Bioefficacy of *Trichoderma* as biological control agent against sugarcane wilt

*Ram Ji Lal¹, Rashmi Nigam², Joginder Singh³*

¹Division of Crop Protection, ICAR-Indian Institute of Sugarcane Research, Lucknow, U.P., India  
²Department of Plant Pathology, Janta Vedic College, Baraut (Baghpat) U.P., India  
³Department of Horticulture, Janta Vedic College, Baraut (Baghpat) U.P., India  
*Corresponding email: lalramji054@gmail.com*

**ABSTRACT**

Sugarcane is one of the most important cash crops grown in India. Diseases cause deterioration in the juice quality of infected canes and reduce extraction of sugar. Among the various diseases reported from India, wilt is important disease in sugarcane. The disease is soil as well as sett borne in nature. In recent years, the use of bio-pesticides has gained attention in the management of diseases because of their non-hazardous nature, easily biodegradable and also do not cause bioaccumulation. Some of the most potent isolates of *Trichoderma* spp. identified from study were tested in lab as well as field condition for disease management of wilt. In a field experiment it was observed that the wilt incidence in the susceptible variety CoLk 97169 was considerably reduced and yield was enhanced in soil application of *Trichoderma* (20 kg TMC/ha) followed by sett dipping in *Trichoderma* spore suspension (10⁶ spores/ml) before planting. *Trichoderma* can be multiplied either on FYM or press mud for its incorporation in sugarcane fields for the management of wilt disease.

**KEYWORDS**

Bioefficacy, *Trichoderma*, Disease, Biological Control, Sugarcane Wilt

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Sugarcane (*Saccharum* L. interspecific hybrid) is one of the most important cash crops grown in India. It is extensively cultivated in an area of about 4.2 million hectares with 281.12 million ton cane production, 19.27 million ton sugar production and average productivity of about 1 t/ha. It plays a prominent role in Indian agriculture and continued to be a sole source of sugar besides *khandasari*, *gur* (*jaggery*) *etc.*, in the country. Its significance is increasing in view of expanding horizon of agro-industrial uses of sugarcane, particularly in cogeneration of electricity and production of ethanol for blending in petrol. However, sugarcane production does not commensurate with the cultivated area in India. Several factors, *viz.*, high cost of cultivation, inadequate irrigation facilities, breakdown of resistance of varieties due to diseases, insect-pests and other natural calamities *etc.*, are responsible for low cane productivity. It has been estimated that sugarcane diseases alone reduce 19% crop yield every year. Diseases cause deterioration in the juice quality of infected canes and reduce extraction of sugar. Among the various diseases reported from India, wilt is next to red rot in importance. It causes 5-100% loss in yield and deterioration of juice quality. Butler and Khan (1913) first reported this disease from Bihar. But, now it is prevalent in many sugarcane-growing areas of U.P., Gujarat, Haryana, Punjab and Bihar. The occurrence of this disease has also been reported from several other sugarcane-growing countries of the world, like Bangladesh, Pakistan, Philippines, Uganda, South Africa, West Indies, Mexico, Barbados, Columbia, Argentina, Trinidad, United States, Zimbabwe (Agnihotri, 1990).
In the past, several commercial genotypes/varieties viz., Co 245, Co 312, Co 419, Co 453, Co 658, Co 775, Co 951, Co 975, Co 997, Co 1107, Co 1122, Co 1336, CoC 671 etc., have been abandoned or phased out of cultivation due to this disease. The disease is often found in association with red rot, pineapple sett rot, stalk borer, scale insect and root borer (Agnihotri, 1990). Thick caned and high sugar varieties/genotypes are more vulnerable to this disease (Lal et al., 1998).

Symptoms

Symptoms of wilt disease appear in the month of July/August or in 4-5 months old crop. The affected canes or whole clump display a conspicuous stunting and unthrifty appearance followed by yellowing and or withering of crown leaves. The midrib of all leaves in a crown generally turns yellow, while the leaf lamina may remain green. The canes become light and hollow resulting in total loss for milling. On splitting the canes, at early stage of infection, the pith is found to have a diffuse purple or dirty red discoloration that is more prominent at the nodes. The vascular tissues are of dark colour and appear as reddish brown streaks passing through the internodal tissues from one internode to another.

The odor of wilt affected stalks disagreeable but distinct from the characteristic sour smell of cane stalks affected with red rot. Due to loss of moisture, shrinkage of internodal tissues takes place resulting in the formation of longitudinal spindle shaped cavities in the central portion of the internode, these tapers towards nodes. Sometimes, cavities also develop in the nodal tissues, thus cane appears tubular (Srinivasan, 1964). The intensity of tissue discoloration and formation of the cavities is more in the basal portion of the affected cane stalks than in the upper portion. Histopathology of internal tissues of wilt affected cane stalks show the discolouration of the walls of xylem vessels, presence of fungal hyphae and formation of gum in their lumen. In the advance stage of disease development, the pathogen grows and sporulate inside the cavities of the internal tissues.

Causal Organism

The disease is considered to be a syndrome and chiefly caused by a fungus but the symptom production depends on several factors. The pathogen is originally identified as *Cephalosporium sacchari* (Butler and Khan, 1913) and subsequently *Fusarium sacchari*, *F. moniliforme var subglutinans*, *Acremonium implicatum*, *F. oxysporum* have been also reported to be associated with this disease (Sinha, et al., 2007).

**Biological Control**

There were many approaches to control this dreaded malady viz., use of disease free seed material, avoidance of ratooning, trash burning, hot water treatment with or without chemical etc. The disease is soil as well as sett borne in nature. The chemicals or fungicides used for the control of this disease are easily degraded in the soil or losses their effectiveness after certain period and are also less economical. Hence, in recent years there is developing consensus that modern chemicals based farming is non-sustainable as a result alternative eco-friendly approaches are now being researched to achieve not only food security and safety but also economical viability by employing effective measures for the biological control of plant pathogens in agriculture.

In recent years, the use of bio-pesticides (bioagents, botanical and their derivatives) has gained attention in the management of diseases because of their non-hazardous nature, easily biodegradable and also do not cause bioaccumulation (Harman et al., 2004 and Lal, 2004). Even the earliest farmers practiced biological control by rotating their crops, burrowing disease infested crop residues and fertilizing with organic manures. Biological control can be achieved either by introducing biocontrol agents directly into natural ecosystem or by adopting practices which favour population build up of biocontrol agents under natural condition or both. The biocontrol agents provide protection against plant disease either through direct action against the pathogen or indirectly reducing the host susceptibility towards the pathogen.

*Trichoderma* spp. has been known since 1920s for their ability to act as biocontrol agents against plant pathogens. Until recently, the principal mechanisms for control have been assumed to be those primarily acting upon the pathogens and induced mycoparasitism, antibiosis and competition for resources and space. Recent advances demonstrate that the effects of *Trichoderma* on plants, including induced systemic or localized resistance, are also very important. *Trichoderma* spp. colonizes the root epidermis and outer cortical layer and releases bioactive molecules that cause walling off of the *Trichoderma* thallus. At the same time, the transcriptome and the proteome of plants are substantially altered.
As a consequence of pathways for resistance in plants, increased plant growth and uptake of phosphate, MnO$_2$, Fe$_2$O$_3$ and Zn occur (Harman, 2006). Sugarcane is an ideal crop for the use of bioagent(s), especially *Trichoderma* spp. to enhance the productivity and yield because the crop continues in the same field for 2 to 3 years by way of ratoon(s). Monoculture of sugarcane also helps the multiplication of *Trichoderma* without interruption. Commercial cultivation of crop on large scale enables application of *Trichoderma* very easy (Mohanraj et al., 2002). Studies conducted so far have shown that the use of *Trichoderma* spp. (*T. viride* and *T. harzianum*) in sugarcane not only reduce incidence of diseases, like root rot, sett rot, red rot, wilt, smut etc., but also accelerate the degradation of trash, increases availability of locked-in nutrients, and improves the yield of plant and ratoon(s). Bhatti and Chohan (1990) found that the strains of *Streptomyces* and *Bacillus* to be highly antagonistic to *Cephalosporium sacchari* and indicated the possibilities of suppressing it in soil through their use. Ramu (1994) investigated in depth some aspects of biological control of sugarcane wilt and reported that the isolates of *T. viride* and *Pseudomonas* were inhibitory to wilt fungi *in vitro* and also reduced their population in soil. In addition, the antagonists markedly reduced colonization of the host by the pathogens in infested soil. Both the mechanisms *i.e.* competition and antibiosis were involved in the inhibitory action of the antagonist against the wilt fungi. In *vitro*, *T. viride* and *T. harzianum* were highly antagonistic against wilt pathogen (*Fusarium moniliforme* var. *subglutinans*) (Singh *et al.*, 2004).

**Table 1. Effect of mixed inoculum of *Trichoderma viride* and *T. harzianum* on wilt incidence and yield parameters (CoLk 97169)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination (%)</th>
<th>No. of Millable Canes (000 ha$^{-1}$)</th>
<th>Wilt Incidence (%)</th>
<th>Yield (t ha$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planting of healthy sets (Healthy check)</td>
<td>30.6</td>
<td>78.2</td>
<td>1.4</td>
<td>58.5</td>
</tr>
<tr>
<td>Planting of inoculated sets (Diseased check)</td>
<td>20.9</td>
<td>64.5</td>
<td>11.3</td>
<td>42.3</td>
</tr>
<tr>
<td>Soil application of <em>Trichoderma</em> plus planting of healthy sets</td>
<td>27.1</td>
<td>87.3</td>
<td>0.0</td>
<td>67.9</td>
</tr>
<tr>
<td>Sett dipping in <em>Trichoderma</em> followed by inoculation with wilt pathogen</td>
<td>23.7</td>
<td>80.5</td>
<td>3.5</td>
<td>59.6</td>
</tr>
<tr>
<td>Soil application of <em>Trichoderma</em> plus planting of inoculated sets</td>
<td>27.0</td>
<td>81.3</td>
<td>3.0</td>
<td>60.5</td>
</tr>
<tr>
<td>Soil application of <em>Trichoderma</em> plus sett dipping of inoculated sets in <em>Trichoderma</em> spore suspension before planting</td>
<td>26.4</td>
<td>83.8</td>
<td>2.6</td>
<td>62.3</td>
</tr>
<tr>
<td>Soil application of <em>Trichoderma</em> plus sett dipping of healthy sets in <em>Trichoderma</em> spore suspension before planting</td>
<td>32.5</td>
<td>88.5</td>
<td>0.0</td>
<td>70.1</td>
</tr>
</tbody>
</table>

Application of *T. harzianum*, *T. viride* and *T. longibrachiatum* in the sick soil was also found effective in the management of sugarcane wilt (Solanki *et al.*, 2001). Fifty isolates of *Trichoderma* spp. were isolated from the rhizosphere of various crops including sugarcane and their antagonistic activity was tested *in vitro* against *F. moniliforme* var. *subglutinans*, associated with wilt. The antagonistic activity was recorded on scale given by (Bell, 1972). It was observed that the *Trichoderma* isolates tested, neither completely masked the growth of the test pathogen (scale 1) nor the pathogen overgrew the antagonist. Eight *Trichoderma* isolates viz., TR-10, TR-16, TR-23, TR-26, TR-31, TR-34, TR-35 and TR-38 overgrew more than 2/3rd growth of the pathogen (scale 2), manifesting maximum antagonistic activity. Thirty eight isolates covered 50 per cent of the mycelial growth of the pathogen (scale 3), while 4 isolates viz., TR-4, TR-17, TR-20 and TR-25 were grouped into scale-4 i.e. when pathogen overlapped and the antagonist locked at the point of contact (Singh *et al.*, 2004). Some of the most potent isolates of *Trichoderma* spp. identified from this study were further tested for disease management under field conditions. In a field experiment conducted at this institute it was observed that the wilt incidence in the susceptible variety CoLk 97169 was considerably reduced and yield was enhanced in soil application of *Trichoderma* (20 kg TMC/ha) followed by sett dipping in *Trichoderma* spore suspension (10$^8$ spores/ml) before planting (table 1).
Mass Multiplication of *Trichoderma* for Field Application

*Trichoderma* spp., the widely used fungal antagonist can be grown on solid substrates like sorghum grains, wheat straw, wheat bran, spent tea leaf waste, coffee husk, maize grain/cobs, press mud etc. It may be multiplied as follows either on FYM or press mud for its incorporation in sugarcane fields for the management of wilt disease.

Farm Yard Manure (FYM)

A simple technique for mass multiplication of *Trichoderma* has been standardized. *Trichoderma* is grown on maize-meal bran (2 kg) for one week, subsequently mixed with sterilized 20 kg of farmyard manure (FYM) having about 30% moisture and incubated at room temperature (25+2°C) for 10 days for its multiplication. This *Trichoderma* mixed culture (TMC) is moistened by periodical watering and covered with trash or polythene sheets for better growth. After a month, this stock culture can be added in 8 ton of seasoned press mud and allowed for a week before application in the field (Solanki et al., 2001). Thus, *Trichoderma* multiplied either on FYM or press mud may be supplied to the cane growers or any other agencies for wilt disease management besides enhancing cane yield and sugar production.

Press Mud

Liquid culture of *Trichoderma* (10^8 conidia/ml) @ 2 liter is added in 120 kg press mud and allowed to multiply for 30 days. *Trichoderma* mixed culture (TMC) is moistened by periodical watering and covered with trash or polythene sheets for better growth. After a month, this stock culture can be added in 8 ton of seasoned press mud and allowed for a week before application in the field (Solanki et al., 2001). Thus, *Trichoderma* multiplied either on FYM or press mud may be supplied to the cane growers or any other agencies for wilt disease management besides enhancing cane yield and sugar production.

References


Mohanraj, D., Padammaban, P. and Viswanathan, R. (2002) Biological control of sugarcane diseases, In: Biological Control of Crop Plant Diseases (Eds.).


