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Research Article
Control of Stemphylium Leaf Blight Disease of Onion and Elevation of Seed Production Using Certain Bioagents

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Abstract

Objective: This present study aimed to study the effect of some bioagents against *Stemphylium vesicarium in vitro* and under greenhouse conditions. **Materials and Methods:** The isolate of *Stemphylium vesicarium* used in this study was recovered from a diseased onion in Assiut Governorate (Egypt) and the experiments of biocontrol of the disease were carried out under greenhouse condition at Assiut University. Data were analyzed using one-way ANOVA with the SPSS 10.0 software program. Means were compared by the Duncan's multiple tests and statistical significance was determined at 5% level. **Results:** After preliminary screening on antagonistic activity of 20 fungal isolates recovered from onion plants, two isolates to control the disease under greenhouse conditions were selected namely, *Trichoderma harzianum* isolate 3013 and *Stachybotrys chartarum* isolate 2031. Application of *T. harzianum* isolate 3013 and *Stachybotrys chartarum* isolate 2031 showed, when applied before pathogen infection, a reduction in disease by 67.9 and 71.3%, respectively. As well as after pathogen infection they caused a reduction in the disease by 72.8 and 73.2%. In comparison, the applied fungicide Ridomil gold plus reduced the disease by 84.4 and 95.7% before and after pathogen inoculation, respectively. Spore suspension of *Trichoderma harzianum* isolate 3013 and *Stachybotrys chartarum* isolate 2031 and Ridomil gold plus improved number of seeded fruits, number of seeds per inflorescence and weight of seeds per inflorescence compared to infected control. **Conclusion:** It could be control of SLB under greenhouse condition with this bioagents. The use of *T. harzianum* isolate 3013 and *Stachybotrys chartarum* isolate 2031 could be used to control onion Stemphylium blight disease and elevate the seed production.

Key words: Bioagents, *Stemphylium vesicarium*, onion seed production, Ridomil gold plus, SLB

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In Egypt (Assiut governorate) the first report of Stemphylium leaf blight (SLB) of onion caused by *Stemphylium vesicarium* (Wall.) Simmons was reported by Hassan *et al.*[1]. The disease was reported by Shishkoff and Lorbeer[2] in the main production areas of the world. It is more severe on seed crop compared to bulb crop[3] causing sometimes 100% loose of the seed production[4,5]. Although chemical control of SLB had been practiced, its success depends largely on high number of spraying[6]. Today there are strict regulations on chemical fungicide use, due to residual toxicity problems and environmental pollution[7]. As well as, using of synthetic fungicides can cause development of fungicide-resistant strains of the pathogen. Therefore, there are a large number of studies have been devoted to searching for alternative control strategies to plant pathogens[8-11]. *Trichoderma* spp. are considered most popular and environmentally friendly bio-control agents against numerous phytopathogenic fungi[12], they successfully suppress leaf fungal pathogens[13] and many others soil-borne pathogens[14]. According our information there no study has been done on use of *Stachybotrys chartarum* against SLB under greenhouse condition.

In Egypt, Stemphylium blight is one of the most important disease attacked onion plant especially in seed production. It cause considerable losses in seed crops of onion and sometimes causes 100% loss. For this problem of Stemphylium blight the present study is undertaken to evaluate new strains of local bioagents for the management of Stemphylium blight of onion.

MATERIALS AND METHODS

Source of *Stemphylium vesicarium* and inoculum preparation: The isolate of *Stemphylium vesicarium* used in this study was recovered from a diseased onion in Assiut governorate (Egypt), identified and deposited in Assiut University Mycological center under accession number AUMC10512. The pathogenicity of this isolate was previously tested showing 37.25% disease severity[15]. The inoculums were prepared from stock cultures on PDA media stored at 4°C. A piece of culture medium containing mycelium (approximately 2 mm) of fungi was placed centrally on 9 cm diameter petri dish with PDA medium and incubated at 25±1°C for 15 days under a 12 h photoperiod using a near-ultraviolet light suspended approximately 20 cm above the cultures. Ten milliliters of sterile distilled water was then added to each plate and colonies were scraped with a sterile needle. The resulting conidial suspension (5×10^4 conidia mL^{-1}) inoculated onto leaves and seed-stalks of onion plants (110 days old cv., 'Giza 6') using an atomizer.

In vitro, preliminary determination of antagonistic capability of non-pathogenic fungal against *Stemphylium vesicarium*: Twenty fungal isolates were recovered from onion plants and identified by Abdel-Hafez *et al.*[16] and antagonistic capability of it were carried out using Dual culture method described by Li *et al.*[17].

Petri dishes (9 cm diameter) containing 15 mL PDA medium were seeded with inoculum of the SLB (approximately 5 mm) pathogen on one plate edge, then after 48 h. Incubation at 25±1°C the tested fungal isolates were seeded on opposite position edge. Plates inoculated with *Stemphylium vesicarium* only as well as tested fungi only were used as a control. The observations were recorded when the growth of the pathogen completely covered the plate surface in the control treatment. Three replicates of both control and treated plates were used. An arbitrary scale index was used to show the mechanism of antagonism plus inhibition zone diameter. N: No antagonism (Inhibition zone 0 mm), L: Low antagonism (Inhibition zone less than 3 mm), M: Moderate antagonism (Inhibition zone 3-7 mm), H: High antagonism (Inhibition zone>7 mm) and OG: Over growth (Mycoparastism).

Chitinase activity: Conidia from 4 days old Potato Dextrose Agar (PDA) cultures of the fungal isolate that showed overgrowth on *Stemphylium vesicarium* AUMC10512 were grown on 250 mL Erlenmeyer flasks containing 50 mL liquid culture medium. These medium contains the following components (g L^{-1}), 1.4 $(NH_4)_2SO_4$, 2.0 KH_2PO_4, 6.9 NaH_2PO_4, 0.3 $MgSO_4.7H_2O$, 1.0 colloidal chitin and 10 peptone[18].

Colloidal (acid swollen) chitin was prepared as follows: One gram of chitin (crab shell) was added to 10 mL of 85% orthophosphoric acid. The mixture was stirred to make a gelatinous paste and stored at 0°C for 24 h. The gelatinous mixture was re-precipitated into an excess of cold (15°C) distilled water. It was made to a paste using a pestle and mortar and then re-suspended in Na-acetate buffer (50 mM, pH 4.75). The suspension was stored in a sterile container at 10°C.

Chitinase activity was assayed as follows: a tube containing reaction mixture [0.55 mL of acid swollen chitin (5 gL^{-1}, suspended in 50 mM Na-acetate buffer, pH 4.75), 0.30 mL of acetate buffer (50 mM, pH 4.75) and 0.15 mL of culture filtrate] was incubated at 47°C for 1 h in un-stirred condition. The reaction was terminated by adding 1 mL of

potassium sodium-tartrate reagent to the reaction mixture[19]. A control was taken where the enzyme was deactivated by adding 1 mL of potassium sodium-tartrate reagent before the commencement of incubation. The alkaline tartrate reagent was prepared by mixing 270 g potassium sodium-tartrate dissolved in 400 mL distilled water and a solution of 6.25 g of phenol in 10% sodium hydroxide solution (17.5 g NaOH in 175 mL distilled water). The reagent was stored in a dark bottle. One milliliter of dinitrosalicylic acid (DNS) reagent was added to the tubes and the tubes were kept in a boiling water bath for 5 min. The DNS reagent was prepared by dissolving 1.5 g of 3, 5-dinitrosalicylic acid in 100 mL of distilled water. The reaction mixture was cooled to 30°C. Seven milliliters of distilled water were added to each tube to make up the final volume to 10 mL. The mixture was centrifuged at 5000 rpm for 5 min and the intensity of colour (formed due to the reaction of N-acetyl-D-glucosamine released with dinitrosalicylic acid) was measured at 540 nm by a spectrophotometer[20].

One unit of enzyme activity was defined as the amount of enzyme required for the formation of 1 µg of the product per minute of the reaction, under the standard assay conditions.

Biological control of Stemphylium blight *in vivo*: Two antagonistic fungal isolates *Trichoderma harzianum* isolate 3013 (6×10^8 conidia mL^{-1}) and *Stachybotrys chartarum* isolate 2031 were selected to application for controlling onion SLB disease under greenhouse condition. All treatments were applied by two methods, the first was applied bioagents before 48 h of pathogen's inoculation, while the second was applied after 48 h of pathogen's inoculation. Disease assessment of each treatment was recorded 15 days after inoculation as a percentage disease severity previously described by Hussein *et al.*[21]. The foliar fungicide Ridomil gold plus (2 g L^{-1}) was used as a positive control for the comparison purpose with the biocontrol agents in controlling the disease incidence. Each pot was sprayed with 100 mL of each treatment (conidia of bioagents or fungicide) and three replicates were used to each treatment. Negative control were used and treated with sterile water. The chemical composition of Ridomil gold plus (w/w): Mefenoxam 2.5%, dicopper chloride trihydroxide 69%, adhesive components 6% and carrier 22.5%. Mefenoxam is the common name of methyl N-(methoxy acetyl)-N-(2.6-xylyl)-D-lalaninate; methyl (R)-2-[(2, 6-dimethylphenyl) methoxyacetyl] amino-propionate.

Effect of various treatments on seed production: At the end of experiments, some parameters were determined such as, number of flowers per inflorescence, number of seeded fruits per inflorescence, number of seed per inflorescence and weight of seeds (g) per inflorescence after completion of flowering/harvest season as described by Asaduzzaman *et al.*[22].

Statistical analysis: The data were analysed using an one-way ANOVA with the SPSS 10.0 software program. Mean and standard errors were calculated for three replicate values. Means were compared by the Duncan's multiple tests and statistical significance was determined at 5% level.

RESULTS

Antagonistic effect of some recovered fungal isolates against growth of *Stemphylium vesicarium in vitro*: Data in Table 1 indicated that, seven isolates exhibited antibiosis effect to suppress mycelial growth of pathogen producing an inhibition zones. These isolates included three categories according to diameter of inhibition zone: Low inhibition zone (less than 3 mm), it was shown by *Chaetomium specifer* (No. 2027), moderate inhibition zone (3-7 mm), it was represented by five fungal isolates namely, *Chaetomium globosum* (No. 3003, 3038 and 3045) and *Stachybotrys chartarum* (No. 2032 and 2033) and high inhibition zone (more than 7 mm), it was produced by *S. chartarum* (No. 2031). According to the ability of tested isolates to compete on n utrient and space against *S. vesicarium*, 9 isolates of *Trichoderma* showed more than 50% of inhibition growth of the pathogen. Four isolates of *T. harzianum* exhibited the highest rate of pathogen inhibition contributing 73.1, while five isolates of *T. longibrachiatum* showed inhibition rate equaling 70.3%. Isolate of *Stachybotrys chartarum* isolate 2031 showed inhibition of mycelial growth rate contributing 52.2%. The other remained isolates exhibited inhibition against the pathogen growth ranging between 8.6-43.7% (Table 1).

Chitinase activity: Chitinase activity was tested for antagonistic fungal isolates that showed overgrowth on *S. vesicarium* AUMC10512. These isolates (12 isolates) showed different significant degrees of chitinase activity (Table 2). Isolates of *T. harzianum* (4 isolates) exhibited the highest values of chitinase activity ranging 1.98-2.7 U min^{-1}. Of these isolates, *T. harzianum* isolate 3013 showed the maximum chitinase activity contributing 2.7 U min^{-1}. The remaining isolates were exhibited chitinase activity as the following: *T. longibrachiatum* (5 isolates) showed chitinase activity contributing 1.42-1.73 U min^{-1} and *Gliocladium roseum* (3 isolates) comprising 1.06-1.37 U min^{-1}, showed no chitinase activity.

Table 1: *In vitro*, antagonistic effect of some recovered fungal isolates against growth of *Stemphylium vesicarium*

Species	Isolate No.	AMGI (%)	Inhibition Zone (mm)	
			Values	Mark
Chaetomium				
C. golobosum	3003	43.71	5	M
C. golobosum	3038	43.71	6	M
C. golobosum	3045	43.71	6	M
Cochliobolus				
C. lunatus	2030	8.57	0	N
C. specifer	2027	8.57	2	L
Gliocladium				
G. roseum	3020	38.26	0	OG
G. roseum	3033	38.26	0	OG
G. roseum	3047	38.26	0	OG
Stachybotrys				
S. chartarum	2031	52.18	13	H
S. chartarum	2032	48.57	6	M
S. chartarum	2033	48.57	7	M
Trichoderma				
T. harzianum	3013	73.12	0	OG
T. harzianum	3019	73.12	0	OG
T. harzianum	3032	73.12	0	OG
T. harzianum	3058	73.12	0	OG
T. longibrachiatum	3021	70.30	0	OG
T. longibrachiatum	3055	70.30	0	OG
T. longibrachiatum	3056	70.30	0	OG
T. longibrachiatum	3057	70.30	0	OG
T. longibrachiatum	3058	70.30	0	OG

AMGI: Percentage of average of mycelial growth inhibition, Arbitrary antagonistic scale, N: No antagonistic effect, OG: Over growth on pathogen mycelia, L: Low inhibition zone (less than 3mm), M: Moderate inhibition zone (3-7 mm) and H: High inhibition zone (more than 7 mm)

Table 2: Chitinase activity of antagonistic fungal isolates that showed overgrowth on *Stemphylium vesicarium* AUMC10512

Fungal species	Isolate No.	Chitinase activity (U min^{-1})
Gliocladium		
G. roseum	3020	1.06 ± 0.02^{h}
G. roseum	3033	1.37 ± 0.03^{f}
G. roseum	3047	1.19 ± 0.02^{g}
Trichoderma		
T. harzianum	3013	2.69 ± 0.18^{a}
T. harzianum	3019	2.41 ± 0.03^{b}
T. harzianum	3032	2.07 ± 0.03^{c}
T. harzianum	3058	1.98 ± 0.02^{c}
T. longibrachiatum	3021	$1.56\pm0.27^{d,e}$
T. longibrachiatum	3055	1.73 ± 0.08^{d}
T. longibrachiatum	3056	$1.58\pm0.16^{d,e}$
T. longibrachiatum	3057	1.42 ± 0.20^{e}
T. longibrachiatum	3058	$1.48\pm0.31^{d,e}$

Numbers within column of chitinase activity are means of three replicates. Different letters within column indicate significant differences ($p \leq 0.05$) based on one-way ANOVA analysis

Biological control of Stemphylium blight disease *in vivo*: Data in Table 3 showed that all treatments caused more than 50% disease reduction either before or after inoculation. While, Ridomil gold plus showed the highest percentage of disease reduction either before or after pathogen inoculation comprising 84.4 and 95.8%, respectively followed by *T. harzianum* isolate 3013 *Stachybotrys chartarum* isolate 2031 in both experiments. The best treatment after Ridomil gold plus is *T. harzianum* isolate 3013 since it reduced the disease severity in both experiments to 73% compared to infected control.

Onion seed production: Most of treatments used in this study to control Stemphylium blight disease spore suspension of *Trichoderma harzianum* isolate 3013 and *Stachybotrys chartarum* isolate 2031 and Ridomil gold plus improved number of seeded fruits, number of seeds per inflorescence and weight of seeds per inflorescence (Table 4) the seed production decreased to 0.5 g $inflorescence^{-1}$ in negative control (Fig. 1) compared with healthy floral stalks (2.8 g $inflorescence^{-1}$). Treatments of floral stalks by Ridomil gold plus either before or after the infection by *S. vesicarium* improved the seed production to 2.5 and 2.7 g of seed $inflorescence^{-1}$, respectively. Also application of spore suspension of *T. harzianum* isolate 3013 either before or after the infection by *S. vesicarium* improved the seed production to 2.2-2.4 g of seed $inflorescence^{-1}$. The lowest seed production (1.4 g seed $inflorescence^{-1}$) was recorded by using spore suspension of *Stachybotrys chartarum* isolate 2031 after the infection by *S. vesicarium* (Table 4).

Fig. 1(a-d): Onion Stemphylium blight and seed losses attributed to the infection by *Stemphylium vesicarium* AUMC10512, (a) Healthy onion floral stalks after completion of flowering/harvest season, (b) Infected onion floral stalks by *S. vesicarium* AUMC10512, (c) Healthy Inflorescence containing fruited flowers and (d) Inflorescence showing the huge losses of seed production

Table 3: Effect of application of certain treatments on disease severity of Stemphylium blight under greenhouse condition

Treatments	Treatment before infection	Treatment after infection
Control	43.6±0.3[a]	46.7±0.5[a]
Trichoderma harzianum No. 3013	12.5±0.0[b]	12.5±0.0[b]
Stachybotrys chartarum No. 2031	14.0±0.2[b]	16.7±0.0[b]
Ridomil gold plus (2 g L^{-1})	6.8±0.3[c]	2.0±0.1[c]

Number within columns are means of three replicates. Values within columns that associated with different letters indicate significant differences ($p \leq 0.05$) based on one-way ANOVA analysis

DISCUSSION

Many studies have been carried out to find alternative control strategies of plant pathogens instead of fungicide application as the fungicide has environmental pollution and can lead to development of fungicide resistant strains[23]. In the present study the antagonistic capability of 20 fungal isolates was tested *in vitro* against *S. vesicarium* AUMC10512 among them *T. harzianum* showed highest degree of competition as well as mycoparastism against

Table 4: Effect of application of some treatments on seed production by onion plants infected by *Stemphylium vesicariumi* AUMC10512 under greenhouse condition

	Treatment before infection				Treatment after infection			
Treatments	No. of flowers /inflorescence	No. of seeded fruits/ inflorescence	No. of seed/ inflorescence	Seed weight/ inflorescence	No. of flowers /inflorescence	No. of seeded fruits/ inflorescence	No. of seed/ inflorescence	Seed weight/ inflorescence
Control (healthy)	323.3±0.9	238.7±0.7	556.7±0.6	2.7±0.3	322.2±0.4	268.3±0.3	573.6±0.3	2.8±0.3
Control (diseased by *S. vesicarium*)	366.3±0.3	62.0±0.2	94.3±0.2	0.5±0.2	308.1±0.2	70.0±0.1	102.0±0.2	0.5±0.1
Trichoderma harzianum No. 3013	324.4±0.7	241.2±0.7	471.3±0.6	2.3±0.6	342.1±0.6	243.1±0.6	488.3±0.8	2.4±0.6
Stachybotrys chartarum No. 2031	309.3± 0.6	208..3±0.7	328.3±0.6	1.6±0.3	312.2±0.8	194.6±0.6	292.1±0.6	1.4±0.7
Ridomil gold plus (2 g L^{-1})	322.7±0.8	240.4±0.7	526.2±0.6	2.5±0.5	328.3±0.6	248.3±0.6	566.9±0.6	2.7±0.6

the pathogen. Also, *Stachybotrys chartarum* isolate 2031, as a new bioagents used for foliar diseases, exhibited good antibiosis effect producing highest inhibition zone against *S. vesicarium* AUMC10512. These results were in agreement with laboratory experiment carried out by Rossi and Pattori[24], whereas they reported that production of *S. vesicarium* conidia was significantly reduced by *T. harzianum.* In recent years, *Trichoderma harzianum* (at 1%) exhibited a strong reducing effect on the disease development of Stemphylium blight of onion[13]. The results of this investigation indicated that the mechanism of antagonistic capability of *T. harzianum* depends on competition on nutrient and space (causing inhibition rate up to 73.1%) and mycoparastism (overgrowth and chitinase activity up to 2.7 U min^{-1}). The general mechanism of biological control by *Trichoderma* spp. can be divided into direct and indirect effects on the plant pathogen. Direct effects include competition for nutrients or space, production of antibiotic and lytic enzymes, inactivation of the pathogen's enzymes and parasitism. Indirect effects include all aspects that produce morphological and biochemical changes in the host plant producing induced resistance[25,26].

The results of management of Stemphylium blight disease under greenhouse condition showed that Ridomil gold plus showed the highest disease reduction percentage either before or after inoculation up to 84.4 and 95.8%, respectively and *T. harzianum* isolate 3013 caused disease reductions reached to more than 71.3 and 73.2% before and after pathogen inoculation, respectively. The same results were reported by Prakasam and Sharma[27], mentioned that among 17 fungal isolates and 6 bacterial isolates, *T. harzianum* (Th-3) expressed high level of disease reduction under glasshouse and field conditions. Also, Hussein *et al.*[21] selected several bioagents to control *S. vesicarium* under greenhouse condition, out of them *T. harzianum* showed reduction in diseases severity up to 36.22%. Shahnaz *et al.*[28] studied the management of onion foliar diseased caused by *Alternaria porri* and *S. vesicarium* and other fungal pathogens *in vivo*. They reported that application of *T. harzianum* resulted in significantly reduction of disease intensity as compared to control. *Trichoderma* spp., as a biocontrol agent has gained more importance. These antagonistic organisms act on the pathogen by different mechanisms via competition lysis, antibiosis, siderophore production and hyperparasitism[29]. *Trichoderma harzianum* and its products induce systemic resistance against several plant pathogens[30,31]. Different elicitors, including xylanase, have been isolated from *Trichoderma* and they trigger the synthesis of various defense compounds, including phytoalexins[32] and defense related enzymes viz., phenylalanine ammonia lyase, peroxidase, polyphenol oxidase and β-1-3-glucanase[33].

CONCLUSION

The current study proved that the use of *T. harzianum* isolate 3013 and *Stachybotrys chartarum* isolate 2031 have fungicidal impact to control onion Stemphylium blight disease and elevate the seed production as alternatives to fungicides.

REFERENCES

1. Hassan, M.H.A., A.D.A. Allam, K.A.M. Abo-Elyousr and M.A.M. Hussein, 2007. First report of Stemphylium leaf blight of onion caused by *Stemphylium vesicarium* in Egypt. Plant Pathol., 56: 724-724.
2. Shishkoff, N. and J.W. Lorbeer, 1989. Etiology of Stemphylium leaf blight of onion. Phytopathology, 79: 301-304.
3. Gupta, R.B.L. and V.N. Pathak, 1988. Yield losses in onions due to purple blotch disease caused by *Alternaria porri*. Phytophylactica, 20: 21-23.
4. Daljeet, S., J.S. Dhiman, A.S. Sidhu and H. Singh, 1992. Current status of onions in India: Strategies for disease resistance breeding for sustained production. Onion Newslett. Trop., 4: 43-44.
5. Schwartz, H.F., 2004. Botrytis, downy mildew and purple blotch of onion. Fact Sheet No. 2.941, Colorado State University Cooperative Extension, USA. http://extension.colostate.edu/docs/pubs/crops/02941.pdf

6. Kucharek, T., 2004. Florida plant disease management guide: Okra. Plant Pathology Department Document PDMG-V3-41, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL.
7. Rial-Otero, R., M. Arias-Estevez, E. Lopez-Periageo, B. Cancho-Grande and J. Simal-Gandara, 2005. Variation in concentrations of the fungicides tebuconazole and dichlofluanid following successive applications to greenhouse-grown lettuces. J. Agric. Food Chem., 53: 4471-4475.
8. Iranbakhsh, A., M. Ebadi and M. Bayat, 2010. The inhibitory effects of plant methanolic extract of *Datura stramonium* L. and leaf explant callus against bacteria and fungi. Global Veterinaria, 4: 149-155.
9. Chakraborty, M.R., S. Ojha and N.C. Chatterjee, 2012. Application of biopesticides and fungicides for the control of fruit rot of custard-apple. Indian Phytopathol., 65: 249-252.
10. Madhavi, M., A. Kavitha and M. Vijayalakshmi, 2012. Studies on *Alternaria porri* (Ellis) Ciferri pathogenic to onion (*Allium cepa* L.). Arch. Applied Sci. Res., 4: 1-9.
11. Abdel-Hafez, S.I.I., K.A.M. Abo-Elyousr and I.R. Abdel-Rahim, 2013. Effect of certain plant extracts to control purple blotch disease of onion plants (*Allium cepa* L.). J. Plant Physiol. Pathol., Vol. 1. 10.4172/2329-955X.1000111
12. Abo-Elyousr, K.A.M., S.I.I. Abdel-Hafez and I.R. Abdel-Rahim, 2014. Isolation of *Trichoderma* and evaluation of their antagonistic potential against *Alternaria porri*. J. Phytopathol., 162: 567-574.
13. Nainwal, D. and K. Vishunavat, 2016. Management of purple blotch and Stemphylium blight of onion in Tarai and Bhabar regions of Uttarakhand, India. J. Applied Nat. Sci., 8: 150-153.
14. Ahmed, H.A.M. and N.G. Ahmed, 2015. Management of white rot of onion using composts and *Trichoderma harzianum*. Curr. Life Sci., 1: 63-69.
15. Abdel-Hafez, S.I.I., K.A.M. Abo-Elyousr and I.R. Abdel-Rahim, 2015. Leaf surface and endophytic fungi associated with onion leaves and their antagonistic activity against *Alternaria porri*. Czech Mycol., 67: 1-22.
16. Abdel-Hafez, S.I.I., K.A.M. Abo-Elyousr and I.R. Abdel-Rahim, 2014. Effectiveness of plant extracts to control purple blotch and Stemphylium blight diseases of onion (*Allium cepa* L.) in Assiut, Egypt. Arch. Phytopathol. Plant Protect., 47: 377-387.
17. Li, G.Q., H.C. Huang and S.N. Acharya, 2003. Antagonism and biocontrol potential of *Ulocladium atrum* on *Sclerotinia sclerotiorum*. Biol. Control, 28: 11-18.
18. Kumari, J.A. and T. Panda, 1992. Studies on critical analysis of factors influencing improved production of protoplasts from *Trichoderma reesei* mycelium. Enzyme Microb. Technol., 14: 241-248.
19. Monreal, J. and E.T. Reese, 1969. The chitinase of *Serratia marcescens*. Can. J. Microbiol., 15: 689-696.
20. Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31: 426-428.
21. Hussein, M.A.M., M.H.A. Hassan, A.D.A. Allam and K.A.M. Abo-Elyousr, 2007. Management of Stemphylium blight of onion by using biological agents and resistance inducers. Egypt. J. Phytopathol., 35: 49-60.
22. Asaduzzaman, M., M.M. Hasan, M.M. Hasan, M. Moniruzzaman and M.H.K. Howlander, 2012. Effect of bulb size and plant spacing on seed production of onion (*Allium cepa* L.). Bangladesh J. Agric. Res., 37: 405-414.
23. Soria, S., R. Alonso and L. Bettucci, 2012. Endophytic bacteria from *Pinus taeda* L. as biocontrol agents of *Fusarium circinatum* Nirenberg & O'Donnell. Chilean J. Agric. Res., 72: 281-284.
24. Rossi, V. and E. Pattori, 2009. Inoculum reduction of *Stemphylium vesicarium*, the causal agent of brown spot of pear, through application of *Trichoderma*-based products. Biol. Control, 49: 52-57.
25. Van Loon, L.C., P.A.H.M. Bakker and C.M.J. Pieterse, 1998. Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol., 36: 453-483.
26. Bouziane, Z., L. Dehimat, W.A. Aziz, M. Benabdelkader and N. Kacem Chaouche, 2011. The antagonism between *Trichoderma viride* and other pathogenic fungal strains in *Zea mays*. Agric. Biol. J. North Am., 2: 584-590.
27. Prakasam, V. and P. Sharma, 2012. *Trichoderma harzianum* (Th-3) a potential strain to manage the purple blotch of onion (*Allium cepa* L.) caused by *Alternaria porri* under North Indian plains. J. Agric. Sci., 4: 266-272.
28. Shahnaz, E., V.K. Razdan, S.E.H. Rizvi, T.R. Rather, S. Gupta and M. Andrabi, 2013. Integrated disease management of foliar blight disease of onion: A case study of application of confounded factorials. J. Agric. Sci., 5: 17-22.
29. Vidyasekaran, P., 1999. Biotechnological approaches to suppress seed to plant transmission of seed-borne pathogens. Proceedings of the National Symposium on Seed Science and Technology, August 5-7, 1999, Mysore, India.
30. Howell, C.R., L.E. Hanson, R.D. Stipanovic and L.S. Puckhaber, 2000. Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. Phytopathology, 90: 248-252.
31. Ricard, T. and H. Jorgensen, 2000. Binab's effective, economical and environment compatible *Trichoderma* products as possible Systemic Acquired Resistance (SAR) inducers in strawberries. 17th Danish Plant Protect. Conf. Hortic. Tjele Denmark DJF Rapport Havebrug, 12: 67-75.
32. Calderon, A.A., J.M. Zapata, R. Munoz, M.A. Pedreno and A.R. Barcelo, 1993. Resveratrol production as a part of the hypersensitive-like response of grapevine cells to an elicitor from *Trichoderma viride*. New Phytol., 124: 455-463.
33. Yedidia, I., N. Benhamou, Y. Kapulnik and I. Chet, 2000. Induction and accumulation of PR proteins activity during early stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T-203. Plant Physiol. Biochem., 38: 863-873.