Improving gas exchange characteristics, antioxidant enzymes, yield and yield attributes of black cumin through foliar application of ascorbic acid

Abid Mehmood[1*], Khalid Naveed[1], Muzaffar Iqbal[1], Rashid Ali[1], Malik Faizan Shaukat[2], Haris Shahzad[2], Anees Ur Rehman[1], Ahmad Ayub[2], Mubasir Ahmed[1], Diyan Ahmad[1], Imad Ali[1], Abdul Tawab[1], Saifur Rehman[1], Saad Hasan[3], Sagheer Khan[4], Qammer Shahzad[4], Nauman Shehzad[3], Riaz Hussain[1], Muhammad Umer Khatab[3] and Naveed Ul Haq[4,5]

1. Department of Agronomy, The University of Haripur, Pakistan
2. Department of Horticulture, The University of Haripur, Pakistan
3. Department of Soil & Climate Science, The University of Haripur, Pakistan
4. Department of Food Science and Technology, The University of Haripur, Pakistan
5. Department of Food Science, The University of Guelph, Canada

* Corresponding author's email: abidawan1990@gmail.com

Abstract

Plant growth regulators are known to improve plant's vegetative growth and yield when applied at very low concentrations. Ascorbic acid (Vitamin-C) plays a vital role in sustaining plant growth among the numerous plant growth regulators that control growth under normal or stress conditions. An experiment was conducted during rabi 2018-19 to assess the performance of black cumin crop when sprayed with different ascorbic acid concentrations, with the objective of digging out the best ascorbic acid concentration for enhancing the production of black cumin under climatic conditions of Haripur. The results showed that 100 µM ascorbic acid was found to be effective followed by 150µM ascorbic acid. Photosynthetic rate (m mol (CO_2) m^{-2} s^{-1}), Stomatal conductance (mmol H_2O m^{-2} s^{-1}), Net CO_2 assimilation rate (µmol CO_2 m^{-2} s^{-1}), Transpiration rate (mmol H_2O m^{-1} s^{-1}), Peroxidase (POX), Plant height, number of capsules plant^{-1}, seeds capsule^{-1}, 1000-seed weight (g) and Seed yield (kg ha^{-1}) were significantly affected from ascorbic acid concentrations. However, as this study was conducted with only with ascorbic acid, it is advisable to conduct an experiment with various other growth regulators, their combinations and different concentrations to check out their synergistic effects on production traits of black cumin to make reliable and acceptable recommendations.

Keywords: Black cumin, Photosynthesis, antioxidant enzymes, growth regulator, ascorbic acid

Introduction:

The production of medicinal plants become ultimate goal because of the demand for medicinal plants around the world (Shahzad *et al*., 2020). However, these crops need special attention, using suitable and recommended agronomic practices to obtain maximum income as compared with traditional crops. Black cumin, *Nigella sativa* L. commonly known as kalonji (Rehman *et al*., 2022) is one of the most widely examined plant possessing naturally occurring compounds with anti-cancer potential. The numerous vernacular names indicate the use of the spice in more than 100 countries (Mehmood *et al*., 2021a). The seeds are bitter in taste and consumption of whole seed even in small quantity gives a feeling of constriction of throat (Saul *et al.*, 2013). The seeds of black cumin crop contain approximately 30- 35 % of oil which can be utilized in food and pharmaceutical industries (Mehmood *et al*., 2018). The production of black cumin in Pakistan has not been reported on commercial scale but it is widely used for various purposes in the country. With increase in demand for medicinal plants, black cumin is a potential specie for crop diversification and it can also reduce the risk of crop failure from which economic condition of smaller land holdings could also be improved **(**Hussain *et al*., 2003: Mehmood *et al*., 2021b**)**. The major crops grown in Haripur are wheat, maize and peas thus offering limited choice of seed spice crops for the farmers. Introduction of new seed spices open new avenues for the farmers by not only widening the choice of crops but also improve their revenue from the land (Ayub et al., 2021). In such a scenario, among the several suitable seed spice crops, black cumin demands special attention due to its export value as well as increasing demand as medicinal spice (Khan et al., 2020).

Yield is dependent to a large extent on soil nutrient status and external application of nutrients (Ayub et al., 2020). Beside the importance of synthetic fertilizers, they have many adverse effects environment, human and animals' health, loss of soil fertility and also their cost is very high higher (Boraste *et al*., 2009; Mehmood et al., 2020). Finding the alternate methods for supplying essential nutrients to the crop is need of the hour (Hassan and Ali, 2013). The physiological efficiency of plants is affected by plant growth regulators (Solaimalai *et al*., 2001). They influence growth and yield of plants when applied at a very low concentration (Naeem *et al*., 2004; Ayuba et al., 2020). In addition, the impact of plant growth regulators on crops may differ with the type of plants, their genetic characteristics, variety, stage of development, environmental conditions, physiological and nutritional status, and senescence delays in endogenous hormonal equilibrium (Adebayo *et al*., 2011).

Ascorbic acid (Vitamin C) is synthesized and affects the growth and production of plants in higher plants and plays an important part in the electron transport mechanism Blokhina *et al*., (2003) reported that Ascorbic acid has potential as regulator of cell division and differentiation and to play a significant role in a broad range of functions, such as antioxidant defense, photosynthesis control and development (Allahveran *et al*., 2018). Ascorbic acid is a cofactor for certain enzymes, such as those active in the synthesis of the cell wall and proline residue hydroxylation (Abdel-Hafeez *et al*., 2019).

However, little information seems to be available on the concentration of ascorbic acid for foliar application on black cumin crop to enhance growth and obtain maximum yield. Keeping this in mind, present investigation was performed in order to identify the best ascorbic acid concentration for maximizing yield and its components of black cumin under Haripur conditions.

Materials and methods:

Site description:

The present research was carried out at the Agricultural Farm, Department of Agronomy, The University of Haripur, KP during rabi season 2018-19. This study was aimed find photosynthetic efficiency, antioxidant enzymes and yield and yield attributes of black cumin through foliar application of ascorbic acid under climatic conditions of Haripur.

Soil samples from the depth of 0 and 15 cm were collected and a composite sample was prepared for soil analysis before preparatory cultivation. Standard methods were followed for physio-chemical properties of soil (Table 1).

Experimentation:

RCBD was used for the arrangement of experiment. Each treatment was replicated three times. Black cumin variety NARC-1 kalonji was used. Seeds were sown on first week of November. Seeds were sown in rows having 30 cm row spacing. The plot size was 2 x 3 m^2. NPK was applied at the rate of 40-20-20.

Ascorbic acid was applied as foliar application as T1= 0µM, T2= 50µM, T3= 100µM, T4= 150µM, T5= 200µM, T6= 250µM, T7= 300µM. These were applied 90 days after sowing. 0 µM ascorbic acid plots were sprayed with distilled water only.

Data on Physiological characters like photosynthetic rate, transpiration rate, stomatal conductance, transpiration rate and Net CO_2 assimilation rate, were recorded on youngest leaf which are fully expended from each plant with the help of an open system LCA-4 ADC portable Infrared gas analyzer (Analytical Development Company, Hoddesdon, England). The readings were recorded from 10:15 to 11:45 with the following specifications/adjustments: leaf surface area 3.25 cm^2, temperature of leaf chamber varied from 31.5 to 37.8 C, leaf chamber gas flow rate (v) 392.8 ml min^{-1}, Molar flow of air per unit leaf area (Us) 404.84 mol $m^{-2}sec^{-1}$. Ambient chamber ranged from 20.5–23.1 m bar, PAR (Qleaf) at leaf surface was maximum up to 1048 l mol $m^{-2}s^{-}1$. The peroxidase (POX), catalase (CAT), and ascorbate peroxidase (APX) behaviours were spectrophotometrically determined. Leaves were homogenised as defined by Dixit *et al*., (2001) in a medium composed of 50 mM phosphate buffer with 7.0 pH and 1 mM dithiothreitol (DTT). Plant height, capsules plant^{-1}, capsule^{-1} seeds, 1000-grain weight (g) and seed yield (kg ha^{-1}) were recorded. The analysis of variance (ANOVA) was worked out from collected data to observe difference among various treatments (Gomez and Gomez, 1984).

Table 1; Physico-chemical Profile of Soil

Texture class	**PH value**	**% TSS**	**Organic matter**	**N (%)**	**P (ppm)**	**K (ppm)**	**Ca^+**	**Mg^+**	**HCO_3**	**Cl**
Silty Clay	7.41	1.2	1.71	0.089	9.54	68	5.00	0.95	5.68	0.11

RESULTS AND DISCUSSIONS

Photosynthetic rate (mmol (CO_2) m^{-2} s^{-1}):

Photosynthetic rate of black cumin was significantly affected from ascorbic acid concentrations (table 2). Statistically similar results regarding highest photosynthetic rate was recorded in those plants sprayed with ascorbic acid @ 150 µM (15.5 (mmol (CO_2) m^{-2} s^{-1}) and plants treated with ascorbic acid concentration of 100 µM (15.2(mmol (CO_2) m^{-2} s^{-1}), followed by (13.8(mmol (CO_2) m^{-2} s^{-1})) the plants sprayed with ascorbic acid @ 50 µM, while lowest photosynthetic rate (8.2(mmol (CO_2) m^{-2} s^{-1}) was observed in control treatment (table 03). In photosynthesis, ascorbic acid has a central position, as the high concentration in chloroplasts would suggest. In its three biochemical modes, it functions. Firstly, by removing hydrogen peroxide produced by oxygen photo reduction, it acts as an antioxidant (Akram *et al*., 2017). This series has been dubbed the reaction of Mehler peroxidase (Aziz *et al.*, 2018). Secondly, mono-dehydro ascorbate, formed by ascorbate peroxidase can act as a direct electron acceptor to PSI (Hussein and Mohamed, 2017). Thirdly, it is a violaxanthin de-epoxidase cofactor. In bright sun, or when low temperatures and drought limit carbon dioxide fixation, zeaxanthin dissipates the excess excitation energy as heat in the light harvesting antennae (Mohamed *et al*., 2017).

Table 02: Analysis of variance results (Means squares) of various traits of black cumin as affected from ascorbic acid concentrations

Source	DF	Photosynthetic rate	Stomatal Conductance	Net CO_2 assimilation rate	Transpiration rate	CAT	POX
Replication	2	0.1300	0.00010	0.08020	0.01857	63.4762	92.33
Treatment	6	21.5997	0.00489	3.07004	2.03857	82.8889 ^{NS}	4344.41
Error	12	0.5228	0.00011	0.09592	0.08690	62.1984	54.56
CV %	---	5.81	3.21	3.56	6.92	1.37	0.34
Mean	---	12.443	0.3286	8.704	4.2571	574.67	2162.2

Significant at 0.05 level, NS= Non-Significant

Stomatal conductance (mmol H_2O m^{-2} s^{-1}):

Table 02 depicting analysis of variance of stomatal conductance of black cumin affected from various concentrations of ascorbic acid. Significant differences were observed regarding foliar application of various ascorbic acid concentrations. Statistically similar results regarding highest stomatal conductance was noted in the application of ascorbic acid at concentration of 100 µM (0.38 mmol H_2O m^{-2} s^{-1}) and 150 µM (0.37 mmol H_2O m^{-2} s^{-1}). While statistically lower stomatal conductance was observed 300 µM (0.28 mmol H_2O m^{-2} s^{-1}) and in control treatment (0.27 mmol H_2O m^{-2} s^{-1}). To reduce transpiration rate of leaf and to maintain turgor of cell for maintaining normal biochemical processes, regulation of stomatal conductance is an initial defense mechanism of plants to various stresses (Mehmood *et al.*, 2021). Studies have shown that ascorbic acid has an effect on stomatal conductance in barley (Habibi, 2013), chickpea (Rahbarian *et al*., 2011), wheat (Moud and Yamagishi, 2005), and in potato (Kawakami *et al*., 2006).

Net CO_2 assimilation rate (µmol CO_2 m^{-2} s^{-1}):

Net CO_2 assimilation rate was affected significantly from different concentrations of ascorbic acid (table 02). Maximum CO_2 assimilation rate (10.3 µmol CO_2 m^{-2} s^{-1}) was observed in plants sprayed with 100 µM, followed by (9.3 µmol CO_2 m^{-2} s^{-1}) plants sprayed with 150 µM. whereas, minimum CO_2 assimilation rate (7.3 µmol CO_2 m^{-2} s^{-1}) was observed in crop plots. Ascorbic acid is considered to have a key role in photosynthesis, stomatal conductance and the net rate of CO_2 assimilation is characterized by its occurrence in all photosynthetic tissues where reversed hydration of CO_2 is catalyzed. In the present study, plants treated with medium ascorbic acid concentration have possessed greater CO_2 assimilation rate as compared to the control plants. This effect is likely caused by influence of ascorbic acid on the CO_2 assimilation rate, which involves translation/transcription (Alam *et al*., 2012).

Table 03: Means of photosynthetic rate, stomatal conductance, transpiration rate, CO_2 assimilation rate, POX and CAT of black cumin as affected from ascorbic acid concentrations

	Photosynthetic rate	**Stomatal Conductance**	**Net CO_2 assimilation rate**	**Transpiration rate**	**CAT**	**POX**
T1	8.2 e	0.27 d	7.3 d	2.7 e	574.3 ^{NS}	2096.3 f
T2	13.8 b	0.34 b	9.0 b	4.5 bc	577.3	2193.3 b
T3	15.2 a	0.38 a	10.3 a	5.1 a	581.0	2211.3 a
T4	15.5 a	0.37 a	9.3 b	4.9 ab	577.3	2179.3 c
T5	12.6 bc	0.34 b	8.8 b	4.4 bc	577.0	2156 d
T6	11.5 c	0.31 c	8.2 c	4.2 cd	565.3	2162.3 d
T7	10.2 d	0.28 d	7.8 c	3.7 d	570.3	2137 e
LSD	**1.28**	**0.01**	**0.55**	**0.52**	**14.03**	**13.14**

NS= Non-significant

Transpiration rate (mmol H_2O m^{-1} s^{-1}):

Transpiration rate (mmol H_2O m^{-1} s^{-1}) was affected significantly from different concentrations of ascorbic acid (table 02). Highest Transpiration rate (5.1 mmol H_2O m^{-1} s^{-1}) was observed in plants sprayed with 100 µM, followed by (4.9 mmol H_2O m^{-1} s^{-1}) plants sprayed with 150 µM. whereas, minimum transpiration rate (2.7 mmol H_2O m^{-1} s^{-1}) was observed in crop plots. Plants sprayed with ascorbic acid exhibited higher transpiration rate than the control. Medlyn *et al.*, (2013) proposed that function of stomata may be governed by mesophyll activities; hence, reduced transpiration rate facilitates the free exchange of gases from plants.

Catalase (CAT):

Table 02 depicting analysis of variance results of catalase activity of black cumin as affected from various ascorbic acid concentrations. Results regarding catalase activity showed non-significant differences. Highest CAT was seen in plants sprayed with ascorbic acid @ 100 µM while lowest was noted in plants sprayed with ascorbic acid @ 250 µM.

Peroxidase (POX):

Analysis of variance for peroxidase showed significant differences with various concentrations of ascorbic acid application (table 02). Highest POX (2211.3 min^{-1} g^{-1} FW) was observed in ascorbic acid concentration of 100 µM, followed by (2193.3 min^{-1} g^{-1} FW) plants sprayed with ascorbic acid @ 50 µM, whereas, lowest POX value (2096.3 min^{-1} g^{-1} FW) was observed in control treatment. Increased POX activation / levels were reported by Khan *et al.*, 2012 in the *Pteris vittata* L ascorbic acid accumulator. Using the incorporation of ascorbic acid. POX enzyme activation has been documented in many plants treated with ascorbic acids exposed to different stresses, for example in case of rapeseed seedlings when subjected to drought stress (Hasanuzzaman and Fujita, 2014), Phytotoxicity in wheat seedlings under cold stress and in barley under ascorbic acid, which could be directly related to oxidative stress caused by production of ROS, i.e. H_2O_2 and superoxide anion O_2 or, indirectly, by decreasing the content of glutathione (Benavides *et al.,* 2005).

Plant height (cm):

Plant height of black cumin was significantly affected from ascorbic acid concentrations (Table 04). Tallest plants (86.63 cm) were recorded with foliar application of 150 µM ascorbic acid concentration followed by 83.9 cm when ascorbic acid was sprayed @ 200 µM. Whereas, shortest plants were observed in plots sprayed with distilled water only (64.3 cm) (table 05). Plant growth regulators are chemicals which influence plant growth and yield when applied at a very low concentration. It is very well known that major development processes in plants are controlled by various group of plant hormones which sometimes act as growth promotor when applied in lower concentrations while in higher concentrations they act as growth retarders. It was thus apparent that these two treatments among other treatments caused maximum plant height. ascorbic acid is known to be a significant plant growth regulator that has been documented to promote a broad variety of processes, including cell elongation, contributing to increased development and growth (Sadak *et al*., 2015).

Capsules plant^{-1}:

Significant difference among various ascorbic acid concentrations was observed during the study (table 04). Maximum number of pods/capsules plant^{-1} (43.6) was produced by the plants sprayed with 100 µM ascorbic acid followed by (40.66) when ascorbic acid was applied @ 150 µM. Whereas, minimum capsules plant^{-1} (28.6) were recorded in those plants which were not treated with growth hormones (Table 05). It is interesting to note here that lower concentration of ascorbic acid (100 µM) increased the capsules number plant^{-1} than higher concentrations. Maximized photosynthesis and improved sink capability can be related to these important results by providing photo-assimilates from leaves and translocation to create high-quality fruit and yield as reported by Thomas and Howarth, 2000 also revealed that the application of growth hormones induced bio-synthesis of cytokinin in turned maximum number of active photosynthetic leaves and that is apparent from the number and area of leaves per plant retaining higher concentrations of chlorophylls reflecting on plant yield.

Table 04: Analysis of variance results (Means squares) of various yield related traits of black cumin as affected from ascorbic acid concentrations

Source	DF	Plant height	Capsules plant^{-1}	Seeds capsule^{-1}	1000-seed weight	Seed yield kg ha^{-1}
Replication	2	20.195	8.1905	28.5962	0.00123	687.83
Treatment	6	202.863	94.6349	58.7638	0.19682	6679.89
Error	12	1.582	1.3016	5.9962	0.00458	224.87
CV %	---	1.68	3.28	2.63	2.61	2.73
Mean	---	74.952	34.762	93.195	2.5910	549.21

Significant at 0.05 level

Seeds capsule^{-1}:

Table 04 showed that all levels of ascorbic acid lead to significant increases in seeds capsule^{-1} as compared to the control treatment. Data also cleared that spraying ascorbic acid @ 100 µM on black cumin plants was sufficient to produce the superiority results of seeds capsule^{-1} as compared to all other concentrations. ascorbic acid foliar application with low concentration provides adequate quantities of stimulant substances promoted to increase the rate of cell division, cell enlargement, growing plants, gradually generating more and higher seed. These results are in full agreement with those obtained by Kandil *et al*., (2007) who found that exogenously applied ascorbic acid 100 ppm maximize seed yield and its components due to reducing flower, shedding percentage, lodging of plants and an escalating yield component.

Table 05: Effect of **different concentrations of ascorbic acid on growth and yield of Black cumin**

	Plant height	**Number of capsules plant^{-1}**	**Number of seeds capsule^{-1}**	**1000-seed weight**	**Seed yield kg ha^{-1}**
T1	64.3 f	28.66 e	86.8 e	2.25 e	488.89 e
T2	72.36 d	36.33 c	89.1 de	2.30 e	500.00 de
T3	66.7 c	43.66 a	97.2 bc	2.91 a	605.5 a
T4	86.63 a	40.66 b	99.4 a	2.73 bc	577.7 b
T5	83.93 b	31.66 d	94.6 bc	2.82 ab	600.00 ab
T6	75.26 c	31.00 d	93.6 bc	2.63 c	522.22 d
T7	75.46 c	31.33 d	91.5 cd	2.46 d	550.00 c
LSD	**2.23**	**2.02**	**4.35**	**0.12**	**26.67**

1000-seed weight:

1000-seed weight of black cumin was affected significantly from various ascorbic acid concentrations. Application of ascorbic acid @ 100 µM showed promising results and was significantly on par, followed by ascorbic acid concentration of 150 µM. While least 1000-seed weight were observed in plots sprayed with distilled water only. Chattha *et al*. (2015) also found the increased 1000-grain weight due to application of growth enhancing substances applied at different concentrations.

Seed yield kg ha^{-1}:

All concentrations of ascorbic acid improve seed yield. However, highest seed yield (605.5 kg ha^{-1}) was recorded with 100 µM ascorbic acid, followed by (600 kg ha^{-1}) 200 µM ascorbic acid, whereas, lowest seed yield was observed in control plots (488.8 kg ha^{-1}). The increase in seed yield may be due to the defensive effect of ascorbic acid in increasing assimilates and their translocation from leaves to fruits where the weight of the seeds increased (Ibrahim *et al*., 2007).

Conclusion:

Form this study it can be concluded that ascorbic acid @ 100 µM was more effective for enhancing the growth, yield and yield components of black cumin under the climatic conditions of Haripur Hazara, Khyber Pakhtunkhwa, Pakistan.

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