Biological Control of Botrytis allii by Trichoderma viride on Onion Allium cepa

M.A.M. Hussein, M.H.A. Hassan and K.A.M. Abo-Elyoussr

Department of Plant Pathology,
Faculty of Agriculture, Assiut University, Assiut, 71526- Egypt

Abstract: Several pathogens attack Egyptian onion crop causes many losses in yield, one of those important causal pathogens is Botrytis allii the causal pathogen of umbel “Head” blight disease. B.allii was isolated from infected onion plants grown in an open field in Assiut Governorate, Egypt. All the tested isolates infected onion plants with different degrees of disease severity. The antagonistic efficiency of Trichoderma spp evaluated in in vitro study. All Trichoderma spp. suppressed the growth of B.allii with different degree. Results of antagonistic capability showed that Trichoderma viride caused the highest reduction of the growth compared to other species, 86%, 84% and 85% for T. viride and T. harzianum respectively. Under greenhouse conditions the confrontation of T. viride affected on the causal pathogen growth with different rates decreased the disease severity around 45% in 2010 season and around 30% during 2011 season. Results can be suggested that use of T. viride as biocontrol agents under greenhouse condition but these results need to confirm by field study.

Key words: Biological control • Botrytis allii • Trichoderma viride • Onion Umbel blight disease

INTRODUCTION

Onion (Allium cepa, L.) is one of the most important commercial vegetable crops in the world, it reached 63,000 (Ha) harvested area in 2011 with onions and it produced 36159 (Kg/Ha) in 2011, gave total yield of 2,304207 tones (Ha) harvested area in 2011 with onions and it produced 36159 (Kg/Ha) in 2011, gave total yield of 2,304207 tones of Onions[1]. Several fungal disease attack Allium cepa such as Stemphylium vesicarium, Alternaria porri and Sclerotium cepivorum [2-4]. The umbel blight disease is an important disease that attack the plant in the most important stage of producing the seeds, this disease can devastating unprotected crops up to 70% [5]. In Egypt, onion is one of the main crops grown for seed production [6]. The wide usage of the fungicides increased the resistance of the pathogens, additionally is not safe over public concern over food and environmental safety, therefore we need alternative methods which potentially safe to human health and the environment for control the diseases [7]. The biological control is the alternative method of the fungicides, that achieved remarkable success to control the plant pathogens by their rule and with their impact as antagonistic agents [8].

Umbel blight disease is an old disease in Egypt studied because of the high lose in the yield of onion seeds [9]. It is considered a destructive disease affecting the crop causing serious damage to the plant and consequent decrease of the seed yield production.. A number of fungi are known to be very effective against seed borne diseases. Many researchers have shown that foliar diseases can also be managed effectively through microorganisms [10]. Among the different fungi, Trichoderma spp. has been reported to have greatest impact on the pathogens [11]. Trichoderma sp plays an important role in biological control and it represents 60% of other biofungicides and was used as a pesticide and herbicide, use of this fungus contributes to the improvement of plant growth promotion [12].

The objective of the study is to isolate the pathogen and Trichoderma spp. and the antagonistic effect of Trichoderma sp against Botrytis allii, in vitro and in vivo.

MATERIALS AND METHODS

Isolation and Identification of the Causal Pathogen: Onion plants showed umbel blight symptoms collected from different places in Assiut governorate, Egypt, the collected plants were cleaned and washed by sterilized
water then surface sterilized with 2% sodium hypochlorite solution for two minutes, then rinsed several times in sterilized water and dried. The surface sterilized samples were placed on to Potato Dextrose Agar (PDA) medium and incubated at 27°C. After 4-5 days incubation period, the developed fungal colonies were purified by hyphal tip and single spore isolation technique. Identification of the fungal isolates was carried out by using the morphological characteristics of mycelia and spores as described by [13-15].

**Isolation of Fungal Antagonists:** Five isolates of *Trichoderma harzianum*, two isolates of *Trichoderma viride* were isolate from the onionphilosopher and rhizosphere of the onion plants in Egypt - Assiut.

**Pathogenicity Tests:** Onion (cv. Giza 6 and Giza 20) plants were grown in a greenhouse maintained at 20°C during the day and 18°C at night. The onion plants were transplanted into 30 cm-diameter pots containing a mixture of sandy loam soil.

*Botrytis allii* isolates used under greenhouse conditions in 2009 season. The inocula prepared by adding 10 ml of sterilized water and scratch the growth of the fungi in the Petri dishes of PDA after 15 days of incubation on 25°C then complete to 100 of suspension which adjusted to 10⁷ cfu/ml, the suspension of 10 ml sprayed on each umbel of onion plants (120-day, cv. Giza 6 and Giza 20) followed by coverage with polyethylene for 48 hours to maintain suitable humidity.

For pathogenicity tests and in vivo study at seasons 2010 and 2011 disease severity first not was after eight days and confirmed after fourteen days from the infection with the causal pathogen. Three replicates were used. The disease severity determined using:

Disease severity percentage on umbels “flower Heads” of onion recorded as average percentage of infected umbels and readings were converted to disease index using the following procedure. The number of plants of each symptoms category was multiplied by the corresponding numerical grade and the resulting figures were collected the summations were converted to disease index values by dividing each of them by the maximum numerical for the given number of plant and multiplying the resulting reading by 100. This could be simplified by the following equation: Disease severity % = \( \frac{\sum (n \times V)}{5 \times N} \) x 100, Where: n = Number of seed-stalk within each infection category = Numerical values of infection categories = Total number of Umbels examined, S = Constant, highest numerical value, Scales of 0 - 4 was used, 0 = No infection, 1 = 25% seed-stalk area infected, 2 = 50% seed-stalk area infected, 3 = 75% seed-stalk area infected, 4 = 100% seed-stalk area infected.

**In vitro Antagonistic Effect of Fungal Antagonists on the Pathogenic Fungus:** Antagonistic effect of *T. harzianum* and *T. viride* which were cultured on PDA medium for 7 days at 28-30°C. Then used on the linear growth of *B. allii* was investigated in Petri dishes containing PDA medium. Each plate was divided into equal halves, one half was inoculated with a disc (5-mm-diameter) of the antagonistic fungus taken from 7-day-old cultures, the opposite half was inoculated with a disc taken from 10-day-old culture of the pathogenic fungus. Three plates were used for each treatment. Plates were then incubated at 25°C for 7 days. Percentage of reduction in linear growth of the tested fungi was determined using the following formula: \( R = \frac{(C - T/C) \times 100}{100} \) whereas: R= Percentage of growth reduction, C= Diameter of the control hyphal growth and T=Diameter of the treated hyphal growth [16].

**Evaluation of Antagonistic Microorganisms In vivo Preparation of Antagonists Inocula:** Inoculum of *T. viride* was prepared in the form of a conidial suspension by hemocytometer slide (10⁹ spores/ml) [17] it was grown on PDA then the highly antagonistic isolates of *T. viride* were used to study their effect against *B. allii* on onion plants under greenhouse conditions. The fungal isolates were used at concentration of 5x10⁴cfu/ml prepared from 15-day cultures grown on PDA. The bioagent was sprayed, on onion plants (120 Day) by using a hand atomizer. Bioagents were applied before inoculation and after two days of inoculation with the pathogen [18, 19].

The disease severity was recorded after 8 days of infection with the causal pathogen as mentioned in pathogenicity test.

Obtained data were statistically analyzed using complete randomized block designs suggested by [20] and treatments means were compared using L.S.D. test at 5%.

**RESULTS AND DISCUSSION**

**Isolation and Identification of the Causal Pathogen:** Three isolates isolated from naturally infected umbels of onion plants showing blight symptoms were identified as *B. allii* based on the morphological characteristics [13, 14]. If the humidity was very high it can be seen the grey mycelium
of the causal pathogen \textit{B. allii}. Umbel suddenly turn dry debris can be broke even the stalk still fresh and alive. During this experiment the causal pathogen \textit{B. allii} isolated and studied the pathogenicity and disease severity on the host plant \textit{A. cepa} and this isolates was confirmed before as a causal pathogen [21].

**Pathogenicity Test:** Data showed in Table (1) refer to that the isolates of \textit{B. allii} caused the umbel “head” blight symptoms by different ratios. The tested \textit{B. allii} isolates showed the highest disease severity by first isolate by 71.7\%, second isolate was moderate by 65\% and the lowest isolate was 58.9\%, while the used isolate during the experiments was the highest isolate disease severity on the onion plants. The causal pathogen \textit{B. allii} isolated and studied the pathogenicity and disease severity on the host plant \textit{A. cepa} and this isolates was mentioned before as a causal pathogen [21].

**Effect of Trichoderma spp. On Growth of Botrytis allii, in vitro:** The inhibition percentage of \textit{B. allii} caused by different \textit{Trichoderma sp.} ranged from 55-85\%, the inhibition ratio of mycelia growth was varied among the tested isolates it was 86\% and 84\% for \textit{T. viride} 1, 2; and 85\% for \textit{Trichoderma harzianum}, against \textit{B. allii} as shown in Table 2. According to these results we chose isolate No 1 from the pathogen and isolate No 1 from \textit{Thrichoderma viride} for the following experiments.

Results of the study showed that \textit{T. viride} has highest inhibition against the causal pathogen (Table 2) which match the results reported by [22, 23], studying \textit{in vitro} antagonism between isolates of antagonistic fungi \textit{T. harzianum} and \textit{T. viride} against the used pathogenic isolate of \textit{Botrytis allii} on potato dextrose agar medium \textit{T. viride} caused inhibition of causal pathogen by different rates 86, 85, 84\% this result refer to the inhibition by the different antagonistic isolates that affected on the growth of mycelia and the spores formations of the causal pathogen. The results are in agreements with the results obtained by [24] who found the inhibition rate of \textit{Fusarium} basal rot infection of onion by \textit{T. harzianum} was 73.3 \%.

**Effect of Trichoderma spp. on the Disease Severity under Greenhouse Conditions 2010 Season:** Data in Table (4) showed that the disease severity \% of the causal pathogen \textit{B. allii} infection followed by the \textit{Trichoderma viride} application reduced to 36.1\% and 55.6\% for Giza 6 and Giza 20 varieties, respectively during 2010 season that mean a reduction in the disease by 63\% and 44\%. In Giza 6 and Giza 20 respectively. While the disease severity percentage of \textit{T. viride} application followed by the causal pathogen \textit{B. allii} infection reduced to 53.3 \% and 52.8\% for Giza 6 and Giza 20 varieties, respectively during 2010 season, by reduction in disease with 46\% and 47\% in both varieties. This result refer to that Giza 6 variety was the most durable and acceptable of the \textit{T. viride} application.

**Effect of Trichoderma spp. on the Disease Severity under Greenhouse Conditions 2011 season:** In Table (4) Disease severity \% of the causal pathogen \textit{Botrytis allii} infection followed by the \textit{Trichoderma viride} application reduced to 58.3\% and 50.0\% for Giza 6 variety and Giza 20 varieties respectively during 2011 with percentage of reduction in the disease by 51\% and 50\% in Giza 6 and Giza 20 respectively. While disease severity \% of \textit{Trichoderma viride} application followed by the causal pathogen \textit{Botrytis allii} infection reduced to 61.1 and 66.7 for Giza 6 variety and Giza 20 varieties respectively during 2011 season with percentage of reduction in the disease by 38\% and 23\%. In Giza 6 and Giza 20 respectively. This result refer to that Giza 6 variety was the most durable and acceptable of the \textit{T. viride} application.

\textit{T. viride} decreased the disease severity of the umbel blight disease on the host plant \textit{A. cepa} when \textit{T. viride} applied before the infection with the causal pathogen \textit{Botrytis allii} during in both seasons of experiment 2010 for Giza 6 variety and 2011 for variety Giza 20 by 36.1\% and 50.0\% respectively, \textit{T. harzianum} and \textit{T. atroviride} decreased the symptoms on rose flower caused by \textit{Botrytis spp.} \textit{Trichoderma spp.} can gave plants a glucose oxidase and growth stimulating compounds molecules increase vigor as resistance consequence against the pathogen beside the antibiotics such as gliotoxin, viridian wich involved in disease suppression [25, 26]. The treatment of seeds with suspension of \textit{Trichoderma spp.} eliminated the disease incidence significantly [12]. On rose flowers which treated before harvesting [27, 28]. \textit{T. viride} decreased the disease severity of the umbel blight disease on the host plant \textit{Allium cepa} when \textit{T. viride} applied before the synthetic infection with the causal pathogen \textit{B. allii} during in both seasons of experiment 2010 for Giza 6 variety and 2011 for variety Giza 20 by 36.1\% and 50.0\% respectively. This result can be related with previous results of studies said that treatment of seeds with \textit{Trichoderma spp.} reduce the disease severity because of the systematic resistance that gave by \textit{Trichoderma spp.} application against the foliar diseases [29] \textit{Trichoderma spp.} can gave plants a glucose oxidase and growth stimulating compounds molecules.
Table 1: Pathogenicity test of three isolates of *Botrytis allii* on Giza 6 variety during 2009 season:

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source of isolate</th>
<th>Disease severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Botrytis allii</em> 1</td>
<td>El-Fath-Assiut</td>
<td>71.7</td>
</tr>
<tr>
<td><em>Botrytis allii</em> 2</td>
<td>El-Zawia-Assiut</td>
<td>65</td>
</tr>
<tr>
<td><em>Botrytis allii</em> 3</td>
<td>Reefa-Assiut</td>
<td>58.9</td>
</tr>
</tbody>
</table>

L.S.D. at 0.05 = 7.1

Table 2: Reduction percentage of mycelia growth of *Botrytis allii* by different *Trichoderma* spp.

<table>
<thead>
<tr>
<th>No.</th>
<th>Isolates names</th>
<th>Mycelial growth inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Trichoderma harzianum</em> 1</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>T. harzianum 2</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>T. harzianum 3</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>T. harzianum 4</td>
<td>56</td>
</tr>
<tr>
<td>5</td>
<td>T. harzianum 5</td>
<td>55</td>
</tr>
<tr>
<td>6</td>
<td><em>Trichoderma viride</em> 1</td>
<td>86</td>
</tr>
<tr>
<td>7</td>
<td><em>Trichoderma viride</em> 2</td>
<td>84</td>
</tr>
</tbody>
</table>

L.S.D. at 0.05 = 5.3

Table 3: *T. viride* effects on disease severity of *B. allii* under greenhouse conditions 2010 season:

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Giza 6</th>
<th>Giza 20</th>
<th>Total Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection with <em>B. allii</em> Before Application</td>
<td>36.1</td>
<td>55.6</td>
<td>45.8</td>
</tr>
<tr>
<td>Infection with <em>B. allii</em> after Application</td>
<td>53.3</td>
<td>52.8</td>
<td>53.1</td>
</tr>
<tr>
<td><em>B. allii</em> Control</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

L.S.D. at 0.05 A(Varaities) = .6.5
L.S.D. at 0.05 B(Treatments) = 4.2

Table 4: *T. viride* effects on disease severity of *B. allii* under greenhouse conditions 2011 season:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Giza 6</th>
<th>Giza 20</th>
<th>Total Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection with <em>B. allii</em> Before Application</td>
<td>58.3</td>
<td>50.0</td>
<td>54.2</td>
</tr>
<tr>
<td>Infection with <em>B. allii</em> after Application</td>
<td>61.1</td>
<td>66.7</td>
<td>63.9</td>
</tr>
<tr>
<td><em>B. allii</em> Control</td>
<td>77.8</td>
<td>88.9</td>
<td>83.3</td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

L.S.D. at 0.05 A(Varaities) = 8.1
L.S.D. at 0.05 B(Treatments) = 7.2

increase vigor as resistance consequence against the pathogen beside the antibiotics such as gliotoxin, viridian wich involved in disease suppression [25, 26]. The treatment of seds with suspension of *Trichoderma spp.* eliminated the disease incidence significantly [12]. On rose flowers which treated before harvesting [27, 28]. The local isolates of *Trichoderma viride* a reduction in the infection percentage which promised with effective application can used for low cost and safely.

REFERENCES

1. FAOSTAT F. Statistics Division 2012.


