Evaluation of antagonistic microbes against peduncle blight caused by *Lasiodiplodia theobromae* (PAT.) Griffon & Maubl in tuberose

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ABSTRACT

The effects of eight native bacterial isolates were tested *in vitro* for their ability to inhibit the growth of *Lasiodiplodia theobromae*, the causal agent of peduncle blight of tuberose. The studies revealed that *Pseudomonas fluorescens* (PFC-I_8) and *Bacillus subtilis* (BSC-I_1) showed the highest inhibition of mycelial growth of (70.60%; 66.41%, respectively) *L. theobromae*. Both the antagonists were compatible with each other and they were tested alone and together *in vivo* for the control of *L. theobromae*. The combined application of *P. fluorescens* + *B. subtilis* (T_6) through bulb treatment followed by soil and foliar application recorded minimum incidence of peduncle blight and maximum plant growth and flower yield.

Keywords: Tuberose, Peduncle blight, *Lasiodiplodia theobromae*, Bacterial biocontrol agents, Disease management.

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INTRODUCTION

Tuberose (*Polianthes tuberosa* L.) is one of the most important tropical ornamental bulbous flowering plant cultivated for production of long lasting flower spikes. It is popularly known as Rajanigandha or Nishigandha which means night fragrant. It belongs to the family Amaryllidaceae and is native of Mexico. Tuberose is an important commercial cut as well as loose flower crop due to pleasant fragrance, longer vase-life of spikes, higher returns and wide adaptability to varied climate and soil. They are valued much by the aesthetic world for their beauty and fragrance (Biswas *et al*., 2002). Tuberose is grown commercially in a number of countries including China, Egypt, France, Hawaii, India, Italy, Kenya, Mexico, Morocco, North Carolina, South Africa, Taiwan, USA and many other tropical and sub-tropical areas in the world (Khan and Pal 2001). In India, commercial cultivation of tuberose is popular in Krishnanagar of West Bengal, Karnataka, Maharashtra, Pune, Punjab, Rajasthan and Uttar Pradesh, Coimbatore and Madurai districts of Tamil Nadu. The total area under tuberose cultivation in the country is about 7.95 lakh ha. The production of loose and cut flower is estimated to be 27.71 000 MT and 1560.70 lakh No's respectively (Anonymous, 2015).

The major constraints in the production of tuberose are the diseases *viz*., tuber rot incited by *Fusarium oxysporum* (Muthukumar *et al.,* 2005); leaf spot caused by *Alternaria polyanthi* (Muthukumar *et al*., 2007); collar rot caused by *Sclerotium rolfsii* (Behera *et al*., 2015) and peduncle blight, blossom blight caused by *Lasiodiplodia theobromae* (Durgadevi and Sankaralingam, 2012) are the other diseases recorded in tuberose. The only viral disease reported so far is mosaic caused by *Tuberose mild mosaic poty virus* (Raj *et al*., 2009). Among various diseases infecting tuberose, peduncle blight caused by *L. theobromae* is a serious problem in tuberose

growing areas of Tamil Nadu and causing considerable economic loss to the farmers. The symptoms included blighting of flowers followed by die-back of peduncle from tip to downwards. Several pycnidia were observed over the infected flowers and spike.

Lasiodiplodia theobromae is a plant pathogen with wide host range. It is a facultative pathogen which grows and reproduces on dead and senescing plant tissues and plant debris but can also rapidly invade healthy or wounded plant tissue when environmental conditions are favourable (Punja and Utkhede, 2003). The pathogen thrives under high humid conditions and cools to moderate temperature; conidia require free water to germinate and infect plant tissues. Ornamental plants are under constant disease pressure from fungal pathogens which infect roots, stem, leaves, flowers and fruits (Punja and Utkhede, 2003). Plant health management is a major concern for ornamental growers since all parts of the plant-flowers, leaves, stem and roots-must be of high quality for sale on the market and the economic threshold for pest damage is very low (Daughtrey and Benson, 2005).

Control of these diseases is currently achieved through the use of chemical fungicides but there is increasing interest in utilizing alternative approaches such as biological control agents (Belanger, 2006). Due to the increasing concern about potentially harmful effects of chemical pesticides on agricultural land, water and soil pollution as well as other health problems have demanded that agricultural scientist pursue alternative controls that are more environmentally friendly, ecologically viable, medically safe and specific for controlling pathogens (EI-Kassas and Khairy, 2009). More attention has been given to using biological control agents to manage diseases of flower crops (Belanger, 2006; Preethi *et al*., 2016). Alternatively, antifungal agents produced by microorganisms may be used as biocontrol agent (Chitarra *et al*., 2003), as the materials based on micro organisms have properties such as: high specificity against target plant pathogens, easy degradability and low cost of mass production. Biological control offers an important alternative to synthetic chemicals. The successful application of antagonistic micro organisms (*Pseudomonas* and *Bacillus*) for the control of *L. theobromae* has been previously reported by several workers in various crops (Adeniyi *et al*., 2013; Kedar *et al*., 2014; EI-Banna *et al*., 2015).

The investigation of mechanisms of biological control by bacterial antagonists also revealed that biocontrol agents, which protect the plants from various pathogens in several crops, activate the defense-related enzymes including phenylalanine ammonia-lyase (PAL), peroxidase (PO), etc., which are involved in synthesis of phytoalexins (Van Peer and Schippers, 1992). Besides, early and increased expression of defense-related genes in induced systemic resistance (ISR) is very important in protecting the crops against several pathogens (Xue *et al*., 1998; Muthukumar *et al*., 2012; Muthukumar and Venkatesh, 2014). However, in the case of biological control of foliar diseases, knowledge of ISR for the management of peduncle blight of tuberose disease is lacking. The objectives of the present study are: (1) to evaluate the antagonistic activity of bacterial isolates against *L. theobromae* in *in vitro*, (2) to test the compatibility between effective bacterial antagonist, and (3) to study the role of effective antagonist in consortium for the control of peduncle blight of tuberose.

MATERIALS AND METHODS

Tuberose, variety Pajwal single bud was obtained from farmer's field at Karmangudi village belongs to Cuddalore district, Tamil Nadu, India, and used for the greenhouse experiments in the entire period of investigation.

Isolation, maintenance and identification of pathogen

The peduncle blight symptoms were collected from the farmer's field of Karmangudi village of Cuddalore District. They were washed, cut into 5 mm segments including the advancing margins of infection. The segments were surface-sterilized in 0.5 % sodium

hypochlorite solution for 5 min and rinsed in three changes of sterile distilled water. The segments were separately dried in between sheets of sterile filter paper and placed (three pieces per plate) on fresh potato dextrose agar (PDA) medium (Ainsworth, 1961) impregnated with streptomycin, and incubated for 7 days at 28 ± 2 ℃.

The emerging colonies were sub cultured onto PDA plates. Single spore isolation of the fungus or single hyphal tip method was followed for making the pure cultures and maintained on PDA slants (Aneja, 2003). The culture thus obtained was stored in refrigerator at 5 ℃ for further studies and was sub cultured periodically. They were identified based on morphological and colony characteristics (Goos *et al*., 1961; Punithalingam, 1976) and further (ID.NO. 3/426/2016/766) confirmed by National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute, Pune.

Isolation and identification of bacteria

Soil samples were collected from major tuberose (rhizosphere) growing areas of Tamil Nadu (Coimbatore, Dharmapuri, Dindigul, Erode, Krishnagiri, Madurai, Namakkal, and Cuddalore). After removing the loosely adhering soil from freshly excised roots, root segments (1 g) were taken and isolation was done as per the method described by Elad and Chet (1983) using King's B medium (King *et al.,* 1954). Totally eight bacterial isolates were obtained. These isolates were identified according to Bergey's Manual of Systematic Bacteriology.

Screening of bacterial isolates against *Lasiodiplodia theobromae*

The antagonistic activity of bacterial isolates against *L. theobromae* was tested by dual culture technique (Dennis and Webster, 1971) using PDA medium. Each treatment was replicated four times with five plates per replication and experiment was repeated four times. The plates were incubated at room temperature (28 ± 2 ℃) for 48 h. The radial growth (in mm) of the pathogen was measured after incubation. The per cent inhibition of mycelial growth was also calculated. Based on the dual culture technique the most effective isolate of *P. fluorescens* (PFC) and *B. subtilis* (BSC) was used for subsequent studies.

Bioformulations of PFC and BSC

The talc-based formulation of PFC and BSC was prepared by following the method described by Papavizas *et al*. (1984), Vidhyasekaran and Muthamilan (1995). The bacterial strain was grown separately in conical flasks (250 mL) containing 100 mL of King's broth (KB) and Nutrient broth and kept for 48, 72 h on a rotary shaker (150 rpm) at (28 ± 2 ℃). Bacteria were subsequently pelleted by centrifugation at 8,000 ×g for 10 min at 4 ℃. The pellets were washed with sterile distilled water three times, and the concentration of cells adjusted to 3×10^{8} cfu mL^{-1} by dilution to give the suspensions an optical density of 0.45 (A610 nm) using UV-visible spectrophotometer (Mortensen, 1992). Ten grams of carboxy methyl cellulose (CMC) was added to 1 kg of talc and mixed well and the mixture was autoclaved for 30 min on each of two consecutive days. For PFM and BSC 100 mL of 48 h grown bacterial suspension containing 3×10^{8} cfu mL^{-1} was mixed with carrier mixture under aseptic conditions. The formulations thus prepared were allowed to dry aseptically (approximately 35 % moisture content) and were then ground to powder. They were then packed in sterile polythene bags and stored at 4 ℃.

Compatibility bioassay

The compatibility of the two antagonistic organisms was tested *in vitro* through two methods. The mutual compatibility of two antagonistic organisms was tested by dual culture method, and the plates were assessed for inhibition zone after 48 h. In another method *P. fluorescens* culture was streaked on King's B medium. After 2 days, the *B. subtilis* suspension was sprayed over the *P. fluorescens* colonies. Similarly, the *P. fluorescens* suspension was sprayed over *B. subtilis* colonies and the plates were assessed for the inhibition zone after 48 h (Bharathi *et al*., 2004).

Evaluation of biocontrol agents for their efficacy against peduncle blight of tuberose on plant growth and flower yield under glasshouse condition

A pot culture experiment was conducted using two selected bio-control agents *viz., P. fluorescens* (PFC) and *B. subtilis* (BSC) and their combinations along with fungicide namely carbendazim was laid out with 9 treatments replicated three times in completely randomized design. A single bulb of tuberose was planted in each pot containing sterile potting medium (red soil: sand: FYM @ 1:1:1 w/w/w). The method of application included bulb treatment (BT), soil application (SA), soil drenching (SD) and foliar application (FA). Talc based bioformulation of *P. fluorescens* (PFC) and *B. subtilis* (BSC) were applied as bulb treatment @ 11 g per kg of bulb before planting. Soil application with talc based bioformulation of *P. fluorescens* (PFC) and *B. subtilis* (BSC) were applied @ 2.5 kg/ha on 60, 90 and 110 DAS and foliar application was given @0.2 per cent on 60, 90 and 110 DAS. The fungicide carbendazim was applied as bulb treatment @ 0.1 per cent, soil drenching and foliar application (SD; FA) @ 0.1 per cent on 60, 90 and 110 DAS (days after sowing). *L. theobromae* was inoculated on the peduncle region in all the treated plants on 120 DAS. Plants inoculated with the pathogen alone served as control. Healthy controls were also maintained. Disease incidence was recorded on 120 DAI (days after inoculation). The plant height was recorded on 120 days after sowing and yield parameters such as spike length, number of flowers/spike, weight of 10 flowers and flower yield was also recorded at that time of harvest. The per cent disease incidence was calculated as follows

$$\text{Disease incidence (\%)} = \frac{\text{Number of flower buds affected}}{\text{Total Number of flower buds / peduncle}} \times 10$$

The treatment details are furnished below:

T_1	*P. fluorescens* (PFC)	-BT+SA+FA
T_2	*B. subtilis* (BSC)	-BT+SA+FA
T_3	*P. fluorescens* (PFC) + *B. subtilis* (BSC)	-BT
T_4	*P. fluorescens* (PFC) + *B. subtilis* (BSC)	-SA
T_5	*P. fluorescens* (PFC) + *B. subtilis* (BSC)	-FA
T_6	*P. fluorescens* (PFC) + *B. subtilis* (BSC)	-BT+SA+FA
T_7	Carbendazim	-BT+SD+FA
T_8	Pathogen inoculated Control	-
T_9	Healthy control	-

Experimental design and statistical analysis

All the experiments were carried out in a CRD. For the data on the effect of biocontrol agents on mycelial growth, percent reduction over control was calculated. The data on disease incidence were arcsine transformed before undergoing statistical analysis. The data were analyzed using the IRRISTAT version 92-1 program developed by the biometrics unit, International Rice Research Institute, Metro Manila, The Philippines. Data were subjected to analysis of variance (ANOVA). The treatment mean values were compared by Duncan's multiple range test (DMRT) at 5 % significance level (Gomez and Gomez, 1984).

RESULTS

The results presented in the table 1 revealed varying degree of antagonism by the bacterial isolates against *L. theobromae*. Among the isolates tested isolate-I_8 (PFC) recorded the maximum inhibition zone of 12.33 mm and a minimum of 26.30 mm mycelial growth of *L. theobromae* accounting for 70.66 per cent reduction in the mycelial growth over control. This was followed by isolate-I_1 (BSC) and I_3 (PFDC). The least growth inhibition was observed with the isolate-I_2 (PFD) and I_6 (PFK).

Table 1. *In vitro* inhibition of mycelial growth of *L. theobromae* by native bacterial isolates

I. No.	Location	Isolates	Mycelial growth (mm)	Per cent inhibition over control	Inhibition zone (mm)
I_1	Coimbatore	BSC	30.00 b	66.41	10.6 b
I_2	Dharmapuri	PFD	40.00 f	55.22	7.3 d
I_3	Dindigul	PFDC	32.00 c	64.17	9.3 c
I_4	Erode	PFE	35.66 e	60.10	9.0 c
I_5	Madurai	BSM	34.33 d	62.00	9.0 c
I_6	Krishnagiri	PFK	40.00 f	55.22	7.0 d
I_7	Namakkal	BSN	36.00 e	60.00	8.9 c
I_8	Cuddalore	PFC	26.33 a	70.60	12.3 a
	Control	-	89.33 g	-	-

BSC-*Ba. subtilis* (Coimbatore) PFD-*Ps. fluorescens* (Dharmapuri) PFK-*Ps. Luorescens* (Krishnagiri)
BSM-*Bacillus subtilis* (Madurai) PFE-*Ps. fluorescens* (Erode) PFD-*Ps. Fluorescens* (Dindigul)
BSN-*Ba. subtilis* (Namakkal) PFC-*Ps. fluorescens* (Cuddalore)

Mean of three replications; Values in each column followed by the same letter are not significantly different according to the DMRT method (P = 0.05)

Compatibility studies

The bacterial antagonists were tested for its compatibility. The results showed that there was no inhibition zone between *P. fluorescens* and *B. substilis* indicating the compatible nature of both the antagonists (data not shown).

Plant growth and peduncle blight

Studies on the effect of antagonists under glasshouse condition revealed that combined application of *P. fluorescens* + *B. subtilis* (T_6) through bulb treatment followed by soil and foliar application recorded minimum incidence of peduncle blight. This was on par with carbendazim which recorded 6.66% disease incidence after inoculation with pathogen. This was followed by foliar application with *P. fluorescens* + *B. subtilis* (T_5) which recorded 7.33% disease incidence (Table 2). The maximum disease incidence was observed in control.

Generally all the treatments with bioformulations showed increased plant height and flower yield when compared to control (Table 3). Of these, the treatment consisting of T_6-*P. fluorescens* + *B. subtilis* (BT+SA+FA) recorded maximum plant height at 120 days after sowing. This was on par with the treatment T_7 - Carbendazim which recorded a plant height of 58.00 cm. It was followed by the treatment T_5- *P. fluorescens* + *B. subtilis* (FA). In general all the treatments except control recorded lowest plant height.

Spike emergence and spike length

The number of days taken for spike emergence was recorded during the emergence of spike and the data on length of spike were recorded at the time of harvest under glasshouse condition (Table 3). In general all the treatments were significantly superior over untreated control which took 128 days for spike emergence and recorded spike length of 65.70 cm. However, early emergence of spike was seen in treatment T_6 - *P. fluorescens* + *B. subtilis* (BT+SA+FA) and which recorded spike length of 84.00 cm. This was followed by the treatment T_5- *P. fluorescens* + *B. subtilis* (FA).

Number of flowers and flower yield

The effect of consortium of bioagents on number of flowers per spike and yield of tuberose were recorded at the time of harvest under glasshouse condition and data presented in Table 3. In general maximum number of flowers which received through the treatment T_6 -*P. fluorescens* + *B. subtilis* (BT+SA+FA) followed by T_5. Weight of ten flowers was significantly higher in all the treatments compared to control. The maximum weight of ten flowers and highest flower yield (68.0 g/plant) were recorded in

Table 2. Effect of consortium of selected bio-control agents on peduncle blight of tuberose under glasshouse condition, Mean of three replications

Treatments	Incidence of peduncle blight (%)	Percent decrease over control
T_1- *P. fluorescens* (BT+SA+FA)	11.66 d	82.00
T_2- *B. subtilis* (BT+SA+FA)	12.33 e	81.00
T_3- *P. fluorescens* + *B. subtilis* (BT)	10.33 c	84.00
T_4- *P. fluorescens* + *B. subtilis* (SA)	9.00 b	86.00
T_5- *P. fluorescens* + *B. subtilis* (FA)	7.33 a	89.00
T_6- *P. fluorescens* + *B. subtilis* (BT+SA+FA)	6.00 a	91.00
T_7-Carbendazim (BT+SD+FA)	7.00 a	89.11
T_8-Healthy control	19.33 f	70.00
T_9-Inoculated control	64.33 g	-

Values in each column followed by the same letter are not significantly different according to the DMRT method ($P = 0.05$)

plants treated with consortium of bioagents (T_6) through bulb treatment plus soil application plus foliar application.

DISCUSSION

All the eight bacterial isolates showed varying degrees of antagonism to *L. theobromae*. Among these, PFC (I_8) recorded maximum inhibition zone with minimum mycelial growth of *L. theobromae.* Similarly, Govindaiah *et al*. (2003) and Sharma *et al*. (2009) reported that *P. fluorescens* (Pf-1) was found most effective bacterial bioagent which inhibited 84.8 percent growth of *B. theobromae* (stem end rot of citrus) followed by *B. subtilis* (64.05%). Kedar *et al*. (2014) reported that five known bio-agents tested by dual culture technique showed that *P. fluorescens* and *B. subtilis* were strong antagonism to *L. theobromae* (banana fruit rot) by inhibiting the mycelial growth up to 75.83 and 70.50%, respectively.

Production of siderophores and chitinases are two factors that may be involved in biological control activity. Indeed, it is known that chitinolytic activity and siderophore production are correlated with antifungal activity (Castoria *et al.,* 2001; Kamensky *et al.,* 2003; Quecine *et al.,* 2008). In addition, *P. fluorescens* is capable of solubilizing phosphate and producing IAA, characteristics that may enhance its potential use as an effective biological control agent to contribute to the control of *L. theobromae*. Mahesh (2007) suggested that fungal growth is mainly inhibited by HCN production and siderophore production. All these earlier results lend support to the present findings.

In addition to this, *Pseudomonas* spp. are well known for production of broad spectrum antibiotics such as phenazine by *Pseudomonas* sp. B-109 in tomato (Chin-A-Woeng *et al.,* 1998); 2, 4-diacetylphloroglucinol (2,4-DAPG) by *Pseudomonas* sp. 28r/-96 in wheat (Raaijmakers and Weller, 2001); Pyoluteorin by *P. fluorescens* CHAO in tobacco (Keel *et al.,* 1992); Pyrrolnitrin by *P. fluorescens* BL 915 in cotton (Ligon *et al*., 2000); Viscosinamide by *P. fluorescens* D1254 in sugar beet (Nielsen *et al*., 1998) and Zwittermycin A by *B. cereus* UW in alfalfa (Silo-Such *et al.,* 1994) which proved to be a major mechanism involved in their biocontrol activity. Moreover, Baker *et al.* (2003) reported that ability of some *Pseudomonas* spp. in producing siderophores, antibiotics and lipopolysaccharides as important factors in improving the effectiveness of the antagonist. All the above reports were in line with the present observations.

The compatibility study revealed that there was no inhibition zone between *P. fluorescens* and *B. subtilis* indicating the compatible nature of both the antagonists. Earlier studies also reported that the both bacteria are compatible and the combination was highly successful in controlling crop diseases (Thilakavathi *et al*., 2007; Salaheddin *et al*., 2010; Sivakumar *et al*., 2012; Sundaramoorthy and Balabaskar, 2013;

Table 3. Effect of consortium of selected bio-control agents on plant growth and flower yield of tuberose under glasshouse condition

Treatments	Plant height at 120 DAS (cm)	Days taken for spike emergence	Spike length (cm)	Number of flowers/spike	Weight of 10 Flowers (g)	Flower yield (g/plant)
T_1-*P. fluorescens* (BT+SA+FA)	52.40 d	110.00 e	75.00 e	38.70 d	13.70 cd	61.70 e
T_2- *B. subtilis* (BT+SA+FA)	51.00 e	112.33 f	73.70 f	38.00 d	14.00 bc	60.00 f
T_3- *P. fluorescens* + *B. subtilis* (BT)	54.40 c	104.00 c	78.70 c	41.00 c	14.70 ab	64.40 c
T_4- *P. fluorescens* + *B. subtilis* (SA)	53.00 d	106.70 d	77.33 d	40.70 c	15.00 a	63.00 d
T_5- *P. fluorescens* + *B. subtilis* (FA)	56.00 b	102.33 b	81.33 b	43.33 b	14.33 bc	65.33 b
T_6- *P. fluorescens* + *B. subtilis* (BT+SA+FA)	58.70 a	100.00 a	84.00 a	46.00 a	15.70 a	68.70 a
T_7-Carbendazim (BT+SD+FA)	58.00 a	100.70 a	83.33 a	45.33 a	15.00 a	68.00 a
T_8-Healthy control	48.33 f	117.33 g	70.33 g	34.00 e	12.33 e	43.33 g
T_9-Inoculated control	42.33 g	128.00 h	65.70 h	32.00 f	11.70 f	38.33 h

Mean of three replications; DAS-Days after sowing

Values in each column followed by the same letter are not significantly different according to the DMRT method (P = 0.05)

Khabbaz *et al*., 2015). These earlier reports corroborates with the present observations. The combined application of *P. fluorescens* + *B. subtilis* (T_6) through bulb treatment followed by soil and foliar application recorded minimum incidence of peduncle blight. Sivakumar *et al.* (2012) reported that peat formulations of both strains (*P. fluorescens* strain Pf51; *B. subtilis* B45) were applied as rhizome bacterization and soil application which resulted in 54% reduction of rhizome rot over control as compared to individual treatments as rhizome bacterization and soil application.

Enhanced disease suppression with combination of bacterial bio-agents through combined delivery system was effective in controlling tomato damping off (Nakkeeran *et al*., 2006), dry root rot of green gram (Thilakavathi *et al.,* 2007) ground nut root rot (Ramesh and Korikanthimath, 2010); bacterial blight of cotton (Salaheddin *et al*., 2010); tomato *Fusarium* wilt (Sundaramoorthy and Balabaskar, 2013). The combined application of bio-control agents provides an alternative to chemical fungicides by offering environmentally safer management practice in tuberose. The foregoing results have also indicated the possibility of using bio inoculants as supplement for chemical fungicides in the control of peduncle blight caused by *L. theobromae.*

The combined application of *P. fluorescens* + *B. subtilis* (BT+SA+FA)-T_6 showed increased plant height, spike length, number of flowers/spike and flower yield. Rhizome bacterization and soil application with *P. fluorescens* strain Pf51 and *B. subtilis* strain B45 recorded maximum height (167.21 cm) and number of tillers in (30.14) cardamom (Sivakumar *et al.,* 2012). The combined application of EPI (Pf-5) +KGI (Bs-4) +KPI (Pf-7) antagonistic bacterial formulation significantly increased the plant height (by 73.62 cm), dry weight (by 127 mg) and fruit yield (by 288.389 g) of tomato when compared to individual strains and untreated control (Sundaramoorthy and Balabaskar, 2013). Similar results on increased plant growth due to combined application of Pf1+Py15+Bs16+Zimmu in tomato (Latha *et al*,. 2009). Similar observations were made by several workers in various crops (Dey *et al*., 2004; Valverde *et al*,. 2006; Gopalakrishnan *et al*,. 2015). The increase in plant growth might be due to the growth-promoting compounds such as auxins, gibberellins and cytokinins (Pal *et al*. 2000; Gholami *et al*., 2009; Son *et al*., 2014).

These results are consistent with Hatayama (2005), that PGPR such as *Bacillus* sp. and *Pseudomonas* sp. capable of providing a direct influence that can trigger the growth of plants (biostimulant), while the indirect effect that the bacteria is able to inhibit the growth of harmful microbes such as disease-causing (pathogenic plan). Therefore, the plants were given the treatment of bacterial antagonists gave high yield than the control. Mukaromah (2005), states that the siderophore role in the mechanism of Induced Systemic Resistance (ISR). In this condition, siderophore induce the plant to produce salicylic acid, which acts as signal transduction genes that reduces the disease incidence.

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