



Research Article

Evaluating Soil Amendments for the Management of Bacterial Wilt Disease of Tomato

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Abstract | Two organic composts i.e. green manure (GM) and farmyard manure (FYM) were added to the soil at three concentrations i.e. C1= 1%, C2=5%, and C3=10%, to amend topsoil. Results revealed that increase in amendment's concentrations to 10% decreased the chance of plant survival to 68.06% while maximum (89.72%) number of plants survived for 1% amendments concentration. Significantly high number (87.22) of plants survived for green manure as compared to FYM with 78.47. C2 amended was found to be best with lowest (1.19) disease ratings. Significantly higher (i.e. 1.75 and 1.89) disease was observed for C1 and C3 amended soils. FYM was better as it produced significantly less (1.14) disease as compared to green manure with 1.35 disease severity. On the other hand, among concentrations, 5% was the best yielding with 2.17kg plant⁻¹, while the other concentrations i.e. 1% and 10% gave statistically similar and less yield (1.83 and 1.77kg plant⁻¹, respectively). Thus, it was concluded, that the application of green manure compost (used in 1% and 5% V/V concentration) to the soil, suppress the disease more and produced more yield than FYM-treated plants, however, the higher concentration i.e., 10% soil amendments had negative effect.

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Introduction

Tomato (*Lycopersicon esculentum* Mill.), belonging to family *solanaceae* (night shade family), is one of the important vegetable throughout the world. Average per hectare yield of tomato in Pakistan was 9864 kg.ha⁻¹ (MINFAL, 2013). In average per hector production of tomato Pakistan ranks 142th (FAOSTAT, 2015). The major cause of low production of tomato are different tomato diseases, caused by viruses, nematodes, fungi and bacteria. Tomato blight, wilting and cankers are very common and important diseases. Tomato bacterial wilt caused by *Ralstonia solanacearum* (Smith) is considered to be one of the most important diseases due to its economic impact.

Management of tomato wilt requires a high level of understanding of the pathogen, early and correct diagnosis and adoption of cultural and plant host resistance based strategies.

Due broad range of host plants availability it is very difficult to control bacterial wilt disease completely. Soil fumigants tested by Murakoshi and Takahashi (1984) were found non-effective against bacterial wilt pathogen. Antibiotics are good but their applications are sometimes restricted and cannot be applied to field, tetracycline, penicillin, ampicillin and streptomycin have little effect (Farag *et al.*, 1982). Biological control has also been investigated, but not satisfactory. In some previous researches

avirulent pathogen strains of *Ralstonia solanacearum* and *Pseudomonas fluorescens* were also used by some scientists to support the biological method of control but the results were not satisfactory or limited only to laboratories (Gallardo and Panno, 1989; Ciampi-Panno *et al.*, 1989; Hartman and Elphinstone, 1994).

As pathogen attack the plants from the soil it is difficult to control the disease using chemicals, it has been known that certain composts used as soil amendments or in container media may protect plants from disease causing pathogen (Hoitink and Kuter, 1986) Soil amendments that enhance host plant resistance have been given due consideration (Datnoff *et al.*, 2001). A very little research has been done on bacterial wilt in Khyber Pakhtunkhwa (KP). Keeping in view the importance of the disease, its destructive nature, and the chance of its presence in KP, it was decided to initiate the present research with the aim to investigate the effect of farmyard manure (FYM) compost and green compost (GM) for the management of the disease.

Materials and Methods

For the determination of the effects of soil amendments on the control of tomato wilt, pot experiments were conducted in the green house at the University of Agriculture, Peshawar, using completely randomized design, replicated three times. Farmyard manure (FYM) and green manure (GM) (composed of green and fallen leaves of different non-solanaceous, naturally and locally grown trees and grasses), were the two composts, used to amend topsoil (i.e. 5-10cm). Each amendment was added to the soil (volume/volume) at three concentrations i.e. C1= 1%, C2=5%, and C3=10%. A non-amended and un-inoculated soil was also used as control i.e. C0=control. Additionally, the experiments were performed on five tomato varieties i.e. Lyreka, Rio-Grand, Yaqui, Red-Stone, Money-maker and Rio-Fuegd in order to chance of natural disease escape or tolerance by a specific variety. The effect of amendments on disease severity and plant survival after inoculation was investigated.

Preparation of bacterial inoculum

Due to high virulence based on colony morphology (French *et al.*, 1995) bacterial isolate RS14-PES1 (Junaid *et al.*, 2018) was grown on Nutrient Agar for 24h, scrapped off the plates and stored in solution of 100g skimmed milk and 10g Na-glutamate per liter

at -20 °C for (24 h) to avoid possible development of non-pathogenic mutants. The stock culture was thawed, streaked on the rich agar medium and incubated at 30°C for 48 hours. Bacteria were collected with sterilized scalpel and suspended into 0.85% saline sterilized distilled water. The concentration was adjusted to 10⁷ cells.ml⁻¹ (using photo-spectrometer with OD₆₀₀) for inoculation to plants.

Preparation of plant material

Seedlings of each variety were separately raised in peat soil in green house at 25±5°C. Thirty days after sowing, the seedlings were ready to be transplanted to the earthen pots. Environmental and soil condition were kept same for all varieties. Similarly, plant nutrition, water requirements and cultural practices were also kept constant.

Inoculation of the pathogen

Pots were inoculated by pouring 100 ml of *R. solanacearum* culture containing 10⁷ cells.ml⁻¹ into the pot soil (top 2/3), by doing so the final bacterial density in soil was ca. 5x10⁵ CFU g⁻¹ dry soil. Within one hour of inoculation fifteen days old seedlings were transferred to pots. All the pots were watered independently using a hose with fine nozzle. To avoid cross-contamination a distance of 1.5 feet were kept between pots during watering.

The plants were regularly observed for the symptoms development. For recording the data, the three most important parameters i.e. percent survival of plants, Disease severity and yield of the plants were used. For percent survival of the plants number of plants were counted after one week of inoculation. For disease severity recording the rating scale (0-4) of Swanson *et al.* (2005) was used with some minor modifications (Table 1).

Table 1: Disease rating scale for bacterial wilt of tomato.

S.No	Rating	Description
1	0	no symptoms of wilting
2	1	0 to 25% of plant showing wilting
3	2	26 to 50% of plant showing wilting
4	3	51 to 75% of plant showing wilting
5	4	76 to 100% showing wilting

Results and Discussion

Survival percentage

The effects of bacterium was quite adverse on survival of

the plants that were inoculated just before transplanting (Table 2). Differences among the amendments, their concentrations and varieties were significant. Increase in amendment's concentrations decreased the chance of plant survival. Maximum (89.72) number of plants survived for 1% amendments concentration. The decrease (80.83%) in survival was significant for 5% concentration, while the lowest (68.06%) number of plants survived when amendments concentration was 10%. Significantly high number (87.22%) of plants survived for green manure as compared to FYM with 78.47%. On the other hand M maker and Rio-fuegd were the varieties in which maximum (91.25% and 89.58%, respectively) numbers of plants survived, followed by Yaque with 82.5% plants survived.

Disease severity

Data (Table 3) regarding disease severity of bacterial wilt of tomato was found to be quite significant as the

pathogen affected the soil all the disease ratings for soil amendments, their concentrations and varieties. All interactions and main effects were found to be significant. For concentrations, 5% had significantly lowered (1.19) disease while the other two i.e. 1% and 10% were statistically similar and produced 1.75 and 1.89 disease severity respectively. Here the un-inoculated control also produced some (0.14) disease but it was negligible. For soil amendments, FYM was better as it produced significantly less (1.14) disease as compared to green manure with 1.35 disease severity. On the other hand, highest (2.46) disease rating was recorded for varieties R. Grand. Results of Lyreka, Red stone and Rfuegd (1.12, 1.21 and 1.33, respectively) were statistically similar (non-significant) to each other. However, M maker was the variety proved to be somewhat resistant against the disease with lowest disease ratings (0.37).

Table 2: Effect of soil amendments, their concentration and varieties on survival of tomato plants inoculated with *R. solanacearum*.

Amendments (A)	Concentrations (C)	Varieties						A x C
		Lyreka	R. Grand	Yaqui	R. Stone	M. maker	R. Fuegd	
FYM	1 %	73.33	80.00	86.67	80.00	96.67	90.00	84.44
	5 %	73.33	73.33	73.33	70.00	86.67	80.00	76.11
	10 %	70.00	60.00	53.33	53.33	66.67	76.67	63.33
	Control	80.00	83.33	96.67	90.00	100.00	90.00	90.00
GM	1 %	93.33	86.67	96.67	93.33	100.00	100.00	95.00
	5 %	80.00	83.33	86.67	73.33	100.00	90.00	85.56
	10 %	73.33	63.33	66.67	63.33	80.00	90.00	72.78
	Control	93.33	86.67	100.00	93.33	100.00	100.00	95.56
Amendments x Varieties								Means
FYM		74.17	74.17	77.50	73.33	87.50	84.17	78.47 B
GM		85.00	80.00	87.50	80.83	95.00	95.00	87.22 A
Concentrations x Varieties								Means
	1 %	83.33	83.33	91.67	86.67	98.33	95.00	89.72 A
	5 %	76.67	78.33	80.00	71.67	93.33	85.00	80.83 B
	10 %	71.67	61.67	60.00	58.33	73.33	83.33	68.06 C
	Control	86.67	85.00	98.33	91.67	100.00	95.00	92.78 A
Means		79.58 BC	77.08 C	82.50 B	77.08 C	91.25 A	89.58 A	

Alphabetical letters with means in categories shows significant differences at 5% probability. LSD values at P≤0.05; Varieties(V): 4.40, Amendments(A): 2.54, Concentrations(C): 3.59, A×V: 6.22, C×V: 8.80, A×C: 5.08, A×C×V: 12.44.

Yield (kg. plant⁻¹)

Significant effect of bacterial inoculation was observed for varieties and amendments with concentrations in terms of yield data (Table 4). Interactions as well as main effects all were found highly significant. 5%

concentration of amendments was found to be the best with a yield of 2.17 kg.plant⁻¹, while the other concentrations i.e. 1% and 10% gave statistically similar and less yield (1.83 and 1.77kg.plant⁻¹, respectively). Control plants gave good yield (2.99kg.

plant⁻¹) as no inoculation was done to these plants. Green manure give good yield (2.25kg.plant⁻¹) as compared to FYM with 2.13kg.plant⁻¹. Among varieties M.maker proved to somewhat resistant

to the disease and hence produced maximum (2.78 kg.plant⁻¹) yield, followed by Yaqui (2.42 kg.plant⁻¹) and Lyreka (2.24kg.plant⁻¹). Variety R Grand gave minimum (1.33kg) yield per plant.

Table 3: Effect of soil amendments with different concentration and varieties on disease severity of tomato plants inoculated with *R. solanacearum*.

Amendments (A)	Concentrations (C)	Varieties						A x C
		Lyreka	R. Grand	Yaqui	R. Stone	M.Maker	R. Fuegd	
FYM	1 %	1.33	4.00	1.00	1.33	0.33	1.67	1.61
	5 %	1.33	2.00	0.67	1.33	0.00	1.00	1.06
	10 %	1.33	3.33	1.33	1.33	1.33	1.67	1.72
	Control	0.00	0.00	0.33	0.00	0.33	0.33	0.17
GM	1 %	2.00	4.00	1.67	1.67	0.00	2.00	1.89
	5 %	1.67	2.67	1.00	1.67	0.00	1.00	1.33
	10 %	1.33	3.67	1.67	2.33	1.00	2.33	2.06
	Control	0.00	0.00	0.00	0.00	0.00	0.67	0.11
Amendments x Varieties								Means
FYM		1.00	2.33	0.83	1.00	0.50	1.17	1.14 B
GM		1.25	2.58	1.08	1.42	0.25	1.50	1.35 A
Concentrations x Varieties								Means
	1 %	1.67	4.00	1.33	1.50	0.17	1.83	1.75 A
	5 %	1.50	2.33	0.83	1.50	0.00	1.00	1.19 B
	10 %	1.33	3.50	1.50	1.83	1.17	2.00	1.89 A
	Control	0.00	0.00	0.17	0.00	0.17	0.50	0.14 C
Means		1.12 BC	2.46 A	0.96 C	1.21 BC	0.37 D	1.33 B	

Alphabetical letters with means in categories shows significant differences at 5% probability. LSD values at P≤0.05; Varieties(V): 0.33, Amendments(A): 0.19, Concentrations(C): 0.27, A×V: 0.46, C×V: 0.65, A×C: 0.38, A× C× V: 0.92.

Table 4: Effect of soil amendments, their concentration and varieties on yield (kg.plant⁻¹) of tomato plants inoculated with *R. solanacearum*.

Amendments (A)	Concentrations (C)	Varieties						A x C
		Lyreka	R. Grand	Yaqui	R. Stone	M. Maker	R. Fuegd	
FYM	1 %	1.85	0.30	2.02	1.94	2.63	1.57	1.72
	5 %	1.97	1.39	2.32	1.85	2.88	2.09	2.08
	10 %	2.04	0.68	1.90	1.67	2.11	1.49	1.65
	Control	2.97	3.33	3.14	2.64	3.25	3.00	3.06
GM	1 %	2.01	0.41	2.27	2.16	2.76	1.97	1.93
	5 %	2.11	1.47	2.36	2.22	2.88	2.46	2.25
	10 %	2.20	0.80	2.15	1.98	2.34	1.90	1.90
	Control	2.76	2.25	3.17	2.88	3.38	3.07	2.92
Amendments x Varieties								Means
FYM		2.21	1.43	2.35	2.03	2.72	2.04	2.13 B
GM		2.27	1.24	2.49	2.31	2.84	2.35	2.25 A
Concentrations x Varieties								Means
	1 %	1.93	0.36	2.15	2.05	2.70	1.77	1.83 C
	5 %	2.04	1.43	2.34	2.04	2.88	2.27	2.17 B
	10 %	2.12	0.74	2.03	1.83	2.22	1.70	1.77 C
	Control	2.87	2.79	3.16	2.76	3.31	3.04	2.99 A
Means		2.24 BC	1.33 D	2.42 B	2.17 C	2.78 A	2.19 C	

Alphabetical letters with means in categories shows significant differences at 5% probability. LSD values at P≤0.05; Varieties(V): 0.19, Amendments(A): 0.11, Concentrations(C): 0.15, A×V: 0.26, C×V: 0.37, A×C: 0.21, A×C×V: 0.52.

In our studies, the application of green manure to potted soil was found to be more effective than farm yard manure (FYM). In our case it was insured that green manure wasn't composed of solanaceous plants (host plants) however it is quite possible that the green manure, consist of solanaceous crops or its residues might not give the same results of our research. We found that green-manured treatments suppressed the disease more and produced more yield than FYM-treated ones. Similar results were reported by other scientists (Ooshiro *et al.*, 2004; Arthy *et al.*, 2005; Matsushita *et al.*, 2006; Olivier *et al.*, 2006; Almeida *et al.*, 2007; Posas *et al.*, 2007; Acharya and Srivastava, 2009; Paret *et al.*, 2010; Amorim *et al.*, 2011; Yuan *et al.*, 2012). Concentration of the soil amendments is however important. In the present study, both 1% and 5% concentration of soil amendments generally decreased disease severity and increased yield and yield parameters. The higher concentration i.e., 10% soil amendment had negative effect. Similar results, regarding concentration effect, were reported by Yadessa *et al.* (2010) who noted that there was significantly higher area under disease progress curve for soil amended 10% GM compared to 1% GM. They also noted that total N, NO₃:N, NH₄:N and soluble N contents were more in 10% GM amended soil as compare to 1% GM amended soil. Availability of N sources, if exceed than crop requirement cause more vegetative growth at the result this soft and succulent vegetation appeal pests and diseases.

Soil amendments can improve the physical, chemical and biological structure and properties of the soil, thus very beneficial and affect plant growth positively (Bailey and Lazarovits, 2003). Due organic matter degradation sometimes the soil micro flora and so the pathogens present in soil are effected in terms of its viability and survival as some availability of the nutrients decreases and sometimes even inhibitory effects do occur (Bailey and Lazarovits, 2003). In the same degrading process of organic matter release of carbon triggers, the active competition in the soils (Bailey and Lazarovits, 2003). Organic amendments in the soil also increase the activities of beneficial or neutral microbes at the result they become antagonistic to the target bacterium (Akhtar and Malik, 2000). In addition, vitamins, growth regulators, and toxins (biologically-active molecules) are also present organic amendments and can affect soil microbes. Application of the compost to soils

increase resistance in plants against the wilt disease of tomato. The soils were tested positive for the presence of mono-dehydro-ascorbate reductase, dehydro-ascorbate-reductase, glutathione reductase and scorbateperoxidase after compost's applications (Youssef and Tartoura, 2013).

Conclusions and Recommendations

It can be concluded, that the application of green manure compost of non-solanaceous plants (used in 1% and 5% V/V concentration) to the soil, suppress the disease more and produced more yield than FYM-treated plants, however, the higher concentration i.e., 10% soil amendments had negative effect. Thus, soil amendments like FYM and green manure are recommended to be used in 1-5% V/V concentrations and well composted condition for minimizing the disease and increasing the yield.

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Novelty Statement

Bacterial wilt disease in tomato can be minimized by adding a Non-solanaceous green manure compost to the soil.

Author's Contribution

Junaid, M. performed the experiment. Junaid, M. and Ahmad, M. wrote the first draft of the manuscripts. Saifullah designed the experimentation and Junaid, M. analysed the data. The final manuscript is approved by all authors.

Conflict of interest

The authors have declared no conflict of interest.

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