J. Soils and Crops 31 (2) 219-224, December 2021

ISSN 0971-2836 (Print)
ISSN 2582-2756 (Online)

IN VITRO EVALUATION ON POPULATION DYNAMICS OF *Pseudomonas fluorescens* IN SUPPRESSION OF BACTERIAL BLIGHT OF RICE (*Xanthomonas oryzae* PV. *oryzae*) ENRICHED WITH MICRONUTRIENTS

P. Sharma[1], L.C. Bora[2] and P. Bora[3]

ABSTRACT

Bacterial blight (BB) incited by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is regarded as one of the most important disease of rice crop in Assam. Management practices, such as chemical control, host-plant resistance, modification of cropping systems, and biological control have been employed to reduce damage caused by BB, but most of these practices have their own limitations. The effect of few micronutrient supplements on growth and aggressiveness of *Pseudomonas fluorescens* was evaluated in the present investigation during 2015-16 at department of Plant Pathology, Assam Agricultural University, Jorhat. Four micronutrients *viz*., Zinc (Zn), Boron (B), Molybdenum (Mo) and Iron (Fe) were tested *in vitro* at different concentrations *viz.*, 5, 10, 50, 100 and 500 ppm for their relative effect on aggressiveness of *P. fluorescens* against the bacterial pathogen. Zn (50 ppm) was found to support the growth and activity of *P. fluorescens* AS1 showing higher population dynamics ($7.55x10^9$ cfu ml^{-1}) followed by Fe @ 50 ppm (71.66×10^8 cfu ml^{-1}) up to 60 days.

(Key words: Rice, bacterial blight, *Xanthomonas oryzae* pv. *oryzae*, *Pseudomonas fluorescens*, micronutrient)

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops in the world, feeding about half of humanity with a worldwide production of 755.47 million metric tons in an area of 162.06 million hectares with the productivity of 4.66 metric tons hectare $^{-1}$ (Anonymous, 2020). Among the rice growing countries in the world, India has the largest area under rice crop and ranks second in production next to China. In India, rice is an important part of the diet of its people and cultivation of rice is the main occupation of those engaged in agriculture. The crop is regarded as the second major cereal crop of India after wheat with a production of 116.42 million tonnes in an area of 43.79 million hectares with a productivity of 2.66 tonnes hectare^{-1} (Anonymous a , 2020), which share 43 % of total food grain production and 46 % of total cereal production in the country. In Assam rice occupies about two-third of the total cropped area in the state. Rice is highly vulnerable at all stages of growth to different pathogens that affect the quality and quantity of its yield. Bacterial blight caused by *X. oryzae* pv. Oryzae is one of the most important and oldest known diseases of rice. The bacterial pathogen infects at the maximum tillering stage of the crop, resulting in 20-40 per cent reduction in yields. Various disease management practices, such as chemical control, host-plant resistance, modification of cropping systems, and biological control have been employed to reduce damage caused by bacterial blight (BB).

Indiscriminate use of synthetic pesticides in crop protection programmes around the world resulted in disturbances of the environment (Samrit *et al*., 2020), and host-plant resistance which is based on a single gene may not be durable in the field leading to frequent resistance breakdowns. Biological control is an ecology-conscious, cost-effective, and sustainable alternative method in BB management. This approach can also be integrated with other management practices to afford greater levels of protection and sustain rice yields. Antagonistic bacteria used in biological control are easy to handle, grow rapidly, and colonize the rhizosphere aggressively (Weller, 1988). Research of Duffy and Défago (1997) has been identified zinc as a factor to improve the biocontrol ability of *Pseudomonas fluorescens* . Study of Sharma *et al*. (2020) revealed that *P. fluorescens* strain PfAs1 enriched with 50 ppm Zn could suppress the blight pathogen by 45.92% compared to control and other micronutrients under *in-vitro* condition. Similarly Iron (Fe) is important for siderophore production, a major mechanism of antagonism by *P. fluorescens* ((Bora *et al*., 2016). There are other reports of micro and macro element amendments used commercially on a limited scale to manage certain soil borne diseases (Engelhard, 1989). Trace mineral amendments may appear as an inexpensive way to improve the biocontrol activity of

1. Asst. Professor, Dept. of Plant Pathology, SCSCA Assam Agricultural University, Dhubri-783371, Assam
2. Ex. Professor and Head, Dept. of Plant Pathology, Assam Agricultural University, Jorhat-785013, Assam
3. Jr. Scientist, Dept. of Plant Pathology, Assam Agricultural University, Jorhat-785013, Assam

certain bacterial strains. Formulations that efficiently supply minerals to the target strain may further improve their availability and effect on biocontrol, which means lower doses and reduced costs. In our present study effect of few micronutrient supplements on growth and aggressiveness of the antagonist *P. fluorescens* was evaluated.

MATERIALS AND METHODS

Isolation of *P. fluorescens* was made from rhizospheric soils collected from different rice fields located under different geographical locations of Assam. Soil samples loosely adhering to the roots were used to isolate different isolates of *P. fluorescens* following serial dilution and streak plate technique (Vincent, 1970). The bacterial blight pathogen *X. oryzae* pv. *oryzae* (*Xoo*) was isolated following standard protocols using modified Wakimoto's and sucrose peptone agar (SPA) media specific for *Xoo* (Fahy and Persley, 1983). All the isolated organisms were preserved in refrigerator at 4°C for subsequent experimentations.

Effect of different micronutrients on population dynamics of *P. fluorescens* and *Xoo* after different incubation periods

The population dynamic of *P. fluorescens* and *Xoo* was tested at five different concentrations, *viz*., 5, 10, 50, 100 and 500 ppm in SPA broth as basal with four sets of incubation periods (7, 30, 60, and 90 days). The given concentrations of each micronutrient was tested in three experimental sets *viz.,* micronutrient + *P. fluorescens* alone; micronutrients + *Xoo* alone and micronutrients + *P. fluorescens* + *Xoo.* The stock solutions of each of the micronutrient were prepared separately by dissolving the required quantity in distilled water and sterilized by filtration (0.2 µm pore). SPA broth was prepared and dispensed into 252 culture tubes @ 30 ml each and were sterilized by autoclaving. To these tubes suitable amount of micronutrient solution from stock was mixed to get the required concentration. To this 1 ml aliquot each of *Xoo* and *P. fluorescens* from 48 h old stock bacterial suspensions were dispensed representing separate treatment combination. The culture tubes were then incubated at 28 ± 1^0C for 7 days, 30 days, 60 days and 90 days respectively. The microbial population in the culture tubes were enumerated by dilution plate technique.

RESULTS AND DISCUSSION

Effect of different micronutrients on population dynamic *P. fluorescens* AS1 and *Xoo* after different incubation periods

The micronutrient enrichment of *P. fluorescens* AS1 at different concentrations significantly affected the population count of *P. fluorescens* AS1 incubated for 7, 30, 60, and 90 days (Table 1a). Significant increase in population of the bacterial antagonist was observed in plates enriched with Zn at a concentration of 5, 10, 50 and 100 ppm after various incubation periods. However, a decline in the population of *P. fluorescens* AS1 was observed after addition of Zn @ 500 ppm in different days of incubation. At 60 days after incubation 62.87, 69.85, 75.50 and 62.66 x 10^8 cfu ml^{-1} was recorded in plates enriched with Zn @ 5, 10, 50 and 100 ppm respectively. Whereas, in untreated plates the population was 37.56, 49.66, 56.50 and 48.85 (x 10^8 cfu ml^{-1}) after 7, 30, 60, and 90 days of incubation respectively. Highest population of *P. fluorescens* AS1 was recorded in Zn @ 50 ppm (75.50 x 10^8 cfu ml^{-1}) followed by Fe @ 50 ppm (71.66 x 10^8 cfu ml^{-1}) after 60 days of incubation. In plates enriched with B, a slight increase in population observed at the concentrations of 5 and 10 ppm in all sets of incubation periods after which it declined. Similarly Fe @ 5, 10 and 50 ppm could also be enhanced the population of *P. fluorescens* AS1 in all the four incubation periods tested. In contrast to this, Mo showed incompatibility with *P. fluorescens* AS1 and reduced the bacterial population over control in all concentrations and incubation periods. From the data it is apparent that after 90 days of incubation there was a slight decline in population count irrespective of the micronutrients and their concentrations (Table 1a).

Effect of micronutrients on population dynamics of *Xoo*

All the tested micronutrients have negative effects on growth of *Xoo* at all concentrations and incubation periods (Table 1b). Maximum growth inhibition was observed in Zn at all the concentrations (@ 5, 10, 50, 100 and 500 ppm), where population count of *Xoo* reduced to 20.81, 21.50, 19.50, 23.33 and 25.05 (x 10^8 cfu ml^{-1}) respectively as compared to untreated control (61.45 x 10^8 cfu ml^{-1}) after 90 days of incubation. This was followed by Fe with population count of 25.17, 23.20, 21.93, 25.52 and 26.13 x 10^8 cfu ml^{-1}; B with population count of 29.56, 30.06, 29.63, 30.86 and 33..54 x 10^8 cfu ml^{-1} over control. Mo showed less suppression of *Xoo* populations at all concentrations and incubation periods.

Effect of micronutrients on population dynamic of *P. fluorescens* AS1 and *Xoo* in co-culture

Highest inhibition of *Xoo* growth in broth was recorded in all the tested incubation periods when *Xoo* containing flasks were inoculated with *P. fluorescens* AS1 along with Zn 50 ppm. In this treatment considerably high population of *P. fluorescens* AS1 (60.50, 76.50, 120.50 and 105.52 x 10^8 cfu ml^{-1}) was observed in all four incubation periods (7, 30, 60, and 90 days respectively). A lower population count of 12.81, 11.11, 9.19 and 6.03 x 10^8 cfu ml^{-1} of *Xoo* was recorded in treatment with *P. fluorescens* AS1 + Fe @ 50 ppm respectively at 7, 30, 60, and 90 days of incubation. In this treatment also significantly high *P. fluorescens* AS1 population was recorded after all periods of incubations. It is apparent from the data that combining *P. fluorescens* AS1 with Zn (50 ppm) or Fe (50 ppm) augments the inhibitory properties against the BLB pathogen than they were applied alone (Table 1c).

It was reported that mineral amendments can create a more favourable environment for biocontrol to occur; leading to disease management with modification of mineral

Table 1a. Population of *P. fluorescens* AS1 on micronutrients enriched media at various concentrations and at different days of incubation

Treatments	Population of *P. fluorescens* (x10^8cfu ml^{-1})			
	Days of incubation			
	7 days	30 days	60 days	90 days
Pf AS1 (Control)	37.56	49.66	56.50	48.85
	(6.13)	(7.05)	(7.52)	(6.99)
Pf AS1 + Zn (5ppm)	43.37	51.66	62.87	54.66
	(6.59)	(7.19)	(7.93)	(7.39)
Pf AS1 + Zn (10ppm)	45.66	53.66	69.85	58.66
	(6.76)	(7.33)	(8.36)	(7.66)
Pf AS1 + Zn (50ppm)	50.07	58.66	75.50	60.66
	(7.08)	(7.66)	(8.69)	(7.79)
Pf AS1 + Zn (100ppm)	37.66	50.93	62.66	55.00
	(6.14)	(7.14)	(7.92)	(7.42)
Pf AS1 + Zn (500ppm)	31.64	43.66	51.80	45.86
	(5.63)	(6.61)	(7.20)	(6.77)
Pf AS1 + Mo (5ppm)	33.66	41.66	52.86	44.50
	(5.80)	(6.45)	(7.27)	(6.67)
Pf AS1 + Mo	35.33	49.66	55.92	38.50
(10ppm)	(5.94)	(7.05)	(7.48)	(6.20)
Pf AS1 + Mo (50ppm)	32.94	38.66	45.66	30.33
	(5.74)	(6.22)	(6.76)	(5.51)
Pf AS1 + Mo	31.16	34.83	42.16	32.50
(100ppm)	(5.58)	(5.90)	(6.49)	(5.70)
Pf AS1 + Mo	28.69	32.33	31.35	25.93
(500ppm)	(5.36)	(5.69)	(5.60)	(5.09)
Pf AS1 + B (5ppm)	39.5	51.0	62.29	54.66
	(6.28)	(7.14)	(7.89)	(7.39)
Pf AS1 + B (10ppm)	40.33	53.66	59.92	48.50
	(6.35)	(7.33)	(7.74)	(6.96)
Pf AS1 + B (50ppm)	36.72	42.66	48.58	39.50
	(6.06)	(6.53)	(6.97)	(6.29)
Pf AS1 + B (100ppm)	33.82	37.80	42.16	34.00
	(5.82)	(6.15)	(6.49)	(5.83)
Pf AS1 + B (500ppm)	29.84	30.0	32.16	26.89
	(5.46)	(5.48)	(5.67)	(5.19)
Pf AS1 + Fe (5ppm)	41.33	49.0	61.15	53.02
	(6.43)	(7.00)	(7.82)	(7.28)
Pf AS1 + Fe (10ppm)	43.57	52.64	65.11	55.00
	(6.60)	(7.26)	(8.07)	(7.42)
Pf AS1 + Fe (50ppm)	47.12	56.06	71.66	58.88
	(6.86)	(7.49)	(8.47)	(7.67)
Pf AS1 + Fe (100ppm)	35.78	47.29	60.99	54.58
	(5.98)	(6.88)	(7.81)	(7.39)
Pf AS1 + Fe	30.16	41.90	50.03	39.91
(500ppm)	(5.49)	(6.47)	(7.07)	(6.32)
SEd±	0.045	0.037	0.037	0.037
CD 5%	0.075	0.062	0.062	0.062

* Data in the parenthesis are root over transformed values

Pool means of 2 years data

Table 1b. Population of *X. oryzae* pv *oryzae* on micronutrients enriched media at various concentrations and at different days of incubation

Treatments	Population of *Xoo* (x 10^8cfu ml^{-1})			
	Days of incubation			
	7 days	30 days	60 days	90 days
Xoo (Control)	39.50	52.70	75.11	61.45
	(6.29)	(7.26)	(8.67)	(7.84)
Xoo + Zn (5 ppm)	34.50	31.66	29.83	20.81
	(5.87)	(5.63)	(5.46)	(4.56)
Xoo + Zn (10 ppm)	31.66	30.66	28.73	21.50
	(5.63)	(5.54)	(5.36)	(4.64)
Xoo + Zn (50 ppm)	33.53	30.00	27.72	19.50
	(5.79)	(5.48)	(5.26)	(4.42)
Xoo + Zn (100 ppm)	34.94	31.80	29.87	23.33
	(5.91)	(5.64)	(5.46)	(4.83)
Xoo+ Zn (500 ppm)	36.45	33.24	30.87	25.05
	(6.04)	(5.77)	(5.56)	(5.00)
Xoo + Mo (5 ppm)	38.77	37.79	35.04	31.01
	(6.23)	(6.15)	(5.92)	(5.57)
Xoo + Mo (10 ppm)	38.40	36.86	36.08	34.44
	(6.20)	(6.07)	(6.01)	(5.87)
Xoo + Mo (50 ppm)	37.59	35.98	34.66	31.43
	(6.13)	(5.60)	(5.89)	(6.61)
Xoo + Mo (100 ppm)	38.89	37.00	35.94	32.37
	(6.24)	(6.08)	(5.99)	(5.69)
Xoo + Mo (500 ppm)	39.75	39.19	38.94	34.56
	(6.30)	(6.26)	(6.24)	(5.88)
Xoo + B (5 ppm)	37.00	35.73	30.15	29.56
	(6.08)	(5.98)	(5.49)	(5.44)
Xoo + B (10 ppm)	36.71	35.19	31.98	30.06
	(6.06)	(5.93)	(5.66)	(5.48)
Xoo + B (50 ppm)	35.87	33.19	31.73	29.63
	(5.99)	(5.76)	(5.63)	(5.44)
Xoo + B (100 ppm)	38.72	37.67	35.15	30.86
	(6.22)	(6.14)	(5.93)	(5.56)
Xoo + B (500 ppm)	38.83	37.48	35.02	33.54
	(6.23)	(6.12)	(5.92)	(5.79)
Xoo + Fe (5 ppm)	35.90	32.91	30.51	25.17
	(5.99)	(5.74)	(5.52)	(5.02)
Xoo + Fe (10 ppm)	35.80	31.79	29.47	23.20
	(5.98)	(5.64)	(5.43)	(4.82)
Xoo + Fe (50 ppm)	34.66	31.27	29.23	21.93
	(5.89)	(5.59)	(5.41)	(4.68)
Xoo + Fe (100 ppm)	38.65	34.18	30.26	25.52
	(6.22)	(5.85)	(5.50)	(5.05)
Xoo + Fe (500 ppm)	39.15	34.43	31.77	26.13
	(6.26)	(5.87)	(5.64)	(5.11)
SEd±	0.037	0.037	0.026	0.045
CD 5%	0.062	0.062	0.044	0.075

* Data in the parenthesis are root over transformed values

Pool means of 2 years data

Table 1c. Population of *P. fluorescens* isolates and *X. oryzae* pv *oryzae* assayed from different micronutrient enriched treatments after different days of incubation

Treatments		Population of *Xoo* (x 10^8cfu ml^{-1})			
		Days of incubation			
		7 days	30 days	60 days	90 days
Pf AS1 + *Xoo* + Zn (5 ppm)	Pf	44.32 (6.66)	58.66 (7.66)	69.90 (8.36)	56.10 (7.49)
	Xoo	30.50 (5.52)	29.50 (5.43)	23.43 (4.84)	19.96 (4.47)
Pf AS1 + *Xoo* + Zn (10 ppm)	Pf	46.33 (6.81)	65.66 (8.10)	72.82 (8.53)	61.50 (7.84)
	Xoo	29.20 (5.40)	23.76 (4.87)	14.48 (3.81)	8.50 (2.92)
Pf AS1 + *Xoo* + Zn (50 ppm)	Pf	60.50 (7.78)	76.50 (8.75)	120.50 (10.98)	105.52 (10.27)
	Xoo	0.50 (0.71)	0.50 (0.71)	0.50 (0.71)	0.50 (0.71)
Pf AS1 + *Xoo* + Zn (100 ppm)	Pf	43.20 (6.57)	59.88 (7.74)	69.22 (8.32)	58.50 (7.65)
	Xoo	30.15 (5.49)	22.83 (4.78)	19.12 (4.37)	11.56 (3.40)
Pf AS1 + *Xoo* + Zn (500 ppm)	Pf	35.54 (5.96)	48.50 (6.96)	61.38 (7.83)	45.78 (6.77)
	Xoo	31.50 (5.61)	29.81 (5.46)	26.04 (5.10)	23.21 (4.82)
Pf AS1 + *Xoo* + Mo (5 ppm)	Pf	34.00 (5.83)	44.66 (6.68)	57.82 (7.60)	49.23 (7.02)
	Xoo	33.20 (5.76)	31.88 (5.65)	30.17 (5.49)	29.87 (5.47)
Pf AS1 + *Xoo* + Mo (10 ppm)	Pf	37.50 (6.12)	53.50 (7.31)	62.88 (7.93)	52.50 (7.25)
	Xoo	35.26 (5.94)	33.17 (5.76)	32.09 (5.66)	31.00 (5.57)
Pf AS1 + *Xoo* + Mo (50 ppm)	Pf	34.58 (5.88)	42.50 (6.52)	53.50 (7.31)	40.66 (6.38)
	Xoo	34.62 (5.88)	33.08 (5.75)	32.00 (5.66)	31.17 (5.58)
Pf AS1 + *Xoo* + Mo (100 ppm)	Pf	35.91 (5.99)	40.95 (6.40)	52.41 (7.24)	41.00 (6.40)
	Xoo	35.00 (5.92)	36.00 (6.00)	34.29 (5.86)	32.14 (5.67)
Pf AS1 + *Xoo* + Mo (500 ppm)	Pf	31.69 (5.63)	36.50 (6.04)	37.55 (6.13)	29.91 (5.47)
	Xoo	31.50 (5.61)	30.20 (5.50)	29.72 (5.45)	28.13 (5.30)
Pf AS1 +*Xoo* + B (5 ppm)	Pf	41.66 (6.45)	57.50 (7.58)	70.39 (8.39)	57.50 (7.58)
	Xoo	34.29 (5.86)	33.97 (5.83)	30.20 (5.50)	27.63 (5.26)
Pf AS1 + *Xoo* + B (10 ppm)	Pf	46.50 (6.82)	59.50 (7.71)	76.78 (8.76)	62.50 (7.91)
	Xoo	34.95 (5.91)	32.99 (5.74)	30.09 (5.49)	26.79 (5.18)
Pf AS1 + *Xoo* + B (50 ppm)	Pf	43.88 (6.62)	56.50 (7.52)	68.63 (8.28)	49.50 (7.04)
	Xoo	35.06 (5.92)	33.24 (5.77)	30.99 (5.57)	28.54 (5.34)
Pf AS1 + *Xoo* + B (100 ppm)	Pf	39.99 (6.32)	43.76 (6.62)	52.10 (7.22)	43.79 (6.62)
	Xoo	37.66 (6.14)	36.50 (6.04)	34.31 (5.86)	31.10 (5.58)

PTO

PfAS1 + *Xoo* + B (500 ppm)	Pf	36.89 (6.07)	40.00 (6.32)	47.06 (6.86)	36.66 (6.05)
	Xoo	35.67 (5.97)	36.35 (6.03)	34.12 (5.84)	32.91 (5.74)
PfAS1 + *Xoo* + Fe (5 ppm)	Pf	47.67 (6.90)	59.66 (7.72)	71.26 (8.44)	62.79 (7.92)
	Xoo	30.15 (5.49)	29.89 (5.47)	27.88 (5.28)	23.14 (4.81)
PfAS1 + *Xoo* + Fe (10 ppm)	Pf	48.42 (6.96)	62.72 (7.92)	75.14 (8.67)	67.50 (8.22)
	Xoo	31.74 (5.63)	30.79 (5.55)	29.05 (5.39)	19.94 (4.47)
PfAS1 + *Xoo* + Fe (50 ppm)	Pf	49.12 (7.01)	66.08 (8.13)	94.85 (9.74)	81.50 (9.03)
	Xoo	12.81 (3.58)	11.11 (3.33)	9.19 (3.03)	6.03 (2.46)
PfAS1 + *Xoo* + Fe (100 ppm)	Pf	38.90 (6.24)	51.43 (7.17)	62.13 (7.88)	55.65 (7.46)
	Xoo	33.51 (5.79)	31.26 (5.59)	23.21 (4.82)	19.46 (4.41)
PfAS1 + *Xoo* + Fe (500 ppm)	Pf	33.96 (5.83)	44.92 (6.70)	56.16 (7.49)	41.14 (6.41)
	Xoo	36.07 (6.01)	33.64 (5.80)	28.63 (5.35)	24.38 (4.94)
SEd±		0.026	0.026	0.037	0.026
CD 5%		0.043	0.043	0.062	0.043

* Data in the parenthesis are root over transformed values Pool means of 2 years data

fertilization regimes. Moreover, Jondhale *et al.* (2021) reported that grain and straw yield of rice significantly increased to the tune of 15 and 16% respectively with the application of zinc.

Hence, integration of mixture of bio-agents and antibiotic biosynthesis are the main tools for improvement of the efficacy of *P. fluorescens* and other biocontrol agents. In the present experiment we introduced a new research area for integration of micronutrients for enhancement of aggressiveness of bioagents. Zinc improved the biocontrol activity of *P. fluorescens* and enhanced the efficacy in performance of *P. fluorescens*.

REFERENCES

Anonymous, 2020.Food and Agriculture Organization of United Nations. www.fao.org

Anonymous a, 2020. Agriculture statistics at a glance 2019. Directorate of Economics and Statistics. Govt. of India.www.agricoop.nic.in pp.48-49.

Bora, P., L. C. Bora and P. C. Deka, 2016. Efficacy of substrate based bioformulation of microbial antagonists in the management of bacterial disease of some solanaceous vegetables in Assam. J. Biol. Cont. **30**(1): 49–54.

Duffy, B.K. and G. Défago, 1997. Zinc improves biocontrol of Fusarium crown and root rot of tomato by *Pseudomonas fluorescens* and represses the production of pathogen metabolites inhibitory to bacterial antibiotic biosynthesis. Phytopathology **87**:1250-1257.

Engelhard, A.W. 1989. Soilborne Plant Pathogens: Management of Diseases with Macro and Microelements. APS press, St. Paul.

Fahy, P. C., and G. J. Persley. 1983. Plant Bacterial Diseases, A Diagnostic Guide. Academic Press, New York. pp.393.

Jondhale, D.G., T.J. Bedse, M.R. Wahane, and N.H.Khobragade, 2021. Influence of zinc nutrition on yield, yield attributing characters and economics of rice cultivation. J. Soils and Crops. **31**(1): 83-88.

Samrit, R.M., B.N. Choudhari, G.R.Shankuwar, P.S. Neharkar and H.R.Sawai, 2020.Evaluation of Biopesticides, botanicals and Plant extracts against rice stem borer, Scripophaga incertulus (Walker). J. Soils and Crops. **30** (1): 124-128.

Sharma, P., L.C Bora, P.D Nath, S. Acharjee, P. Bora, and J. M. Vasantrao, 2020. Zinc enriched *Pseudomonas fluorescens* triggered defense response in rice against bacterial leaf blight. Indian J. Agric. Sci. **90**(3): 593-596.

Vincent, J.M. 1970. A manual for Practical Study of Root Nodule Bacteria. Blackwell Scientific Publishing, Oxford.

Weller, D.M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Ann. Rev. Phytopathol .**26** : 379–407.

Rec. on 25.05.2021 & Acc. on 09.06.2021