Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-7: 656-664

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 Special Issue-7 pp. 656-664
Journal homepage: http://www.ijcmas.com

Original Research Article

Effect of *Bacillus subtilis* var. *amyloliquefaciens* on Growth, Yield and Control of Early Blight in Tomato (*Solanum lycopersicum* L.)

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ABSTRACT

An experiment to study the effect of taegro as bio-fungicide (*Bacillus* var. *amyloliquefaciens* strain FZB24) on yield and control of early and late blight diseases in tomato (*Solanum lycopersicum* L.) cv. Hybrid NS -501 was conducted at the Department of Horticulture, Gandhi Krishi Vigyan Kendra, University of Agricultural Science, Bangalore during *rabi* season of 2013-2014. The study included seed treatment with taegro alone (4g/kg), seedling dip with taegro alone (4g/l), both seed treatment with taegro (4g/kg) and soil drenching (250g/ha), both seedling dip with taegro (4g/l) and soil drenching (500g/ha) and both soil drenching with taegro (250g/ha and 500g/ha) and foliar spray (250g/ha and 500g/ha) against mancozeb @ 2g/l as standard fungicide. The maximum plant height, number of leaves, number of branches, fresh weight of leaves and total fresh weight per plant and dry weight of leaves per plant were observed in the treatment taegro application of both soil drenching and foliar spray @ 500 g/ha. The same treatment has recorded maximum number of clusters per plant, flowers per cluster and fruits per cluster. However, the standard chemical mancozeb (2g/l) recorded highest number of fruits per plant (110.07), yield per plant (6.42 kg), yield per plot (179.42 kg), yield per hectare (117.33 t) and least diseases incidence of *Alternaria solani* of 8.97 and 15.79 PDI at 65 DAT and 90 DAT, respectively.

Keywords

Bio-fungicide, Taegro, *Bacillus* sp Mancozeb, *Alternaria solani*

Introduction

Tomato (*Solanum lycopersicum* L.) is most important and remunerative vegetable crop in India with good source of income for small and marginal farmers. In India tomato is grown in an area of 0.879 million hectare with a production of 18.22 million tonnes and the productivity being 20.72 tonnes per hectare. The leading tomato growing states are Andhra Pradesh, Karnataka, Orissa, West Bengal, Maharashtra, Haryana, Uttar Pradesh, Punjab and Bihar. In Karnataka, it occupies an area of 0.57 lakh hectares with a production and productivity 19.16 lakh tonnes and 33.15 tonnes/ha respectively (Anonymous, 2013). The leading tomato growing districts in the state are Belgaum, Dharwad, Kolar, Bengaluru and Bellary.

Tomato plant is attacked from many serious diseases under greenhouse and field conditions. Several important diseases of tomato reduce crop yield and the most devastating plant pathogens are fungi and oomycetes (Agrios, 2005). For example, the early blight, caused by *Alternaria solani* (Ellis & Martin) Sorauer, (Chaerani and

Voorrips, 2006) and late blight, incited by *Phytophthora infestans* (Mont.) deBary (Deahl, *et al.,* 2008) are economically important diseases of tomato worldwide including India causing crop losses up to 100% (Chaerani and Voorrips, 2006). Since commercial cultivars do not have sufficient resistance to leaf blights, cultural practices and fungicides applied at 5–7 days intervals form the basis for leaf blight management programs (Tumwine *et al.,* 2002). However, development of fungicide resistance, accumulation of residues in fruits, reduction of beneficial phylloplane and soil microbes and environmental pollution are associated problems (Akinnifesi *et al.,* 2005). Considering the seriousness of the problem, the present investigation was carried out. The hazardous effects of chemicals used in plant disease management have diverted plant pathologists to find out the alternative techniques of plant disease control which may cause little or no adverse effect on environment. Notable success of disease management through the use of antagonistic bio-agents in the laboratory, glass house and field has been achieved during past several years. 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 bio-control agents has already become available in the market. In the present study, attempts have been made to identify antagonistic bio-agents against early and late blight diseases in field condition.

Taegro is novel bio-fungicide released by the company Novozymes South Asia Pvt. Ltd. Taegro has been commercially tested on different vegetable crops in controlling diseases like tomato (late blight, bacterial wilt and bacterial spot diseases), cucumber (*Rhizoctonia*), lettuce (bottom rot and downey mildew) and pepper (powdery mildew) at the rate of 180-360 g/ha at 7-28 days interval for soil borne diseases and 7-14 days interval for foliar diseases with 1-12 number of applications during crop season. Taegro is a bio-fungicide which contains *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24, which is used to suppress soil-borne diseases like *Fusarium* spp., *Rhizoctonia* spp. and *Phytophthora* spp. and also the active ingredient *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 also acts as a plant growth promoter. It also produces various antifungal agents and enzymes. Taegro involves in more than 8.5 per cent production of secondary metabolites (peptides, lipopeptides, polyketides and siderophores) through pathways that do not involve ribosomes. The genome contains nine giant gene clusters directing the synthesis of Lipopeptides (Surfactin, Iturins, Fengycin, Bacillibactin and Bacilysin) and Polyketides (Bacillene, Difficidin, Macrolactin). These compounds are known to suppress bacteria and fungi within the plant rhizosphere. At present agriculture / horticulture ecosystem contains high toxicity fungicides and is leading to environmental impact and also residues remaining in fruits and vegetables. The aim of this study was to determine the effects of commercially available *Bacillus amyloliquefaciens* strain FZB24 on tomato (*Solanum lycopersicum* L.) growth, yield and early blight in field condition.

Materials and Methods

The present investigation was conducted to test the new bio-fungicide (taegro) on growth, yield, quality and cost economics of hybrid tomato cv. NS-501 was carried out under field condition. Research was undertaken at the Department of Horticulture, Gandhi Krishi Vignana Kendra, University of Agricultural Science, Bengaluru during *rabi* season of 2013-2014.

The experiment was carried out in Randomised Completely Block Design (RCBD) with eight treatments and three replications. The study included three levels of taegro (*Bacillus subtilis* var. *amyloliquefaciens* strain FZB24) *viz.*, 4g/kg seed, 250g and 500g per hectare through four methods of application *viz.*, seed treatment, seedling dip, soil drenching and foliar spraying in various combinations. Overall different levels and methods of application were done for T_1 to T_6 and T_7 Standard chemical control by mancozeb @ 2 g/l and T_8 is absolute control.

The observations with regard to growth, yield and disease were recorded from the five randomly selected and tagged plants. Observations were recorded on growth parameters like plant height, number of branches and number of leaves at 30, 60 and 90 days after transplanting (DAT), fresh and dry weight of plant at final harvest. Yield and quality parameters like days to first flowering, days to fifty per cent flowering, number of fruits per plant, fruit yield per plant, per plot, per hectare.

Disease incidence was observed on five randomly chosen plants per plot at 65 and 90 days after transplanting. Five leaves were selected from different positions of each plant and the leaf area infested by each disease was measured on 0-5 scale (Datar and Mayee, 1981) (Table 1). Each disease was identified on the basis of following symptoms and expressed as Per cent Disease Index (PDI).

Per cent disease index (PDI) was calculated as per the formula of Jones, (2011) and the data was analyzed statistically.

Per cent disease index = (Sum of individual ratings / No. of leaves assessed) X (100 / Max. Disease Grade)

Results and Discussion

Effect of different methods and levels of application of *Bacillus* var. *amyloliquefaciens* strain FZB24 on plant height, number of leaves per plant and per branches at 30, 60 and 90 days after transplanting

Treatment T_6 *i.e.*, both soil drenching and foliar spray @ 500g/ha resulted in significantly highest plant height (76.35, 99.42 and 117.03 cm.) at 30, 60 and 90 DAT, respectively (Table 2). It was *on par* with T_1, T_2, T_3, T_4, T_5, and T_7 at all the three stages. 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 confirmation with studies conducted by Vessey, (2003) in tomato. Similar such observations were obtained by Diaz, (2006) and Jones, (2011) in tomato crop due to spray of *B. subtilis* application. The ability of taegro *i.e. B. subtilis* var. *amyloliquefaciens* FZB24 to enhance growth and control plant disease could be as a result of production of plant hormones such as indole-3-acetic acid (IAA) (Bottini *et al.*, 2004). Lowest plant height (63.25, 84.11, 92.93 cm) was observed in T_8 *i.e.*, untreated control at 30, 60, and 90 DAT, respectively.

Treatment T_6 *i.e.*, both soil drenching and foliar spray @ 500g/ha recorded the highest number of leaves/plant (13.76 and 55.81) at 30 and 60 DAT, respectively and it was *on* *par* with T_1, T_2, T_3, T_4, T_5 and T_7 at both 60 and 90 DAT. At 90 DAT highest no. of leaves/plant was found in the treatment T_7 *i.e.*, standard chemical control (35.73) which was *on par* with T_1, T_2, T_3, T_4, T_5 and T_6. The least no. of leaves/plant were found in the treatment T_8 (10.27, 43.57, and 27.16) at 30, 60 and 90 DAT, respectively. Significantly highest number of branches/plant (10.38 and 19.81) were recorded by T_6 *i.e.*, both soil drenching and foliar spray @ 500g/ha at 30 and 90 DAT, respectively. T_6 was *on par* with the treatments T_4, T_5, T_1, T_7 and T_2 (10.16, 10.08, 9.95, 9.47 and 9.24 per plant, respectively) at 30 DAT. At 90 DAT T_6 was followed by T_1, T_2, T_3, T_4, T_5 and T_7. At 60 DAT T_5 *i.e.*, both soil drenching and foliar spray @ 250g/ha recorded the maximum number of branches (14.98/plant) and it was *on par* with treatments T_6, T_4, T_2 and T_1 (14.71, 13.80, 13.68 and 13.68 per plant, respectively), That may be due to the ability to produce phytohormones, vitamins and solublizing minerals and inhibition of pathogen growth. Similar such results were obtained by Morsy *et al.*, (2009) and Monaim *et al.*, (2012).

Enhancement of plant growth by root colonizing species of *Bacillus* and *Paeni bacillus* is well known. Chowdappa *et al.*, (2013) first demonstrated the production of reasonable quantities of IAA from Gram-positive bacterium *B. amyloliquefaciens* FZB42 and IAA production was enhanced when the bacterium was fed with tryptophan. Production of IAA was dramatically reduced in the mutants deficient in trp gene responsible for biosynthesis of IAA, suggesting that main route of IAA biosynthesis in this bacterium was dependent on tryptophan.

The minimum number of branches (6.74, 9.79 and 14.40/ per plant) was recorded in treatment T_8, *i.e.*, untreated control at 30, 60 and 90 DAT, respectively.

Effect of different methods and levels of application of *Bacillus* var. *amyloliquefaciens* strain FZB24 on number of fruits per plant, yield per plant, per plot, per hectare and diseases incidence of *Alternaria solani* at 65 and 90 days after transplanting

The treatment T_7 (Standard chemical control by spraying Mancozeb @ 2 g/l) recorded the maximum number of fruits per plant (110.07), yield per plant (6.42kg), yield per plot (179.42kg) and yield/ha (112.13t), which was *on par* with T_6, for number fruits per plant (107.40) and T_6 and T_5 for yield per plant (6.31 and 6.18kg/plant, respectively) (Table 3).

The probable reason for such finding may be that, mancozeb 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 may be the reason for minimum disease intensity and maximum yield as compared to other treatments. Similar trends were reported by Hooda *et al.*, (2008).

They also reported that mancozeb was the most effective fungicide in minimizing disease intensity against early blight of tomato. This was also supported by the findings of Chourasiya *et al.*, (2013) against early blight of tomato. Treatment T_7 was *on par* with T_1, T_2, T_3, T_4, T_5 and T_6 with respect to fruit yield per plot and fruit yield per hectare. The minimum number of fruits per plant (65.52), yield per plant (3.42kg), yield per plot (96.29kg) and yield per hectare (60.18t) was recorded in treatment T_8 *i.e.*, untreated control.

Table.1 Per cent Disease Index (PDI)

Per cent leaf area covered	Severity scale	Disease reaction
<1	0	Immune
1-5	1	Resistant
6-20	2	Moderately resistant
21-40	3	Moderately susceptible
41-70	4	Susceptible
71-100	5	Highly susceptible

Table.2 Effect of different methods and levels of *Bacillus* var. *amyloliquefaciens* strain FZB24 on plant height, number of leaves and branches per plant at 30, 60 and 90 days after transplanting in hybrid tomato cv. NS -501

Treatments	Plant height (cm) at			Number of leaves per plant at			Number of branches per plant at		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T_1	74.16 ^{b}	95.38 ^{b}	112.45 ^{b}	12.26 ^{b}	55.39 ^{b}	36.61^b	9.95 ^{b}	13.66 ^{b}	17.36 ^{b}
T_2	71.86^b	96.96 ^{b}	112.33 ^{b}	12.66 ^{b}	55.52 ^{b}	36.47 ^{b}	9.24 ^{b}	13.68 ^{b}	17.56 ^{b}
T_3	72.93 ^{b}	92.63^b	105.21^b	11.72 ^{b}	55.50 ^{b}	36.68 ^{b}	8.76 ^{b}	12.10 ^{b}	16.79 ^{b}
T_4	72.30 ^{b}	97.40^b	109.77^b	12.55 ^{b}	54.62 ^{b}	35.86 ^{b}	10.16 ^{b}	13.80 ^{b}	17.62 ^{b}
T_5	75.80 ^{b}	97.95 ^{b}	111.52 ^{b}	12.70 ^{b}	55.41 ^{b}	36.14 ^{b}	10.08 ^{b}	14.98 ^{c}	18.43 ^{b}
T_6	76.35 ^{b}	99.42 ^{b}	117.03 ^{c}	13.76 ^{c}	55.81 ^{b}	37.57 ^{b}	10.38 ^{b}	14.71 ^{c}	19.81 ^{c}
T_7	75.52 ^{b}	96.07 ^{b}	111.62 ^{b}	12.01 ^{b}	52.80 ^{b}	35.73 ^{b}	9.46 ^{b}	12.38 ^{b}	17.02 ^{b}
T_8	63.25^a	84.11 ^{a}	92.93 ^{a}	10.27 ^{a}	43.57 ^{a}	27.16 ^{a}	6.74 ^{a}	9.79 ^{a}	14.40 ^{a}
F test (p=0.05)	*	*	*	*	*	*	*	*	*
S Em ±	2.52	2.77	3.60	0.37	2.04	1.07	0.49	0.57	0.68
CD @ 5 %	7.65	8.41	10.92	1.12	6.18	3.24	1.50	1.73	2.05
CV %	6.00	5.06	5.72	5.23	6.58	5.24	9.16	7.52	6.73

Note: T_1- Seed treatment with taegro @ 4g/kg seed, T_2- Seedling dip with taegro @ 4g/L of water for 25 minutes, T_3- Both seed treatment with taegro @ 4g/kg seed and soil drenching twice (*i.e.*, 15 and 30 DAT) @ 250g/ha, T_4- Both Seedling dip with taegro @ 4g/L of water and soil drenching twice (*i.e.*, 15 and 30 DAT) @ 500 g/ha, T_5- Both soil drenching and foliar spray with taegro @ 250g/ha on 15 and 30 DAT, T_6- Both soil drenching and foliar spray with taegro @ 500g/ha on 15 and 30 DAT, T_7- Standard chemical control by spraying mancozeb @ 2 g/l 10 days interval throughout crop growth period, T_8- Untreated control; DAT: Days after transplanting; In a column means followed by same letter (s) are not significantly different as per DMRT; * Significant at 5 % level

Table.3 Effect of different methods and levels of *Bacillus* var. *amyloliquefaciens* strain FZB24 on number of fruits per plant, yield per plant, per plot and per hectare and diseases incidence of *Alternaria solani* at 65 and 90 DAT

Treatments	Total of all harvests				PDI of *A. solani*	
	Number of fruits per plant	Yield per plant (kg)	Yield per plot (kg)	Yield per hectare (t)	At 65 DAT	At 90 DAT
T_1	98.38 [bc]	6.12 [b]	170.47 [b]	106.54 [b]	13.88 [bc]	20.49 [b]
T_2	94.45 [ab]	5.77 [bc]	161.26 [b]	100.78 [b]	13.67 [bc]	25.23 [c]
T_3	89.79 [a]	5.30 [b]	147.93 [b]	92.45 [b]	15.02 [c]	27.64 [c]
T_4	98.32 [bc]	6.12 [bc]	171.80 [b]	107.37 [b]	13.20 [b]	21.82 [b]
T_5	102.94 [cd]	6.18 [bc]	173.56 [b]	108.47 [b]	12.45 [bc]	20.24 [b]
T_6	107.40 [de]	6.31 [c]	177.40 [b]	110.87 [b]	11.15 [ab]	19.46 [b]
T_7	110.07 [e]	6.42 [c]	179.42 [b]	112.13 [b]	8.97 [a]	15.79 [a]
T_8	65.52 [a]	3.42 [a]	96.29 [a]	60.18 [a]	20.03 [d]	33.26 [d]
F test (p=0.05)	*	*	*	*	*	*
S Em ±	2.83	0.31	12.00	7.50	1.05	0.88
CD @ 5 %	5.58	0.93	36.38	22.74	3.19	2.67
CV %	5.11	9.26	13.00	13.00	13.43	6.64

Plate.1 View of tomato plot- Foliar spray with mancozeb @ 2 g/l. (T_7)

Plate.2 View of tomato plot- Untreated control. (T_8)

Effect of different methods and levels of application of *Bacillus* var. *amyloliquefaciens* strain FZB24 on diseases incidence of *Alternaria solani* at 65 and 90 DAT

Significant difference in per cent diseases index of *A. solani* were observed among the treatments at both 65 and 90 DAT (Plate.1). The treatment T_7, *i.e.*, standard chemical control by mancozeb @ 2g/l recorded the lowest per cent diseases index of *Alternaria solani* of 8.97 and 15.79 PDI at 65 DAT and 90 DAT (Table 3). The probable reason for such finding may be that, mancozeb 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 may be the reason for minimum disease intensity and maximum yield as compared to other treatments. The results are in agreement with the findings of Salman and Abuamsha, (2012), who reported that metalaxyl acts mainly by inhibiting fungal growth and sporulation through the inhibition of RNA synthesis.

Treatments T_1, T_2, T_4, T_5, and T_6 were the next best in recording less per cent disease index of *A. solani* at both 65 and 90 DAT. The highest per cent disease index of *A. solani* (20.03 and 33.26) were noticed in T_8 *i.e.,* untreated control. The results obtained in this experiment are in accordance with the findings of Chowdappa *et al.,* (2013) 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. This was in conformity with the findings of Kader *et al.,* (2012); Nihorimbere *et al.,* (2010); Tan *et al.,* (2013) in tomato. *B. subtilis* strains could be effective biocontrol agents against soil fungi plant pathogens and could have a potential bio-fertilizer effect, since they stimulated growth and yield of tomato plants Suarez *et al.,* (2011). *B. subtilis* B1, B6, B28 and B99 significantly promoted growth and biocontrol activity against *F. oxysporum* f.sp *ciceris* in chickpea. They were observed to produce IAA, HCN and antifungal volatiles among others Karimi *et al.,* (2012). It may be due to the influence of plant growth promotion and induced systemic resistance (ISR) in enhancing the disease resistance in tomato plants. This is in confirmation with studies conducted by Latha *et al.,* (2009). The result shows that *Bacillus* spp are very effective biocontrol agents and should be harnessed for further biocontrol applications (Ajilogba *et al.,* 2013). Available reports suggest that specific strains of the species *Bacillus amyloliquefaciens, B. subtilis, B. pasteurii, B. cereus, B. pumilus, B. mycoides,* and *B. sphaericus* elicit significant reductions in the incidence or severity of various diseases on diversity of hosts including greenhouse studies or field trials on tomato, bell pepper, muskmelon, watermelon, sugarbeet, tobacco, Arabidopsis species, cucumber, loblolly pine and tropical crops Kloepper *et al.,* (2004).

The present investigation revealed that, apart from the standard chemical control through mancozeb treatments, the application bio-fungicide (taegro) by both soil drenching and foliar spray @ 500 g per hectare has proved to be the most appropriate and economically viable treatment in enhancing growth, yield and disease control. The next best treatment was soil drenching and foliar spray of taegro @ 250 g per hectare.

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