTGTGACATTAAGCATCGTTTTCTGCTGCT
GGCCTGCCTGATCGGCTGTTGCGAATCGTCCAGAAC
CACGCCTTGGTCGTGAGCTTCGCTGCAAGGCACCCC
CTCGATATCTCTTGGCCTCGGCCAACCCTGGCGCTT
CTCCCCTGAT.

The Genome of Isolated Bacterium:
GCAACCGCTCGACACTGGTCACCAGCCAGATGCCC
GGTGGACAAATGGACAAATGGCATGCGCTAATCGG
CGATCCCACCTTGGGCGACGCGATCCTCGACCGGCT
GGTGCACAACGCTTATCGGATCGAACTGAAGGGCG
ATCGAACTGAAGGGCGATCGAGTCGATGCCGCAGA
CGCGCAACGAAAAATTGACGACGGCAGGGACTTCA
GAGTAATGCGAACCTGCGTCGCTGCTGCGCTCCGAC
TGCCTGTCCGGATGATCGTTTAACGGGTGTCCGGAT
GTTGGTGGGCTGAGTGTCCAGATGGCGTGGAATCCGC

ACACCATGAACGGAACTGCAAAAGCAAGGTAGCC
CTCCTGCGTCCAGGTTGACCGGTAATAGGGTCGGCT
GATTCGTTCGCTCGTTGCATGGGCACTTTACTTTTG
CGTGCTGTTAAATGTGTTCGTTATGGTTTGGGTCAT
GGCCAGTGAGCGGGTTCGCACTCAATGGTATCGGCG
GTGAGTTTTGCTCTGTATCAGGTGGCAAGGCCCCGA
TCGCCAGTCCCGATTTCTCTHACCAHTCHCCCAHC
GGTCGTTCGCTCGTTGCATGGGCACTTACTTTTGCC
TGCTGTTAAATGTGTTCGCTTGGTTTGGGTCATGGC
CAGATGAGCGGGTTCGCACTCAATGGTATCGGCGG
TGATTTTGCTCTGTATCAGGTGGCAAGGCCCCGATC
GCCAGTGCGGATTTCTCTGAGGAGTCGCCCAGCGGT
TGGGCCGGTAGTCTTGTTCTGCCCTATCTGTTCTGCT
GTGACATTAAGCATCGTTTTCTGCTGCTGGCCTGCC
TGATCGGCTGTTGCGAATCGTCCAGAACCACGCCT
TGGTCGTGTCGTCGCTGCAAGGCACCCCCTCGATAT
CTCTTGGCCTCGGCCAACCCTGGCGCTTCTCCCCTG
AT.

Investigating the Effect of the Microbial Suspension on the Plant Seeds and Their Germination: It was observed after washing the seeds (of weed and rice) with the microbial suspension, that this suspension has no effect on the seeds and the germination stage and that the seeds washed with this microbial suspension, germinate and grow completely.

Investigation the Effect of Microbial Suspension on the Plant Leaves and Stems: After treating the leaves and stems (of weed and rice) with the microbial suspension, it was observed that this suspension has no effect on the plant leaves and stems.

Investigation the Effect of Microbial Suspension on the Plant Roots: It was observed after adding the microbial suspension to the sterile soil and planting the weed in the soil, that the plant start to dry after about 48 hrs.

Whereas, the planted rice in this soil, is not damaged and continues to grow completely.

DISCUTION

It should be noted that the weeds which dried due to their normal condition, whereas a series of the blank weeds which were dried intendedly, returned again to the normal condition by irrigation, after a while.

This history of weed control is as long as the history of cultivating farms plants. Human being has learned to apply tools, tractor power and chemicals in order to control weeds and fight against them.

Using chemical herbicides to control weeds leads to the reduce of soil quality and consequently a decrease in the yield of agricultural products, on the other hand, improving the resistance against chemicals, has resulted in increasing the amount and the dosage of these compounds used, which is an important issue from the economical aspect. The use of biological herbicides, especially microbial herbicides, to control weeds has been very effective; meanwhile these compounds are environmentally friendly and no specific equipment is required for their production. Echinocloa cruss-galli is a type of annual weed with creeping nature, whose height sometimes more than 90 centimeter and is young plant resemble rice, therefore hand weeding of it in the initial stage of growth is difficult.On the other hand, as the use of chemical toxin and herbicides in order to fight against this kind of weed, has deteriorated the quality of rice product, applying microbial herbicides which somehow have specific function, is very important. The result obtained from this research are somehow in accordance with the above items, from the aspect of the bacterium effect on the plants.

Vassilev [8]. isolated Pseudomonas syringae from wheat and barley, in 1996.

Gross and DeVay [9]. Isolated some strains of the mentioned bacterium from pear, peach, millet and citrus in 1997.

Strobel, Garry [1] isolated this bacterium from the sick plant tissues in 2005.

Tomihama T. [13] isolated the noted bacterium from the contaminated tissues of tea in 2008.

The pathogenic effect of this bacterium on plants was studied in all cases, which was in accordance with the obtained result from this test and the effect of the isolated bacterium from soil in this study research.

In addition, Vassilev [8] stated that this kind of bacterium creates necrotic symptoms in the wheat seedling but there are differences in the intensity and the time of sign appearance following the inoculation of the bacterium.

Ninak Zidack [13] demonstrated that this bacterium is responsible for burning and local death of bean and in these conditions, the bacterium contaminates the plant root and once.

In 2002, Grünwald [14] studied the effect of the mentioned bacterium, as a biocontrol substance, on Camel thorn and found that applying this bacterium for 4-5 weeks on the plant, reduces the dry weight of the germ up to 52%.

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