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# Integrated Management of Garlic White Rot (*Sclerotium cepivorum* Berk) Using Some Fungicides and Antifungal *Trichoderma* Species

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## Abstract

White rot (S*clerotium cepivorum* Berk), is one of the most destructive soil borne pathogens that pose significant threat to production of garlic and other *Allium* species in Ethiopia and all over the world. Since most of the conventional control methods are not effective, the development of eco-friendly and cost effective integrated management method is critically required. A study was then conducted with completely randomized design and three replications that consist of all possible combinations of 31 treatments. The study was conducted during 2013/14 under greenhouse condition with the objective of evaluating the effect of two fungicides, Apron Star 42 WS and Tebuconazole, and in combination with four *Trichoderma* species namely *T. hamatum, T. harzianum, T. oblongisporum* and *T. viride.* The results of this study revealed that the efficacy of both fungicides, when tested alone, against *S. cepivorum* was lower than those treated with *Trichoderma spp*. alone and the fungicide combined treatments. Among all treatments, T16 (Apron Star 42 WS fungicide combined with *T. hamatum* and *T. viride*) has provided the best antagonistic activity against *S. cepivorum* with no disease incidence, followed by *T. viride* (T8) alone and Tebuconazole combined with *T. hamtum* (T21) (both 11.1% incidence). This was well correlated to the level of foliar, stem base and bulb rots symptoms as well as to plant growth and biomass of garlic plant parts. The results suggested that integration of fungicides and *Trichoderma* species is better than applying them alone, which could be attributed to the synergistic and additive growth promoting effects of combined treatments besides controlling the disease. This integrated approach appears to be the first report in Ethiopia, which has never been tested before.

**Keywords:** Garlic; White rot (*Sclerotium cepivorum); Trichoderma spp*; Fungicides

## Introduction

Garlic (*Allium sativum* L.) is a monocotyledonous plant and belongs to the family *Alliaceae.* It is the second most widely cultivated vegetable next to onion and widely produced for its medicinal and nutritional properties and has been recognized in almost all the cultures for its culinary properties. Garlic is an excellent source of several minerals and vitamins that are essential for health and has medicinal role for centuries such as antibacterial, antifungal, antiviral, antitumor and antiseptic properties [1]. In Ethiopia, the total area under garlic production in 2011/12 reached 13,278.55 ha and the production is estimated to be over 123,961.46 tons annually [2]. Production of garlic is done on sandy soil with higher organic matter content, pH 6-7 at altitude of 1800-2500 m.a.s.l, rainfall 600-700 mm and temperature of 15-24°C [3,4]. Economic significance of garlic in Ethiopia is fairly considerable and contributes to the national economy as export commodity [5] and important for small holder farmers [6]. It was reported that heavy damage to garlic due to fungal diseases, in later years, has become very important in major production areas of garlic [7-11].

Of the fungal diseases, white rot (*Sclerotium cepivorum* Berk) is the most destructive disease of garlic, and other *Allium* species throughout the world. It attacks leaves, roots, and bulbs of *Allium spp.* and can survive in the soil for nearly 20 years. Sclerotia are the only reproductive structures of *S. cepivorum* has no perfect stage has not yet been described and no asexual spores are produced. The sclerotia are stimulated to germinate only by *Allium*-specific root exudates (alkyl-cysteine sulphoxides) which are broken down by soil microorganisms to form thiols and sulphide compounds and then stimulate *S. cepivorum* sclerotia to germinate, indicating that the host range is limited to *Allium* species [10]. White rot causes important economic losses in garlic production worldwide and can cause losses from 1 to 100% [11]. In Ethiopia, the yield loss has been found to range between 20.7% and 53.4 % [12]. Once it is established permanently renders a field unusable for a garlic production. In spite of its importance garlic productivity in many parts of the world, is low due to the lack of improved variety and, traditional production system besides diseases and pest problems. The use of low quality seeds, imbalanced fertilizers, inappropriate agronomic practices, uneven irrigations and marketing facilities are the main constraints [9,10].

Management of diseases caused by soil borne pathogens like *S. cepivorum,* is very difficult and need a multi-pronged management strategy [13]. The earliest methods used to control garlic and onion white rot were cultural and physical practices of field hygiene and sanitation and crop rotation were used for primary inoculums reduction. These have been viewed as impractical for *Allium* white rot control due to long persistence nature of the sclerotia for more than 20 years. Soil flooding, soil solarisation and sterilization, biological control agents,

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*sclerotia* germination stimulants (diallyl disulfides, DADS), composted onion waste, host resistant were also found moderately effective at varying degrees [14-16]. It has been found that systemic as well as non-systemic fungicides significantly reduced garlic white rot disease development and resulted in improved garlic yield. Several effective fungicides have been recommended against this pathogen. Among these, Tebuconazole was also effective in reducing the incidence and in increasing the yield when applied as a clove treatment [17,18].

Recent efforts have focused on developing economically safe, long lasting and effective bio-control methods for the management of plant diseases. Use of biocontrol agents has been shown to be eco-friendly and effective against many plant pathogens. Among the fungal antagonists, *Trichoderma* is considered as the most important because it controls various soil borne and seed diseases caused by a wide range of fungal pathogen [19,20]. *Trichoderma* grows rapidly when inoculated in the soil as it is naturally resistant to many toxic compounds including herbicides, fungicides and insecticides such as DDT and phenolic compounds. The resistance to toxic compounds may be due to the presence of ABC transport systems in *Trichoderma* strain. The biocontrol mechanisms exercised by *Trichoderma* could be attributed to mycoparasitism, competition for nutrients, release of toxic metabolites and extra cellular hydrolytic enzymes [21].

In Ethiopia, research effort on host resistant against garlic white rot is very limited. It was reported that systemic as well as non-systemic fungicides significantly reduced incidence of white rot, its progress rate and severity that also resulted in improved garlic yield [7,9]. Study revealed that some of the *Trichoderma* species are endowed with great potential in controlling the garlic white rot [22].

The most effective control systems to date have involved the integration of a number of systems for managing garlic white rot [13,23]. The combined use of biocontrol agents and chemical pesticides has attracted much attention as a way to obtain synergistic or additive effects in the control of soil-borne pathogens. Seed treatment with *Trichoderma* along with compatible fungicide is common practice among the farmers for economic and effective management of seed and soil-borne plant diseases. Combination of *Trichoderma* with reduced levels of fungicide promotes the degree of disease suppression without risk on non- target organisms similar to that achieved with full dose of fungicide application [24-26]. *Trichoderma harzianum* C52 was found to be compatible with some fungicides and determined to be effective biocontrol agent of the onion white rot pathogen [27]. It was found that *T. viride* combined with either Tebuconazole or onion compost resulted in enhanced white rot control (>90%) and was better than any treatment alone [23,28].

However, attempt has not been made in Ethiopia to determine the effect of integrating various control measures with *Trichoderma* species for the management of white rot in garlic. Hence, the present study was undertaken on the management of garlic white rot with the integration of four selected *Trichoderma spp*. (*T. hamatum, T. harzianum, T. oblongisporum* and *T. viride.*) and two recommended fungicides (Apron Star 42 WS and Tebuconazole) under pot culture condition. In this paper the results of this integrated management of garlic white rot under pot culture condition is described.

## Materials and Methods

### Experimental design

The experiment was conducted in a Completely Randomized Design (CRD) with three replications and 31 treatments consisting of all possible combinations with the objective to achieve integrated management of garlic white rot using four *Trichoderma spp* of PPRC isolates and two recommended fungicides [Apron Star 42 WS and Tebuconazole (Folicur 250 EC] under greenhouse condition. The *Sclerotium cepivorum* sclerotia propagules were maintained and undertaken in pot experiment (Seedling bioassay), as described earlier by [23,29]. Inoculated local garlic clove with *S. cepivorum* and un-inoculated alone were used as positive and absolute control, respectively.

### Culturing of *Sclerotium cepivorum*

Culture specimens of *S. cepivorum* preserved in the Mycology Section of Ambo Plant Protection Research Centre (APPRC) were used for this study. Stock culture was inoculated onto sterile potato dextrose agar (PDA) plates and incubated at 25°C for 2 days and then examined for the growth of the fungus. After incubation, the appearance of colonies on the medium was observed which proved the viability of preserved isolates of the *S. cepivorum*. The well-grown mycelium was selected for further study.

### Mass production of *Sclerotium cepivorum*

The sclerotia of *S. cepivorum* isolate were first produced on PDA in 9-cm diameter petri dishes by incubating at 20°C for 5 days. Since the pathogen doesn't have functional spores, a small, round, seed-like structure known as sclerotia was initially produced. The refreshed *S. cepivorum* sclerotia were further inoculated on whole wheat grains [30]. Fifty grams of the inoculated whole wheat grains were added to each of twenty, 250 ml conical flasks, the content of the flasks were treated with 45 millilitres of 0.0025 % (w/v) Chloramphenicol and the flasks were left overnight at room temperature. The treated flasks of wheat (50 g each) were autoclaved at 121°C and 15 psi for 30 min, and this was repeated for three consecutive days. After cooling to room temperature, each flask was inoculated with four, 5 mm disks of *S. cepivorum* taken from the actively grown edge of a 5 day old culture grown on PDA. The flasks were incubated at 20°C in the dark for 6 to 8 weeks and shaken at weekly intervals to ensure an even distribution of mycelium. During the first three weeks of incubation, 0.5 ml of sterile distilled water (SDW) was added if the flasks appeared dry, to encourage mycelia growth .

### Harvesting *Sclerotium cepivorum* sclerotia

The sclerotia of *S.cepivorum* were harvested from the wheat grains using progressive wet sieving through 850 µm, 500 µm and 250 µm sieves [31,32]. Only healthy sclerotia was retained on the 500 µm sieve which was air dried on sterilized Whatman No. 1 filter paper for 24 h before they were used or conditioned. The sclerotia used after this stage was termed "fresh". Before using for the greenhouse study, both the fresh and conditioned sclerotia viability were resolved by taking a sample of 100 sclerotia and surface sterilized in 0.25% sodium hypochlorite (NaOCl) for 1min. Subsequently, it was washed in five changes of sterile distilled water (SDW), then spreaded over Whatman No. 1 filter paper to absorb excess liquid. Then, it was placed onto PDA in petri-dishes. The petri-dishes were sealed with polythene wrap and then incubated at 20°C in the dark and the sclerotial viability/ germination was examined for 10 days. The number of germinated sclerotia was recorded to reach >96%. Once the viability of the sclerotia germination percentage and competence were decided, 100 g of sclerotia/kg of sterilized moist soil was incorporated into the *in vivo* experiment. This is based on the fact that 0.01-0.1 g sclerotia/g of soil resulted in infection of less than or equal to 85-100% and 100% incidence of disease in onion and garlic plants, respectively. This is

similar to the finding that only one sclerotia per kilogram of soil can provoke a 50%, and 10-20 sclerotia per kilogram can result in infection of essentially all plants (as the disease severity depends on sclerotia levels in the soil at the time of planting [33].

## Mass production of *Trichoderma spp.*

The *Trichoderma spp*. used in this study were obtained from the culture specimen collections of APPRC, that, previously isolated from soils characterized in Ethiopia and preserved in culture collection [34]. These *Trichoderma spp*. were found to be effective in controlling faba bean fungal disease, *Fusarium solani* [35]. Furthermore, out of seven *Trichoderma* species tested under *in vitro* and *in vivo* antifungal activities against white rot of garlic, four of them registered high percentage inhibition zone ranging from 51.7 to 59.3%. [26] Therefore these four potent species were selected for the present study viz., *T. hamantum, T. harzianum, T. oblongisporum and T. viride*.

Mass multiplications of *Trichoderma spp*. were carried out according to standard procedures [36,37]**.** Thus, spore suspensions of *Trichoderma spp*. were prepared by adding 20 ml sterile distilled water to a three-week-old petri-dish cultures and scraping gently with a sterile spatula. The harvested spore suspension of *Trichoderma spp*. were inoculated into a sterilized one litre jar containing wheat bran, sand and water medium or sorghum grain and incubated for three days at 20°C.

## *In vivo* efficacy test

The experiment was conducted under greenhouse condition using the local cultivar of garlic. The appropriate soil composition were made proportionally with the composition of sand, compost and sandy clay loam soil mixed at (1:1:2 ratios) and then sterilized. Each pot (21 cm top diameter and 9 cm height) were filled with 3 kg of mixed soil. The pots were arranged and placed in saucers so that all watering were from below, then after, the cloves of garlic were first surface sterilized using 70% ethanol for five mins and rinsed three times with SDW. Then cloves were dressed with recommended fungicides (Apron Star 42 WS (3gm of Apron Star 42 WS powder with 10 ml of water) and Tebuconazole (2.1 ml of Tebuconazole with 15 ml water) [4] by partial and/or with combinations of both fungicides and then soaked for one hour.

The treated cloves were planted at 3 cm depth into the moist soil thoroughly incorporated with 100 g sclerotia propagules/kg of soil in the pot (5 cloves/pot were planted and two of them were thinned after germination) immediately under greenhouse condition at 12-15°C minimum and 26-30°C maximum temperature. Each *Trichoderma spp*. spore suspension were prepared by diluting with SDW at the rate of 10 g *Trichoderma spp*. mass produced/2 litre of water were mixed. Subsequently, 300 ml adjusted spore suspension of *Trichoderma spp* were drenched on the planted soil of each pot after seven days and continued within three days intervals [38]. The emerging garlic plants were assessed for symptoms of white rot every week up to 18 weeks. The treatments were arranged as (i) four *Trichoderma spp*. each alone or (ii) two fungicides each alone or (iii) fungicides combined with one or more *Trichoderma spp*. These were evaluated for their potential to control garlic white rot on garlic under greenhouse condition. Thus, the effect of partial and combined treatments for the control of *Sclerotium* cepivorum was examined and the result was compared with un-inoculated treatment. White rot disease incidence and severity was recorded in each pot.

The following treatments were applied for the experiment:

1. -(ve) absolute control
2. +(ve) control (inoculated with *S. cepivorum)*
3. Apron Star 42 WS+*S. cepivorum*
4. Tebuconazole+*S. cepivorum*
5. *T. hamatum*+*S. cepivorum*
6. *T. harzianum*+*S. cepivorum*
7. *T. oblongisporum*+*S. cepivorum*
8. *T. viride*+*S. cepivorum*
9. *S.cepivorum*+*T. hamatum*+*T. harzianum*+*T. oblongisporum*+ *T. viride*
10. Apron Star 42 WS+*S. cepivorum*+*T. hamatum*
11. Apron Star 42 WS+*S. cepivorum*+*T. harzianum*
12. Apron Star 42 WS+*S. cepivorum*+ *T. oblongisporum*
13. Apron Star 42 WS+*S. cepivorum*+ *T. viride*
14. Apron Star 42 WS+*S. cepivorum*+*T. hamatum* and *T. harzianum* combination
15. Apron Star 42 WS+*S. cepivorum*+ *T. hamatum* and *T. oblongisporum* combination
16. Apron Star 42 WS+*S. cepivorum*+ *T. hamatum* and *T. viride combination*
17. Apron Star 42 WS+*S. cepivorum*+ *T. harzianum* and *T. oblongisporum* combination
18. Apron Star 42 WS+*S. cepivorum*+ *T. harzianum* and *T. viride combination*
19. Apron Star 42 WS+*S. cepivorum*+ *T. oblongisporum* and *T.viride combination*
20. Apron Star 42 WS+*S. cepivorum*+ *T. hamatum*+ *T. harzianum*+ *T. oblongisporum*+ *T. viride*
21. Tebuconazole+*S. cepivorum*+ *T. hamatum*
22. Tebucunazole+*S. cepivorum*+ *T. harzianum*
23. Tebucunazole+*S. cepivorum*+ *T. oblongisporum*
24. Tebucunazole+*S. cepivorum*+ *T. viride*
25. Tebucunazole+*S. cepivorum*+ *T. hamatum* and *T. harzianum* combination
26. Tebucunazole+*S. cepivorum*+ *T. hamatum* and *T. oblongisporum* combination
27. Tebucunazole+*S. cepivorum*+ *T. hamatum* and *T.viride combination*
28. Tebuconazole+*S. cepivorum*+ *T. harzianum* and *T. oblongisporum* combination
29. Tebuconazole+*S. cepivorum*+ *T. harzianum* and *T. viride combination*
30. Tebuconazole+*S. cepivorum*+ *T. oblongisporum* and *T. viride combination*
31. Tebuconazole+*S. cepivorum*+ *T. hamatum*+ *T. harzianum*+ *T. oblongisporum*+ *T. viride*

### Data analysis

Data on initial and final plant stand count at emergence, disease incidence and severity were collected every week from the experiment. All garlic bulbs were hand-harvested from each pot. Average of plant height, shoot length, root length and bulb biomass were recorded at soggy/moist phase and also after drying the samples in air for 7 days. Furthermore, 5 bulbs were randomly collected from which bulb diameter were measured, weight of cloves per bulb/plant were determined and number of cloves per bulb/plant were counted as described by [39]. Severity was assessed using a scale from 0 to 5 [40] and Disease Severity Index (DSI) was calculated.

Plants were uprooted separately from pots of each replication and determined for *mycelium expansion*; bulb and root rots were undertaken. A disease severity index based on symptoms observed and a disease severity formula was used to rate garlic treatments for their resistance to *S. cepivorum*. The Analysis Of Variance (ANOVA) of the data was separately subjected to SAS version 9.0 for further analysis and also the treatment mean were further separated by Duncan's Multiple Range Test (DMRT) at 5% significance level.

## Results and Discussion

### Effect of treatments on the foliar, stem base and bulb rot symptoms

The treated garlic plants showed slightly yellowing and wilting of delicate leaves and thin stems appeared after germination and also very few elongated roots developed on bulbs. White rot incidence was evaluated every week from the first appearance of the disease. Infected plants were examined as a small patch of plants or single plant more chlorotic than surrounding plants 45 days after artificial inoculation. The symptoms appeared as chlorosis as of lower leaves beginning at the tips, followed by a necrosis and collapse of the affected leaves of the aerial parts of the seedlings (Figure 1).

Observation of bulbs infections were carried out after harvest 127 days after artificial inoculation. Thereafter, the development of mycelia mat around stem base and sclerotial emerged on the bulbs of different treatments were seen (Figure 2). The seedlings exhibited characteristic garlic white rot symptoms including the blueing foliage, leaf tip dieback and a patchy distribution of diseased seedlings within each pot as shown in (Figure 3).

The disease symptom was initiated early in the trial just three weeks after planting. Some seedlings were discoloured, collapsed and lying on the soil surface of the pot. White rot symptoms appeared at about 45 days after planting the inoculated garlic bulbs and it's foliar and stem base symptoms incidence assessment was recorded weekly. Initially, disease symptoms were observed in all treatments, while the disease increased slowly in treatments and then become conspicuous just at the three to five leaves stage. The observations of assessments made at three stages (i). Foliar symptoms (every week until 98 days), (ii) Stem base symptoms (84-98 days), and (iii) Bulb rots symptoms (126 days) are presented in Table 1. When there is no stunting, no leaves colour change, no chlorosis, no wilting and collapsing and no stem base rotting, the bulb is designated as "Healthy".

The diseased tissues were diagnosed and the pathogen was re-isolated as an evidence of the disease development in the trial. Over 50% of the total white rot infections were recorded in the first seven weeks. The number of diseased seedlings in all treatments increased slowly for the duration of the trial and after 12 weeks. Among 279 seedlings, 126 were infected with garlic white rot. These seedlings showed characteristic garlic white rot symptoms including the blueing foliage, leaf tip dieback and a patchy distribution of diseased seedlings within each pot. After 16 weeks, more than 80% of the seedlings were diseased in the pathogen control; a significantly greater ($p>0.05$) amount of disease than on both fungicides treatment applications (Table 2). In the positive control (T2), the whole plants were extremely affected within less than 6 weeks and the seedlings were completely died.

### Effect of treatments on the growth and biomass of garlic plants

The effect of different treatments on the Shoot length (cm), Plant height (cm), Fresh biomass wt. (g), Root length (cm), Fresh bulb biomass (g), Bulb diameter (cm), plant dry biomass (g), Number of cloves/bulb, Wt. of cloves/bulb revealed that weight of cloves/bulb

**Figure 1:** Symptoms of garlic white rot appeared early on the seedlings.

(2a) 2b)

**Figure 2:** Mycelia mat around stem base and emergence of *Sclerotia* on the bulbs. (2a) top view of the mycelia mat, (2b) closer view of stem base and bulbs.

**Figure 3:** Blueing foliage, leaf tip dieback and a patchy distribution of diseased seedlings.

| Assessment time after inoculation | Score | Description | Treatments |
|---|---|---|---|
| 1. Foliar symptoms (every week until 98 days) | 0 | Healthy leaves (no disease symptom) | T8, T16 and T21 |
| | 1 | one to two leaves infected | T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, T12, T13, T14, T15 and T21 |
| | 2 | Two to three leaves infected | T1,T2, T3, T4, T5, T6, T7, T9, T10, T11, T12, T13, T14, T15,T17, T18, T19, T20, T22, T23…T31. |
| | 3 | Three to four leaves infected | T1,T2, T3, T4, T5, T6, T7, T9, T10, T11, T12, T13, T14, T15,T17, T18, T19, T20, T22, T23, T31. |
| | 4 | Four to five leaves infected | T2, T3, T4, T5, T6, T7, T9, T10, T11, T12, T13, T14, T15, T17, T18, T19, T20, T22, T23…T31 |
| | 5 | Five leaves infected | T1,T2, T3, T4, T5, T6, T7, T9, T10, T11, T12, T13, T17, T18, T19, T20, T22, T23, T31. |
| 2. Stem base symptoms (84-98 days) | 0 | Stem base free from mycelium and sclerotia | T8, T16 and T21 |
| | 1 | Mycelium and sclerotia absent on stem base | T2, T3, T4, T5, T6, T7, T9, T10, T11, T12, T13, T14, T15, T17, T18, T19, T20, T22, T23…T31. |
| | 2 | Mycelium present, sclerotia absent on stem base | T2, T3, T4, T5, T6, T7, T9, T10, T11, T12,T13, T14, T15, T17, T18, T19, T20, T22, T23….T31 |
| | 3 | Mycelium and sclerotia present on stem base | T2, T3, T4, T5, T6, T7, T9, T10, T18, T19, T20, T22,. T31 |
| | 4 | Mycelium absent, sclerotia present on stem base | T2, T3, T4, T5, T6, T7, T9, T10, T18, T19, T20, T22, T23. |
| | 5 | Only sclerotia present on stem base | T2, T3, T4, T5, and T6. |
| 3. Bulb rot symptoms (126 days) | 0 | Healthy stem base and bulbs | T8, T16 and T21 |
| | 1 | Mycelium present, sclerotia absent on bulbs | T1,T9, T10, T11, T12, T13, T14 |
| | 2 | Mycelium absent, sclerotia present on bulbs | T2, T3, T4, T5, T19, T20, T22… T30. |
| | 3 | Mycelium and sclerotia present on bulbs | T2, T3, T4, T5, T6, T7, T9, T10, T11, T12, T13, T14, T18, T19, T20, T22, T23…T31 |
| | 4 | Only mycelium presents on all bulbs | T1,T2, T3, T4, T5, T6, T7, T9, T10, T11, T12, T13, T14, T15, T17, T18, T19, T20, T22, T23, T31 |
| | 5 | Only sclerotia present on all bulbs | T2, T3, T4, T5, T6, T20, T23, T24 T25, T26, T27, T28, T29 and T31 |

**Table 1:** Assessment of foliar, stem base and bulb rot symptoms observed in different treatments.

| Treatments | Disease severity score/ Replication | | | Total plants infected | Sum of disease severity score | Mean of disease severity | Disease Incidence (%) |
|---|---|---|---|---|---|---|---|
| | Rep. I | Rep. II | Rep. III | | | | |
| T1 | 5.000 | 4.160 | 5.000 | 8.5 | 14.160 | 1.6 | 94.4 |
| T2 | 5.000 | 5.000 | 5.000 | 9 | 15.000 | 1.7 | 100 |
| T3 | 3.333 | 5.000 | 5.000 | 8 | 13.333 | 1.5 | 88.9 |
| T4 | 3.333 | 5.000 | 5.000 | 8 | 13.333 | 1.5 | 88.9 |
| T5 | 1.666 | 3.333 | 3.333 | 5 | 8.332 | 0.9 | 55.6 |
| T6 | 1.666 | 1.666 | 3.333 | 4 | 6.665 | 0.7 | 44.4 |
| T7 | 0.000 | 1.666 | 3.333 | 3 | 4.999 | 0.6 | 33.3 |
| T8 | 0.000 | 0.000 | 1.666 | 1 | 1.666 | 0.2 | 11.1 |
| T9 | 1.666 | 0.000 | 3.333 | 3 | 4.999 | 0.6 | 33.3 |
| T10 | 1.666 | 1.666 | 3.333 | 4 | 6.665 | 0.7 | 44.4 |
| T11 | 0.000 | 3.333 | 3.333 | 4 | 6.666 | 0.7 | 44.4 |
| T12 | 3.333 | 3.333 | 0.000 | 4 | 6.666 | 0.7 | 44.4 |
| T13 | 1.666 | 0.000 | 5.000 | 4 | 6.666 | 0.7 | 44.4 |
| T14 | 0.000 | 1.666 | 3.333 | 3 | 4.999 | 0.6 | 33.3 |
| T15 | 1.666 | 1.666 | 1.666 | 3 | 4.998 | 0.6 | 33.3 |
| T16 | 0.000 | 0.000 | 0.000 | 0 | 0.000 | 0.0 | 0 .0 |
| T17 | 1.666 | 3.333 | 3.333 | 5 | 8.332 | 0.9 | 55.6 |
| T18 | 1.666 | 5.000 | 5.000 | 7 | 11,666 | 1.3 | 77.8 |
| T19 | 0.000 | 5.000 | 3.333 | 5 | 8.333 | 0.9 | 55.6 |
| T20 | 0.000 | 1.666 | 5.000 | 4 | 6.666 | 0.7 | 44.4 |
| T21 | 1.666 | 0.000 | 0.000 | 1 | 1.666 | 0.2 | 11.1 |
| T22 | 1.666 | 5.000 | 3.333 | 6 | 9.999 | 1.1 | 66.7 |
| T23 | 5.000 | 3.333 | 1.666 | 6 | 9.999 | 1.1 | 66.7 |
| T24 | 0.000 | 5.000 | 5.000 | 6 | 10.000 | 1.1 | 66.7 |
| T25 | 1.666 | 5.000 | 3.333 | 6 | 9.999 | 1.1 | 66.7 |
| T26 | 1.666 | 0.000 | 5.000 | 4 | 6.666 | 0.7 | 44.4 |
| T27 | 1.666 | 3.333 | 3.333 | 5 | 8.332 | 0.9 | 55.6 |
| T28 | 3.333 | 5.000 | 0.000 | 5 | 8.333 | 0.9 | 55.6 |
| T29 | 1.666 | 5.000 | 3.333 | 6 | 9.999 | 1.1 | 66.7 |
| T30 | 3.333 | 5.000 | 3.333 | 7 | 11.666 | 1.3 | 77.8 |
| T31 | 5.000 | 1.666 | 1.666 | 5 | 8.332 | 0.9 | 55.6 |

**Table 2:** Disease incidence (%) and severity of garlic seedlings bioassay recorded in each treatment.

in the uninoculated control T1 is 1.12 whereas T16 (Apron star 42 WS+*S.cepivorum*+ *T. hamatum* & *T. viride* combination) has yielded 8.44 g, suggesting that, it may have synergistic and additive growth promoting effect on garlic in addition to controlling the white rot disease (Table 3). The same beneficial growth promoting effect can also be clearly deduced in treatment T8 (*T. viride*+*S. cepivorum)* and T21 (Tebuconazole+*S.cepivorum*+ *T. hamatum*). It is to be noted that growth promotion effect is one of the mechanism of *Trichoderma spp* exerted for control of phytopathgenic diseases [41-43].

## Effect of treatments on the disease incidence and severity on garlic plants

Significant differences on disease incidence was observed at all assessment times among the treatments (p>0.05) (Table 2). The highest incidence and severity was recorded on negative and positive control (T1 and T2) (94.4% and 100%, respectively) and with both fungicides (T3, T4) (88.9%) and Apron Star 42 WS combined with *T. harzianum* and *T. viride* (T18) (77.8). Tebuconazole combined with *T. oblongisporum* and *T. viride* (T30) has (77.8%) disease incidence under similar conditions. The highest disease incidence and severity observed in uninoculated (-ve absolute control) treatment (T1) was assumed to occure from mycelia remained in the cloves after surface sterilization. Apron Star 42 WS treated with both *T. hamatum* and *T. viride* (T16) has provided efficient and highly significant disease control as compared with uninoculated check. Whereas, Tebuconazole combined with *T. hamatum* (T21) and *T. viride* alone (T8) were the next treatments that showed lower percentage (11.1%) of disease incidence as compared to all other treatments except "Apron Star 42 Ws combined with *T. hamatum* and *T. viride*". Therefore, these two treatments are relatively the promising bioagent for antagonising garlic white rot next to Apron Star 42 WS combined with *T. hamatum* and *T.viride* (T16).

When the Tebuconazole combined with *T. harzianum, T. oblongisporum* and *T. viride* alone (T22 and T23) and Tebuconazole integrated with *T. harzianum* and *T. viride* combination (T29) exhibited disease incidence of 66.6%. On other hand, Apron Star 42 WS combined with *T. harzianum* and *T. viride,* (T18) and Tebuconazole integrated with *T. hamatum* and *T. harzianum* (T25) and *T. oblongisporum* and *T. viride* (T30) disease incidence of (77.8%) were recorded, while both fungicides alone (T3, T4) provided 88.9%. However, garlic cloves treated by *T. harzianum* alone (T6) and the mixed-up of Apron Star

| Treatments | Shoot length (cm) | Plant height (cm) | Fresh biomass wt. (g) | Root length (cm) | Fresh bulb biomass (g) | Bulb diameter (cm) | Dry biomass wt. of plants (g) | Number of cloves bulb$^{-1}$ | Wt. of cloves/bulb/ plant (g) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.57fg | 11.57cd | 10.43c | 6.50c | 12.50d | 0.70klm | 6.93c | 0.80lm | 1.12kl |
| 2 | 0.00g | 0.00h | 0.00n | 0.00h | 0.00e | 0.00m | 0.00h | 0.00m | 0.00 l |
| 3 | 9.90abcdef | 5.90efg | 5.57defghij | 2.67defg | 0.87e | 2.57lm | 0.38h | 1.78jkl | 1.21ijk |
| 4 | 7.10bcdef | 6.93defg | 0.87mn | 0.60gh | 0.10e | 0.53lm | 0.47h | 1.22kl | 1.12jk |
| 5 | 6.67bcdef | 9.63cdef | 3.73ijklm | 1.97defgh | 0.50e | 2.57bcd | 0.35h | 1.67jkl | 1.50fghijk |
| 6 | 9.83abcdef | 10.27cdef | 3.10jklmn | 1.93defgh | 0.33e | 2.87b | 0.93h | 2.34hijk | 1.38ghijk |
| 7 | 11.53abc | 7.23defg | 7.77cdef | 3.30de | 1.13e | 2.80bc | 1.29h | 3.78defg | 2.07cdefghij |
| 8 | 13.23a | 34.67ab | 38.23ab | 13.57b | 26.07b | 5.50a | 27.72b | 8.89c | 7.69a |
| 9 | 7.43abcdef | 7.70defg | 5.10fghijk | 1.93defgh | 0.77e | 2.53bcd | 2.21defgh | 3.67defg | 2.39bcdefgh |
| 10 | 11.33abcd | 10.20cdef | 5.37efghij k | 2.13defg | 0.80e | 2.80bc | 3.71defg | 4.22def | 2.59bcdef |
| 11 | 8.90abcdef | 11.10cde | 7.40cdefg | 2.00defgh | 1.57e | 2.23bcdef | 3.85def | 4.55d | 2.69bcde |
| 12 | 6.50bcdef | 11.77cd | 8.80 cd | 3.27de | 1.57e | 2.40bcde | 4.27cde | 4.11defg | 2.96bcd |
| 13 | 5.50defg | 8.63cdefg | 7.10defgh | 2.20defg | 1.00e | 2.67bc | 4.54cd | 3.67defg | 2.52bcdef |
| 14 | 5.97bcdefg | 11.93cd | 8.63cde | 2.83def | 1.77e | 2.65bcde | 4.08de | 4.22def | 3.42b |
| 15 | 7.77abcdef | 13.43 C | 6.73defghi | 3.30de | 1.57e | 2.43bcde | 4.19cde | 4.33de | 3.17bc |
| 16 | 11.83ab | 38.27a | 40.87a | 14.57b | 33.93a | 5.57a | 34.40a | 11.78a | 8.44a |
| 17 | 8.10abcdef | 10.37cdef | 2.97jklmn | 1.83defgh | 0.97e | 1.47ghij | 2.07defgh | 4.00 defg | 2.50bcdef |
| 18 | 5.67cdefg | 5.97efg | 2.37jklmn | 1.00fgh | 0.23e | 1.40hijk | 1.17fgh | 4.33de | 3.15bc |
| 19 | 9.70abcdef | 8.00defg | 5.23fghijk | 2.60defg | 0.87e | 1.57fghij | 1.74efgh | 4.34de | 2.42bcdefg |
| 20 | 10.67abcde | 8.63cdefg | 8.67cd | 2.70def | 1.07e | 2.17fbcdefg | 4.34cde | 4.34efghi | 2.98bcd |
| 21 | 11.07abcd | 32.83b | 37.43b | 20.70a | 18.50c | 5.10a | 29.80b | 10.11b | 7.46a |
| 22 | 4.67efg | 5.77fg | 1.63lmn | 1.17fgh | 0.27e | 1.13ijkl | 0.70h | 3.44defgh | 1.90defghijk |
| 23 | 7.97abcdef | 8.47cdefg | 2.60jklmn | 1.23efgh | 0.37e | 1.07jkl | 1.24fgh | 3.33fghi | 2.40bcdefgh |
| 24 | 7.13abcdef | 4.30h | 2.40jklmn | 1.10fgh | 0.30e | 0.57lm | 1.13fgh | 3.44defgh | 2.00defghij |
| 25 | 9.07abcdef | 9.40cdefg | 2.23klmn | 1.07fgh | 0.70e | 0.53lm | 0.88h | 3.00ghi | 2.29defghi |
| 26 | 10.87abcd | 9.30cdefg | 4.33hijkl | 3.33d | 0.57e | 1.50fghij | 2.37defgh | 3.22efghi | 1.68efghijk |
| 27 | 9.83abcdef | 7.33defg | 3.13 jklmn | 1.53defgh | 0.37e | 1.77fghij | 0.95h | 3.22efghi | 1.92defghij |
| 28 | 7.90abcdef | 5.57fg | 3.83ijklmn | 1.47defgh | 0.50e | 2.13bcdefgh | 2.08defgh | 2.44hij | 1.62efghijk |
| 29 | 5.67cdefg | 6.85defg | 2.57jklmn | 1.23efgh | 0.33e | 2.00cdefgh | 1.21fgh | 1.67jkl | 1.49fghijk |
| 30 | 5.40defg | 5.40fg | 2.10lkmn | 0.87fgh | 0.30e | 1.77efghij | 0.98h | 2.22ijkl | 1.31hijk |
| 31 | 10.23abcdef | 7.83defg | 4.10hijkl | 1.33defgh | 0.77e | 1.83efghi | 2.11defgh | 3.78defg | 2.41bcdefgh |
| LSD (5%) | 6.0146 | 2.001 | 3.2858 | 2.084 | 3.0388 | 2.003 | 2.7703 | 1.1034 | 2.002 |
| CV (%) | 45.30 | 30.19 | 25.42 | 37.34 | 52.17 | 21.72 | 34.58 | 25.71 | 16.53 |

Means in every column with the same letters are not significantly different at 5% significance level.
LSD: Least Significant Difference; CV: Coefficient of Variation.

**Table 3:** Effects of different treatments on the growth and biomass of garlic plant parts.

42 WS (T11) with individual four *Trichoderma spp.* (T10, T11, T12, T13) and also T20 integrated with all four bioagents as one and Tebuconazole combined with *T. hamatum* and *T. oblongisporum* (T26) all showed medium disease control (44.4%). In the garlic plants treated with Tebuconazole combined mutually with all four *Trichoderma spp.* (T31) had the disease incidence of (55.6%).

The results revealed that there was no significant difference in the percentage of diseased seedlings (88.9%-77.8%) between the garlic applied with both fungicides partly and with combination of *Trichoderma spp.* in disease control measures. In the remaining treatment, disease prévalence percentage range were categorized in ascending order as : 0% (T16); 11.1% (T8 and T21); 33.3% (T9, T14 and T15); 44.4% (T10, T11, T12, T13, and T26); 55.6% (T6, T17, T20, T27, T28 and T31); 66.7% (T5, T7, T19, T22, T23, T25 and T29); 77.8% (T18, T24 and T30) ; 88.9% (T3 andT4) ; 94.4% - 100% (T1 and T2) (Figure 4)

The combination of fungicide with one or two species of *Trichoderma* bioagent has provided similar effective control of *Allium* white rot (AWR) caused by *S.cepivorum* pathogen under greenhouse condition. *T. harzianum* has earlier been reported to reduce *S. cepivorum* infection from 84% to 29% in greenhouse trials [44]. Similarly, *T. koningii* was reported to reduce onion white rot disease by 60% when incorporated in a millet formulation and added to soil at seed planting [45] and *T. koningii*, the same isolate as used in biological control study, provided 79% disease control of onion white rot, when incorporated into the soil in a sand: bran mix [32]. Similar to previous research results, this study has also showed the best significant disease control when combining Apron Star WS fungicide with two *Trichoderma species* (*T. hamatum* and *T.viride*) (T16); Tebuconazole fungicide along with *T. hamatum* integration (T21) and *T. viride* alone (T8) gave a better result in greenhouse trial (Figure 5).

It was observed that cloves/seedlings treated with Tebuconazole showed a suppressive effect on the developments of the whole plant and roots formation and even delayed the germination by a week. In all treatments receiving Tebuconazole, the plant stands was weak, very thin and fragile. The roots were very few in numbers and very thin, shrivelled, elongated and sheath paled off easily and bulbs were tiny.

The beneficial effect of the fungicide in combination with *Trichoderma spp.* at planting was effective to suppress the sclerotial germination. These results indicate that, combining of fungicide with the bio-agent(s) provides a good prospect for garlic growers as *Trichoderma spp.* are safe for animals, human beings and environment. The results indicate a break from susceptible cropping, and integration of fungicide with biocontrol agents was the strategy providing greater reduction in suppressing the viability of *S. cepivorum* inocula in the soil resulting in minimum incidence of white rot (T16) (Figure 6). The magnitude of economic benefit and synergistic value of *Trichoderma spp.* after combination remains to be determined, as does the ecological one, when combining these practices. It can be seen that, the degree of disease control achieved by treatment with Tebuconazole alone was lower than that of all the treatments combined with Apron Star 42 WS fungicide, and mixed-up of one or more mixture of four *Trichoderma spp.* each other when compared to un-inoculated and T16 which has absolutely controlled the pathogen.

It was reported that treatment of garlic cloves with Tebuconazole and base spray provided significant reduction in the rate of disease progress and the final of plant mortality by *S. cepivorum* [17]. Eighty five percent

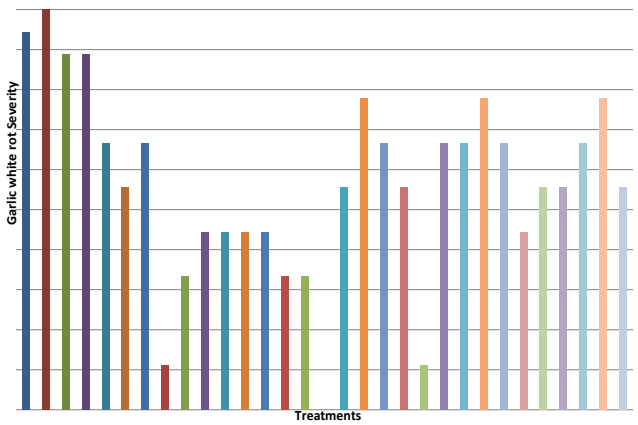


**Figure 4:** Effect of different treatments on disease severity of *S. cepivorum*.

**Figure 5:** Effect of different treatments T8, T3 and T1 on disease severity of *S. cepivorum*. (5a)=T. *viride* (T8), (5b)=Tebuconazole (T3) and (5c)=Un-inoculated (T1).

**Figure 6:** Effect of treatment T16 on disease severity of *S. cepivorum*.

disease incidence reduction was reported in Tebuconazole treated plots compared with untreated plots in onion [46]. Other researchers also reported that combinations of Tebuconazole and a biocontrol agent enhanced the control of onion white rot [23]. Even though an indication of antagonism has been obtained from this greenhouse study and previous literature based on similar isolates [32], the level of control did vary with the same isolates when similar methodology was used. In general, an important factor in biocontrol agent effectiveness is the rate at which the propagules/mycelium dilution amounts proliferate when applied to the potting mix. To predict and successfully use biological control agents for soil borne disease control, it is critical that their biology and ecology be more completely understood. It was beyond the scope this study to determine the individual components of the three types of potting mixed-up in relation to microbial carrying capacity as an indicative difference to antagonize the white rot pathogen activities. Thus, integration of fungicides and biological control agents may enable the number of fungicide sprays to be reduced, while providing control of garlic white rot.

## Conclusion

The results obtained in this study revealed that the two *Trichoderma spp*. (*T. hamatum* and *T. viride*) in combinations with two fungicides can have substantial antagonistic activity against garlic white rot pathogen. This could be attributed to their synergistic and additive growth effects that yielded better biomass besides controlling the disease. The findings also suggest that *T. hamatum* and *T. viride* are playing an important role in controlling garlic white rot pathogen better than the two fungicides alone. This is highly advantageous in light of the fact that the use of *Trichoderma*-based products is not only safe for the farmers and consumers but is also environmentally friendly. Tebuconazole has been frequently reported as effective fungicide against this aggressive pathogen worldwide, including in Ethiopia, whereas Apron Star 42 WS is reported here in Ethiopia for the second time, while it is not common elsewhere. Since the compatibility of *Trichoderma spp* with these two fungicides is now proved in this study, the method can be tested for control of other diseases.

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