

Impact of eco-friendly treatments on managing onion downy mildew

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ABSTRACT

Onion (*Allium cepa* L.) is a significant agricultural crop in Egypt, valued for their contributions to both domestic consumption and export markets. However, onion cultivation faces considerable challenges, notably from downy mildew, caused by *Peronospora destructor* (Berk.) Casp. Serenade ASO 1.34% SC (*Bacillus subtilis* QST 713 1×10^9 cfu/g) and Tricho Nitro Plus WP (*Trichoderma* spp. 10^7 spores/ml) were applied at a concentration of 1 L/100 L water/feddan, while Bio Control T34 12% WP (*Trichoderma asperellum* 10^7 spores/g) and Champ DP 57.6% WG, a copper hydroxide-based fungicide were applied at a concentration of 85 and 180 g/100 L water/feddan respectively to study their effect on onion downy mildew. Champ DP emerging as the most effective solution, significantly reducing the severity of disease by 80.3% and 76.4% after two and three months of application during the 2022-23 and 2023-24 growing seasons. Tricho Nitro Plus followed closely, demonstrating a reduction of 80.2% and 76.3% in disease severity, highlighting its potential as an effective biocide. Notably, all treatments resulted in increasing in onion bulbs yield and enhanced total carbohydrate, total soluble solids and plant enzymes defenses. In conclusion, the application of all treatments, particularly Champ DP and Tricho Nitro Plus, significantly mitigates onion downy mildew, thereby enhancing the yield and quality. This research underscores the potential of integrated management approaches that combine chemical and biological treatments to sustainably address the challenges posed by plant pathogens in onion cultivation, fostering both local food security and agricultural export opportunities in Egypt.

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

INTRODUCTION

Onion (*Allium cepa* L.) is the greatest commonly grown *Allium* specie. Egypt relies heavily on this crop for both domestic and export markets. It contains a high concentration of phytochemicals, specifically medicinal flavonoids (Javadzadeh *et al.*, 2009).

Downy mildew caused by *Peronospora destructor* is a common fungus-like disease associated with onions and other *Alliums*. Infections generally damage onion plants, although they do induce early leaf dieback and infect the neck of the bulb, providing greens unmarketable and decreasing bulb size and storage quality (Pablo and Colnago, 2011). Downy mildew infection makes crops more sensitive to other diseases (Cook, 2015; Kamel *et al.*, 2017 and Ankita *et al.*, 2019).

Humans have recently noticed that the high toxicity of many fungicides might disrupt the delicate balance of ecology and have negative effects on both human health and the environment (Getinet *et al.*, 2024).

Research suggests that using antagonistic microorganisms can reduce pathogen inoculum levels and effectively control downy mildew disease in onions without the use of chemicals (Ahmed *et al.*, 2017).

Biofungicides treatments like bio arc, bio Zeid, clean root, blight stop, and plant guard outperformed the recommended fungicide, amistar top 32.5% EC, in terms of reducing the severity of downy mildew in onion plants (Shahin, 2017).

Also increased onion bulb yield, chemical components and enzymes activity related to defense mechanisms (Khan *et al.*, 2020, Bhatti *et al.*, 2021 and Getinet *et al.*, 2024).

The aim of this research is to utilize biological control as an eco-friendly and acceptable alternative to chemical methods for managing onion downy mildew disease and its effects on the production of healthy, toxin-free onions in the food chain, ensuring high yield and quality.

MATERIALS AND METHODS

The fungicides:

Three biocides and one natural fungicide (Champ DP 57.6% WG) were used for this investigation to study their effect on onion downy mildew under field conditions. The details and specifications for all used fungicides are listed in table (1)

Table (1): Details and specification of used fungicides.

Common name	Active ingredient	Conc.	Dose/100 L/feddan	Company
Serenade ASO 1.34% SC	<i>Bacillus subtilis</i> QST 713	1x10 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

Field experiment:

This study conducted in fields infected with *Peronospora destructor* during two winter growing seasons (2022-2023 and 2023-2024) at Riyadh Al-Saleheen experimental farm, Sohag Governorate, Egypt, using drip irrigation method. Complete randomized blocks were used. Three replicate plots were used for each treatment and control; each plot measured 10.5 m² (3.0 × 3.5 m). Onion transplants 'Giza 6' 60 days old were transplanted on October 15 in both seasons, with about 75 plants/ row totaling 450 plants/ plot. Early foliar spraying before sunrise was performed at regular intervals and repeated 7 times every two weeks from the first of January to the end of March, whereas un-sprayed control plants were treated with distilled water. The different treatments mixed with 50 ml/100 L of surfactant and sticker material. All treatments received the recommended farming procedures and irrigation till harvest at May.

Disease assessment:

The methodology developed by Mohibullah (1992), disease severity (DS%) was rated on a scale of 1 to 9, and the following formula was used to assess the disease severity index of downy mildew:

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

Where: DSI= Disease severity index, n = Number of leaves in each category, v = Numerical value of each category, z = Numerical value of highest category and N = Total number of leaves in the sample.

Also, the efficacies of the treatments were calculated according to the following formula:

$$Efficacy = \frac{Control - Treatment}{Control} \times 100$$

Onion bulbs yield:

Yield of onion bulbs, weighted after harvest from each treatment, expressed as kg/plot.

Biochemical analysis:

Total soluble solids (TSS) were measured using a Carl Zeiss hand refractometer at the end of each season, and total carbohydrates were measured in the onion bulb's liquid extract in accordance with Dubois *et al.* (1956).

Enzymatic activity:

Samples were collected one week after last spray. The biochemical activities were assessed using the Maxwell and Bateman (1967) method for peroxidase (PO) and the Thimmaiah (1999) approach for polyphenol oxidase (PPO).

Statistical analysis:

The collected data was statistically analyzed using MSTAT software. The least significant difference (LSD) at a 0.5 level of probability was used to compare treatment means in accordance with the guidelines provided by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Effectiveness of different treatments on *Peronospora destructor*:

The data presented in Table (2) indicates that various treatments were effective in mitigating the severity of onion downy mildew over two consecutive growing seasons (2022-23 and 2023-24). Notably, the analysis reveals that the percentage of disease severity was significantly higher three months post-planting than at the two-month mark across both seasons. This observation underscores the importance of timely intervention in managing the disease. Among the treatments assessed, Champ DP emerged as the most efficacious natural fungicide, achieving a remarkable reduction in disease severity at 80.3% after two months and 76.4% after three months of application. Following closely was Tricho Nitro Plus, a biocide, which also demonstrated impressive efficacy with reductions of 80.2% and 76.3%, respectively, highlighting its viability as a treatment option, followed by Bio Control T34 as the second most effective biocide. Conversely, Serenade ASO 1.34% SC displayed relatively poor performance compared to the other treatments, ranking as the least successful over the duration of the study.

The biocontrol agents *Trichoderma* spp. or *Bacillus* spp. may have caused the host plant to develop resistance against onion downy mildew, which could account for the bioagents' beneficial effect (Kamel *et al.*, 2017 and Raziq *et al.*, 2018). These findings are consistent with those of Shahin (2017) who attributed these findings to the combined action of bioagents that generate growth regulators and the chemical action of antioxidants, both of which are known to enhance plant physiology, metabolism, and generate systemic resistance (ISR). These results also are in line with those of Ankita *et al.* (2019) who confirmed that all of the fungicides tested were successful in controlling onion downy mildew (*Peronospora destructor*) due to their diverse levels of effectiveness. Disease severity increases with age; this also suggests that the pathogen is a low-sugar fungus, translocation from source to sink raised disease severity (Amin and Ahmed, 2023). Also, increase the witness between plants at high plant densities, resulting in a higher percentage of downy mildew disease (Getinet *et al.*, 2024).

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 2022-23 and 2023-24 growing seasons.

Treatments	Dose/feddan	Disease severity (%)							
		After 2 months				After 3 months			
		2022-23	2023-24	Mean	Efficacy	2022-23	2023-24	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 spraying start from the first of January to the end of March.

Effects of treatments on onion chemical components:

The results presented in Tables 3, 4, and 5 illustrate an effort to understand the impact of mentioned treatments on disease reduction, as well as their effects on treated plants, including total carbohydrates, total soluble solids, defense enzymes, and subsequent onion bulbs yield.

The results presented in Table (3) indicate that Tricho Nitro Plus emerged as the most efficacious treatment, demonstrating significant enhancements in total carbohydrates recorded 1.87 and 1.92 mg/g dry weight, and TSS values were noted at 15.72% and 15.98%, in both successive growing seasons of 2022-23 and 2023-24, surpassing those of the control treatment. This underscores the potential of Tricho Nitro Plus in augmenting plant health and productivity.

Also, Bio Control T34 ranked as the second most effective option compared to untreated onion plants. Conversely, Serenade ASO was identified as the least effective treatment, correlating with lower yield components across both experimental seasons.

These findings are consistent with those of Ahmed *et al.* (2017) due to *Trichoderma* spp. enhanced the previously indicated crop characteristics. These findings showed that all treatments maintained the health and encouraged the best growth of onion plants, which is highlighted by the control treatment's excessively low chemical content (Bhatti *et al.*, 2021). Regulators like IAA may be the cause of growth promotion (Amin and Ahmed 2023). Additionally, they ensure that treatments with significant plant protection and disease reduction were paired with increased total carbohydrates, total nitrogen, and TSS levels.

Table (3): The impact of foliar spraying* of five eco-friendly fungicides on the content of total carbohydrates and total soluble solids (TSS) under field conditions at Riyadh Al-Saleheen experimental farm, Sohag Governorate, Egypt during 2022-23 and 2023-24 growing seasons.

Treatments	Dose/feddan	2022 - 23 growing season		2023 - 24 growing season	
		Total carbohydrates (mg/g dry weight)	TSS (%)	Total carbohydrates (mg/g dry weight)	TSS (%)
Serenade ASO	1 L	1.44	11.87	1.53	12.13
Tricho Nitro Plus	1 L	1.87	15.72	1.92	15.98
Bio Control T34	85 g	1.83	14.21	1.88	14.46
Champ DP	180 g	1.58	12.26	1.65	12.52
Control(Untreated)	-	0.16	10.15	0.18	10.87
LSD at 5%		0.11	0.49	0.14	0.51

* As described under Table (2).

Enzymatic activities:

The findings presented in Table (4) indicate that the application of all treatments considerably raised the peroxidase (PO) and polyphenol oxidase (PPO) activity of onion plants in comparison to untreated plants in two seasons in a field setting. In this regard, Tricho Nitro Plus was the most effective treatment in increasing PO (0.225 and 0.231 unit/mg protein) and PPO (0.047 and 0.049 unit/mg protein) in the two seasons, surpassing Champ DP, Bio Control T34, and Serenade ASO. Applying biocide and natural fungicides greatly enhanced peroxidase and polyphenol oxidase activities in onion plants compared to untreated plants over the 2022-23 and 2023-24 growth seasons. Plants treated with biocide showed a significant increase in peroxidase and polyphenol oxidase activity.

Increased enzyme activity is linked to host plant defense mechanisms (Develish and Sugh, 2016; Bhatti *et al.*, 2021). The same findings were observed by Amin and Ahmed in 2023, who demonstrated that *B. subtilis* isolates were able to positively control white rot disease on onion plants.

They attributed this to their direct suppression of the fungal pathogen, *S. cepivorum*, as well as the increase in plant resistance brought on by the activation of defensive enzymes as a result of control.

Table (4): The impact of foliar spraying* of five eco-friendly fungicides on enzymatic activity** under field conditions at Riyadh Al-Saleheen experimental farm, Sohag Governorate, Egypt during 2022-23 and 2023-24 growing seasons.

Treatments	Dose/feddan	2022-23 growing season		2023-24 growing season	
		Peroxidase (unit/mg protein)	PPO (unit/mg protein)	Peroxidase (unit/mg protein)	PPO (unit/mg protein)
Serenade ASO	1 L	0.167	0.026	0.172	0.028
Tricho Nitro Plus	1 L	0.225	0.047	0.231	0.049
Bio Control T34	85 g	0.199	0.038	0.204	0.041
Champ DP	180 g	0.181	0.031	0.187	0.034
Control(Untreated)	-	0.063	0.010	0.065	0.011
LSD at 5%		0.021	0.007	0.022	0.008

* As described under Table (2).

** Samples were collected one week after last spray.

Onion bulbs yield:

Table (5) presents compelling evidence that the application of fungicides and biocides through foliar treatment has significantly improved onion yield during the 2022-2023 and 2023-2024 growing seasons. Specifically, overall yield per plot experienced substantial enhancement, with figures escalating to 43.3 kg/plot in the 2022-2023 and reaching 44.6 kg/plot in the following season with Tricho Nitro Plus, followed by Bio Control Plus with 40.9 and 41.3 kg/ plot. In contrast, Serenade ASO was found to be the least effective treatment across both growing seasons.

In general, these results suggest the potential for employing biological control in the prevention and control plant diseases, or in conjunction with chemical pesticides, as part of integrated control and sustainable agriculture. This approach aims to achieve positive outcomes, safeguard the environment, minimize pesticide use, and mitigate their harmful effects.

These findings are consistent with those of Develish and Sugh (2016) and Narla *et al.* (2016) who attributed these findings to the combined action of bioagents that generate growth

regulators and the chemical action of antioxidants, both of which are known to enhance plant physiology, metabolism, and generate systemic resistance (ISR).

Table (5): 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 2022-23 and 2023-24 growing seasons.

Treatments	Dose/feddan	2022-23 growing season	2023-24 growing season
		Yield (kg/plot)	Yield (kg/plot)
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

* As described under Table (2).

When applied to various field crops, their surplus production of phenolic compounds results in a higher yield (Fira *et al.*, 2018 and Khan *et al.* 2020). They are credited with their ability to promote plant growth by solubilizing phosphate and producing phytohormone indole 3-acetic acid, which impacts plant growth and yield (Getinet *et al.*, 2024).

Conclusion:

The effectiveness of four biocides including Serenade ASO 1.34% SC (*Bacillus subtilis* QST 713 1×10^9 cfu/g), Tricho Nitro Plus WP (*Trichoderma* spp. 10^7 spores/ml) and Bio Control T34 12% WP (*Trichoderma asperellum* 10^7 spores/g), and in addition to the natural fungicide Champ DP 57.6% WG (Copper hydroxide 57.6 %) were evaluated for controlling downy mildew disease on onion plants under field conditions. All treatments proved effective in controlling downy mildew disease, increased onion bulbs yield, total carbohydrates, total soluble solids, and activity of PO and PPO. Tricho Nitro Plus as a biocide gave better results than the control as well as Champ DP.

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