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Assessment of Hexaconazole Fungicide's Impact on Root Growth and Stomatal Characteristics of *Allium cepa* L.

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ABSTRACT

Fungicides play a crucial role in modern agriculture by effectively managing fungal diseases that can severely impact crop yield and quality. Hexaconazole, a widely used fungicide, has been proven effective against various fungal pathogens, making it an essential tool for crop protection and disease management strategies. Understanding the effects of Hexaconazole on plant physiology, particularly root growth and stomatal characteristics, is crucial for optimizing its application and ensuring sustainable agricultural practices growth. Therefore, his study aimed to investigate the effects of Hexaconazole (HEX), a fungicide, on root growth and stomatal characteristics of *Allium cepa* L. Different concentrations of Hexaconazole (0.0 as control treatment with only distilled water, 0.5, 1, and 1.5 ml/L) were utilized. The parameters examined included root length, root percentage, number of roots, and germination percentage. Additionally, stomatal features such as stomata number per mm^2, stomata length (µm), stomata width (µm), stomata index, and number of epidermal cells per mm^2 of the abaxial surface of the tubular leaf were analyzed. The results revealed that the control group exhibited the highest root length (7.11 cm), followed by T1 (4.27 cm), while the other treatments (T2 and T3) displayed significantly lower values (3.44 cm and 2.83 cm, respectively). In terms of root number, the T3 group recorded the highest number (67.75), whereas the control group exhibited the lowest root number (38.25). A highly significant correlation coefficient was observed between the number of roots and the root percentage. Regarding stomatal features, *Allium cepa* L. exhibited similar internal structural characteristics, but variations were observed in the measurements of stomatal traits. In general, the findings of this study contribute to the understanding of the impact of hexaconazole on root growth and stomatal characteristics in *Allium cepa* L. However, further research is warranted to elucidate the underlying mechanisms and potential implications of these findings.

Keywords: *Allium cepa* L; Fungicide; Hexaconazole; Stomata; Root Growth.

INTRODUCTION

Fungal diseases pose a significant threat to crop production worldwide, leading to substantial yield losses and reduced agricultural productivity. Among the various strategies employed for disease management, the use of fungicides has become indispensable in modern agriculture. Hexaconazole, a broad-spectrum fungicide, has gained prominence for its efficacy in controlling fungal pathogens in a wide range of crops. Hexaconazole, belonging to the triazole group of fungicides, has been reported to induce various physiological and morphological changes in plants. Hexaconazole acts by inhibiting the biosynthesis of ergosterol, a crucial component of fungal cell membranes, leading to the disruption of cell integrity and subsequent inhibition of fungal growth. Its effectiveness against a diverse array of fungal species has made it a valuable tool in crop protection and disease management programs. Studies have shown that it can lead to reduced stimulation of rooting, inhibition of gibberellin synthesis, alterations in biochemical constituents including defense-related enzymes and carbohydrate status, as well as a transient increase in abscisic acid content (Gopi et al., 2007). Triazoles, in general, have found commercial applications in enhancing lodging resistance, cold hardiness, and yield in important crop plants, as well as in regulating growth (Johnson et al., 2008).

The onion plant (Allium cepa L.), belonging to the Alliaceae family, is renowned as one of the most popular spice crops, particularly for its bulb. It serves as a common vegetable and is widely cultivated in various Asian nations, also being a favored spice in regions like Bangladesh. Notably, regions such as Diaper, Comilla, Faridpur, and Manikgonj are prominent areas for onion cultivation and development, alongside Pabna, Jessore, Jabalpur, Rajshahi, Mymensingh, and Rangpur. Unfortunately, *Allium cepa* L., commonly known as onion, is susceptible to various fungal diseases, posing significant threats to its production. These fungal infections can have devastating consequences, negatively impacting bulb quality, storage life, and market value. To ensure sustainable onion production, effective management strategies for fungal diseases are of paramount importance.

In the context of pesticide usage, it has been observed that pesticides can enter plant leaves through stomata, subsequently penetrating the cells. This infiltration can disrupt normal respiration processes and induce changes in photosynthetic mechanisms, including electron transport, oxidative phosphorylation, and alterations in chlorophyll levels. Furthermore, pesticides can also influence

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biochemical activities such as carbohydrate and amino acid synthesis. As a result, plants may exhibit weakened resilience and diminished capacity to effectively utilize their environment, especially when exposed to unfavorable conditions (Berova, 2002). However, it is worth noting that there is limited research available on the specific impact of fungicides on root growth and stomatal characteristics in onions (*Allium cepa* L.). Further exploration in this area would provide valuable insights into the potential effects of fungicides on onion plants, aiding in the development of more comprehensive management approaches. Understanding the effects of fungicides like Hexaconazole on plant physiology and growth is of utmost importance for optimizing their use in agricultural practices. Therefore, our study was carried out to gain insights into the physiological and morphological changes induced by Hexaconazole on root growth and stomata characteristics in onion plants. This will help to develop strategies to minimize potential adverse effects while maximizing its beneficial impacts on crop productivity and disease management.

MATERIALS AND METHODS

Evaluation the impact of Hexaconazole Fungicide

Rooting Percentage and Root Length

This study was conducted in October 2022 using equal-sized common onion bulbs (*Allium cepa* L.) with a diameter of 5 cm. The bulbs were carefully selected and each one was placed in a separate glass bottle with a wide bottleneck. The bottles contained 200 ml of a fungicide solution, which was the tested chemical Hexaconazole at concentrations of 0.0, 0.5, 1, and 1.5 ml L^{-1}. To prepare the bulbs for the experiment, the outer scales were removed, and the bulbs were cut into discs using sharp and clean blades while ensuring that the primordial roots remained intact. For the experimental analysis, five bulbs were initially chosen for each dose. After a period of 2 weeks, the length of the rooted bulbs' roots was measured using a ruler. The rooting percentage of the bulbs was determined using the following equation, as described by Noori and Ertushi (2021):

$$R\ (\%) = Rb/Tb \times 100$$

Where, R is the rooting (%); Rb is the rooted bulbs; and Tb is the total bulbs as mentioned by Çavuşoğlu, (2020).

Stomata

For the investigation of stomatal characteristics, leaves were selected as samples. The cuticular structures were examined through a series of steps. Firstly, the samples were dehydrated using 90% ethyl alcohol and then stored in 70% ethanol. They were subsequently washed with distilled water, dried, and immersed in a solution of glacial acetic acid and hydrogen peroxide in equal volumes (1:1). The samples were then placed in an oven at 60°C for a duration of 20-40 hours, depending on the species under study. To further analyze the stomatal features, abaxial peelings from the macerated tubular leaves were stained with safranin-glycerin jelly and mounted on microscopic slides, following the methodology described by Darwesh and Mustafa (2012). A total of 25 observations were recorded for each of the following measurements, and the averages were calculated.

These measurements were:

a. Epidermal cell density =

$$= \frac{Epidermal\ cell\ number}{mm^2}$$

b. Stomatal dimensions (width and length).

c. Stomata index% =

$$\frac{Stomatal\ density}{(stomata\ density + epidermal\ cell\ density)}$$

Where stomatal density can be calculated as follow:

$$= \frac{Stomatal\ number}{mm^2}$$

The prepared slides were meticulously examined under a magnifying microscope. A 4x magnification was used to count the number of stomata and epidermal cells, while a 10x magnification was employed to measure the dimensions of the stomata. The regions of interest were thoroughly studied, and the observations were carefully documented. High-resolution photographs of the stomatal structures were captured using a digital camera attached to the eyepiece of the microscope, specifically the Dino-Eyes microscope eyepiece digital camera.

Statistical analysis

The collected data were analyzed using the SPSS program (SPSS, 2019) to conduct statistical analysis. Descriptive statistics were employed to summarize the data, while one-way analysis of variance (ANOVA) was utilized to compare the different treatment groups for the studied characteristics. In order to determine significant differences between means within the ANOVA, Duncan's Multiple-Range Test (Duncan, 1955) was applied. Furthermore, correlation analysis was conducted using the same SPSS program to examine the relationships between pairs of the studied characteristics.

RESULTS

Analysis of Root Growth Characteristics

The data presented in Table (1) clarifies the impact of Hexaconazole treatments on the studied characteristics of root growth. It is evident that both root length and the number of roots exhibited significant variations ($p \leq 0.05$) across the different treatments. However, the other two parameters, namely root percentage and germination percentage, did not show any significant differences ($p > 0.05$) among the treatments.

Regarding root length, the control group exhibited the highest recorded value (7.11 cm), which was significantly different from T1 (4.27 cm). The other treatments, T2 and T3, showed significantly lower values (3.44 cm and 2.83 cm, respectively). In terms of the number of roots (Fig.1), a reverse trend was observed. T3 had the highest number of roots (67.75), followed by T1 (60.25). T2 had 44.25 roots, while the control group had the lowest number of roots (38.25). However, the differences among the studied treatments for both root percentage and germination percentage were non-significant ($p > 0.05$), as shown in

Table (1). Moreover, number of roots recorded revealed that the control group had a mean of 38.25 roots, which was significantly lower compared to treatments T1, T2, and T3, with means of 60.25 roots, 44.25 roots, and 67.75 roots, respectively. The statistical analysis indicated a significant difference in the number of roots among the treatments, as denoted by the *p*-value of 0.42.

For rooting percentage, the recoded data showed that the percentage was relatively consistent across all treatments, with values ranging from 24.50% to 24.75%. The statistical analysis did not show a significant (*p*-value, 1.0) difference among the treatments, which suggests no significant effect of the different concentrations of fungicide on rooting percentage. However, the germination percentages varied among the treatments. The control group had a mean germination percentage of 10.50%, while treatments T1, T2, and T3 had mean percentages of 6.50%, 11.00%, and 5.25%, respectively. The statistical analysis did not show a significant difference in germination percentage among the treatments (*p*-value, 0.238). In general, the results suggest that the treatments had a significant impact on root length and the number of roots, but did not significantly affect the rooting percentage or germination percentage.

Correlation analysis of measured root parameters

The correlation analysis between the studied parameters characteristics to root growth (root length, number of roots, root percentage, and germination percentage; Table 2) revealed the following findings: root length showed a weak negative correlation with the number of roots (r =-0.347), indicating that as root length increased, the number of roots tended to decrease, although this relationship was not statistically significant ($p > 0.05$). However, the number of roots exhibited a moderate positive correlation with root percentage (r =0.708) and high negative correlation with germination percentage (r =-0.902). This suggests that as the number of roots increased, both root percentage and germination percentage tended to increase significantly ($p \leq 0.01$).

For root percentage, it showed a negligible negative correlation with root length (r =-0.034) and a strong positive correlation with the number of roots (r =0.708). However, the relationship between root percentage and germination percentage was highly negative (r =-0.791), indicating that as root percentage increased, germination percentage tended to decrease significantly ($p \leq 0.01$).

Meanwhile, germination percentage demonstrated a slight positive correlation with root length (0.160) and a strong negative correlation with both the number of roots (r =-0.902) and root percentage (r =-0.791). This implies that as germination percentage increased, the number of roots and root percentage decreased significantly ($p \leq 0.01$).

Table (1): Effect of different concentration (0.0, 0.5, 1, and 1.5 ml L^{-1}) of Hexaconazole fungicide on growth parameters of *Allium cepa.*

Measured parameters	Treatments[†]	Mean	Minimum	Maximum	*p*-value
Root Length (cm)	Control	7.11±1.98 [a]	3.79	12.5	*.047
	T1	4.27±0.08 [ab]	4.07	4.42	
	T2	3.44±0.30 [b]	2.55	3.87	
	T3	2.83±0.11 [b]	2.66	3.14	
No. of Roots	Control	38.25±6.01 [b]	27	51	*.042
	T1	60.25±7.83 [ab]	41	73	
	T2	44.25±9.48 [b]	17	59	
	T3	67.75 ±3.71[a]	60	77	
Rooting Percentage	Control	24.50±4.09	17	33	NS 1.0
	T1	24.75±3.19	17	30	
	T2	24.50±5.44	9	33	
	T3	24.50±1.32	22	28	
Germination percentage	Control	10.50±1.76	7	14	NS 0.238
	T1	6.50±0.96	5	9	
	T2	11.00±4.02	6	23	
	T3	5.25±0.48	4	6	

[†]Control, no fungicide add; T1, T2, and T3 are treatments with Hexaconazole Fungicide at concentration 0.5, 1, and 1.5 ml L^{-1}, respectively.

Evaluation the impact of Hexaconazole fungicide on stomata

The results of the effects of different dosages of the Hexaconazole fungicide on the stomata characteristics and epidermal cells on the abaxial leaf surfaces of *Allium cepa* L., showed variations among the studied treatments (Fig. 2). The study revealed notable variations in the shape and size of stomata, while the abaxial leaf epidermal cells exhibited a rectangular structure resembling the onion bulb. Significant differences were observed in the measurements of stomatal components among the different treatments.

For stomatal length, the treatment with Hexaconazole at a concentration of 0.5 ml/L resulted in the highest measurement (31.715 µm) on the lower (abaxial) epidermis of *Allium cepa* L. In contrast, the control treatment showed the lowest value (17.272 µm). Similarly, the treatment with Hexaconazole at a concentration of 0.5 ml/L induced the maximum stomatal width (10.785 µm), while the minimum width was observed in the treatment with Hexaconazole at a concentration of 1 ml/L. It is worth noting that the control treatment performed better in terms of stomatal number, epidermal cell count, and stomatal index, exhibiting the lowest values for stomatal number and epidermal cell count.

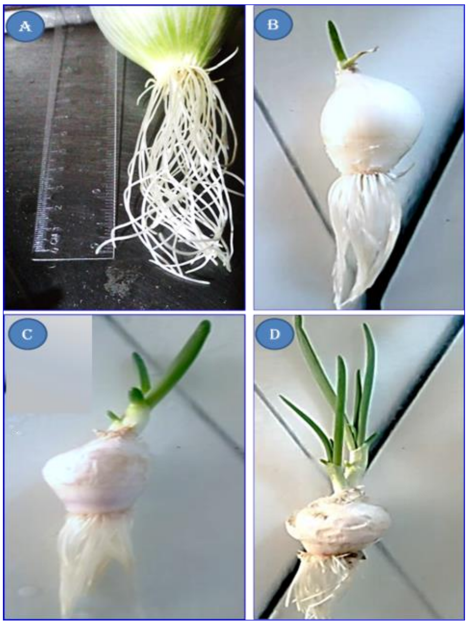

Figure (1): Impact of Hexaconazole fungicide on growth parameters of onion: a comparative visual analysis of root measured parameters at different concentations. A. 0.0; B, 0.5; C, 1.0 and D,1.5 ml L^{-1}.

Generally, the study provides valuable insights into the nuanced effects of Hexaconazole on stomatal features and epidermal cell properties, contributing to our understanding of the fungicide's influence on the leaf surfaces of *Allium cepa* L. These findings may have implications for understanding the plant's adaptive responses to fungicide application and the potential impact on leaf physiology.

DISCUSSION

Fungicides can play a crucial role in managing fungal diseases in onion crops. They are typically used to prevent or control fungal infections that can cause significant damage to onion plants and reduce crop yields. In the present investigation, it has been demonstrated that the growth patterns of onion bulbs are subject to variations depending on the treatments administered. Conspicuous differences can be observed in the morphological attributes of root growth, including parameters such as root length, root count, root percentage, and germination percentage. Additionally, the structural composition of stomata, including dimensions such as stomatal length, stomatal width, stomatal density per mm^2, epidermal cell count per mm^2, and stomatal index, has proven to be instrumental in discerning these disparities.

The present study revealed that the control group exhibited the greatest root length, measuring at 7.11 cm, which was significantly ($p \leq 0.05$) higher than the measurements obtained from T1 (4.27 cm). Particularly, the T2 and T3 treatments resulted in significantly ($p \leq 0.05$) reduced root lengths that measured 3.44 cm and 2.83 cm, respectively. These findings highlight the susceptibility of roots to environmental disturbances and emphasize the crucial role of apical meristems in facilitating the plant's response to stress stimuli. This response involves intricate signaling pathways that communicate with various plant organs, as indicated by Jiang (2014). In a study conducted by Maznah *et al*. (2017), Hexaconazole was investigated as a fungicide due to its potential to inhibit fungal growth, specifically targeting *Ganod-erma* species. Their research focused on elucidating the effects of Hexaconazole on fungal control.

Furthermore, Shahid *et al*. (2018) conducted a study that demonstrated the negative impact of fungicides, including Hexaconazole, on the biological characteristics of *P. sativum* plants. They found that fungicide

Table (2): Correlation Coefficients among the studied characteristics for root growth

Parameters measured	Root Length	No. of Root	Root Percentage	Germination percentage
Root Length	1	-0.347	-0.034	0.16
No. of Root	-0.347	1.00	.708*	-.902**
Root Percentage	-0.034	0.708*	1.00	-.791*
Germination percentage	0.16	-.902**	-.791*	1

-, negative correlation; +, positive correlation; ≤ 0.5 represents weak to moderate correlation; > 0.5-0.7; moderate to strong correlation; >0.8 to 1.0 very strong correlation.

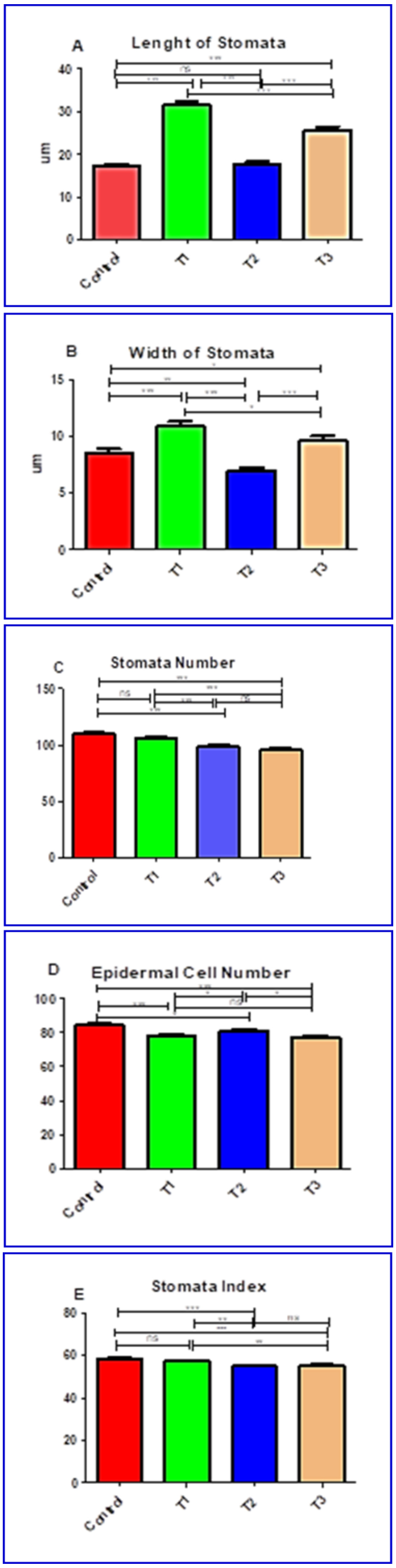

Figure (3): Effect of different concentration (0.0, 0.5, 1.0, and 1.5 ml L^{-1}) of Hexaconazole fungicide on measured stomata parameters, length, wideth, number of stomta. Epidermal cell number as well as stomatal index were calculated. The control and experimental groups (T1, T2 and T3). A, Length of stomata; B, Width of stomata; C, Stomata number; D, Epidermal cell number; E, Stomata index.

application induced stress, leading to a series of systematic changes in physiology, anatomy, cytotoxicity, cellular damage, and distortion in root morphology. In an additional investigation done by Roy *et al*. (2018) described Hexaconazole as a systemic fungicide that has been examined for its impact on a range of plant species. According to their findings, Hexaconazole can affect the balance of hormones, including auxin and cytokinin levels, which can have an effect on plant growth and development. These hormonal changes can potentially influence apical meristem activity and subsequent signaling pathways. Gorshkov *et al.* (2023) performed an additional analysis and discovered that the Hexaconazole negatively impacted on legume-Rhizobium symbiosis including nodule number, dry weight, and ultra-structure.

Regarding the dimensions of stomata, the treatment of Hexaconazole at a concentration of 0.5 ml/L exhibited the highest stomatal length measurement (31.715 µm), while the control treatment recorded the lowest value (17.272 µm). Similarly, the treatment of Hexaconazole at a concentration of 0.5 ml/L yielded the highest stomatal width value (10.785 µm), whereas the treatment of Hexaconazole at a concentration of 1 ml/L resulted in the lowest value. Moreover, the control treatment demonstrated the highest values for stomatal number per mm^2, epidermal cell count per mm2 on the abaxial surface, and stomatal index, measuring at 109.92, 84.36, and 58.093, respectively. These findings align with the observations made by Berova (2002), who highlighted that pesticides can permeate leaves through stomata, consequently impacting normal respiration and leading to modifications in chlorophyll composition, electron transport, oxidative phosphorylation, and disruption of biochemical processes. Under unfavorable environmental conditions, the plant's vigor diminishes, impeding opt-imal utilization of its surroundings. In agreement with our study, Ashok, *et al*. (2006) proved in their study the differential effects of Hexaconazole and paclobutrazol on the foliage characteristics of Chinese potato (*Solenostemon rotundifolius* Poir., in which this compound affected stomatal pore length and width, stomatalpore size, thickness of upper and lower epidermis and thenumber of stomata, palisade, spongy cells, chloroplast perpalisade and spongy cells. Our data also is in accordance with the previous reports of Bora *et al.* (2002) and Gupta *et al*. (2004).

In general, the observed inhibition of root growth in *Allium cepa* L. plants treated with the Hexaconazole fungicide suggests that the fungicide may interfere with root cell division and elongation processes. This inhibition can ultimately affect nutrient uptake, water absorption, and overall plant productivity. Furthermore, the alterations in stomatal characteristics indicate that Hexaconazole can impact the plant's ability to regulate gas exchange, transpiration, and water loss. These effects may disrupt the plant's normal physiological functions and compromise its adaptation to environmental changes.

CONCLUSION

In conclusion, this study provides valuable insights into the effects of Hexaconazole fungicide on root growth and stomatal characteristics in. *Allium cepa* L. The observed inhibition of root growth and alterations in stomatal characteristics indicate that this fungicide can have significant effects on the overall health and functioning of plants. Future research is in need to investigate the long-term repercussions of Hexaconazole exposure and devise effective strategies to minimize any associated risks. By enhancing our understanding of the potential consequences of fungicide application, we can better develop appropriate management strategies to mitigate any potential risks, inform agricultural practices and ensure the sustainable management of plant health in agricultural ecosystems.

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