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The Effectiveness of Some Safety Compounds in Controlling *Botrytis* Neck Rot of Onion

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ABSTRACT

Onion (*Allium cepa* L.) is one of the major vegetable crops in Egypt that are damaged and lost by Botrytis neck rot. The aim of this study was to determine the efficacy of silica gel, acetic acid, fulvic acid and charcoal as protective agents against *Botrytis allii* under both *in vitro* and in greenhouse and field conditions. In *vitro*, the maximum disease reduction was observed by 1%, 0.8% and 0.5% acetic acid (73.7%, 63.7%, and 61.2%, respectively), followed by 2% of charcoal (52%) and 3% of silica gel (47.5%). In the greenhouse and field conditions, silica gel (3%) and acetic acid (1%) were the best treatments and were shown to inhibit the disease up to 85.7% in green house and up to 70.4% and 69% in field, respectively compared to untreated control, therefore, we considered that the two treatments would be suitable applications as a safe and cheap alternative to fungicides.

Keywords: Onion; *Botrytis* neck rot; Silica gel; Acetic acid; Fulvic acid; Charcoal.

فعالية بعض المركبات الآمنة في مكافحة مرض عفن الرقبة في البصل

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الملخص: البصل من أهم محاصيل الخضر في مصر التي تتعرض للتلف بسبب الأصابه بمرض عفن الرقبة المتسبب له فطر البوتريتيس ألاي , تم دراسة تأثيرفعالية بعض المركبات العضوية مثل السيليكا جيل وحامض الخليك وحامض الفولفيك والفحم النباتي لمقاومة الفطرفي المعمل وايضاً تحت ظروف الصوبة الزجاجية والحقل وأوضحت النتائج ان أستخدام حمض الخليك بتركيز 1 % أدت إلي خفض شدة الأصابة بنسبة تزيد عن 73% مقارنة بالكنترول في المعمل. بينما تحت ظروف الصوبة الزجاجية و الحقل وجد ان السيليكا جيل بتركيز 3 % وحامض الخليك بتركيز 1 % افضل المعاملات حيث قللوا شدة الأصابة المرضية بنسبة تزيد عن 85 % في الصوبة الزجاجية و بنسبة تتراوح ما بين 70.4 % , 69 % علي التوالي في الحقل. ولذلك يقترح استخدام هذين المركبين بشكل فعال وآمن للسيطرة على مرض عفن الرقبة فى البصل كبديل آمن ورخيص بدلاً من المبيدات الفطرية.

الكلمات المفتاحية: البصل، عفن الرقبة ، السيليكا جيل، حمض الخليك، حمض الفولفيك، الفحم النباتي.

1. Introduction

Egypt ranks among the top ten onion-producing nations globally. In 2019, the country cultivated approximately 209,400 feddan, resulting in a total harvest of 3,081,047 tons and an average yield of 16.219 tons per feddan [1, 2]. *Botrytis* neck rot is caused by *B. acclada* (Fresenius), *B. allii* (Munn), and *B. byssoidea* (Walker), and are considered specific to members in the Alliaceae (onion and garlic). They can be found in all onion producing regions around the world [3,4]. Onion neck rot is considered a storage disease, although infection may occur in the field when necks are injured or are mature and become infected by spores blown from infested onion debris and improperly disposed cull piles [5]. If onions are harvested immature, *Botrytis* spp. sporulates on the surface of infected bulbs and can result in more than 30% yield loss [6]. Silicon (Si) increases the plant defence mechanisms by increasing nutrient availability in the rhizosphere and root uptake. It also increases the activity of antioxidants enzymes and enhances non-enzymatic mechanisms. Silicon also binds with OH^- of proteins and interferes with cationic co-factors of the enzymes which influence pathogenesis related (PR) events. [7] reported that Silicon (Si) exerted a protective influence on plants by enhancing the production of antimicrobial compounds. This effect was attributed to silicons's ability to modulate the intricate network of signal pathways, leading to the activation of defense-related gene expression.

Acetic acid is commonly used as an antifungal and inhibits both conidial germination and appressorium formation of fungal growth [8]. It is effective in controlling common bunt in wheat (*Tilletia tritici*), leaf stripe in barley (*Pyrenophora graminea*) [9, 10] and other pathogens in seeds [11]. Acetic acid and fulvic acid are considered as chemical fungicides for the control of some plant diseases such as *Fusarium* wilt, root rot, powdery and downy mildew [12, 13]. [14] found that fulvic acid increases some antioxidants such as α-tocopherol, superoxide dismutase, α-carotene and ascorbic acid which induce systemic resistance in plants. [15] also reported that fulvic acid contains phenolic compounds that played an important role in inducing systemic resistance in plants. Additionally, elevated levels of antioxidants like α-tocopherol, α-carotene, superoxide dismutase, and ascorbic acid have been reported [14]. The application of hardwood biochar has been shown to enhance systemic resistance [16] and promote the proliferation of beneficial organisms such as Pseudomonas and arbuscular mycorrhizal fungi (AMFs) [17]. The application of acetic acid has a positive influence on the germination parameters of carrot seeds contaminated with impurities. Nevertheless, adverse effects on seed germination ability were observed at the highest concentration, as indicated by [18]. In contrast, salicylic acid did not exhibit any detrimental impact on the assessed vegetative growth characteristics. However, elevated doses of salicylic acid were found to adversely affect the healthy flowering capacity of plants in outdoor conditions, as reported by [19]. The objective of this work was to derive an effective treatment method using fulvic acid, silica gel, acetic acid and charcoal at different concentrations on reducing the disease severity of neck rot disease of onions.

2. Material and methods

2.1 Preparation and culturing of pathogen

Brown scales at the neck of diseased onion were collected from random regions in Assiut governorate, washed in running tap water then the leaves were cut using sterilized scalpel into small pieces (1 – 2 cm^2). The fragments were subjected to sterilization in a 1% hypochlorite solution for three minutes, followed by multiple rinses with sterilized water to eliminate any residual disinfectant solution. Afterward, the pieces were air-dried on sterile filter paper before being transferred to Petri dishes containing Potato Dextrose Agar medium (PDA) supplemented with an antibacterial agent. These dishes were then placed in an incubator at 25 °C for three days. The identified fungi were cross-referenced with the characteristics described in colony descriptions by [20, 21]. To purify the *Botrytis allii* isolates, a single spore technique was employed to cultivate them into pure cultures, following the methodology outlined by [22].

2.2 Pathogenicity test

Onion bulbs, seemingly free of any infection by the *B. allii* Giza 6 cultivar, underwent a thorough cleansing with tap water. Subsequently, they were surface sterilized by immersing them in a 1% sodium hypochlorite solution for 2-3 minutes. Afterward, the bulbs were rinsed again with sterile distilled water and left to air-dry at room temperature. Spores of 4-6 days of *Botrytis allii* colony grown on PDA were placed on pathogen-free bulbs which have been cut at the neck of the diseased onion with sterile scissor. Inoculated bulbs coating with Parafilm and incubated at 24°C for two weeks. Controls were prepared by cut bulbs having no pathogen and kept under similar conditions [5].

2.3 *In vitro* studies

The aim was to investigate the antagonistic effect of fulvic acid (3%, 5% and 7% conc.), silica gel (1 %, 2 % and 3 % conc.), acetic acid (0.5 %, 0.8 % and 1 % conc.) and charcoal (0.5%, 1% and 2 % conc.) on mycelial growth of *Botrytis allii*. These compounds were obtained from Sigma-Aldrich Co., St. Louis, MI, USA and made in different concentrations from each other according to previous reports and different in mode of actions of these compounds on plant and fungal pathogens. Also, the highest concentrations of the tested compounds were those reported to negatively affect seed germination ability and healthy flowering capacity ([18, 19]. Various concentrations of each treatment, in ten millilitres of solution, were added to the PDA medium prior to solidification. The plates were then inoculated at the centre with 5-mm discs of *B. allii* derived from a 7-day-old culture. *Botrytis allii* without any treatments served as the control. Following incubation, the mycelial linear growth diameter (cm) was measured to assess the inhibition of the causal pathogen, as per the equation outlined by [23].

R=(C–T/C) x 100.   (1)
Where, R is the percentage of growth reduction, C is control hyphal growth diameter, and T is treated hyphal growth diameter.

2.4 In green house studies

The following experiment was carried out in the open greenhouse of Agricultural Assiut University, during the 2022/2023 growing seasons. The onion seedlings were treated with 5% fulvic acid, 3% Silica gel, 1% acetic acid and charcoal (2%). These concentrations were chosen based on previous studies as the optimal concentrations with antifungal activity. In this investigation, 40-day-old Onion seedlings of the Giza-6 cultivar underwent surface sterilization through immersion in a 0.1% sodium hypochlorite solution for 3 minutes. Following sterilization, the seedlings were rinsed three times with sterile distilled water, left to air dry at room temperature, and subsequently planted in formalin-sterilized plastic pots with a diameter of 30 cm. Each pot was filled with 5.0 kg of formalin-sterilized loam soil, accommodating 4 seedlings per pot. Four weeks after transplantation, the neck of the onion bulbs was inoculated by spraying 10 ml of a spore suspension using a hand atomizer, following the method outlined by [24]. For the control, only 10 ml of sterilized distilled water (the untreated control) was also sprayed onto the seedling surfaces. Three replicate pots were used for each isolate tested. One month post-inoculation, we documented visual manifestations of neck rot symptoms. The individual bulbs underwent assessment for disease severity (DS) on a scale of 0-4: 0 denoting no rot, 1 indicating rot confined to the neck, 2 representing upper third with rot, 3 signifying upper two-thirds with rot, and 4 indicating more than two-thirds with rot [25]. Subsequently, the percentages of disease incidence (DI) and disease severity (DS) were computed using the following formulas:

$$DI\% = \text{No. of infected plants} / \text{Total plants} \times 100$$

$$DS\% = (\Sigma\, Si \times Ni) / (4 \times Nt) \times 100$$

Where Si corresponds to the severity of rot on a scale of 0-4, Ni represents the number of plants in each rotting category, and Nt stands for the total number of rotted plants.

2.5 In field studies

The experiment design in each treatment was done in open Agricultural Assiut University; during the 2022/2023 growing seasons. Three plots 3.5×3.5 m each were used as replicates. Each experimental plot consisted of three rows spaced 70 cm apart, with 50 seedlings planted in each row. Control plots were left untreated. In this study, onion seedlings underwent treatment with a solution containing 5% fulvic acid, 3% silica gel, 1% acetic acid, and 2% charcoal. The treatment involved spraying 10 ml of each concentration on seedlings at ten days of age. Thirty days after transplanting, the neck of onion bulbs was inoculated with *B. allii* by applying 10 ml of inoculum per plant using a hand atomizer. After one month of inoculation, the percentages of DI and DS were recorded, following the methodology outlined by [2].

3. Results and discussion

3.1 Pathogenicity test

The information provided in Table 1 reveals that all tested *Botrytis allii* isolates are capable of inducing neck rot disease in onions. Additionally, the data illustrates that isolate No. 3 exhibited a particularly high severity, reaching 62%. *Botrytis allii* the main causal agent of neck rot disease on onion [25, 26].

3.2 *In vitro* studies

Treating onion seedling with 7% fulvic acid, 3% silica gel, 1% acetic acid and 2% charcoal reduced the mycelial linear growth of *B. allii* at all tested concentrations. The highest reduction of the pathogen growth was detected by 1%, 0.8 % and 0.5 % acetic acid (73.7%, 63.7 %, and 61.2%, respectively), followed by 2% of charcoal (52%) and 3% of Silica gel (47.5%) . In contrast, fulvic acid at 3% concentration caused the lowest inhibitory effect and reduced the mycelial linear growth by 20% compared to the control (Table 2, Figure 2). Acetic acid is a carboxylic acid known for its antimicrobial properties which inhibited both conidial germination and appressorium formation of fungal growth [8]. Acetic acid is considered a chemical fungicide and is effective in controlling the growth of bacteria causing soft root rot disease in sugar beet [27]; on grey mould in fruits (*Botrytis cinerea*) [28]; seeds of onion (*Alternaria alternata, Botrytis cinerea, Cladosporium* spp., *Penicillium* spp. and *Stemphylium botryosum*) [11], wheat (*Tilletia tritici*), leaf stripe in barley (*Pyrenophora graminea*) [9, 10]. The use of hardwood biochar increased systemic resistance [16]. Salicylic acid induced systemic resistance after an initial pathogen attack [29]. Salicylic acid contains mono hydroxyl benzoic acid with an ortho and para position of OH- group and can be very toxic to fungi [30]. [2] reported that salicylic acid at 20 mM caused the highest reduction against *Botrytis allii* causing neck rot disease of onion *in vitro*.

Table 1. Pathogenicity test of *Botrytis allii* isolates causal pathogen of neck rot of onion.

Isolates	No. of isolate	Disease severity %
Botrytis allii	1	45
Botrytis allii	2	51
Botrytis allii	3	62
Botrytis allii	4	44

Table 2. Effect of different concentrations of fulvic acid, salicylic acid, acetic acid and charcoal on the mycelial linear growth of *B. allii in vitro*.

Compounds tested	Concentrations (%)	Mycelial linear growth (cm)	% Growth inhibition of the pathogen
Acetic acid	1	2.1	73.7±1.2
	0.8	2.9	63.7±2
	0.5	3.1	61.2±3
Charcoal	2	4	52±2.8
	1	4	50±2.1
	0.5	3.9	51.2±2
Fulvic acid	7	4.0	50±3.8
	5	4.2	47.5±3.8
	3	6.4	20±8
Silica gel	3	4.2	47.5±1.2
	2	5	37.5±4
	1	6.2	22.5±3.8
Control		8	0

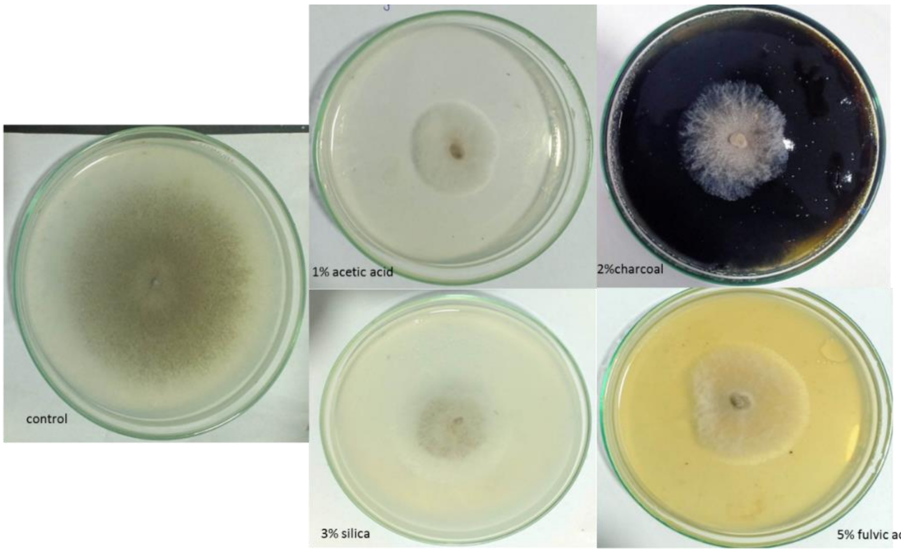

Figure 1. Effect of fulvic acid, silica gel, acetic acid and charcoal on the mycelial linear growth of *B. allii in vitro*.

3.3 In green house and field conditions

The results showed that in green house and field conditions, silica gel and acetic acid with concentration of 3%, and 1%, respectively were the best treatments and were shown to inhibit the disease up to 85.7 % in green house and up to 70.4% and 69 % in field, respectively, compared to control, while fulvic acid with concentration 5% had the lowest disease reduction up to 33.3% in green house and charcoal with concentration in field by 40.1% reduction compared to control (Table 3, Figure 3). Silicon improves the activities of chitinases, peroxidases, polyphenol oxidases and flavonoid phytoalexins, these enzymes play an important role of increased the natural defence system of the plant therefore the resistance of the plant to fungal pathogens [31]. [2, 32] showed that salicylic acid was the best treatment in reducing the mycelial growth of *B. allii* the causal agent of neck rot diseases of onion in both greenhouse and field trials. [27] reported that 5% acetic acid controlling the growth of bacteria responsible. [11] found that treatment onion seeds with 1 and 2% acetic acid reduced the growth of *Alternaria alternata*, *Botrytis cinerea*, *Cladosporium* spp., *Penicillium* spp. and *Stemphylium botryosum* after storage. In the study conducted by [33], it was revealed that the prevalence of *Botrytis aclada*, a prominent fungal pathogen during low-temperature storage, exhibited a significant reduction of up to 96% when treated with a thymol solution compared to the untreated control. Furthermore, [5] demonstrated that incorporating a novel Iranian isolate of Streptomyces into onion plants enhanced the resistance of the bulbs against *Botrytis allii*, the causative agent of neck rot. This innovative approach not only provides a sustainable alternative but also contributes to a reduction in the reliance on environmentally harmful fungicides in agricultural fields.

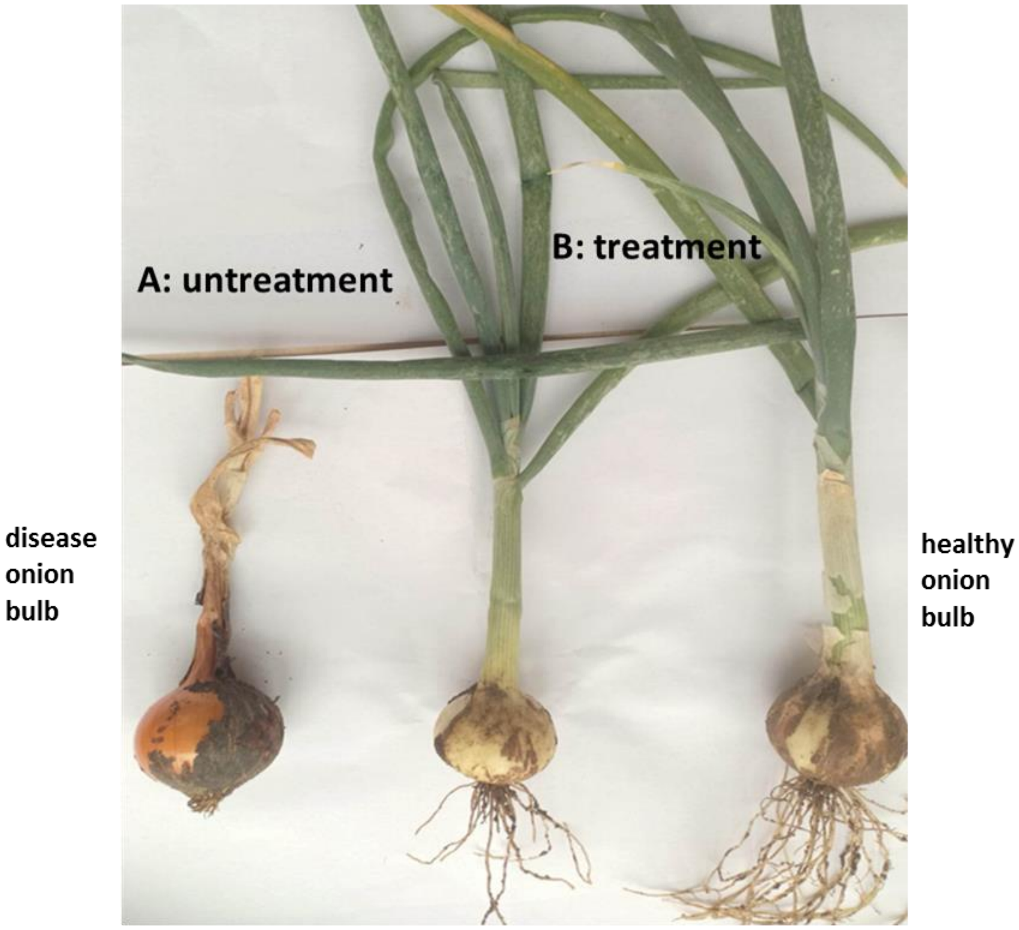

Figure 3. Showing (A) Untreated onion bulbs, (B) Treated onion bulbs.

Table 3. Effect of fulvic acid, salicylic acid, acetic acid and charcoal on the percentage of disease severity and inhibition in greenhouse and field conditions.

Compounds tested	Concentration (%)	**Greenhouse conditions**	**Field conditions**
		% inhibition	% inhibition
Acetic acid	1	85.7±1.2	69±2
charcoal	2	57.1±3	40.1±4
Fulvic acid	7	57.1±3.6	46.7±2.2
Salicylic acid	3	85.7±1.2	70.4±2.1
control		0	0

4. Conclusion

The results showed that silica gel and acetic acid with concentration of 3%, and 1%, respectively were the best treatment and were shown to inhibit the disease up to 85.7 % in green house and up to 70.4% and 69 % in field, respectively compared to control so that we considered that the two treatments would be suitable applications in the limited and available against Botrytis that causes onion neck rot.

Conflict of interest

The authors declare no conflict of interest.

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References

1. FAOSTAT. (2021). Rome, Italy: Food and Agriculture Organization (FAO); Available from http://www.fao.org/faostat/en/#data/QC.
2. Salama, M., Asran, M.R. and Moharam, M. Toxicity of some nanomaterials against *Botrytis allii*, the causal

pathogen of neck rot disease of onion (Allium cepa L.). *Journal of Sohag Agriscience*, 2021, **6(1)**, 8-19.
3. Yohalem, D.S., Nielsen, K. and Nicolaisen, M. Taxonomic and nomenclatural clarification of the onion neck rotting *Botrytis* species. Mycotaxon, 2003, **85**, 175-182.
4. Delahaut, K. and Stevenson, W. Onion disorders: *Botrytis* leaf blight, leaf fleck, and neck rot. A3803 University of Wisconsin Extension publication, USA. 2004.
5. Jorjandi, M., Bonjar, G.S., Baghizadeh, A., Sirchi, G.S., Massumi, H., Baniasadi, F. and Farokhi, P.R. Biocontrol of *Botrytis allii* Munn the causal agent of neck rot, the post harvest disease in onion, by use of a new Iranian isolate of *Streptomyces*. *American Journal of Agricultural and Biological Sciences*, 2009, **4(1)**, 72-78.
6. Ibrahim, S.D. Fungal pathogens Associated with Stored Onions in Maiduguri and Bama Towns of Borno state. Unpublished M.Sc. thesis, University of Maiduguri, 35-40.
7. Wang, M., Gao, L., Dong, S., Sun, Y., Shen, Q. and Guo, S. Role of silicon on plant–pathogen interactions. *Frontiers in Plant Science*, 2017, **8**, 701.
8. Kuroki, M., Shiga, Y., Narukawa-Nara, M., Arazoe, T. and Kamakura, T. Extremely low concentrations of acetic acid stimulate cell differentiation in rice blast fungus. *Iscience*, 2020, **23(1)**, 27.
9. Borgen, A. and Nielsen, B. Effect of seed treatment with acetic acid in control of seed borne diseases. In Proceedings of the BCPC Symposium No. 76: Seed Treatment: Challenges and Opportunities, 2001, 76(135-140).
10. Borgen, A., Klaedtke, S., Feher, J., Thüringer, A., Petcu, V., Boffin, L. and REY, F. Vinegar seed treatment to control common bunt in wheat. *EUCARPIA Breeding and Seed Sector Innovations for Organic Food Systems*, 2021, **1**, 111-112.
11. Dorna, H., Rosińska, A. and Szopińska, D. The Impact of Acetic Acid Treatments on Stored Onion (Allium cepa L.) Seeds' Quality. *Agriculture*, 2023, **13(7)**, 1327.
12. Abdel-Monaim, M.F., Abdel-Gaid, M.A. and Zayan, S.A. Effectiveness of organic compounds in controlling root rot/wilt diseases, growth and yield parameters of pepper. *International Journal of Agricultural Sciences*, 2014, **3**, 81-89.
13. Kamel, S. M., Afifi, M. M., El-shoraky, F. S. and El-Sawy, M. M. Fulvic acid: a tool for controlling powdery and downy mildews in cucumber plants. *International Journal of Phytopathology*, 2014, **3(2),** 101-108.
14. Zhang, X. (1997). Influence of plant growth regulators on turfgrass growth, antioxidant status, drought tolerance (Doctoral dissertation, Virginia Polytechnic Institute and State University).
15. Hahlbrock, K. and Scheel, D. Physiology and molecular biology of phenyl propanoid metabolism. *Annual Review of Plant Biology*, 1989, **40(1)**, 347-369.
16. Poveda, J., Abril-Urias, P., and Escobar, C. Biological control of plant-parasitic nematodes by filamentous fungi inducers of resistance: *Trichoderma*, mycorrhizal and endophytic fungi. *Frontiers in Microbiology*, 2020,11, 992.
17. Elmer, W.H. Effect of leaf mold mulch, biochar, and earth worm son mycorrhizal colonization and yield of asparagus affected by Fusarium crown and root rot. *Plant Disease*, 2016, **100**. 2507-2512.
18. Othmen, S., Elimem, M., Abir, H., Abbes, K. and Chermiti, B. The effect of acetic acid treatment on germination parameters of carrot seeds infected with *Candidattus Liberibacter solanacearum*, 2019, **10(1)**, 207-212.
19. Cavusoglu, A., Ozer-Uyar, G. and Unal, F.İ.L.İ.Z. An abiotic stress factor: Phytotoxicity of high doses of salicylic acid on growth parameters of *Tulip* sp. Under glasshouse and outdoor conditions. *Applied Ecology and Environment Research*, 2022, **20(6)**.
20. Chilvers, M.I and dutoit, L.J. Detection and Identification of *Botrytis* species associated with Neck Rot, Scape Blight and Umbel Blight of onion. *Plant Health Progress*, 2006, **7(1)**, 38.
21. Robert, A.S and Ellen, S.V.G. Introduction of Foodborne Fungi, Pomsen and Looijen, B.W, Wageningen, 1988.
22. Shabana, Y., 1987. Biological control of water weeds by using plant pathogens. M.Sc. thesis, Faculty of Agriculture, Mansoura University, Egypt. Pp. 78.
23. Hussein, M.A.M., Hassan, M.H.A., Allam, A.D.A. and Abo-Elyousr, K.A.M. Management of *Stemphylium* blight of onion by using biological agents and resistance inducers. *Egypt Journal of Phytopathology*, 2007, **35(1)**, 49-60.
24. Kaufman, J. and Lorbeer, J.W. Control of *Botrytis* neck rot of onions by fungicidal dusts and desiccant chemicals. *Plant Disease Reporter*, 1967, **51**, 696-699.
25. Köhl, J., Molhoek, W.M.L. and Fokkema, N.J. Biological control of onion neck rot (*Botrytis aclada*): Protection of wounds made by leaf topping. *Biocontrol Science and Technology*, 1991, 261-269.
26. Nielsen, K., Justesen, A.F., Jensen, D.F. and Yohalem, D.S. Universally primed polymerase chain reaction alleles and internal transcribed spacer restriction fragment length polymorphisms distinguish two subgroups in Botrytis aclada distinct from B. byssoidea. *Phytopathology*, 2001, **91(6)**, 527-533.
27. Abdalla, M.E., Seadh, S.E. and Hamza, S. Inhibitory effect and morphological changes by organic acids to bacterial strains causing sugar beet soft root rot in vitro. *Journal of Plant Protection and Pathology*, 2019, **10(3)**, 187-193.
28. Ruiz-Moyano, S., Hernández, A., Galvan, A.I., Córdoba, M.G., Casquete, R., Serradilla, M.J., and Martín, A. Selection and application of antifungal VOCs-producing yeasts as biocontrol agents of grey mould in fruits. *Food Microbiology*, 2020, **92**, 103556.
29. Hammerschmidt R., Metraux, J.P. and van Loon L.C.The role of salicylic acid in disease resistance. Slusarenko A., R.S.S. Fraser, Van Loon L.C. (Eds.), In Mechanisms of Resistance to Plant Diseases, Kluwer Academic Publisher, 2000, 37-53.

30. Huang, W.Y., Cai, Y.Z. and Zhang, Y. Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. *Nutrition and Cancer*, 2009, **62(1)**, 1-20.
31. Fawe, A.B., Abou-Zaid, M., Menzies, J.G. and Bélanger, R.R. Silicon-mediated accumulation of flavonoid phytoalexins in cucumber. *Phytopathology*, 1998, **88(5)**, 396-401.
32. El-Babley and Hala F.M. Integrated control of onion black mould and neck rot diseases. Ph.D. Thesis, Faculty of Agriculture, Minoufiya University, Minoufiya, Egypt, 132 pp, 2012.
33. Ji, S.H., Kim, T.K., Keum, Y.S. and Chun, S.C. The major postharvest disease of onion and its control with Thymol fumigation during Low Temperature Storage. *Mycobiology*, 2018, **46(3)**, 242-253.