

Comparative Management Studies on Banded Leaf and Sheath Blight of Maize Caused by *Rhizoctonia Solani F.SP. Sasakii*

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Received October 16, 2020; Revised November 17, 2020; Accepted November 26, 2020

Abstract Bioassay of biocontrol agents and fungicides against Maize Banded Leaf and Sheath Blight caused by *Rhizoctonia solani* showed inhibition. Among the biocontrol agents, *Bacillus subtilis* and among the fungicides Hexaconazole was found to be effective under field conditions with the percent disease incidence of 2.25% and 3.00% respectively over control 10.45%. Seed treatment (4g/kg) and soil application (2.5kg/ha) of *Bacillus subtilis* recorded 78.46% reduction in sheath blight incidence and highest grain yield of 6.7t/ha. Foliar spraying of Hexaconazole (0.2%) showed 71.30% reduction and grain yield of 6.4t/ha. Hence, seed treatment and soil application of *Bacillus subtilis* is considered to be effective against BLSB under field condition. Seed treatment and soil application of *Bacillus subtilis*, Foliar spraying of Hexaconazole may be recommended for Banded Leaf and Sheath Blight of Maize.

Keywords: *Rhizoctonia solani*, banded leaf and sheath blight, biocontrol agents, fungicides, *Bacillus subtilis*, *Trichoderma asperellum*

Cite This Article: Radhajeyalakshmi Raju, Sethuraman Kandhasamy, Lakshmi Narayanan Subramanian, and Arivudai Nambi Marisamy, "Comparative Management Studies on Banded Leaf and Sheath Blight of Maize Caused by *Rhizoctonia Solani F.SP. Sasakii*." *Applied Ecology and Environmental Sciences*, vol. 9, no. 1 (2020): 53-57. doi: 10.12691/aees-9-1-7.

1. Introduction

Maize is one of the important crops in India occupying fifth place in area and 3rd place in production. In India, maize is cultivated in an area of about 8.26 m ha with the production of 19.73 million tonnes and productivity of 2295 kg/ha [1]. Maize banded leaf and sheath blight (BLSB) caused by *Rhizoctonia solani f.sp sasakii* (*Thanatephorus cucumeris*) is considered as one of the most important disease and major constraint for low yields. The banded leaf and sheath blight pathogen is soil-borne and its occurrence has also been recorded several maize growing areas. In India the disease was first recorded in the Tarai (foot hill plain areas) region of Uttar Pradesh [2]. Yield losses vary from 11 to 40 per cent [3]. The disease causes direct losses, resulting in premature death, stalk breakage and ear rot and indirect losses by not only reducing the grain yield but also grain quality in terms of human consumption. In India, losses in grain yield have been estimated in the range of 23.9% and 31.9% [4]. Singh and Sharma [3] estimated 40.5% loss in grain yield with 71% disease index. This pathogen causes losses in grain yield to the extent of over 90 per cent [5]. In view of increasing importance of banded leaf and sheath blight of

maize, the present study was undertaken to investigate the efficacy of fungicides and biocontrol agents against the disease.

2. Materials and Methods

2.1. *In vitro* Antifungal Assays

The present investigation was carried out during 2017 - 2019 at Maize Research Station, Vagarai, Tamil Nadu Agricultural University, which is located at 10.58°N latitude, 77.57°E longitude with an altitude of 254 MSL in the Dindigul region of Tamil Nadu state. The BLSB infected leaf sheaths were collected from the field and the pathogen was isolated. The isolated pathogen was again inoculated in the plants raised in pots for confirmation of the pathogenicity and *In-vitro* experiments were conducted in complete randomized design with three replications.

The biocontrol agents *Trichoderma asperellum* (Tv1) ITCCNO:6914 and *Bacillus subtilis* (Bs1) (Strain No: EPC5) were obtained from Department of Plant Pathology, TNAU, Coimbatore-641003, Tamil Nadu, India for testing against Banded Leaf and Sheath Blight Pathogen. *In vitro* evaluation of bio control agents was

assessed by Dual Culture Plate Technique. An agar disc (6 mm) was taken from 4-day-old PDA culture plates of *Trichoderma asperellam* and a loop of *Bacillus subtilis* placed at the periphery of the PDA plates (9 mm). Another agar disc of the same size of *R. solani* was also placed at the periphery but on the opposing end of the same Petri dish and as a control, *R. solani* was placed alone at the periphery of the PDA plates (9 mm). All pairings were carried out in quadruplicate and incubated at 28°C. Antagonistic activity was tested 4 days after incubation by measuring the radius of the *R. solani* colony in the direction of the antagonist colony (R_2) and the radius of the *R. solani* colony in the control plate (R_1). The two readings were transformed into percentage inhibition of radial growth (PIRG) using the formula developed by Skidmore and Dickinson [23].

On the basis of formulation, five fungitoxicants viz., Carbendazim, Mancozeb, Copper oxychloride, Copper hydroxide, Hexaconazole were evaluated against *R. solani* isolated from Maize Research Station, Vagarai following poisoned food technique [6], percent inhibition of fungal growth by biocontrol agents was calculated against *R. solani* using dual culture technique. Radial growth of the test fungus as well as bioagent was measured after 72hr to determine the antagonistic potential of the bioagents. Percent inhibition of fungal growth was calculated by using formula given by Mc Kinney [7] (Table 1).

Table 1. In vitro efficacy of biocontrol agents and fungicides on Maize Banded Leaf and Sheath Blight caused by *Rhizoctonia solani*

Sl.No.	Treatments	Radial growth (mm)	% Inhibition over control
1	<i>Trichoderma asperellam</i>	66.2	26.44
2	<i>Bacillus subtilis</i>	72.3	19.66
3	Carbendazim	0.0	100
4	Mancozeb	0.0	100
5	Copper oxychloride	20.68	77.02
6	Copper hydroxide	10.23	88.63
7	Hexaconazole	0.0	100
8	Control	90.0	-
	CD (p=0.05)	0.032	

2.2. *Rhizoctonia Solani* Inoculum Preparation and Soil Application

Pure culture of *R. solani* was obtained from infected maize leaf sheaths by following standard isolation procedures. Sand-maize meal medium was prepared and placed 100 g in 250 ml flask. After Autoclaving the medium the flasks at 15 lb for 20 minutes, inoculated each flask with a bit of actively growing fungal culture and incubated at 30°C for 15 days. A fungus-soil mixture was prepared by hand mixing the pathogen @ 50 g/kg autoclaved soil [8]. The field was Inoculated with approximately 2 kg of soil/plot will be required to spread each plot.

2.3. Field Experiments on the Efficacy of Bioagents against Banded Leaf and Sheath Blight of Maize

To assess the field performance, the trials were conducted at two seasons prevailing at Dindigul region of

Tamil Nadu namely *Kharif* and *Rabi* in randomized block design with three replications maintaining spacing of 60 cm between rows and 20 cm between plants. The bioagents *T.asperellam* (1×10^4 cfu/g) and *B.subtilis* (1×10^5 cfu/g) were applied in the soil before sowing @ 200g/m². Unamended plots served as check. Seeds were treated with the slurry of carbendazim (0.2%) and bioagents of talc based formulation viz., *T.asperellam* (4g/kg), *B.subtilis* (10g/kg). Soil application of *T.asperellam* and *B.subtilis* was done @ 2.5kg/ha just before sowing. After treating with fungicides, maize seeds were allowed to air dried before sowing. Bioagent treated seeds were kept overnight in moist chamber so as to enable the antagonists to establish on seed surface. The fungitoxicants were applied in the form of spray on the above ground parts of the plants. Two foliar sprays, first two days after inoculation followed by the second 15 days later were made. Observations on disease severity were recorded 45 days after soil inoculation of the pathogen following 1-9 scale devised by Ahuja and Payak [9] as follows.

Scale 1: Disease on one leaf sheath only; few small, noncoalescent lesions present. Scale 2: Disease on two sheaths; lesions large and coalescent. Scale 3: Disease up to four sheaths, lesions many and always coalescent. Scale 4: As in scale 3 + rind discolored with small lesions. Scale 5: Disease on all sheaths except two internodes below the ear. Scale 6: Disease up to one internode below the ear shoot; rind discoloration on many internodes with large depressed lesions. Scale 7: Disease up to internode bearing the ear shoot but shank not affected. Scale 8: Disease on the ear; husk leaves show bleaching, bands and caking among them-selves as also of silk fibers; abundant fungal growth between and on kernel rows; kernel formation normal except their being lusterless; ear size less than normal; some plant prematurely dead. Scale 9: In addition to scale 8, shrinkage of stalk; reduced ear dimensions; wet rot and disorganization of ear; kernel formation absent or rudimentary; premature dead plants common; abundant sclerotia production on husk leaves, kernels, ear tips or silk.

$$P = \frac{\sum(n \times v)}{Z \times N} \times 100\%$$

P = disease severity n = number of samples in each category v = numerical value of each category Z = the highest numerical value of scale N= total number of samples.

2.4. Statistical Analysis

Statistical analyses were conducted using the IRRISTAT version 92-1 programme developed by biometrics unit at International Rice Research Institute, The Philippines. Differences between treatment mean values were determined following LSD test at 0.05 probability level.

3. Results

In-vitro experiments revealed the highest percent inhibition by fungicides and bioagents towards *R. solani*.

Bacillus subtilis inhibited 20% and Hexaconazole inhibited the mycelium about 100%. Field experiments conducted during 2017 and 2018 revealed the reduced severity of BLSB (2.25 and 2.99 grades) by Hexaconazole compared to control (16.25 and 16.44 grades) and seed treatment, soil application of *Bacillus subtilis* reduced the disease severity (4.00 & 4.82 grades) compared to control (16.33 and 17.15 grades).

During *Rabi* 2017 seed treatment and soil application of *B. subtilis* increased seed germination (98.67%), lowered disease incidence (4.82%) and increased the yield (7.3t/ha). Foliar spraying of Hexaconazole lowered the disease incidence (2.99%) and increased yield (7.6t/ha) (Table 2 & Table 3)

During *Kharif* 2018, the results of the experiments confirmed the suitability of *B. subtilis* with increased seed germination of 95.60%, lowered disease incidence (4.00%) and increased yield (6.4t/ha). Foliar spraying of Hexaconazole increased the yield (6.7t/ha) with lower disease incidence (2.25%) (Table 4 & Table 5).

Confirmative field experiments conducted during *Kharif* 2019 with the best treatments showed maximum disease reduction by *Bacillus subtilis* and increased yield (Figure 1 & Figure 2). Seed treatment and soil application of *Bacillus subtilis* recorded 78.46% reduction in disease incidence and higher grain yield (6-7t/ha). Foliar spraying of Hexaconazole (0.2%) showed 71.30% reduction and grain yield (6-7t/ha).

Table 2. In vivo efficacy of biocontrol agents on Maize Banded Leaf and Sheath Blight caused by *Rhizoctonia solani* during *Rabi* 2017

Sl.No.	Treatments	Germination (%)	Percent incidence (%)	Yield (kg/ha)
1	ST of <i>T. asperellam</i> *	96.75	10.10	7059
2	SA of <i>T. asperellam</i> *	99.05	7.87	7144
3	ST+SA of <i>T. asperellam</i> *	100	7.61	7561
4	ST of <i>B. subtilis</i> *	100	5.23	7144
5	SA of <i>B. subtilis</i> *	98.90	5.84	7222
6	ST+SA of <i>B. subtilis</i> *	98.67	4.82	7305
7	Farm Yard Manure	98.57	11.10	6383
8	Neem Cake	95.23	9.49	6844
9	Control	94.17	17.15	5705
	CD (p=0.05)	2.10	1.12	313.85

*ST: Seed Treatment; SA: Soil Application.

Table 3. In vivo efficacy of fungicides on Maize Banded Leaf and Sheath Blight caused by *Rhizoctonia solani* during *Rabi* 2017

Sl.No.	Treatments	Germination (%)	Percent incidence (%)	Yield (kg/ha)
1	Carbendazim	98.57	2.18	7033
2	Mancozeb	99.29	4.68	7099
3	Copper oxy chloride	98.57	9.80	6420
4	Copper hydroxide	99.29	12.58	6458
5	Hexaconazole	97.41	2.99	7637
6	Control	85.71	16.44	5953
	CD (p=0.05)	2.411	1.47	333.4

Table 4. In vivo efficacy of biocontrol agents on Maize Banded Leaf and Sheath Blight caused by *Rhizoctonia solani* during *Kharif* 2018

Sl.No.	Treatments	Germination (%)	Percent incidence (%)	Yield (kg/ha)
1	ST of <i>T. asperellam</i> *	100	7.33	6119
2	SA of <i>T. asperellam</i> *	99.33	8.00	6180
3	ST+SA of <i>T. asperellam</i> *	100	4.00	6661
4	ST of <i>B. subtilis</i> *	100	5.00	6283
5	SA of <i>B. subtilis</i> *	98.33	4.33	6267
6	ST+SA of <i>B. subtilis</i> *	95.66	4.00	6423
7	Arbuscular mycorrhizae	100	9.60	6229
8	Farm Yard Manure	93.66	9.00	5343
9	Neem Cake	94.33	6.66	5660
10	Control	94.33	16.33	4954
	CD (p=0.05)	2.13	1.15	315.80

*ST: Seed Treatment; SA: Soil Application.

Table 5. In vivo efficacy of fungicides on Maize Banded Leaf and Sheath Blight caused by *Rhizoctonia solani* during *Kharif* 2018

Sl.No.	Treatments	Germination (%)	Percent incidence (%)	Yield (kg/ha)
1	Carbendazim	98.57	1.75	6526
2	Mancozeb	98.00	3.00	6226
3	Copper oxy chloride	98.50	7.00	5557
4	Copper hydroxide	99.75	9.00	5631
5	Hexaconazole	98.50	2.25	6749
6	Control	95.00	16.25	5041
	CD (p=0.05)	2.42	1.48	323.4

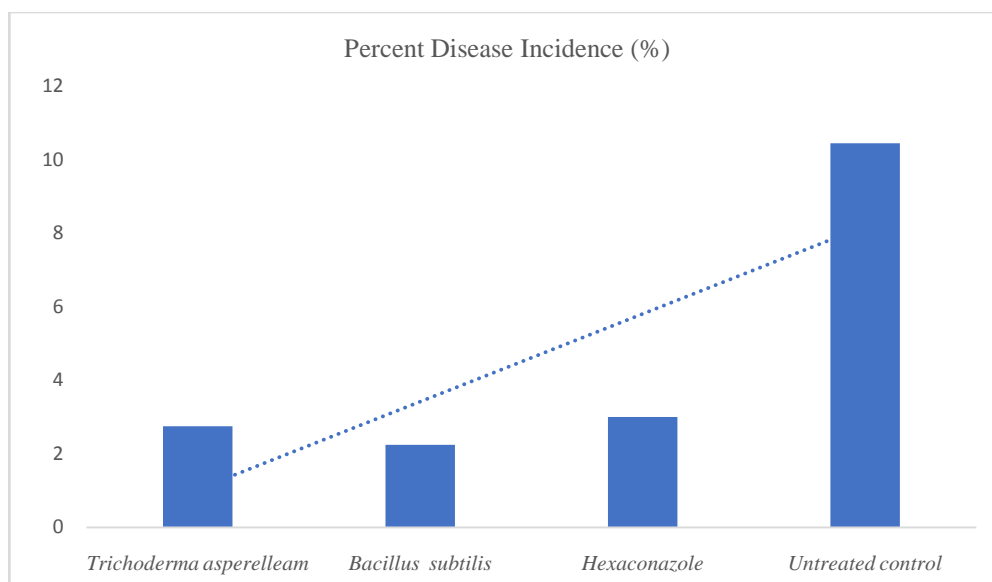


Figure 1. Effect of fungicides and biocontrol agents on Banded Leaf and Sheath Blight (BLSB) incidence (Kharif 2019)

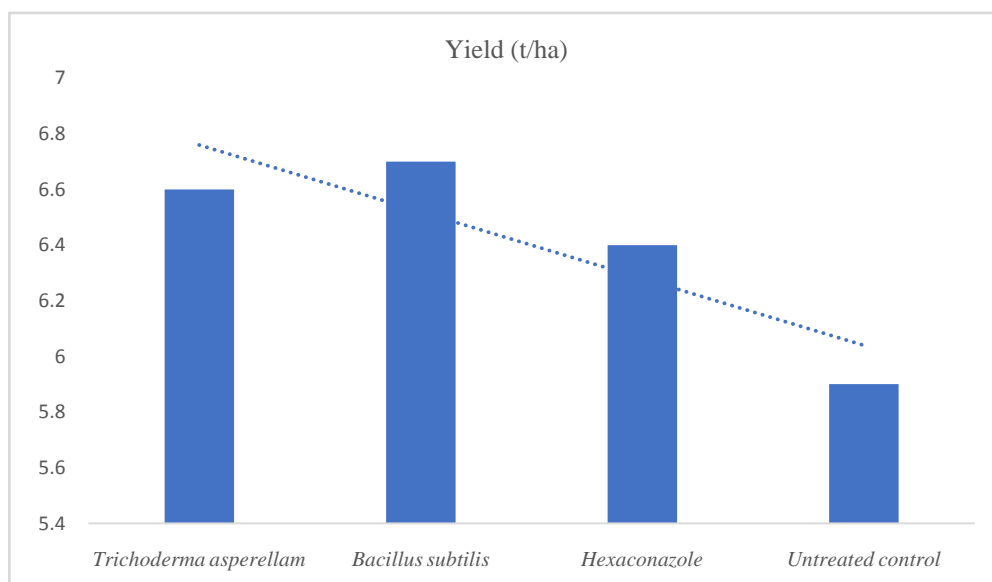


Figure 2. Effect of fungicides and biocontrol agents on the Yield of Maize (Kharif 2019)

4. Discussion

Banded Leaf and Sheath Blight of maize is one of the serious problems in dryland areas of Tamil Nadu, India. The yield losses recorded from 10 - 50 % with poor stalk quality which fetches low market value. The pathogen is a soil - borne nature and survives in soil and in stubbles in a persistent manner as dormant sclerotia. Hence, it is difficult to eradicate the pathogen with chemicals applied in soil. *Bacillus subtilis* is one of the rhizosphere bacteria that compete and colonize well on *Rhizoctonia solani*. Application of talc-based formulation will help to increase the population of *Bacillus* in the soil and can manage the pathogen throughout the cropping period.

The present findings under *in-vitro* conditions are in accordance with the findings of Meena *et al.* [10], Sharma and Saxena [11] with regard to efficacy of *T. harzianum*, *T. viride* and *T. virens* against *R. solani*. *Trichoderma harzianum*, showed 68% of inhibition of the mycelia of *R. solani*, under *in vitro* conditions [11]. Bacterial strains from the corn rhizosphere soil were isolated and screened

in vitro for antagonistic activity against *F. graminearum* [12]. Among biocontrol microbes, *Bacillus* spp. exerted their biocontrol capability predominantly through inhibitory activity on the growth of plant pathogens, as well as inducing systemic resistance in plants and competing for ecological niches with plant pathogens [13]. *Bacillus* spp. have been the most frequently exploited bacteria for commercial development of biocontrol agents owing to their ability to form endospores, which can survive heat exposure and desiccation, and their capacity to be formulated into stable dry powders with a long shelf life [1,14]. *Bacillus* spp. have been reported as plant growth promoter, systemic resistance inducer, and used for production of a broad range of antimicrobial compounds (lipopeptides, antibiotics and enzymes) and competitors for growth factors (space and nutrients) with other pathogenic microorganisms through colonization [15,16].

Similar findings were observed that *Bacillus velezensis*, the strain exhibited remarkable antifungal activity against *Fusarium graminearum*, a pathogenic fungus that causes

Corn Stalk Rot [12] and *Bacillus subtilis* BR23 strain controlled BLSB, when applied as Seed Treatment and Soil Application on corn crop [17]. As per the findings of McMullen and Lamey [18], *B.subtilis* used as seed treatment colonize the developing root system, suppressing disease organisms such as *Fusarium* and *Rhizoctonia*. Convincing evidences were obtained from several biocontrol studies using *B.subtilis* as seed treatment material have shown its effectiveness against *R. solani* causing root rot of soybean [19], potato black scurf, damping-off of mungbean, and rice sheath blight [20], root cancer of peanut [21], and root rot of sugarbeet [22].

5. Conclusion

The present investigation highlighted the potentialities of talc-based formulations of *Trichoderma asperellum* and *Bacillus subtilis* obtained from Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India in managing Banded Leaf and Sheath Blight disease of maize. Seed treatment and soil application of *Trichoderma asperellum* and *Bacillus subtilis* reduced disease incidence under *in vivo* conditions with high yield and the same isolate may be recommended for ecofriendly management of maize pathogens in dryland ecosystems. Whenever the incidences are severe, foliar spraying of Hexaconazole may be recommended.

Acknowledgements

The author is thankful to Tamil Nadu Agricultural University for funding through Non-Plan Scheme and providing field to carry out experimental trials at Maize Research Station, Vagarai.

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