



Plant Pathology

ORIGINAL ARTICLE

Performance of *Trichoderma* fortified composts in controlling collar rot caused by *Sclerotium rolfsii* of soybean

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ARTICLE INFORMATION

Article History

Submitted: 18 Jul 2019

Revised: 25 Jul 2019

Accepted: 30 Jul 2019

First online: 30 Aug 2019

Academic Editor

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ABSTRACT

Seedling diseases and collar rot/stem rot (*Sclerotium rolfsii*) are the main constraints for soybean production in Bangladesh. Number of experiments were undertaken to control seedling mortality and stem rot of soybean using *Trichoderma* fortified compost at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) research field. Pathogenicity test confirmed that *S. rolfsii* (isolate SR4) causes 95% seedling mortality therefore, considered as the most aggressive isolate. *Trichoderma harzianum* (Isolate-Chan 6) was used for inocula preparation with different composting substrates like cow dung, saw dust, rice straw, water hyacinth and poultry manure to prepare *Trichoderma* fortified composts. Among the composts, *Trichoderma* fortified poultry manure was found as the best treatment in reducing 75.45% pre- and post-emergence seedling mortality and diseases severity (84.53% reduction) as well as increased yield (80.91% increase) and yield attributing characters such as 55% increase of plant height and 27% increase of 1000-grain weight compared to the control. However, other composts also showed good response in reducing seedling mortality and growth promotion though they were not similar in performance with *Trichoderma* fortified poultry manure. Therefore, *Trichoderma* fortified composts have the immense potentiality to suppress diseases and improve yield of soybean. These studies should be conducted at the farmer's field before validating the substrates as *Trichoderma* fortified composts.

Keywords: *Trichoderma*, compost, growth promotion, pathogenicity

Cite this article: Akter R, Bhuiyan MKA, Hossain MM, Rayhanur J, Bhuiyan MAB. 2019. Performance of *Trichoderma* fortified composts in controlling collar rot caused by *Sclerotium rolfsii* of soybean. Fundamental and Applied Agriculture 4(3): 943–949. doi: 10.5455/faa.57557

1 Introduction

Soybean (*Glycine max* L. Merr.) is an important crop around the world for its important protein source which is used to reduce protein deficiency (Kaul and Das, 1986). In Bangladesh soybean suffers from many

diseases which includes rust, leaf spots, rot (seed and seedling rots, stem or collar rot, root rot, charcoal rot), wilt, powdery mildew, bacterial and viral diseases. Among the diseases, soybean subjects substantial damage from seedling diseases and collar rot incited by *Sclerotium rolfsii* which is soil borne pathogen

and it has vast range of hosts. This pathogen is distributed worldwide (Singh and Thapliyal, 1998). The yield reduction by soybean diseases is reported as an average of 8-10% across the world (Ivancovich, 2005). Although chemical fungicides sometimes found effective against *R. solani* and *S. rolfsii* but it causes environmental pollution that is why it is desirable to find out eco-friendly diseases control measures. Bio-control with *Trichoderma*, a well-known antagonistic fungus against *R. solani* and *S. rolfsii*; which are sclerotia forming fungi (Elad et al., 1982). Therefore, biocontrol of plant pathogens by antagonistic fungus *Trichoderma* is considered as one of the best alternatives to chemical due to the advantages such as cost-effective, eco-friendly, enhanced penetration and composting (Saba, 2012). The filamentous *T. harzianum* is used as a biological control agent. Judicious application of *Trichoderma*-rich biofertilizer could reduce the infestation of soil borne pathogens therefore, yield is increased and less requirement of N fertilizers (Rodd et al., 2002; Rahman K M, 2006). In addition, *Trichoderma*-based biofertilizers could convert unavailable nutrient elements to available form by the biological mechanisms. Ultimately, the yield of crop is increase (Hegde et al., 1999); these biofertilizers acted for long time in the soil and they release macro-nutrients such as N, P and K slowly into the soil (Sullivan et al., 2002). Number of experiments have been undertaken to control seedling diseases (caused by *R. solani* and *S. rolfsii*) of crops using *Trichoderma* both *in-vitro* and *in-vivo* (Begum and Bhuiyan, 2007; Islam and Bhuiyan, 2006; Rahman, 2004). However, scanty of researches have been performed in Bangladesh on use of *Trichoderma* fortified compost to reduce the seedling diseases of soybean and its growth promotion. Therefore, it is necessary to exploit the potentiality of *Trichoderma*-fortified compost at field condition to control soil-borne pathogens and also in increasing yield potentiality of soybean.

2 Materials and Methods

Experiments were performed in the laboratory and research fields of the Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU). The experimental location is 24°2'15.1"N, 90°23'53.7"E. The field belongs to Shallow Red Brown Terrace type of agro ecological zone (AEZ) Madhupur Tract. The soil type was silty clay with pH 6.5. The sub-tropical climatic zone was characterized by less rainfall, almost clear sun shine and moderate temperature.

2.1 Isolation and storing of *S. rolfsii*

Forty fungal isolates were isolated from the rhizosphere and rhizoplane of Bush bean (*Phaseolus vulgaris*) and Soyaben (*Glycine max*) grown in the re-

search field of BSMRAU by both soil dilution plate (SDP) (Mian, 1995) and root washing methods (RWM) (Hyakumachi, 1994). Among the isolates, five isolates namely SR1, SR2, SR3, SR4, and SR5 were identified as *S. rolfsii* following the standard identification key (Barnet, 1980). The pure culture of *S. rolfsii*, was preserved by using PDA slants in refrigerator at 15 °C as stock culture for further use.

2.2 Pathogenicity test of the isolates

The pathogenicity test of collected isolates of *S. rolfsii* was tested using soybean cultivar 'Shohag' in earthen pots containing sterilized soil. Each pot was filled with 0.5 kg autoclaved soil. Inoculum of *S. rolfsii* was accomplished by adding the sclerotia of *S. rolfsii* from a 10-day-old dried PDA culture with final concentration was 0.1 g (dry wt) kg⁻¹ sterilized soil. The pot prepared with only sterilized soil was served as control. In each pot, 8-soybean seeds were sown. Each isolate was replicated in 5 earthen pots. Data on disease development was recorded at 15-30 d after sowing (DAS) to determine the effect of pathogens responsible for pre- and post-emergence seedling mortality. The causal agent of seedling mortality was performed by re-isolation of the pathogen from the infected seedlings.

2.3 Preparation of inoculum

The inoculum of the *S. rolfsii* using isolate-SR4 was prepared on sterilized moistened wheat grain in an Erlenmeyer flask (500 mL). Wheat grains were previously soaked in sterilized distilled water for 12 h then excess water was drained out then poured into the Erlenmeyer flask (500 mL). Mycelial blocks (5 mm diameter) were cut from the margin of 7 d old pure PDA culture of *S. rolfsii*. In each flask about 5 to 7 mycelial disks were added followed by incubation at 25 °C for 21 d. Proper colonization was confirmed by shaken the flask by hand at 2-3 d interval. Then it was air dried for 2 d. The inocula were stored at 10 °C for future use.

2.4 Collection of *Trichoderma harzianum*

An antagonistic isolate of *T. harzianum* (Chan 6) was obtained from the stock culture that were preserved at 15 °C in refrigerator on PDA slants at microbiology laboratory of Plant Pathology department, BSMRAU. Before commencing experiment antagonism properties of collected *Trichoderma* isolate was confirmed by dual culture technique with *S. rolfsii* (SR4).

2.5 Preparation of composts

Five different substrates such as saw dust, poultry manure, rice straw, water hyacinth and cow dung were

used to make *Trichoderma* fortified compost. To do this, each substrate of 20 kg was placed in separate pit for decomposition and 2.5 kg wheat grain colonized *Trichoderma* was mixed with 45-day old compost in a single pit. The pit was left for 90 d to decompose the substrate and to make *Trichoderma* fortified compost.

2.6 Performance of composts in the field

A field experiment was undertaken to assess the most suitable compost using the following treatments. Seed samples of soybean was collected from Bangladesh Agricultural Research Institute (BARI). The fertilizer dose was fertilized @ 30-35-50-20 kg NPKS ha⁻¹ (Saha and Sultana, 2008). The experiment was carried out by RCBD with three replications. Above mentioned *Trichoderma* fortified composts were used in the field under following treatments:

T1= Untreated control without pathogen and *Trichoderma* fortified compost (substrate: wheat grain colonized *Trichoderma* = 8:1), T2 = Inoculation of *S. rolfisii* (SR4) without *Trichoderma* fortified compost, T3 = Inoculation of *S. rolfisii* (SR4) + *Trichoderma* fortified in saw dust, T4 = Inoculation of *S. rolfisii* (SR4) + *Trichoderma* fortified in cow dung, T5 = Inoculation of *S. rolfisii* (SR4) + *Trichoderma* fortified in rice straw, T6 = Inoculation of *S. rolfisii* (SR4) + *Trichoderma* fortified in water hyacinth, T7 = Inoculation of *S. rolfisii* (SR4) + *Trichoderma* fortified in poultry litters.

2.7 Observation of disease development

The growth of soybean plants were monitored regularly from the date of seed sowing till the appearance of pre- and post-emergence seedling mortality.

2.8 Data analysis

Data were recorded on seed germination, number of healthy and infected plants. Symptomatic seedlings were scored following the scale proposed by Fery and Dukes (2002). The scores were 1 – no stem lesion, 2 – small stem lesion (0-25% of the stem circumference), 3 – moderate stem lesion (26–50% of the stem circumference), 4 – large stem lesion (>51% of the stem lesion), and 5 – dead plant (lesion girdled the stem 100%). The formulae for calculation the disease incidence and disease severity were (Seem, 1984):

$$DI = \frac{P_I}{P_T} \times 100 \quad (1)$$

where, *DI* = disease incidence (%), *P_I* = number of infected plants, and *P_T* = number of total plants.

$$PDI = \frac{\sum R_i}{N \times R_{max}} \times 100 \quad (2)$$

where, *PDI* = percent disease index, *R_i* = summation of ratings, *i* = 1, . . . *N*, *N* = number of plants observed, and *R_{max}* = maximum rating, i.e. 5.

After necessary transformation, data recorded on various disease components were analyzed statistically using the MSTAT-C computer program. The means were separated and compared using Duncan's Multiple Range Test (DMRT).

3 Results

3.1 Pathogenicity test of *S. rolfisii*

The highest pre-emergence mortality (95%) of soybean was caused by the isolate SR4 followed by isolate SR5 (91.66%) (Fig. 1 and Table 1). All the tested isolates of *S. rolfisii* caused more than 70% total mortality.

3.2 Performance of *Trichoderma* fortified composts

The highest reduction (75.45%) of total seedling mortality was found with the *Trichoderma* fortified compost (where poultry manure was substrate) however, significantly similar consequence was also found in *Trichoderma* fortified compost using saw dust (71.73%) and water hyacinth (70.85%) as the substrates. The other treatments T4 (cow dung) and T5 (rice straw) were also significantly reduced the seedling mortality 68.76% and 67.26%, respectively compared to the control (T2) (Table 2).

3.3 Incidence and severity of collar rots

The lowest incidence of collar rot disease was found for *Trichoderma* fortified poultry manure (5.98%) followed by *Trichoderma* colonized water hyacinth (19.75%) and rice straw substrate colonized by *Trichoderma* (19.25%) compared to control (39.30%). Other substrates also reduced disease incidence significantly compared to control. The maximum reduction (85.0%) of percent disease index was observed for *Trichoderma* fortified poultry manure (T7) substrate than any other substrate used. But all the substrate gave significant reduction of percent disease index compared to control (Table 3).

3.4 Effect of composts on soybean crop

The increase of yield of soybean is maximum (80.91%) with poultry manure substrate colonized by *Trichoderma* than all other substrates. But all the *Trichoderma* fortified compost treatment significantly increased the characters such as plant height, pods/plant and branch/plant in comparison to control (T1) and (T2) (Table 4).



Figure 1. Pathogenicity test of five isolates of *S. rolfsii* in pot culture

Table 1. Pathogenicity test of *Sclerotium rolfsii* isolates on Soybean variety ‘Shohag’

| Isolates of <i>S. rolfsii</i> | % Mortality of soybean seedlings | | |
|-------------------------------|----------------------------------|----------------|-------|
| | Pre-emergence | Post-emergence | Total |
| SR1 | 62.5 | 20.8 | 83.3 |
| SR2 | 75.0 | 12.5 | 87.5 |
| SR3 | 79.2 | 0.0 | 79.2 |
| SR4 | 95.0 | 0.0 | 95.0 |
| SR5 | 83.3 | 8.3 | 91.6 |
| Control | 0.0 | 0.0 | 0.0 |

Table 2. Effect of *Trichoderma* fortified compost in controlling seedling mortality of soybean in the field

| Treatment | % Mortality of soybean seedlings | | | |
|-----------|----------------------------------|----------------|-------|--------------------------|
| | Pre-emergence | Post-emergence | Total | % reduction of mortality |
| T1 | 10.33 | 3.33 | 13.67 | 69.50 b |
| T2 | 29.31 | 15.51 | 44.82 | |
| T3 | 11.0 | 1.67 | 12.67 | 71.73 a |
| T4 | 10.33 | 3.67 | 14.0 | 68.76 b |
| T5 | 10.33 | 4.33 | 14.67 | 67.26 b |
| T6 | 9.67 | 3.67 | 13.33 | 70.25 a |
| T7 | 8.0 | 3.0 | 11.0 | 75.45 a |

T1 = Untreated field (control 1); T2 = Untreated Field + *S. rolfsii* (control 2); T3 = *S. rolfsii* + Colonized *Trichoderma* with Saw dust; T4 = *S. rolfsii* + Colonized *Trichoderma* with Cow dung; T5 = *S. rolfsii* + Colonized *Trichoderma* with rice straw; T6 = *S. rolfsii* + Colonized *Trichoderma* with water hyacinth; T7 = *S. rolfsii* + Colonized *Trichoderma* with poultry manure. Means within same column with common letter(s) are significantly similar (P = 0.05) by multiple range test-DMRT.

Table 3. Effect of *Trichoderma* fortified compost on disease incidence and disease severity (PDI) of collar rot/stem rot disease caused by *S. rolfsii* of soybean in the field

| Treatment | <i>Sclerotium rolfsii</i> | | | |
|-----------|---------------------------|---------------------|---------|-----------------|
| | % Disease incidence | % disease reduction | PDI | % PDI reduction |
| T1 | 25.91 b | 34.07 e | 23.88 b | 41.74 d |
| T2 | 39.30 a | – | 40.99 a | – |
| T3 | 16.83 d | 57.17 c | 13.11 d | 68.02 b |
| T4 | 12.42 e | 68.39 b | 14.14 d | 65.50 b |
| T5 | 19.25 c | 51.01 d | 16.09 c | 60.75 c |
| T6 | 19.75 c | 49.75 d | 16.27 c | 60.31 c |
| T7 | 5.988 f | 84.76 a | 6.34 e | 84.53 a |

T1 = Untreated field (control 1); T2 = Untreated Field + *S. rolfsii* (control 2); T3 = *S. rolfsii* + Colonized *Trichoderma* with Saw dust; T4 = *S. rolfsii* + Colonized *Trichoderma* with Cow dung; T5 = *S. rolfsii* + Colonized *Trichoderma* with rice straw; T6 = *S. rolfsii* + Colonized *Trichoderma* with water hyacinth; T7 = *S. rolfsii* + Colonized *Trichoderma* with poultry manure. Means within same column with common letter(s) are significantly similar ($P = 0.05$) by multiple range test-DMRT. PDI = percent disease index

Table 4. Effect of *Trichoderma* fortified compost on growth promotion components and yield of soybean

| Treatments | Plant height (cm) | No. of pod plant ⁻¹ | No. of branch plant ⁻¹ | 1000-seed weight (g) | Seed yield (t ha ⁻¹) | Yield increment (%) |
|------------|-------------------|--------------------------------|-----------------------------------|----------------------|----------------------------------|---------------------|
| T1 | 46.40* c | 39.25 b | 3.0 b | 103.81 b | 1.64 c | 25.19 |
| T2 | 42.25 c | 26.75 c | 1.83 c | 88.53 c | 1.31 d | – |
| T3 | 59.64 ab | 54.25 a | 3.85 a | 104.82 b | 1.94 b | 48.09 |
| T4 | 59.63 ab | 54.50 a | 4.00 a | 105.54 b | 1.94 b | 48.09 |
| T5 | 63.50 a | 59.50 a | 3.85 a | 106.31 b | 1.95 b | 48.85 |
| T6 | 60.13 ab | 59.25 a | 3.87 a | 105.21 b | 1.96 b | 49.61 |
| T7 | 65.50 a | 63.75 a | 4.00 a | 112.81 a | 2.37 a | 80.91 |

T1 = Untreated field (control 1); T2 = Untreated Field + *S. rolfsii* (control 2); T3 = *S. rolfsii* + Colonized *Trichoderma* with Saw dust; T4 = *S. rolfsii* + Colonized *Trichoderma* with Cow dung; T5 = *S. rolfsii* + Colonized *Trichoderma* with rice straw; T6 = *S. rolfsii* + Colonized *Trichoderma* with water hyacinth; T7 = *S. rolfsii* + Colonized *Trichoderma* with poultry manure. Means within same column with common letter(s) are significantly similar ($P = 0.05$) by multiple range test-DMRT.

4 Discussion

In the present study, all the *Trichoderma* (Isolate-Chan 6) fortified compost compositions reduced seedling mortality more than 70% compared to control. The best performance (84.76% disease reduction) was recorded in case of *Trichoderma* fortified compost where poultry manure was used as substrate. Same composition also reduced (84.53%) the percent disease index (PDI) compared to control. The in-vitro study of *T. harzianum* against *S. rolfsii* reported 64–99% inhibition of growth of *S. rolfsii* (A. Kashem et al., 2016). *Trichoderma* fortified composts suppressed fungal pathogens (A Gharib et al., 2008; Naidu et al., 2010). The mode of inhibition in mycelial growth of *S. rolfsii* could be through various mechanisms such as mycoparasitism, antibiosis, lysis, competitive, metabolites secretions, competition and modulation of induced resistance (Fotoohiyani et al., 2015).

Moreover, organic amendment produces volatile organic components (VOC) and non-volatile substances (NVOC) during their decomposition which helps to introduce and establish antagonists into the soil (Hoitink and Boehm, 1999).

Poultry manure based-*Trichoderma* fortified compost produced the highest yield (80.91%) compared to control (untreated soil + *S. rolfsii*) may be due to higher NPK contents in poultry manure. According to Siddiqui (2004) the content of NPK in poultry manure was higher as compared to cowdung, goat dung, horse dung. Yield attributing characters such as height, pod plant⁻¹, 1000-grain weight also significantly higher in *Trichoderma* fortified poultry manure in comparison to all other treatment combinations and control (untreated soil + *S. rolfsii*).

Trichoderma fortified composts were considered as bio-fertilizers which also enhanced plant growth as composting substrates increases the status of soil

nutrients and improves the efficacy of antagonist (A Gharib et al., 2008; Naidu et al., 2010). It could be concluded that poultry manure based *Trichoderma* fortified compost could be used in soybean field to reduce the collar rot/stem rot as well as to improve the yield and yield attributing characters of soybean. Meanwhile, further study could be undertaken to validate the results of this study using multiple varieties of soybean; also, the experiments should be performed in different locations of Bangladesh.

5 Conclusions

Among the treatments *Trichoderma* fortified poultry manure has the great potentiality in controlling collar rot/stem rot diseases and increasing the yield and yield attributing characters of soybean. Other *Trichoderma* fortified composts (saw dust, cow dung, water hyacinth and rice straw) also have the potentiality to suppress collar rot and increase yield of soybean in compare to control treatment. Based on these results we may conclude that *Trichoderma* fortified poultry manure is recommended as bio-pesticide as well as a bio-fertilizer for the production of soybean.

Acknowledgements

The authors acknowledged the financial supports from HEQEP-UGC, Bangladesh. The first author got stipend from this project.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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The Official Journal of the
Farm to Fork Foundation
ISSN: 2518–2021 (print)
ISSN: 2415–4474 (electronic)
<http://www.f2ffoundation.org/faa>