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EVALUATION OF SOAKING AND SPRAY TREATMENTS WITH GA_3 TO BLACK CUMIN (*NIGELLA SATIVA* L.) IN RELATION TO GROWTH, SEED AND OIL YIELDS

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Summary. The efficiency of two modes of GA_3 application (spraying or seed soaking) was compared in pot experiments conducted with *Nigella sativa* L. cv. KJ-125. Different concentrations of the hormone, 10^{-6}, 10^{-5} or 10^{-4} M were used for 10 h seed soaking or sprayed on 40-day-old seedlings. At day 70 after sowing (DAS), leaf area (LA), plant height (PH), total dry matter (TDM), net photosynthetic rate (P_N), relative growth rate (RGR), net assimilation rate (NAR) and nitrogen, phosphorus and potassium contents of the test plants were found to be significantly enhanced by both treatments. Likewise, at harvest (130 DAS), an increase was also observed in the number of capsules, seed yield and biological yield per plant as well as in oil and essential oil contents, the concentration of 10^{-5} M proving to be most stimulatory. The spray application was found to be more effective in enhancing all plant parameters studied in comparison to the soaking treatment, thereby proving to be a better mode of hormone application for practical use.

Keywords: Black cumin; foliar spray; gibberellic acid; seed soaking; yield.

Abbreviations: LA – Leaf area; P_N – Net photosynthetic rate; PH – Plant height; TDM – Total dry matter; RGR – Relative growth rate; NAR – Net assimilation rate.

INTRODUCTION

Black cumin is an integral component of the Greco-Arab and traditional Indian systems of medicine. The seeds are used as astringent, stimulant, diuretic, emmenagogue, and anthelminthic, while the seed oil is remedial to numerous ailments, such as pityriasis, leucoderma, ringworm, eczema, chest congestion, migraine, jaundice, intermittent fever, dyspepsia, piles, paralysis and rheumatism (Babayan et al., 1978). In the premises of the recent shift towards herbal health care products, considerable research has been directed towards comprehensive

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evaluation of the therapeutic properties of this herb. However, concurrent to elucidation of therapeutic benefits, it is imperative to develop methods of boosting cultivation, to capitalize on the research findings, especially when the growing demand for this herb has made the black seed and oil a valuable source of foreign exchange.

Among the plethora of techniques advocated to enhance plant performance and productivity, exogenous hormone supplementation has been found to hold a particularly promising potential. With regard to *N. sativa* L., our previous studies have proven the efficacy of foliar GA_3, KIN (Shah, 2007), and 4-Cl-IAA (Shah, 2011), in improving morpho-physiological characteristics. However, as the response to exogenous growth regulators varies depending on the mode of application, it is of relevant interest to compare the efficiency of different methods for possible practical use, especially in relation to economically significant yield characteristics. Moreover, as yield is the culmination of growth processes occurring over the life of the plant, a concurrent evaluation of morpho-kinetic parameters is also desirable. Therefore, in the present investigation a comparative analysis of two modes of GA_3 supplementation (foliar spray or seed soaking) was carried out in relation to growth, seed and oil yields of *Nigella sativa*, with the aim to ascertain the relative efficacy of the two modes of treatment.

MATERIALS AND METHODS

Homogenous seeds of *Nigella sativa* L. cv. KJ-125 were obtained from the Regional Research Institute of Unani Medicine, Aligarh (UP), India. They were surface sterilized with mercuric chloride solution (0.01%), followed by repeated washes with double distilled water. Gibberellic acid (GA_3) was obtained from Sigma Chemicals Co., St. Louis, USA. The pre-sowing seed treatment was done by soaking the seeds in water (control) or aqueous solutions of 10^{-6}, 10^{-5} or 10^{-4} M GA_3 for 10 h. The seeds were then sown in earthen pots filled with sandy loam soil (pH 8.1 and available N, P and K: 196.00, 25.20 and 175.00 kg ha^{-1}, respectively) and farmyard manure, mixed in a ratio of 9:1. A uniform basal dose (45, 300 and 78 mg, respectively) of N, P and K, in the form of urea, single superphosphate and muriate of potash (IFFCO, New Delhi, India) was applied at the time of sowing to each pot. After germination only 5 uniform seedlings were left in each pot. The foliar treatment was carried out 40 days after sowing (identified as the most suitable stage for hormone application to *Nigella sativa* (Shah, 2007). Each plant was sprayed with 5 cm^3 $plant^{-1}$ of 10^{-6}, 10^{-5} or 10^{-4} M GA_3. Control plants were sprayed with double distilled water only. The pots were irrigated with tap water as required. The experiment was carried out on a completely randomized block design, under natural day/night conditions: average 12 h photoperiod, photosynthetically active radiation (PAR)>1100 μmol $m^{-2}s^{-1}$; temperature 22±3°C and relative humidity (RH) 66–53%. Each treatment was replicated five times.

Five plants from each replicate were randomly selected for the measurements of leaf area (LA), net photosynthetic rate (P_N), plant height (PH), total dry matter (TDM), relative growth rate (RGR), net

assimilation rate (NAR) and N, P and potassium K contents at 70 days after sowing (DAS). LA was measured using a portable LA meter (LI-COR-3100 Lincoln, Nebraska, USA). P_N was recorded using a portable infrared gas analyzer (*LICOR-6200*, Lincoln, NE, USA) on the fully expanded, uppermost leaf of the main plant axis, at saturating irradiance between 11:00 and 12:00 h under atmospheric conditions: PAR – 1,012–1,084 μmol $m^{-2}s^{-1}$, atmospheric CO_2 – 350 μmol mol^{-1} , relative humidity 62±2%, and temperature 23±2°C. Total dry matter (g $plant^{-1}$) of all above-ground plant tissues was recorded by drying the plants at 80°C for 24 h. RGR and NAR were determined as: RGR = ln DW_1 – ln DW_2 / $t_2 – t_1$ (g $g^{-1}d^{-1}$), and NAR = [(DM_2 – DM_1) ($LA_2 – LA_1$)$^{-1}$] [(ln LA_2 – ln LA_1) ($t_2 – t_1$)$^{-1}$] (g $m^{-2}d^{-1}$), where DM_1 is the initial total dry mass, DM_2 the final total dry mass, LA_1 the initial leaf area, LA_2 the final leaf area, and ($t_2 – t_1$) the difference in time interval between the final (t_2 = 70 DAS) and initial (t_1 = 50 DAS) harvests. Leaf N content was estimated according to the method of Lindner (1944). The leaf dried powder was digested in H_2SO_4 in a digestion tube. A 10 ml aliquot (peroxide-digested-material) was poured into a 50 ml volumetric flask where 2 ml of 2.5 N sodium hydroxide and 1 ml of 10% sodium silicate solutions were added to neutralize the acid excess and prevent turbidity. A 5 ml aliquot of this solution was poured into a 10 ml graduated test tube and 0.5 ml Nessler's reagent was added. The OD of the solution was recorded at 525 nm using a spectrophotometer. The method of Fiske and Subba Row (1925) was used to estimate leaf P content in the digested material. A 5 ml aliquot was poured into a 10 ml graduated test tube with 1 ml of molybdic acid (2.5%), followed by addition of 0.4 ml 1-amino-2-naphthol-4-sulphonic acid. When the color became blue, the volume was made 10 ml with the addition of double distilled water. The OD of the solution was recorded spectrophotometrically at 620 nm. Potassium content in the leaves was estimated in the aliquot with the help of emission spectra using specific filters (Hald, 1946). In the flame-photometer, the solution (peroxide-digested-material) was discharged through an atomizer in the form of a fine mist into a chamber, where it was drawn into a flame. Readings of the light emitted were recorded with the help of emission spectra using a specific filter (Model, C150, AIMIL, India).

At maturity (130 DAS), the number of capsules $plant^{-1}$ and seed yield $plant^{-1}$ were determined using 5 plants per each treatment. The plant material remaining after threshing the seeds was sundried and weighed for determination of biological yield per plant. Oil was extracted three times with a chloroform/methanol (2:1, v/v) mixture, according to the method of Kates (1972). 25 g of ground seed meal was transferred to a Soxhlet apparatus and sufficient quantity of petroleum ether was added. The apparatus was kept in a hot water bath running at 60°C for about 6 h for complete extraction of oil. The petroleum ether from the extracted oil was evaporated after sometime. The extracted oil was expressed as a percentage by mass of the seeds and was calculated by the following formula:

$$\text{Oil content [\%]} = (m_0/m_s) \times 100$$

where:

m_0 – sum of the mass of oil

m_S – seed sample mass

The percent of oil content in the seeds was then multiplied by seed yield to obtain oil yield. Essential oil was extracted following the method of Kantar et al. (2003). Seeds were powdered in a mixer. 20 g of the powdered seeds were added to 400 ml of distilled water and the extraction was carried out by steam distillation. The process of distillation continued until about 200 ml of the distillate were collected. The distillate was extracted three times with chloroform. Moisture was removed by anhydrous sodium sulphate and the resultant extract was evaporated using a water bath (40°C), leading to the appearance of the volatile oil.

For the calculation of the index of relationships, the index number was expressed as a percentage relative to the maximum value obtained for each treatment.

Statistical analysis

Statistics were performed using analysis of variance (ANOVA) and means were separated using the least significant difference (LSD) (P = 0.05) calculated according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Application of GA_3 resulted in an appreciable enhancement of growth and yield of black cumin, the sprayed plants showing a better response as compared to those arising from the treated seeds. Maximum enhancement was noted after foliar application of 10^{-5}M GA_3 followed by the seed treatment with the same concentration. LA, PH, and DM were elevated in the sprayed plants by 49, 59, and 47%, respectively as compared to 37, 48, and 38% in plants emerging from the treated seeds (Table 1). RGR and NAR of the 10^{-5} M sprayed plants increased by 38 and 45 % respectively (Fig.1), concurrent to enhancement of 53 % in P_N (Table 1). Comparable enhancements by 26, 34 and 39 % were recorded in the soaking treatment. Likewise, an increase in leaf contents of N, P and K by 14, 23 and 12 %, respectively was observed after the spray application, against 9, 16 and 7 % enhancement due to the soaking treatment (Table 1). Consequently, at harvest, number of capsules, seed yield and biological yield of the sprayed plants was enhanced by 47, 44 and 54 % over the control as compared to 33, 30 and 46% increase in the seed-treated plants (Table 2). Moreover, as relevant economically, the hormone application also resulted in a 12 and 7% increase in the fixed and essential oil content in the foliar treated plants, in comparison to respective increases of 10 and 5% following the pre-sowing seed treatment; subsequently, oil yield was enhanced by 55 and 66%, respectively following the two treatments (Table 2).

Though a general enhancement in growth and yield of *N. sativa* L. was noted following exogenous supplementation of GA_3, consistently better results were obtained after spraying than the soaking treatment (Tables 1, 2). Similar results have been reported in Okra dwarf by Unamba et al. (2009). Apparently, in case of foliar application, availability of a greater surface area, and potential for ready absorption/translocation of the hormone to active physiological sites contributed to an advantage over gradual imbibition through the tough *Nigella* seed coat. In addition, the variable nature of predominant physiological processes

Table 1. Effect of GA_3 seed soaking (for 10 h) or spray (at 40 days after sowing) on growth, photosynthesis and nutrient contents of black cumin.

GA_3 concentrations [M]	GA_3 application	
	Seed soaking	Spray
	Leaf area [cm^2 $plant^{-1}$]*	
0	281.20±13.7^e	290.21±12.2^e
10^{-6}	321.11±16.1^d	351.23±18.3 ^{c}
10^{-5}	385.21±22.2^b	429.17±24.3^a
10^{-4}	376.61±23.4^b	436.45±21.1^a
	Total dry matter [g $plant^{-1}$]*	
0	1.68±0,12^e	1.72±0.14^e
10^{-6}	1.91±0.16^d	2.10±0.27^c
10^{-5}	2.33±0.21^b	2.56±0.28^a
10^{-4}	2.28±0.19^b	2.59±0.22^a
	Plant height [cm $plant^{-1}$]*	
0	41.12±2.8^d	44.51±2.9^d
10^{-6}	50.95±3.2^c	60.94±3.7^b
10^{-5}	61.38±4.2^b	70.76±4.8^a
10^{-4}	58.20±3.9^b	68.51±4.3^a
	Net photosynthetic rate [mmol CO_2 $m^{-2}s^{-1}$]*	
0	13.8±0.9 ^{e}	14.4±1.2 ^{e}
10^{-6}	16.3±1.3 ^{d}	18.4±1.6 ^{c}
10^{-5}	19.2±1.8 ^{b}	22.0±2.1 ^{a}
10^{-4}	19.4±1.7 ^{b}	21.5±1.9 ^{a}
	Nitrogen content [mg g^{-1}]*	
0	27.19±2.2^d	27.50±2.4^d
10^{-6}	28.41±2.7^c	29.28±3.2^b
10^{-5}	29.69±3.4^b	31.29±3.7^a
10^{-4}	29.57±3.1^b	31.21±3.5^a
	Phosphorus content [mg g^{-1}]*	
0	2.85±0.40^d	2.94±0.43^d
10^{-6}	3.10±0.49^c	3.25±0.52^b
10^{-5}	3.34±0.56^b	3.63±0.59^a
10^{-4}	3.35±0.55^b	3.58±0.57^a
	Potassium content [mg g^{-1}]*	
0	26.09±2.4^d	26.51±2.6^d
10^{-6}	26.87±2.5^c	27.93±2.8^b
10^{-5}	27.89±3.2^b	29.59±3.5^a
10^{-4}	27.78±3.1^b	29.49±3.4^a

*Determinations were done 70 days after sowing.

Each value is a mean ± SE, (n = 5). Different superscripts (a, b, c, d, e) represent the values significantly different at $P \leq 0.05$.

Table 2. Effect of GA_3 seed soaking (for 10 h) or spray (at 40 days after sowing) on yield characteristics of black cumin.

GA_3 concentrations [M]	GA_3 application	
	Seed soaking	Spray
	Capsules plant^{-1*}	
0	15.21±1.2^{e}	15.41±1.3^{e}
10^{-6}	17.31±1.5^{d}	19.05±1.8^{c}
10^{-5}	19.77±1.9^{b}	22.66±2.2^{a}
10^{-4}	19.80±1.7^{b}	22.81±2.0^{a}
	Seed yield [g plant^{-1}]*	
0	1.12±0.14^{d}	1.16±0.15^{d}
10^{-6}	1.23±0.16^{c}	1.39±0.17^{b}
10^{-5}	1.45±0.21^{b}	1.67±0.25^{a}
10^{-4}	1.41±0.19^{b}	1.65±0.23^{a}
	Biological yield [g plant^{-1}]*	
0	4.95±0.28^{e}	5.11±0.31^{e}
10^{-6}	5.98±0.32^{d}	6.38±0.38^{c}
10^{-5}	7.22±0.40^{b}	7.86±0.48^{a}
10^{-4}	7.11±0.36^{b}	7.95±0.46^{a}
	Oil content [%]*	
0	34.54±2.6^{e}	34.71±2.7^{e}
10^{-6}	35.41±2.8^{d}	36.46±2.9^{c}
10^{-5}	37.30±3.4^{b}	38.18±3.8^{a}
10^{-4}	36.80±3.1^{c}	37.71±3.6^{b}
	Oil yield [g plant $^{-1}$]*	
0	0.39±0.016^{e}	0.40±0.018^{e}
10^{-6}	0.45±0.018^{d}	0.56±0.022^{b}
10^{-5}	0.54±0.021^{b,c}	0.64±0.025^{a}
10^{-4}	0.52±0.019^{c}	0.62±0.023^{a}
	Essential oil content [%]*	
0	0.710±0.024^{e}	0.713±0.027^{e}
10^{-6}	0.730±0.027^{d}	0.750±0.029^{b}
10^{-5}	0.745±0.028^{b}	0.762±0.033^{a}
10^{-4}	0.738±0.026^{c}	0.759±0.030^{a}

*Determinations were done 130 days after sowing.

Each value is a mean ± SE, (n = 5). Different superscripts (a, b, c, d, e) represent the values significantly different at $P \leq 0.05$.

characteristic of each formative stage at which the hormone was supplemented could also contribute to the differential responses obtained in the two treatments. In other words, while the GA_3 spray acted in full potency precisely at the phase of profuse vegetative growth (by influencing dynamic acquisition processes and active growth sites), influence of seed treatment on the other hand, was apparent only in its waning phase as manifested through its impression on early metabolic processes and key enzyme activities (Shah, 2007).

Concurrent to the stimulation of absolute growth, as evidenced by the increase in LA and PH, RGR of the treated plants was also higher (Table 1, Fig. 1). Gibberellins plays an essential role in attaining maximum growth as they cause an increase in elongation of stem nodes by enhancing cell division and cell wall extensibility (Taiz & Zeiger, 1998). In fact, GA_3 has been shown to cause elevation in the levels of transcripts and enzymes conducive to cell expansion (Huttly and Phillips

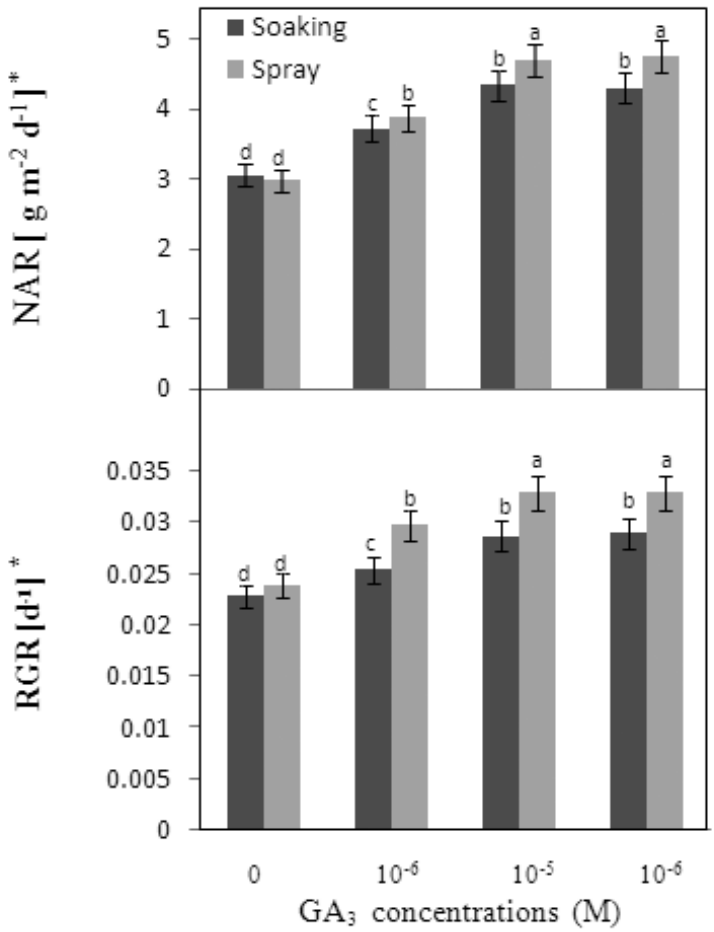

Fig.1. Effect of GA_3 seed soaking (for 10 h) or spray (at 40 days after sowing) on relative growth rate (RGR) and net assimilation rate (NAR) of black cumin.

*Determinations were done at 50 and 70 days after sowing.

Values represent means of five replicates ± SE. Bars with different superscripts (a, b, c, d, e) are significantly different ($P < 0.05$).

1995, Mendu and Silflow 1993, Potter and Fry 1994). Moreover, besides thus contributing towards growth and lamina expansion, this hormone also increases the sink strength of the leaves (Sanz et al., 1987) and hence, favors the uptake of nutrients (Table 1). Meanwhile, an increased efficiency to assimilate the absorbed nutrients is indicated by the enhanced P_N of the treated plants (Table 1). Our earlier studies have demonstrated the stimulation of photosynthesis by GA_3, consequent to enhancement in the activity of key photosynthetic enzymes, and optimization of the photosynthetic area (Shah et al., 2007, Shah and Samiullah, 2007). In this context, to account for the differences in responses to the two modes of treatment, the immediate effect of the spray on photosynthetically active tissues may be contrasting to the indirect influence of the soaking treatment through the modification of embryonic physiological processes. On the whole, augmentation of photosynthetic assimilation and up-gradation of nutrient status could have in turn made possible, the simultaneous elevation observed in RGR and NAR (Figs. 1, 2).

Early vegetative growth affects the

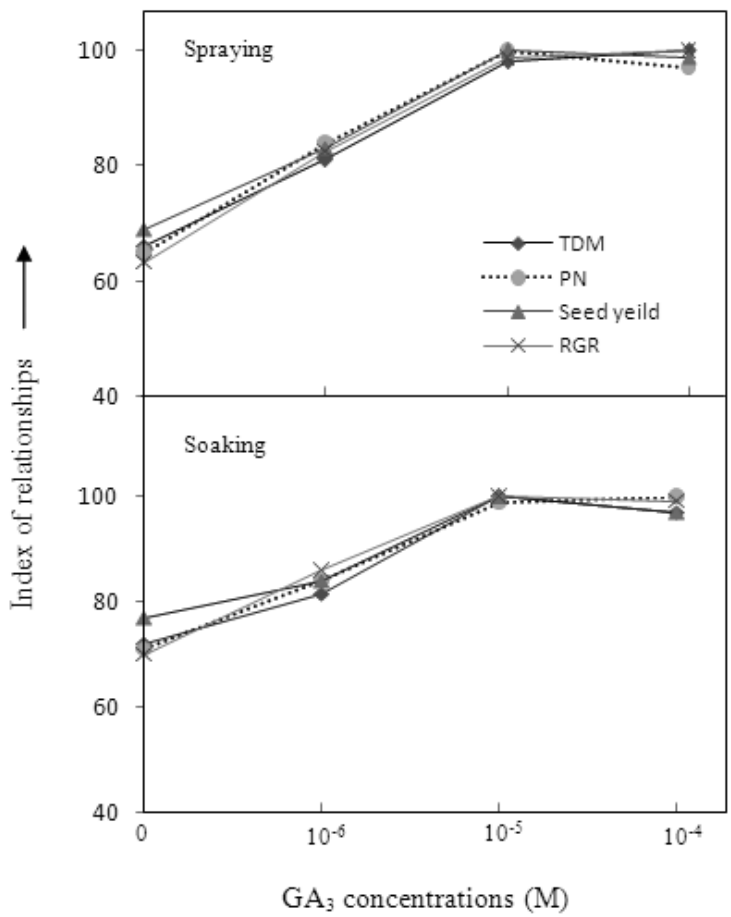

Fig. 2. Index of relationships among total dry matter (TDM), net photosynthetic rate (P_N), seed yield, and relative growth rate (RGR) of black cumin after seed soaking (for 10 h) or spraying (at 40 day after sowing) with GA_3.

number and size of photosynthesizing sites, which remain available for photosynthetic activity after flowering. It is this surface that provides the products to the sink (seed) and determines the quantity and quality of yield. The observed relationships between growth, photosynthesis and yield of the treated plants further confirm these facts (Fig. 2). Consequent to enhanced dry matter production, efficient translocation and partitioning of assimilates caused by the hormone treatment (Peretó and Beltrán, 1987), resulted in an increase in the number of capsules and seed yield at harvest (Table 2). Simultaneously, the content of fixed and essential oils in seeds also increased (Table 2). Such a concurrent enhancement in biomass as well as in oil production indicated that the treated plants were in a sound state of metabolism, and with their morphological and physiological constraints relaxed, could efficiently engage in their natural "manufacturing" processes, to the extent dictated by their inherent genetic potentials. In addition, oil content could also have been affected by GA_3, by a possible modulation of the biosynthesis of the oil components; the black seed oil constitutes primarily of mono-terpenes (D'Antuono et al., 2002) which share a common synthetic pathway with gibberellins (Akhila, 2007). As such, it may be postulated that exogenous gibberellin supplementation may have modulated biosynthetic process to probably result in a redirection towards oil (monoterpene) synthesis. However, this speculation needs to be testified in order to ascertain the exact mechanism of such an influence of the hormone on oil content of the herb. Similar results have been reported by Mostafa et al. (2005).

In general, among different concentrations of GA_3 applied, 10^{-5} M proved to be most stimulatory, the other dosages being either too mild or supra-optimal. In concept, exogenous hormone supplementation is intended to achieve only a limited desired deviation in the pattern of hormone levels, and in supra-optimal dosages induces a feedback inhibition of hormone action (Weyers and Paterson, 2001), the non-physiological levels proving to be relatively less effective.

In conclusion, through its prominent effects on growth rate and assimilate production, foliar treatment was found to result in higher yield of black cumin at harvest, and was hence established to be a more productive method for exogenous supplementation of GA_3 for potential practical use.

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