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RESEARCH ARTICLE

NEW WAYS FOR CONTROLLING ONION WHITE ROT DISEASE (*Sclerotium ceviporum*) IN NORTH EGYPT.

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Abstract

Sclerotium cepivorum, is worldwide destructive soil borne fungal pathogen and has been reported attacking onion and other *Allium* species causing considerable damage to the host under congenial environments in Egypt and other regions. Recently, this species was isolated from North Egypt after long settlement in south Egypt. Many attempts were used to control onion white rot disease caused by this pathogen. In the present study three fungicides viz. procimidone (sumisclex®25), vinclozolin (ronilan) and tolclofos-methyl (rizolex) were used against *Sclerotium cepivorum* and compared with a range of non chemical treatments including five antagonistic fungi, two antagonistic bacteria, two essential oils and fine red onion waste (ROW) in naturally infested area to evaluate their effects on the incidence and severity of onion white rot disease under field conditions. Among all fungicides tested, it was found that sumisclex®25 was the best chemical treatment giving 14.81% reduction in disease incidence and 13.38% severity. While, *Gliocladium virens* achieved the best results of reducing disease incidence giving 8.89% and disease severity by 8.38%. On the other hand, *Pseudomonas fluorescens* and *Bacillus subtilis* reduced disease incidence, giving 21.48 and 22.96%, respectively and high reduction in disease severity by 19.01 and 21.23% respectively. Among all tested essential oils, cinnamon oil considered the best treatment giving 32.59% disease incidence and 28.88% severity. While, ROW was the best treatment reducing the disease incidence, giving 8.15% disease severity.

Introduction:-

Sclerotium cepivorum Berk, is a soil-borne fungal pathogen infesting *Allium* spp., including onion, garlic, and leeks. As a member of the Sclerotiniaceae (**Carbone and Kohn, 1993; Willetts, 1997**), disease symtom appears as a white fluffy mycelial growth at the base of the stem plate as a soft mould. The pathogen survives in a vegetative state in the absence of a host plant as small dark sclerotia, which remain dormant in soil for many years (**Coley-Smith et al., 1990**). So the fungus becomes a serious problem found all over the world on *Allium* spp. that occurring large crop losses. In Egypt this pathogen appears after the high dam building, because the recent agriculture production system depends on the permanent surface irrigation instead of the temporary irrigation system. This situation helps so much the onion white rot disease to be more established in the soil especially in upper Egypt mainly in onion and garlic producing fields(**Abd El-Moity, 1976 and 1981; Abd El-Razik, 1993 and Embaby**, **2005**) and now it jumped to north Egypt **(El-Sheshtawi et al., 2009).** Sclerotia germinate only in the presence of root exudates of *Allium* spp. means sclerotial germination stimulants. Root exudates contain a natural products (e.g., diallyl disulfide) which stimulate sclerotia to germinate, recently a sclerotial germination stimulant, di allyl di sulphide, has become available **(Tyson et al., 1999)**. Many strategies were used for controlling onion white rot disease by decaying the sclerotia such as crop rotation with non host crops, and other integrated control means i..e. soil solarization, biological control agents, sclerotial germination stimulants. Other tools such as the use of antioxidants and composted onion waste have been tried with varying levels of success, compared with chemical fungicides **(Ouf, et al. 2008 and Whipps, et al. 2008**). However, such attempts were not effective for the management of white rot under onion and garlic production conditions (**Tyson, et al. 1999)**. We think that there is urgent and essential need for starting a serious research programme on this pathogen to find the relationship between the distribution of the onion white rot disease and the other environmental conditions helping for the epidemiology of this disease. Control of this disease could be achieved by finding safe means to solve such problem to have cure quality products for export and local consumption. The non-chemical and biological control means by the use of antagonistic fungi, antagonistic bacteria and other means such as essential oils and fine red onion waste (ROW) should help in this way under field conditions.

Field experiments:-

Experiments were conducted under natural conditions in an naturally infested area to evaluate the effect of biological agents i.e.*Trichoderma viride*, *T. harzianum*, *Coniotherium minitans*, *Gliocladium virens*, *Penicillium janthinallum*, *Bacillus subtilis* and *Pseudomonas fluorescens*; two essential oils (cinnamon oil and garlic oil) and onion waste compared with sumisclex®25, ronilan and rizolex-T as standard chemical treatments on the incidence and severity of onion white rot disease under field conditions.

Experiments were carried out in infested field in Dakahlia Governorate, Egypt, at 70% onion white rot disease incidence from January to May 2014/2015. Plots were established each consisted of three rows (9m X 1m) and each treatment was replicated three times. Root dipping application of onion seedlings cv, Giza 20 was used for each antagonistic fungus and bacteria treatments, according to the method reported by **El-Sheshtawi et al. (2009)**. While fine red onion waste was incorporated into the soil, On the other hand, essential oils i.e. cinnamon oil and garlic oil were used as root seedlings dipping.

Onion seedlings (30 days old) were dipped in 1 % sodium hypochlorite for 1 min., and then washed several times in sterilized distilled water. Seedlings roots were then treated with talc powder and dipped in suspension of antagonistic fungi (10^9 spore/ ml^{-1}) and **in** antagonistic bacteria (10^8 spore/ ml^{-1}) for 5min.; then transplanted in the soil. Planting as 45seedling /$1m^2$, planting distances were 10 cm. (**Clarkson, et al., 2006**)
Treatments of essential oils were done by soaking seedlings roots in 0.5% emulsion of essential oils (oil emulsions prepared by adding 0.5 ml of essential oil to 99.5ml distilled water and 0.5ml tween-80) for 2h.,. Fine red onion wastes were incorporated into soil at $1.5k/M^2$. Each treatment was compared with four controls, three of them as standard fungicides (procimidone (Sumisclex®25), vinclozolin (ronilan) and tolclofos-methyl (rizolex)) applied at 3g/1L, then seedlings were dipped in for 5min., **(Pung, et al., 2004)**. However, absolute control seedlings were dipped in sterilized distilled water for 5min. Seedlings were then left to dry and transplanted as described before. The percentage of disease incidence and severity were recorded after 60, 90 and 120 days as follows:

$$PI = \left[C - I / C \right] \times 100$$

(PI) = Disease incidence %., (I)=number of replicates and (C)=mean number of survived plants in the control **(Brix and Zinkernagel, 1992).**

Disease severity was calculated according to **Zewide et al. (2007)** as followed this scale and formula by: 0=Healthy plants, 1=The outer leaves are yellow and roots brown instead of white, 2=Leaf tip die backed and roots felt soft firm and 3=Leaves were wilted and collapsed of stem base and roots had collapsed when touched with tweezers.

$$\text{Disease severity} = \left[\frac{\text{Sum of all ratings}}{\text{Total number of plant} \times \text{maximum score}} \right] \times 100$$

Statistical analysis:-

All experiments were repeated twice with three replicates for each treatment. All data were subjected to one-way analysis of variance (ANOVA) and significant differences between treatment means were determined using

Duncan's Multiple Range Test (**Duncan, 1955**) and Tukey's HSD test at P<0.05. The data were analyzed by SAS (version 9.1, SAS Institute, Cary, NC, USA).

Results:-

Effect of some antagonistic fungi on disease incidence and disease severity of onion white rot caused by *S. cepivorum*:-

G. virens treatment gave significantly results in reducing disease incidence (8.89%) and disease severity (8.38%) compared with natural control (89.63 and 80% respectively). While chemical controls reached 14.81% disease incidence and 13.38% severity in case of sumisclex®25. This was followed by *P. janthinallum, C. minitans*, *T. harzianum* and *T. viride* giving 14.81, 23.33, 27.40 and 28.88% reduction of disease incidence, respectively. While gave 14.32, 21.73, 24.94 and 27.16% reduction rates in disease severity respectively, when compared with natural controls (Table 1).

Effect of some antagonistic bacteria on disease incidence and disease severity of onion white rot caused by *S. cepivorum*:-

B .subtilis (21.48%) and *P. fluorescens* (22.96%) treatments significantly reduced disease incidence.While both of them gave 19.01 and 21.23% disease severity, respectively. However, natural control gave 89.63% disease incidence while chemicals control gave 14.81% disease incidence in case of sumisclex®25). (Table 2).

Effect of some essential oils and onion waste on disease incidence and disease severity of onion while rot caused by *S. cepivorum*:-

ROW was the best treatment in reducing disease incidence and disease severity, giving 8.15%. While among the essential oils treatments, the cinnamon oil considered the best treatment giving 32.59% disease incidence and 28.88% disease severity compared with untreated control (89.63% disease incidence) and chemical controls (14.81% disease incidence in case of sumisclex®25). (Table 3).

Table 1: Effect of some antagonistic fungi on disease incidence and disease severity of onion white rot caused by *S. cepivorum.*

Treatments	Disease incidence %	Disease severity %		
		60 days	90 days	120 days
Control	89.63a[a]	34.57 a	64.45a	80.00a
Rizolex-T	38.52b	14.57 b	26.92 b	38.41 b
Ronilan	22.96c	0.00 c	16.79cd	22.22c
Sumisclex®25	14.82d	0.00 c	11.60def	13.83e
C.minitans	23.33c	0.00 c	13.08de	21.73cd
G. virens	8.89d	0.00 c	4.94g	8.39e
P. janthinallum	14.81d	0.00 c	9.38efg	14.32de
T .harzianum	27.41c	0.00 c	16.54cd	24.94c
T .viride	28.89c	1.48 c	19.01c	27.16c

[a]Values in the same column followed by a different letter are significantly different according to Tukey's HSD test at P<0.05.

Each number is a mean of three replicates.

Table 2: Effect of two antagonistic bacteria on disease incidence and severity of onion white rot caused by *S. cepivorum.*

Treatments	Disease incidence %	Disease severity %		
		60 days	90 days	120 days
Control	89.63a[a]	34.57 a	64.45a	80.00a
Rizolex-T	38.52b	14.57 b	26.92 b	38.41 b
Ronilan	22.96c	0.00 c	16.79c	22.22c
Sumisclex®25	14.82d	0.00c	11.60c	13.83c
B. subtilis	21.48c	0.00 c	12.84c	19.01c
P. fluorescens	22.96c	0.00 c	14.57c	21.23c

[a]Values in the same column followed by a different letter are significantly different according to Tukey's HSD test at $P<0.05$

Each number is a mean of three replicates.

Table 3: Effect of two essential oils and onion waste on disease incidence and severity of onion white rot caused by *S. cepivorum*

Treatment	Disease incidence %	Disease severity %		
		60 days	90 days	120 days
Control	89.63a[a]	34.57 a	64.45a	80.00a
Rizolex-T	38.52b	14.57b	26.92b	38.41 b
Ronilan	22.96c	0.00 c	16.79cd	22.22de
Sumisclex®25	14.82d	0.00 c	11.60de	13.83ef
Cinnamon Oil	32.59b	0.00 c	21.48bc	28.89cd
Garlic Oil	37.78b	0.00 c	25.43b	35.31bc
Onion Waste	8.15e	0.00 c	6.17e	8.15f

[a]Values in the same column followed by a different letter are significantly different according to Tukey's HSD test at $P<0.05$.

Each number is a mean of three replicates.

Discussion:-

The antifungal activity of *Trichoderma* spp. could be attributed to mycoparasitism (**Inbar, et al., 1996 and Shaigan, 2008),** fungitoxic cell-wall-degrading enzymes (**Chet et al., 1998**), and probably to peptaibol antibiotics. These lytic enzymes, act also as fungal cell-wall degrading agents such as N–acetyl-β-D–gluocosedeaminidase, chitinase, endochitinase, β-1,3gluocose, β-1,4gluocose, β-1,6gluocose, chitobiosidase and protease **(Harman, 2000 and Harman et al., 2004**). Evaluation was done under field conditions to measure the efficiency of the examined antagonistic bioagents on onion white rot disease incidence and disease severity. *G. virens* performed the best results in reducing the disease incidence and severity. These results are in agreement with **Howell (1990)** who stated that *Glicoladium* spp. as biocontrol agent was useful against soil borne pathogens and its application as seed or seedling treatment should be able to colonize extensive parts of the root system. Colonization of roots by *Glicoladium* spp. showed that both tap roots and secondary roots were colonized when *Glicoladium* spp. was introduced as seed or seedling treatment, so *Glicoladium* spp. is in a position to interact with seedling pathogens on the rhizoplane. This may explain why *G. virens*. has been reported to be an effective biocontrol agent for plant diseases caused by *Rhizoctonia solani* and other soil borne fungal pathogens (**Howell, et al. 1993 and Wei, et al., 2005**). In addition, the mechanisms of *Glicoladium* spp. may be due to competition, antibiosis or a combination of these two mechanisms.

The mechanisms of bioantagonistic fungi included an important role in induction resistance by producing peptaibols of many amino acids and salicylic acid which increase some compounds in plants such as poly phenols e.g. terpenes and flavonoids (**Howell et al., 2003)**. Certain antagonistic microorganisms produce separate substances that reduce and chelate iron (Fe^{+3}) and other nutrients to make it in available from (Fe^{+2}) to plants (**Altomare et al., 1999**). Under field conditions, *B. subtilis* and *P. fluorescens* showed high effect on reducing disease severity and incidence, these results are in agreement with **Hu et al. (2005)** who found that *B. subtilis* significantly reduced the incidence of white rot disease caused by *S. sclerotiorum* on oil seed rape under field conditions, when iron availability in the soil was reduced by the addition of an iron chelator and chelating agents which are not equally effective at all pH values.

Competition for iron is supposed to be the mechanism by which bacteria suppress disease. Siderophores such as pseudobactin are special case of Fe-specific chelating agents produced by plants, fungi, bacteria, function in rhizosphere to help Fe transportation may also help to solubilize Fe from solid phases, disease suppression occurrence in soil with low iron availability**.** The addition of iron in a form that can be assimilated by the pathogens ($FeCl3$ or chelated ethylenediaminetetraacetic acid, Fe EDTA) also reduces the capacity of the bacteria to suppress disease **(Hammer, et al. 2004).** *P. fluorescens* may increase synthesis of phenolic compounds such as Tannic, Gallic, Caffeic, chlorogenic and cinnamic acids. Treatment with *P. fluorescens* was found to be more effective in inducing phenolic compounds, the hyphae of the pathogen surrounded by phenolic substances exhibited considerable morphological changes including cytoplasmic disorganization and loss of protoplasmic content.

Plant synthesizes defense compounds as a greater accumulation of defense compounds when *P. fluorescens*-treated plants were challenged with the pathogen during the experimental period **(Basha et al. 2006)**. Under field conditions, ROW was the best treatment reducing the disease incidence, while among the essential oils, cinnamon oil considered as the best treatment for reducing disease incidence and severity as well. This finding agrees with **Coventry et al. (2002 &2006)**. They affirmed that application of the red onion waste alone or combined with *T. viride* could eliminate *S. cepivorum*. Onion wastes are not suitable for phytopathogens, e.g. *S. cepivorum* (white rot). (**Lecain et. al., 1999**). Processing and stabilizing onion wastes (residues and surpluses of onion) could represent both advantages: a solution of the environmental problem derived from the great onion wastes disposal and the obtaining of stabilized onion by-products as natural antioxidant ingredients. Under field conditions, the sumisclex®25 chemical fungicide treatment showed the best reduction in disease incidence and severity. This result agrees with (**Stewart and Fullerton (1991)** who reported that procymidone gave a reduction in disease incidence and disease severity of *S. cepivorum*.

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References:-
1. Abd El-Moity, T.H. (1976). Studies on the biological control of white rot disease of onion. M.Sc. Thesis Plant Pathology Dept., Fac. Agric. Menoufeia University.
2. Abd El-Moity, T.H. (1981). Further studies of the biological control of white rot disease of onion. Ph.D. Thesis Plant Pathology Dept. Fac. Agric. Menoufeia University.
3. Abd El-Razik, A.A. (1993). Integrated control of onion white rot in Upper Egypt. Final Report Project in Plant Pathol. Fac. Agric. Assiut University.
4. Abd El-Razik, A. A., Shatla, M. N., & Rushdi,M. (1973). Studies on the infection of onion plants by *Sclerotium cepivorum* Berk. Phytopathologische Zeitschrift, 76, 108–116.
5. Altomare, C.; Norvell, W. A.; Bjorkman, T. & Harman, G. E. (1999). Solubilization of phosphates and micronutrients by the plant growth promoting and biocontrol fungus *Trichoderma harzianum* .Appl. Eviron. Microbiol .62:2926-2933.
6. Basha, S.A.; Sarma, B.K.; Singh, D.P.; Annapurna, K. & Singh, U.P. (2006). Differential methods of inoculation of plant growth-promoting rhizobacteria induce synthesis of phenylalanine ammonia-lyase and phenolic compounds differentially in chickpea. Folia Microbiologica. 51(5):463-468.
7. Brix H. D. & V. Zinkernagel (1992) Effects of cultivation, conditioning and isolate on sclerotium germination in *Sclerotium cepivorum* .Plant Pathology 41, 13-19.
8. Carbone I., Kohn L.M.(1993). Ribosomal DNA sequence divergence within internal transcribed spacer 1 of the Sclerotiniaceae. Mycologia, 85:415–27.
9. Chet, I., Benhamou, N. & Haran, S. (1998). In *Trichoderma* and *Gliocladium*. Taylor and Francis, London .Vol. 2 (eds Kubicek, C. P. & Harman, G. E.) 153–172.
10. Clarkson, J. P., Scruby, A., Mead, A., Wright, C., Smith, B., & Whipps, J. M. (2006). Integrated control of *Allium* white rot with *Trichoderma viride*, tebuconazole and composted onion waste. Plant Pathology, 55, 375–386.

11. Coley-Smith, J. R.; Christine, M. M. and Claire, E. S. (1990). Long-term survival of sclerotia of *Sclerotium cepivorum* and *Stromatinia gladioli* Plant Pathology.39, 58-69.
12. Coventry, E.; Noble, R.; Mead, A. and Whipps, J. M. (2002). Control of *Allium* white rot (*Sclerotium cepivorum*) with composted onion waste. Soil Biology and Biochemistry 34 (2002)1037-1045.
13. Coventry, E.; Noble, R.; Mead, A.; Marin, F. R.; Perez, J. A. and Whipps, J. M. (2006). *Allium* white rot suppression with composts and *Trichoderma viride* in relation to sclerotia viability. Phytopathology 96, (9), 1009-1020.
14. Duncan, D. B. (1955). Multiple ranges and multiple F test. Biometrics11:1-42.
15. El-Sheshtawi, M., El-Gazzar, T., & Saad, A. S. (2009). Comparative study between chemical and non-chemical control against *Sclerotium cepivorum*, the causal white rot of onion under Egyptian condition. Journal of Agricultural Science, Mansoura University, 34, 2169–2182.
16. Embaby, M. (2006). Using a Biofungicide (*Coniothyrium minitans* Campbell.) In controlling some soil-borne plant pathogenic fungi in Egypt. Research Journal of Agriculture and Biological Sciences, 2(6): 423-432.
17. Harman, G. E. (2000). Myths and dogmas of biocontrol. Changes in perceptions derived from research on *Trichoderma harzianum* T22. Plant Dis. 84, 377–393.
18. Harman, G. E.; Howell, C. R.; Viterbo, A.; Chet, I. & Lorito, M. (2004). *Trichoderma* species-opportunistic, avirulent plant symbionts. Nat. Rev. Microbial. 2 (1), 43–56.
19. Howell, C. R. (1990). Fungi as biological control agents, in biotechnology of plant microbe interactions (NAKAS,J.P. & HAGEDORN, C., Eds) McGraw-Hill, New York, NY, USA, pp. 257-286.
20. Howell, C.R. (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant Disease 87:4-10.
21. Howell, C.R.; R. D. Stipanovic & R. D. Lumsden (1993). Antibiotic production by strains of *Gliocladium* virens and its relation to the biocontrol of cotton seedling diseases. Biocontrol Science and Technology 3: 435-441.
22. Hu, X.; Roberts, D.P.; Jiang, M. & Zhang, Y. (2005).Decreased incidence of disease caused by Sclerotinia *sclerotiorum* and improved plant vigor of oilseed rape with *Bacillus subtilis* Tu-100. Applied Microbiology & Biotechnology. 68(6):802-807.
23. Inbar, J.; Menendez, A. & Chet, I.(1996). Hyphal interaction between *Trichoderma harzianum* and *Sclerotini*a sclerotiorum and its role in biological control. Soil Biol. Biochem. 28, 757–763.
24. Lecain, S., N. g, A.; Parker, M. L.; Smith, A. C. and Waldron, K. W. (1999). Modification of cell-wall polymers of onion waste – Part I. Effect of pressure-cooking. Carbohydrate Polymers, 38, 59–67.
25. Ouf, S.; Ali, A. M. I. & A. E. Ibrahem. (2008). *In vitro* evaluation of the biocontrol activity of some biofungieides on *Sclerotium cepivorum*. International Journal of Agriculture and Biology 10 (3): 241-248.
26. Pung, M.; Smolinska, U. & M. Horbowicz (2004). Fungicidal activity of volatiles from selected cruciferous plants against resting propagules of soil-borne fungal pathogens. J. Phytopathology 147,119-124.
27. Shaigan, M.A. (2008). Competitiveness of a genetically engineered strain of *Trichoderma virens*. Mycopathologia. 166(1):51-59.
28. Stewart, A. & Fullerton, R.A., (1991). Additional studies on the control of onion white rot (*Sclerotium cepivorum* Berk) in New Zealand. NZ J. Crop Horticultural Science. 19, 129–134.
29. Tyson, J.L.; Fullerton, R. A. & Stewart, A. (1999). Changes in the efficacy of fungicidal control of onion white rot. Proc. 52nd N.Z. Plant Protection Conference:171-175.
30. Wei, X.; Yang, F. & Straney, D.C. (2005). Multiple non-ribosomal peptide synthetase genes determine peptaibol synthesis in *Trichoderma virens*. Canadian Journal of Microbiology. 51(5):423-429.
31. Whipps, J. M.; Sreenivasaprasad, S.; Muthumeenakshi, S.; Rogers, C. W. & Challen, M. P. (2008). Use of *Coniothyrium minitans* as a biocontrol agent and some molecular aspects of sclerotial mycoparasitism . Eur. J. Plant Pathol. 121:323–330.
32. Willetts H.J. (1997). Morphology, development and evolution of stromata/ sclerotia and macroconidia of the Sclerotiniaceae. Mycological Research. 101:939–52.
33. Zewide, T., Fininsa, C., & Sakhuja, P. K. (2007). Management of white rot (*Sclerotium cepivorum*) of garlic using fungicides in Ethiopia. Crop Protection, 26, 856–866.