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Responses of Black Cumin (*Nigella sativa* L.) To Applied Nitrogen with or without Gibberellic Acid Spray

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Abstract: Field experiments were conducted during two successive winter seasons at the Agricultural Farm of Aligarh Muslim University, Aligarh, India, to study the effect of foliar spray of 10^{-5} M gibberellic acid (GA_3) at vegetative stage along with basally applied 0, 40, 60, 80 and 100 kg N ha^{-1} on chlorophyll (Chl) content, net photosynthetic rate (P_N), stomatal conductance (g_s), leaf N content, leaf area (LA) and total dry matter (TDM) production (monitored at 30 days after spray application) and number of capsules $plant^{-1}$, 1000-seed weight, seed yield ha^{-1}, biological yield ha^{-1}, harvest index (HI), seed yield merit (SYM). Results indicated that, at 0, 40 or 60 kg N ha^{-1}, GA_3 did not produce any significant effect, but at basal 80 kg N ha^{-1}, GA_3 affected the parameters favourably with the exception of HI. A level of 100 kg N ha^{-1} proved supra-optimal. GA_3 sprayed plants exploited nitrogen from the soil more effectively and resulting in enhanced morphophysiological and yield responses.

Key words: Growth · gibberellic acid · *Nigella sativa* · nitrogen · physiology · productivity

INTRODUCTION

The genus *Nigella* belongs to the family Ranunculaceae. Many of the plants of this family have remarkable aromatic properties and medicinal value. *Nigella sativa* L., an indispensable constituent of medicinal and food formulations for centuries, is widely cultivated throughout South Europe, Syria, Egypt, Saudi Arabia, Turkey, Iran and Pakistan [1]. This herb is an important valuable object of the Greco-Arab/Eastern system of Medicine's Pharmacopoeia with interesting ethonobotanical and ethnopharmacological data. In their external use the seeds have been found to give relief when applied on pityriasis, leucoderma, ringworm, eczema, alopecia, freckles and pimples. Besides, the seed oil also alleviates asthma, chronic headache, migraine, chest congestion, rheumatism, paralysis [2, 3] and is even appreciably effective against Dalton's lymphoma ascites [4].

Keeping these medicinal properties in view, the present authors realized the relevance of investigating suitable methods, which can augment the yield of this medicinal crop with better utilization of input fertilizers, especially basal nitrogen (N). Use of N to control crop growth and productivity has been one of the key contributing factors for the incremental improvement not only in agricultural but also in medicinal crop production [5]. Nitrogen is used in crop cultivation to enable full exploitation of the genetic potential of the crop. It is the nutrient that has the largest effect on plant physiology [6] and is probably the single most important limiting nutrient for crop growth [7]. However, agricultural soils are often deficient in N and hence, to ensure adequate N supply to crops and prevent nutrient deficiencies, increasingly large amounts of inorganic N are employed, which remain unassimilated and result instead, in toxicity for the plant and soil alike [5].

Therefore, an approach, which can result in efficient manupulation, exploitation and assimilation of the available N by the crop plants may be explored. In this context, the use of phytohormones, particularly gibberellic acid (GA_3), may be considered to be a prospective solution. GA_3 is an essential growth hormone, that is known to be actively involved in various physiological activities such as growth, flowering and ion-transport [5, 8-10]. Moreover, GA_3 acts as a mediator for acclimation of plants to leaf canopy, stimulates leaf area expansion [11, 12] and induces elongation and osmoregulation in internodes [13], in addition to increasing dry matter and biomass production [14] and greatly enhancing the sink potential [15].

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Therefore, the aim of this study was to investigate the effects of N fertilization rate and foliar application of 10^{-5} M GA_3 at vegetative stage on *N. sativa* with respect to morphophysiological changes and yield.

MATERIALS AND METHODS

Crop cultivation: Completely Randomized Field experiments were conducted on *Nigella sativa* L., during the winter seasons of 2000-2001 and 2001-2002 at the Agricultural Farm of Aligarh Muslim University, Aligarh, India to study the response of the medicinal crop to basal 0, 40, 60, 80 and 100 kg N ha^{-1} applied with or without 10^{-5} M GA_3 spray (de-ionized water as control). The basal application of N in the form of urea was done at the time of sowing whereas, at 40 days after sowing (pre-flowering stage), GA_3 was sprayed at the rate of 600 l ha^{-1} along with 0.5% teepol. The control plots were sprayed with de-ionized water and teepol. The soil of the experimental field was sandy loam type with pH 8.1 and available N for both the years was 185.6 and 191.7 kg N ha^{-1}, respectively. Seeds were obtained from the Regional Research Institute of Unani Medicine, Aligarh, India. They were surface sterilized with mercuric chloride solution (0.01%), followed by repeated washings using double distilled water. The seeds were then sown in 24 m^2 plots and at the seedling stage, maintaining a distance of 30 cm between rows and 15 cm between plants in a row. Each treatment was replicated three times.

Variables and their determination: At 30 days after spray treatment, net photosynthetic rate (P_N) and stomatal conductance (g_s) were recorded by a LI-6200 portable photosynthesis system (LI-COR, Lincoln, NE, USA). The fully expanded top leaf of main axis was selected and photosynthesis measurement was carried out at about 1100 m mol $m^{-2}s^{-1}$ photosynthetically active radiation at 1100-1200 h. Chlorophyll (Chl) content was estimated according to Mackinney [16]. Leaf area was calculated according to Watson [17]. Total dry matter (TDM) $plant^{-1}$ was recorded by drying the plants at 80°C for 24 h. N content was determined as a product of the concentration of N (estimated by micro-Kjeldahl's method) [18] and the dry weight of the plant. For yield attributes twenty plants from each plot were removed and capsule number $plant^{-1}$ was recorded. Random samples were taken from threshed seeds for determination of the 1000-seed weight. The seed yield from a 24 m^2 plot was noted after threshing the seeds. The remaining plant material was sun dried and weighed and biological yield was calculated. Harvest index (HI) was determined by dividing the seed yield by the biological yield. Moreover, seed yield merit (SYM) was obtained by using the method given by Imsande [19]. Analysis of variance was carried out on the data obtained and L.S.D. ($p=0.05$) was calculated according to Gomez and Gomez [20].

RESULTS AND DISCUSSION

The data presented in Tables 1 & 2 show the average of the data for the two successive years. As is evident, growth response of *N. sativa* to applied N was linear, being maximum to the application of 80 kg N ha^{-1}. Higher dosage of 100 kg N ha^{-1} proved supra-optimal. The observed favourable effect of soil applied N is in conformity with innumerable reports on various plants, including those of Singh [21], Hashmi [22], Cooke *et al.* [23] Ashraf and Noman [24] and Ashraf *et al.* [25]. Nitrogen is known to be the most important growth limiting factor, as it forms an integral part of biologically indispensable molecules, such as purines, pyrimidines, enzymes, co-enzymes, structural and catalytic proteins as well as chlorophyll [26]. Implicably, an abundant supply of N increases the number of meristems, which may compete strongly with each other for organic and mineral nutrients, especially for N and are responsible for increasing the leaf number, leaf area and branch number $plant^{-1}$ (Table 1). The stimulatory effect of N on the vegetative growth may be additionally attributed to the increased production of cytokinins under ample N supply. Cytokinins are amino acid derivatives and are known to promote growth of buds and tillers [27]. Moreover, it is not surprising that the effect of N on crop production is via the expansion of more leaf area by affecting the cellular mechanisms driving expansion [23], which ultimately culminates into increased production of photoassimilates. The recovery of leaves as dry mass (Table 1) hence, owes much to the better management of source-sink relationship under the administration of N. This finding is corroborated by the strong positive correlation between nitrogen and dry matter ($r = 0.993^{**}$).

The above-mentioned growth attributes were also significantly affected, in addition to N, by the application of GA_3. It was also noted that at 0, 40 and 60 kg N ha^{-1} GA_3 did not show much effect, however, along with 80 kg N ha^{-1} results of GA_3 application were most pronounced. Probably, during the vegetative phase GA_3 must have triggered a better utilization and partitioning of optimally

Table 1: Effect of 10^{-5} M GA_3 spray on growth and physiological traits of black cumin (*Nigella sativa* L.) grown at a range of nitrogen levels (average of 1999-2000 and 2000-2001)[A]

	-GA_3[C]					+GA_3[D]					
Variables[B]	N_0	N_{40}	N_{60}	N_{80}	N_{100}	N_0	N_{40}	N_{60}	N_{80}	N_{100}	L.S.D. at 5%
LN ($Plant^{-1}$)	28.500	40.210	46.710	53.240	54.410	32.200	49.720	59.900	70.540	74.150	3.15
LA ($Plant^{-1}$)	228.300	350.700	398.700	500.400	510.200	250.900	424.900	514.500	680.900	692.200	19.25
BN ($Plant^{-1}$)	7.200	9.550	10.410	11.600	11.740	7.800	10.930	12.700	15.080	15.190	1.15
N content (%)	2.450	2.810	3.210	3.640	3.790	2.500	2.950	3.500	4.120	4.210	0.12
P_N (μ_mol (CO_2) m^{-2} s^{-1})	13.100	15.110	17.810	20.730	20.910	14.500	17.910	21.670	26.710	27.110	1.79
g_s (mol $m^{-2}s^{-1}$)	0.251	0.288	0.335	0.374	0.381	0.273	0.339	0.417	0.497	0.502	0.02
Chl (g kg^{-1})	1.410	1.620	1.740	1.880	1.910	1.450	1.840	1.950	2.150	2.210	0.07
TDM (g $plant^{-1}$)	1.970	2.690	3.150	3.710	3.820	2.240	3.250	3.970	4.940	4.750	0.51

[A]Determinations were carried out at 30 days after spraying, [B]LN, leaf number; LA, leaf area; BN, branch number; N, nitrogen content; P_N, net photosynthetic rate; g_s, stomatal conductance; Chl, chlorophyll content; TDM, total dry matter, [C]Without GA_3 spray, [D]With GA_3 spray

Table 2: Effect of 10^{-5} M GA_3 spray on yield characteristics of black cumin (*Nigella sativa* L.) grown at a range of nitrogen levels (average of 1999-2000 and 2000-2001)[A]

	-GA_3[C]					+GA_3[D]					
Variables[B]	N_0	N_{40}	N_{60}	N_{80}	N_{100}	N_0	N_{40}	N_{60}	N_{80}	N_{100}	L.S.D. at 5%
Capsule number $Plant^{-1}$)	15.21	19.20	22.30	26.92	27.11	17.11	23.50	29.42	36.04	37.05	2.47
1000-seed weight (g)	2.40	2.61	2.72	2.85	2.91	2.45	2.73	2.88	3.21	3.01	0.10
Seed yield (q ha^{-1})	8.54	10.21	12.81	15.71	15.46	8.61	11.45	15.12	20.28	19.87	2.12
Biological yield (q ha^{-1})	28.18	32.01	39.41	46.96	47.17	28.32	34.80	44.66	57.71	58.21	3.45
HI (%)	30.30	31.90	32.50	33.45	32.91	30.40	32.91	33.85	35.10	34.21	N.S.[E]
SYM	258.80	325.70	416.30	525.50	508.70	261.70	376.70	511.80	711.90	679.70	110.20

[A]Determinations were carried out at harvesting (130 DAS), [B]HI, harvest index; SYM, seed yield merit, [C]Without GA_3 spray, [D]With GA_3 spray
[E]Not significant

available N, hence leading to enhancement in the levels of key constituents for the building blocks (organs) of the plant infrastructure maximally, resulting in turn, in profuse vegetative growth. In other words, the synergistic interplay of the applied N and phytohormones at optimum levels may be the cause for the positive results expounded by the plants in the response to combined treatment. Further, the GA_3 - induced wall extensibility [28], leading to cell expansion and ultimately to elongation of internodes [29, 30] may be assigned as another probable cause for the increase in the shoot length. Needless to say, increased shoot length enhances the opportunity for the formation of more leaves and branches, as observed (Table 1). Moreover, the aforesaid stimulation of cell expansion and division due to GA_3 contributes to an increase in the leaf area [12, 31], which in turn manifests ultimately in the form of more dry matter (Table 1). This stance is further strengthened by the strong positive correlation between maximum leaf area and dry matter (r=0.975**).

N is intimately related with photosynthesis, stomatal conductance and total leaf Chlorophyll Content [32]. Analysis of all these attributes revealed that they were significantly enhanced by N application, especially 80 kg N ha^{-1} (Table 1), which proved optimum. This increase can well be ascribed to the higher mineral status especially N (Table 1) of the test plants and larger leaf area, which implies a more efficient photosynthesizing machinery, thereby affecting photosynthesis. Synchronously, the stimulatory effect of 80 kg N ha^{-1} was most prominently expressed in combination with the GA_3 spray. The leaves of plants receiving the exogenous GA_3 treatment had a higher Chl content, which may be explained on the basis of the GA_3 generated enhancement of ultra structural morphogenesis of plastids [33], coupled with retention of Chl and delay of senescence due to the hormone treatment [15] and an efficient utilization of optimally available N - which is a key constituent of Chl. Further, these test plants also expounded a lesser stomatal resistance, implying a greater

g_s and resulting in a free exchange of gases, as per Arteca and Dong [34]. A subsequent expression of the cumulative effects of increased levels of Chl and g_s was the high P_N of the treatment subjected crops. Various other factors, such as adequate supply of CO_2, under a better g_s along with the GA_3-induced promotion of the rates of cyclic and non-cyclic phosphorylation [35, 36] and the activity of CA [37] and RuBPCO [38], may also have supplemented the above stance.

Yield is the final manifestation of several intricate morpho-physiological traits, initiated at germination and terminated at harvest [39]. In this study, all the yield attributes were significantly affected by the application of N, with 80 kg N ha^{-1} proving optimum in increasing the seed yield (Table 2), as a result of cumulative effect of the enhancement of various other yield characteristics i.e., biological yield ha^{-1}, number of capsules $plant^{-1}$ and 1000-seed weight (Table 2). Further, this increase was synergistically enhanced upon the combined application of 80 kg N ha^{-1} and the 10^{-5} M GA_3 spray (Table 2). Apparently, the availability of sufficient amount of nutrients in the soil medium, coupled with their efficient manipulation, absorption and utilization due to the phytohormone, resulted in intense vegetative growth, leading to substantial increase in the size and stature of the reproductive sink (i.e. number of flowers and capsules) to attract more photosynthates. This, along with increase in the sink strength due to GA_3 [15], provides a greater potential for translocation of assimilates from the vegetative structures to capsules, thereby increasing their number [40]. Ample availability of the photosynthates subsequently leads to enhanced seed filling and culminates into increased seed weight, seed yield and seed yield merit (Table 2). In addition, the increase in the economic yield of the crop may also be an outcome of the effect of GA_3 in checking the pre-mature drop of the reproductive organs [41, 42]. Simultaneously, it is assumed that seed yield and harvest index frequently do not provide satisfactory measures of plant yield efficiency because a large plant may have high yield and low harvest index, whereas a small plant may have vice-versa. Seed yield merit, which combines the merits of these two traits [19], was also found to be influenced by GA_3 spray.

Summarily, we can say that the observed enhancement of the yield attributing characters is an expression of the cumulative effect of the treatment on the leaf metabolism, re-directed mobilization of metabolites and delayed senescence of the plant organs. Similar results have also been reported by Hayat *et al.* [43], Mousa *et al.* [44] and Khandelwal *et al.* [45]. It is therefore, concluded that addition of N at 80 kg N ha^{-1}, along with spraying of *N. sativa* plants with 10^{-5} M GA_3 had the most beneficial effects, among all the treatments examined and characters evaluated in this study.

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