
Evaluation of two species of *Trichoderma* as compost activator and bio-control agents

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The two *Trichoderma* species were found to be viable in improving the composting efficiency of the compost especially when the *Trichoderma* sp. 2 was inoculated on the compost. *Trichoderma* sp. 2 increased the total NPK content of the compost at 4.88% with the C: N ratio of 6:1. Combination of the two species of *Trichoderma* could also increase the nutrient content of the compost with 4.48% with the high percentage recovery of 67%. However, compost inoculated with *Trichoderma* sp. 1 had the lowest total NPK content of 3.68% as compared with the compost not inoculated with activator (3.93%). The two *Trichoderma* species had the ability to inhibit the mycelia growth of *S. rolfsii* under in-vitro condition by 34.92% and 31.44%, respectively. Different mycoparasitic activity was observed among the two species of *Trichoderma* and this could be attributed to the released of different metabolites against *S. rolfsii*. The two *Trichoderma* were able to produce metabolites, by inhibiting the growth of *S. rolfsii* by 10.46% to 29.03% in volatile, 3.74% to 5.59% in non-volatile, and 11.3% to 12.94% in direct-diffusile metabolite. Biological activity of *Trichoderma* on seed coating treatment showed enhancement of the seed germination of cucumber when the seeds were coated with *Trichoderma* sp. 1 (93.75%) alone, or coated with *Trichoderma* sp. 1 + *Trichoderma* sp. 2 (84.38%). Seeds not coated with any of the *Trichoderma* increased the percentage of disease incidence as compared with the seeds coated with *Trichoderma* either alone or in combination. Biological activity of the *Trichoderma* on soil treatment revealed that seeds sowed in any of the infected or non-infected soil treated with the two species of *Trichoderma* either alone or in combination enhanced the seed germination of cucumber by 87.50% to 93.75%. Disease incidence in cucumber was high even in the presence of *Trichoderma* sp. 1; however application of the two species of *Trichoderma* reduced the incidence of damping off disease by 6.25%. *Trichoderma* species were found effective as compost activator in rice straw-based compost as it enhance the nutrient content of the compost. Likewise it produce different amount of metabolites that inhibit the growth of *S. rolfsii*. The two species of *Trichoderma* also helped to improve seed germination and reduce the disease incidence of cucumber in seed and soil treatment application.

Key words: *Trichoderma*, compost activator, bio-control agent

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Introduction

Large amounts of chemical fertilizers are used for crop production but continued use of chemical fertilizer leads to serious nutrient imbalances and soil degradation. The integrated nutrient management and soil fertility challenges for sustainable agriculture in the Philippines promote the practice of organic agriculture, and introduction of the use of compost fungus activators in addition to organic fertilizer (Ponce, 2004) as substitute for chemical fertilizer in crop production by small farmers. Likewise, biological control method has been highly appreciated around the world as an eco-friendly alternative to agrochemicals (Tayung *et al.*, 2010). Several workers have demonstrated that many antagonistic strains of bacteria and fungi are used as biological control of postharvest diseases of fruits and vegetables.

The genus *Trichoderma* is very popular in organic farming. It is employed widely in agriculture because it releases a variety of compounds that induce systemic resistance against soil borne pathogen, and enhances crop productivity (Harman, 2000). Furthermore, the biological mechanism of *Trichoderma* may be due to multiple factors as they have the ability to produce a variety of extracellular lytic enzymes and different metabolites.

This study aimed to evaluate two species of *Trichoderma* previously isolated from carabao manure as compost activators in terms of the compost quality and as bio-control agent by determining the effects of the two species of *Trichoderma* when applied singly or in combination, in reducing the incidence of damping off due to *Sclerotium roflsii* both on seed and soil treatment. It also determines the production of metabolites of the two species of *Trichoderma*, since different *Trichoderma* species have different characteristics and mode of action.

Materials and methods

Study 1. Evaluation of the Two Species of Trichoderma as Decomposer of Rice Straw-Based Compost

The influence of *Trichoderma* species as compost activator in rice straw based formulation was evaluated based on the chemical attributes such as temperature, moisture content, pH, microbial load, C: N ratio, NPK content and percent recovery of harvested compost. A composite lot of rice straw was mixed with carabao manure and carbonized rice hull at a ratio of 3:2:0.5 (v/v) and divided into several lots and subsequently prepared and inoculated with the following treatments: T₁- *Trichoderma* sp1; T₂- *Trichoderma* sp2; T₃- *Trichoderma* sp1 and sp2; T₄- Commercially available compost fungus

activator (positive control) and T₅- Without compost fungus activator (negative control)

Statistical Analysis

The experiment was laid down in Randomized Completely Blocked Design (RCBD). The data were statistically analyzed using Sirichai Statistic Version 6.00 (Sirichai Unsrisona, Maejo University, 2007). Comparison among means was done using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

Study 2. Evaluation of Bio-control Activity of the Two Trichoderma species against S. rolfsii

2.1 In-vitro Antagonism Assay of the Two Species of Trichoderma against *S. rolfsii*

2.1.1 Dual culture test for growth inhibition

The two species of *Trichoderma* were evaluated in vitro for their ability to inhibit the growth of *S. rolfsii* using the dual culture test as described by Alvindia and Natsuaki (2008). The percent growth inhibition of Korsten et al., (1995) as cited by Alvindia and Natsuaki (2008) was computed after 14 days of incubation as follows:

$$GI = \frac{Kr-r1}{Kr} \times 100$$

where: Kr - the distance (measured in mm) of fungal growth from the point of inoculation to the colony margin of the control plates;
r1 - distance of fungal growth margin in the direction of the antagonist

Agar block test for mycoparasitism

Interaction of the two species against *S. rolfsii* was determined using the slide culture technique as cited by (Alvindia and Natsuaki, 2008). Mycoparasitism such as coiling of the hypae and penetration structures, or

plasmolysis of the cell as a result of wall disintegration were observed (Alvindhia and Natsuaki, 2008).

2.2 Influence of the metabolites released by two species of *Trichoderma* on the growth of *S. rolfsii*

The influence of volatile, non-volatile and direct-diffusible metabolites of *Trichoderma* on the growth of *S. rolfsii* was determined by the following procedure of Dennis and Webster (1971) as cited by Alvindhia and Natsuaki (2008).

Each test on volatile, non-volatile and direct-diffusible metabolites was conducted in three replicates, and repeated twice. All the cultures were incubated at 25°C. The colony diameter of the pathogen, and inhibition percentage were measured daily following the formula of Edington *et al.* (1971) as follows: $1\% = [(C2-C1)/C2] \times 100$.

2.3 In -vivo Test on the Antagonistic Effect of the Two Species of *Trichoderma* Against *S. rolfsii*

Biological Activity of the Two Species of Trichoderma Applied as Seed Treatment Against S. rolfsii

Following the procedure of Manjula *et al.* (2004), the effect of the two species of *Trichoderma* on cucumber seeds was observed. Eight seeds of cucumber coated or non-coated with *Trichoderma* were sown in each pot with *S. rolfsii* infected soil. Incidence of damping off was evaluated 7-15 days after sowing. This study was set up with the following treatments:

Factor A (Inoculant)	Factor B (Activator)
A ₁ - inoculated with <i>S. rolfsii</i>	B ₁ - coated with <i>Trichoderma</i> sp1
A ₂ - uninoculated with <i>S. rolfsii</i>	B ₂ -coated with <i>Trichoderma</i> sp2
	B ₃ - coated with <i>Trichoderma</i> sp1 and sp2
	B ₄ -no antagonist

2.4 Biological Activity of the Two Species of *Trichoderma* Applied as Soil Treatment Against *S. rolfsii*

Five kilograms of pasteurized soil artificially inoculated with *S. rolfsii* at the rate of 2.0 g dry mycelium/kg. Each pot was watered for 7 days before the inoculation of two *Trichoderma* species. Eight cucumber seeds previously

disinfected were sown per pot. Percent seed germination was recorded 7 days after sowing (DAS). Damping off disease incidence was recorded at 14 DAS. The experiment included the following treatments:

T ₁ - non inoculated soil (without antagonist and pathogen)	T ₅ - <i>S. rolfsii</i> - infected soil + <i>T. sp1</i>
T ₂ - <i>S. rolfsii</i> non-infected soil + <i>T. sp1</i>	T ₆ - <i>S. rolfsii</i> - infected soil + <i>T. sp2</i>
T ₃ - <i>S. rolfsii</i> non-infected soil + <i>T. sp2</i>	T ₇ - <i>S. rolfsii</i> - infected soil + <i>T. sp1</i> + <i>T. sp2</i>
T ₄ - <i>S. rolfsii</i> - infected soil	T ₈ - <i>S. rolfsii</i> - none infected soil + <i>T. sp1</i> + <i>T. sp2</i>

Formula for disease incidence was computed using the following formula:

$$\% \text{Damping off} = \left(\frac{\text{No. of infected seedlings per pot}}{\text{Total number of seedlings per pot}} \right) \times 100$$

Data Analysis

The study on seed treatment was laid out in a 2 x 4 factorial in Completely Randomized Design with four replicates while Randomized Completely Block Design was used in soil treatment test with four replicates.

All the data were collected and analyzed using Sirichai Statistic Version 6.00 (Sirichai Unsrisona, Maejo University, 2007). Means were compared using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

Results and discussion

Study 1. Evaluation of the Two Species of Trichoderma as Decomposer of Rice Straw-Based Compost

Chemical Attributes of Compost

The chemical characteristics of the harvested compost such as C: N ratio, total organic carbon, total organic matter, total nitrogen, total phosphorus and total potassium (Table 1).

C: N ratio. All the compost from the different treatments had stable C: N ratio ranging from six to nine which were all comparable with each other. Matured compost should have 10 to 20 C: N ratio to be considered stable. Stability of the compost as indicated by a C: N ratio of at most 20 is important. The low C: N ratio of all the compost from the different treatments indicates that the inoculated microorganisms decomposed the material efficiently (Goyal and Sindhu, 2011).

Table 1. Influence of *Trichoderma* as inoculants on the chemical characteristics and percent recovery of the compost after 33 days of composting

Treatment	Total organic carbon (%)	Total organic matter (%)	N	Total (%) P ₂ O ₅	K ₂ O	C:N Ratio	Percent recovery
T1= <i>T. sp1</i>	9.55 ^{ab}	16.43 ^{ab}	1.18	0.65 ^{ab}	1.85 ^b	8:1	58.97 ^{ab}
T2 = <i>T. sp2</i>	8.63 ^b	14.85 ^b	1.41	0.85 ^a	2.62 ^a	6:1	51.59 ^b
T3 = <i>T. sp1</i> + <i>T. sp2</i>	8.90 ^b	15.30 ^b	1.07	0.69 ^{ab}	2.52 ^{ab}	8:1	67.94 ^a
T4 = CCA (positive control)	10.80 ^a	18.58 ^a	1.14	0.71 ^{ab}	2.08 ^{ab}	9:1	61.96 ^{ab}
T5= Uninoculated (negative control)	8.22 ^b	14.04 ^b	1.07	0.66 ^b	2.04 ^{ab}	8:1	49.35 ^b

Means in column followed by a common letter are not significantly different from each other at 5% level using DMRT.

Total Organic Carbon

The rice straw-based compost inoculated with commercial compost activator had the highest total organic carbon content which was comparable to the compost inoculated with *T. sp1*. The total organic carbon from the combination of the two species of *Trichoderma* (*sp1* + *sp2*), *T. sp2* and uninoculated compost was comparable but was significantly lower than the commercial compost activator (Table1). Degradation of complex organic matter into simple ones with the evolution of CO₂ gas and energy occurred during composting (Adani *et al.*, 1997).

Total Organic Matter

The organic matter content of the compost was significantly affected by the inoculation of *Trichoderma*. The commercial compost activator had significantly the highest organic matter content of 18.58% which was comparable with the compost inoculated with *T. sp1* having 16.43%. On the other hand, compost inoculated with *T. sp2*, combination of the two species of *Trichoderma* and uninoculated compost obtained comparable organic matter content of 15.30%, 14.85% and 14.04%, respectively (Table 1). Organic matter content of the compost produced from composting was one of the important indicators of the quality of organic fertilizer.

Total NPK

The nitrogen content of the compost produced from the different treatments was not significantly affected by inoculation of compost activator (Table 1). This means that the activities of the microorganisms during composting process did not alter the final nitrogen content of the compost. However, numerical value of the nitrogen content of the compost inoculated with *Trichoderma* enhanced the N content of the compost considering the fact that nitrogen was used by the microorganism for the synthesis of protein they needed for their growth and development. The nitrogen assimilated by the microorganisms became part of their tissues which would eventually become a component of the compost once these organisms died. Thus, it was possible to have comparable N content of the compost since the substrates used were all the same.

On the other hand, the phosphorus and potassium contents of the compost varied significantly among treatments. Inoculation of compost activators irrespective of the source or strains used in the study generally increased the phosphorus content of the compost relative to the uninoculated or negative control. Within the inoculated treatments, no significant mean variation on P content was noted. Among the treatments evaluated, the highest P content was noted on the use sp1 of *Trichoderma*. It could be that this species was more efficient in solubilizing organic P during the composting process.

According to Tallapragada and Gudimi (2009) in their study on phosphate solubility and biocontrol activity of *Trichoderma*, this organism utilizes phosphatases for their growth resulting in P solubilization. This might be the reason on the increase P content in compost inoculated with *Trichoderma* as compost activator. In terms of the potassium content of the compost, all the treatments had comparable K content with the un-inoculated negative control as well as with the use of commercial compost activator. The highest K content of 2.62 % was noted from compost inoculated with *T. sp2* and the lowest from *T. sp1*.

As far as the total NPK content was concerned, the compost applied with *T. sp2* had the highest value of 4.88%, followed by compost with combined application of *Trichoderma*, and compost applied with commercially available compost activator having 4.28% and 3.94%, respectively. Lowest NPK of 3.77% and 3.36% was noted from the compost without compost activator and compost applied with *T. sp1*, respectively.

Percent Recovery

The percent recovery of the compostable materials applied with two species of *Trichoderma* significantly increased the percentage recovery (67.94%). Meanwhile, compost applied with commercially available compost activator (61.96%) and compost applied with *T. sp1* (58.97%) were comparable with each other. Lowest percentage recovery was recorded on the compost applied with *T. sp2* and un-inoculated compost 58.97% and 49.35%, respectively (Table 2). Sancom *et al.* (2006) also reported that compost inoculated with *T. aureviride* Rifai, *T. koningii* Oudemans, *T. pseudokoningii* Rifai, *T. reesi* Simmons, *T. viridae* Preson ex Fries and *T. harzianum* were found viable for improving compost efficiency.

Study 2. Evaluation of Bio-control Activity of Two Trichoderma species against S. rolfsii

In vitro antagonistic assay of the two species of Trichoderma against S. rolfsii

T. sp2 and *T. sp1* inhibited the radial mycelia growth of *S. rolfsii* by 34.92% and 31.44%, respectively (Figure 1). Percent growth inhibition of mycelia was based on the scale of 2 which indicated moderate inhibition. This showed that the two species of *Trichoderma* were able to suppress the growth of *S. rolfsii*.

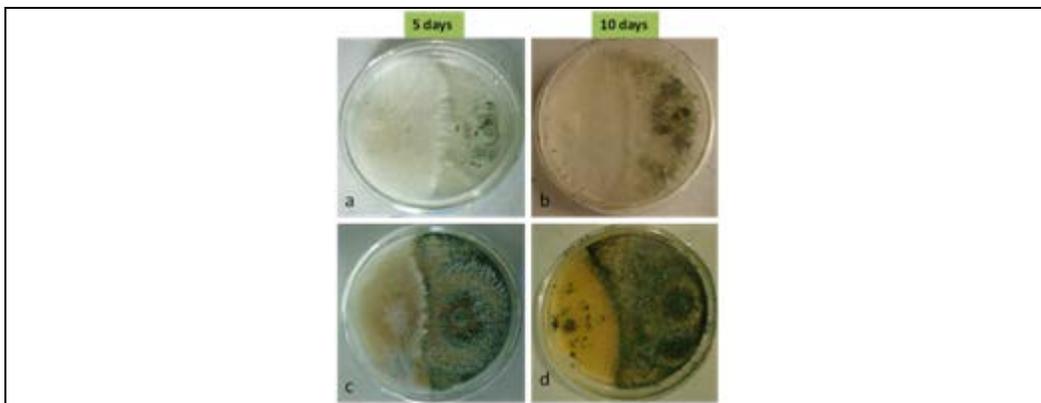


Fig 1. In vitro antagonistic assay of the *T. sp1* (a and b) and *T. sp2* (c and d) against *S. rolfsii* by dual culture on PDA at 5 days to 10 days of incubation period

It has been reported that *Trichoderma* species is known as mycoparasites to a number of plant pathogens including *S. rolfsii* (Jegathambigai *et al.*, 2010). *Trichoderma* are well known in presenting bio-control activity against several plants pathogenic fungi through various mechanisms: antibiosis, competition, mycoparasitism, and enzymatic hydrolysis (Mascarin *et al.*, 2012).

Effect of the Metabolites Released by Two Species of Trichoderma on the Mycelial Growth of S.rolfsii

Volatile metabolites, non-volatile metabolites and diffusion test showed that species of *Trichoderma* produced metabolites that inhibit the growth of *S. rolfsii*. Volatile test in *T. sp1* and *T. sp2* varied from 10.46% to 29.03% while non-volatile metabolites inhibited the growth of *S. rolfsii* by 3.74% and 5.69%. Diffusion test reduced mycelia growth of the pathogen by 12.94% and 11.3% indicating that *Trichoderma* species produce both volatile and non-volatile metabolites which affect the growth of different fungi (Choudary *et al.*, 2007). This suggests that different species often produced different compounds and reaction against pathogens (Choudary *et al.*, 2007).

Determination of interaction between the two species of Trichoderma and S. rolfsii

Direct antagonism of *T. sp2* against *S. rolfsii* was observed (Figure 2). Once the antagonist coiled on the host hyphae, it attached to the host and coiled around it to form appressoria on the host surface. As described by Gajera (2013), remote sensing was used by the antagonist to detect the host pathogen. Once the antagonist was attached to the host pathogen, it subsequently coiled and penetrated through the formation of appressoria on the host hyphae resulting in the maceration and cell lysis of the host hyphae and eventually, disintegration and destruction of the resting structures.

T. sp1 showed different mechanisms against *S. rolfsii*, with no coiling or penetration of the host hyphae (Figure 3). *Trichoderma* hyphae simply grew and touched the host's hyphae which secreted an enzyme that degraded the host cell wall (Gajera, 2013). This suggests that various antagonists have different mechanisms of host recognition (Silva *et al.*, 2004; Gajera *et al.*, 2012).

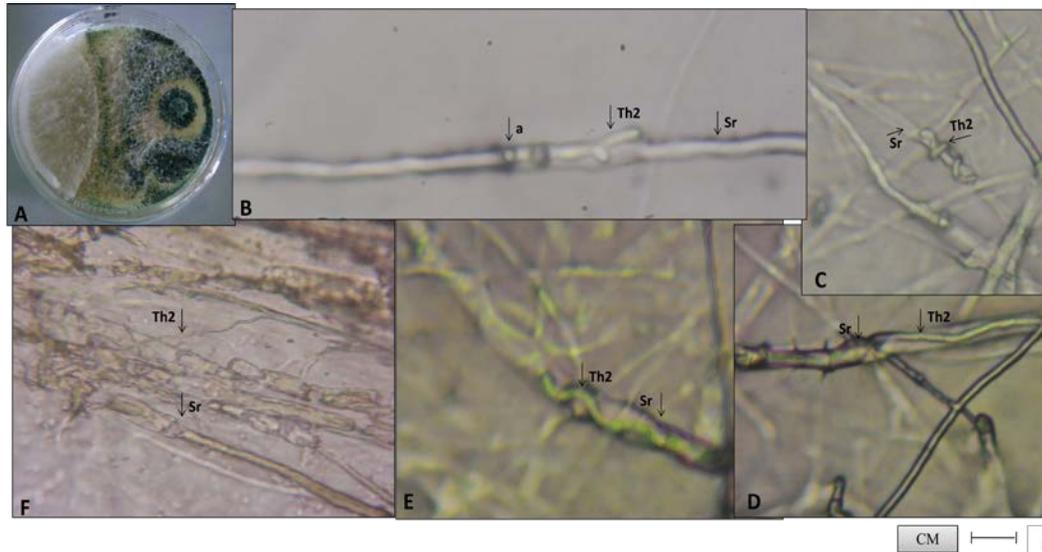


Fig. 2. Mycoparasitism interactions of *T. sp2* (Th2) against *S. rolfsii*, 14 days after incubation

Antagonism of *T. sp2* against *S. rolfsii* (A), hyphal contact with the host forming haustoria (a) (B), formation of coil structure (C), penetration of the host cell wall (D-E) and cell lysis of the host cell wall (F).

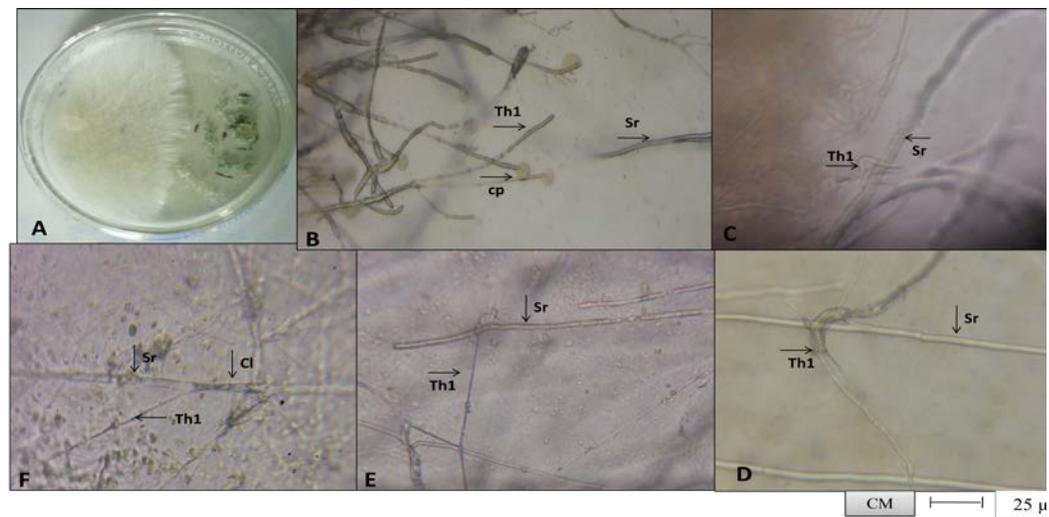


Fig 3. Mycoparasitism interactions of *T. sp1* (Th1) against *S. rolfsii* (Sr) after 14 days of incubation

Antagonism of *Trichoderma* against *S. rolfsii* (A), growth inhibition (B), hyphal contact on the host hyphae (C-E) and cell lysis of host mycelium (F).

In -vivo Test of the Antagonistic Effect of the Two Trichoderma Species Against S. rolfsii

Biological Activity of the Two Species of Trichoderma on Seed Coating Treatment

Biological activity of *Trichoderma* species on seed coating treatment increased the seed germination of the seeds coated with *T. sp1* (93.75%), and two species of *Trichoderma* (84.38%). Lowest seed germination was obtained on seeds not coated with *T. sp1* & *T. sp2* (46.78%). Highest disease incidence of seeds was recorded on seeds not coated with *T. sp1* & *T. sp2* (43.74%), and lowest percentage of disease incidence was obtained in seeds coated with *T. sp2* (9.38%). It has been reported that some *Trichoderma* species significantly decreased infection and disease through antibiosis making the seed to grow and germinate (Motlagh and Samimi, 2013).

Biological Activity of Two Species of Trichoderma on Soil Treatment

Biological activity of *Trichoderma* on soil treatment showed the highest seed germination on the soil inoculated with *S. rolfsii* + *T. sp1* + *T. sp2* (96.87%), while lowest seed germination was observed on the soil inoculated with *S. rolfsii* (62.5%). Highest percentage of damping off disease on seeds sowed in non-inoculated soil, soil inoculated with *S. rolfsii* and soil with *S. rolfsii* + *T. sp1* (18.75%) were observed while seeds sowed from the non-inoculated soil *T. sp1* + *T. sp2* (6.25%) reduced the percentage of disease incidence. The possible response mechanisms employed by *Trichoderma* to control pathogen could be the nutrient and niche competitions, antibiosis producing volatile components and non-volatile antibiotics (Harman *et al.*, 2008).

Conclusion

Based on the study, the two species of *Trichoderma* were found to be viable in improving the composting efficiency of the compost especially when

the *T. sp2* was inoculated on the compost. The addition of *T.sp2* increased the total NPK content of the compost at 4.88% with the C: N ratio of 6:1. Combination of the two species of *Trichoderma* could also increase the nutrient content of the compost with 4.48% with the high percentage recovery of 67%.

The two species of *Trichoderma* had the ability to inhibit the mycelia growth of *S. rolfsii* under in-vitro condition by 34.92% and 31.44%, they have also the ability to produce metabolites that inhibit the growth of *S. rolfsii* .

Biological activity of *Trichoderma* on seed coating treatment showed enhancement of the seed germination of cucumber when the seeds were coated with *T. sp1* (93.75%) alone, or coated with *T. sp1* + *T. sp2* (84.38%). Biological activity of the *Trichoderma* on soil treatment revealed that seeds sowed in any of the infected or non-infected soil treated with the two species of *Trichoderma* either alone or in combination enhanced the seed germination of cucumber by 87.50% to 93.75%. Disease incidence in cucumber was high even in the presence of *T. sp1*; however application of the two species of *Trichoderma* reduced the incidence of damping off disease by 6.25%.

Recommendations

Based on the study, the following are recommended: Different substrates and ratios must be used for further evaluation of the efficacy of the two species of *Trichoderma* as compost activators; Changes in organic carbon, total NPK, C: N ratio during the various stages of composting must be employed to determine the efficiency of the two species of *Trichoderma*; Identification and determination of the metabolites released by the two species of *Trichoderma*; Experiments must be done to verify other bio-control mechanisms and specificity of systemic induced resistance of the two species of *Trichoderma* on different fungal diseases under in-vitro and in-vivo test; Different test crops must be used to determine the long term response of the two species of *Trichoderma* against disease-causing pathogens on certain crops; Finally, a study on the identification and molecular characterization of two species of *Trichoderma* to determine its species and molecular features must be made.

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