Print ISSN : 0972-8813
e-ISSN : 2582-2780

[Vol. 19(3), September-December, 2021]

Pantnagar Journal of Research

(Formerly International Journal of Basic and Applied Agricultural Research ISSN : 2349-8765)

G.B. Pant University of Agriculture & Technology, Pantnagar

PANTNAGAR JOURNAL OF RESEARCH

Vol. 19(3) September-December, 2021

CONTENTS

In vitro evaluation of endophytes and consortium for their plant growth promoting activities on rice seeds

DAS, J., DEVI, R.K.T. and BARUAH, J.J.[1]

College of Post Graduate Studies in Agricultural Sciences, Umiam, Meghalaya, [1]Assam Agricultural University, Jorhat

ABSTRACT: The present study entitled was conducted with an aim to find out the efficacy of endophytes and their Microbial Consortium (MC) on per cent germination of rice seeds and effect on vigour index of rice seedlings. The parameters for vigour index were recorded on 7th, 14th and 21st days after germination. Rice seeds of CAU-R1 variety was used for determining the plant growth promoting (PGP) activity of the endophytes alone and in consortium under *In vitro*. It was found that Microbial Consortium (MC) of endophytes showed more effectiveness than the individual endophytes on Per cent seed germination (98.33%) and vigour index after 7(778), 14(2134.50) and 21(2541.42) days of seed germination.

Key words: Consortium, endophytes, PGP activity

For a majority of the people of Asian and African countries, rice (*Oryza sativa* L.) is an important staple food. In India, more than 70% of people are directly or indirectly dependent on rice cultivation for their livelihood. Disease and pest infestations are one of the major constraints for increasing rice production. Diseases of rice are mainly caused by bacteria, fungi, virus, nematodes and as well as by phytoplasma. The seed borne pathogens may be both internally and externally associated within the rice grains (Kalyanasundaram, 1988). Reduction in seedling vigour and seedling mortality is also seen (Das and Narrain, 1988). In addition to these, toxins produced by different types of micro-organisms further deteriorates the quality of rice grains (Udagawa *et al*., 1979). Endophytes are found everywhere in plant ranging from leaves, seeds, roots and even in the rhizospheric soil. In addition to providing resistance against various diseases to plants, it also helps in better growth of the plant (Lee *et al*., 2004). Raupach and Kloepper (1998) reported that, combined application of antagonistic strains *viz*., *Bacillus pumilus, B. subtilis* and *Curtobacterium flaccumfaciens* enhanced growth promotion when used as seed treatment, as compared to the strains tested singly. Microbial consortium consisting of superior and efficient strains for growth promotion and biological control may prove to be a superior technique compared to traditional seed treatment by chemicals.

MATERIALS AND METHODS

Isolation of bacterial endophytes

The present study was carried out in the Department of Plant Pathology, School of Crop Protection, College of Post Graduate Studies in Agricultural Sciences, (Central Agricultural University), Imphal, Umiam, Meghalaya in the year 2019-20. Isolation was done by following the method of Zinniel *et al*. (2002). Five *Bacillus* spp. were isolated from healthy rice leaves. Identification of the isolates was done by referring the guidelines described in the Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

Functional attributes of screened bacterial endophytes

Functional attributes were studied for ammonia production (Thomas 1912, cellulose degradation (Gupta *et al*., 2012), HCN production (Lorck, 1948), IAA production (Ehmann, 1977) and phosphate solubilization (Pikovskaya, 1948).

Development of Microbial Consortium (MC)

Compatibility test among the isolates

The 5 isolates were tested for their compatibility among each other following the method of Fukui *et*

al., 1994.

Preparation of microbial formulation and consortium

Preparation of the consortia was done following the method described by Nandakumar *et al*., 2001.

***In vitro* evaluation of endophytes alone and in consortium for their PGP activities**

For the seed treatment by endophytes alone and consortium *In vitro,* rice seeds of CAU-R1 variety was used. The observations for per cent seed germination were recorded at 7 days after incubation.

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds kept for test}} \times 100$$

The observations for root length, shoot length and corresponding vigour index were calculated at 7, 14 and 21 days after germination by taking the method of Haque *et al*. (2007).

$$\text{Vigour Index (Vi)} = (RL + SL) \times GP$$

Where, RL = Root length (cm); SL = Shoot length (cm); GP = Germination per cent

RESULTS AND DISCUSSION

Functional attributes of bacterial endophytes

Ammonia production test

Four isolates of *Bacillus* spp. produced ammonia which was indicated by deep yellow to brownish colour of the broth culture tube (Fig 1).

Cellulose degradation test

Out of 5 isolates, 4 *Bacillus* spp. isolates showed positive reaction to cellulose degradation test which was indicated by formation of clear zone around the colonies (Fig 1).

HCN production test

All the isolates of *Bacillus* spp. showed negative reaction to HCN production test (Fig 1).

IAA production test

Out of 5 isolates of *Bacillus* spp., only two isolates *viz*., BC 3 and BC 8 showed positive reaction to IAA production test which was indicated by the appearance of pink colour of the broth culture (Fig 1).

Phosphate solubilization test

All the isolates of *Bacillus* spp. showed positive reaction to phosphate solubilization test which was indicated by the formation of clean zones around the spot inoculated colonies in Pikovskaya media (Fig 1).

Table 1: Cultural and morphological characters of 5 isolates of *Bacillus* spp.

Isolates/ Characters	BC 3	BC 6	BC 8	BC 10	BC 15
Cell shape	Rod	Rod	Rod	Rod	Rod
Colony colour	DW	DW	PW	C	C
Colony elevation	H	H	R	R	R
Colony margin	U	U	S	S	S
Colony shape	F	F	R	R	R
Gram reaction	-	-	-	-	-
KOH Test	-	-	-	-	-
Odour	+	+	+	+	+

C- Creamish; DW- Dull White; F- Flat; H- Hilly; PW- Pure White; R- Raised; S- Smooth; U- Undulated; (+): Positive result; (-): Negative result

Table 2: Functional attributes of 5 isolates of *Bacillus* spp.

Isolates/ Tests	BC 3	BC 6	BC 8	BC 10	BC 15
Ammonia production test	+	-	+	+	+
Cellulose degradation test	+	+	-	+	+
HCN production test	-	-	-	-	-
IAA production test	+	-	+	-	-
Phosphate solubilization test	+	+	+	+	+

(+) : Positive test; (-) : Negative test

Development of Microbial Consortium (MC)

Compatibility test among the efficient endophytes

The 5 isolates of *Bacillus* (BC 3, BC 6, BC 8, BC 10, BC 15) when streaked horizontally and vertically with each other in a nutrient agar plate, showed no zone of inhibition at the point of contact between each other after 72 h of incubation. Hence it can be said that the 5 isolates of *Bacillus* were compatible with each other and a Microbial Consortium (MC) was prepared (Fig 2).

***In vitro* evaluation of endophytes alone and in consortium for their PGP activities**

Rice seeds treated with the 5 isolates of *Bacillus* (BC 3, BC 6, BC 8, BC 10, BC 15) and MC showed good germination Per centage (Fig 3) of 98.33% over

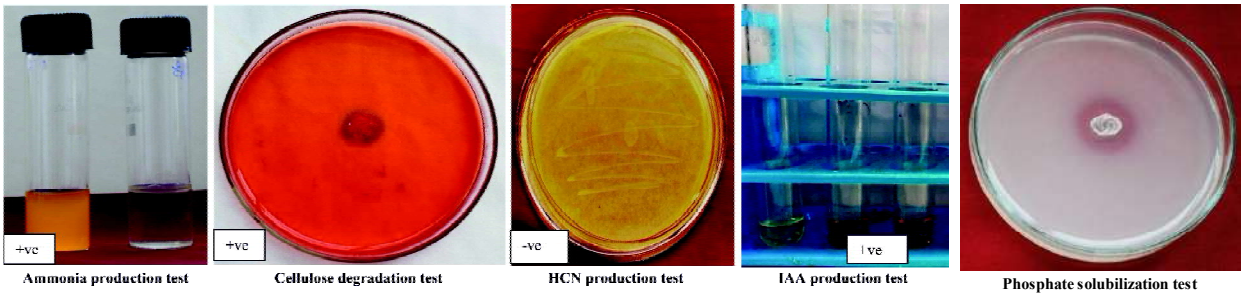

Fig 1: Functional attributes of 5 isolates of *Bacillus*

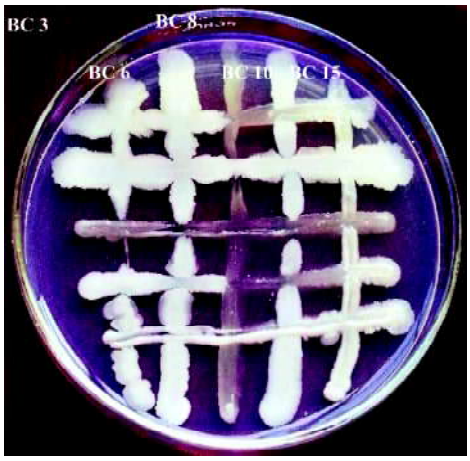

Fig 2: Compatibility test

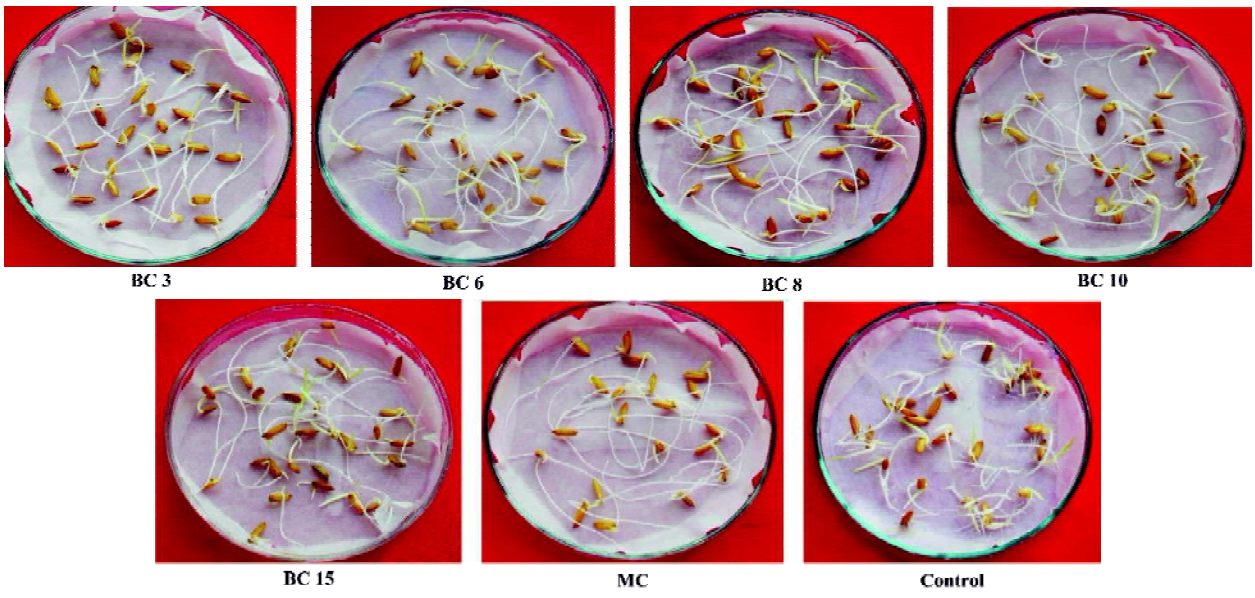

Fig 3: Efficacy of endophytes and consortium on rice seed germination

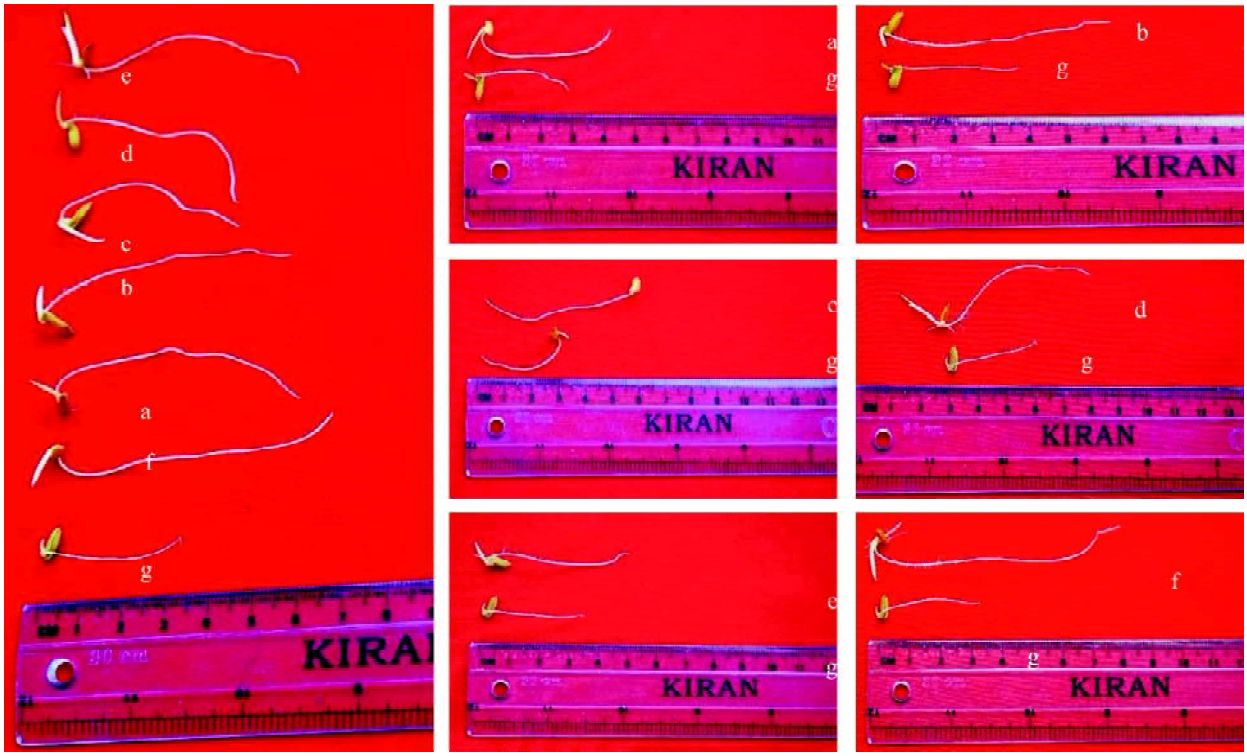

Fig 4a: Efficacy of endophytes and consortium on seedling vigour of rice seeds at 7 days after germination [a) BC 3 b) BC 6 c) BC 8 d) BC 10 e) BC 15 f) MC g) Control]

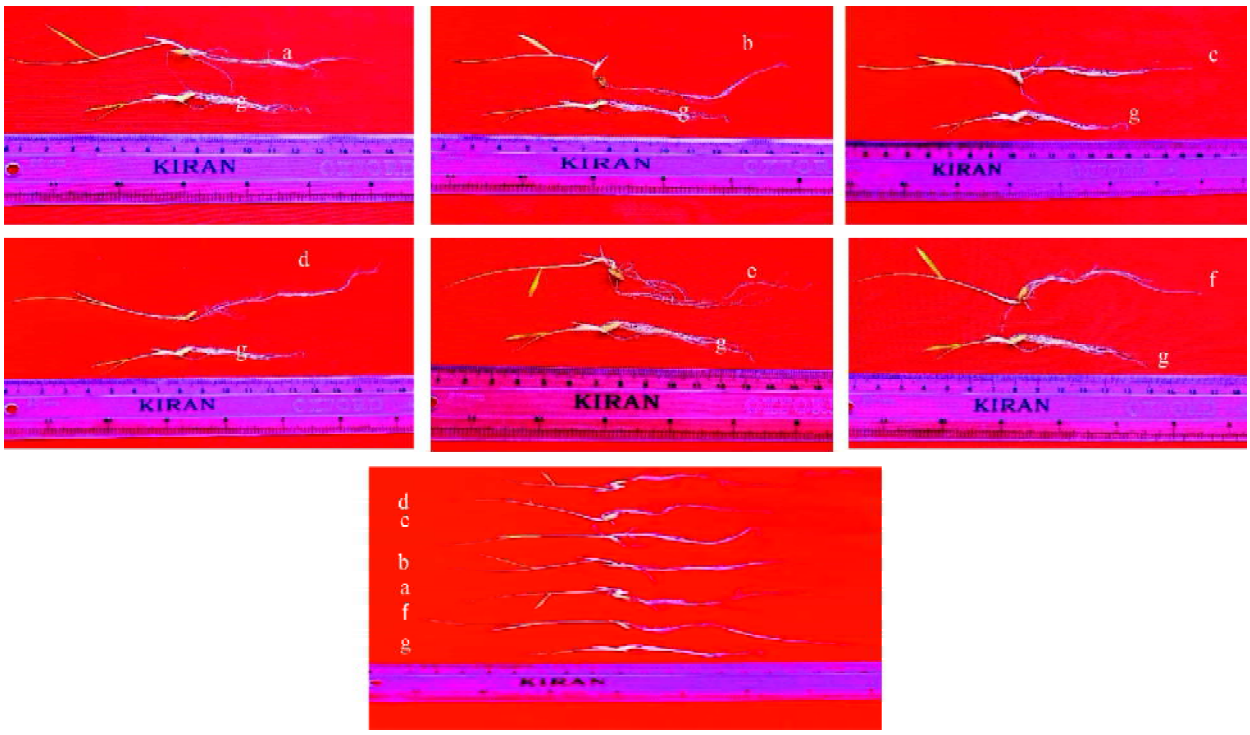

Fig 4b: Efficacy of endophytes and consortium on seedling vigour of rice seeds at 7 days after germination [a) BC 3 b) BC 6 c) BC 8 d) BC 10 e) BC 15 f) MC g) Control]

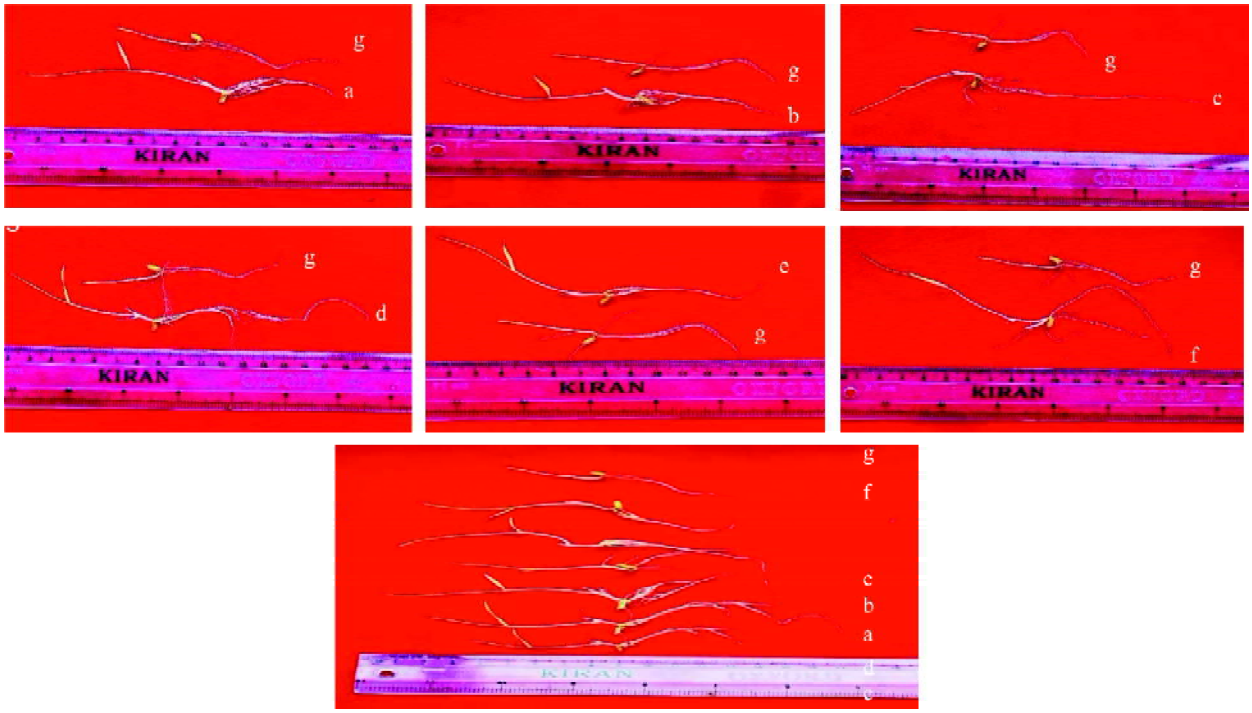

Plate 4 c: Efficacy of endophytes and consortium on seedling vigour of rice seeds at 7 days after germination [a) BC 3 b) BC 6 c) BC 8 d) BC 10 e) BC 15 f) MC g) Control]

control (81.66%). The individual isolates and MC showed maximum vigour index of rice seeds over control. MC exhibited maximum vigour index for 3 weeks after sowing (Table 3 and Fig 4).

CONCLUSION

The 5 endophytes were found to be compatible and a Microbial Consortium (MC) was formulated. Seed treatment of CAU-R1 with MC and individual 5 endophytes were found superior in maximum Per centage of seed germination and vigour index over control. So, further study can be carried out on field condition by testing the Microbial Consortium (MC) and mass production of the formulation can be taken up with efficient carrier system.

REFERENCES

Das, A.N. and Narrain, A. (1988). Detection of grain discoloring fungal organisms of rice and production of disease free seeds. *J. Mycol. Pl. Pathol.*, 18: 24-30.

Ehmann, A. (1977). The Van Urk- Salkowski reagent- a sensitive and specific chromogenic reagent for silica gel thin-layer chromatographic detection and identification of indole derivatives. *J. Chromatogr.,* 132(2): 267-276.

Fukui, R., Schroth, M.N., Handson, M., and Hancock, J.G. (1994). Interaction between strains of Pseudomonads in sugar beet spermospheres and the relationship to pericarp colonization by *Pythium ultimum* in soil. *Phytopathol.*, 84: 1330-1332.

Gupta, P., Samant, K., and Sahu, A. (2012). Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. *Int. J. Microbiol.,* 4: 1-5.

Haque, A.H.M.M., Akon, M.A.H., Islam, M.A., Khalequzzaman, K.M., and Ali, M.A. (2007). Study of seed health, germination and seedling vigour of farmers produced rice seeds. *Int. J. Sustain. Crop Prod.*, 2(5): 34-39.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T.,

Table 3: *In vitro* evaluation of PGP activities of the 5 isolates of *Bacillus* and Microbial Consortium (MC)

S. No.	Isolates	Germination (%)	Shoot length (cm)			Root length (cm)			Vigour Index		
			7	14	21	7	14	21	7	14	21
			(Days after germination)			(Days after germination)			(Days after germination)		
1	BC 3	91.66±1.66	1.31±0.08	8.27±0.14	9.79±0.12	5.84±0.08	8.60±0.16	9.82±0.18	655.93±9.90	1546.80±27.30	1798.16±42.32
2	BC 6	93.33±1.66	1.56±0.08	7.78±0.19	8.93±0.12	5.91±0.09	8.16±0.15	9.84±0.26	697.50±20.89	1487.26±13.59	1752.85±63.01
3	BC 8	91.66±1.66	1.18±0.08	8.18±0.24	9.95±0.12	6.02±0.03	8.54±0.30	9.26±0.14	661.26±22.59	1532.33±30.33	1853.70±44.95
4	BC 10	95.00±0.00	2.01±0.14	8.32±0.05	10.26±0.18	5.56±0.12	9.11±0.20	11.18±0.10	719.46±25.23	1656.80±21.90	2037.11±26.61
5	BC 15	93.33±1.66	1.40±0.17	8.00±0.07	9.66±0.29	6.22±0.07	9.18±0.16	11.05±0.13	711.90±15.33	1633.05±10.42	1932.96±30.15
6	MC	98.33±1.66	2.38±0.13	10.77±0.12	13.00±0.18	7.39±0.32	10.99±0.08	12.82±0.13	978.00±45.82	2134.50±48.85	2541.41±69.41
7	Control	81.66±1.66	0.70±0.10	5.97±0.32	6.32±0.12	4.33±0.03	7.92±0.17	8.64±0.17	411.43±12.90	1136.60±60.71	1222.22±49.21
CD (0.05)		4.72	0.34	0.58	0.78	0.43	0.58	0.61	74.74	106.79	171.86
Sem (±)		1.90	0.11	0.19	0.26	0.12	0.19	0.23	12.81	34.81	58.51

Data in the table are mean values of 3 replicates

and Williams, S.T. (1994). Bergey's manual of determinative bacteriology, 9th edn. Williams & Wilkins, Baltimore, 787p.

Kalyanasundaram, I.G.J. (1988). Storage fungi in rice in Karnataka, India. *Microb. Biotechnol.*, 14(2): 67-76.

Lee, S., Encarnacion, M.F., Zentella, M.C., Flores, L.G., Escamilla, J.E., and Kennedy, C. (2004). Indole-3-acetic acid biosynthesis is deficient in *Gluconacetobacter diazotrophicus* strains with mutations in cytochrome c biosynthesis genes. *J. Bacteriol.*, 186: 5384-5391.

Lorck, H. (1948). Production of hydrocyanic acid by bacteria. *Pl. Physiol.*, 1: 142-146.

Nandakumar, R., Babu, S., Viswanathan, R., Raguchander, T., and Samiyappan, R. (2001). Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens. Soil Biol. Biochem.*, 33(4-5): 603-612.

Pikovskaya, R.I. (1948). Mobilization of phosphorous in soil in connection with vital activity of some microbial species. *Mikrobiologiya*, 17: 362-370.

Raupach, G.S. and Kloepper, J.W. (1998). Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. *Phytopathol.*, 88: 1158-1164.

Thomas, P. (1912). Surune reaction coloree del'ammoniaque. *Bull. Soc. Chemique. Ser.*, 4(11): 796-799.

Udagawa, S., Muroi, T., Kurata, H., Sekita, S., Yoshihira, K., Natori, S., and Umeda, M. (1979). The production of chaetoglobosins, sterigmatocystin, O-methylsterigmatocystin and chaetocin by *Chaetomium* spp. and related fungi. *Can. J. Microbiol.*, 25(2): 170-177.

Zinniel, D.K., Lamrecht, P., Harris, N.B., Feng, Z., Kuczmarski, D., Higley, P., Ishimaru, C.A., Arunakumari, A., Barletta, R.G., and Vidaver, A.K. (2002). Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. *Appl. Environ. Microbiol.*, 68(5): 2198-2208.

Received: December 9, 2021
Accepted: December 12, 2021