MANAGEMENT OF FUSARIUM WILT OF TOMATO BY WEEDS AND MYCOFLORA PROCESSED WEEDS COMPOST

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f.sp. lycopersici
Weed extracts

INTRODUCTION
Pathogenic formae specialis of the soil inhabiting fungus, Fusarium oxysporum (Sacc.) W.C. Snyder and H N Hans., cause severe losses in various crop plants. Fusarium oxysporum f.sp. lycopersici (FOL) is known to cause Fusarium wilt of tomato throughout the tomato growing areas and devastates the crop. In addition, the incidence and severity of Fusarium wilt are also increased due to the interaction of nematode with root system (Moorman et al., 1980). Although, various management strategies have been proposed by several workers, yet occurrence and development of new pathogenic races is a continuing problem. Biological controlling agents are being used for the management of various diseases as they contribute to disease suppression through; competition, antibiosis, parasitism/predation and induced resistance (Hoitink and Boehm, 1999). Trichoderma virens, T. harzianum and Aspergillus niger are widely used as effective biological control agents against soil borne pathogens (Papavizas, 1985; Ullah et al., 2011). Paecilomyces lilicanus, an egg parasite of root knot and cyst nematodes also inhibit the growth of Fusarium oxysporum (Mansoor et al., 2007) whereas, Cladosporium cladosporioides secretes acid phosphatase and other phytohormones (El-Shora and Metwally, 2009) facilitate the multiplication of rhizospheric microbes. Botanicals have low mammalian toxicity, target specificity, biodegradability and biocidal activity against several insect-pests and pathogens (Harish et al., 2008) can serve as safe fungicide against many pathogenic fungi. Futhermore, triterpenoid saponin secreted by Launaea pinnatifida fairly showed antifungal activity against Fusarium oxysporum (Yadav and Chakravarti, 2009). Presence of cannabidiol (CBD) in different species of Cannabis decides the antimicrobial activity (Leizer et al., 2000). NpPDR1 (ATP binding cassette) produced by Nicotiana plumbaginifolia plays a major role in both constitutive and jasmonic acid dependent induced defense (Stukkens et al., 2005) against plant pathogens. Prasad and co-workers (2009) documented that chloroform extracts of Physalis minima exhibited antifungal and antibacterial properties. Therefore, the combinations of more than one method provide more potential control than either component alone (Ehteshmul-Haque et al., 1995).

In the light of above literature, the work was initiated with a hypothesis to exploit the antimicrobial activity of weeds alongwith beneficial microbes as an alternate method to chemical control. Hence, the present investigation was undertaken with an objective to evaluate the effective and compatible bioagents with different weed plants having allelopathic effect as substrate and use of such combination (bioagent-weed) for the management of Fusarium wilt of tomato.

MATERIALS AND METHODS

Microbial and plant material
Five beneficial fungal strains, Trichoderma virens (ITCC 7109),...
Trichoderma harzianum, Aspergillus niger, Cladosporium cladosporioides (ITCC 7116) and Paecilomyces lilacinus (ITCC 7115) were isolated from rhizosphere of different crops through dilution plate technique on Rose Bengal Agar (RBA) medium. All fungi were maintained on Potato Dextrose Agar (PDA) medium at 4°C.

Qualitative attributes of beneficial fungi as antagonist (Papavizas, 1985 and Ullah et al., 2011), phosphorus solubilizer (Dey et al., 2001), IAA producer (Sarwar and Kremer, 1995) and decomposers (Nair et al., 2008) were tested by standard methods. The fungi Trichoderma virens, T. harzianum and Aspergillus niger were established as antagonists, A. niger and C. cladosporioides as phosphorus solubilizer, C. cladosporioides as IAA producer and all the tested fungi as good decomposers.

Fusarium oxysporum f. sp. Lycopersici was isolated from wilted tomato plant and its selectivity for tomato plant was established through pathogenicity test. The fungus was cultured and maintained on PDA at 4°C.

Seven common weed plants (Table 1), tested for their antifungal and substrate compatibility with beneficial fungi were collected from Research Farm of Rajendra Agricultural University, Pusa (Bihar) (25°-59’N; 84°-48’E) in north-eastern part of India.

Preparation of extract

Aqueous extracts of weed plants were obtained with minor modifications as described by Tiwari and co-workers (2011). Fresh plants of selected weeds were washed with distilled water and then chopped into small pieces of 10 cm length. Pieces weighing 100 gm were ground with pestle and mortar and kept in stoppered container for 12 hours on rotatory shaker at 150 rpm. The remnant obtained was strained through cheese cloth followed by centrifugation at 5000 rpm for 15 minutes to get ethanol free aqueous plant extract. These extracts were filter sterilized using 0.22 mm MEP filter (Hi media, Mumbai, India). The concentrated extracts were designated as crude extract and stored at 4°C for further study.

Preparation of fungus loaded weed compost

Seven weed plants used under present investigation were pre decomposed with five fungal strains with minor modifications as described by Espiritu (2011). Weeds were chopped in to 15 cm long pieces and loaded individually with Trichoderma virens, T. harzianum, Aspergillus niger, Cladosporium cladosporioides and Paecilomyces lilacinus, respectively. Conical flask of one litre capacity were filled with 500 gm of chopped weeds and loaded with 10 ml of 10² propagules/ml of fungal suspension. These flasks were incubated at 25 ± 2°C for 30 days with periodic turning at every seven days interval. At the end of 15 days, liquid materials collected at the bottom of flask from decomposing weeds, were collected and filter sterilized by using 0.22 mm MEP filters. The extracts finally obtained were used for preparing 20 per cent dilution under anti fungal study and the solid material (decomposed weeds) was used as growth media for tomato plant.

Antifungal activity

Microfiltered (0.22 mm) aqueous extract of weeds and fungus loaded weeds were supplemented with 20 per cent (v/v) in potato dextrose medium to determine the mycelial growth of fungi by poison food technique (Nene and Thapilyal, 2000). A centrally placed 10 mm² size of mycelia disc (from seven days old culture) of Trichoderma virens (TV), T. harzianum (TH), Aspergillus niger (AN), Cladosporium cladosporioides (CC), Paecilomyces lilacinus (PL) and Fusarium oxysporum f. sp. lycopersicae (FOL) were placed individually on amended Petri-plates in triplicate at 25 ± 1°C along with control (without amendment). The observations of mycelial growth of fungi were recorded after five days of incubation. The experiments were repeated thrice.

Mass multiplication of pathogenic fungi

Fusarium oxysporum f. sp. lycopersicae (FOL) was mass multiplied on sand + maize flour mix. The inoculum of fungus was produced on sand + maize flour mix (9:1), moistened with water and autoclave twice for 90 minutes on two consecutive days. One week old culture of fungi on potato dextrose agar medium was inoculated in sand + maize flour mix and incubated at room temperature for four weeks with repeated shaking at one week interval. Fungal inoculums prepared on sand + maize flour mix was used @ 15 gm in 500 gm of potting mix.

Assessment of disease severity

Tomato cv. Pusa Ruby is known to be susceptible to Fusarium spp. was used as test plant. On the basis of in-vitro performance of fungus on weeds and fungus loaded weeds extract, their (weed’s) availability, the pre-decomposed weeds were selected for the study of disease management. Composts prepared from different fungus loaded weeds were used as biological tool for the management of soil borne pathogen. For this purpose, tomato plants were grown in (pre-inoculated Fusarium oxysporum f. sp. lycopersicae) plastic pots (20 cm diameter) containing potting mix (soil:sand::1:1). The fungi loaded weed composts were filled in each pot contains potting mix (1:3) as growth medium for plants along with control (without amended compost). Each treatment consisted of three pots (10 plants per pot) in triplicate and experiment was repeated thrice. Post-emergence disease severity was evaluated by the number of surviving plants, starting from 10 days of the established plants up to 60 days at every 10 days interval. Disease severity was calculated by the per cent wilted plants/ premature falling of leaves of each plant as given by Chaube and Singh (1990).

Statistical analysis

Homogeneity of the data were first tested through chi-square test and then were subjected to Analysis of variance (ANOVA) for different treatments using Fisher’s protected least significant

Table 1: List of weed plants tested for antifungal activities

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Botanical name</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Launea pinnatifida Cass.</td>
<td>Compositae</td>
</tr>
<tr>
<td>2.</td>
<td>Cannabis sativa L.</td>
<td>Cannabaceae</td>
</tr>
<tr>
<td>3.</td>
<td>Nicotiana plumbaginifolia Vv.</td>
<td>Solanaceae</td>
</tr>
<tr>
<td>4.</td>
<td>Parthenium hysterophorus L.</td>
<td>Asteraceae</td>
</tr>
<tr>
<td>5.</td>
<td>Desmodium triflorum (L.)</td>
<td>Fabaceae</td>
</tr>
<tr>
<td>6.</td>
<td>Physalis minima L.</td>
<td>Solanaceae</td>
</tr>
<tr>
<td>7.</td>
<td>Chenopodium album L.</td>
<td>Chenopodiaceae</td>
</tr>
</tbody>
</table>
difference (LSD) test. Duncan’s multiple range test (DMRT) was used to indicate the difference between the treatments at the probability level of \( p < 0.05 \) using the GLM procedure of SAS software for windows (version 9.3). This software is available with the Department of Maths., Statistics and Computer Applications, RAU, Pusa.

**RESULTS AND DISCUSSION**

**Antifungal activity of weed’s extracts on fungi**

Weeds extracts significantly reduced the mycelial growth of *T. virens* compared to control (Table 2). Extracts of *L. pinnatifida* had minimum (34.3 mm) inhibitory effect on *T. virens* followed by *C. sativa* and *N. plumbaginifolia*. Similarly, weed extracts also had inhibitory effect on *T. harzianum*, extracts of *P. minima* exhibited minimum (46.7 mm) inhibitory effect while maximum (16.0 mm) was recorded in *D. triflorum*. This inhibition may have originated from the release of different alkaloids from weed plants having antifungal properties. It has been well documented by several workers that the antifungal compounds present in weed plant’s extracts have inhibitory effect on the growth of pathogens (Leizer et al., 2000; Prasad et al., 2009).

In contrast to *Trichoderma* spp., weed extracts significantly promoted the mycelial growth of *P. lilacinus* except *P. minima*, compared to control. The highest (34.3 mm) growth was recorded in *L. pinnatifida* and the least (6.7 mm) in *D. triflorum*. Likewise, there was a mixed response on mycelial growth of *A. niger* on different weed extracts. The maximum (41.0 mm) growth was recorded in *D. triflorum* followed by *N. plumbaginifolia*. The effect of *C. sativa*, *P. hysterophorus*, *C. album* and *D. triflorum* were identical and also superior to control in promoting the growth of *A. niger*. Moreover, weed extracts markedly supported the growth of *C. cladosporioides* compared to control, but the efficacy of *P. hysterophorus* (32.0 mm) and *P. minima* (31.7 mm) was alike and superior to rest of the treatments. However, inhibitory effect of weed extracts on *A. niger*, *P. lilacinus* and *C. cladosporioides* was comparatively lower, indicates that the fungal metabolites produced by tested fungi might countered the anti-microbial properties of weeds. Lytic enzymes produced by *A. niger*, *P. lilacinus* and *C. cladosporioides* (Lahoz et al., 1983; Khan et al., 2004; Hong et al., 2011) are responsible for hydrolysis of disaccharides into glucose, that regulates the growth of pathogen by making the carbon source in more available form.

Inhibitory effect of all weeds extracts on mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* was significant to unamended treatment (control). The maximum growth (22.0 mm) was recorded in *L. pinnatifida* followed by *N. plumbaginifolia* (25.7 mm), *C. sativa* (27.7 mm) and *D. triflorum* (29.0 mm), respectively. Extract of *L. pinnatifida* was highly suppressive towards FOL possibly due to the presence of saponins. This finding corroborates with those of Yadav and Chakravarti (2009), who reported that the triterpenoid saponins have antimicrobial activity against various fungi.

**Table 2: Effect of weed extracts on mycelial growth of beneficial fungi and *Fusarium oxysporum* f. sp. *lycopersici* (FOL)**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mycelial growth of fungi (mm)</th>
<th><em>T. virens</em></th>
<th><em>T. harzianum</em></th>
<th><em>P. lilacinus</em></th>
<th><em>A. niger</em></th>
<th><em>C. cladosporioides</em></th>
<th>FOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>34.3 ± 3.2</td>
<td>33.0 ± 3.0</td>
<td>34.3 ± 2.1</td>
<td>21.3 ± 1.5</td>
<td>24.3 ± 1.5</td>
<td>22.0 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>26.0 ± 1.0</td>
<td>26.7 ± 1.5</td>
<td>27.0 ± 1.0</td>
<td>27.0 ± 3.0</td>
<td>22.7 ± 1.5</td>
<td>27.7 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>26.7 ± 1.5</td>
<td>28.3 ± 7.6</td>
<td>27.7 ± 3.2</td>
<td>33.7 ± 2.5</td>
<td>26.0 ± 3.6</td>
<td>25.7 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>22.0 ± 1.0</td>
<td>36.0 ± 1.0</td>
<td>22.7 ± 2.5</td>
<td>27.0 ± 1.0</td>
<td>32.0 ± 1.0</td>
<td>31.7 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>5.3 ± 1.2</td>
<td>16.0 ± 1.0</td>
<td>16.0 ± 1.0</td>
<td>24.3 ± 4.0</td>
<td>25.7 ± 1.0</td>
<td>29.0 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>T6</td>
<td>22.0 ± 3.0</td>
<td>46.7 ± 1.5</td>
<td>6.7 ± 0.6</td>
<td>41.0 ± 3.6</td>
<td>31.7 ± 0.6</td>
<td>36.7 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>T7</td>
<td>21.0 ± 3.6</td>
<td>32.0 ± 1.0</td>
<td>21.0 ± 1.0</td>
<td>26.0 ± 1.0</td>
<td>24.3 ± 2.1</td>
<td>36.7 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>T8</td>
<td>41.7 ± 1.5</td>
<td>62.7 ± 2.5</td>
<td>14.0 ± 2.0</td>
<td>19.3 ± 1.2</td>
<td>14.3 ± 2.1</td>
<td>47.0 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>CD at 5%</td>
<td>3.9</td>
<td>5.5</td>
<td>3.3</td>
<td>4.3</td>
<td>3.3</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>9.03</td>
<td>9.07</td>
<td>8.89</td>
<td>9.14</td>
<td>7.57</td>
<td>4.60</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3: Effect of weed compost prepared from beneficial fungi on wilt disease incidence**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Disease incidence (per cent) at different time intervals</th>
<th>10 days</th>
<th>20 days</th>
<th>30 days</th>
<th>40 days</th>
<th>50 days</th>
<th>60 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>TV (CS)</td>
<td>2.50</td>
<td>5.00</td>
<td>10.00</td>
<td>16.67</td>
<td>26.67</td>
<td>23.37</td>
<td></td>
</tr>
<tr>
<td>TH (CS)</td>
<td>2.50</td>
<td>5.00</td>
<td>7.50</td>
<td>20.00</td>
<td>46.79</td>
<td>48.58</td>
<td></td>
</tr>
<tr>
<td>TH (PH)</td>
<td>5.00</td>
<td>13.35</td>
<td>13.35</td>
<td>15.01</td>
<td>18.36</td>
<td>20.02</td>
<td></td>
</tr>
<tr>
<td>PL (PM)</td>
<td>2.50</td>
<td>7.50</td>
<td>10.86</td>
<td>15.01</td>
<td>21.69</td>
<td>23.34</td>
<td></td>
</tr>
<tr>
<td>AN (PH)</td>
<td>5.00</td>
<td>7.50</td>
<td>7.50</td>
<td>11.67</td>
<td>16.67</td>
<td>18.34</td>
<td></td>
</tr>
<tr>
<td>CC (NP)</td>
<td>2.50</td>
<td>5.00</td>
<td>7.50</td>
<td>23.36</td>
<td>33.34</td>
<td>35.00</td>
<td></td>
</tr>
<tr>
<td>TV (PH)</td>
<td>2.50</td>
<td>5.00</td>
<td>7.50</td>
<td>16.68</td>
<td>35.03</td>
<td>38.39</td>
<td></td>
</tr>
<tr>
<td>Control (compost)</td>
<td>10.00</td>
<td>16.68</td>
<td>23.36</td>
<td>37.71</td>
<td>53.42</td>
<td>56.76</td>
<td></td>
</tr>
<tr>
<td>CD at 5%</td>
<td>3.76</td>
<td>8.23</td>
<td>9.14</td>
<td>8.11</td>
<td>9.64</td>
<td>10.83</td>
<td></td>
</tr>
</tbody>
</table>

**Notes:** *T1 = Launea pinnatifida, T2 = Cannabis sativa, T3 = Nicotiana plumbaginifolia, T4 = Parthenium hysterophorus, T5 = Desmodium triflorum, T6 = Physallis minima, T7 = Chenopodium album, T8 = Potato Dextrose Agar. Data reported are the mean values of three replications ± standard deviation. Value marked with common letters was not statistically different (p < 0.05) in Duncan’s multiple range test.
Suppression of Fusarium oxysporum f sp. lycopersicae by fungus loaded weeds extract

Different fungus loaded weed extracts showed variable response in suppressing the mycelial growth of FOL (Fig. 1). Extract of *P. minima* loaded with *P. lilacinus* was highly suppressive towards test pathogen while *L. pinnatifida* weed extracts promoted the growth of pathogenic fungi. However, extract obtained from weeds loaded with *T. virens* had significantly reduced the growth of FOL but *C. album* that supported the growth of pathogenic fungi. *C. sativa* (22.3 mm) loaded with *T. viriden* had markedly reduced the growth of test pathogen followed by *N. plumbaginifolia* (25.2 mm), *P. hysterophorus* (25.5 mm), *D. triflorum* (27.2 mm) and *P. minima* (26.0 mm), later treatments were similar in their effect. Similarly, extracts of *T. harzianum* loaded on *C. sativa* (22.0 mm) had significantly inhibited the mycelial growth of FOL. Although, all weed extracts loaded with *A. niger* was highly suppressive towards the mycelial growth of the FOL but *Cannabis sativa* (23.8 mm) was superior to all. In addition, extracts of *C. sativa* (21.8 mm) loaded with *C. cladosporioides* had also inhibited the mycelial growth of FOL followed by *N. plumbaginifolia*, *L. pinnatifida* and *P. hysterophorus*, respectively. *C. sativa* loaded individually with *T. virens*, *T. harzianum*, *A. niger*, and *C. cladosporioides* was highly suppressive towards FOL. This increase in suppressing ability of weed extracts fortified with beneficial fungi against FOL, suggest that there was synergistic action between toxins produced by tested beneficial fungi and cannabinooids of *C. sativa*. It has been reported that prenyl moiety of cannabinooids (Appendino et al., 2008) have inhibitory effect against the several fungi.

**Figure 1**: Effect of extracts of weeds loaded with beneficial fungi on mycelial growth of *Fusarium oxysporum* f sp. *lycopersicae* (FOL)

**Disease Incidence**

Disease incidence was significantly less on weed composts prepared from beneficial fungi at different time intervals compared to control (Table 3). The result indicates that the accumulation of phytoalexins produced by beneficial fungi and, easily available nutrients from the decomposed substrates had strongly induced the host defence systems. Benitez and co-workers (2004) reported that the ability of *Trichoderma* spp. to control plant pathogens have been associated with production of lytic enzymes such as chitinase, b-1, 3 glucanase and proteases. Besides, composts prepared from weed species before flowering stage had more beneficial effect than prepared at later stages because of higher nutrient contents.
Maximum disease incidence (10%) on 10th day was recorded in control while rest treatments were similar in their effect on reducing the disease incidence. On 20th day, disease incidence in *P. hysterophorus* compost prepared from *T. harziana* and control (unamended compost) was much pronounced whereas rest treatments were superior in reducing the disease incidence that ranged between 5-7.5 per cent. Effect of all the treatments except control was significant and almost identical on 30th day in reducing the disease incidence. However on 40th day, *P. hysterophorus* compost (11.67 per cent) prepared from *A. niger* was superior to other tested composts in inhibiting the disease progress. Manifestation of the disease was the maximum on 50th day, that ranged between 16.67 - 53.42 per cent and, had not varied (18.34 - 56.76 per cent) much up to 60th day. Variation in suppression of disease incidence at different time intervals, suggest that extent of decomposition of substrate inoculated with beneficial fungi and C:N ratio decides the Fusarium wilt suppression. Similar results were reported by Hoitink et al. (1997), who observed that compost with low C:N ratio do not suppress Fusarium wilt even when inoculated with effective bioagents. Compost of *P. hysterophorus* prepared individually from both *T. harziana* (20.0 per cent) and *A. niger* (18.34 per cent); *P. minima* compost prepared from *P. lilacinus* (23.34 per cent) had markedly reduced the wilt incidence in tomato plant followed by *C. sativa* compost prepared from *T. virens* (23.37 per cent) on the last day (60th) of observation. This probably due to the presence of antifungal compounds in weed composts, its impact on soil microbial community structure and their synergistic interaction for production of secondary toxic metabolite/induction of SAR in host plant. It has been shown that phenolic compounds in *P. hysterophorus* (Belz et al., 2007) and alkaloids in *P. minima* (Prasad et al., 2009) have antifungal activity. In addition, *Trichoderma* sp. and *A. niger* produces b-1,3 glucanase, protease, polygalacturonidase and a-amyrase (Benitez et al., 2004; Lahoz et al., 1983) acts as elicitor for induction of SAR in host plant. Thus, it can be concluded that incorporation of weed composts inoculate with potential bioagents can be a novel strategy in plant disease management.

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