Egypt. J. Phytopathol., Vol. 35, No. 1, pp. 49-60 (2007)

Management of Stemphylium Blight of Onion by using Biological Agents and Resistance Inducers

M.A.M. Hussein; M.H.A. Hassan; A.D.A. Allam and K.A.M. Abo-Elyousr

Plant Pathol. Dept., Fac. of Agric., Assiut Univ., Egypt.

Bacillus subtilis, ***Pseudomonas fluorescens***, ***Trichoderma harzianum***, ***Gliocladium*** sp. and ***Saccharomyces cerevisiae*** were evaluated for their efficacy in controlling Stemphylium blight on onion plants. *In vitro* study, the highest inhibition of *Stemphylium vesicarium* mycelial growth was achieved by *P. fluorescens*, *B. subtilis* and *T. harzianum*. In greenhouse experiment, application of Ridomil gold plus on onion plants resulted in significant reduction of disease severity percentage. The bioagents, *i.e. B. subtilis*, *Sacc. cerevisiae* and *P. fluorescens* exhibited the highest reduction in disease severity. On the other hand, *T. harzianum* gave the lowest reduction in the disease severity. All resistance inducers (*i.e.* Bion, K_2HPO_4 and salicylic acid) treatments resulted in significant reduction in disease severity.

Keywords: Biological control, induced resistance, onion and *Stemphylium vesicarium*.

Onion, (*Allium cepa* L.) is one of the main important and oldest vegetable crops grown in Egypt. The Egyptian onion is famous allover the world for its superior quality and early appearance in European markets. Onion although primarily is grown for food, it is also used as traditional medicine.

During the last years the international cultivated area with onion profoundly has been decreased because of the serious damage of the crop yield due to infection by several diseases that attack onion during the growing season (Schwartz and Mohan, 1996). Stemphylium blight disease, incited by different species of *Stemphylium* is one of the serious diseases that attacks onion leaves causing apical choler spots which become necrotic and covering the leaves. It can cause severe damage, especially to the onion seed crop and losses of about 80-85% on the crop by affecting leaves and seed stalk (Tomaz and Lima, 1988).

In 2004, onion plants (*Allium cepa*) cv. Giza 6 grown in several commercial fields in Upper Egypt (Assiut Governorate) exhibited symptoms of blight on the leaves and seed-stalks. Initial symptoms on leaves consisted of tip necrosis followed by small white and/or large purple spots. Such observed symptoms resembled Stemphylium blight symptoms on onion leaves, which are caused by *S. vesicarium* (Wallr.) Simmons. The disease is widespread in Asia and Europe and has been recorded previously on onion plants in South Africa (Verwoperd and Du Plessis, 1931) and in New York (Shishkoff and Lorbeer, 1989).

Many attempts were carried out for controlling Stemphylium blight on onion using cultural practices and chemical control (Aveling and Snyman, 1993).

Complete control of airborne pathogens is often difficult to achieve. In recent years, the goal of biological control as viable and reliable in modern agriculture has increased dramatically. The application of biological control using microorganisms proved to be successful for controlling various plant diseases in many countries (Sivan, 1987).

One of the potential methods of reducing the severity of plant pathogenic diseases is the induction of plant resistance. Also, pretreatment of susceptible plants with avirulent pathogens (biotic inducers) can enhance resistance to subsequent attack (Ryals *et al.*, 1996). In addition to biotic inducers, certain chemicals, such as salicylic acid (SA) and 2,6-dichloroisonicotinic acid (INA), potassium salts, amino butyric acid (BABA) and Bion were reported to induce systemic acquired resistance (SAR) in plants (Oostendorp *et al.*, 2001).

The present work was planned to throw light on the causal pathogen of onion Stemphylium blight in Assiut Governorate, as well as control of the disease using bioagents and resistance inducers.

Materials and Methods

Isolation and identification of the causal pathogen:

Naturally diseased onion plants, showing leaf blight symptoms were collected from different localities of Assiut Governorate in 2004 growing season. They were cut in small pieces, thoroughly washed with tap water, surface sterilized for two minutes with 2% sodium hypocholorite solution, then rinsed several times in sterilized distilled water and dried between folds of sterilized filter papers. The surface sterilized samples were plated onto 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 techniques. Identification of the fungal isolates was carried out by using the morphological characteristics of mycelia and spores as described by Ellis (1971).

Fungal and bacterial bioagents:

Five bacterial isolates, *i.e. Bacillus subtilis* (4 isolates) and *Pseudomonas fluorescens* (one isolate) and nine fungi, *i.e. Trichoderma harzianum* (5 isolates), *Gliocladium* sp. (one isolate) and *Saccharomyces cerevisiae* (3 isolates) were obtained from the stock cultures collection of the Department of Plant Pathology, Faculty of Agriculture, Assiut University. Such bacterial and fungal isolates were previously isolated from soil rhizosphere of onion cultivars areas of Assiut Governorate during seasons of 2003-2004.

Pathogenicity tests:

Pathogenicity tests of Stemphylium isolates were carried out under greenhouse conditions in 2004-2005 experiments in the greenhouse of Plant Pathol. Dept., Fac. of Agric., Assiut Univ. Inocula were prepared by growing each of the tested isolates on PDA medium at 27°C for 15 days. Then ten ml of sterile distilled water were

added to each plate and colonies were carefully scraped with a sterile needle. The resulting conidial suspension from each isolate was adjusted to 10^4 cfu/ml and used for inoculation of leaves and seed-stalks of 12 onion plants (110-day-old cv. Giza 6), using an atomizer. After inoculation, plants were covered with polyethylene bags for 48 hours to maintain high humidity. After this period, the bags were removed and plants were kept under normal conditions. Fifteen days after inoculation, disease severity was recorded. The experiment was repeated twice under greenhouse condition at 2004 and 2005. Autoclaved pots (20-cm-diameter) were filled with autoclaved clay soil and each pot was planted by two saddling. Three replicates were used. Each replicate consisted of four pots.

Biological control:

In vitro antagonistic effect of fungal bioagents on the pathogenic fungus:

Antagonistic effect of *T. harzianum* and *Gliocladium* sp. on the linear growth of *S. vesicarium* 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 7-day-old culture of the pathogenic fungus. Eight 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: $R = (C - T/C) \times 100$

Whereas: R= Percentage of growth reduction, C= Diameter of the control hyphal growth and T= Diameter of the treated hyphal growth

In vitro antagonistic effect of bacteria on the pathogenic fungus:

Agar disks carrying mycelium of *S. vesicarium*, each was placed at the centre of a PDA plate between two parallel streaks of the tested bacteria which were 5 cm apart. Plates inoculated with the fungi alone served as control. When the fungal growth of the control approached the edge of the plates. *In vitro* antagonistic effect was assessed by relating mycelial diameters on plates inoculated with bacteria to mycelial diameter on control plates and computing percentage inhibition. Eight plates were used for each treatment.

In vivo evaluation of antagonistic microorganisms:

The highly antagonistic isolates of *B. subtilis*, *Sacc. cerevisiae*, *P. fluorescens* and *T. harzianum* were used against *S. vesicarium* on onion plants.

Onion plants (110-day-old) were sprayed with each of the bacterial suspensions alone at the concentration of 10^8cfu/ml, the yeast, *Sacc. cerevisiae* isolates were tested at the concentration of $4x10^4$ cfu/ml. Inoculum was prepared by growing the bacteria and yeasts cultures in nutrient yeast extract broth, incubated at 25°C on an orbital shaker at 200 rpm for 24 h. Bacteria and yeasts were subsequently pelleted by centrifugation at 15000 rpm for 5 min and washed in 0.1% saline solution. The fungal isolates were used at concentration of $5x10^8$ cfu/ml prepared from 10-day-old cultures grown on PDA. The bioagents were sprayed, each alone, on onion plants by using a hand atomizer. Bioagents were applied at the same time of inoculation and/or three days before inoculation with the pathogen.

The foliar fungicide Ridomil gold plus (2g/liter) was used for comparison purpose with the biocontrol agents in controlling the disease incidence. The experiment was repeated twice under greenhouse condition in 2004 and 2005. Autoclaved pots (20-cm-diameter) were filled with autoclaved clay soil. Three replicates were used. Each replicate consisted of four pots and each pot consisted of 2 plants. Disease assessment was recorded at 15 days after inoculation.

In vitro tests of induced reststance:

The effect of different concentrations of three chemical compounds, *i.e.* Bion (62.5, 125 and 250 ppm), salicylic acid (1, 2 and 5 mM) and K_2HPO_4 (10, 25 and 50 mM) were added to PDA medium and then purred in Petri dishes (8-cm-diameter) and inoculated in the centre with disks (3-mm-diameter) from 7-day-old culture of isolates No. (1) of *Stemphylium vesicarium*. Plates were incubated at 27°C till the control plates were filled up the fungus.

The diameter of pathogen radial growth was measured and percentage of inhibition growth was calculated as mentioned previously.

In vivo tests of induced resistance:

The effect of different concentrations of three chemical compounds, on the induction of resistance against the pathogen was *in vivo* studied. They were sprayed, each alone, on 110-day-old onion plants, 2 days before inoculation with the pathogen. The experiment was repeated twice under greenhouse conditions, with 3 replicates. Each replicate consisted of four pots and each pot consisted of 2 plants. Disease assessment of each treatment was recorded after 15 days from inoculation date.

Disease assessment:

Disease severity on seed-stalks of onion was recorded as the average percentage of infected seed-stalk area and readings were converted to disease index using the following procedure.

The disease severity on each seed-stalk was scored on 0-4 scale as follows: 0= No visible infection, 1= >1-25% seed-stalk area infected, 2= 26-50% seed-stalk area infected, 3= 51-75% seed-stalk area infected and 4= 76-100% seed-stalk area infected. Obtained data were statistically analyzed using complete randomized block design suggested by Gomez and Gomez (1984) and treatments means were compared using L.S.D. test at 5%.

Results

Isolation and identification of the causal pathogen:

Fifteen fungal isolates were isolated from naturally diseased onion leaves and seed-stalks showing blight symptoms and identified as *Stemphylium vesicarium* (Wallr) Simmons, based on the morphological characteristics (Ellis, 1971). These identified isolates were confirmed by Assiut Univ. Mycol. Centre, Assiut, Egypt.

Disease symptoms:

The disease symptoms (Fig. 1) started as small pale yellow lesion on onion leaves then enlarge and becoming ovate-elongate and dark brown to black when sporulation occurs. Severe infection was observed on the seed-stalk weakening it and finally causing its collapse.

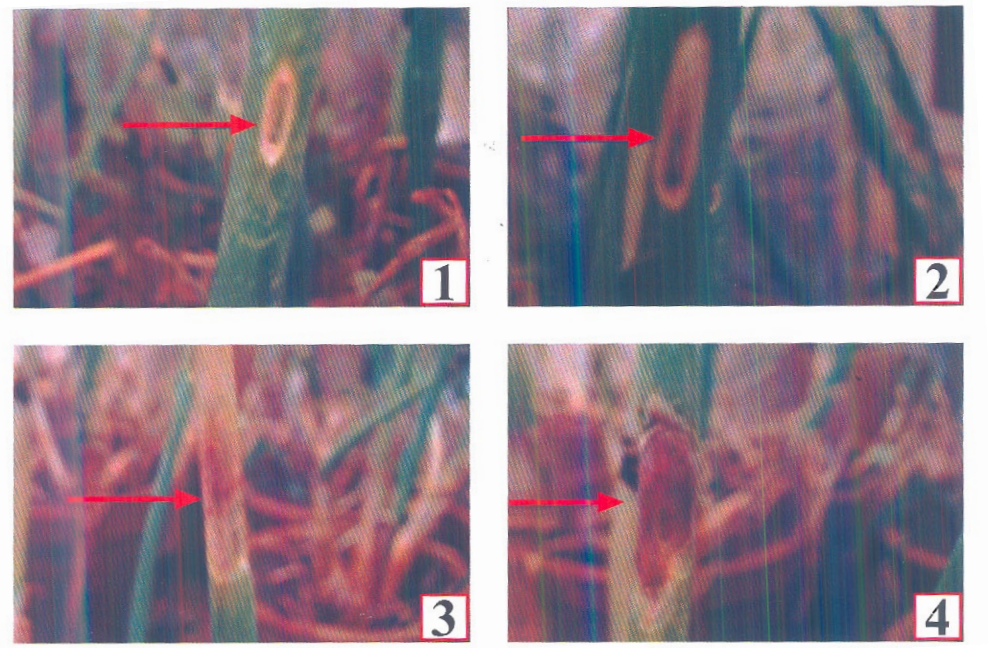

Fig. 1. Different stage of artificial disease development: 1- After 6 days from inoculation. 2- After 10 days from inoculation. 3- After 14 days from inoculation. 4- After 23 days from inoculation.

Pathogenicity tests:

Results illustrated in Fig. (2) indicate that all the tested isolates of S. vesicarium were able to infect onion plants causing typical Stemphylium blight symptoms with different degrees of disease severity. Data indicate that isolates Nos. 1, 4, 8, 11, 12, 14 and 15 were highly pathogenic and caused the highest disease severity. Isolate No. 5 exhibited the lowest disease severity on onion seed-stalk followed by isolates Nos. 6 and 7. Other tested isolates showed moderate infection.

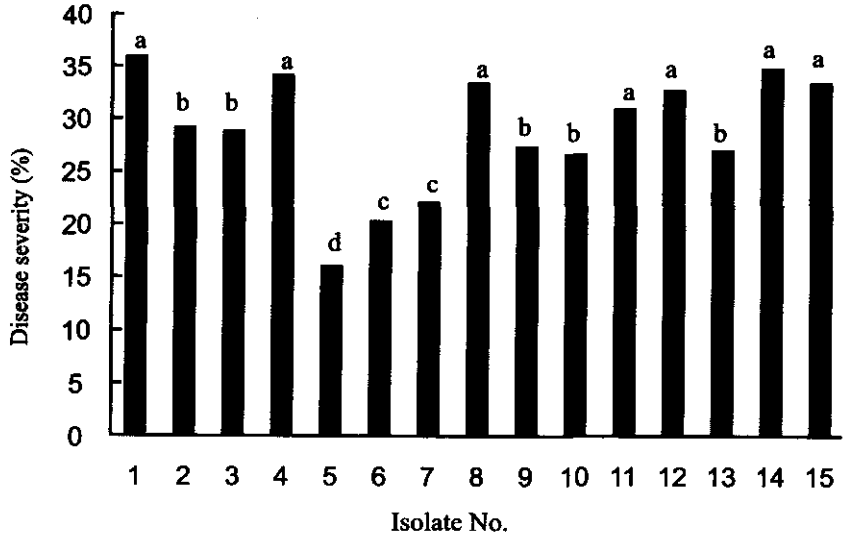

Fig. 2. Pathogenic variations among *Stemphylium vesicarium* isolates on the seed-stalks of Giza 6 onion cultivar. Different letters indicate significant differences among treatments according to the least significant difference test (P= 0.05).

In vitro evaluation of antagonistic isolates:

Data presented in Table (1) indicate that all tested microorganisms were able to inhibit the mycelial growth of the causal pathogen with variation in their antagonistic capability. The highest inhibition of mycelial growth was caused when Ridomil gold plus was used, being (99.6%) followed by *Pseudomonas fluorescens* (84.4%) followed by *Bacillus subtilis* No. 1 (79.6%), *T. harzianum* No. 1 (78.1%) and *B. Subtilis* No.2 without significant differences. Data also indicate that *Saccharomyces cerevisiae* No. 2 and No. 3 showed the lowest mycelial growth inhibition, being 22.44 and 20.9 % respectively without significant differences.

Effectiveness of certain microorganisms in controlling Stemphylium blight on onion, under greenhouse conditions:

Data presented in Table 2 show that all tested bioagents and Ridomil gold plus fungicide treatments significantly reduced Stemphylium blight of onion plants when any of the two tested methods of application of bioagents was used as compared with control plants. The highest significant reduction in disease severity was observed in case of *B. subtilis* (Bs 1) treatment. Application of *P. fluorescens* and *T. harzianum* (Th1) showed the lowest reduction in diseases severity in both experiments. In the absence of the bioagents, *S. vesicarium* was highly pathogenic to onion plants causing 41.66 and 42.54% disease severity during 2004 and 2005 on the average, respectively.

Table 1. Antagonistic effect of the tested isolates against *in vitro* mycelial growth of *S. vesicarium*

Tested isolate name and number	Mycelial growth inhibition (%)
Trichoderma harzianum (1) (Th1)	78.1
T. harzianum (2) (Th2)	65.1
T. harzianum (3) (Th3)	60.3
T. harzianum (4) (Th4)	60.6
T. harzianum (5) (Th5)	56.8
Gliocladium catenulatum (1) (GC1)	52.1
Bacillus subtilis (1) (Bs1)	79.6
B. subtilis (2) (Bs2)	73.6
B. subtilis (3) (Bs3)	63.3
B. subtilis (4) (Bs4)	57.7
Pseudomonas fluorescens (Pf1)	84.4
Saccharomyces cerevisiae (1) (Sc1)	35.9
S. cerevisiae (2) (Sc2)	22.4
S. cerevisiae (3) (Sc3)	20.9
Fungicide (Ridomil gold plus)	99.6
L.S.D. at 0.05	6.39

Table 2. Effect of some antagonistic microorganisms and Ridomil Gold plus, fungicide, and time application dates on Stemphylium blight in 2004 and 2005 experiments under greenhouse conditions

Treatment	Experiment year	Time of application				Mean
		3 days before inoculation		At inoculation time		
		Disease severity	Efficacy (%)	Disease severity	Efficacy (%)	
P. fluorescens	2004	27.33	32.78	26.16	38.68	26.75
B. subtilis (1)		18.91	53.49	11.50	73.04	15.21
S. cerevisiae (1)		19.83	51.23	15.66	63.29	17.75
T. harzianum (1)		27.41	32.59	25.66	39.85	26.54
Ridomil (fungicide)		12.25	69.87	6.83	83.99	9.54
Control		40.66	-	42.66	-	41.66
Mean		27.40	-	21.41	-	
P. fluorescens	2005	24.08	44.75	25.83	37.76	24.96
B. subtilis (1)		17.83	59.09	12.16	70.70	15.00
S. cerevisiae (1)		19.00	56.40	16.50	60.24	17.75
T. harzianum (1)		27.66	36.53	26.00	37.35	26.83
Ridomil (fungicide)		9.91	77.26	3.83	90.77	6.86
Control		43.58	-	41.50	-	42.54
Mean		23.68		20.97		
L.S.D. at 0.05 for:		2004		2005		
Treatment (A) =		4.82		4.83		
Application time (B) =		3.41		2.24		
Interaction (AB) =		8.35		5.5		

In general, in 2004 and 2005 experiments the significant different was detected between the two tested methods of application and the application of bioagents at the same time of pathogen inoculation showed the highest effect as compared with the application 3 days before inoculation.

Effect of chemical resistance inducers on in vitro pathogen mycelial growth:

Data in Table (3) show that no significant differences were detected between all chemical inducers and its concentrations on the mycelial growth of *Stemphylium vesicarium* on PDA medium.

Table 3. Effect of chemical inducers on *in vitro* radial growth of *Stemphylium vesicarium* (Isolate No. 1)

Chemical inducer	Concentration	Mycelia growth diameter (cm)	Mycelia growth inhibition (%)
Bion	62.5 ppm	7.26	0.4
	125 ppm	7.23	0.9
	250 ppm	7.30	0.0
K_2HPO_4	10 mM	7.26	0.4
	25 mM	7.30	0.0
	50 mM	7.30	0.0
SA	1 mM	7.30	0.0
	2 mM	7.30	0.0
	5 mM	7.23	0.9
Control		7.30	0.0
L.S.D. at 0.05			N.S.

Effect of chemical inducers on induction of systemic acquired resistance against the disease:

Data in Table (4) show that all chemical inducers treatments significantly reduced Stemphylium blight of onion in both tested experiments except in case of SA treatment at 1mM in 2004 experimental season. The highest significant reduction in disease severity was observed in case of Bion at 250 ppm and 125 ppm concentration where the recorded percentages of disease severity in 2004 experiment were 79.1 and 69.10 % respectively followed by SA at 5 mM (21.66 %) without significant differences. In 2005 experiment, the highest reduction in disease severity was observed when K_2HPO_4 was used at 50 mM and Bion 250 ppm and SA at 5 mM where the reductions percentage in the disease severity recorded 66.81, 65.71 and 64.8% respectively, followed by SA 2 mM, Bion 125 ppm and K_2HPO_4 at 25 mM without significant difference. SA at 1 mM did not give any reduction of disease severity in 2004 season. The high concentrations of all inducers gave the highest reduction in the disease severity in both tested experiments.

Table 4. Effect of different resistance inducers on Stemphylium blight in 2004 and 2005 experiments under greenhouse conditions

Tested inducer	Concentration	2004 experiment		2005 experiment	
		Disease severity (%)	Efficacy (%)	Disease severity (%)	Efficacy (%)
Bion	62.5 ppm	37.83	31.22	55.00	32.65
	125 ppm	17.00	69.10	35.00	57.14
	250 ppm	11.50	79.10	28.00	65.71
K_2HPO_4	10 mM	35.00	36.36	37.33	54.29
	25 mM	26.16	52.44	35.00	57.14
	50 mM	22.83	58.49	27.33	66.81
SA	1 mM	60.00	9.10-	55.00	32.65
	2 mM	40.83	25.76	31.00	62.04
	5 mM	21.66	61.22	29.33	64.08
Control		55.00		81.66	
L.S.D. at 0.05:		10.35		10.01	

Discussion

Stemphylium blight disease, incited by different species of *Stemphylium* is one of the diseases which attack onion plants causing severe damage, especially to onion seed crop (Tomaz and Lima, 1988). In Egypt, during the last few years, new disease symptoms on onion plants were observed, showing different symptoms from the traditional Stemphylium blight symptoms caused by *Stemphylium botryosum*. These symptoms were virulent on onion foliage, killing the leaves progressively and caused the black stalk rot disease of onion (Schwartz and Mohan, 1996). The causal pathogen was isolated and identified as *S. vesicarium* (Wallr.) Simmons, produced the telemorphic state *Pleospora allii* (Rabenh.) Ces. & De Not in culture. These results are similar to the findings obtained by Basallote *et al.* (1999), Suheri and Price (2000) and Cedeño *et al.* (2003). According to the available literature this is the first record of such disease on onion plants in Egypt caused by *Stemphylium vesicarium*.

Pathogenicity tests of 15 isolates of *S. vesicarium* obtained from naturally diseased onion plants indicted that all tested isolates were able to infect onion plants causing Stemphylium blight disease with different degrees of disease severity. Such results are in agreement with those reported by Verwoperd and Du Plessis (1931) in South Africa and Shishkoff and Lorbeer (1989) in New York.

One of the alternative control methods of plant diseases is the use of biological control agents. This is the first study in Egypt on the potential biocontrol agents for controlling *S. vesicarium* on onion.

Testing antagonistic capabilities of certain microorganisms was *in vitro* investigated. Data revealed that all the tested microorganisms were able to inhibit the mycelial growth of the causal pathogen with different degrees of antagonistic capability. Several researchers have successfully employed antagonistic bacteria,

streptomyces and fungi including yeasts to control plant diseases (Fokkema and Lorbeer, 1974; Abd El-Megid *et al.*, 2001 and Hassan and Abd El-Rehim, 2002).

Application of certain antagonistic microorganism's isolates for controlling onion Stemphylium blight under greenhouse conditions affected significantly the disease severity. Application of *Trichoderma harzianum* and *P. fluorescens* gave the highest reduction in the disease severity. In most cases application of these bioagents at the same time of inoculation with the pathogen gave higher reduction in disease severity than the application 3 days before inoculation. The obtained results are in agreement with those obtained by many other researchers (Leeman *et al.*, 1995; Paulitz and Bèlanger, 2001 and Hassan and Abd El-Rehim 2002).

Results reported herein, also indicate that no significant effects of all tested inducers at all tested concentrations on the *in vitro* pathogen radial growth. These findings are agreement with Kessmann *et al.* (1994) who reported that inducers must be lack of direct antimicrobial activity and no conversion of the compound in plant into antimicrobial metabolites. Data indicate that, in both of the greenhouse experiments, all chemical inducers treatments gave significant reduction in disease severity. These results are in agreement with those obtained by other researchers working on the induced resistance with various chemicals inducers (Hammerschmidt and Smith-Becker, 1999; Mosa, 2002 and Geetha and Shetty, 2002).

References

Abd El-Megid, A.H.; Mitwally, S.M.; Abdel-Momen, A. and Hilal, A.A. 2001. A Preliminary field study on the possibility of controlling foliar diseases of onion using some Egyptian medicinal plants extracts in comparison with a fungicide. *Egypt. J. Phytopathol.*, **29** (1): 21-31.

Aveling, T.A.S. and Snyman, H.G. 1993. Infection studies of *Stemphylium vesicarium* on onion leaves. *Mycological Res.*, **97: 984-988.**

Basallote, M.J.; Prados-Ligero, A.M. and Melero-Vara, J. M. 1999. Aetiology of leaf spot of garlic and onion caused by *Stemphylium vesicarium* in Spain. *Plant Pathol.*, **48** (1): 139-45.

Cedeño, L.; Carrero, C.; Quintero, K.; Pino, H. and Espinoza, W. 2003. *Stemphylium vesicarium*, causal agent of severe foliar blight on garlic and onion in Mérida, *Venezuela. Interciencia*, **28** (3): 174-177.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, England.

Fokkema, N.J. and Lorbeer, J.W. 1974. Interactions between *Alternaria porri* and the saprophytic mycoflora of onion leaves. *Phytopathology*, **64:** 1128-1133.

Geetha, H.M. and Shetty, H.S. 2002. Induction of resistance in pearl millet against downy mildew disease caused by *Sclerospora graminicola* using benzothiadiazole, calcium chloride and hydrogen peroxide a comparative evaluation. *Crop Protection*, **21: 601–610.**

Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedures for Agriculture Research.* 2nd Ed. John Willey. New York. 680pp.

Hammerschmidt, R. and Smith-Becker, J.A. 1999. The role of salicylic acid in disease resistance. Pages: 37-53. In: *Induced Plant Defenses Against Pathogens and Herbivores*. Agrawal, A.A.; Tuzun, S. and Bent, E. (eds.). APS Press, St. Paul, MN, USA.

Hassan, M.H.A. and Abd El-Rehim, G.H. 2002. Yeast application as a biofertilizer and biocontrol agent for onion neck rot disease in relation to bulb productivity and quality. *Assiut J. Agric. Sci.*, 33 (1): 241-251.

Kessmann, H.; Staub, T.; Hofmann, C.; Maetzke, T.; Herzog, J.; Ward, E.; Uknes, S. and Ryals, J. 1994. Induction of systemic acquired resistance in plants by chemicals. *Annu. Rev. Phytopathol.*, 32: 439-459.

Leeman, M.; Van Pelt. J.A.; Hendrickx, M.J.; Scheffer, R.J. and Bakker, P. 1995. Biocontrol of Fusarium wilt of radish in commercial greenhouse trials by seed treatment with *Pseudomonas fluorescens* WCS374. *Phytopathology*, 85: 1301-1305.

Mosa, A.A. 2002. Induced resistance in rice against blast disease using abiotic and biotic agents. *Ann. Agric. Sci., Ain Shams Univ., Cairo*, 47 (3): 993-1008.

Oostendorp, M.; Kunz, W.; Dietrich, B. and Staub, T. 2001. Induced disease resistance in plants by chemicals. *Europe. J. Plant Pathol.*, 107: 19-28.

Paulitz, T.C. and Bélanger, R.R. 2001. Biological control in greenhouse systems. *Annu. Rev. Phytopathol*, 39: 103-133.

Ryals, J.A.; Neuenschwander, U.H.; Willits, M.G.; Molina, A.; Steiner, H. and Hunt, M.D. 1996. Systemic acquired resistance. *Plant Cell*, 8: 1809-1819.

Schwartz, H.F. and Mohan, S.K. 1996. *Compendium of Onion and Garlic Diseases.* APS Press. St. Paul, MN, USA.

Shishkoff, N. and Lorbeer, J.W. 1989. Etiology of Stemphylium leaf blight of onion. *Phytopathology*, 79: 301-304.

Sivan, A. 1987. Biological control of Fusarium crown rot of tomato by *Trichoderma harzianum* under field conditions. *Plant Dis.*, 71: 587-592.

Suheri, H. and Price, T.V. 2000. Infection of onion leaves by *Alternaria porri* and *Stemphylium vesicarium* and disease development in controlled environments. *Plant Pathol.*, 49: 375-82.

Tomaz I.I. and Lima A. 1988. An important disease of onion caused by *Stemphylium vesicarium* (Wallr.) Simmons in Portugal. *Horticultural Abstracts*, 58: 618.

Verwoperd, L. and Du Plessis, S.J. 1931. Description of some new species of South African fungi and of species not previously recorded in South Africa. III. *South Africa J. Science*, 28: 290-297.

(Received 06/03/2007;
in revised form 24/04/2007)

مقاومة مرض لفحة الاستمفليوم على اوراق البصل باستخدام عوامل بيولوجية ومستحثات المقاومة

محمد عبدالمنعم حسين ، محمد حسن عبد الرحيم حسن ،
على دياب علام ، كمال أحمد محمد أبوالبشر
قسم أمراض النبات – كلية الزراعة – جامعة اسيوط – أسيوط.

أجريت هذه الدراسة بهدف عزل وتعريف المسبب المرضى وتأكيد القدرة المرضية لتلك العزلات على إصابة البصل تحت ظروف محافظة اسيوط وكذلك دراسة فاعلية بعض عوامل المكافحة سواء الحيوية أو غير الحيوية كمحثات للمقاومة الطبيعية للنبات ويمكن تلخيص أهم النتائج فيما يلى:

1- عزل وتعريف المسبب المرضى لمرض لفحة الاستمفليوم وقد ثبت أنه فطر إستمفليوم فوسيكاريم *Stemphylium vesicarium* (Wallr) Simmons والذى كون الطور الجنسى *Pleospora allii* (Rabenh). Ces & De Not. تحت ظروف البيئات الصناعية.

2- اختبار كفاءة التضاد لعديد من الكائنات الحية الدقيقة (فطريات– بكتريا– خمائر) ضد النمو الميسليومى للفطر الممرض على الأطباق فى المعمل وقد أظهرت النتائج أن هذه الكائنات الحية الدقيقة لها القدرة على تثبيط نمو ميسليوم الفطر فى الأطباق بدرجات مختلفة وكانت أكثرها تثبيطا لنمو الفطر الممرض هى عزلات:
Bacillus subtilis, Gliocladium catenulatum, Pseudomonas fluorescens and *Trichoderma harzianum.*
وكانت اقلها تثبيطا لنمو الفطر عزلات *Saccharomyces cerevisiae*

3- اختبار كفاءة العزلات عالية القدرة التضادية لتثبيط نمو الفطر الممرض تحت ظروف الصوبة فى مقاومة المرض حيث أظهرت العزلات *Bacillus subtilis, Saccharomyces cerevisiae* قدرة عالية على خفض شدة الإصابة بالمرض ويليها البكتريا *Pseudomonas fluorescens* ثم عزلة فطر *Trichoderma harzianum* .

4- إستخدام عوامل المقاومة الحيوية السابقة متزامنة فى نفس وقت العدوى بالمسبب المرضى كان أفضل من استخدامها قبل ثلاثة أيام من العدوى بالمسبب المرضى.

5- ظهر ان اختبار بعض محفزات المقاومة الكيماوية (البيون، حمض السلسليك، فوسفات البوتاسيوم ثنائية القاعدية) على حث العائل لمقاومة المرض تحت ظروف الصوبة بين أن المعاملة بأى من هذه المواد قد أدى إلى خفض شدة المرض. وكان البيون وحمض السلسليك أكثرها فاعلية فى خفض شدة المرض فى موسم 2004 أما فى موسم 2005 فكانت جميعها لها قدرة عالية على خفض شدة الإصابة.