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Bioefficacy Study of *Bacillus subtilis* Based Biofungicide on Leaf Spot Disease, Growth and Yield Attributes of Tomato [*Solanum lycopersicum* L.] CV. Arka Vikas

Ajay Kumar Tiwari[1*], B. K. Singh[1], Trivikram[1], A. K. Singh[1] and Pankaj Kumar Singh[1]

[1]Department of Horticulture, Institute of Agricultural Sciences, BHU, Varanasi–221005 (UP), India.

Authors' contributions

This work was carried out in collaboration among all authors. Author AKT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author BKS guided the author AKT during the whole research period and edited the manuscript. Authors AKS and Trivikram managed the analyses of the study. Author PKS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The present study entitled "Bioefficacy study of *Bacillus subtilis* based biofungicide on leaf spot disease, growth and yield attributes of tomato [*Solanum lycopersicum* L.] cv. ArkaVikas" was conducted at Vegetable Research Farm, Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during rainy season of 2016-17 in Randomized Block Design (RBD) with three replications. The treatments included foliar spray of chemical fungicide (Copper oxychlooride) and biofungicide (Taegro® and Trichoderma) either alone or in combination. Taegro® is a bacterial biofungicide containing 1×10^{10} CFU/g (13%w/w) of *Bacillus amyloliuefaciens* strain FZB24 formulated as WP. A total of 12 characters including disease, growth and yield parameters were studied. Disease parameter included leaf spot disease incidence (%) and disease severity (%) whereas growth and yield parameters included days to 50% flowering, height, number

**Corresponding author: E-mail: ajayhorti15@gmail.com;*

of branches per plant, fruit length (cm), fruit width (cm), number of fruits per plant, average fruit weight (g), number of seed per fruit, fruit yield per plot (kg), fruit yield per hectare (kg) were studied. The biofungicide Taegro exhibited significant potential in reducing the leaf spot in tomato and improving the growth and yield attributes of tomato as compared to control. But combined used of Taegro with standard chemical copper oxychloride gave better result than Taegro alone. As a consequence, this may be used as part of an integrated disease management approach so as to minimizes the use of standard fungicides and also protect the environment from pollution and maintenance of the human health.

Keywords: Bacillus subtilis; biofungicide; copper oxychloride; leaf spot; Taegro.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is grown in India in a wide range of climatic conditions across states of Uttar Pradesh, Andhra Pradesh, Odisha, Karnataka, Maharashtra, West Bengal, Bihar, Gujarat, Madhya Pradesh and Chhattisgarh, accounting for a total production of 19.69 million tonnes from an area of 0.81 million hectares with an average productivity of 24.4 tonnes per ha [1]. In Uttar Pradesh, the crop is cultivated during rainy, winter and summer seasons and occupies an area of 20.88 thousand hectares with production of 826.32 thousand tonnes [1]. Tomato is attacked by many serious diseases under greenhouse and field condition. Diseases are one of the major factors that affect quality and yield of crops. Tomato suffers from number of diseases caused by bacteria, fungi and viruses, of them bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria,* bacterial speck caused by *Pseudomonas syringae* pv. *syringae,* bacterial wilt caused by *Pseudomonas solanacearum*, tomato spotted wilt, tomato leaf curl, mosaic, early blight caused by *Alternaria solani*, late blight caused by *Phytophthora infestans* are important. Since commercial cultivars do not have sufficient resistance to disease, cultural practices and fungicides form the basis for disease management programs [2]. However, development of fungicide resistance, accumulation of residues in fruits, reduction of beneficial phylloplane and soil microbes and environmental pollution are associated problems [3]. Considering the seriousness of the problem, the present investigation was carried out to divert the hazardous effects of chemicals used in disease management to find out the alternative techniques of disease control which may cause little or no adverse effect on environment. Notable success in disease management has been achieved during past several years through the use of antagonistic bio-agents in the laboratory, glass house and field. On the basis of this information, there is possibility of development of biological control for plant diseases. Now a day, the commercial formulation of some of the biocontrol agents has already available in the market. In the present study, attempts have been made to identify antagonistic bio-agents against leaf spot diseases in field condition. Plant Growth Promoting Rhizobacterium (PGPR) such as *Bacillus subtilis* is used in a wide range of plants as biocontrol agent for management of different pathogens. Induced systemic defense responses in plants have been reported as one of the mechanisms by which these organism reduce the diseases in plants in conjunction with other mechanisms including direct antagonism, antibiosis and siderophore production. Induction of defense responses by *Bacillus spp.* is largely associated with production of pathogenesis related proteins like b-1,3-glucanase and the defense enzyme phenylalanine ammonia-lypase and oxidative enzymes like peroxidase, polyphenol oxidase and superoxide dismutase. Apart from controlling diseases, these bio-control organisms also promote plant growth and yield by production of plant growth hormones like IAA and GA3 in combination with increased availability of nutrients [4]. At present agriculture ecosystem contains high toxicity fungicides and is leading to harmful environmental impact. The aim of this study was to determine the effects of commercially available *Bacillus subtilis var. amyloliquefaciens* strain FZB24 viz. Taegro on growth, yield and control of leaf spot diseases in field condition.

2. MATERIALS AND METHODS

The present experiment was conducted to test the biofungicide (Taegro) on control of leaf spot disease of tomato cv. Arka Vikas was carried out under field condition. Research was undertaken at the Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during rabi season of 2016-2017. Thirty days old healthy and uniform seedlings were transplanted to 9 m^2 experimental plots at a spacing of 60×60 cm with a population of 30 plants per plot. Before transplanting well decomposed farm yard manure at the rate of 35 tonnes per hectare was applied along with the fertilizer dose of 180:120:150 kg NPK per hectare. Total P and K and half dose of nitrogen was applied as basal dose and the remaining half dose of nitrogen was top dressed after one month of transplanting and other management practices were undertaken as per the package of practices for tomato crop. The experiment was carried out in Randomized Completely Block Design (RBD) with three replications comprising ten treatment viz. T_1: Control, T_2: Copper oxychloride @ 2 g/litre × 8 Sprays at weekly interval, T_3: Trichoderma viridae @ 2 g/litre × 8 applications at weekly interval, T_4: Taegro @ 185 g/ha total 8 application at weekly interval, $T_{5:}$ Taegro @ 370 g/ha total 8 application at weekly interval, $T_{6:}$ Taegro @ 500 g/ha total 8 application at weekly interval, $T_{7:}$ Copper oxychloride @ 2 g/litre of water alternated with Taegro @370 g/ha × 8 applications at weekly interval, $T_{8:}$ Copper oxychloride @ 2 g/litre × 4 applications only coinciding with the 1^{st}, 3^{rd}, 5^{th} & 7^{th} sprays, $T_{9:}$ Copper oxychloride @ 2 g/l×2 applications, alternated with Taegro @370 g/ha×2 applications, in 2 phases totaling 8 applications, $T_{10:}$ Copper oxychloride @ 2 g/litre × 4 applications only conciding with 1^{st}, 2^{nd}, 5^{th} & 6^{th} spray. The observations were recorded with regard to growth parameter, yield parameter and incidence of leaf spot disease of tomato. Growth and yield parameters included height, days to 50% flowering, number of branches per plant, fruit length (cm), fruit width (cm), number of fruits per plant, average fruit weight (g), fruit yield per plot (kg), fruit yield per hectare (kg), number of seed per fruit. Disease incidence was observed on five randomly chosen plants per plot at 0, 14, 21, 28, 35, 42, 49 and 56 days after spraying. Five leaves were selected from different positions of each plant and the leaf area infested by each disease was measured using 0-5 scale of Horsefall and Barette, [5] (Table 1). Disease was identified on the basis of symptoms and expressed as Disease Incidence (%) and Disease Severity Index (%) was calculated as per the formula of and the data was analyzed statistically.

Disease incidence (%)

$$=\frac{\text{Number of Diseased leaves}}{\text{Total Number of Leaves Inspected}}\times 100$$

Disease severity index (%)

$$=\frac{\text{Sum of Individual numerical rating}}{\text{Total Number of Leaves assesed} \times \text{Maximum score in scale}}\times 100$$

Table 1. Disease rating scale for assessment of leaf spot disease of tomato

Scale	Disease severity (%)	Description
0	0.0	Leaf free from leaf spot
1	0%-5%	0-5% leaf area infected and covered by spots
2	5.1%-10%	5.1-10% leaf area infected and covered by spots
3	10.1%-25%	10.1-25% leaf area infected and covered by spots
4	25.1%-50%	25.1-50% leaf area infected and covered by spots
5	>50%	>50% leaf area infected and covered by spots

3. RESULTS AND DISCUSSION

3.1 Influence of Fungicides on Disease Parameters

The role of fungicides in disease control has been well documented. The fungicides kill or inactivate the spores of fungi and hence help in effective control of disease. The spore becomes unviable or gets killed by application of fungicides and ultimately, the disease is controlled. The fungicides, both in chemical form as well as biological agents help in disease control. The present study revealed that the untreated plants showed highest level of disease incidence as compared to treated plants. Among different treatments it was found that leaf spot incidence was much more pronounced in case of control (untreated plants). The alternated spray of copper oxychloride with *Bacillus subtilis* helped much in disease control. The plants expressed some symptoms of leaf spot in this treatment. The biological agent used for leaf spot control, namely *Trichoderma* was not found upto the mark in disease control.

The most effective treatment to control leaf spot in this study was found to be the standard chemical copper oxychloride. The maximum leaf spot incidence (53.48%) and disease severity (24.80%) was recorded with treatment T_1 (control) while Minimum leaf spot incidence (20.62%) and disease severity (6.58%) recorded in treatment T_2 followed by T_7 having 24.37%

and 6.61% disease incidence and disease severity respectively. The results obtained in this experiment are in accordance with the findings of Chowdappa et al. [6] in tomato by enhancing systemic resistance in tomato seedlings through induction of growth hormones like indole-3-acetic acid (IAA) and gibberellic acid (GA_3) and defense enzymes like peroxidase, polyphenol oxidase and superoxide dismutase. *B. subtilis* strains could be effective biocontrol agents against soil fungi, plant pathogens and could have a potential biofertilizer effect, since they have stimulated growth and yield of tomato (Suarez et al., 2011) [7]. *B. subtilis* B1, B6, B28 and B99 significantly promote growth and biocontrol activity against *F. oxysporum* f. sp*ciceris* in chickpea. They were observed to produce IAA, HCN and antifungal volatiles (Karimi et al.) [8]. It can be due to the influence of plant growth promotion and induced systemic resistance in enhancing the disease resistance in tomato plants. Minimum leaf spot incidence (20.62%) recorded in treatment T_2 (copper oxychloride). Similar findings were also reported by Adinarayana et al. [4], Abbasi and Weselowski [9] and Roberts et al. [10]

3.2 Influence of Fungicides on Growth Parameters

The beneficial effect of fungicides in disease control has been well documented. In the present investigation, two biofungicide formulations namely *Bacillus subtilis* and *Trichoderma*, and one standard chemical fungicide (copper oxychloride), either alone or in combination, were evaluated for their role in affecting plant growth. Among the treatments, maximum plant height (124.07 cm) was recorded in chemical control T_2 followed by alternated use of chemical and biological fungicide T_7 having height 120.67 cm. It may be due to the plant growth promoting rhizobacteria (PGPR) that grow in association with a plant host that stimulates its growth through direct and indirect mechanisms. Direct mechanisms such as production of phytohormones, solubilization of phosphates and increased uptake of iron. Indirect effects can be antibiotic production, nutrient competition, parasitism and inhibition of pathogen toxins or induced resistance. This is in accordance with studies conducted by Adinarayana et al. [4] and Jones [11] in tomato crop. The ability of taegro i.e. *B. subtilis* var. *amyloliquefaciens* FZB24 to enhance growth and control plant disease could be a result of production of plant hormones such as indole-3-acetic acid (IAA). Minimum plant height (98.67 cm) was found in controlled plot.

Significantly highest number of branches per plant (4.73) were recorded in chemical control T_2 and the result is par with alternated use of chemical and biological fungicide T_7 having 4.60 branches per plant, that may be due to the ability to produce phytohormones, vitamins and solublizing minerals and inhibition of pathogen growth. Similar results were also obtained by Adinarayana et al. [4] and Abdel-Monaim et al. [12]. The minimum number of branches (3.00) was recorded in controlled plot.

Among the treatments minimum number of days to attain 50 percent flowering was noticed in treatment T_7 (37.00). This is at par with T_9, T_6, T_5 having 50% flowering in 37.67 days each. While, the maximum number of days to attain 50% flowering was recorded under treatment T_1-untreated control in 39.67 days. The induction of early flowering was due to better nutritional status of the plants. Increased production of leaves might have helped to elaborate more photosynthates and induced flowering, thus effecting early initiation of flower bud. Similar such findings were reported by Adinarayana et al. [4].

3.3 Influence of Fungicides on Yield Parameters

Yield is the manifestation of morphological, physiological, biochemical and growth parameters and is considered to be the result of trapping and conversion of solar energy efficiency. Yield is polygenic character and is influenced by several internal and external factors throughout the crop growth period. Increased plant height and early flowering diverted photosynthates towards formation of fruits and increased fruit yield.

From the present investigation, it was found that more height of plants due to application of copper oxychloride led to increase the number of branches in such plants. The branches in turn produced the higher number of fruits per plant in such treated plants (application of copper oxychloride). Hence, maximum number of fruits per plant was observed in chemical control T_2 which is at par with alternated use of chemical and biological fungicide T_7. Similar such observation is recorded by Adinarayana et al. [4].

In the present study, fruit size of tomato was significantly influenced by different biological and chemical fungicides. Fruit size is attributed to individual fruit weight and the receipt of the food material. The maximum fruit length (4.26 cm) was noted in chemical control T_2 followed by

Table 2. Effect of different methods and levels of Taegro as bio-fungicide on disease incidence (%), disease severity index (%), growth, yield attribute and yield of tomato cv. ArkaVikas

Treatment	Disease incidence (%)	Disease severity index (%)	Plant height (cm)	Days to 50% Flowering	No. of branches per plant	No. of fruits per plant	Fruit length (cm)	Fruit width (cm)	Average fruit weight (g)	No. of seed per fruit	Fruit yield (kg/plot)	Fruit yield (kg/ha)
T_1	53.48	24.8	98.67	39.67	3.00	28.93	3.02	4.13	40.06	72.73	22.40	24888.15
T_2	20.62	6.58	124.07	38.00	4.73	38.60	4.26	5.00	50.04	113.33	38.34	42600.00
T_3	42.94	17.58	103.00	39.33	3.73	29.93	3.19	4.15	41.50	81.20	25.44	28266.67
T_4	38.93	14.46	104.33	39.00	3.80	30.53	3.22	4.26	42.77	79.47	26.43	29368.52
T_5	35.55	11.74	106.73	37.67	3.87	32.33	3.56	4.60	43.90	113.27	27.60	30670.37
T_6	32.63	11.66	109.47	37.67	3.93	33.60	3.87	4.71	44.22	134.13	31.45	34948.15
T_7	24.37	6.61	120.67	37.00	4.60	37.60	3.88	5.01	48.04	100.40	36.49	40548.15
T_8	29.75	9.32	114.53	38.33	4.00	34.53	3.40	4.41	45.42	88.75	34.98	38870.37
T_9	25.07	7.46	117.17	37.67	4.53	36.13	3.53	4.80	47.04	109.93	35.24	39155.56
T_{10}	30.71	9.51	113.20	38.00	3.93	34.33	3.53	4.26	44.08	81.60	34.42	38248.89
SE.m.±	0.59	0.52	2.48	0.48	0.17	1.38	0.13	0.13	1.51	4.60	0.66	737.75
CD	1.74	1.55	7.38	1.42	0.51	4.11	0.39	0.38	4.49	13.67	2.70	3003.20
CV%	3.04	7.55	4.32	2.17	7.42	7.12	6.43	4.90	5.85	8.18	3.68	3.68

T_1: Control, T_2: Copper oxychloride @ 2 g/litre × 8 Sprays at weekly interval, T_3: Trichodermaviridae @ 2 g/litre × 8 applications at weekly interval, T_4: Taegro @ 185 g/ha total 8 application at weekly interval, T_5: Taegro @ 370 g/ha total 8 application at weekly interval, T_6: Taegro @ 500 g/ha total 8 application at weekly interval, T_7: Copper oxychloride @ 2 g/litre of water alternated with Taegro @370 g/ha × 8 applications at weekly interval, T_8: Copper oxychloride @ 2 g/litre × 4 applications only coinciding with the 1st, 3rd, 5th & 7th sprays, T_9: Copper oxychloride @ 2 g/l×2 applications, alternated with Taegro @370 g/ha×2 applications, in 2 phases totaling 8 applications, T_{10}: Copper oxychloride @ 2 g/litre × 4 applications only conciding with 1st, 2nd, 5th & 6th spray

alternated use of chemical and biological fungicide T_7. The minimum fruit length (3.02 cm) was noted under T_1. Maximum fruit width (5.01 cm) was observed in T_7 followed by T_2 and minimum was under T_1-controlled plot. Similar observations were recorded by Abdel-Monaim et al. [12] and Dursun et al. [13].

The maximum average fruit weight (50.04 g) was recorded in case of chemical control T_2 followed by alternated use of chemical and biological fungicide T_7 having 48.04 g average fruit weight. Diversion of photosynthates towards fruit formation causes increase in fruit weight with the application of biofungicides. Similar finding were also reported by Basamma et al. [14] and Abdel-Monaim et al. [12].

All the treatments show a significant effect on number of seeds per fruit. The maximum number of seeds per fruit was recorded in T_6, followed by T_2 and T_5. Minimum number of seeds per fruit was recorded in T_1 that was control treatment. Production of growth substances by fungicides might have increased the availability and uptake of nutrients through plant roots and produce large size fruits which bear more number of seeds. More biomass was produced by the plants which resulted in the sustainable healthy plant system consequently increased fresh weight of plant, fruits and seeds.

The maximum fruit yield per plot (38.34) was observed in chemical control T_2 followed by alternated use of chemical and biological fungicide in T_7. Similar trend was also observed in case of fruit yield per hectare. Increased plant height and early flowering diverted photosynthates towards formation of fruits and increased fruit yield. The copper oxychloride treated plot gave best result then others but alternate use of copper oxychloride and taegro also gave satisfactory result. Similar results have been also reported by Adinaryana et al. [4] and Abbasi and Weselowski [15]. The probable reason for such finding may be that, copper oxychloride would have affected the spore germination and mycelial development, which may have resulted in the inhibition of disease producing activity of pathogen in the plant and induced resistance in plant. This can be the reason for minimum disease intensity and maximum yield as compared to other treatments.

4. CONCLUSION

The present investigation revealed that, apart from chemical control through copper oxychloride, the application of *Bacillus subtilis* based biofungicide taegro has proved to be effective among other bioagent. But combined use of taegro alternated with chemical fungcide copper oxychloride give better result than taegro alone in promoting growth and yield of tomato apart from controlling disease. As a consequence, this may be used as part of an integrated disease management approach so as to minimizes the use of standard fungicides and also protect the environment from pollution and maintenance of the human health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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