



Effect of eco-friendly treatments on onion downy mildew in a sustainable agricultural system

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Abstract

Onion (*Allium cepa* L.) is an important agricultural crop in Egypt, valued for its contributions to both domestic consumption and exportation. However, onion cultivation faces significant challenges, particularly from downy mildew, which is caused by *Peronospora destructor* (Berk.) Casp. To evaluate the efficacy of various eco-friendly treatments in field trials against onion downy mildew, a range of biological control agents (Serenade ASO, Tricho Nitro Plus, Bio Control T34) and a natural fungicide (Champ DP) were applied. The results indicated that Champ DP was the most effective treatment, significantly reducing the severity of the disease by 80.3% and 76.4% after two and three months of application during the 2020-21 and 2021-22 growing seasons, respectively. Tricho Nitro Plus also performed well, demonstrating a reduction of 80.2% and 76.3% in disease severity. Importantly, all treatments led to an increase in onion bulb yield, as well as enhanced total carbohydrates, total soluble solids, and plant enzyme defenses. This research underscores the potential for integrating biological and natural fungicides to sustainably address the challenges posed by plant pathogens in onion plants, ultimately supporting local food security and agricultural export opportunities in Egypt.

Keywords: Onion, *Allium cepa*, *Peronospora destructor*, bio-fungicide, Champ DP.

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1. Introduction

Onion (*Allium cepa* L.) is the most commonly grown species in the *Allium* family. In Egypt, this crop plays a crucial role in both domestic consumption and export markets. Onions are rich in phytochemicals, particularly medicinal flavonoids (Javadzadeh et al., 2009), which contribute to their defense mechanisms and overall quality. Downy mildew, caused by the pathogen *Peronospora destructor*, is a widespread disease affecting onions and other *Alliums*. This infection can significantly damage onion plants by inducing early leaf dieback and infecting the neck of the bulb, leading to unmarketable greens and reduced bulb size and storage quality (Pablo and Colnago, 2011). Additionally, downy mildew infections can make crops more susceptible to other diseases (Ankita et al., 2019; Kamel et al., 2017; Cook, 2015). Recent concerns have emerged about the high toxicity of many fungicides, which can disrupt ecological balance and negatively impact both human health and the environment (Turini, 2004). Research indicates that utilizing antagonistic microorganisms can effectively reduce pathogen levels and control downy mildew in onions without relying on chemical treatments (Ahmed et al., 2017). Biofungicide treatments,

such as Plant Guard, Blight Stop, Bio Zeid, Bio Arc, and Clean Root have shown greater efficacy than the recommended fungicide Amistar Top in reducing the severity of downy mildew in onion plants (Shahin, 2017). These treatments also increase onion bulb yield and enhance the chemical components and enzyme activity associated with defense mechanisms (Bhatti et al., 2021; Khan et al., 2020; Turini, 2004). The goal of this research is to promote biological control as an eco-friendly and acceptable alternative to chemical methods for managing downy mildew disease in onions. This study specifically investigates the efficacy of several biocides and a natural fungicide. This approach aims to enhance the production of healthy, toxin-free onions, ensuring high yield and quality in the food chain.

2. Materials and methods

2.1 Treatments

In this study, three biocides and one natural fungicide (Champ DP 57.6% WG) were utilized to investigate their efficacy against onion downy mildew under field conditions. The details and specifications of all the fungicides used are provided in Table (1).

Table 1: Details and specifications of used fungicides.

Common name	Active ingredient	Concentration	Dose/100 L/feddan*	Company
Serenade ASO 1.34% SC	<i>Bacillus subtilis</i> QST 713	10 ⁹ cfu/g	1 L	Bayer
Tricho Nitro Plus WP	<i>Trichoderma</i> spp.	10 ⁷ spores/ml	1 L	Bio Egypt
Bio Control T34 12% WP	<i>Trichoderma asperellum</i>	10 ⁷ spores/gm	85 g	Shoura
Champ DP 57.6% WG	Copper hydroxide	57.6 %	180 g	Nofarm

* Feddan = 4200 m² = 0.420 hectares = 1.037 acres.

2.2 Field experiment

This study was conducted in fields infected with *Peronospora destructor* during the 2020-21 and 2021-22 growing seasons at the Riyadh

Al-Saleheen experimental farm in Sohag Governorate, Egypt, using a drip irrigation method. A complete randomized block design was employed, with three replicate plots for each treatment and control. Each plot

measured 10.5 m² (3.0 × 3.5 m). Onion transplants of the 'Giza 6' variety, 60 days old, were transplanted on October 15th in both seasons, with approximately 75 plants per row, totaling 450 plants per plot. Early morning foliar spraying was conducted at regular intervals, repeated seven times every two weeks from January 1st to the end of March. Control plants were sprayed with distilled water only. The different treatments were mixed with 50 ml of surfactant and sticker material per 100 liters. All treatments received the recommended farming procedures until harvest in May.

2.3 Disease assessment

One hundred leaves from each plot were randomly collected to determine the disease severity after 2 and 3 months of treatment. Severity was rated on a scale of 1 to 9, where 1 represents no disease symptoms and 9 represents severe disease symptoms with extensive leaf damage, and the disease severity index was assessed using the following formula as developed by Mohibullah (1991):

$$DSI = \frac{\sum (n \times v)}{ZN} \times 100$$

Where: n = number of leaves in each category, v = numerical value of each category, Z = maximum category value, N = total number of leaves sampled.

2.4 Biochemical changes

One week after the last spray, onion plant samples were collected, and peroxidase (PO) and polyphenol oxidase (PPO) activities were assessed according to the methods described by Maxwell and Bateman (1967) and Thimmaiah (1999), respectively. While, at the end of each season, total soluble solids (TSS) were measured using a Carl Zeiss hand

refractometer, and total carbohydrates were determined in the liquid extract of onion bulbs following the procedure outlined by Dubois et al. (1956).

2.5 Onion bulbs yield

At the end of each season, onion bulbs yield was weighed for each treatment and expressed as kg/plot.

2.6 Statistical analysis

The collected data were statistically analyzed using MSTAT software. The least significant difference (LSD) at a 0.05 level of probability was employed to compare treatment means, following the guidelines established by Snedecor and Cochran (1989).

3. Results

3.1 Effectiveness of different treatments on *Peronospora destructor*

The data presented in Table (2) indicate that various treatments were effective in reducing the severity of onion downy mildew during the 2020-21 and 2021-22 growing seasons. Notably, the analysis shows that the percentage of disease severity was significantly higher three months after planting compared to two months across both seasons. This observation emphasizes the importance of timely intervention in managing the disease. Among the treatments assessed, Champ DP stood out as the most effective natural fungicide, achieving a remarkable reduction in disease severity of 80.3% after two months and 76.4% after three months of application. Following close behind was Tricho Nitro Plus, a biocide, which also demonstrated impressive efficacy with reductions of 80.2% and 76.3%, respectively,

further underscoring its potential as a treatment option. Bio Control T34 is ranked as the second most effective biocide. In contrast, Serenade ASO 1.34% SC performed relatively

poorly, showing the least effectiveness with a disease reduction of onion downy mildew in the respective seasons, ranking as the least effective treatment throughout the study.

Table 2: The impact of foliar spraying* of five eco-friendly fungicides on onion downy mildew disease severity under field conditions at Riyadh Al-Saleheen experimental farm, Sohag Governorate, Egypt during 2020-21 and 2021-22 growing seasons.

Treatments	Dose/feddan**	Disease severity (%)							
		After 2 months				After 3 months			
		2020-21	2021-22	Mean	Efficacy***	2020-21	2021-22	Mean	Efficacy
Serenade ASO	1 L	12.4	12.7	12.6	77.8	15.4	15.7	15.6	74.1
Tricho Nitro Plus	1 L	11.1	11.3	11.2	80.2	14.1	14.3	14.2	76.3
Bio Control T34	85 g	11.3	11.5	11.4	79.8	14.3	14.5	14.4	76.0
Champ DP	180 g	11.0	11.3	11.2	80.3	14.0	14.3	14.2	76.4
Control (Untreated)	-	50.0	63.0	56.5	0.0	55.0	65.0	60.0	0.0
LSD at 5%		1.2	0.9	-	-	1.3	1.1	-	-

* Seven sprays, one spray every two weeks, started on the first of January to the end of March. ** Feddan = 4200 m² = 0.420 hectares = 1.037 acres. *** Efficacy = $\frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$.

3.2 Biochemical changes

The results presented in Table (3) and Figure (1) illustrate the impact of various treatments on several biochemical changes, including total carbohydrates, total soluble solids (TSS), peroxidase (PO), and polyphenol oxidase (PPO). All treatments significantly increased the tested biochemicals in treated onion plants compared to untreated ones over the two growing seasons under field conditions. Among the treatments, Tricho Nitro Plus

outperformed Champ DP, Bio Control T34, and Serenade ASO as the most effective treatment, leading to significant improvements in all tested parameters, highlighting the potential of Tricho Nitro Plus to enhance plant health and productivity. Bio Control T34 is ranked as the second most effective compound when compared to untreated plants. Conversely, Serenade ASO was identified as the least effective treatment, which correlated with lower yield components across both experimental seasons.

Table 3: The impact of foliar spraying* of five eco-friendly fungicides on some biochemical changes under field conditions at Riyadh Al-Saleheen experimental farm, Sohag Governorate, Egypt during 2020-21 and 2021-22 growing seasons.

Treatments	Dose/feddan**	Total carbohydrates (mg/g dry weight)		TSS (%)		PO (unit/mg protein)		PPO (unit/mg protein)	
		2020-21	2021-22	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22
Serenade ASO	1 L	1.440	1.530	11.870	12.130	0.167	0.172	0.026	0.028
Tricho Nitro Plus	1 L	1.870	1.920	15.720	15.980	0.225	0.231	0.047	0.049
Bio Control T34	85 g	1.830	1.880	14.210	14.460	0.199	0.204	0.038	0.041
Champ DP	180 g	1.580	1.650	12.260	12.520	0.181	0.187	0.031	0.034
Control (Untreated)	-	0.160	0.180	10.150	10.870	0.063	0.065	0.010	0.011
LSD at 5%	-	0.110	0.140	0.490	0.510	0.021	0.022	0.007	0.008

* Seven sprays, one spray every two weeks, started on the first of January to the end of March. Samples for PO and PPO were collected one week after the final spray, and at the end of the season for total carbohydrate and TSS. ** Feddan = 4200 m² = 0.420 hectares = 1.037 acres.

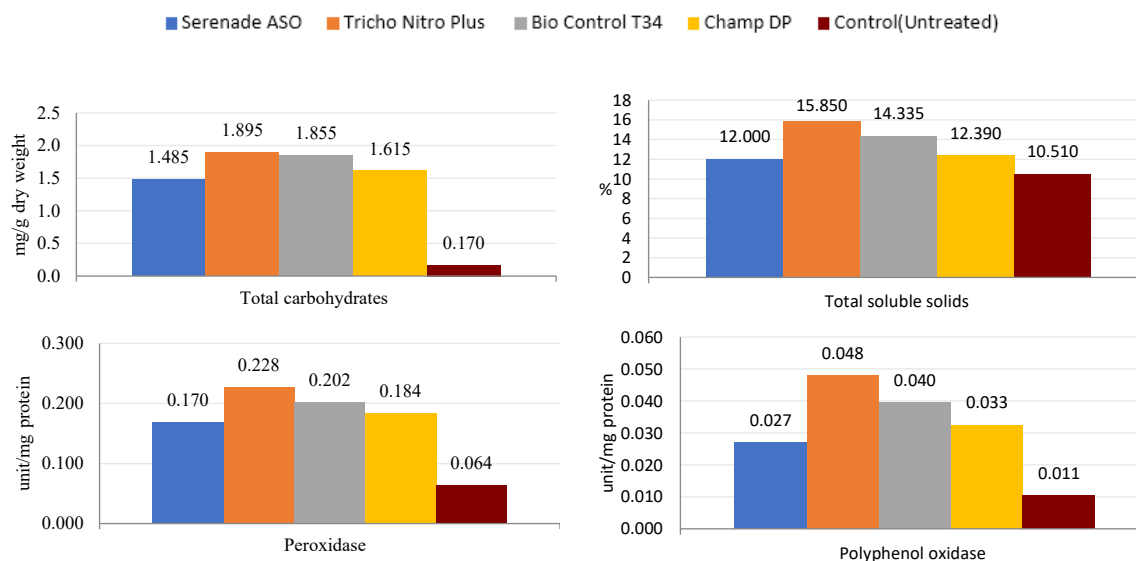


Figure 1: The effect of foliar spraying with Serenade ASO, Tricho Nitro Plus, Bio Control T34 and Champ DP compared to untreated plants as means of the 2020-21 and 2021-22 growing seasons under field conditions at Riyadh Al-Saleheen experimental farm, Sohag Governorate, Egypt.

3.3 Onion bulbs yield

Table (4) provides data indicating that applying fungicides and biocides through foliar treatment has significantly improved onion yields during the 2020-21 and 2021-22 growing seasons. The overall yield per plot showed substantial improvement, increasing to 43.3 kg per plot in the 2020-21 season and reaching 44.6 kg per plot in the following season when using Tricho Nitro Plus. Bio Control T34 also showed promising results with yields of 40.9 kg and 41.3 kg per plot in

the respective seasons. In contrast, Serenade ASO was the least effective treatment across both growing seasons. These results suggest that employing biological control methods can be beneficial for preventing and managing plant diseases. Integrating these eco-friendly approaches with conventional pest management strategies could form part of a sustainable agriculture strategy. This approach aims to achieve positive agricultural outcomes, protect the environment, reduce reliance on synthetic pesticides, and mitigate their harmful effects.

Table 4: The impact of foliar spraying* of five eco-friendly fungicides on onion yield under field conditions at Riyadh Al-Saleheen experimental farm, Sohag Governorate, Egypt during 2020-21 and 2021-22 growing seasons.

Treatments	Dose/feddan**	Yield (kg/plot)	
		2020-21 growing season	2021-22 growing season
Serenade ASO	1 L	32.8	33.2
Tricho Nitro Plus	1 L	43.3	44.6
Bio Control T34	85 g	40.9	41.3
Champ DP	180 g	35.7	36.9
Control (Untreated)	-	13.2	14.5
LSD at 5%		1.7	1.8

* Seven sprays, one spray every two weeks, started on the first of January to the end of March. ** Feddan = 4200 m² = 0.420 hectares = 1.037 acres.

4. Discussion

This study aims to increase the use of biocides and reduce the use of hazardous chemicals in agriculture, while producing high-quality food in sufficient quantities and enhancing biodiversity within the framework of sustainable agriculture with increasing effective non-chemical strategies to protect onion plants from downy mildew disease. These strategies include the use of biocides and natural fungicides, such as Serenade ASO 1.34% SC (*Bacillus subtilis* QST 713 10^9 cfu/g), Tricho Nitro Plus WP (*Trichoderma* spp. 10^7 spores/ml), Bio Control T34 12% WP (*Trichoderma asperellum* 10^7 spores/g), and Champ DP 57.6% WG (Copper hydroxide 57.6%). Previous treatments have successfully reduced the severity of *Peronospora destructor* the causal pathogen of onion downy mildew over 2020-21 and 2021-22 growing seasons. These findings align with those of Develish and Sugh (2016), as well as Narla et al. (2016), who attribute the effectiveness of these treatments to the combined action of bioagents that produce growth regulators and the chemical action of antioxidants. These factors are known to enhance plant physiology, metabolism, and induce systemic resistance (ISR). The biocontrol agents, *Trichoderma* spp. or *Bacillus* spp., may have helped the host plant develop resistance against the pathogen, contributing to the beneficial effects of these bioagents (Kamel et al., 2017; Raziq et al., 2008). Also, Shahin (2017) similarly noted that the combined action of these bioagents, responsible for generating growth regulators and antioxidants, enhances plant physiology, metabolism, and ISR. These results are consistent with findings by Ankita et al. (2019), who confirmed that all fungicides tested were effective in controlling onion downy mildew (*Peronospora destructor*) due to varying levels of effectiveness. Disease

severity tends to increase with plant age, suggesting that the pathogen behaves like a low-sugar fungus. Additionally, translocation from the source to the sink can elevate disease severity (Wong et al., 1983). Decreased spacing between plants at high densities can also lead to a higher incidence of downy mildew (Turini, 2004). Among the treatments, Tricho Nitro Plus was the most effective, resulting in the highest yield components, including total carbohydrates, total soluble solids (TSS), PO, PPO and onion bulb yield, compared to the control treatment over the two growing seasons (2020-21 and 2021-22). Bio Control T34 was the second most effective treatment, and these findings are consistent with those of Ahmed et al. (2017), which indicated that *Trichoderma* spp. enhance the aforementioned crop characteristics, and surplus production of phenolic compounds leads to higher yields when applied to various field crops (Khan et al., 2020; Fira et al., 2018). All treatments effectively maintained plant health and promoted optimal growth in onion plants, as evidenced by the control treatment's notably low chemical content (Bhatti et al., 2021). Growth regulators like IAA may contribute to this growth promotion (Wong et al., 1983). Furthermore, treatments that provided significant plant protection and disease reduction were associated with increases in total carbohydrates, total nitrogen, and TSS levels. These treatments promote plant growth by solubilizing phosphate and producing the phytohormone indole-3-acetic acid, which positively impacts growth and yield (Turini, 2004). The application of biocides and Champ DP significantly enhanced PO and PPO activities in onion plants compared to untreated plants. Plants treated with biocides showed a notable increase in the activity of these enzymes, which are linked to the host plant's defense mechanisms (Bhatti et al., 2021; Develish and

Sugh, 2016). Wong et al. (1983) observed similar findings, demonstrating that *B. subtilis* isolates effectively controlled white rot disease on onion plants. They attributed this success to the direct suppression of the fungal pathogen *Sclerotium cepivorum* and the increased plant resistance resulting from the activation of defense-related enzymes.

5. Conclusion

In the context of sustainable agriculture, the application of biocides such as Serenade ASO 1.34% SC (*Bacillus subtilis* QST 713, 10^9 cfu/g), Tricho Nitro Plus WP (*Trichoderma* spp., 10^7 spores/ml), and Bio Control T34 12% WP (*Trichoderma asperellum*, 10^7 spores/g), along with the natural fungicide Champ DP 57.6% WG (Copper hydroxide, 57.6%), on onion plants demonstrated effectiveness in managing onion downy mildew and enhancing onion bulb yield, total carbohydrates, total soluble solids, and the activity of PO and PPO. Among the biocides tested, Tricho Nitro Plus demonstrated superior results compared to both the control group and Champ DP.

References

- Ahmed MFA, Amin MM, El-Fiki IAI, 2017. Efficacy of bioagents against *Alternaria porri* incitant of purple blotch of onion in Egypt. *Egyptian Journal of Phytopathology* **45**(1):17–29.
- Ankita CS, Kumar M, Meena VK, 2019. Ecofriendly approach of managing onion downy mildew. *International Journal of Current Microbiology and Applied Sciences* **8**(12): 2474–2480.
- Bhatti TA, Nizamani ZA, Gadhi MA, Soomro F, Kumar R, Abro SA, Soomro AH, Qazi S, Kandhro UJA, Khan M, 2021. Management of downy mildew of onion through selective fungicides in the field condition. *Journal of Applied Research in Plant Sciences* **2**(1), 92–107.
- Cook HT, 2015. Downy mildew disease of onion. New York Agricultural Experiment Station, Ithaca, New York, USA, Memoir No. 143, pp. 1–40.
- Develish RK, Sugh SK, 2016. Management of downy mildew (*Peronospora destructor*) of onion (*Allium cepa*). *Journal of Plant Pathology* **16**(1): 63–67.
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F, 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**: 350–356.
- Fira D, Dimkić I, Berić T, Lozo J, Stanković S, 2018. Biological control of plant pathogens by *Bacillus* species. *Journal of Biotechnology* **10**(285): 44–55.
- Javadzadeh AA, Ghorbanihagho S, Bonyadi MR, Rashidi M, Mesgari M, Rashtchizadeh N, Argani H, 2009. Prevention effect of onion juice on selenite-induced experimental cataract. *Indian Journal of Ophthalmology* **57**:185–189.
- Kamel SM, Ismail AM, Omara RI, Ahmed MFA, 2017. Influence of humate substances and fungicides on the control of onion downy mildew. *Egyptian Journal of Phytopathology* **45**(1): 31–44.
- Khan N, Bano AM, Babar A, 2020. Impacts of plant growth promoters and plant growth regulators on rainfed agriculture. *PloS One* **15**(4): e0231426.
- Maxwell DP, Bateman DF, 1967. Changes in the activities of some oxidase in extracts of *Rhizoctonia* infected bean hypocotyls in relation to lesion maturation. *Phytopathology* **57**: 132–136.

- Mohibullah K, 1991. Studies on major diseases of bulb vegetables (onion and garlic) in NWFP. Final Technical Report Agricultural Research Institute Tarnab, Peshawar, Khyber Pakhtunkhwa, Pakistan.
- Narla RD, Muthomi JW, Gachu SM, Nderitu JH, Olubayo FM, 2016. Effect of intercropping bulb onion and vegetables on purple blotch and downy mildew. *Journal of Plant Science* **45**(1): 154–162.
- Pablo HG, Colnago P, 2011. Quantitative studies on downy mildew (*Peronospora destructor* Berk. Cusp.) affecting onion seed production in Southern Uruguay. *European Journal of Plant Pathology* **129**: 303–314.
- Raziq F, Alam I, Naz I, Khan H, 2008. Evaluation of fungicides for controlling downy mildew of onion under field conditions. *Sarhad Journal of Agriculture* **24**(1): 85–92.
- Shahin SI, 2017. Effect of some biocides on development of the onion downy mildew disease, caused by *Peronospora destructor* (Berk.). *Egyptian Journal of Biological Pest Control* **27**(1): 71–77.
- Snedecor GW, Cochran WG 1989. Statistical methods, 8th Edition. Iowa State University Press, Ames, Iowa, USA, pp. 503
- Thimmaiah SK, 1999. Standard methods of biochemical analysis. Kalyani Publishers, New Delhi, India, pp. 545.
- Turini TA, 2004. Comparison of fungicides for control of onion downy mildew, 2004. *Plant Disease Management Report* **60**: V124.
- Wong J, Utkhede RS, Rahe JE, 1983. Biological control of onion white rot. *Soil Biology and Biochemistry* **15**(1): 101–104.