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Research article

Exogenous application of ascorbic acid improves physiological and productive traits of Nigella sativa

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ABSTRACT

For thousands of years, plants have been utilized for medicinal purposes. For its naturally existing antibacterial properties, *Nigella sativa* is one of the most researched herbs. A study was conducted during rabi 2020-21 at The University of Haripur in order to evaluate the potential of ascorbic acid as plant growth enhancer. Two concentrations of ascorbic acid i-e 350 μm and 400 μm were sprayed along with control and water only spray on *Nigella sativa* crop. The study was arranged in RCBD two factor factorial arrangement. Factor A: ascorbic acid concentrations along with control and water spray, factor B: Growth stages (Stage1 = 40 days after sowing, Stage 2 = 80 DAS, Stage 3 = 120 DAS, Stage 4 = 40 + 80 DAS, Stage 5 = 40 + 120 DAS, Stage 6 = 80 + 120 DAS, Stage 7 = 40 + 80 + 120 DAS). Crop was sown in first week of November. Results reviled that chlorophyll *b* content, fixed oil content, 1000 seed weight, grain yield, Photosynthetic rate (μ mole $m^{-2}s^{-1}$), Transpiration rate (mmole $m^{-2}s^{-1}$), photosynthetic water use efficiency, Internal CO_2 concentration (*Ci*) of leaf tissue and Stomatal conductance (mmole $m^{-2}s^{-1}$) were significantly affected

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by ascorbic acid concentrations and stage of application. Crop growth rate increased by 19.88% and 17.29%, chlorophyll *b* by 12.3% and 11.2%, fixed oil by 11.7% and 9%, grain yield by 10.29% and 9.8%, harvest index by 4% and 5.7% photosynthetic rate by 33%, 20% and stomatal conductance by 24.24% and 24.25 with application of ascorbic acid @ 350 μm, over control and water spray respectively. On the basis of these results it is concluded that application of ascorbic acid at the rate of 350 μm, followed by ascorbic acid at the rate of 400 μm significantly improves black cumin (*Nigella sativa*) yield and production. Hence it is recommended to apply ascorbic acid at the rate of 350 μm at 40 + 80+120 days after sowing of *Nigella sativa* crop for obtaining maximum results.

1. Introduction

Black cumin is one of the most studied plants for its naturally occurring anti-cancer chemicals. It's a buttercup-like field crop from the Ranunculaceae family [1]. It has been used to treat a range of health conditions, including respiratory issues, digestive problems, and skin conditions. The seeds have a bitter flavor, and even a tiny amount of entire seed causes a sense of tightness in the throat [2]. The seeds of Nigella sativa contain bioactive compounds such as thymoquinone, which has demonstrated anti-inflammatory, anti-oxidant, and antimicrobial properties. These properties contribute to its potential medicinal benefits. The seeds of Nigella sativa are a common spice in Pakistani cuisine. They are used to add flavor and aroma to various dishes, including bread, curries, and pickles. The distinct taste of the seeds contributes to the culinary diversity of the region [3]. Nigella sativa is mentioned in Islamic traditions, and its seeds are referred to as "Habbat al-Barakah" or the blessed seed. It is believed that the Prophet Muhammad spoke about the therapeutic properties of black seed, adding to its cultural and religious significance. The major black cumin producers are India, Pakistan, Syria, Turkey, Saudi Arabia, Egypt, and Bangladesh, although comparative production data are not available in open-access online sources [4].

Plant growth regulators have been used exogenously in conjunction with specific nutrients, antioxidants, organic and inorganic substances to accelerate plant growth and development, resulting in higher economic returns [5]. Ascorbic acid holds a crucial role as the predominant antioxidant compound indispensable for diverse biological functions within plants [6]. Exogenous application of ascorbic acid enhances crop development and yield in a variety of crop species in both normal and stressed conditions [7]. Ascorbic acid serves as a cofactor for enzymes participating in the processes of photosynthesis and hormone synthesis in plants [8]. By raising the activities of catalase and ascorbate peroxidase, as well as lowering chlorophyll degradation, ascorbic acid reduces oxidative stress-induced senescence in wheat leaves [9]. Ascorbic acid plays a pivotal role in the initial detoxification and neutralization of superoxide radicals [10]. The external application of antioxidants has been documented to significantly enhance the inhibitory effects of water stress on plant growth and metabolism [11]. As an antioxidant, ascorbic acid (AA) positively influences cell growth, division, differentiation, and metabolism in plants [12]. Ascorbic acid interacts with H_2O_2, O_2, OH, and lipid hydroperoxidases, among others. Ascorbate peroxidase utilizes ascorbic acid in chloroplasts, which reduces the chance of ROS. Additionally, ascorbate preserves and regenerates oxidized carotenoids and tocopherols [13].

The potential effects of ascorbic acid on plant structure and physiological activities have motivated numerous researchers to test it on a variety of crops with the goal of increasing growth and production while also improving tolerance and resistance to various damaging agents [14]. Depending on the dosage, plant species, developmental stage, and environmental circumstances, ascorbic acid stimulates some physiological processes while inhibiting others. Ascorbic acid contributed to enhanced vegetative as well as

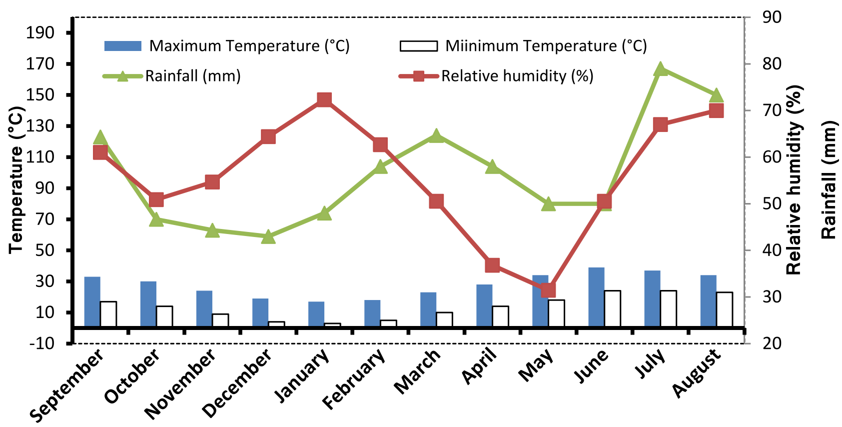

Fig. 1. Monthly climate trends at Agricultural Research Farm, The University of Haripur, Pakistan, in 2020–21. Blue bars represent maximum temperature (°C), white bars indicate minimum temperature (°C), an orange line with square dots illustrates relative humidity (%), and a grey line with triangle dots portrays monthly rainfall (mm) (Fig. 1). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

reproductive development of various crops [15–17]. Ascorbic acid reduces the adverse effect of salt stress on chlorophyll *a* and protected from it oxidative stress [18], by improving the photosynthetic pigments and the nutritional status in crops [19]. Application of ascorbic acid increased the total carbohydrates, crude protein, N, P, K, and Ca contents [20]. The present investigation was carried out with the objective to determine the best level of ascorbic acid and best stage for application of ascorbic acid which will result in optimum productivity of black cumin under climatic conditions of Haripur.

2. Materials & methods

2.1. Experimental site and plant material

The study was carried out at Agricultural Research Farm, The University of Haripur, Pakistan 33.9781° N, 72.9128° E, during rabi 2020–21. The soil at the experimental site was sandy clay loam and had the following chemical characteristics: pH = 7.9; electrical conductivity = 0.25 s m^{-1}, N = 0.038%; P = 11.5 ppm; K = 145 ppm; organic matter = 0.61%, Ca = 5.20%, Mg = 0.70%. Therefore, the soil was mildly alkaline, had low organic C and N, had moderate phosphorus concentrations, but had sufficient potassium concentration. All agronomic operations were consistently performed across all treatments throughout the study period, adhering to the experimental protocol..

The experimental plots were ploughed for three times across the slope and prepared to a fine tilth. Subsequently cross-wise harrowing was done to level the field properly and for provision of drainage. The field was divided into plots as per the layout of experiment. Black cumin variety NARC-1-Kalonji was used in the experiment. The seeds were sown @ 5 kg ha^{-1} on start of November. After 25 days of seedling emergence, the seedlings were thinned to maintain required spacing. The field was inspected from time to time to discover visual variations between the treatments as well as any weed, insect, or disease infestations, in order to reduce pest losses. Four levels i-e, ascorbic acid @ 350 μm, ascorbic acid @ 400 μm, control (no water no ascorbic acid) and water only (no ascorbic acid) was applied at seven stages i-e Stage-1: 40 Days after sowing (DAS), stage-2: 80 DAS, stage-3: 120 DAS, Stage-4: 40 + 80 DAS, stage-5: 40 + 120 DAS, Stage-6: 80 + 120 DAS and stage-7: 40 + 80+120 DAS. Observations recorded during the study are as under.

2.2. Plant analysis

Plants were chosen at random from each treatment and plant height was measured with a measuring tape. Crop growth rate was figured out by sun drying five plants uprooted at 40 days after sowing and finding out initial dry weight (W1) with electronic balance. Similarly, final dry weight (W2) was calculated in the same way after 50 days after sowing. Crop growth rate was measured with formula:

$$CGR = W2–W1/T2-T1$$

Leaf chlorophyll content of leaves at 50% flowering were observed by using method of [21] Arnon (1974). Fresh leaves (0.5 g) were

Table 1
Plant height (cm), Crop growth rate (g m^{-2} day^{-1}), Chlorophyll *a* content (mgg^{-1}), Chlorophyll *b* content (mgg^{-1}), and Essential oil content (% vw^{-1}) of black cumin as affected by ascorbic acid applied at different growth stages.

	Plant height (cm)	Crop growth rate (g m^{-2} day^{-1})	Chlorophyll *a* content (mgg^{-1})	Chlorophyll *b* content (mgg^{-1})	Essential oil content (% vw^{-1})
Growth stages					
Stage 1	52.2^{e}	1.99^{c}	30.41^{c}	27.86^{f}	0.35^{d}
Stage 2	63.35^{c}	2.16^{b}	32.18^{b}	$28.4d^{e}$	0.34^{d}
Stage 3	49.85^{f}	1.89^{d}	29.93^{c}	29.5^{c}	0.36^{c}
Stage 4	66.75^{b}	2.29^{a}	33.28^{a}	30.55^{b}	0.39^{b}
Stage 5	56.58^{d}	2.03^{c}	30.06^{c}	28.68^{d}	0.35^{cd}
Stage 6	66.2^{b}	2.21^{b}	32.4^{b}	28.13^{e}	0.36^{c}
Stage 7	71.26^{a}	2.35^{a}	32.88^{ab}	33.26^{a}	0.42^{a}
LSD (0.05)	**0.62**	**0.05**	**0.66**	**0.35**	**0.01**
Ascorbic Acid concentrations					
AA @ 350 μm	61.19^{NS}	2.17^{a}	31.74^{NS}	29.76^{a}	0.37^{NS}
AA @ 400 μm	60.58	2.09^{b}	31.44	29.2^{b}	0.36
LSD (0.05)	**NS**	**0.01**	**NS**	**0.1**	**NS**
Control vs Rest treatments					
Control	42.16	1.81	29	26.5	0.32
Rest treatments	59.71	2.11	31.46	29.3	0.36
Significance	**	**	**	**	**
Water only vs Rest treatments					
Water only	43.33	1.85	29.56	26.76	0.33
Rest treatments	60.88	2.13	31.59	29.48	0.37
Significance	**	**	**	**	**
Interaction	**Significance**				
Stages x Concentrations	**NS**	**NS**	**NS**	Fig. 1	**NS**

grounded in 80% acetone and centrifuged for 5 min at 5000 rpm using a centrifuge (Biobase H-2018j China). Absorbance was observed using a spectrophotometer (Robus Technologies UV-1100 Canada). Essential oil content of was determined by following [22] (Remmal et al., 1993) method. Four grams of powdered black cumin seeds sample were extracted with n-hexane for 6 h using soxlet apparatus for determining the fixed oil content (%). 1000-seed weight was measured after taking hand sample of 1000 seeds from each treatment. The weight of the complete plant except roots was measured after harvesting. It was expressed as kgha^{-1}. After threshing and winnowing, weight of clean seeds obtained from individual plots were recorded in grams per plot. The data was then converted into kg ha^{-1}. Gas exchange characteristics were determined by using portable infrared gas analyzer (CI-340 handheld photosynthesis system CID Bio-science USA). Statistical analysis was done using computer based software Excel 2016. Means were separated at 5% probability [23] (Gomez and Gomez 1984).

3. Results

3.1. Plant height (cm)

Analysis of variance results showed that stage of application of ascorbic acid had significant effect on plant height of black cumin crop whereas, concentration and their interaction remained non-significant during the study. Taller plants (71.26 cm) were observed with the application of ascorbic acid at stage-7 followed by (66.2) stage-6, whereas lowest plant height (49.85 cm) was observed with application of ascorbic acid at stage-3.

3.2. Crop growth rate (g m^{-2} day^{-1})

Significant differences among various concentrations of ascorbic acid and various stages of application of ascorbic acid was observed for crop growth rate but non-significant interaction was observed during the study (Table 1). Highest CGR (2.17 g m^{-2} day^{-1}) was recording in plants treated with ascorbic acid at the rate of 350 μm, whereas, lowest CGR (2.09 gm^{-2} day^{-1}) of black cumin was observed with application of ascorbic acid @ 400 μm. Regarding application of ascorbic acid at various growth stages, highest crop growth rate (2.35 gm^{-2} day^{-1}) of black cumin was observed when ascorbic acid was sprayed at 40 + 80 + 120 DAS, whereas, lowest crop growth rate (1.89 g m^{-2} day^{-1}) was observed in plants sprayed with ascorbic acid at 120 days after sowing.

3.3. Chlorophyll *a* content (mgg^{-1})

The effect of ascorbic acid applied at various growth stages of black cumin showed significant result ($P \leq 0.05$) regarding chlorophyll *a* content, whereas, ascorbic acid concentrations as well as their interaction remained non-significant. Data presented in Table 1 indicated that statistically similar chlorophyll *a* content (33.28 mgg^{-1}) was recorded with application of ascorbic acid at stage-4, followed by (32.88 mgg^{-1}) stage-7 and (32.18 mgg^{-1}) stage-2, whereas, statistically similar and lower chlorophyll *a* content (29.93 mgg^{-1}) was observed in stage-6 and (30.06 mgg^{-1}) stage-5 (40 + 120 days after sowing).

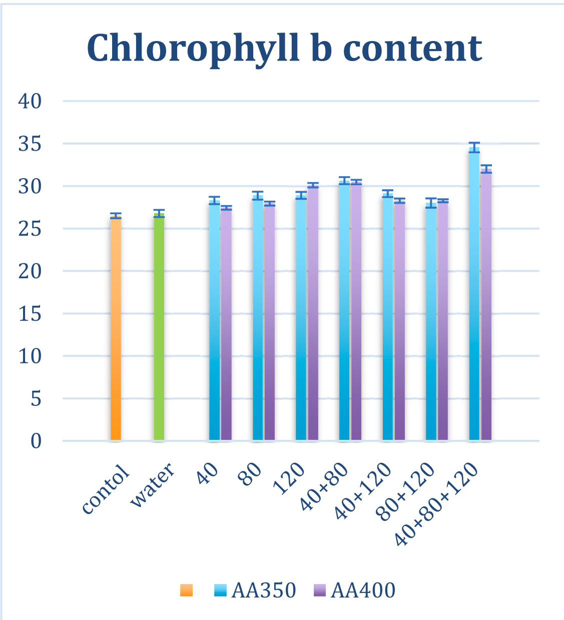

Fig. 2. Chlorophyll *b* content of black cumin as affected by foliar application of ascorbic acid applied at various growth stages.

3.4. Chlorophyll b content (mgg^{-1})

The amount of chlorophyll in plants is a measure of how well they convert solar energy into chemical energy. Table 1 shows chlorophyll *b* values. The analyzed data revealed that various growth stages, both concentrations of ascorbic acid as well as their interaction pronounced impact on concentration of chlorophyll *b* in flag leaf of black cumin. Computed means for chlorophyll *b* content showed that maximum value (29.76 mgg^{-1}) was observed with application of ascorbic acid @ 350 μm, whereas, lowest (29.2 mgg^{-1}) was recorded of ascorbic acid @ 400 μm. Regarding stage of application, maximum value of chlorophyll *b* content (33.26 mgg^{-1}) was observed with application of ascorbic acid at stage-7, followed by (30.55 mgg^{-1}) stage-4, whereas lowest chlorophyll *b* content (27.86 mgg^{-1}) was recorded in stage-1. Concerning the interaction, maximum chlorophyll *b* content (34.53 mgg^{-1}) was recorded with application of ascorbic acid at the rate of 350 μm applied at stage-7, whereas, lowest chlorophyll *b* content (27.43 mgg^{-1}) was observed with application of ascorbic acid at the rate of 400 μm applied at stage-1 (Fig. 2).

3.5. Essential oil content (% vw^{-1})

It can be inferred from the data presented in Table 1 that essential oil content of black cumin seeds was significantly affected ($P \leq 0.05$) from ascorbic acid concentrations. Maximum essential content (0.42 % vw^{-1}) was recorded with application of ascorbic acid at stage-7, followed by growth stage-4 whereas, lowest essential oil content (% vw^{-1}) was observed in stage-3.

3.6. Fixed oil content (% vw^{-1})

Statistical analysis of the data given in Table 2 indicates that both concentrations of ascorbic acid, stage of application of ascorbic acid had significant ($P \leq 0.05$) effect on fixed oil content of black cumin crop. It was evident from the data that highest fixed oil content (34.75 vw^{-1}) was recorded from the plots sprayed with ascorbic acid at the rate of 350 μm, while, lowest fixed oil content (33.53 vw^{-1}) was recorded when ascorbic acid was given at the rate of 400 μm. The data further reviled that application of ascorbic acid at stage-7 (40 + 80+120 DAS) showed promising results and was at par regarding fixed oil content (36.5 % vw^{-1}), followed by (34.98 % vw^{-1}) stage-4 and (34.67% vw^{-1}) stage-6, while lowest fixed oil content (32.03% vw^{-1}) was recorded in stage-1.

3.7. 1000-Seed weight (g)

Data presented in Table 2 illustrated that 1000 seed weight was significantly affected from ascorbic acid concentrations and application stage application, whereas, the interaction had non-significant differences. Table depicting that maximum 1000 seed weight (2.44 g) was recorded with application of ascorbic acid at the rate of 350 μm whereas, lower 1000 seed weight (2.32 g) was observed with application of ascorbic acid @ 400 μm. Regarding growth stages, maximum 1000 seed weight (2.96 g) was recorded with application of ascorbic acid at stage-7, followed by (2.69 g) stage-6. Minimum 1000 seed weight (2.02 g) was recorded in stage-1.

Table 2
Fixed oil content (% vw^{-1}), 1000-seed weight (g), Grain Yield (kg ha^{-1}), Biological yield (kg ha^{-1}) and Harvest index (%) of black cumin as affected by ascorbic acid applied at different growth stages.

	Fixed oil content (% vw^{-1})	1000-seed weight (g)	Grain Yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index
Growth stages					
Stage 1	32.03^{e}	2.02^{f}	881.16^{e}	2108.16^{d}	41.79^{d}
Stage 2	33.71^{c}	2.21^{e}	905.16^{d}	2141.16^{b}	42.28^{c}
Stage 3	32.99^{d}	1.76^{g}	839.66^{g}	2082.33^{e}	40.32^{e}
Stage 4	34.67^{bc}	2.61^{c}	924.66^{b}	2159^{a}	42.82^{b}
Stage 5	34.1^{c}	2.39^{d}	845.66^{f}	2132.83^{c}	39.64^{f}
Stage 6	34.98^{b}	2.69^{b}	919.33^{c}	2137.33^{bc}	43.01^{b}
Stage 7	36.5^{a}	2.96^{a}	942.66^{a}	2165.83^{a}	43.52^{a}
LSD (0.05)	**0.64**	**0.07**	**3.8**	**7.96**	**0.25**
Ascorbic Acid concentrations					
AA @ 350 μm	34.75^{a}	2.44^{a}	901.47^{a}	2136.8^{NS}	42.17^{a}
AA @ 400 μm	33.53^{b}	2.32^{b}	886.61^{b}	2127.95	41.65^{b}
LSD (0.05)	**0.18**	**0.02**	**1.08**	**NS**	**0.07**
Control vs Rest treatments					
Control	31.11	1.65	817.33	2030.66	40.24
Rest treatments	33.99	2.33	889.13	2127.33	41.78
Significance	**	**	**	**	**
Water only vs Rest treatments					
Water only	31.88	1.69	820.33	2056.66	39.88
Rest treatments	34.14	2.38	894.04	2132.38	41.91
Significance	**	**	**	**	**
Interaction	**Significance**				
Stages x Concentrations	**NS**	**NS**	Fig. 2	**NS**	**NS**

3.8. Grain yield (kgha^{-1})

Grain yield was significantly affected from ascorbic acid concentrations, application stage as well as their interaction (Table 2). Maximum grain yield (901.47 kgha^{-1}) was recorded with application of ascorbic acid at the rate of 350 μm whereas lowest grain yield (886.61 kgha^{-1}) was recorded in plots sprayed with ascorbic acid concentration of 400 μm. Regarding stage of application of ascorbic acid, maximum grain yield (942.66 kgha^{-1}) was recorded in stage-7, followed by (924.6 kgha^{-1}) stage-4, whereas, lowest grain yield (839.6 kgha^{-1}) was recorded with application of ascorbic acid at stage-3. Concerning the interaction, Fig. 3 showed that maximum grain yield (945 kgha^{-1}) was recorded with application of ascorbic acid at the rate of 350 μm at stage-7 (40 + 80 + 120 DAS), whereas, lowest grain yield (831 kgha^{-1}) was observed with application of ascorbic acid @ 400 μm applied at stage-5 (40 + 120 DAS).

3.9. Biological yield (kg ha^{-1})

Biological yield, which includes stalk yield, grain yield, and cob pith, is an indication of photosynthetic activity. It depicts a plant's physiological and physical characteristics. Analysis of variance for biological yield (kgha^{-1}) revealed that application of ascorbic acid at various growth stages significantly influenced biological yield (kgha^{-1}) of black cumin. While non-significant results were obtained from various concentrations of ascorbic acid. The interaction also showed non-significant results. Table 2 depicted that maximum biological yield (2165.8 kgha^{-1}) was observed in stage-7, followed by (2159 kg ha^{-1}) in stage-4 (40 + 80 DAS), whereas, lowest biological yield (2082.3 kg ha^{-1}) was recorded in stage-3 (120 DAS).

3.10. Harvest index

Analysis of variance for harvest index content revealed that application of ascorbic acid at different stage and various concentrations of AA had significant effect while the interaction showed non-significant results (Table 2). Highest harvest index (42.17) was observed with ascorbic acid at the rate of 350 μm as foliar application, followed by (41.65) application of ascorbic acid @ 400 μm. Regarding growth stages, highest harvest index was observed in stage-7, followed by stage-6, whereas, lowest harvest index was recorded in stage-5.

3.11. Photosynthetic rate (μ mole m^{-2}s^{-1})

The effect of ascorbic acid concentrations and their stage of application are stated in Table 3. Ascorbic acid concentrations and their stage of application were found to have radically changed photosynthetic rate. Interactions of ascorbic acid concentrations and their stage of application was found significant for photosynthetic rate (μ mole m^{-2}s^{-1}) of black cumin. Means for photosynthetic rate (μ mole m^{-2}s^{-1}) showed that maximum value (9.66 μ mole m^{-2}s^{-1}) was observed with application of ascorbic acid @ 350 μm, whereas, lowest (9.38 μ mole m^{-2}s^{-1}) was observed with application of ascorbic acid @ 400 μm. Regarding stage of application, maximum value of photosynthetic rate (10.63 μ mole m^{-2}s^{-1}) was observed with application of ascorbic acid at stage-7, followed by (9.91 μ mole m^{-2}s^{-1}) stage-6, whereas lowest photosynthetic rate (7.63 μ mole m^{-2}s^{-1}) was recorded in stage-1. Concerning the interaction,

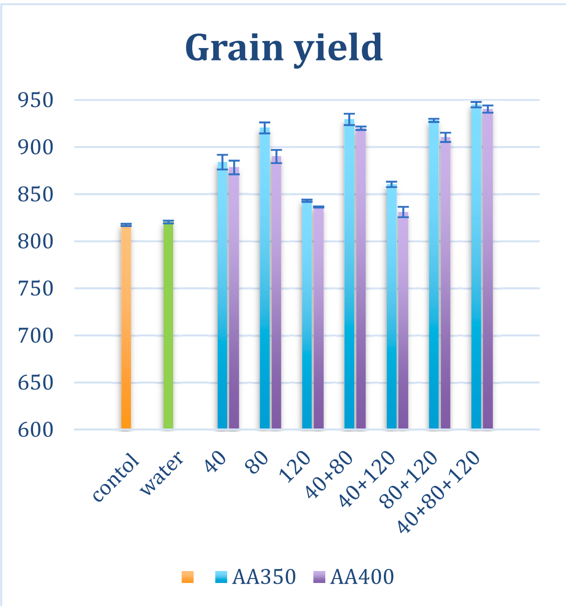

Fig. 3. Grain yield (kg ha^{-1}) of black cumin as affected by foliar application of ascorbic acid applied at various growth stages.

Table 3
Photosynthetic rate (μ mole $m^{-2}s^{-1}$), Transpiration rate (mmole $m^{-2}s^{-1}$), photosynthetic water use efficiency, Internal CO_2 concentration (*Ci*) of leaf tissue and Stomatal conductance (mmole $m^{-2}s^{-1}$) of black cumin as affected by ascorbic acid applied at different growth stages.

	Photosynthetic rate (μ mole $m^{-2}s^{-1}$)	Transpiration rate (mmole $m^{-2}s^{-1}$)	Photosynthetic water use efficiency	Internal CO_2 concentration (*Ci*) of leaf tissue	Stomatal conductance (mmole $m^{-2}s^{-1}$)
Growth stages					
Stage 1	7.63[d]	4.16[e]	1.83[e]	9.61[d]	0.34[g]
Stage 2	8.88[c]	4.87[b]	1.82[f]	9.86[c]	0.35[f]
Stage 3	9.88[b]	4.72[c]	2.1[b]	9.39[e]	0.39[d]
Stage 4	9.88[b]	5.23[a]	1.88[d]	10.2[a]	0.44[b]
Stage 5	9.81[b]	4.39[d]	2.23[a]	9.9[b]	0.37[e]
Stage 6	9.91[b]	5.19[a]	1.91[d]	9.26[f]	0.41[c]
Stage 7	10.63[a]	5.26[a]	2.01[c]	10.22[a]	0.46[a]
LSD (0.05)	**0.19**	**0.08**	**0.09**	**0.04**	**0.007**
Ascorbic Acid concentrations					
AA @ 350 μm	9.66[a]	4.86[NS]	1.99[a]	9.8[NS]	0.41[a]
AA @ 400 μm	9.38[b]	4.8	1.95[b]	9.75	0.38[b]
LSD (0.05)	**0.05**	**NS**	**NS**	**NS**	**0.002**
Control vs Rest treatments					
Control	7.26	3.9	1.86	9.34	0.33
Rest treatments	9.41	4.78	1.97	9.75	0.39
Significance	**	**	**NS**	**	**
Water only vs Rest treatments					
Water only	8	4.09	1.95	9.43	0.33
Rest treatments	9.52	4.83	1.97	9.78	0.39
Significance	**	**	**NS**	**	**
Interaction	**Significance**				
Stages x Concentrations	Fig. 3	**NS**	Fig. 4	**NS**	**NS**

maximum photosynthetic rate (10.69 μ mole $m^{-2}s^{-1}$) was recorded with application of ascorbic acid at the rate of 350 μm applied at stage-7, whereas, lowest photosynthetic rate (8.4 μ mole $m^{-2}s^{-1}$) was observed with application of ascorbic acid @ 400 μm applied at stage-1 (Fig. 4).

3.12. Transpiration rate (mmole $m^{-2}s^{-1}$)

Data concerning transpiration rate is given in Table 3. Results reviled that foliar application of ascorbic acid concentrations showed non-significant results, their stage of application showed significant effect on transpiration rate of black cumin whereas, their interaction showed non-significant results. Maximum Transpiration rate (mmole $m^{-2}s^{-1}$) was observed with application of ascorbic acid at stage-7, followed by stage-4, whereas, transpiration rate (mmole $m^{-2}s^{-1}$) was recorded in stage-1.

3.13. Photosynthetic water use efficiency

Photosynthetic water use efficiency is the ratio of photosynthetic rate and transpiration rate. Photosynthetic water use efficiency of black cumin was significantly affected from ascorbic acid concentrations, application stages along with their interaction. Mean comparison of control with ascorbic acid application and water spray with ascorbic acid application showed non-significant results. Maximum photosynthetic water use efficiency was recorded with application of ascorbic acid @ 350 μM, followed by 400 μM. Regarding application stages, maximum photosynthetic water use efficiency was observed with application of ascorbic acid at stage-5, whereas, minimum photosynthetic water use efficiency was observed in stage-1. Concerning the interaction (Fig. 5), maximum photosynthetic water use efficiency was observed with application of ascorbic acid @ 350 μM applied at stage-5, whereas, lowest photosynthetic water use efficiency was recorded with application of ascorbic acid @ 400 μM applied at stage-1.

3.14. Internal CO_2 concentration (Ci) of leaf tissue

Perusal of mean comparison Table 3 depicts that effect of application stage of ascorbic acid was significant on internal CO_2 concentration of leaf tissues, whereas, various concentrations as well as interaction of concentrations of ascorbic acid and application stage of ascorbic acid showed non-significant effect on internal CO_2 concentration. Statistically similar results for higher net CO_2 intake was recorded in stage-7 and stage-4. Lower CO_2 intake was observed in stage-3.

3.15. Stomatal conductance (mmole $m^{-2}s^{-1}$)

Data (Table 3) showed that ascorbic acid concentrations and application stage of ascorbic acid had significant effect on stomatal conductance (mmole $m^{-2}s^{-1}$) of black cumin crop. Interactive effect of ascorbic acid concentrations and application stage of ascorbic

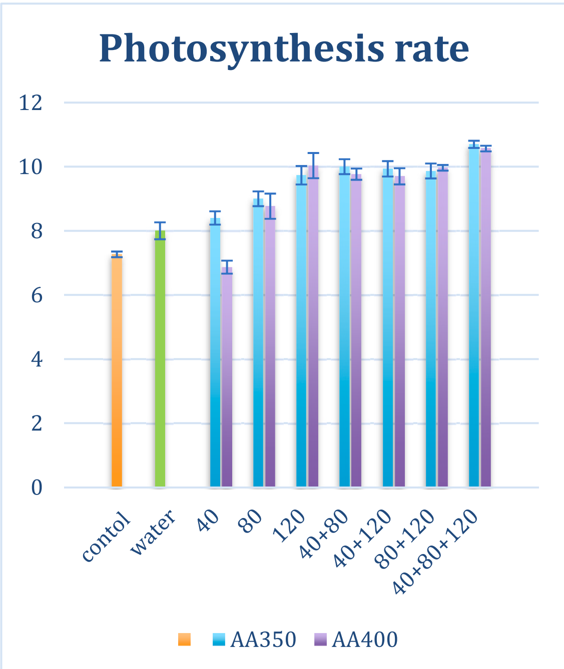

Fig. 4. Photosynthesis rate of black cumin as affected by foliar application of ascorbic acid applied at various growth stages.

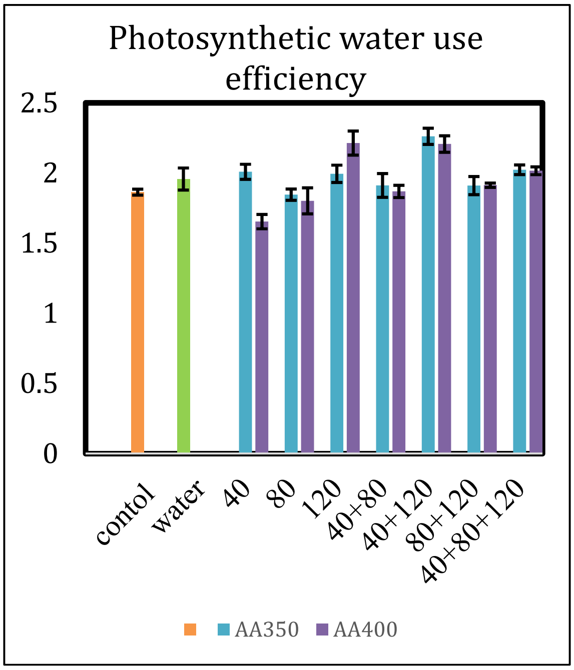

Fig. 5. Photosynthetic water use efficiency of black cumin as affected by foliar application of ascorbic acid applied at various growth stages.

acid was non-significant. Application of ascorbic acid @ 350 μm showed statistically higher stomatal conductance (0.41 mmol $m^{-2}s^{-1}$), while application of 400 μm showed lower results for stomatal conductance (0.38 mmol $m^{-2}s^{-1}$). Regarding stage of application of ascorbic acid, higher stomatal conductance (0.46 mmol $m^{-2}s^{-1}$) was observed with the application of ascorbic acid at stage-7, followed by (0.44 mmol $m^{-2}s^{-1}$) stage-4 while lower stomatal conductance (0.34 mmol $m^{-2}s^{-1}$) was recorded in stage-1.

3.16. Discussion

It was observed that the Ascorbic acid foliar spraying had a good influence on black cumin vegetative development metrics, and spraying transplants with dramatically enhanced plant height. These increases in the parameter may be owing to the fact that ascorbic acid, as an anti-oxidant, acts as a plant growth regulator [24–26]. At the vegetative stage, adding ascorbic acid decreased the inhibitory effect of stress on pigment concentration while marginally increasing overall pigment amounts. At two phases of wheat plant growth [15], found a progressive rise in chl. a and chl. b with increasing concentrations of ascorbic acid above their corresponding control. Similarly [27,28], discovered that ascorbic acid improved chlorophyll *a*, *b*, and total chlorophylls in soybean and sunflower plants. The obtained data show that the foliar spray of ascorbic acid by any concentration gave the highest significant value of chlorophyll *b* content when applied multiple times. Several researchers have indicated that ascorbic acid improves plant tolerance to abiotic stress [29]. The application of ascorbic acid to the leaves of the treated plants was more successful in improving physiological parameters [30]. further suggested that ascorbic acid's beneficial effect on chlorophyll content might be owing to their significant function in chlorophyll molecule production. According to Ref. [31], ascorbic acid has a positive influence on the vegetative development, photosynthetic pigments, herb productivity, and essential oil percentage of plants cultivated under normal circumstances or under stress. In a similar manner, when ascorbic acid was sprayed on basil plants at concentrations of 100, 150, and 200 ppm [32]. also observed that all ascorbic acid concentrations tested had a positive effect on growth, herb fresh and dry weights, oil percentage, and concentrations of photosynthetic pigments. Ascorbic acid has been shown to improve vegetative growth, productivity, and the proportion of essential oil on several medicinal and aromatic plants [33]; on *Thymus vulgaris* L. [34], on *Foeniculum vulgare* and [35] on *Calendula officinalis*.

Ascorbic acid is a recognized plant growth regulator that may affect the pace of development, as well as the quality and amount of essential oils produced by medicinal and aromatic plants [36]. [37] reported that 100 grain weight of pea plants was increased by foliar spray with growth hormones at various growth stages [38]. mentioned that treated foliar sprayed pea plants with growth hormones produced the highest 1000 grains weight. Ascorbic acid is a natural and safe bio-regulator molecule that has dramatic effects on a variety of physiological processes at relatively low doses, operating as a hormone signaling modulator and as a coenzyme in processes that metabolize carbs, lipids, and proteins [39].

It's possible that ascorbic acid's beneficial influence on biological and grain yields when given at crucial growth phases is due to its participation in metabolite translocation from leaves to reproductive organs. Furthermore, protein and nucleic acid production, which boost grain and straw yields. These findings corroborated those of [40]. The exogenous administration of ascorbic acid at critical times significantly boosted the biological yield in the current investigation. These findings are consistent with previous studies in which external AA supply resulted in a considerable increase in wheat and sorghum plant yields [41]. The ascorbic acid-induced growth and yield improvement found for black cumin plants during this study might be owing to an AA-induced increase in leaf area, which is the photosynthetic tissue of the plants, which resulted in a final increase in yield [29]. Plant traits are influenced by gas exchange characteristics. AsA-induced changes in chlorophyll pigments are associated to this modification. Activated oxygen compounds overabundance occurs during stress, and antioxidant synthesis mitigates the negative effects of water stress by scavenging H_2O_2, O_2, and OH^-, so they can help preserve the photosynthetic equipment from oxidative damage [42,43]. In the present research, the highest number of branches, seed yield and harvest index were obtained in with application of ascorbic acid @ 350 μm and at stage-7. The reason might be the better remobilization that occurs in black cumin causes the seeds to be filled better. Exogenous treatment of ascorbic acid increases yield qualities in corn [44], and barley [45]. High concentrations of ascorbic acid might be toxic to plants, causing damage to cell membranes or interfering with cellular processes [46]. Paradoxically, while ascorbic acid is an antioxidant, at high concentrations, it could potentially lead to oxidative stress in plants, disrupting normal cellular functions [47]. Excessive ascorbic acid may interfere with the uptake of essential nutrients by the plant roots, leading to nutrient imbalances and stunted growth [48].

4. Conclusions

Ascorbic acid (AsA), also known as vitamin C, plays a fundamental role in regulating plant development by actively participating in key physical, physiological, and biochemical processes. Internal factors, such as growth and development, significantly impact AsA levels in plants. The application of AsA offers several advantages, including involvement in redox reactions within cell compartments, regulation of cell division and expansion, preservation of photosynthetic pigment levels, modulation of chlorophyll fluorescence, maintenance of membrane permeability, and improvement in overall yield. It was concluded from the study that application of ascorbic acid at different times/stages resulted in better morphology and higher yield of black cumin under climatic conditions of Haripur.

CRediT authorship contribution statement

Abid Mehmood: Investigation, Conceptualization. **Khalid Naveed:** Supervision, Investigation, Formal analysis, Data curation. **Ke Liu:** Resources, Software, Validation, Visualization, Writing – original draft. **Matthew Tom Harrison:** Writing – review & editing, Methodology, Project administration, Validation. **Shah Saud:** Project administration, Methodology, Funding acquisition, Formal analysis. **Shah Hassan:** Project administration, Methodology, Funding acquisition, Investigation. **Taufiq Nawaz:** Visualization, Validation, Software. **Bikram Dhara:** Resources, Methodology, Data curation. **Dong-Qin Dai:** Visualization, Validation, Project administration. **Iftikhar Ali:** Writing – review & editing, Validation. **Muhammad Adnan:** Funding acquisition, Formal analysis. **Khaled El-Kahtany:** Software, Resources, Project administration, Methodology. **Shah Fahad:** Writing – review & editing, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Appendix A. Supplementary data

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