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Influence of Elicitors on Growth and Yield of Black Cumin (*Nigella sativa* L.) Varieties

H. S. Arpitha [a++*], K. Umesha [b#] and G. S Anilkumar [c++]

[a] Department of PSMA, KRCCH Arabhavi, College of Horticulture, University of Horticultural Sciences Campus, GKVK Post, Bengaluru – 560 065, India.
[b] College of Horticulture, GKVK Post, Bengaluru, University of Horticultural Sciences Campus, GKVK Post, Bengaluru – 560 065, India.
[c] Department of PSMA, College of Horticulture, GKVK Post, Bengaluru, University of Horticultural Sciences Campus, GKVK Post, Bengaluru – 560 065, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Black cumin is a miracle herb, an emerging seed spice and a medicinal plant of great demand. Black cumin has been used since thousands of years as a spice and food preservative as well as a protective and curative remedy for several disorders. A field experiment was conducted at the Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Horticulture, UHS Campus, GKVK, Bengaluru, from November 2018 to March 2019. The experiment followed a randomized complete block design (RCBD) with three replications and included two factors: varieties and elicitors. Among the various treatment combinations, foliar application of methyl jasmonate at 100 ppm resulted in the highest recorded values for plant height (46.7 cm), primary

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[++] Ph.D. Scholar;
[#] ICAR- Emeritus Professor;
**Corresponding author: Email: arpithabhat15@gmail.com;*

branches (5.43), stem diameter (4.62 mm), dry weight of plants (8.42 g), and plant spread (397 cm^2) when applied with nitric oxide (Sodium Nitroprusside (SNP)) at 2 mM on Pant Krishna variety. Conversely, the significantly highest chlorophyll content in leaves was observed in the Azad Kalonji variety sprayed with salicylic acid at 100 ppm. Furthermore, the significantly highest numbers of capsules (22.60), seeds per capsule (84.28), and seed yield per hectare (12.40 q) were recorded in Pant Krishna sprayed with methyl jasmonate at 100 ppm. The present study concludes that all varieties, including Azad Kalonji, AN-20, and Pant Krishna, performed equally well regarding growth and yield attributes in this location. Among the elicitors tested, methyl jasmonate and salicylic acid at 100 ppm show promise for commercial cultivation of black cumin.

Keywords: Nigella sativa L.; elicitors; salicylic acid; methyl jasmonate; nitric oxide; hydrogen peroxide.

1. INTRODUCTION

Black cumin (*Nigella sativa* L.), belonging to the family Ranunculaceae (2n=12), is a significant emerging seed spice and medicinal plant native to Southern Europe, North Africa, and Southwest Asia. Commonly known as Kalonji in Hindi and by various names in English such as black cumin, black caraway, black seed, and fennel flower, it is also referred to as Krishna jeerige in Kannada [1]. This annual flowering plant typically grows to a height of 40-60 cm and features finely divided leaves. Its flowers are hermaphrodite, solitary, and can be white, pale blue, or pale purple in colour, with 5-10 petals and notable nectarines. The fruit, a large inflated capsule, comprises 5-7 united follicles, each containing several seeds.

Black cumin has been utilized for thousands of years both as a spice and food preservative, as well as a protective and curative remedy for various disorders. Its medicinal properties have been documented in religious and ancient texts dating back over 2000 years. In traditional Indian systems of medicine such as Unani and Ayurveda, black cumin holds significant importance. Both the seeds and oil exhibit efficacy against numerous diseases including cancer, cardiovascular complications, diabetes, asthma, and kidney diseases [2].

The seeds contain approximately 32 to 40% fixed oil and 0.4 to 0.45% volatile oil (comprising compounds such as nigellone, thymoquinone, thymohydroquinone, dithymo-quinone, thymol, carvacrol, α and β-pinene, d-limonene, d-citrlnellol, and p-cymene), along with proteins (16-19.9%), minerals (1.79-3.74%), carbohydrates (33.9%), fiber (5.50%), and water (6.0%) [1].

In recent years, the use of elicitors has emerged as a promising strategy for significantly enhancing the production, productivity, and quality of seed spices. Elicitors are substances that induce physiological changes in plants, triggering an array of mechanisms similar to defence responses against pathogen infections or environmental stimuli. These elicitors stimulate plant metabolism and enhance the synthesis of phytochemicals, ultimately influencing the production of commercially important compounds that contribute to the quality of raw materials [3,4]. Methyl Jasmonate is a plant signaling molecule derived from jasmonic acid. It plays a crucial role in plant defense responses against herbivores, pathogens, and environmental stresses. MeJA induces the production of defense-related compounds such as secondary metabolites, proteins, and enzymes, thereby enhancing plant resistance. Salicylic Acid is a key signaling molecule involved in systemic acquired resistance (SAR) in plants. It activates defense responses against pathogens, including viruses, bacteria, and fungi. SA induces the expression of pathogenesis-related (PR) genes and triggers the accumulation of antimicrobial compounds, contributing to plant immunity. Nitric Oxide (NO) is a gaseous signaling molecule that regulates various physiological processes in plants, including defense responses. It functions as a signaling molecule in both basal and induced defense mechanisms. NO participates in the modulation of stomatal closure, reactive oxygen species (ROS) signaling, and the activation of defense genes. Hydrogen Peroxide (H_2O_2) is a reactive oxygen species (ROS) that serves as a signaling molecule in plant defense responses. It is involved in the oxidative burst, a rapid production of ROS during pathogen attack. H_2O_2 acts as a secondary messenger, mediating the activation of defense genes, cell wall reinforcement, and programmed cell death (PCD) to limit pathogen spread.

Black cumin (*Nigella sativa* L.) is an important spice cum medicinal crop mostly grown in northern parts of India. The same is being grown

on small scale in south India, more specifically in certain areas of Karnataka as a rabi season crop owing to its high medicinal value. It can be used for safe and effective herbal medicine for human benefit. Hence, the demand is on the rise with premium price in the market and the requirement is met out of material procured from distant north Indian markets. For profitable cultivation of this crop under Bangalore conditions, choosing a right variety of high intrinsic value with good adaptability is of paramount importance. Furthermore, efforts are needed to cultivate this crop on sound scientific lines are also important to realize maximum returns per unit area and time. The use of elicitors may be one of the best possible ways to achieve spectacular progress in increasing production, productivity and quality of seed spices. Hence, the present investigation was taken up to study the performance of already released varieties for traditional areas under Bangalore conditions which represents the eastern dry zone of Karnataka and also the effect of different elicitors on growth, yield and quality of this valuable crop.

2. MATERIALS AND METHODS

A field experiment was conducted at the Department of Plantation, Spices, Medicinal, and Aromatic Crops, College of Horticulture, Bengaluru. The location is situated at an altitude of 930 m above Mean Sea Level (MSL), positioned at 12º 58¹ North Latitude and 77 º 35¹ East Longitude, falling within the Eastern Dry Zone (zone-5) of Karnataka. The experiment followed a Factorial Randomized Block Design with three replications and fifteen treatments.

The treatments included three varieties: Azad Kalonji (V_1), Ajmer Nigella-20 (V_2), and Pant Krishna (V_3), along with five elicitors: Control (E_1), Salicylic acid at 100 ppm (E_2), Methyl jasmonate at 100 ppm (E_3), Nitric oxide (utilizing Sodium nitroprusside as an NO donor) at 2mM (E_4), and Hydrogen peroxide at 50mM (E_5).

Prior to sowing, the experimental area was prepared by bringing it to a fine tilth. Plots measuring 1.5 X 2.4 m were laid out, and farmyard manure at a rate of 5 tonnes per hectare, along with the full dose of NPK (40:20:20 kg/ha), was applied. Seeds were sown at a depth of 1-1.5cm in shallow furrows spaced at 30 X 10 cm. Germination occurred within 12 days of sowing, with the entire process completed within 15 days. The elicitors were applied 50 days after sowing, primarily through foliar spray, except for hydrogen peroxide, which was applied as a soil drench. Harvesting took place when capsules reached full maturity and transitioned from green to brownish in colour [5].

Statistical analysis is done using Fisher method of analysis of variance as given by Panse and Sukhatme [6] was applied for analysis and interpretation of data. The level of significance used in 'F' test was at P = 0.05 and critical difference (CD) values were worked out wherever 'F' test was significant.

3. RESULTS AND DISCUSSION

The results of the present investigation on the growth attributes of black cumin (*Nigella sativa* L.) are summarized in Fig 1. There were no significant differences observed in plant height among the various treatments. However, a consistent increasing trend in plant height was noted across different crop growth stages, with the maximum height achieved at harvest. Specifically, the Pant Krishna variety treated with Methyl jasmonate at 100 ppm exhibited the highest plant height (46.7 cm), closely followed by the Azad Kalonji variety treated with salicylic acid at 100 ppm (44.7 cm) at harvest. These findings align with previous studies by Hesami et al. [7] in coriander, Assefa et al. [8] and Emran et al. [9] in black cumin varieties.

Significant variations were observed in plant spread among the different varieties. The Pant Krishna variety recorded the maximum plant spread of 349.5 cm^2. This difference in plant spread can be attributed to the cessation of vertical growth in all varieties by 90 days after sowing, leading to a diversion of vigour towards horizontal growth. These results are consistent with studies conducted by Anilkumar et al. [10] in coriander and Anitha et al. [11] in fenugreek. Moreover, foliar application of methyl jasmonate at 100 ppm resulted in a significantly higher plant spread (368.6 cm^2), comparable to salicylic acid at 100 ppm (349.3 cm^2). The positive effect of methyl jasmonate may be attributed to its role in cell division, elongation, differentiation, nutrient uptake, as well as physiological and biochemical processes.

There were no significant variations observed in primary branches among different varieties, elicitors, or their interaction. This finding is consistent with the results reported by Hesami et al. [7] in coriander and Assefa and Girma [12] in black cumin varieties.

Table 1. Yield parameters of black cumin varieties as influenced by different elicitors

Treatments	Days to first flowering	Days to 50% flowering	Days to crop maturity	No. of capsules per plant	No. of locules per capsule	No. of seeds per capsule	1000 seed weight (g)	Seed yield (q/ha)	Harvest index (%)
V_1	46.5	57.9	108.33	13.53	5.23	75.27	2.23	9.16	32.13
V_2	47.7	57.3	111.67	13.87	5.26	77.42	2.24	9.41	31.11
V_3	47.0	57.5	111.67	16.87	5.26	76.74	2.26	9.57	28.81
S.Em. ±	0.7	0.7	0.9	0.99	0.03	1.2	0.04	0.65	1.18
CD at 5%	NS	NS	2.8	2.05	NS	NS	NS	NS	NS
E_1	47.1	56.8	111.00	14.71	5.24	75.11	2.17	8.08	31.26
E_2	46.4	56.8	109.89	16.67	5.24	77.42	2.40	10.12	28.55
E_3	45.5	58.0	109.33	16.07	5.36	79.27	2.22	11.52	32.31
E_4	47.9	57.4	110.44	13.38	5.28	77.16	2.15	8.59	30.91
E_5	48.3	58.8	112.11	12.96	5.23	73.44	2.27	8.61	30.39
S.Em. ±	0.9	0.9	1.2	1.29	0.04	1.6	0.05	0.84	1.52
CD at 5%	NS	NS	NS	2.65	NS	3.3	0.15	2.44	NS
V_1E_1	47.0	57.7	109.33	11.93	5.20	74.43	2.12	6.35	33.07
V_1 E_2	46.0	56.3	107.67	20.37	5.20	80.24	2.32	11.51	31.34
V_1E_3	43.3	59.3	106.00	14.00	5.33	76.39	2.22	11.92	30.94
V_1 E_4	48.0	57.5	106.00	10.73	5.32	74.42	2.19	8.12	33.17
V_1E_5	48.0	58.3	112.67	10.73	5.12	70.91	2.29	7.91	32.14
V_2E_1	46.7	55.3	109.33	16.40	5.21	75.43	2.18	8.21	32.41
V_2 E_2	46.0	56.0	111.00	13.13	5.27	77.53	2.30	9.05	24.13
V_2E_3	47.0	58.7	112.67	11.60	5.27	77.13	2.20	10.22	33.24
V_2 E_4	50.7	58.0	114.33	12.93	5.25	80.20	2.24	9.22	34.90
V_2E_5	48.3	58.3	111.00	15.27	5.30	76.83	2.27	10.36	30.89
V_3E_1	47.7	57.3	114.33	15.80	5.33	75.47	2.21	9.67	28.31
V_3 E_2	47.3	58.0	111.00	16.60	5.24	74.49	2.56	9.79	30.17
V_3E_3	46.3	56.0	109.33	22.60	5.48	84.28	2.25	12.40	32.76
V_3 E_4	45.0	56.7	111.00	16.57	5.26	76.87	2.03	8.43	24.68
V_3E_5	48.7	59.7	112.67	12.87	5.30	72.60	2.26	7.56	28.15
S.Em. ±	1.6	1.5	2.1	2.23	0.07	2.8	0.09	1.45	2.63
CD at 5%	NS	NS	NS	4.58	NS	5.8	NS	NS	NS

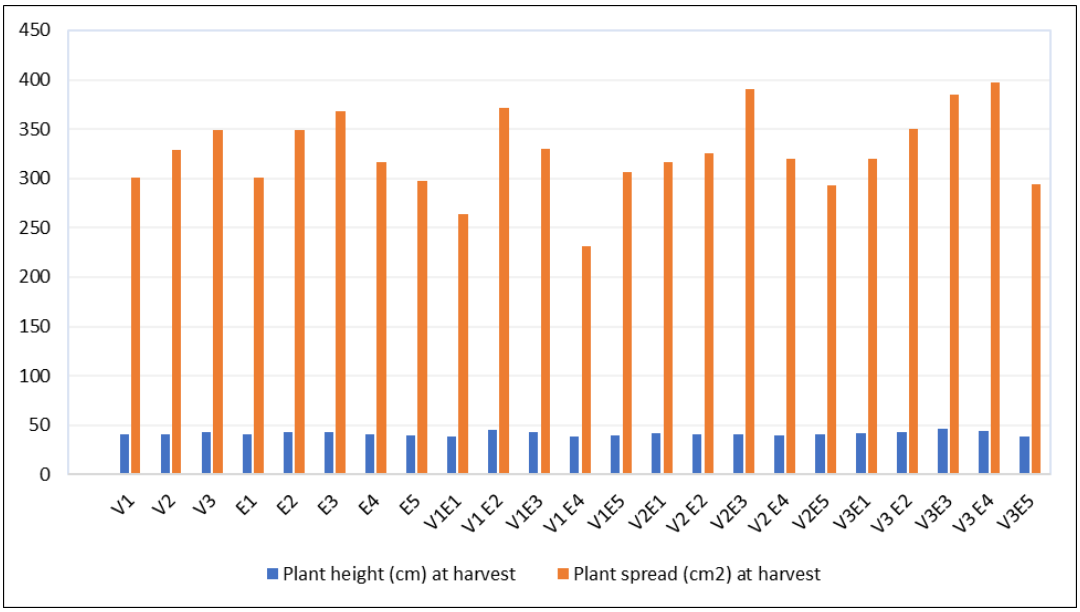

Fig. 1a. Growth attributes of black cumin varieties as influenced by different elicitors

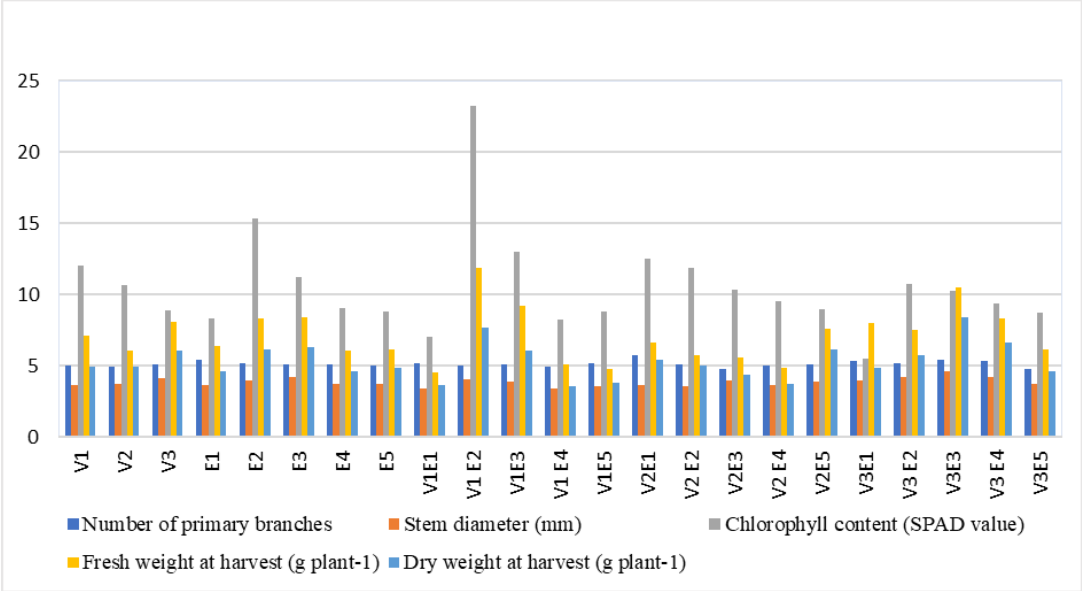

Fig. 1b. Growth attributes of black cumin varieties as influenced by different elicitors

Stem diameter showed significant differences among varieties, with the Pant Krishna variety recording a maximum stem diameter of 4.14 mm. Varietal differences in stem diameter may be attributed to genetic variation in the production, translocation, and accumulation of carbohydrates in the stem. Similar variations in stem diameter were reported by Salih et al. [13] in Hibiscus cannabinus.

Chlorophyll content at 60 days after sowing (DAS) was highest in the Azad Kalonji variety (12.08 SPAD value) and with foliar application of salicylic acid at 100 ppm (15.32 SPAD value) and their interaction (23.28 SPAD value). The increased chlorophyll content may be attributed to genetic factors, with salicylic acid potentially playing an indirect role in enhancing chlorophyll content by regulating the uptake of nutrient elements required for chlorophyll synthesis. These results are consistent with previous studies conducted by Gharib [14] in sweet basil and marjoram, Yildrium and Dursan (2009), Kazemi et al. [15] in tomato, and El-Gamal and Ahmed [16] in coriander.

Fresh weight and dry weight varied significantly among the interactions of varieties and elicitors, with the maximum fresh weight recorded in Azad Kalonji plants sprayed with salicylic acid at 100 ppm (11.85 g plant^{-1}) and the maximum dry weight in Pant Krishna treated with methyl jasmonate at 100 ppm (8.42 g plant^{-1}). The variation in fresh weight among varieties may be attributed to genetic factors influencing leaf turgidity and phenotypic variation. The positive effect of methyl jasmonate may be due to its modulating effect on gene expression and specific aspects of plant growth and development. Similar variations among different varieties were reported by Aggrawal et al. [17] and Anitha et al. [11] in fenugreek, Kazemi [15] in tomato, and Ali et al. [18] in fennel.

The data regarding yield parameters in black cumin are presented in Table 1. Varieties, elicitors, and their interaction did not exhibit significant differences concerning days to first flowering and days to fifty percent flowering. On average, plants took between 43.3 to 53.7 days to exhibit first flowering and 55.3 to 59.7 days to reach fifty percent flowering, consistent with findings reported by Assefa and Girma [12] in black cumin.

Days to crop maturity showed significant variation among varieties, with Azad Kalonji maturing earlier at 108.33 days compared to Ajmer Nigella-20 and Pant Krishna, which took 111.67 days to mature, indicating a significant delay. This variation in crop maturity among varieties can be attributed to their genetic factors interacting with environmental conditions during the cropping period. This interaction with various phytohormones might have accelerated the reproductive phase relative to the vegetative growth phase, leading to early initiation and completion of the flowering period, promoting ethylene synthesis and enhancing plant senescence. Conversely, a prolonged vegetative and reproductive phase with increased cell division and elongation could account for the extended time taken for maturity. Similar results were reported by Assefa et al. [8] in black cumin.

The number of capsules per plant was significantly higher in Pant Krishna (16.87) and in plants treated with salicylic acid at 100 ppm (16.67), which was comparable to methyl jasmonate at 100 ppm (16.07). Differences in capsule numbers among varieties may be attributed to variations in growth attributes such as plant height, spread, stem girth, fresh weight, and dry weight, with higher values observed in Pant Krishna, likely contributing to a higher capsule count, a key yield-contributing factor. Similar variations in capsule numbers per plant were reported in black cumin genotypes in Ethiopia by Assefa and Girma [12] and Ali et al. [18] in fennel. Salicylic acid plays a crucial role in regulating physiological processes such as glycolysis, photosynthesis, stomatal conductance, and flowering in thermogenic plants, influencing nutrient uptake. Comparable results were obtained in cumin, where the maximum number of umbels per plant was recorded with salicylic acid treatment followed by methyl jasmonate [19], and in coriander where foliar application of methyl jasmonate resulted in the maximum capsules per plant (22.60) in Pant Krishna [16].

The number of locules per capsule showed no significant variation among varieties, elicitors, and their interaction.

Elicitors and their interaction with varieties significantly increased the number of seeds per capsule in plants treated with methyl jasmonate at 100 ppm (79.27) and in the interaction of Pant Krishna treated with methyl jasmonate at 100 ppm (84.28).

Salicylic acid at 100 ppm recorded the maximum 1000 seed weight (2.40 g), followed by hydrogen peroxide (2.27 g), which were significantly different from other treatments. The higher thousand seed weight with salicylic acid treatment may be attributed to its ability to mobilize more photosynthates into the seeds, resulting in larger seeds as evidenced by the higher fresh and dry weights. These results are consistent with salicylic acid treatment in cumin [20]. Varieties did not significantly differ with respect to 1000 seed weight, consistent with findings reported by Motamedi-Mirhosseini et al. [21] in cumin.

Seed yield differed significantly among different elicitor treatments, with the maximum seed yield per hectare recorded with foliar application of methyl jasmonate at 100 ppm (11.52 q) while the lowest seed yield (8.08 q) was recorded in untreated control plants. However, there was no significant difference among varieties and their interaction with elicitors concerning seed yield. The positive effect of methyl jasmonate at 100 ppm on higher seed yield may be attributed to its promotion of higher plant spread, chlorophyll content, capsule number per plant, seeds per capsule, and thousand seed weight. Elicitors such as salicylic acid and methyl jasmonate have

bio-regulator effects on physiological and biochemical processes in plants, including ion uptake, cell elongation, cell differentiation, sink and source regulation, photosynthetic activity, and antioxidant properties, as well as defense mechanisms against abiotic and biotic stress [19]. These results align with findings in tomato treated with elicitors [15], cumin [20], and fennel [18].

4. CONCLUSION

Based on the results of this investigation, it can be concluded that all varieties, namely Azad Kalonji, Ajmer Nigella-20, and Pant Krishna, performed equally well with respect to growth and yield attributes under Bengaluru conditions. Among the elicitors, methyl jasmonate and salicylic acid at 100 ppm show potential for commercial cultivation of black cumin. Further research could involve multi-location trials of the varieties examined in this study, as well as exploring additional commercial elicitors and their application methods, along with pinching studies, for future investigation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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