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Use of *Trichoderma virens* in the Control of Tomato Sclerotinia in Côte d'Ivoire

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Abstract: ***A biological control approach using Trichoderma virens has been considered in the suppression of sclerotinia in tomatoes in Côte d'Ivoire. In vivo evaluations by amendment with different doses of a formulation of T. virens (TSM) in one or two applications on naturally infested Songon soils and artificially inoculated soils with an isolated strain in the Songon market garden perimeter. Growth parameters, flowering time and disease severity were noted to see the effect of the different treatments. The results showed that the incidence of sclerotinia is reduced in both types of soil amended with T. virens. In addition, transplants on soils treated with T. virens have better vegetative growth compared to transplants on control soils. The 200 g dose of TSM applied once or twice was more effective in both reducing disease and stimulating plant growth. These results are of interest for the use of this biological fungicide as an alternative to chemical control of Sclerotium rolfsii in tomato cultivation.***

Keywords: Biological control, *Sclerotiumrolfsii*, *Trichoderma virens*, Tomato, Ivory Coast

1. Introduction

In Côte d'Ivoire, tomatoes are grown in all agricultural production areas **[18]**and are the main source of income for farmers.However, this crop is becoming increasingly complex because of the many biotic and abiotic constraints that limit its production **[7]**. Among the most aggressive biotic agents, *Sclerotiumrolfsii* Sacc, a fungal parasite causing sclerotiniosis or white rot, is one of the most damaging and destructive soil-borne plant pathogens in market-growing areas **[14; 18]**. Infected plants initially have a wilting of one or more branches, but the young plant can wither and die within weeks of infection **[15; 16]**. Control of this fungal pathogen remains very difficult due to its survival structure (sclerotia) which remains viable in the soil for several years**[12]**. The usual methods used by producers are based on the use of non-conventional pesticides and sometimes at non-recommended rates and frequencies of application. However, the excessive use of chemical fungicides leads to the development of resistant mutants and has harmful consequences for humans and the environment. In this context, the search for alternatives to synthetic products is necessary. According to Demol *etal*., (2002)**[5]**, the creation of resistant varieties is the best solution to avoid damage caused by pests. However, it must be noted that selection always comes up against fluctuations in the strength properties of the selected material, particularly when it is deployed in production areas other than the area of selection **[11]**. Biological control could be considered a very promising alternative because of its sustainability **[13; 14]**. One of the preferred approaches to this control is the use of natural antagonists. The use of microbiological agents has been the subject of increasing study in recent decades due to their considerable agronomic interest in the control of crop diseases and pests.In addition, they are characterized by their great diversity, ease of dissemination, specificity of action and persistence in the environment **[4]**. Among these microorganisms, the genus *Trichoderma* sp. Pers. Fr. (Hypocreaceae) is one of the most studied fungi for its antagonistic effects on crop pathogens. This fungus is very effective in stimulating growth and protecting plants **[2]**.

The objective of this work is to study the effect of a formulation based on *Trichoderma virens* on the incidence of tomato sclerotinia.

2. Material and Methods

2.1 Material

2.1.1. Tomato Cultivar

The variety Tropimech susceptible to sclerotinia was used for in vivo evaluations. The seeds were purchased in Semivoire in Abidjan (Côte d'Ivoire).

2.1.2. Fungal material

A strain of *S. rolfsii* isolated from tomato plants showing symptoms characteristic of sclerotinia and originating from the Songon market garden perimeters was used.

A strain of *Trichoderma virens*, from the Industrial Research Unit on Biopesticides of the Felix Houphouët BOIGNY University in Abidjan (Côte d'Ivoire), was used as fungal

material for biological control. It consists of *Trichoderma virens* spores formulated on corn bran called TSM.

2.2 Methods

2.2.1. Obtaining doses of tomato growing substrates and setting up nurseries

Applications of the TSM formulation to sterile soil were made by amendments to the doses of 50; 100; and 200 g of the formulation per 3 kg of sterile soil. After the various doses of TSM were added, the whole was carefully mixed in order to allow a better colonization of the culture substrate by the fungus. The soil amended with TSM was noted as T0. For each dose of TSM, the resulting substrate was distributed in honeycomb plates. Seeding was then carried out in the plates containing the doses of compost. Regular watering of the plants was done every day.

2.2.2. Preparation of plant transplanting substrates and soil inoculation by *S. rolfsii*

The transplanting substrates for tomato plants were Songon soil naturally infested by *S. rolfsii* (Sn) and sterilized and artificially infested soil (Sa). These two soil categories were amended at different frequencies or not by the TSM. The same amendment rates (50, 100, and 200 g of TSM per 3 kg of soil) used for the nursery were used for transplanting the plants. Thus, transplant substrates amended once with TSM were noted T1 while those amended twice were designated T2. Seven days after the first application of TSM (substrate T1), a second application at the same amendment rates was made on one of the batches of culture substrate T1 and homogenized as previously (Substrate T2). Three other batches of transplanting substrates without amendment with TSM were also formed with sterilized and artificially infested soil on the one hand and naturally infested soil on the other hand. These transplant substrates without amendment with TSM were rated TeSa (Sterilized Soil Control), TeSa+Scl (Artificially infested Soil Control *with* S. *rolfsii*) and TeSn (Naturally infested Soil Control). In total, 5 treatment groups of the tomato growing substrate were formed:

- Sterilized soil not inoculated and not amended with TSM (negative control or TeSa)
- Sterilized soil inoculated (artificially infested) and unamended with TSM (artificial positive control or TeSa+Scl)
- Naturally infested and unamended soil with TSM (natural positive control or TeSn)
- Soil sterilized, inoculated and amended with TSM
- Soil naturally infested and amended with TSM

S. rolfsii was inoculated to the sterilized soil 7 days after the first amendment of the transplant substrates with TSM due to 267 sclerotia per 3 kg of soil.

2.3. Transplantation of tomato plants

The tomato plants were transplanted three weeks after sowing. These plants have beentransplanted into 500 mL polyethylene pots containing 400 mL of the culture substrate. The pots containing the plants were placed under the greenhouse in a three-factor split-plot system: Soil type (Sa and Sn), number of applications of TSM (T0, T1 and T2) and doses of TSM (0, 50; 100 and 200 g of TSM per 3 kg of culture substrate). Five (05) plants by elemental treatment were used in this trial. The experiment was repeated three times.

2.4. In vivo evaluation of the effect of the application of the TSM

The effect of TSM on tomato plant growth parameters, plant flowering time and sclerotinia development was evaluated. Growth parameters were recorded two weeks after transplanting. The ratings were made weekly. These growth parameters concerned:

- The height of the plants from the cotyledon leaves to the V formed between the last unopened leaf and the second last fully opened leaf,
- The number of sheets;
- The diameter of the rods using a caliper

For each of these parameters, averages were calculated by treatment and soil type. The evaluation of treatments on flower appearance times was carried out over a two-week period. The flowering time variable was evaluated in number of days after sowing**[6]**. The average flowering day (Fm) was calculated by treatment.

The effect of TSM on sclerotinia was assessed by calculating severity. The severity index expresses the intensity of symptoms observed on tomato plants. The assessment of the development of sclerotinia on each plant was made using a symptom rating scale proposed by Vakalounakis and Fragakiadakis (1999)**[19]**:

- 0: healthy plant;
- 1: slight yellowing, slight rot of the pivot and secondary roots and rot of the collar;
- 2: yellowing of the leaves with or without wilting or stunting of the plants, significant rot of the neck and browning of the stem vessels;
- 3: Death of the plant.

The severity index (SI) of the disease was calculated according to the formula proposed by Song *etal*.,2004)**[17]**.

2.5. Statistical analysis

The data collected were processed using STATISTICA 7.1 software. A one-factor analysis of variance was used for growth parameters and flowering time. In case of significant difference between the averages, the Newman-Keuls test at the 5% threshold was performed for the separation of the averages.

3. Results

3.1. Effect of TSM on the onset of sclerotinia symptoms

Five weeks after transplantation, the first symptoms of the disease were observed on sterilized soil control plants inoculated (Te+scl). These symptoms were also observed on control plants in unsterilized soil. Characteristic symptoms were seen four weeks later on 50T0 treated plants in both soil types. These symptoms are characterized by the

presence of light brown lesions on the collar (**Figure 1A**). This browning of the neck is followed by the mass growth of a whitish mycelium, followed by the formation of mature brown-black sclerotia. After the pathogen appears, the disease begins with crown rot (**Figure 1B**), extending along the stem (**Figure 1C**). At the same time, the yellowing of the old leaves follows, which spreads a few days later to the entire foliar system (**Figure 1D**). The plants contaminated by the fungus eventually dry out completely within a few days.

3.2 Effect of TSM biofungicide on disease severity.

The severity percentages were statistically different depending on the treatment. The severity percentages were higher for plants that were transplanted onto unsterilized soil regardless of the type of treatment. The Te control of unsterilized soil had the highest percentage of severity (50%) compared to all plants treated on sterilized and unsterilized soil. For all treated plants, severity increased with the dose and number of SST contributions. The severity percentages were high with T1 treatments on substrates amended in proportion 50 g/3kg. The low percentages of severity in transplanted plants on unsterilized soil were recorded with T1 and T2 treatments for the 200 g/3kg soil amendment rate and with T2 treatment for the 100 g/3kg amendment rate. On sterilized soil, the lowest severity indices (%) were obtained with T1 and T2 treatments at a dose of 200 g/3kg soil (**Figure 2**).

3.3 Effect of TSM biofungicide on plant growth parameters

The application of TSM fungicide according to treatment type and soil type showed variable effects on the evolution of tomato plants. A significant difference in growth parameters of transplanted plants on sterilized and unsterilized soil was noted (**Tables I and II**).

3.3.1. Effect of TSM biofungicide on plant height.

Plants transplanted onto sterilized soil that were amended at different doses of TSM showed an increase in stem height compared to the Te (19.06 cm) and Te+scl (18.85) control. Plants on 200T1 and 200T2 treated soils had the highest heights (24.57, 25.42 Cm respectively) (Table I). For unsterilized soil, transplants transplanted on 200T1 and 200T2 treated substrates obtained values of 19.41 and 20.08 cm respectively. On these soils, plants treated with 50T0 and 50T1 recorded the lowest heights with values of 14.99 and 15.10 cm respectively (Table II).

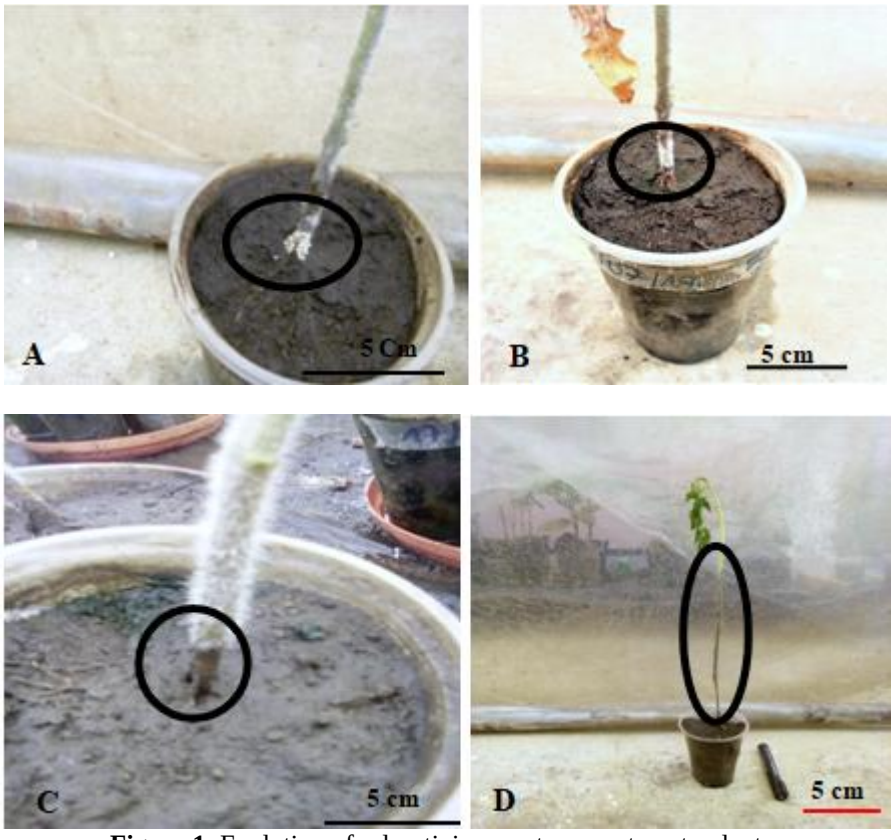

Figure 1: Evolution of sclerotinia symptoms on tomato plants.
A: presence of mycelium and onset of sclerotia formation; **B**: formation of sclerotia at the collar; **C**: rot of the collar and **D**: rot and drying of the stem

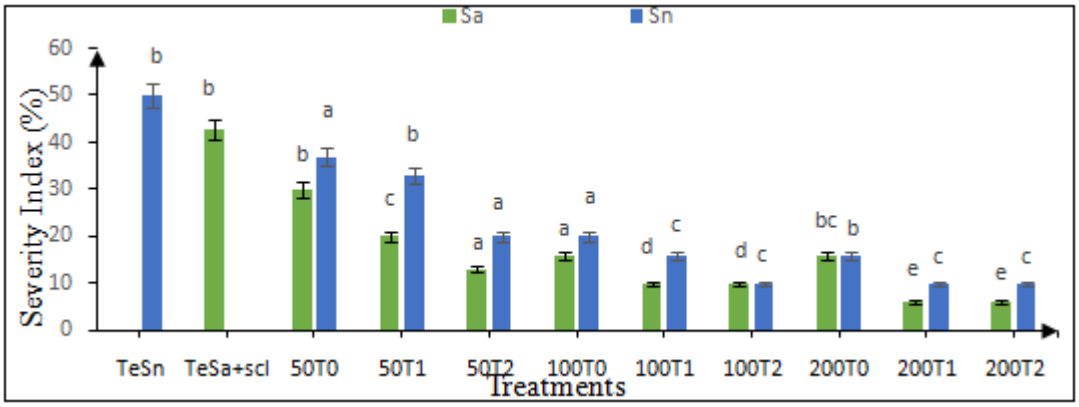

Figure 2: Evolution of disease severity after application of the biofungicide TSM on sterilized (Sa) and unsterilized (Sn) soil
50; 100 and 200 g : dose of TSM ; TeSn : Control naturally infested and unamended ; TeSa+scl : Control artificially infested and unamended ; T0 : amended at the nursery ; T1 : amended once at the nursery and growing substrate and T2 : amended once at the nursery and twice at the growing substrate.
Figures assigned the same letter are not significantly different at the 5% threshold using the Newman and Keuls test for the same soil type.

3.3.2. Effect of TSM biofungicide on average plant diameter

Tables I and II showed that tomato plants recorded larger diameters on sterilized soil than on unsterilized soil. With 200T1 and 200T2 treatments, plant diameters are high on both types of soil. The average diameters were 0.29 and 0.23 respectively on sterilized and non-sterilized soil.

3.3.3. Effect of TSM biofungicide on the number of leaves in plants.

Significant differences were observed between the different treatments on both sterilized and non-sterilized soil. On soil sterilized with 100T2 treatments, plants recorded the highest average number of leaves (8.82) (Table 1). Unlike sterilized soil, plants transplanted into unsterilized soil with 200T1 and 200T2 treatments had the highest leaf values of 7.03 and 7.16 leaves respectively (Table 2). With the exception of soils (sterilized or not) with 50T0 and 50T1 treatments, all plants showed an average leaf count higher than the control.

3.3.4. Effect of TSM biofungicide on flowering time of tomato plants

The analysis of variance showed that there is a significant difference between the flowering time of the different treated plants. Flowering of the plants began between the 50th and 63rd day of planting (Table 3 and 4). For both soil types, plants treated with 200T1, 200T2 and 100T2 were the first to flower. On soil sterilized with 200T1, 200T2 and 100T2 treatments, the flowering time of the plants was 52 days respectively. This time was average for 100T1 and 50T2 plants (56 and 54 days) and slightly high for 50T1 treated plants (57 days). For other treated plants, absence or late flowering is noted 14 days after observations. On unsterilized soil with 200T1, 200T2, 100T1 and 100T2 treatments, flowering times were 58, 56, 64 and 57 days respectively.

4. Discussion

The results of the in vivo tests demonstrated that the application of the biofungicide TSM can have a protective effect against the expression of sclerotinia symptoms on tomato plants. Indeed, the evaluation of the effect of the TSM formulation revealed a significant reduction in the severity of the disease after application compared to the untreated control. The lowest severity percentages are obtained with TSM at a rate of 200 g per 3 kg soil in one or two applications. These results are consistent with those of Yedidia *et al* (1999)**[20]** who showed that the application of *T. harzianum* to the melon culture substrate resulted in activation of the plant's defence system. This activation would be due to an increase in the activity of chitinase, peroxidases and an increase in enzymatic activity in the leaves inducing systemic resistance in these plants.

Table 1: Effect of TSM biofungicide on growth parameters of transplanted tomato plants on sterilized soil (Sa)

Type of treatment	Height (cm)	Number of sheets	Diameter (cm)
Te	19,06 ± 5,79a	6,90± 1,90a	0,22 ± 0,08a
Te+Scl	18,87 ± 5,23a	6,80 ± 2,18a	0,22 ± 0,09a
50T0	19,19 ± 5,82a	6,80 ± 1,44a	0,21 ± 0,06a
50T1	19,04 ± 7,31a	6,87 ± 1,96a	0,22 ± 0,07a
50T2	21,20 ± 7,06ab	7,17 ± 1,58b	0,23 ± 0,08ab
100T0	19,64 ± 4,95a	6,82 ± 1,46a	0,21 ± 0,06a
100T1	19,89 ± 5,78a	6,97 ± 1,88ab	0,24 ± 0,07ab
100T2	22,70 ± 6,61b	8,82 ± 3,08d	0,27 ± 0,07bc
200T0	20,61 ± 6,28a	7,63 ± 1,83c	0,24 ± 0,07ab
200T1	24,57 ± 7,60c	7,63 ± 1,69c	0,29 ± 0,07c
200T2	25,42 ± 8,08c	7,70 ± 2,11cd	0,29 ± 0,10c

50; 100 and 200 g : dose of TSM ; TeSn : Control naturally infested and unamended ; TeSa+scl : Control artificially infested and unamended ; T0 : amended at the nursery ; T1 : amended once at the nursery and growing substrate and T2 : amended once at the nursery and twice at the growing substrate.

Table 2: Effect of TSM biofungicide on growth parameters of tomato plants transplanted into unsterilized soil (Sn)

Type of treatment	Height (cm)	Number of sheets	Diameter (cm)
Te	14,75 ± 1,86a	4,93 ± 1,05a	0,14 ± 0,05a
50T0	15,10 ± 2,09a	4,83 ± 1,08a	0,14 ± 0,05a
50T1	14,99 ± 1,96a	4,83 ± 0,91a	0,14 ± 0,06a
50T2	15,16 ± 2,20a	5,70 ± 0,95b	0,16 ± 0,07a
100T0	15,00 ± 2,19a	4,93 ± 1,17a	0,15 ± 0,05a
100T1	17,14 ± 2,25b	6,10 ± 1,91bc	0,19 ± 0,06b
100T2	18,07 ± 5,02b	6,37 ± 1,81c	0,20 ± 0,07b
200T0	15,13 ± 2,23a	5,16 ± 1,76a	0,14 ± 0,05a
200T1	19,41 ± 5,07c	7,03 ± 2,06d	0,23 ± 0,09c
200T2	20,08 ± 4,74c	7,16 ± 2,29d	0,23 ± 0,11c

50; 100 and 200 g : dose of TSM ; TeSn : Control naturally infested and unamended ; TeSa+scl : Control artificially infested and unamended ; T0 : amended at the nursery ; T1 : amended once at the nursery and growing substrate and T2 : amended once at the nursery and twice at the growing substrate.

Numbers assigned the same letter are not significantly different at the 5% threshold using the Newman and Keuls test for the same parameter

Table 3: Effect of TSM biofungicide on the average flowering time of tomato plants transplanted onto sterilized soil (Sa)

Type of treatment	Flowering time (day)
Te	-
Te+Scl	-
50T0	-
50T1	57 ± 2,52b
50T2	54 ± 1,00bc
100T0	-
100T1	55 ± 1,53bc
100T2	52 ± 2,07c
200T0	-
200T1	52 ± 1,92c
200T2	52 ± 1,58c

The numbers assigned to the same letter are not significantly different at the 5% threshold using the Newman and Keuls test
50; 100 and 200 g : dose of TSM ; Te+scl artificially infested and unamended ; Te : Control without amendment and without *S. rolfsii* inoculation ; T0 : amended at the nursery ; T1 : amended once at the nursery and growing substrate and T2 : amended once at the nursery and twice at the growing substrate - Flowering time undetermined (late flowering)

Table 4: Effect of TSM biofungicide on average flowering time of unsterilized soil transplanted tomato plants (Sn)

Type of treatment	Flowering time (day)
Te	-
50T0	-
50T1	-
50T2	-
100T0	64 ± 1,41a
100T1	57 ± 0,71b
100T2	-
200T0	58 ± 1,41b
200T1	56 ± 1,53c
200T2	56 ± 1,50c

The numbers assigned to the same letter are not significantly different at the 5% threshold using the Newman and Keuls test 50; 100 and 200 g : dose of TSM ; Te+scl artificially infested and unamended ; Te : Control without amendment and without *S. rolfsii* inoculation ; T0 : amended at the nursery ; T1 : amended once at the nursery and growing substrate and T2 : amended once at the nursery and twice at the growing substrate- Flowering time undetermined (late flowering).

For all treatments studied, the antagonistic activity was greater in sterilized soil than in unsterilized soil. Similar observations have also been reported by Yeo (2018)**[21]**, in his work on the effect of biofungicides based on plant extracts against sclerotinia. Other authors have shown that the development and activity antagonist of *T. harzianum* is higher when treated seeds are sown in sterilized soil **[1]**. The reduction in the activity of the antagonist in unsterilized soil would be due to a limitation in the colonization of the root system, resulting in its inability to compete with the soil microflora. According to these authors, the lack of colonization is caused by the presence of antagonistic activity inhibiting substances and by the presence of certain agents, such as *Pseudomonassp*. which produces toxic metabolites against *Trichodermasp*.

Thus, the contribution of the biological control agent in sufficient quantity in the nursery substrate at least two weeks before infection by the pathogen is the preferred approach. During this period, *Trichoderma* sp. multiplies and colonizes the rhizosphere, which allows it to take advantage of one of its modes of action, competition **[11]**, while occupying the ecological niche as a priority. Our results also showed that all TSM applications were able to stimulate the growth and development parameters of tomato plants to varying degrees. This stimulation mainly resulted in a better height growth of the stem, its diameter, the number of leaves and a short flowering period.

Thus, biofungicide at doses of 100 and 200 g TSM per 3 kg of cultivated soil in one or two applications had the most significant effects on growth and flowering time of tomato plants. This stimulating effect was less with 50g doses of TSM per 3 kg of soil applied only once to the growing substrates for nursery and transplanting tomato plants. Similar growth promotion effects have already been reported on other strains of *Trichoderma***[3; 9]**which have shown that the contribution of *T. harzianum* to previously autoclaved soil would increase the dry weight of the roots as well as that of the aerial parts of the tomato compared to the untreated control. By colonizing the roots, Trichoderma sp. increases root and whole plant growth, developing a symbiotic relationship, rather than parasitism with the plants **[8]**. This phenomenon could be induced by hormone production by the plant and a better supply of mineral elements**[10]**. According to Harman *et al* (2004)**[8]**, beneficial fungi such as *Trichoderma* sp. can stimulate plant growth by increasing nutrient availability, increasing efficiency in nitrogen use, and solubilizing nutrients in the soil.

5. Conclusion

This work showed that the doses and number of contributions of the biofungicide based on *Trichoderma virens* have a considerable influence on the growth of tomato plants and the reduction of the aggressiveness of *Sclerotiumrolfsii*. The 200 g dose of TSM applied once or twice was more effective in both reducing disease and stimulating plant growth. These results are of interest for the use of this biological fungicide as an alternative to chemical control of *Sclerotiumrolfsii* in tomato cultivation.

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