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Effect of Microalgae and *Bacillus* on Purple Blotch (*Alternaria porri*) of Onion (*Allium cepa* L.)

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A B S T R A C T

Onion (*Allium cepa* L.) is a biennial bulb crop, produced all over the world. It is attacked by many diseases, out of which Purple blotch of onion caused by *Alternaria porri* (Ellis) Neerg causing heavy yield loss in both bulb production and seed production. Native *Bacillus* were isolated from the rhizosphere of vegetables and ornamental crops of the SHUATS campus and were identified through bio-chemical tests. *In vitro* the *Bacillus* isolates were screened for antagonism on *Alternaria porri* by dual culture technique. The growth of *A. porri* was recorded at 48, 72 and 96hrs after incubation and found that all the six isolates tested significantly inhibited the growth of *A. porri* as compared to the control. For seedlings preparation Nashik red variety were grown in nursery bed in the garden, Department of Plant Pathology and then 28days old plants were transplanted to the field in 2×2 m plots. Seven days after transplanting microalgae was applied @ 3kg /ha through irrigation and after45days 2nd application of the same dosage of microalgae was given and isolates of *Bacillus* spp. was sprayed @ 1.71 × 10^{8} c.f.u/ml. Results showed that all the treatments significantly reduced the disease intensity as compared to control.

Introduction

The onion (*Allium cepa* L.) which is also commonly called as "bulb onion", is one of the important cultivated crops belonging to the genus *Allium* and family *Amaryllidaceae.* Among all the diseases Purple blotch (*Alternaria porri*) is one of the major constrains in onion cultivation. The pathogen is polyphagus infecting crop like onion, Garlic, Shallot and other *Allium* crops. High relative humidity (80 to 90%) and optimum temperature (24+10˚C) are favorable for the development of this disease. Sandhu (1981) reported that this disease causes up to 20-60 per cent loss in bulb yield and extent of loss depends on time of infection and stage of crop growth.

Bacillus species are Gram-positive, endospore-forming, chemo-heterotrophic, rod-shaped bacteria which are usually motile with peritrichous flagella; they are aerobic or facultative anaerobic and catalase positive (Mansour *et al.*, 2015). Members of the *Bacillus* genus are generally found in all

agricultural soils and in other environments. Biological control activities of most bacterial BCAs have been attributed to cell wall-degrading enzymes, antimicrobial peptides, cyclic lipopeptides (CLPs) such as iturins, fengycins, and surfactins, low-molecular-weight metabolites, volatile organic compounds, and induction of systemic resistance in host plants (Ali *et al.,* 2016). Kokalis-Burelle *et al.,* (2004) studied the effect ofsome plant growth-promoting rhizobacteria on seedling growth and naturally occurring diseases on tomato in Florida. They used different strains of *Bacillus subtilis*, *B. amyloliquefaciens*, *B. pumilis* and *B. cereus* and found that, all bacterial strains significantly increased plant growth forall parameters measured.

Micro-algae have been used for the production of plant bio stimulants, i.e. of bio products which contain substances "whose function, when applied to plants or the rhizosphere, is to stimulate natural processes, to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality"(European Bio stimulant Industry Council, 2014). Micro-algae contain important quantities of plant growth hormones, auxins, abscisic acid, cytokinins, gibberellins (Tarakhovskaya *et al.,* 2007). Kulik (1995) stated that protection against soil-borne pathogens that attack seeds and seedlings, when microalgae applied in high volume to the seeds they remain competitive to pathogens.

Materials and Methods

Fresh rhizosphere soil samples were collected from Lady's finger, Spinach, Cabbage, Onion, Tomato, Mustard, Drumstick, Turnip and Rose crops were used and processed according to Gomes *et al.,* (2001). *Bacillus* species were isolated using dilution method with Nutrient Agar medium. Rhizosphere soil samples (1 g) were suspended in 9 mL sterile water and shaken vigorously. The resulting slurry was serially diluted and appropriate dilution (10^{-4} and 10^{-5}) of this suspension (1 mL) was spread on sterilized plates under laminar air flow and further 5ml of Nutrient Agar medium poured to the plates and plates were incubated at 37°C±2 for 2 days.

According to Francis (1993) members of the bacterial genus *Bacillus,* which are ubiquitous in the environment, are aerobic or facultative anaerobic gram-positive or gram-variable spore-forming rods. The vegetative cells range from 0.5 by 1.2 to 2.5 by 10 µm in diameter and can grow at optimal temperatures ranging from 25 to 37°C, most strains are catalase positive, possess peritrichous flagella, and sporulate in air.

After identification of the isolates they were further proceeded to various classical biochemical tests in order to obtain the accurate identification of gram positive rod bacteria. Biochemical methods *viz.,* Gram Staining, KOH test, Catalase, Growth in 7% NaCl, Gelatin Hydrolysis, Casein Decomposition, Starch Hydrolysis, Growth at 50˚C and 65˚C were carried out. Biochemical tests for identification were done by following the Bergey's Manual of Determinative Bacteriology (1930). Isolated bacteria were sub cultured on Nutrient agar medium for 36 hrs. Twenty four hours Old-cultures were used for each test with three replicates for all biochemical tests. The biochemical tests were carried out with appropriate controls following the standard procedures (Table 2).

Gram's reaction

This test is essential to differentiate bacteria into gram positive and gram negative bacteria. A loopfull of bacterial suspension was smeared on to a glass slide. It was air dried and heat fixed by passing the slide rapidly

two to three times on Bunsen burner. The smear was flooded with crystal violet solution for 1 min. The slide was washed with a gentle stream of distilled water blot dried and flooded with iodine for 1 min. Again the slide was washed with distilled water and blot dried, and decolorized by washing in a gentle stream of 95% ethyl alcohol for 30 sec to remove excess stain that will easily wash away, counter staining was done by dipping in safranin for 20 sec (Table 1).

The slide was again washed with distilled water and dried. The preparation was observed under compound microscope at different magnifications for blue-violet stained bacteria representing gram-positive nature (Plate 1) (James and Tod, 1952).

Catalase test

Cultures grown on nutrient agar slants for 24hrs were flooded with 3ml of 3% hydrogen peroxide (H_2O_2) and were observed for the production of gas bubbles. The effervescence indicated the positive catalase activity (Plate 2) (Clarke and Cowan, 1952).

Gelatin hydrolysis

This test was used to determine whether an organism can hydrolyze gelatin by the action of gelatinase enzyme. Test tubes containing gelatin medium was stabbed with loop containing *Bacillus* isolate is inoculated. Uninoculated control and inoculated tubes were incubated at 37˚C for 2-3 days (Plate 3)(Clarke and Cowan, 1952).

Growth in 7% Sodium Chloride

Inoculate tubes of nutrient broth (3 ml/tube) containing 7% (w/v) sodium chloride with a small loopful of *Bacillus* isolates grown in nutrient broth and incubate in slanted position to improve aeriation. Observe for growth after 7 and 14 days incubation (Plate 4) (Jadav *et al.,* 2010).

KOH solubility test

The main principle behind this test is that, the lipopolysaccharides present in the bacterial cell wall gets dissolved in 3% KOH and forms a mucoid thread. A loopfull of *Bacillus* isolate from a well grown colony was mixed in a drop of 3% aqueous KOH solution for not more than 10 seconds with the help of a toothpick. Tooth pick was raised a few centimeters from the slide and was observed for the formation of a mucoid thread. The gram positive *Bacillus* isolates don't produce strands even on repeated strokes of the toothpick (Kumar *et al.,* 2019).

Casein hydrolysis

This test was used to determine whether the bacteria can hydrolyze casein by the action of enzyme casein hydrolase. Nutrient agar medium and skimmed milk powder solution (10%) was sterilized in two different flasks. Both were mixed well before pouring into sterile plates.

The plates were streak inoculated with the *Bacillus* isolates and incubated at 37˚C for 48h. Bacterial cultures were recorded positive if zone of hydrolysis was seen around the colonies (Plate 6) (Osborne and Guest, 1911).

Antimicrobial activity of *Bacillus* isolates

Isolates of *Bacillus* from different crop plants was screened by dual culture technique on the mycelial growth of *Alternaria porri*. The *Bacillus* isolates were initially streaked on the sides of the Potato dextrose agar plates of pH 6.5 as a single line and 5mm diameter mycelia of *Alternaria porri* were kept inoculated on the opposite sides of the streak line of each *Bacillus* isolate. The plates were then

incubated at 30°C for 3 days and further it was observed for the inhibition of the fungal mycelium as described by Berg *et al.,* (2000).

$$Inhibition(\%) = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

Field trials of microalgae and *Bacillus* isolates on onion

Field experiments were carried out in *Rabi* season during the year 2019-20 in the central field at Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj. The experimental layout was a Random Block Design with three replications and six treatments along with control. The seeds were first sown and raised in nursery bed till seedling stage. Twenty eight days old seedlings were transplanted to the main field with 10cm×30cm spacing in 2×2 mplots.

Microalgae was acquired from Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology And Sciences and applied twice @ 3 kg per ha, one week after transplant of seedlings and after 45 days through irrigation. *Bacillus* isolates were sprayed after 45 days of transplanting. Observations were recorded on plant height (cm) at 45 and 60 DAT, bulb radius (cm), bulb weight (g) and disease intensity (%) at 45 and 60 days of transplanting.

Isolation, purification and identification of pathogen

Small pieces of tissues about 3mm from infected collar region with some healthy tissue where cut with sterile scalpel. Microscope was used for the examination of morphology and culture characteristics of fungal structures. The tissues were surface sterilized with one percent sodium hypochlorite solution for 30 sec. The tissues were subsequently washed in three changes of sterile distilled water to eliminate excess sodium hypochlorite and then pieces were transferred to PDA plated petri dishes in presence of laminar air flow chamber. Plates were incubated at 28± 2^0C and were observed periodically for growth of the fungus.

The conidia of *A. porri* is obclavate, borne singly on the tip of conidiophores, rarely in chains. The main body of the conidium was brown, transversely as well as longitudinally septate, number of transverse septa varied in the range of 10-12. The beak was sub-hyaline, simple or forked. The beak was sub-hyaline, simple or forked. The size of conidia from stem and leaf lesion ranged from 105µ x 220.5µ, 17.5µ x 26.0µ Rands (1917).

Rao (1964) observed conidiophores as brown, straight or slightly bent, 2-6 septate, measured. Conidia were pale brown, 7-9 transverse septa, 0-3 vertical septa, thin beak.

Disease intensity

According to Singh *et al.*(1982) disease intensity given on scale 0-9 was used to record. Disease intensity (%) was calculated by using the following formula:

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of rating} \times \text{Maximum disease grade}} \times 100$$

Score Description

0 No incidence
1 1 – 10 %
3 11 – 25 %
5 26 – 50 %
7 51 – 75 %
9 76 - 100 %

Results and Discussion

In vitro evaluation of *Bacillus* native isolates on the radial growth (mm) of *Alternaria porri*

The antagonistic effects of six native *Bacillus* isolates *viz.*Bs1, Bto2, Bs3, Bt4, Bd5 and Bc6on growth of *A. porri* was studied *in vitro* by dual culture method as explained under. The results are presented in Table 3.

The results revealed that the antagonists significantly reduced the growth of *A. porri* either by competition (over growing) or by antibiosis (exhibiting inhibition zones) or by and cell wall degrading enzymes.

Mycelial growth (mm) of *Alternaria porri*

The data presented in table 3 and depicted in figure 1 reveals that at 48h all the *Bacillus* isolates significantly reduced the radial growth (mm) of *Alternaria porri* from control. Anyway these isolates are (T_2, T_4, T_5) (T_5, T_3) are not significantly differ from each other. However, they are significantly reduced from control.

At 72 hall the *Bacillus* isolates significantly reduced the radial growth (mm) of *Alternaria porri* from control. Anyway these isolates are (T_4, T_2, T_5) (T_5, T_3, T_6) not significantly differ from each other. However, they are significantly reduced from control (Fig. 2).

Table.1 Morphological characterization of *Bacillus* isolates

Sr. No	Isolate	Gram reaction	Cell shape	Surface	Color
1	Br1	Positive	Rods	Irregular & wrinkled	Cream
2	Bto2	Positive	Rods	Dull	White
3	Bs3	Positive	Rods	Rough	Brown
4	Bt4	Positive	Rods	Smooth	White
5	Bd5	Positive	Rods	Moderately dull	White
6	Bc6	Positive	Rods	Wrinkled	Brown

Table.2 Isolation and identification of *Bacillus* isolates through Bio- chemical tests

Bio chemical test	*Bacillus* native isolates					
	Br1	Bto2	Bs3	Bt4	Bd5	Bc6
Catalase	+	+	+	+	+	+
Growth at 50̊c	+	-	-	+	-	+
Growth in 7% NaCl	+	+	+	+	+	+
Starch hydrolysis	+	+	+	-	+	+
Growth at 65̊c	-	-	-	-	-	-
Casein hydrolysis	+	+	+	+	+	+
Potassiumhydroxide (KOH) test	-	-	-	-	-	-
NO_3 reduced to NO_2	+	+	-	-	+	+
Gelatin hydrolysis	+	+	+	+	+	+

+ Positive; - Negative.

(Br1 = *Bacillus* rose, Bto2 = *Bacillus* tomato, Bs3 = *Bacillus* spinach, Bt4 = *Bacillus* turnip, Bd5 = *Bacillus* drumstick, Bc6 = *Bacillus* cabbage crops)

Table.3 Effect of *Bacillus* isolates on the radial growth (mm) of *Alternaria porri* at 48, 72, 96hrs of inoculation

Radial growth (mm) of three replication mean						
Treatments		At 48hrs	At 72hrs	At 96 hrs	Mean radial growth (mm)	% of inhibition over control
		Mean radialgrowth	Mean radial growth	Mean radial growth		
T_0	Control	39.33	51.00	62.16	50.83	
T_1	Br1	28.00	36.33	45.00	36.44	28.27
T_2	Bto2	33.66	41.33	50.33	41.44	18.47
T_3	Bs3	31.00	39.33	47.33	39.22	22.84
T_4	Bt4	33.33	41.50	51.16	41.99	17.39
T_5	Bd5	32.33	40.33	47.50	40.05	21.20
T_6	Bc6	29.50	38.33	46.50	38.11	25.02
S.E(m)±		0.58	0.86	0.79		
C.D		1.32	1.90	1.76		

Table.4 Effect of microalgae and *Bacillus* isolates on disease intensity (%) of purple blotch and plant growth parameters of onion at 45 and 60 days after transplanting

	Treatments	**Mean of three replications**						
		Plant height (cm)		**Bulb radius (cm)**	**Bulb weight (g)**	**Disease intensity**		
		45 DAT	**60 DAT**			**45DAT**	**60DAT**	**% reduction over control**
T_0	Control	39.00	43.66	1.66	38.66	31.65	62.65	
T_1	Ma + Br1	53.33	61.66	2.50	56.80	29.86	37.83	39.61
T_2	Ma + Bto2	49.66	58.00	2.23	51.86	30.86	43.64	30.35
T_3	Ma+ Bs3	53.00	58.66	2.50	56.00	31.99	38.96	37.60
T_4	Ma+ Bt4	49.66	55.33	2.13	49.86	32.54	46.96	25.26
T_5	Ma + Bd5	51.00	56.66	2.35	54.40	35.66	44.54	28.86
T_6	Ma + Bc6	52.66	57.00	2.43	55.66	28.54	41.21	34.22
S.E(m)±		3.05	2.12	0.218	3.32	1.39	1.69	
C.D		6.75	4.54	0.480	7.13	2.37	3.54	

Fig.1 Isolated *Bacillus* spp.

Fig.2 Effect of *Bacillus* native isolates on the radial growth (mm) of *Alternaria porri* after 48, 72, 96 h of dual culture

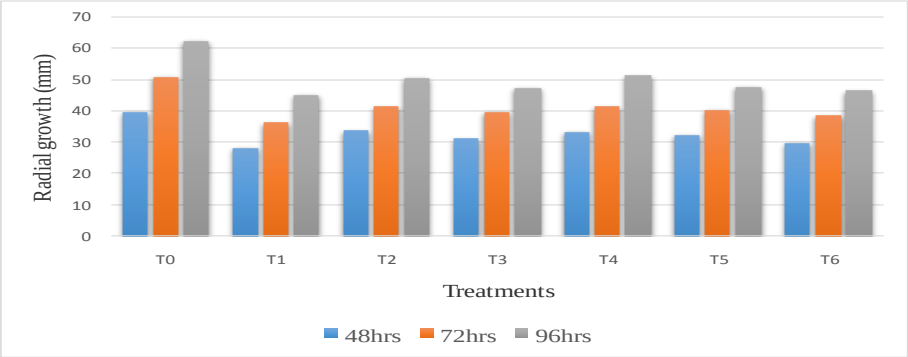


Fig.3 Percent disease intensity of *Alternaria porri* at 45, 60 DAT

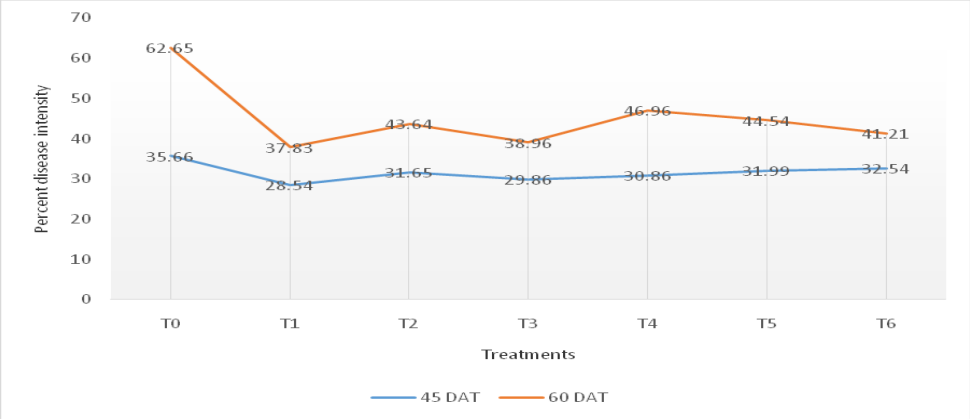


Plate.1 Microscopic view of *Bacillus*

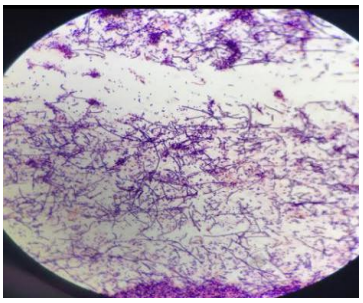


Plate.2 Catalase test

Plate.3 Gelatin Hydrolysis

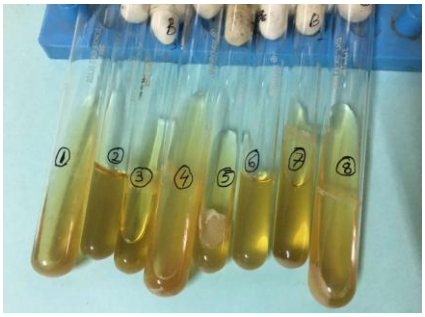


Plate.4 Growth in 7% NaCl

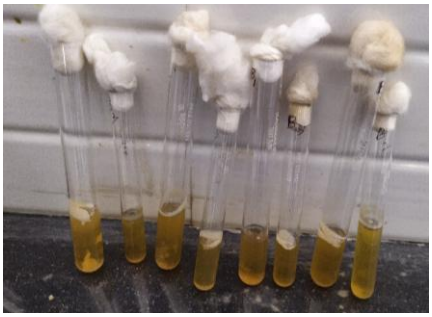


Plate.5 Casein Hydrolysis

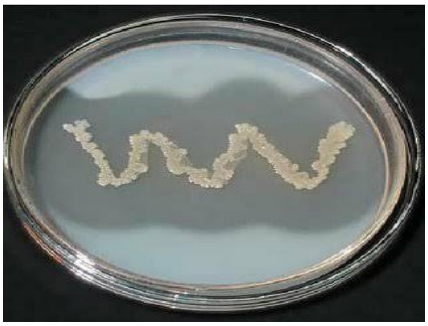
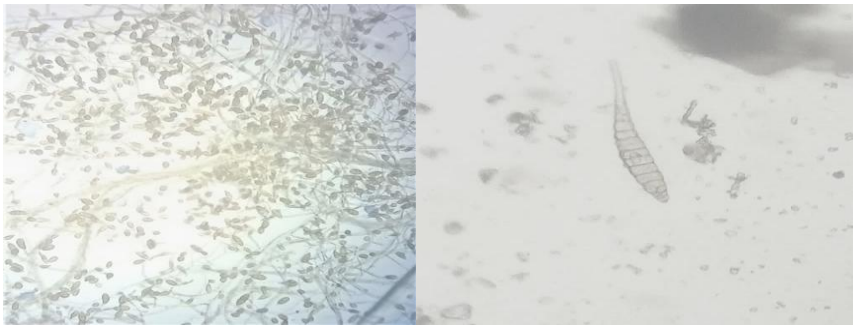


Plate.6 Conidia of *Alternaria porri*



A) Under (10X) **B) Under (40X)**

At 96h all the *Bacillus* isolates significantly reduced the radial growth (mm) of *Alternaria porri* from control. The treatments of *Bacillus* isolates (T_4, T_2) (T_5, T_3, T_6) (T_3, T_6, T_1) not significantly differ from each other. However, they are significantly reduced from control.

Plant height (cm)

The data presented in table 4 reveals that at 45 days all the *Bacillus* isolates significantly increased the plant growth (cm) of onion at 45 DAT from control. Were as among the treatments are found not significant from each other. However, maximum plant height was found in T_1 (Ma+Br)

At 60 days the data presented in table 4 reveals that all the *Bacillus* isolates significantly increased the plant growth (cm) of onion at 60 DAT from control. The treatments of *Bacillus* Isolate (T_4, T_5, T_6, T_2, T_3) (T_5, T_6, T_2, T_3, T_1) are not significantly differ from each other. However, they are significantly increased from control.

The data of bulb radius (cm)presented in table 4 reveals that all the *Bacillus* isolates significantly increased from control under field condition. The treatments of *Bacillus* Isolate (T_4, T_2, T_5, T_6, T_1, T_3) are not significantly differ from each other.

The data of bulb weight (g) presented in table 4 reveals that all the *Bacillus* isolates significantly increased from control under field condition. The treatments of *Bacillus* Isolate (T_4, T_2, T_5, T_3, T_6, T_1) are not significantly differ from each other.

Disease intensity

The data of disease intensity presented in table 4 and depicted in figure 3 reveals that all the *Bacillus* isolates significantly reduced from control. However, (T_1, T_3, T_6) (T_6, T_2, T_5) (T_2, T_5, T_4) are not significantly much differ from each other.

The present study, concluded clearly that all the *Bacillus* isolates performed better but the treatment with Br1 was most significant among all the treatments against *Alternaria porri.* Moreover all the isolates have shown the growth promoting characteristics on the onion seedlings in the field trials with significant growth. Thus *Bacillus* spp. play an important role in controlling the soil borne fungal pathogens which can be used as a bio-agent. It is considered as beneficial and eco-friendly.

References

Ali, G. S., Ashraf, S. A., El-Sayed., Patel, S. J., Green, K. B., Mohamad Ali., Brennam, M., and Norman, D. (2016). *Ex vivo* application of secreted metabolites produced by soil inhibiting *Bacillus* spp. Efficiently Controls Foliar Diseases Caused by *Alternaria* species. *Applied and Environmental Microbiology*, 82(2).

Berg, V. D. H., Faulks, R., Granado, H., Hirschberg, J., Olmedilla, B., Sandmann, G., Southon, S., Stahl, W. (2000). The potential for the improvement of carotenoid levels in food and the likely systemic effects. *Journal of Science, Food and Agriculture*, 80(7): 880-912.

Bergey's manual of determinative bacteriology (1930), 3rd edition, The Williams and Wilkins Co., Baltimore, 589 p..

Clarke, P. H., and Cowan, S. T. (1952). Biochemical Methods for Bacteriology. *Journal of genetic Microbiology*, 6:187-197.

EBIC (2014), http://www.biostimulants.eu/.

Francis, A. D. (1993). *Bacillus cereus* and Related Species. *Clinical Microbiology*

Reviews, 6(4): 324-338.

Gomes, N. C. M., Heuer, H., Schonfeld, J., Costa, R., Mendonc-a-Hagler, L.C., Smalla, K. (2001). Bacterial diversity of maize (*Zea mays*) grown in tropical soil studied by temperature gradient gel electrophoresis. *Plant and Soil*, 232: 167–180.

Jadhav, G. G., Salunkhe, D.S., Nerka,r D.P. and Bhadekar, R.K. (2010). Isolation and characterization of salt-tolerant nitrogen-fixing microorganisms from food. Journal of *European Asian Biological Sciences*, 4: 33-40.

James, W. B., and Tod, M. (1952). The gram stain. Department of Bacteriology, University of Southern California, Los Angeles 7, California.

Katz, E. and Demain, A.L. (1977). The peptide antibiotics of *Bacillus*: chemistry, biogenesis, and possible role. *Bacteriological Reviews*, 41: 449-474.

Kokalis-Burelle, N., Vavrina, C. S., Rosskopf, E. N., and Shelby, R. A., (2002) Field evaluation of plant growth-promoting rhizobacteria amended transplant mixes and soil solarization for tomato and pepper production in Florida. *Plant and Soil*, 238: 257-266.

Kulik, M. M. (1995). The potential for using Cyanobacteria (blue-green algae) and algae in the biological control of plant pathogenic bacteria and fungi. *European Journal of Plant Pathology*,101: 585-599.

Kumar, A. K. M., Chandan, S., and Shivaraju, H. P. (2019). Potential Antimicrobial and Antioxidant Activity of *Saraca indica* and *Clerodendrum paniculatum* from Western Ghats, Shimoga, India. *International Journal of Environmental Health and Technology*, 2581-4516.

Mansour, A., Rakhisi, Z., and Ahmady, A. Z. (2014). Isolation and Identification of *Bacillus* Species from Soil and Evaluation of Their Antibacterial Properties. *Avicenna Journal of Clinical Microbiology Infection*, 2(1): e23233.

Osborne, T. B., and Guest, H. H. (1911). Hydrolysis of casein. *Journal of Biological Chemistry*,9: 333-353.

Rands, R. D. (1917). The production of spores by *Alternaria solani* in pure culture. *Phytopathology*, 7: 316-317.

Rao, V. G. (1964). Two new species of *Alternaria* on economic host from India. *Sydowia Annales Mycological Beihefte*, II. 17: 70-72.

Sandhu (1981).Efficasy of bioagents and plant extract against *Alternaria porri* causing purple blotch of onion. Department of plant pathology, College of Agriculture V.N.M.A.U, Latur, MS.

Tarakhovskaya, E.R., Maslov, Y.I., Shishova, M.F. (2007) Phytohormones in algae. *Russian Journal of Plant Physiology*, 54: 163–170.

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