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Antagonistic activity and Bioefficacy of *Bacillus subtilis* against Sheath Blight of Rice (*Oryza sativa* L.) caused by *Rhizoctonia solani*

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ABSTRACT: *Bacillus subtilis* is a major plant growth promoting rhizobacteria (PGPR) and a potential bio control agents against many plant pathogens of crop plants. In the present study six isolates of *Bacillus subtilis* were collected from SBCL, Chorbhatti Bilaspur while five isolates of *Bacillus subtilis* were isolated from different soils of rice fields representing different locations of Chhattisgarh. All the eleven isolates were tested on their antagonistic activity against *Rhizoctonia solani* under *in vitro*. The isolates showed varied level of inhibition and found the isolates BS1 showed maximum percent growth inhibition (80.73%) against *R. solani* with least mycelial growth (17.33mm). *In vivo* different isolates of *B. subtilis* were evaluated against sheath blight of rice of all the isolates of *B. subtilis* against *R. solani* result indicate that BS4 was most effective isolates for disease controlling potential with minimum percent disease index (33.29%) compared to other isolates. Similarly, the *B. subtilis* isolates BS4 (42.73%) was found to be more effective least percent tillers mortality in sheath blight of rice caused by *R. solani* under pot culture.

Keywords: *Bacillus subtilis, Rhizoctonia solani,* Antagonistic activity.

INTRODUCTION

Rice crop is affected by several biotic factors *i.e.*, viruses, fungus, bacteria, nematodes, and insect pests. Many diseases produced by diverse phytopathogens, such as blast, sheath blight, sheath rot, stem rot, and bacterial leaf blight (BLB), have a significant impact on rice production. Among these diseases, rice sheath blight (ShB) caused by *Rhizoctonia solani* Kuhn, is a devastating disease that causes significant yield loss and quality degradation throughout the world (Eizenga *et al*., 2002; Nagarajkumar *et al*., 2004; Nirmalkar *et al*., 2017[a]). Management of diseases of crop plants is difficult due to absence of resistance in the host and unavailability of resistant cultivars of rice, therefore, chemical control is only effective methods for the management of diseases however, it also causes soil and water pollution. Despite the fact that pesticides are recommended for pathogen reduction, they are not considered to be long-term remedies because of concerns about cost, fungicide residues, exposure risks, toxicity to non-target organisms, and other health and environmental issues. Therefore, current efforts have been concentrated on producing environmentally safe & ecofriendly, long-lasting, and effective therapies against a wide range of plant diseases. Thus, the alternative ways for reducing the use of harmful agrochemicals have become a prominent emphasis in recent years (Boukaew *et al*., 2013)**.** Biocontrol is a critical strategy for reducing the use of chemicals in disease management (Han *et al*., 2015; Nirmalkar *et al*., 2017[a]). The *Bacillus* spp. are classified in the order Bacillales and family Bacillaceae. *Bacillus subtilis* was first discovered by Ehrenberg in 1835. First known as *Vibrio subtilis*, it was renamed in 1872 by Cohn. *Bacillus subtilis* is a gram-positive, rod-shaped bacteria with peritrichous flagella (Nakano and Hulett 1997) and spore-forming bacterium that is widely distributed in the soils and environment, having the characteristics of high thermal tolerance, rapid growth in liquid culture, and readily form resistant spores. Several important plant pathogens, including *Fusarium* sp. (Cao *et al*., 2011), *Rhizoctonia solani* (Kumar *et al*., 2012), and *Sclerotium rolfsii*, can be suppressed by *B. subtilis* (De Curtis *et al*., 2010). *Bacillus subtilis*, which forms endospores, is one of the PGPRs that plays an important

role in plant growth promotion and biocontrol of plant pathogens (Glick, 1995).

Now a days, widely used of biological agent in reducing plant diseases has become more attractive and effective method due to their benefits and advantage of plant growth enhancing besides disease control. Such enhancement and benefits has been found in *Bacillus subtilis*. Therefore, in view of plant growth promotion and disease suppression ability of *Bacillus subtilis* the present study on antagonistic activity and bioefficacy of *Bacillus subtilis* against sheath blight of rice.

MATERIALS AND METHODS

Bacterial strains. Eleven isolates of *Bacillus subtilis* were used for present investigation. Six isolates of *Bacillus subtilis* were procured from State Bio Control Laboratory (SBCL), Chorbhatti, Bilaspur, Chhattisgarh and five isolates were obtained from the rhizosphere soil samples of different rice growing region of Chhattisgarh.

Isolation of *Bacillus subtilis.* One g of soil sample was suspended in 9 ml sterile water and subjected to serial dilution (10^{-1}-10^{-8}). An aliquot of 0.1 ml/ 100µl of each dilution was spread on LB agar medium (Tryptone 10g, Yeast extract 5g, NaCl 10g, Agar agar 15g in 1 L distilled water) by pour plate method (Janisiewicz, 1988 and Roberts, 1990; Pramer and Schmidt 1965). The inoculated plates were incubated at 28° for 24 h. After incubation, the individual colonies were selected based on the their colour, shape, edges further subculture to obtained pure culture (Rangaswami and Mahadevan 2008). The isolated pure colonies were examined to their morphological characteristics and gram staining for the identification of isolates as *Bacillus subtilis*. Rhizospheric isolates designated as BS7 to BS11 and procured isolates designated as BS1 to BS6.

Diseased plant sample collection. Diseased plant samples of sheath blight of rice caused by *Rhizoctonia solani* were used in the present studies was collected from rice field from Sendri, Bilaspur, Chhattisgarh.

Isolation and identification of *Rhizoctonia solani.* For the isolation of *R. solani*, infected leaf sheath/ tillers were used and surface sterilize with 0.5% sodium hypo chlorite for 3 min and washed with distilled water in three changes. Sheath segments were blotted dry with blotting paper and cut into small bits using sterile blade and placed on Potato Dextrose Agar (PDA) medium with the help of forceps. Inoculated plates were incubated at 25±2^{o}C for 7 days. Young active growth of fungal mycelial (5mm) was cut with the help of cork borer and sub-cultured on a new PDA medium to obtained pure culture. Fungal cultures were maintained on PDA slants and stored at 4^{o}C in refrigerator for further use (Killani *et al*., 2011).

Pathogenicity test: Before the experiment was carried out to test the virulence of *R. solani.* Artificial inoculation of rice seedlings sown in earthen pot using sclerotial inoculation method. In this method growing sclerotia was used and placed in between the leaf sheath of rice. After 3 days of inoculation, a typical symptoms of sheath blight were appeared on to the sheath with greyish, water soaked lesion, dark brown margin on the leaf sheath above water line. This confirmed that pathogen (*R. solani*) has ability to cause disease and expressed in the form of symptoms (Nagendran *et al*., 2019).

To screen of the isolates of *Bacillus subtilis* for their antagonistic activity against *Rhizoctonia solani* under *in vitro*

Dual culture method: The antagonistic activity of different isolates of *Bacillus subtilis* against *Rhizoctonia solani* and was investigated performed by dual culture technique under *in vitro* condition (Elkahoui *et al*., 2012). A mycelial disc (5mm), obtained from 5 days old culture of *Rhizoctonia solani* (test pathogen) was cut with the cork borer, and a loopful *Bacillus subtilis* of 24 h old cultures were placed on the *Bacillus* Potato Dextrose Agar medium (Rajkumar *et al*., 2018; Anillo *et al*., 2021) opposite to each other, equidistant from the periphery, and Petri dishes were incubated at 28±2^{o}C. Three replications were maintained for each treatment. The Petri plate containing test pathogen alone served as control. After 7 days of incubation, radial growth of pathogen was recorded, and the percentage inhibition was calculated using following formula (Muthukumar and Venkatesh 2013).

Percentage of mycelial growth inhibition was calculated by using the formula:

$$I = (C\text{-}T/C\times100)$$

where, I is inhibition of radial mycelial growth, C is radial growth measurement of pathogen in control (mm) and T is radial growth measurement of pathogen (mm) in the presence of antagonists.

After 7 days of incubation, number of sclerotia was counted from each treatment (Nagendran *et al*., 2019).

***In vivo* evaluation of isolates of *Bacillus subtilis* against sheath blight of rice**

Mass culture of *Bacillus subtilis.* A single colony of 24 h old culture of test isolates, picked from the culture slants, was transferred and grown to a 250 ml capacity of conical flask containing 100ml LB broth medium and incubated at room temperature for 4 days at 200 rpm using arotatory shaker. The prepared culture being used as a mass culture. Ten % (10ml broth/100ml distilled water) bacterial suspension was used as spray material (Kumar *et al*., 2012; Khedher *et al*., 2015).

Preparation of pathogen inoculums. Five mm diameter of mycelia disc were used from 48 hold active growth culture of *R. solani* was inoculated on PDA medium contained in 30cm diameter of plastic plate seal covered with sterile polythene. The inoculated plates were incubated at 25±2° in BOD incubator for 7 days. After incubation, fungal cultures were cut into rectangular sections (3 cm length× 1.5cm width) and placed into the sheath in between the tillers (Nagendran *et al*., 2019).

Treatments details:

Treatment	Isolates of *Bacillus subtilis*	Formulation	Dose ml/ml of Water
T_1	*Bacillus subtilis* BS1	Liquid 10%	10ml/100ml
T_2	*Bacillus subtilis* BS2	Liquid 10%	10ml/100ml
T_3	*Bacillus subtilis* BS3	Liquid 10%	10ml/100ml
T_4	*Bacillus subtilis* BS4	Liquid 10%	10ml/100ml
T_5	*Bacillus subtilis* BS5	Liquid 10%	10ml/100ml
T_6	*Bacillus subtilis* BS6	Liquid 10%	10ml/100ml
T_7	*Bacillus subtilis* BS7	Liquid 10%	10ml/100ml
T_8	*Bacillus subtilis* BS8	Liquid 10%	10ml/100ml
T_9	*Bacillus subtilis* BS9	Liquid 10%	10ml/100ml
T_{10}	*Bacillus subtilis* BS10	Liquid 10%	10ml/100ml
T_{11}	*Bacillus subtilis* BS11	Liquid 10%	10ml/100ml
T_{12}	Hexaconazole	Liquid 0.1%	0.5ml/500ml
T_{13}	Control(untreated)		

***In vivo* studies:** Twenty days old seedlings were used for transplanting. Five seedlings were transplanted into earthen pots of 9" height with the diameter of 6.3" having loam soil. After the establishment of seedlings and at maximum tillering stage, each pot was inoculated with mass culture of *R. solani*. Fungal inoculums grown on PDA was cut into rectangular bits and placed into the sheath in between the tillers. After inoculation, plants were covered with polythene bag to maintain humidity. After the appearance of first symptoms, liquid formulations (10ml/100ml distilled water) of different strains of *B. subtilis* were sprayed on host plant. Hexaconazole@0.1% was used as positive control while distilled water alone used as a negative control. Two sprays were done, first spray was done after the disease appearance and second spray was done after 10 days interval of first spray. Each treatment was maintained in three replications. Pot experiment were carried out in greenhouse conditions using CRD design for 45 days. The plants were observed critically for visual scoring of disease incidence and severity using the standard evaluation scale (0-9 scale) suggested by the International Rice Research Institute (IRRI), 1996.
Visual scoring of sheath blight incidence rating scale given by International Rice Research Institute (IRRI), 1996.

Scale	Symptoms
0	No infection
1	Vertical spread of the lesions up to 20% of plant height
3	Vertical spread of the lesions up to 21-30% of plant height
5	Vertical spread of the lesions up to31-45% of plant height
7	Vertical spread of the lesions up to 46-65% of plant height
9	Vertical spread of the lesions up 66-100% of plant height

Per cent Disease Index (PDI) was calculated by using the formulas given by IRRI, (1996).

$$\text{PDI} = \frac{\text{Sum of all individual rating}}{\text{Total No.of plant observed}} \times \frac{100}{\text{Maximum disease scale}}$$

Observations recorded. Percent Disease Index (PDI) based on 0-9 scale (Nagendran *et al*., 2019).
Percent tillers mortality: Number of dead tillers/Total number of tillers ×100
Statistical analysis. All the experimental data were statistically analysed using appropriate design *i.e.*, CRD with desired transformation as applicable.

RESULTS AND DISCUSSION

Experiment was conducted under *in vitro* conditions, the antagonist *Bacillus subtilis* exhibited a significant inhibition of the *R. solani* compared to the control (Table 1, Fig. 1). All the eleven *B. subtilis* isolates were tested and the maximum percent growth inhibition was recorded by *B. subtilis* isolate BS1 (88.73%) followed by BS3 (78.84%) which was statistically at par among themselves. Whereas, the other *B. subtilis* isolate showed (60.14%) per cent inhibition by *B. subtilis* isolate BS2, (59.83%) inhibition by *B. subtilis* isolate BS8 (59.83%) which were statistically at par among themselves, whereas, the least per cent inhibition (2.14%) of *R. solani* was recorded by *B. subtilis* isolate BS11 over control. The maximum number of sclerotia was recorded in *B. subtilis* isolate BS11 (52.33) followed by BS10 (48.66), BS9 (47.00), BS4 (45.33), BS5 (41.66), BS6 (33.33), BS2 (25.66), BS8 (21.33), BS7 (19.00) and BS3 (18.66), whereas, minimum number of sclerotia was recorded in *B. subtilis* BS1 (16.33). Severely, Huang *et al*. (2017) also reported such types of results and concluded their findings that *in vitro* antagonistic activity of *Bacillus subtilis* strain SL-44 showed significant antifungal activities with an inhibition rate of 42.3% against *Rhizoctonia solani*. Jamali *et al*. (2019) reported that the *Bacillus subtilis* strain RH5 exhibited significant antagonistic activity (84.41 %) against the fungal pathogen *Rhizoctonia solani*. Nagendran *et al*. (2019) reported that *Bacillus subtilis* strain Bs 7 showed maximum mycelial growth inhibition 51.1% over the control followed by Bs19 observed 49.4% mycelial growth reduction against *Rhizoctonia solani* causing sheath blight diseases. Ghazy and Nahrawy (2021) reported that the antifungal

activity of *Bacillus subtilis* significantly inhibited with inhibition percent was 51.55% and reduced the growth of *Cephalosporium maydis* by 4.36 cm compared to control.

***In vivo* evaluation of isolates of *Bacillus subtilis* against sheath blight of rice.** Pot experiment was carried out in the *in vivo* greenhouse condition for disease controlling potential of different isolates of *Bacillus subtilis* against sheath blight of rice. Different isolates of *B. subtilis* were used as foliar spray@10% concentration in controlling sheath blight of rice. All the isolates of *B. subtilis* were found more effective in plant disease reduction over control.

Results indicate (Table 2, Fig. 2) that the least per cent disease index was recorded in *B. subtilis* treatment T4 (33.29%) which showed maximum disease controlling potential on sheath blight and which was statistically at par with the other treatments *i.e*. T8 (36.28%) and T1 (40.70%), whereas, the isolates *i.e*. T9 (73.32%) was not effective in controlling sheath blight and other *B. subtilis* isolates *i.e*. T5 (64.40%) and T2 (62.29%) which was significantly effective in controlling sheath blight over control (80.73%) which was statistically at par with each other. The significantly less minimum per cent tillers mortality was recorded from *B. subtilis* T4 treatment (42.73%) and treatment T3 (42.80%) compared to control which was statistically at par among themselves which showed the less effective treatment on sheath blight over control (91.26%). Moreover, significant maximum per cent tillers mortality was recorded in *B. subtilis* treatment T9 (89.69%) followed by *B. subtilis* treatment T2 (75.19%) and *B. subtilis* treatment T10 (68.95%), T12 (68.86%), T5 (68.64%), T6 (67.11%) which were statistically at par with each other. Present results confirmed to the finding of Ali and Nadarajah (2013) also reported that the efficacy of *Bacillus subtilis* and *Trichoderma* isolates showed effective is ease reduction under greenhouse conditions against *Rhizoctonia solani*. Combine application of *Bacillus subtilis* and *Trichoderma* isolates T2+Bs and T7+Bs showed significant disease severity and disease incidence (4.33% and 11%), respectively, over the control (42.33% and 67.0%). *B. subtilis* showed 17.67% and 44.33% disease severity and disease incidence against *R. solani* alone. Similarly, Khedher *et al*. (2015) reported the efficacy of *Bacillus subtilis* V26 as a biological control agent against *Rhizoctonia solani* on potato under pot experiment. Here ported that the disease incidence on potato plant roots was significantly lower compared to control. StrainV26 (SPO2-CM) treatment resulted in low disease incidence on potato plant roots, with 63% of reduction. The application of V26 significantly reduced potato tuber caused by black scurf diseases and decrease disease incidence rate to 81% over the untreat done when using strain V26 (SPO_2) treatment. Different bioagents *i.e*., *T. harzianum*, *B. subtilis* and *P. fluorescens* earlier proved as a potential bio-agent of soil borne plant pathogens (Nirmalkar *et al.*, 2017) was found effective against different pathogens *i.e.*, *Rhizotonia solani*, *Fusarium oxysporum f.* sp. *Ciceris* etc may be used alone or in combination with different bioagents as a seed and soil treatment.

Table 1: Efficacy of different isolates of *Bacillus subtilis* on mycelial growth of *Rhizoctonia solani* in dual culture technique.

Isolates of *Bacillus subtilis*	Treatments	Mycelial growth of *R. solani* at 7 DAI	Per cent inhibition over control at 7 DAI	Number of sclerotia
Bacillus subtilis BS1	T1	17.33	80.73 (63.97)	16.33
Bacillus subtilis BS2	T2	35.86	60.14 (50.83)	25.66
Bacillus subtilis BS3	T3	19.03	78.84 (62.64)	18.66
Bacillus subtilis BS4	T4	81.00	9.99 (18.38)	45.33
Bacillus subtilis BS5	T5	76.66	14.81 (22.59)	41.66
Bacillus subtilis BS6	T6	61.33	31.84 (34.33)	33.33
Bacillus subtilis BS7	T7	33.96	52.80 (46.60)	19.00
Bacillus subtilis BS8	T8	35.10	59.83 (50.67)	21.33
Bacillus subtilis BS9	T9	83.20	7.55 (15.86)	47.00
Bacillus subtilis BS10	T10	84.36	6.25 (14.40)	48.66
Bacillus subtilis BS11	T11	88.06	2.14 (8.29)	52.33
Control		90.00	-	57.33
SEm (±)			**1.00**	
C.D. 5%			**2.93**	
CV			**4.91**	

Mean of three replication values in parentheses are arcsine transformed, DAI Days after inoculation

Table 2: *In vivo* efficacy of *Bacillus subtilis* isolates against sheath blight of rice under pot conditions.

Isolates of *Bacillus subtilis*	Designation	PDI		% Tillers mortality	
		Before spray	10th days after 2nd spray	Before spray	10th days after 2nd spray
Bacillus subtilis BS1	T1	8.06	40.70 (39.60)	7.53	61.73 (51.78)
Bacillus subtilis BS2	T2	8.17	62.29 (52.18)	6.41	75.19 (60.13)
Bacillus subtilis BS3	T3	8.23	51.10 (45.62)	6.02	42.80 (40.86)
Bacillus subtilis BS4	T4	7.85	33.29 (35.21)	7.04	42.73 (40.81)
Bacillus subtilis BS5	T5	8.13	64.40 (53.43)	7.14	68.64 (55.95)
Bacillus subtilis BS6	T6	7.88	61.47 (51.79)	8.5	67.11 (55.01)
Bacillus subtilis BS7	T7	8.22	49.88 (44.91)	6.75	58.02 (49.62)
Bacillus subtilis BS8	T8	7.86	36.28 (36.98)	6.46	55.49 (48.16)
Bacillus subtilis BS9	T9	8.08	73.32 (58.92)	7.04	89.69 (71.30)
Bacillus subtilis BS10	T10	8.12	52.59 (46.48)	6.02	68.93 (56.17)
Bacillus subtilis BS11	T11	8.33	43.70 (41.33)	7.44	63.52 (52.85)
Hexaconazole	T12	7.92	54.07 (47.38)	8.38	68.86 (56.09)
Control		8.26	80.73 (63.97)	7.86	91.26 (73.33)
SEm (±)		**-**	**2.60**	**-**	**1.26**
C.D. 5%		**NS**	**7.58**	**NS**	**3.68**
CV		**-**	**9.50**	**-**	**4.00**

*Mean of three replication values in par entheses are arcsine trans formed, PDI Percent disease index

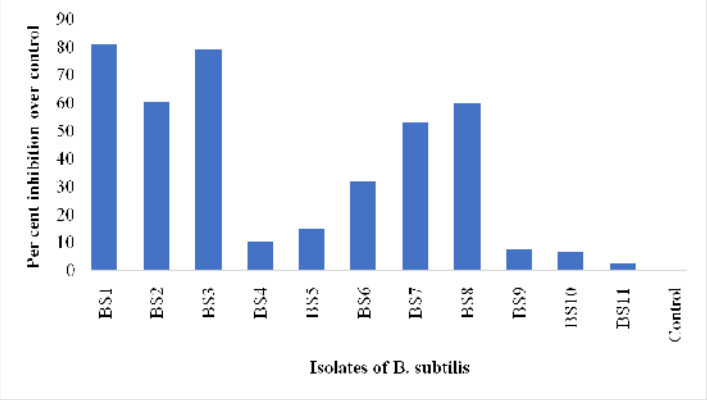

Fig. 1. Efficacy of different isolates of *Bacillus subtilis* on mycelial growth of *Rhizoctonia solani* in dual culture technique.

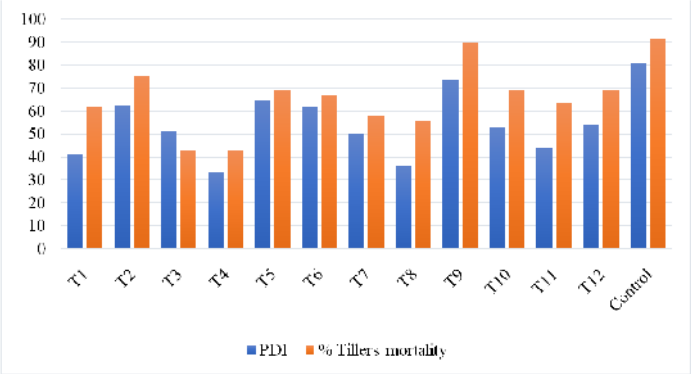

Fig. 2. *In vivo* efficacy of *Bacillus subtilis* isolates against sheath blight of rice under pot conditions.

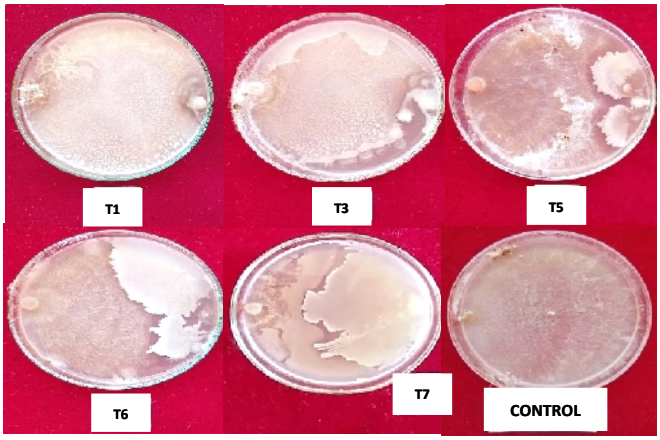

Plate 1. Efficacy of different isolates of *Bacillus subtilis* on mycelial growth of *Rhizoctonia solani* in dual culture technique.

Plate 2. *In vivo* efficacy of *Bacillus subtilis* isolates against sheath blight of rice under pot conditions (a) After inoculation cover with polythene bag (b) First disease symptoms appearance (c) Spraying of different treatments of *B. subtilis* (d) superior treatment (e) Least effective treatment (f) Control.

CONCLUSION

The results suggested that the *Bacillus subtilis* isolates has antimicrobial ability and disease controlling potential. Further, it suggests that *Bacillus subtilis* BS1 showed high antimicrobial activity against *Rhizoctonia solani* and *B. subtilis* BS4 was most effective for the management of sheath blight of rice under *in vivo* condition. Further, it can be used as potential biocontrol agents, which is eco-friendly, long lasting and cost ineffective.

FUTURE SCOPE

B. subtilis isolates have gained worldwide importance for their benefits and advantages. This microorganism have disease controlling ability and enhancing the plant growth. It is used as biocontrol of plant pathogens. Salt and drought tolerance isolates applicable for the disease management in saline soil as well as dessert region. In view of the pot culture, *B. subtilis* is expected to be commercialized for disease management and industrial applications. Need to more further studies and large screening.

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Conflict of Interest. None.

REFERENCES

Ali, H. Z. and Nadarajah, K. K. (2013). Evaluating the Efficacy of *Trichoderma* Isolates and *Bacillus subtilis* as Biocontrol agents against *Rhizoctonia solani*. *Research Journal of Applied Sci*ences, *8*(1): 72-81.

Anillo, H. J. B., Rodriguez, V. E. G., Cantoral, J. M., Sanchez, D. G., Collado, I. G. and Garrido, C. (2021). Endophytic bacteria *Bacillus subtilis*, Isolated from *Zea mays* a Potential Biocontrol Agents against *Botrytis cinerea*. *Journal Biotechnology*, *10*(6): 492.

Boukaew, S., Klinmanee, C. and Prasertsan, P. (2013). Potential for the integration of biological and chemical control of sheath blight disease caused by *Rhizoctonia solani* on rice. *World Journal Microbiology Biotechnology*, *29*(10): 1885-1893.

Cao Y., Zhang Z. H., Ling N., Yuan Y. J., Zheng X. Y., Shen B. A., and Shen Q. R. (2011). *Bacillus subtilis* SQR 9 can control Fusarium wilt in cucumber by colonizing plant roots. *Biology fertility* of *soils*, *47*(5): 495–506.

De Curtis F., Lima G., Vitullo D., and De Cicco, V. (2010). Biocontrol of *Rhizoctonia solani* and *Sclerotium rolfsii* on tomato by delivering antagonistic bacteria through a drip irrigation system. *Crop Protection*, *29*(7): 663–670.

Eizenga, G. C., Lee, F. N. and Rutger, J. N. (2002). Screening *Oryza* species plants for rice sheath blight resistance. *Plant Disease, 86*(7): 808-812.

Elkahoui, S., Djebali, N., Tabbene, O., Hadjbrahim, A., Mnasri, B., Mhamdi, R., Shaaban, M. and Limam, F. (2012). Evaluation of antifungal activity from *Bacillus subtilis* against *Rhizoctonia solani*. *African Journalof Biotechnology*, *11*(18): 4196-4201.

Ghazy, N. and Nahrawy, S. E. (2021). Siderophore production of *Bacillus subtilis* MF497446 and *Pseudomonas koreensis* MG209738 and their efficacy in controlling *Cephalosporium maydis* in maize plant. *Archives of microbiology, 203*(3): 1195-1209.

Glick, B. R. (1995). The enhancement of plant growth promotion by free-living bacteria. *Canadian Journal of Microbiol*ogy, *41*(2): 9-17.

Han, J. H., Shim, H., Shin, J. H. and Kim, K. S. 2015). Antagonistic Activities of *Bacillus* spp. Strains Isolated from Tidal Flat Sediment Towards Anthracnose Pathogens *Colletotrichum acutatum* and *C. gloeosporioides* in South Korea. *Plant Pathology Journal*, *31*(2): 165-175.

Huang, Y., Wu, Z., He, Y., Ye, B. C. and Li, C. (2017). Rhizospheric *Bacillus subtilis* Exhibits Biocontrol Effect against *Rhizoctonia solani* in pepper (*Capsicum annuum*). *Bio Med Research International*, 2017: 9.

IRRI (1996). Standard evaluation system for rice. 4th ed. IRRI, Manila, Phillipine.

Jamali, H., Sharma, A., and Shrivastava, A. K. (2019). Biocontrol potential of *Bacillus subtilis* RH5 against sheath blight of rice caused by *Rhizoctonia solani. Journal of Basic Mircobiology, 60*(3): 268-280.

Janisiewicz, W. J. (1988). Biocontrol of postharvest diseases of apples with antagonistic mixtures. *Phytopathology*, *78*(2): 379-84.

Khedher, S. B., Feki, O. K., Dammak, M., Khiareddine, H. J., Remadi, M. D. and Tounsi, S. (2015). Efficacy of *Bacillus subtilis* V26 as a biological control agent against *Rhizoctonia solani* on potato. *Comptes Rendus Biologies*, *338*(12): 784-792.

Killani, A. S., Abaidoo, R. C., Akintokun, A. K. and Abiala, M. A. (2011). Antagonistic Effect of Indigenous *Bacillus subtilis* on Root-/Soil-borne Fungal Pathogens of Cowpea. *Researcher, 3*(3): 11-18.

Kumar, K. V. K., Yellareddygari, S. K., Reddy, M. S., Kloepper, J. W., Lawrence, K. S., Zhou, X. G., Sudini, H., Groth, D. E., Raju, S. K. and Miller, M. E. (2012). Efficacy of *Bacillus subtilis* MBI 600 against sheath blight caused by *Rhizoctonia solani* and on growth and yield of rice. *Rice Science*, *19*(1): 55-63.

Muthukumar, A. and Venkatesh, A. (2013). Exploitation of fungal and endophytic bacteria for the management of leaf blight of ribbon plant. *Journal of Plant Pathology and Microbiology, 4*(1): 1.

Nagarajkumar, M., Bhaskaran, R. and Velazhahan, R. (2004). Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. *Microbiological Research, 159*(1): 73-81.

Nagendran, S., Kulanthaivelu, S. and Sundararajan, T. (2019). Assessment on antagonistic potential of Bacterial bio agents *Psuedomonas fluorescens* and *Bacillus subtilis* against *Rhizoctonia solani* Kuhn. An incitant of Sheath blight of rice. *Journal of Entomology and Zoology Studies*, *7*(3): 128-142.

Nakano, M. and Hulett, M. (1997). Adaptation of *Bacillus subtilis* to oxygen limitation. *Microbiology*, *157*(1): 1-7.

Nirmalkar, V. K., Said P. P. and Kaushik, D. K. (2017). Efficacy of Fungicides and Bio-Agents against *Pyricularia gresia* in Paddy and Yield Gap Analysis Thought Frontline Demonstration. *International Journal Current Microbiology Applied Sciences, 6*(4): 2338-2346.

Nirmalkar, V. K, Singh, Tiwari, R. S, Said, P. P. and Kaushik, D. K. (2017). Field Efficacy of *Trichoderma harzianum* and *Rhizobium* against Wilt Complex of Chickpea. *International Journal Current Microbiology Applied Sci*ence, *6*(7): 1421-1429.

Pramer, D., Schmidt, E. L. (1965). Experimental Soil Microbiology. Buffer Publication. Co., Minneapolis, (1965), 107.

Rajkumar, K. Naik, M. K. Amaresh, Y. S. and Chennappa, G. (2018). Bioefficacy of *Bacillus subtilis* against Major Pathogen of Chilli *Colletotrichum capsici* Causing Fruit Rot of Chilli. *International Journal Current Microbiology Applied Sciences*, *7*(7): 2681-2686.

Rangaswami, G. and Mahadevan, A. (2008). Diseases of crop plants in India. 4th edn. Prentice Hall of India (Pvt). Ltd, New Delhi, 177-179.

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