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Combined Application of Antagonist and Azoxystrobin on Plant Biometrics and *Fusarium* Wilt Incidence of Tomato

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A B S T R A C T

Tomato (*Solanum lycopersicum* L.) is one of the most widely grown vegetable crops in the world; they are excellent source of various micronutrients and antioxidants. In Tamil Nadu the area under tomato cultivation is 38.78 lakh ha with the production of 841.21 million tonnes. In Tamil Nadu, the major tomato producing districts are Vellore, Salem, Dharmapuri, Coimbatore, Trichy, Thiruvannamalai, Madurai, Dindugul, Erode and Krishnagiri. PKM 1, Marutham, Paiyur1, COTH 2 and Sivam are the important varaities/hybrids grown in Tamil Nadu. Among various diseases, *Fusarium*wilt of tomato caused by *Fusarium oxysporum* f.sp *lycopersici* (Sacc.) Synder and Hansenis considered as one of the major constraints to tomato production. Many effective pesticides have been tested against the pathogen but not considered as long term solution. Therefore, the present investigations were undertaken with an objective to isolate and test effective *B. subtilis* and *Trichoderma viride* along with the new generation fungicide Azoxystrobin 23% SC against wilt disease of tomato and assess the molecular mechanisms involved in the disease suppression. Combined application of seed treatment with consortium (*B.subtilis* and *T.viride*) @ 10.0 ml / Kg of seeds+ seedling root dip of consortium (*B.subtilis and T.viride)* @ 500ml/10 lit of water + Azoxystrobin 23% SC as Foliar Spray @ 0.1% on 45 DAT significantly reduced the tomato *Fusarium*wilt to the minimum and increased the plant growth and yield parameters of tomato to the maximum under pot culture and field condition. Thus, the results of the present study have clearly proved that with the combination of seed treatment seedling root dip of consortium (*B.subtilis* and *T. viride)* along with the new chemical Azoxystrobin 23% SC, it is possible to reduce the amount of chemical being used and also to reduce the number of applications *viz.*, with only one spray of the chemical at 45 DAT the tomato *Fusarium* wilt incidence could effectively be managed with enhanced fruit yield of Tomato.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely grown vegetable crops in the world (Pastor *et al.*, 2012). Tomato is used for consumption due to its high nutritive values, antioxidant and curative properties (Sahu *et al.*, 2013). Tomatoes are excellent source of various micronutrients and antioxidants. Therefore, they are often recommended by dieticians and nutritionists for controlling cholesterol and weight reduction (Lenucci *et al.,* 2006, Keswani, 2015). Tomato contains high levels of vitamin C, lycopene, and ß-carotene, which are anti-oxidants that promote good health. The nutritional quality of tomato is mainly determined by its carotenoid, potassium, vitamin C and vitamin A content. Ripe tomatoes have high levels of carotenoids, of which carotenes make up between 90 and 95% (Guil-Guerrero and Rebolloso-Fuentes, 2009). 'Lycopene' produced only by tomato is a natural antioxidant that works effectively to slow the growth of the cancerous cells (Bhovomik *et al.,* 2012).

China ranks first in the world with an area of 14.5 lakh hectares and 41.626 million tonnes of production per annum (Anon., 2015). India occupies second position in the world with respect to area, but occupies only fifth place in terms of production. Total area under tomato cultivation in India is 773.9 lakh ha with a production of 18732.0 million tonnes (Horticultural Database, 2017 provisional). Tomato plant is susceptible to various diseases caused by different agents such as bacteria, viruses, nematode, fungi and abiotic factors (Sahu *et al.,* 2013). Among the fungal diseases, wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is an economically important and destructive disease of tomato crop worldwide (Jones *et al.,* 1991). The fungus *Fusarium oxysporum* f. sp. *lycopersici* is exerting production losses between 30 to 40% and may even reach up to 80% if, climatic conditions favor the growth of the fungus (Lukyanenko, 1991, Nirmaladevi, 2016). Many effective fungicides have been tested against the *Fusarium* wilt pathogens but not considered as long term solution because of concerns about health and environmental hazards, expensiveness, development of resistance to pesticides (Stammler *et al.,* 2006). Besides, the chemicals pose health hazards to applicators as well as the consumers (Sundaramoorthy, 2013). The non-availability of specific fungicide and lack of resistant varieties also aggravate the problem. Therefore, a need for alternative methods of control of wilt complex pathogens has become vital and several researchers have also reported alternative disease management strategies (Singh *et al.,* 2003).

Biological control of soil borne diseases using antagonistic microorganisms are accepted as a durable and ecofriendly alternative for agrochemicals. The antagonistic fungi especially *Trichoderma* spp viz., *T. harzianum*, *T. atroviride* and *T.longibrachiatum* have been widely used against *F. solani* and *F. oxysporum* f.sp. *lycopersici* (Ahmed, 2011; Hand and Perveen, 2012). *Bacillus subtilis* is also having significant antagonistic activity against *F. oxysporum* in both laboratory and *in vivo* conditions. The *B.subtillis* strain EU07 reduced the incidence of disease caused by *F. oxysporum* f. sp. *lycopersici* by 75% (Rocha, 2017) consortium.

The long term effective management usually uses integration of two or more disease control measures (Fazil and Ishtiaq, 2010).Hence; it was thought that integration of a PGPR with the new fungicide could enhance the disease suppression and also reduce the amount of fungicides applied to the crop. New generation fungicides have proved as a new ray of hope in better management of diseases under field conditions (Ravikumar, 2017). Azoxystrobin is one an efficient fungicide belongs to the strobilurins, group of fungicides (Margot *et al.,* 1998). Azoxystrobin a naturally occurring broad spectrum new generation fungicide extracted from the mushroom fungi *Strobilurus tenacellus* with protectant, curative, eradicant and systemic properties, is found to be very effective in controlling various fungal diseases (Sundravadana *et al.,* 2007) combination with bioagent. Recent works also revealed that the biocontrol agents *T.viride* and *B. subtilis* were used against many soil borne diseases along with the fungicides especially Azoxystrobin (Mohiddin and Khan, 2013; Ranganathswamy *et al.,* 2013; Saxena *et al.*, 2014).

Materials and Methods

Effect of combined application of antagonist and Azoxystrobin on plant growth and *Fusarium* wilt incidence of tomato (pot culture)

Sterilized soil was mixed with the pathogen inoculums @ 5per cent (W/W) level and filled in 1×1×1 feet cement pots. The most effective soil application dosages identified as earlier experiment alone were used for testing the efficacy of soil application of the antagonists. The tomato seeds were treated with the antagonists and Azoxystrobin as seed treatment and after planted in the pot soil mixed with the inoculum of *F.oxysporum* f.sp. *lycopersici* alone served as a control. Carbendazim @ 0.1% was used for comparison. The experiment was conducted with three replication in a randomized block design.

Treatment schedule

T_1 – Seed treatment with consortium (*T.viride* & *B.subtilis*) @ 10ml/kg of seed
T_2– Seedling root dip of consortium (*T.viride* & *B.subtilis*) @ 500ml/10 lit of water
T_3– T_1+ T_2
T_4 – Seed treatment with Azoxystrobin @ 2ml/kg of seed
T_5– Seed treatment with Azoxystrobin @ 2ml/kg of seed+ Foliar spray with azoxystrobin @0.1% @ 45 DAT
T_6– Seed treatment with Azoxystrobin @ 2ml/kg of seed+ Foliar spray with azoxystrobin @0.1% @ 45 DAT and 60 DAT
T_7– T_3+ Foliar spray with azoxystrobin @0.1% @ 45 DAT
T_8 – T_3+ Foliar spray with azoxystrobin @0.1% @ 45 DAT and 60 DAT
T_9 – Seed Treatment with Carbendazim 50% WP @ 2g/ kg of seed+ Foliar spray with azoxystrobin @0.1% @ 45 and 60 DAT
T_{10}- Control

The treatments where given as per the schedule. All the observations *viz., Fusarium* wilt incidence, plant growth parameters viz., plant height, no of fruits per plant and yield per plant were recorded at 30 DAT, 60 DAT, 90 DAT and at final harvest.

Effect of combined application of antagonist and Azoxystrobin on plant biometrics and *Fusarium* wilt incidence of tomato (pot culture)

A field trial were conducted in *Fusarium* wilt prone farmer's field at perungulathur village in Thiruvannamalai district of Tamilnadu 2018-2019, representing irrigated conditions by integrating the best treatments identified in the pot culture experiments. The blanket fertilizer schedule of NPK 75:100:50 kg / ha recommended by the Tamilnadu agriculture university was followed. A plot size of $20m^2$ was used for each treatment. Each treatment was replicated thrice and a suitable control was also maintained.

Treatment schedule

T_1 - Seed treatment with consortium (*T.viride* & *B.subtilis*) @ 10ml/kg of seed
T_2 - Seedling root dip of consortium (*T.viride* & *B.subtilis*) @ 500ml/10 lit of water
T_3 - T_1+ T_2
T_4- Seed treatment with Azoxystrobin @ 2ml/kg of seed
T_5- Seed treatment with Azoxystrobin @ 2ml/kg of seed+ Foliar spray with

azoxystrobin @ 0.1% @ 45 DAT

T_6- Seed treatment with Azoxystrobin @ 2ml/kg of seed+ Foliar spray with azoxystrobin @0.1% @ 45 DAT and 60 DAT

T_7- T_3+ Foliar spray with azoxystrobin @0.1% @ 45 DAT

T_8 - T_3+ Foliar spray with azoxystrobin @0.1% @ 45 DAT and 60 DAT

T_9 - Seed Treatment with Carbendazim 50% WP @ 2g/ kg of seed+ Foliar spray with azoxystrobin @ 0.1% @45 and 60 DAT

T_{10}- Control

The treatments where given as per the schedule. All the observations *viz., Fusarium* wilt incidence, plant growth parameters viz., plant height, no of fruits per plant and yield/ha were recorded at 30 DAT, 60 DAT, 90 DAT and at final harvest.

Results and Discussion

Effect of Consortium (*T. viride* + *B.subtilis*) and Azoxystrobin 23% SC on the biometrics of Tomato var. PKM 1 (Pot culture)

The results obtained on the efficacy of combined application of antagonists and Azoxystrobin 23% SC are furnished in table 1. Among the treatments the combination treatment of seed treatment with consortium (*T.viride& B.subtilis*) @ 10 ml/kg of seed + seedling root dip of consortium (*T.viride& B.subtilis*) @ 500ml/10 lit of water+ foliar spray of Azoxystrobin 23% SC @0.1% @ 45 DAT (T_7) recorded the maximum germination percentage (94.69%), plant height (126.83 cm), no. of fruits per plant (23.45) and fruit yield (2.00kg/plant) which was at par with the treatment T_8 (T3+ foliar spray @ 45 DAT and 60 DAT) recorded germination percentage (93.50%), plant height (117.65 cm), no. of fruits per plant (21.67) and fruit yield (1.73kg/plant) and they were followed by the treatments $T_{6,}T_{3,}T_{5,}T_{4,}T_{7,}T_2$ and T_1 is the decreasing order of merit. The control recorded the least biometric values of germination percentage (64.98%), plant height (89.34 cm), no. of fruits per plant (15.32) and fruit yield (0.89kg/plant).

Combined application of Azoxystrobin 23% SC and consortium (*T.viride* & *B.subtilis*) on the *Fusarium* wilt incidence of tomato var. PKM 1 (field trial)

The results depicted in table 2 of the pot trial showed that the *Fusarium*wilt incidence was effectively reduced by the treatment Seed treatment with Azoxystrobin 23% SC @2ml/kg of seed+ foliar spray @ 45 DAT and 60 DAT(T_6) which recorded the least disease incidence (5.98%, 9.65%, 12.93% and 13.64% at 30DAT, 60DAT, 90DAT and at final harvest respectively). Which was at par with the treatment T3+ Foliar spray of Azoxystrobin 23% SC 0.1% @ 45 DAT and 60 DAT (T8) recorded (6.23%, 9.86%, 13.86% and 14.75% at 30DAT, 60DAT, 90DAT and at final harvest respectively). T3+ foliar spray of Azoxystrobin 23% SC 0.1% @ 45 DAT (T_7) recorded (6.53%, 10.23%, 13.38% and 14.36% at 30DAT, 60DAT, 90DAT and at final harvest respectively). Whereas control recorded the maximum wilt incidence of (20.86%, 34.96%, 50.32% and 63.25% at 30DAT, 60DAT, 90DAT and at final harvest respectively)

Effect of Consortium (*T. viride* + *B.subtilis*) and Azoxystrobin 23% SC on the biometrics of Tomato var. PKM 1 (field trial)

The results obtained on the efficacy of combined application of antagonists and Azoxystrobin 23% SC are furnished in table 3.

Table.1 Combined application of Azoxystrobin 23% SC and consortium (*T.viride* & *B.subtilis*) on the plant biometrics of tomato(Pot culture)

Tr.No	Treatment	Plant biometrics		
		Plant height	No. Of fruits/plant	Yield/ plant
T_1	Seed treatment with consortium (*T.viride* & *B.subtilis*) @ 10ml/kg of seed	95.84^{i}	16.85^{i}	1.22^{i}
T_2	Seedling root dip of consortium (*T.viride* & *B.subtilis*) @ 500ml/10 lit of water	96.54^{h}	16.25^{h}	1.17^{h}
T_3	T_1+ T_2	115.25^{c}	21.34^{c}	1.72^{c}
T_4	Seed treatment with Azoxystrobin 23% SC @2ml/kg of seed	102.35^{f}	20.86^{f}	1.62^{f}
T_5	Seed treatment with Azoxystrobin 23% SC @2ml/kg of seed+ Foliar spray with Azoxystrobin 23% SC @0.1% @ 45 DAT	103.37^{e}	21.85^{e}	1.71^{e}
T_6	Seed treatment with Azoxystrobin 23% SC @2ml/kg of seed+ Foliar spray with Azoxystrobin 23% SC @0.1% @ 45 DAT and 60 DAT	116.79^{c}	22.64^{c}	1.81^{c}
T_7	T_3+ Foliar spray with Azoxystrobin 23% SC @0.1% @ 45 DAT	126.83^{a}	23.45^{a}	2.00^{a}
T_8	T_3+ Foliar spray with Azoxystrobin 23% SC @0.1% @ 45 DAT and 60 DAT	117.65^{a}	21.67^{a}	1.73^{a}
T_9	Seed Treatment with Carbendazim 50% WP @ 2g/ kg of seed+ Foliar spray with Azoxystrobin 23% SC @0.1% @45 and 60 DAT	98.49^{g}	18.64^{g}	1.39^{g}
T_{10}	Control	89.34^{j}	15.32^{j}	0.89^{j}

* Mean of three replications
* In a column, means followed by a common letter are not significantly differ at 5% level by Duncan’s multiple range test (DMRT)

Table.2 Combined application of Azoxystrobin 23% SC and consortium (*T.viride* & *B.subtilis*) on the *Fusarium* wilt incidence of tomato (Field trial)

Tr.No	Treatment	Wilt incidence(%)				Percent decrease over control			
		30DAT	60DAT	90DAT	At final harvest	30DAT	60DAT	90DAT	At final harvest
T_1	Seed treatment with consortium (*T.viride*&*B.subtilis*)@ 10ml/kg of seed	11.96[i] (20.23)	19.55[i] (26.24)	26.25[i] (30.82)	29.25[i] (32.74)	42.58	44.07	47.83	53.75
T_2	Seedling root dip of consortium (*T.viride*&*B.subtilis*) @ 500ml/10 lit of water	11.43[h] (19.76)	17.35[h] (24.61)	22.98[h] (28.64)	27.43[h] (31.58)	45.12	50.76	54.33	56.63
T_3	T_1+ T_2	9.05[f] (17.50)	13.89[f] (21.88)	16.35[f] (23.85)	18.96[f] (25.81)	56.55	60.73	67.50	70.02
T_4	Seed treatment with Azoxystrobin 23% SC @2ml/kg of seed	10.63[g] (19.02)	16.23[g] (23.75)	21.28[g] (27.47)	24.48[g] (29.65)	48.96	53.99	57.71	61.29
T_5	Seed treatment with Azoxystrobin 23% SC @2ml/kg of seed+ Foliar spray with Azoxystrobin 23% SC @0.1% @ 45 DAT	9.85[e] (18.29)	16.05[e] (23.61)	20.25[e] (26.74)	21.78[e] (27.81)	52.71	54.51	59.75	65.56
T_6	Seed treatment with Azoxystrobin 23% SC @2ml/kg of seed+ Foliar spray with Azoxystrobin 23% SC @0.1% @ 45 DAT and 60 DAT	5.98[a] (14.15)	9.65[a] (18.09)	12.93[a] (21.07)	13.64[a] (21.67)	71.33	72.39	74.30	78.43
T_7	T_3+ Foliar spray with Azoxystrobin 23% SC @0.1% @ 45 DAT	6.23[b] (14.45)	9.86[b] (18.30)	13.86[b] (21.85)	14.75[b] (22.58)	70.13	71.79	72.45	76.67
T_8	T_3+ Foliar spray with Azoxystrobin 23% SC @0.1% @ 45 DAT and 60 DAT	6.53 [b] (14.8)	10.23[b] (18.65)	13.38[b] (21.45)	14.36[b] (22.26)	68.65	71.28	73.41	77.28
T_9	Seed Treatment with Carbendazim 50% WP @ 2g/ kg of seed+ foliar spray @45 and 60 DAT	9.46[d] (17.91)	14.56[d] (22.43)	18.89[d] (25.76)	20.89[d] (27.19)	54.58	58.80	62.46	66.97
T_{10}	Control	20.86[j] (27.17)	34.96[j] (36.24)	50.32[j] (45.18)	63.25[j] (52.68)	-	-	-	-

* Mean of three replications

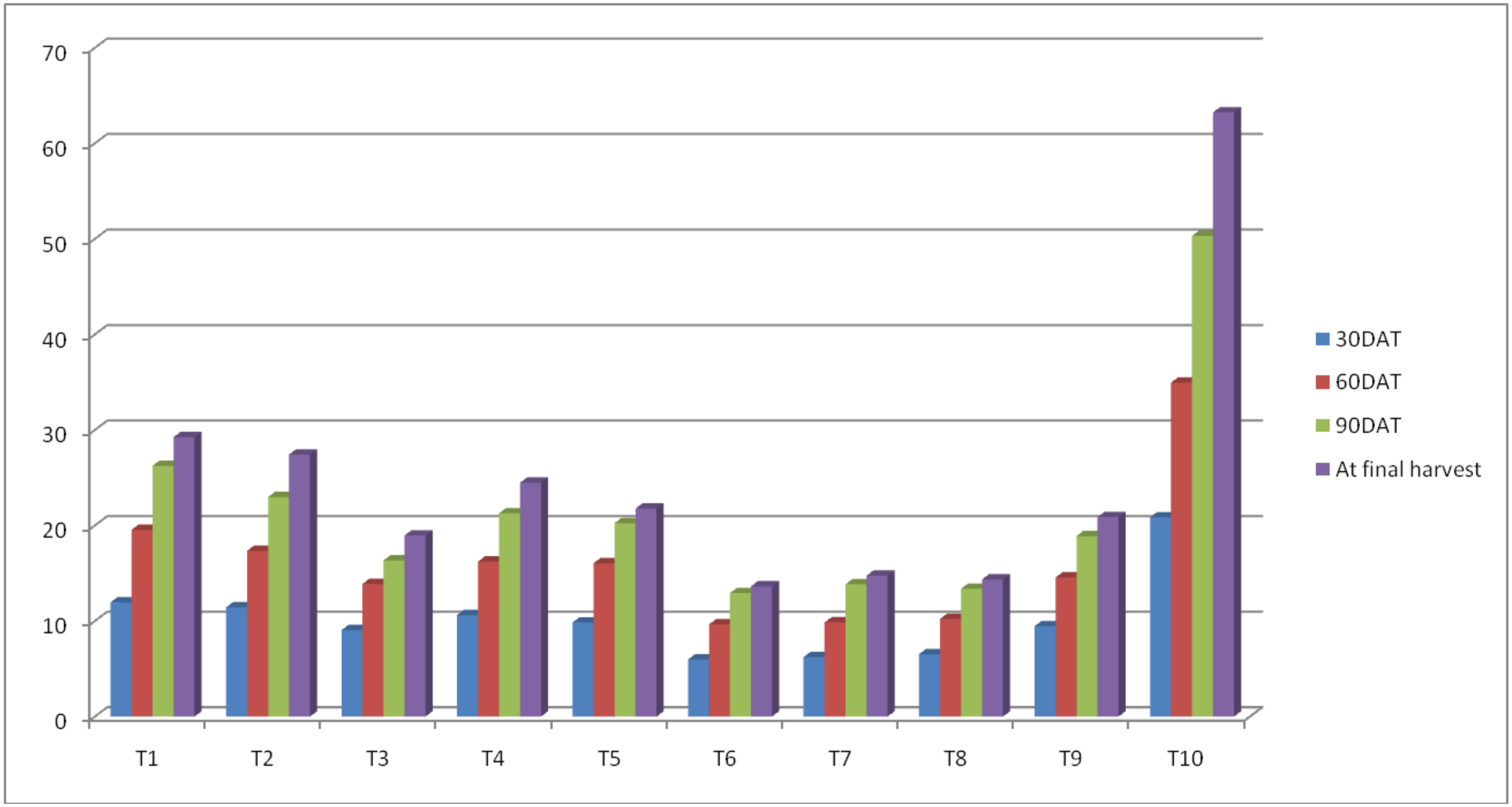
* In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)

Table.3 Combined application of Azoxystrobin 23% SC and consortium (*T.viride* & *B.subtilis*) on the plant biometrics of tomato (Field trial)

Tr.No	Treatment	Plant biometrics		
		Plant height	**No. Of fruits/plant**	**Tonnes / ha**
T_1	Seed treatment with consortium (*T.viride*&*B.subtilis*)@ 10ml/kg of seed	97.33[i]	16.85[i]	25.36[i]
T_2	Seedling root dip of consortium (*T.viride*&*B.subtilis*) @ 500ml/10 lit of water	98.39[h]	17.29[h]	26.24[h]
T_3	T_1+ T_2	115.21[c]	23.64[c]	30.34[c]
T_4	Seed treatment with Azoxystrobin 23% SC @2ml/kg of seed	103.96[f]	21.55[f]	28.76[f]
T_5	Seed treatment with Azoxystrobin 23% SC @2ml/kg of seed+ foliar spray Foliar spray with Azoxystrobin 23% SC @0.1% @ 45 DAT	104.87[e]	22.94[e]	29.64[e]
T_6	Seed treatment with Azoxystrobin 23% SC @2ml/kg of seed+ foliar spray Foliar spray with Azoxystrobin 23% SC @0.1% @ 45 DAT and 60 DAT	117.24[c]	21.69[c]	31.54[c]
T_7	T_3+ foliar spray Foliar spray with Azoxystrobin 23% SC @0.1% @ 45 DAT	131.84[a]	24.72[a]	33.89[a]
T_8	T_3+ foliar spray Foliar spray with Azoxystrobin 23% SC @0.1% @ 45 DAT and 60 DAT	119.57[a]	21.74[a]	32.46[a]
T_9	Seed Treatment with Carbendazim 50% WP @ 2g/ kg of seed+ foliar spray @45 and 60 DAT	99.24[g]	19.87[g]	27.36[g]
T_{10}	Control	89.34[j]	15.47[j]	24.36[j]

* Mean of three replications
* In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)

Fig.1 Combined application of Azoxystrobin 23% SC and consortium (*T.viride* & *B.subtilis*) on the *Fusarium* wilt incidence of tomato (Field trial)



Among the treatments the combination treatment of seed treatment with consortium (*T.viride & B.subtilis*) @ 10 ml/kg of seed + seedling root dip of consortium (*T.viride& B.subtilis*) @ 500ml/10 lit of water+ foliar spray of Azoxystrobin 23% SC @0.1% @ 45 DAT (T_7) recorded the maximum germination percentage (95.87%), plant height (131.84 cm), no. of fruits per plant (24.72) and fruit yield (33.89t/ha) which was at par with the treatment T_8 (T_3+ foliar spray @ 45 DAT and 60 DAT) recorded germination percentage (92.84%), plant height (119.57 cm), no. of fruits per plant (21.74) and fruit yield (32.46t/ha) and they were followed by the treatments $T_{6,}$ $T_{3,}T_{5,}T_{4,}$ $T_{7,}T_2$ and T_1 is the decreasing order of merit. The control recorded the least biometric values of germination percentage (62.98%), plant height (89.34 cm), no. of fruits per plant (15.47) and fruit yield (24.36t/ha).

Thus, it is evident that the integration of bio agent with Azoxystrobin resulted in a synergistic effect and resulted in suppression of the disease. The strobilurins bind to one specific site in the mitochondria, the quinol oxidation (Qo) site (or ubiquinol site) of cytochrome b and thereby stop electron transfer between cytochrome b and cytochrome c, which halts reduced nicotinamide adenine dinucleotide (NADH) oxidation and adenosine triphosphate (ATP) synthesis (Brandt,1993). This leads to the stopping of the energy production and the fungus will eventually die. Also the Strobilurins are having the ability to delay leaf senescence and preserve oxidative balance, which, in turn, can lead to increased yields. Similar to the present observations the mixture *B. subtilis* + azoxystrobin was found very effective and generally more active than the two components applied alone (Gilardi *et al.*, 2008). Also it has been reported that the combinations of biocontrol agents with 50% reduction in the dose of fungicides were effective against the fruit rot of chilli (Anand *et al.*, 2010). Integrated use of biocontrol agents with single dose of fungicide was found effective against *Fusariumm* crown rot and root rot of tomato (Omar *et al.*, 2006). Besides, the synergistic efficacy of antagonists against several pathogens was reported in various crops (Thilagavathi *et al.*, 2007; Rashmi Srivastava *et al.*, 2010; Tayal *et al.*, 2011; Malathi, 2015; Shiva Yendyo *et al.*, 2018).

These reports by the earlier workers lend support to the present investigations. *Trichoderma* species is considered as promising biological control agents against numerous phytopathogenic fungi since it is able to inhibit the phytopathogenic fungi either by developing resistance and plant defense reaction by releasing volatile and non-volatile metabolites including several unknown sesquiterpenes, diterpenes, and tetraterpenes or by direct confrontation through mycoparasitism and competition or by producing antibiotics (Odebode, 2006). It also produce more than 100 metabolites with antibiotic activities including polyketides, pyrones, terpenes, metabolites derived from amino acids and polypeptides (Sivasithamparam and Ghisalberti 1998).

Besides the disease suppression, secondary metabolites produced by *Trichoderma* have been reported to play a role in plant growth promotion, the metabolites produced by *T. koningii* (Koninginin A) and *T. harzianum* (6-pentylalpha-pyrone) that acted as plant growth regulators (Cutler *et al.*, 1989). Vinale*et al*. (2008), reported that azaphilone, butenolide, harzianopyridone, harzianolide, 1-hydroxy-3-methylanthraquinone, 1,8 dihydroxy-3-methyl-anthraquinone and 6-n-pentyl-6H-pyran-2-one (6PP) are the responsible for the plant growth promotion. All these earlier reports corroborates with the present findings.

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