Suitable Integrated Approach for Management of Fusarium Wilt of Tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.)

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http://dx.doi.org/10.22207/JPAM.11.2.36

(Received: 02 August 2016; accepted: 07 October 2016)

Among the different integrated approaches for management of Fusarium wilt and their effect on growth and yield parameters of tomato revealed that soil application of FYM @ 100gm/pot + Neem cake @ 100gm/pot + seedling treatment with bio-formulation of Azotobacter @ 5% + foliar spray of Carbendazim @ 0.1% was showing minimum disease incidence with 6.23, 10.11 and 15.03 per cent at 7, 14 and 21 days after inoculation, respectively. The observations on plant height of tomato was found in T3 treatment representing the value 17.00, 18.85, 20.66, 22.10, 24.10 and 27.30 cm at 5, 10, 15, 20, 25 and 30 days age of seedling, respectively against the minimum plant height i.e. 10.42, 10.92, 11.56, 11.76, and 12.55 in case of control (T0). The effect of integrated approach on branching of shoot in tomato was estimated at 85 days age of plant which revealed the maximum number of branch with 5.00 was found in case of soil application of FYM @ 100gm/pot + Neem cake@ 100gm/pot + seedling treatment with bio-formulation of Azotobacter @ 5% + foliar spray of Carbendazim @ 0.1% whereas, in case of control it was only 2.33. The morphological character of roots was examined and recorded developed robust root system in T3 treatment while the less fibrous, weakly developed roots in control. The maximum yield was recorded per plant in T3 treatment (soil application of FYM @ 100gm/pot + Neem cake@ 100gm/pot + seedling treatment with bio-formulation of Azotobator @ 5% + foliar spray of Carbendazim @0.1%) represented the value 490.30g per plant. Similarly, the maximum large size tuber with 4 in number was recorded in treatment T3 (soil application of FYM@ 100gm/pot+ Neem cake@ 100gm/pot + seedling treatment with bio-formulation of Azotobator(5% + foliar spray of Carbendazim @ 0.1%) followed by treatment T9 (Soil application of FYM @ 100 gm/pot + Neem cake @ 100 gm +seedling treatment with bio-formulation of *T. viride* + foliar spray Carbendazim @0.1%) as 03.

**Keywords:** Fusarium wilt, Biofertilizers, Growth parameters, Disease severity, Root length.

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Tomato (*Lycopersicon esculentum* Mill) belongs to the family Solanaceae and is one of the most remunerable and widely grown vegetable in the world. Among vegetable crops, tomato ranks second important vegetable in the world next to potato and first among the processing crops. The worldwide production of tomato is about 130 million tonnes in the year 2014. China is the largest producer in the world with a production of 48.577 million tonnes (FAOSTAT 2013-14). In India, production of tomato during 2014-15 was 182.86 lakh tones and total area under tomato cultivation was 777.0 ha. This constitutes 9.8 per cent of total vegetable area and 11.2 per cent of total vegetable production. The productivity of tomato in India is 20.7 MT/ha during 2014-15 which is very low.
as compare to other country of the world like USA, 81.0 tones /ha. One of main reasons of low productivity of tomato in India is disease which is caused by fungi, bacteria, virus, nematode and other biotic factors (Blanchard, 1992). Among them, Fusarium wilt incited by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc) is world’s most destructive disease which causes 50 to 86 per cent loss in fruit yield in different parts of country. (Mathur and Shekhawat, 1986) The management of disease can be done through the cultural practices (Tewari *et al.* 2012), biological control (Kokalis Burelle, 2002), chemical measures (Nguyen Khanh *et al.* 2013) etc. But all these methods have some limitations and cannot solely control the disease. Therefore, there is need to integrate all these methods in a suitable and sustainable manner for management of plant diseases in near future. Morajdhwaj *et al.* (2015) reported that soil application of mustard cake along with tuber treatment and foliar spray with *Trichoderma viride* was found best in reducing disease severity of late blight, stimulating germination and increased plant height of potato. Yogesh *et al.* (2015) also reported that among the different integrated approaches, soil application of FYM + seedling treatment with bio-formulation of *Trichoderma harzianum* + foliar spray of mancozeb reduced the disease severity of early blight of tomato and increased the growth parameters of plant. Bio-fertilizers viz., *Azotobacter chroococum*, PGPR, *Trichoderma harzianum*, *Trichoderma viride*, PSB, *Rhizobium*, Carbandazim, have also found effective in management of spot blotch of wheat and increase growth parameters and yield of wheat (Biswas, *et al.* 2015). Keeping all the point in view, the study was under taken in the present investigations as “Suitable Integrated Approach for Management of Fusarium Wilt of Tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.)”

**MATERIAL AND METHODS**

The experiment was conducted in the Wire House Complex Department of Plant Pathology, C.S.A. University of Agriculture & Technology, Kanpur during Rabi season 2014-16.

**Collection of disease sample**

The disease samples were collected from Vegetable Research Farm, C.S.A. University of Agriculture and Technology, Kalayanpur (Kanpur). Infected plants apparently showing wilt like symptoms were collected and bring to the laboratory for initial examination. The specimen diseased sample were pressed in between the fold of sterilized blotting paper and preserved at 4–6°C in refrigerator for further study. The entire specimen were collected and examined in the laboratory for the presence of the causal organism and virulence study.

**Isolation of pathogen**

Infected plant showing typical symptoms was selected for isolation of pathogen. Initially the diseased stem of tomato was washed thoroughly with distilled water to remove dust particles. Then diseased portion of stem were cut in to small pieces by a sterilized knife and each piece is having small bits of disease and healthy tissues. These pieces were dipped in 0.1% mercuric chloride solution for 30 seconds and then thoroughly washed thrice in distilled water to remove the traces amount of mercuric chloride solution. Excess moisture was removed by putting these pieces between the folds of sterilized blotting paper under aseptic conditions. The two pieces were then transferred to sterilized Petri plates containing 2% potato dextrose Agar (PDA) medium in inoculation chamber with the help of sterilized forceps. The Petri plates were then kept at room temperature. The Petri plates were observed daily to notice the presence of mycelia growth around the bits.

The pathogen was then purified by the transfer of mycelium from the tip of the colony to another Petri plate which was previously poured with sterilized PDA in aseptic condition. The purified culture was then characterised based on morphological and cultural behaviour as per described by Sacc. Synder and Hansen (1940).

**Effect of integrated approach on disease incidence, growth parameters and yield of tomato.**

The experiment was conducted at Glass house complex, Department of Plant Pathology, C. S. A. University Agriculture & Technology Kanpur. The Tomato variety ‘Azad T-6’ was sown in the glass house in 30 cm earthen pot, which was previously filled with a mixture of sandy loam and Farm Yard Manure in the ratios of 2:1. The treatments were given as follows-

- **T<sub>1</sub>** = Soil application of FYM @ 100gm/pot + Neem cake @ 100 gm + seedling treatment with formulation of Azotobacter @ 5% + foliar spray of Mancozeb @ 0.25%.
Effect of integrated approach on growth parameters of tomato and development of disease

Growth parameter

Shoot length

The shoot length of tomato was measured after 10 days of transplanting at every 5 days interval up to 30 days age of tomato plant with help of meter scale. This is well to know that same height of seedling was choice for transplanting.

Root length

Prior to measure the root lengths of tomato plants, pots were irrigated and the seedlings were up rooted carefully. The roots of seedling were separated from the shoots and washed with water to remove soil particles and then root length (cm) was measured with the help of meter scale.

Disease development

Inoculation of pathogen

At 45 days after transplanting, plants were inoculated with spore suspension of *Fusarium f. sp. lycopersici* at the concentration of conidia @ 10^6 conidia / ml. The spore suspension was prepared from seven days old culture of pathogen. The homogenized, spore suspensions were inoculated at the base of plant @ 1 ml / plant. The plants were then kept on the bench of wire house. Two controls were kept. In first case, plants were inoculated with pathogen and served as control 1 while in other case; plants were sprayed with distilled water and served as control-2. Three replications were kept for each treatment. Observation on disease severity were recorded at 7, 14, 21 days after final spraying.

Measurement of disease severity

Observation measuring on the disease severity was taken after 10 days of pathogen inoculation. The disease severity was recorded using 0-4 scale (Weitang *et al.* 2004) where zero representing no infection and four denoting completely infected plant. 2 representing moderate infection and 3 denoting extensive infection. The 0–4 scale of the disease incidence was classified as follows:

- No infection.
- Slight infection, where 25% leave become wilted and one or two leaves became yellow.
- Moderate infection, two or three leaves became yellow, 50% of leaves became wilted.
- Extensive infection, all plant leaves became yellow, 75% of leaves become wilted, and growth is inhibited.
- Complete infection, the whole plant leaves

Seedling treatment

Tomato seedlings of a variety were treated by root dip method. The seedlings were dipped in Azotobacter solution @ 20% conc. for a period of four hrs. The packets of Azotobacter containing 200 gm inoculums were obtained from Department of Soil Science, C. S. A. University of Agriculture & Technology Kanpur.

On the other hand, seedling was also treated with bio-formulation of *Trichoderma viride* @ 5% formulation.

Seedling treatment

- T_1 = Soil application of FYM @ 200 gm/pot + seedling treatment with *Trichoderma viride*.
- T_2 = Soil application of FYM @ 100gm/pot + Neem cake@ 100gm/pot + seedling treatment with bio-formulation of Azotobacter @ 5% + foliar spray of Carbenzadim @ 0.1%.
- T_3 = Soil application of FYM @ 100 gm/pot + Neem cake@ 100gm + seedling treatment with bio-formulation *T. viride* + foliar spray of Mancozeb @ 0.25%.
- T_4 = Soil application of FYM @ 100 gm/pot + Mustard cake @ 100 + seedling treatment with *T. viride* + Foliar spray Carbenzadim @ 0.1%.
- T_5 = Soil application of FYM @ 100 gm/pot + Mustard cake @ 100gm + seedling treatment with Azotobacter @ 5%+foliar spray with Carbenzadim @ 0.1%.
- T_6 = Soil application of FYM @ 100 gm/pot + Neem cake @ 100 gm + seedling treatment with bio-formulation of *T. viride* + foliar spray Carbendazim @ 0.1%.
- T_7 = Soil application of FYM @ 200 gm /pot + foliar spray with Mancozeb @ 0.25%.
- T_8 = Soil application of FYM @ 200 gm /pot + seedling treatment with formulation Azotobacter @ 5% + foliar spray with Carbendazim @ 0.1%.
- T_9 = Soil application of FYM @ 200 gm/pot + foliar spray with Mancozeb @ 0.25% only
- T_10 = (Control) Soil application of FYM 200 gm only

In each pot one treated seedling of tomato were sown and irrigated regularly. The experiment design was laid out in simple CRD. Three replications per treatment were kept and three pots were sown with untreated seedling served as control. The foliar spray scheduled was given at 3 days after artificial inoculation. Observation pertaining to effect of different treatment was recorded as per following parameters and days.

- Plant height (cm) at 5 days interval after 10 days of transplanting up to 30 days.
- Disease severity (%) at 50, 60 and 70 days after transplanting.
- Root length (cm) 85 days after transplanting.
- Yield/plant (gm)

### Seedling treatment

Tomato seedlings of a variety were treated by root dip method. The seedlings were dipped in Azotobacter solution @ 20% conc. for a period of four hrs. The packets of Azotobacter containing 200 gm inoculums were obtained from Department of Soil Science, C. S. A. University of Agriculture & Technology Kanpur.

On the other hand, seedling was also treated with bio-formulation of *Trichoderma viride* @ 5% formulation.
become yellow, 100% of leaves become wilted, and the plants die.

The percentage of disease incidence was determined using the formulas as given by Weitang, et al., (2004)

$$\text{disease incidence (\%) = } \left( \frac{\Sigma \text{scale} \times \text{number of plants infected}}{\text{highest scale} \times \text{total number of plants}} \right) \times 100$$

**Yield/plant**

The edible fruits were harvested twice a week from each treated plant and weighted with the help of physical balance. The total weight of all picking was recorded after adding weight of fruits at each picking. The yield of the crop of each treatment was calculated separately.

**RESULTS AND DISCUSSION**

**Effect of integrated approach on growth parameters of tomato**

**Plant height**

The effects of integrated approach on plant height of tomato were studied under Wire House Complex in pot culture experiment, Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur. The observations of plant height were taken after 10 days of transplanting at every 5 days of interval up to 30 days age of tomato plant. The data presented in Table-1 showed that the plant height of tomato was increase in all the treatments over control. The maximum plant height was found in T3 treatment (soil application of FYM@ 100gm/pot+ Neem cake@ 100gm/pot + seedling treatment with bio-formulation of Azotobacter @5% and foliar spray of Carbendazim @0.1%) representing the value 17.00, 18.85, 20.66, 22.10, 24.10 and 27.30 cm at 5, 10, 15, 20, 25 and 30 days age of seedling, respectively. The T9 treatment (soil application of FYM@ 100 gm/pot + Neem cake @100 gm + seedling treatment with bio-formulation of Trichoderma harzianum + foliar spray of mancozeb) was showing 11.53, 12.43, 14.10, 16.60, 21.60, and 26.00 cm at 10, 15, 20, 25 and 30 days age of plant respectively, demonstrating second highest among the treatments. Yogesh et al. (2015) also reported that among the different integrated approaches, soil application of FYM + seedling treatment with bio-formulation of Trichoderma harzianum + foliar spray of Carbendazim reduced the disease severity of early blight of tomato and increased the growth parameters and branching pattern of plant. Tippannavar et al. (2005) observed that the Azotobacter significantly increased the tillering, dry matter accumulation and growth parameter in wheat.

**Root length**

Eighty five days after transplanting, the tomato plant was uprooted and the root length was measured separately by using scale. The data presented in Table-4 showed that all the treatments were able to significantly increased root length over control at 85 days age of tomato (Fig. 1). Among the treatments, the maximum root length was recorded in treatment T3 (soil application of FYM @ 100gm/pot+ Neem cake @100 gm + seedling treatment with bio-formulation of Trichoderma harzianum + foliar spray of Carbendazim @0.1%) representing the value 15.00 cm followed by treatment T9, representing the value 14.00 cm and 10.00 cm respectively. On the other hand, maximum width of root zone was also recorded in T3, representing 16.00 cm.
Table 1. Effect of integrated approach on plant height of tomato at different days

| Treatment | 5      | 10     | 15     | 20     | 25     | 30     | Average no. of branches per plant |
|-----------|--------|--------|--------|--------|--------|--------|----------------------------------|
| T_1       | 14.35  | 15.75  | 17.00  | 18.80  | 21.00  | 24.85  | 3.00                             |
| T_2       | 14.00  | 15.90  | 16.40  | 18.50  | 21.00  | 23.50  | 3.66                             |
| T_3       | 17.00  | 18.85  | 20.66  | 22.10  | 24.10  | 27.30  | 5.00                             |
| T_4       | 15.00  | 16.75  | 19.00  | 20.80  | 21.33  | 23.10  | 4.00                             |
| T_5       | 13.85  | 15.10  | 16.75  | 18.90  | 20.00  | 22.00  | 4.33                             |
| T_6       | 14.20  | 15.75  | 17.53  | 19.00  | 21.22  | 23.10  | 3.66                             |
| T_7       | 12.90  | 14.53  | 16.22  | 18.50  | 20.22  | 22.85  | 4.00                             |
| T_8       | 14.10  | 15.66  | 16.90  | 18.40  | 20.10  | 21.80  | 4.00                             |
| T_9       | 11.53  | 12.43  | 14.10  | 16.60  | 21.60  | 26.00  | 4.48                             |
| T_10      | 10.42  | 10.92  | 11.56  | 11.10  | 11.76  | 12.55  | 2.33                             |
| C.D.      | 1.445  | 1.385  | 1.187  | 1.239  | 1.755  | 2.061  | 0.538                            |
| S.E(diff) | 0.622  | 0.609  | 0.565  | 0.658  | 0.834  | 0.873  | 0.264                            |
| CV%       | 6.495  | 5.756  | 4.145  | 3.70   | 4.362  | 4.112  | 8.416                            |

Table 2. Effect of different treatments on length, weight, branching pattern and morphological characters of tomato root

| Treatment | Root length (cm) | % increase over control | Average no. of branches in Roots | % increase over control | Root width (cm) | Morphological character of roots                      |
|-----------|------------------|-------------------------|----------------------------------|-------------------------|-----------------|-------------------------------------------------------|
| T_1       | 12.00            | 44.00                   | 5.40                             | 50.00                   | 10.66           | Branches are less in number                           |
| T_2       | 11.00            | 32.05                   | 5.00                             | 38.88                   | 10.33           | Less branches, not fibrous                            |
| T_3       | 15.00            | 80.07                   | 7.00                             | 94.44                   | 16.00           | Robust root system, well developed root               |
| T_4       | 9.33             | 12.00                   | 5.00                             | 38.88                   | 15.00           | Branches are less in number, mostly secondary root    |
| T_5       | 10.00            | 20.04                   | 5.00                             | 38.88                   | 10.66           | Roots are fibrous, developed                          |
| T_6       | 8.66             | 3.96                    | 6.00                             | 66.66                   | 14.00           | Thread like roots, tap root branched, many times      |
| T_7       | 11.00            | 32.05                   | 4.00                             | 11.11                   | 8.33            | Poor branches                                         |
| T_8       | 13.00            | 56.06                   | 5.00                             | 38.88                   | 13.66           | Roots are fibrous, developed                          |
| T_9       | 14.00            | 68.06                   | 6.50                             | 80.85                   | 15.32           | Sturdy root, well developed                           |
| T_10      | 8.33             | 00.00                   | 3.60                             | 00.00                   | 11.50           | Branches not developed, weak, less fibrous            |
| C.D.(0.05)| 0.673            | 0.532                   | 1.007                            |                         |                 |                                                       |
| S.E. (Diff)| 0.344          | 0.373                   | 0.478                            |                         |                 |                                                       |
| C.V. %    | 3.504            | 4.872                   | 3.567                            |                         |                 |                                                       |
robust root system is found in T₃ treatment (soil application of FYM @ 100 g/m² + Neem cake @ 100 gm/pot + seedling treatment with bio-formulation of Azotobactor @5% + foliar spray of Carbendazim@0.1% whereas, in case of control, poorly developed, less branching and less fibrous root system are found which is indicated that integrated approach have ability to change the morphology of root.

Table 3. Effect of integrated approach on disease severity Fusarium wilt of tomato

| Treatment | 7 day | 14 days | 21 days |
|-----------|-------|---------|---------|
| T₁        | 11.87 | 18.35   | 21.90   |
| T₂        | 14.35 | 17.75   | 20.12   |
| T₃        | 06.23 | 10.11   | 15.03   |
| T⁴        | 12.30 | 20.02   | 23.08   |
| T₅        | 09.12 | 13.75   | 14.90   |
| T₆        | 14.13 | 20.10   | 24.20   |
| T₇        | 13.05 | 21.02   | 24.08   |
| T₈        | 12.72 | 21.05   | 23.34   |
| T₉        | 08.13 | 14.22   | 17.50   |
| T₁₀       | 64.85 | 81.55   | 95.60   |

S.E.(0.05) 2.658 1.805 4.228
C.D. (Diff.) 1.273 0.865 2.026
C.V . % 9.347 4.454 8.746

Effect of different treatments on development of disease

The data presented in Table-3 found that all treatments were able to reduce the disease incidence over control. The minimum disease severity was recorded in the treatment T₃ (soil application of FYM@ 100 gm/pot + Neem cake@ 100 gm/pot + seedling treatment with bio-formulation of Azotobactor @5% + foliar spray of Carbendazim @0.1%) representing 6.23, 10.11 and 15.03 per cent at 7, 14 and 21 days after inoculation. The treatment T₉ (Soil application of FYM@ 100 gm/pot + Neem cake@100 gm + seedling treatment with bio-formulation of T. viride + foliar spray Carbendazim @0.1%) was showing 8.13, 14.22 and 17.50 per cent disease severity, representing the second lowest among the treatments. Among the treatments, the maximum disease severity was recorded in treatment T₂ (Soil application of FYM @ 200 gm/pot + seedling treatment with Trichoderma viride) i.e. 14.35, 17.75, 20.12 per cent at 7, 14 and 21 days, respectively. Varma et al. (2008) reported that the foliar spray of Trichoderma viride (10⁷ CFU ml⁻¹) 24 hrs before challenge inoculation with the test fungus was found effective in reducing the disease severity under screen house condition.

Yield

The fruits of tomato were harvested and the yield was separated from each treatment (Table-4). It has found that the maximum yield was

Table 4. Effect of different treatments on yield of tomato

| Treatment | Large(>50gm) | Medium(25-50gm) | Small(<25) | Total No. of Fruits / plant | Yield (gm) |
|-----------|--------------|----------------|------------|-----------------------------|------------|
| T₁        | 2            | 102.02         | 3          | 82.35                       | 138.12     | 26 | 320.85 |
| T₂        | 0            | 000.00         | 4          | 138.66                      | 104.77     | 27 | 248.63 |
| T₃        | 4            | 206.74         | 6          | 164.36                      | 112.87     | 40 | 490.30 |
| T₄        | 1            | 50.05          | 4          | 132.25                      | 97.22      | 28 | 260.33 |
| T₅        | 3            | 153.22         | 5          | 140.16                      | 116.11     | 32 | 410.23 |
| T₆        | 0            | 000.00         | 4          | 162.32                      | 64.28      | 13 | 235.50 |
| T₇        | 0            | 000.00         | 5          | 131.23                      | 64.55      | 12 | 233.22 |
| T₈        | 3            | 159.12         | 5          | 141.65                      | 119.31     | 35 | 430.23 |
| T₉        | 0            | 000.00         | 4          | 104.07                      | 78.73      | 20 | 195.60 |
| C.D. (0.05)|              |                |            |                             |            |    | 30.846 |
| S.E.( Diff.) |            |                |            |                             |            |    | 14.784 |
| C.V . %   |              |                |            |                             |            |    | 5.756  |
recorded per plant in T3 treatment (soil application of FYM@ 100gm/pot + Neem cake@ 100gm/pot + seedling treatment with bio-formulation of Azotobacter@5% + foliar spray of Carbendazim@0.1%) represented the value 490.30g per plant. The treatment T4 (Soil application of FYM@ 100 gm/pot + Neem cake@ 100 gm + seedling treatment with bio-formulation of T. viride + foliar spray Carbendazim@0.1%) was showing 430.23g per plant, representing second highest among the treatments. Among the treatments, the maximum large size tuber with 4 in number was recorded in treatment T3 (soil application of FYM@ 100gm/pot + Neem cake@ 100gm/pot + seedling treatment with bio-formulation of Azotobacter@5% + foliar spray of Carbendazim@0.1%) followed by treatment T4 (Soil application of FYM@ 100 gm/pot + Neem cake@ 100 gm + seedling treatment with bio-formulation of T. viride + foliar spray Carbendazim@0.1%) and T5 (soil application of FYM@ 100gm/pot + Neem cake@ 100gm + seedling treatment with bio-formulation of Azotobacter@5% + foliar spray of Carbendazim@0.1%) representing the value 8 and 9, respectively. Among the treatments, the maximum small size tuber was observed in treatment T7 (application of FYM @200 gm/pot + foliar spray with Mancozeb@0.25%) and followed by T8 (Soil application of FYM @200 gm/pot + seedling treatment with formulation Azotobacter@5% + foliar spray with Carbendazim@0.1%25" "HYPERLINK "mailto: Carbendazim@0.1%25"") representing the value 30 and 29, respectively.

Ravindra et al (2015) found that the yield of tomato crop significantly increased by the combine application of seed treatment with Trichoderma viride + soil application of Neem cake powder + foliar spray of Carbendazim. The bio-fertilizers
like Azotobacter, Rhizobium, PGPR, Trichoderma viride, Trichoderma harzianum. Phosphorus solubilising bacteria fix the atmospheric N resulted increased yields and growth in different crops (Singh et al. 2001, Dhawan et al.,2005, Kachroo and Razdan 2006). Mishutin (1966) demonstrated that bacterial fertilizers significantly improved the yield of various crops.

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