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Research Article

Efficacy of some Fungicides, Chemical Inducers, and Their Mixtures in Controlling Downy Mildew in Onion under Field Conditions

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Onion; Fungicides; Chemical inducers; *Peronospora destructor*; Difenoconazole-azoxystrobin.

Abstract:

Onion (*Allium cepa* L.) is among the most significant vegetable crops cultivated commercially worldwide. A major challenge in onion production is downy mildew, caused by *Peronospora destructor*. This common leaf disease impacts both the yield of onion bulbs and the seed production in the second year. One of the key strategies to manage this disease is the application of fungicides. Six fungicides were used in this study *i*.e copper oxychloride, difenoconazole-azoxystrobin, cyazofamid-cymoxanil, mancozeb-dimethomorph, trifloxystrobin–tebuconazole, and dimethomorph-pyraclostrobin. In addition, four chemical inducers, salicylic acid, chitosan, humic acid and ascorbic acid were tested. These inducers were combined with the recommended difenoconazole-azoxystrobin fungicide at the rate of 1:1 individually. These fungicides and chemical inducers were evaluated for their effectiveness in reducing infection with downy mildew diseases on onion caused by *Peronospora destructor* under field conditions. Besides, antioxidant enzymes activities (polyphenol oxidase and peroxidase enzymes) were assessed in onion. Results showed that all the fungicides and chemical inducers significantly reduced the severity of downy mildew and increased the activities of antioxidant enzymes activity (POX and PPO). Moreover, difenoconazole-azoxystrobin, ascorbic acid at conc. 0.704 mg/L, and chitosan at concentration of 2 mg/L combined with difenoconazole- azoxystrobin had the lowest disease severity and the highest efficacy. Meanwhile, copper oxychloride, chitosan, and the combination of difenoconazole-azoxystrobin with chitosan at concentration of 2 mg/L resulted in the highest increase in polyphenol oxidase and peroxidase activities.

1. Introduction

Onion (*Allium cepa* L.) is one of the most important vegetable crops grown commercially worldwide (Mishra et al., 2013). It is widely consumed for its distinctive flavor and health benefits) Malik, 1994) , providing carbohydrates, proteins, vitamin A, thiamine, riboflavin, niacin, and ascorbic acid. Egyptian onions are cultivated globally and known for their superior quality, strong pungency, excellent storability, and early availability in European markets, making them a key source of foreign currency. According to the Ministry of Agriculture and Land Reclamation (2022), the onion crop covers about 152,539 feddans, with an average yield of 10-12 tons of bulbs per feddan. Onion ranks as the first major exportation crop. Bulbs are exported either fresh or dry. Egypt is considered as the fourth principal countries in onion exportation after Netherlands, India, and USA (FAO, 2002). Harrier and Watson (2004) indicated that various plant enzymes play a crucial role in defense mechanisms against plant pathogens and drought stress, such as peroxidase (POD) and polyphenol oxidase (PPO), which catalyze the formation of lignin and other oxidative phenols, helping to the formation of defense barriers that strengthen the plant's cell structure.

Foliar diseases significantly reduce bulb yield and production quality. Among the various diseases affecting onion leaves and bulbs, downy mildew, caused by *Peronospora destructor* (Berk.) Ciferri, is one of the most widespread and destructive (Gupta et al., 2011 and Tripathy et al., 2014). The severity of all these diseases varies considerably based on location and season, largely influenced by the frequency and duration of moisture on onion foliage due to dew (Gupta et al., 2011 and Tripathy et al., 2014).

Downy mildew is a major leaf disease impacting onion bulb seed production in the second year. It can cause local infections on onion leaves or become systemic, affecting the entire plant (Schwartz and Mohan, 2008). Outbreaks of downy mildew can lead to yield reductions in onion bulbs ranging from 30% to 70%, especially under favorable conditions for the disease (Maude, 1990). During seed production, *P. destructor* can cause significant yield losses, with reductions of 60–70% reported in India (Sharma et al., 2002). Moreover, seeds can become infected with *P. destructor*, serving as a primary source of inoculum for the following season (Rondomanski, 1971). One of the important control strategies for managing these diseases involves the use of fungicides. Typically, multiple fungicide applications are necessary to keep onion leaves from reaching damaging levels (Mathur and Sharma, 2006 and Gaikwad et al., 2014). People globally are conscious about environmental deterioration due to use of costly and toxic spray chemicals. So, to promote environmental balance and sustainability, reducing fungicides usage is needed by exploring alternative control methods or in some cases, reduces of the number of fungicides sprays or their concentrations (Akter et al., 2015). Kamel et al. (2017) indicated that the foliar application of two forms of humic substances (HS) individually or in combination with Ridomil Golad 68% (WP) fungicide improving the activity of antioxidant enzymes and reducing disease severity of downy mildew in onion. Farouk et al. (2008) reported that foliar application of chitosan and salicylic acid proved to be effective in reducing severity of downy mildew disease.

Recently, public health and environmental safety have encouraged the use of resistance inducers to enhance growth, nutritional status, and safety foods. Consequently, modern agriculture is seeking new tools to reduce agrochemical inputs without negatively impacting crop yield or the growers' income. The rapidly growing population is raising large pressure on food production in the country. To meet this rising demand, farmers use chemical fertilizers to increase the crop yields. However, the toxins from the chemical fertilizers can accumulate in plant products, causing health issues in humans (Hansra, 1993).

The current study aimed to evaluate the efficacy of some fungicides, chemical inducers, and their mixtures as foliar applications for controlling downy mildew of onion. In addition, biochemical changes in the leaves of onion associated with the application of these treatments were also investigated.

2. Materials and Methods

The present work was carried out at Gemmiza Agricultural Research Station during the 2020/2021 and 2021/2022 seasons under field conditions to control downy mildew disease of onion caused by *Peronospora destructor*. The experiment was conducted in a randomized complete block design (RCBD).

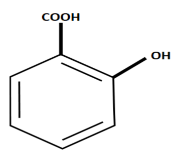
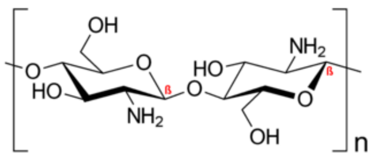
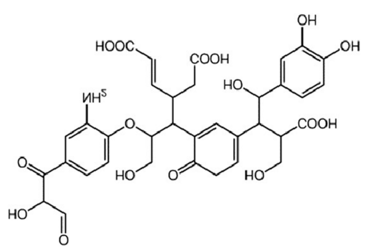
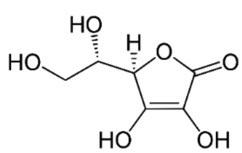
2.1. Pathogenic fungi

Peronospora destructor, a pathogenic fungus, was used in these experiments. The standard culture of this fungus was obtained from the Agricultural Research Center (A.R.C), Plant Pathology Research Institute, Mycology Research & Plant Disease Survey Department, Giza, Egypt.

Table 1. Fungicides used in the present study.

Trade name	Common name	Mol. Formula
Cobox 84% WP	**Copper Oxychloride**	$Cl_2Cu_4H_{12}O_6$
Amistar Top 32.5% SC	**Difenoconazole 12.5%**	$C_{19}H_{17}Cl_2N_3O_3$
	Azoxystrobin 20%	$C_{22}H_{17}N_3O_5$
Tickers 40% WP	**Cymoxanil 30%**	$C_{13}H_{13}ClN_4O_2S$
	Cyazofamid 10%	$C_7H_{10}N_4O_3$
Acrobat MZ 69 % WG	**Mancozeb 60%**	$[C_4H_6MnN_2S_4]xZn$
	Dimethomorph 9%	$C_{21}H_{22}ClNO4$
Nativo 75 WG	**Trifloxystrobin 25 %**	$C_{20}H_{19}F_3N_2O_4$
	Tebuconazole 50 %	$C_{16}H_{22}C_lN_3O$
Ultra Etho 18.7% WDG	**Dimethomorph 12%**	$C_{21}H_{22}ClNO_4$
	Pyraclostrobin 6.7%	$C_{19}H_{18}ClN_3O_4$

Table 2. Chemical inducers used in the present work.

Chemical inducers	Chemical structure	Concentration (mg/L)
Salicylic acid		0.276
		0.552
		0.828
Chitosan		0.50
		1.00
		2.00
Humic acid		0.25
		0.50
		1.00
Ascorbic acid		0.352
		0.704
		1.056

2.2. Host plant

Onion plants of the cultivar Giza 20 were used in the present study as the host plant for downy mildew.

2.3. Tested fungicides

A total of six fungicides, listed in Table (1), were used in the present study.

2.4. Tested chemical inducers

A total of four chemical inducers, listed in Table (2), were used in the present study. Three concentrations of each chemical inducer were tested to reduce downy mildew disease in onion. Three replicates were used for each concentration, in addition to a control treatment.

2.5. Synergistic effects of difenoconazole-azoxystrobin fungicide with chemical inducers for controlling downy mildew disease in onion under field conditions

A mixture of difenoconazole-azoxystrobin fungicide with chemical inducers was tested for controlling downy mildew disease in onion under field conditions. The mixture was applied at the rate of 1:1 mixture of the recommended dose of difenoconazole-azoxystrobin fungicide with each chemical inducer, in addition to a control treatment**.**

2.6. Measurements

2.6.1. Disease severity assessment

Disease severity of downy mildew was recorded three months after transplanting (during the first week of March). Around 10 to 20 plants were randomly collected from each designated plot. One hundred affected leaves from the selected plants were randomly selected to determine disease severity, which was assessed using (0-8) scale and recorded according to the method described by Townsend and Heuberger (1943) as follows:

Scale	Number of colonies per leaf.
0	No infection (leave are completely healthy.
1	1-2 spots per onion leaf**.**
2	3-5 spots per onion leaf**.**
3	6-10 spots per onion leaf**.**
4	25% of leaf surface was attacked.
5	35% < 50% of leaf surface was attacked**.**
6	50% of leaf surface was attacked.
7	75% of leaf surface was attacked.
8	More than 75% of leaf surface was attacked.

Disease severity index of downy mildew was estimated using the following formula:

Disease severity (%) =

$$\frac{[\Sigma\,(\text{rating no.})\,(\text{no. leaves in rating category})\,(100)\,]}{(\text{Total no. leaves})\,(\text{highest rating value})}$$

The efficiency of each treatment in reducing DS (%) compared to the control was calculated according to Townsend and Heuberger (1943), following the followed equation:

$$\textbf{Efficiency \%} = \frac{\text{D.S\% in control} - \text{D.S in treatment}}{\text{D.S\% in control}} \times 100$$

Where D.S = Disease severity

2.6.2. Peroxidase activity (POX; EC 1.11.1.7)

The activity of POX was assayed according to Kato and Shimizu (1987). A sample of 3 ml of the reaction mixture, containing 100 mM sodium phosphate buffer (pH 5.8), 7.2 mM guaiacol, 11.8 mM H_2O_2, and 100 µl enzyme extract, was used for the assay. The reaction was initiated by the addition of H_2O_2, and the change in absorbance was measured at 470 nm. POX activity was expressed as Δ_{470} min^{-1} g^{-1} fresh weight (FW).

2.6.3. Polyphenol oxidase activity (PPO; EC 1.10.3.1)

The activity of PPO was determined according to Mayer et al. (1965). The reaction mixture consisted of 200 µl of the enzyme extract and 1.5 ml of 100 mM sodium phosphate buffer (pH 6.5). For reaction, 200 µl of 100 mM catechol was added. PPO activity was expressed as Δ_{490} min^{-1} g^{-1} FW.

2.7. Statistical analysis

All data were statistically analyzed using CoStat 6.311 software (2005) for the analysis of variance (ANOVA) Gomez and Gomez (1984). All comparisons were first subjected to ANOVA and significant difference among treatments means were determined with Duncan's Multiple Range test at $P \leq 0.05$ Duncan (1955).

3. Results and Discussion

3.1. Effect of some foliar applications on controlling downy mildew disease caused by *P. destrucators* on onion c.v Giza 20 in Gemmiza field during 2020/ 2021 season

The results obtained in Table (3) showed that fungicides foliar applications had a significant effect on disease severity, Data of season 2020/ 2021 showed that the lowest disease severity value of (9.53%) was recorded in the treatment with difenoconazole-azoxystrobin, followed by Cyazofamid-cymoxanil (10.50%) and trifloxystrobin-tebuconazole (14.20%), which were comparable to other fungicides. Therefore, they exhibited the highest efficiencies (85.41, 83.93, and 78.26%, respectively). In contrast, dimethomorph–pyraclostrobin was the least effective in controlling downy mildew caused by *P. destrucators*, resulting in the highest percentage of disease severity (16.42%).

Table 3. Effect of some fungicides (spray treatment) on controlling downy mildew disease in onion c.v Giza 20 in Gemmiza under open field condition (2020/ 2021 season).

Fungicides	Diseases Severity (%)	Efficacy (%)
Copper oxychloride	14.26 ± 0.95 b	78.17
Difenoconazole-azoxystrobin	9.530 ± 0.95 c	85.41
Cyazofamid-cymoxanil	10.50 ± 0.95 c	83.93
Mancozeb-dimethomorph	15.50 ± 0.95 b	76.73
Trifloxystrobin-tebuconazole	14. 20 ±0.95 b	78.26
Dimethomorph-pyraclostrobin	16.42 ± 0.95 b	74.86
Control	65.34 ± 0.95 a	

These results are consistent with findings by Develash and Sugha (1997), O'neill et al. (2002), Romanzzi et al. (2016), and Wright and Beresford (2019), who studied the efficacy of various fungicides in inhibition sporangial germination of *P. destrucators*. Their results indicated that among the tested acylalanine-based fungicides, *P. destrucators was* most sensitive to metalaxyl – mancozeb, followed by oxadixyl+ copper oxychloride, and least sensitive to benalaxyl + mancozeb with ED_{50} value of 2.6, 4.5, and 8.3 mg/ml, respectively. Wright and Beresford (2019) noted that, 12 days after inoculation with *P. destructor* spores, 66% of the control plants (spores only) showed sporulating lesions. Among the 11 experimental treatments, two fungicides showed effective downy mildew control: cymoxanil, with 4% of 100 inoculated onion plants exhibiting sporulating downy mildew infection, and mandestrobin, with 7% infection.. Gupta and Jarial (2014) reported that copper oxychloride demonstrated 67% efficacy in controlling cucumber downy mildew under greenhouse conditions in Yazd.. In their trial, different brands of copper oxychloride showed no significant differences in control efficacy during the first and second evaluations.

3.2. Effect of some fungicides as foliar applications on polyphenol oxidase and peroxidase enzyme activities in onion infected with downy mildew disease

Results in Fig. (1 A and B) revealed that foliar treatments with the tested fungicides affected enzyme activities under controlled conditions. Copper oxychloride and difenoconazole–azoxystrobin led to increases in polyphenol oxidase and peroxidase activities, with increases of 12.3% and 11.58% for polyphenol oxidase and 22.05% and 20.15% for peroxidase, respectively. In contrast, mancozeb–dimethomorph treatment resulted in the lowest increases, with values of 9.42% for polyphenol oxidase and 17.70% for peroxidase.

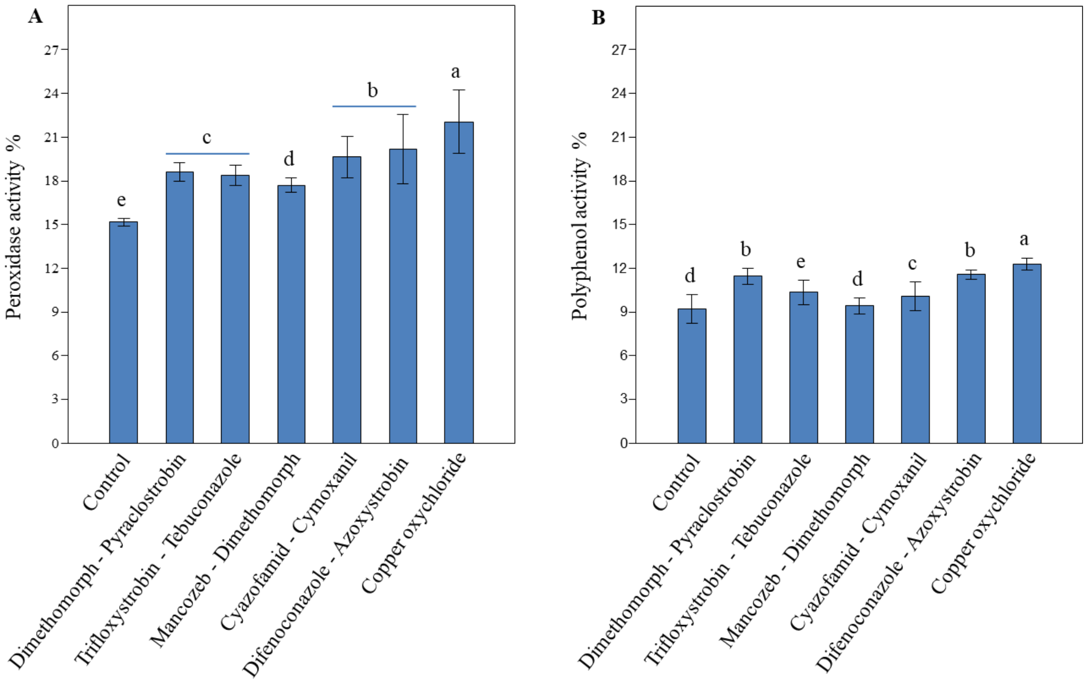


Figure 1. Effect of some fungicides treatment (spray treatments) on (A) peroxidase and **(B)** polyphenol oxidase enzymes activity in leaves of onion c.v. Giza 20 infected with downy mildew during 2020/2021 season.

Several results have been reported on the role of oxidative enzymes during plant infection by fungal pathogens. Peroxidase are oxide reductive enzymes that play a critical role in various metabolic processes, such as the oxidation of phenol to quinones (Bernards et al., 1999) and the reinforcement of plant cell walls (Dean and Kuc, 1987). These findings are consistent with those of Misaghi (1982), who reported that the increase of peroxidase activity helps oxidize phenolics to more fungitoxic compounds, such as quinones. Kafsheer (2016) demonstrated that spray treatment with Metalaxyl-copper oxychloride and azoxystrobin was the most effective in controlling downy mildew disease in maize, while also, showing the highest enzyme activities (polyphenol oxidase and peroxidase) compared to other fungicides and untreated controls. El-Shehawy (2009) studied the effect of various fungicides on downy mildew disease in grain sorghum and their impact on biochemical components, reporting that metalaxyl-copper oxychloride and metalaxyl spray treatment resulted in the highest oxidative enzyme activities (polyphenol oxidase and peroxidase).

3.3. Effect of some chemical inducers on downy mildew disease caused by *P. destrucators* in onion under field conditions during 2020/ 2021 season

Data presented in Table (4) showed that all chemicals inducers significantly reduced disease severity compared to the untreated control when applied as spray treatment. The reduction in disease severity increased with higher concentrations of the inducers. Ascorbic acid at a concentration of 0.704 mg/L was the most effective treatment in reducing disease severity to 10.44%, followed by salicylic acid at 0.828 mg/L (11.06%) and chitosan at 2 mg/L (11.49%). In contrast, foliar application of humic acid at 0.250 mg/L had the least effect, with a disease severity reduction of 17.38%.

Furthermore, data in Table (4) clearly showed that ascorbic acid exhibited the highest efficiency in reducing disease severity, with an efficacy of 83.90% at a concentration of 0.704 mg/L. This was followed by salicylic acid, with 82.95% efficiency at a concentration of 0.828 mg/L, and chitosan at 2 mg/L, showing an efficacy of 82.28%.

Table 4. Effect of some chemical inducers (spray treatment) on controlling downy mildew disease in onion c.v Giza 20 in Gemmiza under open field conditions during 2020/ 2021 season

Inducers resistant	Concentrations (mg/L)	Diseases severity (%)	Efficacy (%)
Salicylic acid	0.276	16.80 ± 0.64 bc	74.10
	0.552	13.35 ± 0.64 de	79.42
	0.828	11.06 ± 0.64 fg	82.95
Chitosan	0.500	12.32 ± 0.64 ef	81.01
	1.00	11.82 ± 0.64 efg	81.78
	2.00	11.49 ± 0.64 efg	82.28
Humic acid	0.250	17.38± 0.64 b	73.20
	0.500	16.55 ± 0.64 bc	74.48
	1.00	15.10 ± 0.64 cd	76.80
Ascorbic acid	0.352	11.66 ± 0.64 efg	82.02
	0.704	10.44 ±0.64 g	83.90
	1.056	11.86 ± 0.64 efg	81.71
Control		64.86 ± 0.64 a	

These results are consistent with those obtained by previous investigators, such as Khalifa et al. (2011), Ata et al. (2012), and Mahmoud et al. (2016), who successfully controlled diseases using inducers. They noted that all plants have the ability to defend themselves against pathogenic infection through a wide variety of mechanisms that can be local or systemic, as well as constitutive or inducible. Systemic acquired resistance (SAR) is a pathogen inducible defense mechanism that depends on salicylic acid (SA) and is associated with the expression of a subset of defense genes and the accumulation of pathogenesis-related (PR) proteins. Kafsheer (2016) indicated that seed treatment with salicylic acid (3 and 5Mm) followed by 5Mm of ascorbic acid, was the most effective treatment in controlling downy mildew disease compared to other treatments. Farouk et al. (2008) reported that foliar application of chitosan and salicylic acid proved to be effective in reducing the occurrence and severity of downy mildew (caused by *Peronospora cogenesis*) in cucumber plants under field conditions. Ahlam et al. (2019) tested the effect of salicylic acid and ascorbic acid on six cultivars of peanut, and the results indicated that salicylic acid at 8mM, followed by ascorbic acid at the same concentration, were the most effective inducers for reducing cercospora leaf spot.

3.4. Effect of some chemicals inducers as a foliar application on polyphenol oxidase and peroxidase enzymes activity in onion infected with downy mildew disease

Data present in Fig. (2 A and B) indicated that chitosan concentrations of 2.00 mg/L and 1.00 mg/L resulted in an increase in polyphenol oxidase activity (14.68 and 13.80%, respectively), followed by humic acid at a concentration of 1.00 mg/L, which recorded an increase of 13. 53%. While ascorbic acid at a concentration of 0.704 mg/L showed the lowest increase in polyphenol oxidase activity (11.40 %).

The data also showed that foliar spray with chitosan at concentrations of 2.00 mg/L, 1.00 mg/L, and humic acid at a concentration of 0.250 mg/L resulted in the highest increase in peroxidase activity (27.12, 25.10, and 23.55%, respectively). In contrast, ascorbic acid at a concentration of 1.056 mg/L showed the lowest in this respect (19.25 %). In general, all the chemical inducers led to an increase in polyphenol oxidase and peroxidase activity compared to the control.

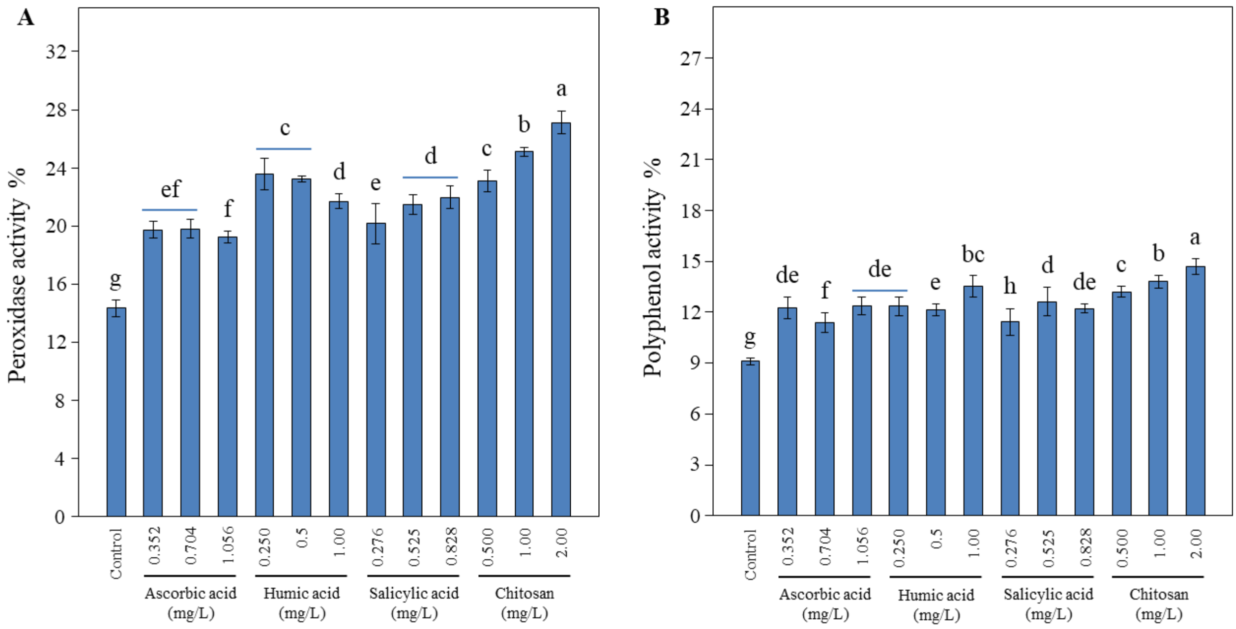


Figure 2. Effect of some chemical inducers (spray treatments) on (A) peroxidase and **(B)** polyphenol oxidase enzymes activity in the leaves of onion c.v. Giza 20 of in response to downy mildew disease during the 2020/2021 season.

These results are in agreement with those obtained by Kamel et al. (2017), who indicated that foliar application of humic acid, either individually or in combination with fungicides, caused an increase in the activity of peroxidase and polyphenol oxidase enzymes in treated on plants compared to the untreated control. Similarly, Manas et al. (2014) found that foliar application of humic acid significantly increased polyphenol oxidase activity of pungent pepper.

In addition, increased peroxidase activity upon infection may be required for additional deposition of lignin around the lesion site induced by the pathogen. Peroxidase is a key enzyme in the biosynthesis of lignin and other oxidized phenols (Bruce and West, 1989). The positive association between the rapid response of peroxidase activity, fungal infection, and disease resistance, as reported by Bi and Zhang (1993), has led to the hypothesis that peroxidase and other oxidative enzymes my bean integral part of the host plant 's defense mechanism.

3.5. Combination effect of difenoconazole-azoxystrobin (1:1 ratio) with chemical inducers on disease severity in onion

Chemical inducers applied as a foliar treatment, combined with difenoconazole-azoxystrobin, were the most effective in reducing downy mildew severity under open field conditions. In the second year (2021 /2022), these experiments were conducted under open field conditions

Data present in Table (5) showed that chitosan concentration of 2.00 mg/L combined with difenoconazole-azoxystrobin was the most effective in reducing downy mildew severityin onion (6.09%), followed by ascorbic acid combined with difenoconazole-azoxystrobin conc. at 1.056 mg/L (6.78%), giving the highest efficacy (91.27 and 90.29%, respectively). Whereas, the combination of humic acid with difenoconazole-azoxystrobin at 0.250 mg/L was the least effective in controlling downy mildew caused by *P. destructor,* showing a disease severity of 10.94%. Generally, the data indicated no significant differences between all treatments.

Table 5. Combination effect of difenoconazole- azoxystrobin with chemical inducers (1:1 Ratio) on controlling downy mildew disease in onion c.v Giza 20 in Gemmiza under open field conditions (2021/ 2022 season).

Combination Difenoconazole- azoxystrobin with chemical inducers	Concentration (mg/L)	Disease severity (%)	Efficacy (%)
Salicylic acid	0.276	10.58± 2.51 b	84.84
	0.552	7.93 ± 2.51 b	88.64
	0.828	7.18 ± 2.51 b	89.71
Chitosan	0.500	8.48 ± 2.51 b	87.85
	1.00	7.88 ± 2.51 b	88.71
	2.00	6.09± 2.51 b	91.27
Humic acid	0.250	10.94 ± 2.51 b	84.33
	0.500	9.31 ± 2.51 b	86.66
	1.00	7.66 ± 2.51 b	89.03
Ascorbic acid	0.352	8.93± 2.51 b	87.21
	0.704	7.45 ± 2.51 b	89.33
	1.056	6.78 ± 2.51 b	90.29
Control		69.83 ± 2.51 a	

These findings align with those reported by Wang (2022), they indicated that the foliar application of chitosan could effectively enhance the efficacy of isopyrazam azoxystrobin against leaf spot disease of kiwifruit, achieving a field control efficacy of 86.83% by spraying 29% isopyrazam- azoxystrobin suspension concentrate (SC) diluted 1500 time +chitosan diluted 100 time. This was significantly ($p < 0.05$) higher than the 78.70% efficacy of the 29% isopyrazam-azoxystrobin (SC) diluted 100 times. Chitosan can activate plant defense responses via inducing various defense related reactions (Verlee et al., 2017; Li et al., 2021; Berger et al., 2016). The effective control effect of isopyrazam- azoxystrobin +chitosan on leaf spot disease was probably due to the excellent preventive and therapeutic properties of isopyrazam –azoxystrobin, as well as the superior induced resistance effect of chitosan. Zhang (2023) reported on the field control of difenoconazole, chitosan and their combination against leaf spot disease in *P. heterophylla* and their effects on the disease resistance. The results showed that a 37% difenoconazole water- dispersible granule diluted 500- times +chitosan diluted 500- times had superior control efficacy against leaf spot disease.

3.6. Combination effect of difenoconazole-azoxystrobin with chemical inducers (1:1 ratio) as a foliar application on polyphenol oxidase and peroxidase enzymes activity in onion infected with downy mildew disease

Data present in Fig. (3 A and B) indicated that the combination of difenoconazole-azoxystrobin with chitosan a concentration of 2.00 mg/L and ascorbic acid at a concentration of 1.056 mg/L resulted in thehighest increase in polyphenol oxidase activities (15.90 and 14.87 %, respectively). In contrast, the combination of difenoconazole- azoxystrobin with salicylic acid at a concentration of 0.828 mg/L showed the lowest polyphenol oxidase activity (12.22%). Additionally, foliar application of difenoconazole-azoxystrobin combined with chitosan at 2.00 mg/L and ascorbic acid at 1.056 mg/L yielded the highest increase in peroxidase activity (27.41 and 26.17 %, respectively). Overall, all combination of chemical inducers with difenoconazole-azoxystrobin significantly enhanced the activities of polyphenol oxidase and peroxidase enzymes compared to the control.

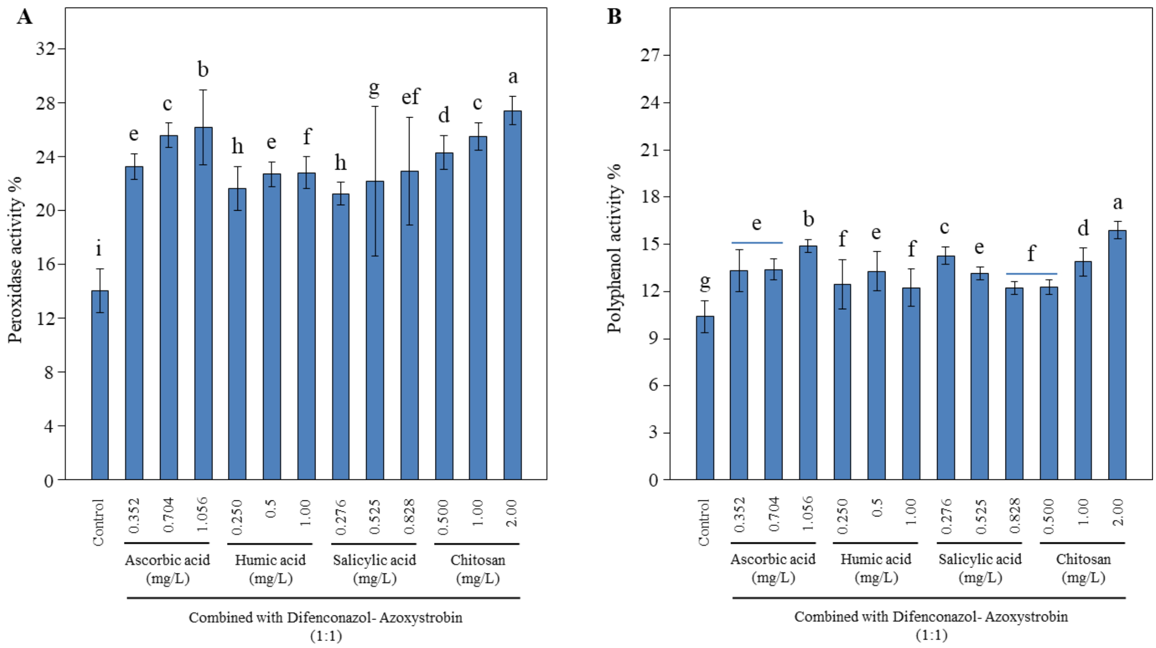


Figure 3. Combination effect of difenoconazole- azoxystrobin with chemical inducers (1:1 ratio) as a foliar application on (A) peroxidase and **(B)** polyphenol oxidase enzymes activity in onion c.v. Giza 20 leaves infected with downy disease (2021/ 2022 season).

These findings are consistent with those reported by Zhang (2023), where the combination of chitosan with difenoconazole-azoxystrobin was shown to be more effectively enhancing polyphenol oxidase activaty in *P. heterophylla* against leaf spot disease.

4. Conclusion

Various field experiments revealed that the combination of difenoconazole-azoxystrobin, ascorbic acid at a concentration of 0.704 mg/L, and chitosan at 2.00 mg/L with difenoconazole-azoxystrobin resulted in the lowest disease severity, thus demonstrating the highest efficacy of these combinations. In contrast, copper oxychloride, chitosan, and the combination of difenoconazole-azoxystrobin with chitosan at 2.00 mg/L led to the highest increase in phenol oxidase and peroxidase enzyme activities.

Conflicts of Interest: The authors declare no conflict of interest.

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