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# Influence of Nitrogen and Phytohormone Spray on Seed, Inorganic Protein and Oil Yields and Oil Properties of *Nigella stiva* L.

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**Abstract:** Two pot trials were carried out on *Nigella sativa* L. at the Department of Botany, Aligarh Muslim University, Aligarh, India, during the winter seasons of 2001-2002 and 2002-2003 to analyze the effect of basal nitrogen at the rate of (0, 40, 60, 80 or 100 kg N $ha^{-1}$) or either of two growth regulators ($GA_3$ or KIN), each applied at $10^{-5}$ M at the vegetative stage (40 days after sowing), on seed, protein and oil yields and oil properties. The experiments were laid down according to randomized complete block design, with three replications. Analysis of the results revealed that, all these treatments significantly influenced capsule number, seed yield, seed protein content, seed oil and protein yields $plant^{-1}$. The response to nitrogen was linear, being maximum with 80 kg N $ha^{-1}$. Also, $GA_3$ was found to produce better results than KIN. However, a decrease was noted in acid, iodine and saponification values of the oil following these treatments. Further, the seed oil content tended to decrease with increasing N dosages, but showed an appreciable increase upon the application of phytohormones.

**Key words:** *Nigella sativa* L., nitrogen, phytohormone, seed oil content, seed oil properties, seed protein content

## INTRODUCTION

*Nigella sativa* L. (black cumin), a native herb of the Eastern Mediterranean region, has been used for centuries in a variety of food as well as medicinal formulations (Janson, 1981). It's seeds are know to possess wonderous remedial properties as digestive stimulants, carminative, aromatic, diuretic, diaphonetic, stomachic, anthelmentic, asthamatic (Salama, 1973; Agarwal *et al*., 1979) and even anticancerous (Salomi *et al*., 1989). Owing to their medicinal and aromatic properties, there is a steadily increasing demand for this herb in the domestic and international markets, which provides excellent scope for the extension of its cultivation to a commercial level.

In this context, the use of plant growth supplements may be considered. Inorganic N fertilizer has been found to incrementally improve both agricultural as well as medicinal crop production (Shah, 2004), as N is the single most important growth-limiting factor for crop plants (Goyal and Huffaker, 1984). Evidently, optimum availability of N liberates a crop plant from impairing nutrient deficiencies and provides a sound platform for superlative growth. It is an essential nutrient for production of plant dry matter, as well as energy-rich compounds that regulate photosynthesis and crop production (FeiBo *et al*., 1998). There is an optimal relationship between plant N and $CO_2$ assimilation (Sinclair and Horie, 1989). Further, plant growth regulators, particularly $GA_3$ and KIN, have also been shown to possess potential to enhance crop productivity (De-La Guardia and Benloch, 1980; El-Keltawi *et al*., 1987; Khan and Samiullah, 2003; Shah and Samiullah, 2006) and manipulate the crop in order to enable the utilization of maximum possible available resources and exploitation of its inherent genetic potential. Therefore, the aim of this study was to investigate the effects of various N fertilization rates and foliar application of $10^{-5}$ M$GA_3$ or KIN at the vegetative stage (40 DAS) on seed, protein and oil yields and oil properties of *N. sativa* L.

## MATERIALS AND METHODS

Two pot experiments were carried out at the Department of Botany, Aligarh Muslim University, Aligarh India, on *Nigella sativa* L. in the winter seasons of 2001-2002 and 2002-2003. The soil of the experimental pots was of sandy loam type, available N for the two years was 195 and 198 kg N $ha^{-1}$, respectively. The basal application of N in the form of urea at the rate of 0, 40, 60, 80 or 100 kg $ha^{-1}$ or $10^{-5}$ M spray concentrations of either $GA_3$ or KIN at the rate of 5 $cm^3$ $plant^{-1}$ was administered at 40 days after sowing (vegetative stage). Water was used as a control treatment. A randomized complete block design with three replications was used. Seeds were obtained from the Regional Research Institute of Unani Medicine, Aligarh, India and were surface sterilized with

0.01% mercuric chloride solution, followed by repeated washings with double distilled water. They were then sown in earthen pots (25 cm in diam.) filled with soil and farmyard manure, mixed in a ratio of 9:1.

At harvest, seed yield from three randomly selected plants was noted after threshing the seeds. Seed protein content was estimated according to AOAC (1985). For determination of seed oil content, oil was extracted three times with a chloroform/methanol (2:1, v/v) mixture, according to the method out lined by Kates (1972). Oil quality characteristics i.e. acid value, saponification value and iodine value, were determined according to methods described by Walker (1985). Analysis of variance was carried out on the data obtained and LSD ($p = 0.05$) was calculated (Gomez and Gomez, 1984).

## RESULTS AND DISCUSSION

**Capsule number and seed yield plant$^{-1}$:** As is evident from Table 1, number of capsules and seed yield plant$^{-1}$ were favourably affected by the application of basal N, with 80 kg N ha$^{-1}$ showing most promising results. This enhancement of yield may be attributed to the ample availability of N, which is an important nutrient that controls growth, prevents abscission and is essential for photosynthetic activity (Reddy *et al*., 1996). Moreover, this essential macronutrient stimulates the mobilization and accumulation of metabolites, thereby increasing the capsule number plant$^{-1}$ and hence seed yield. Results obtained are confirmed by those of Khan (1991).

Seed yield was also found to be significantly enhanced by the application of either of the growth regulators ($GA_3$ or KIN) (Table 1). The results for $GA_3$ were better than those for KIN, probably because endogenous cytokinin is seldom limiting in crop plants (Moore, 1989) and the effect of exogenously applied KIN was therefore, relatively subdued. However, the appreciable increase in number of capsules and seed yield plant$^{-1}$ over the control, as observed herein, may be because both these hormones regulate induction of flower/fruit set (Garcia-Martinez and Carbonell, 1980; Rylott and Smith 1990; Arteca, 1997) and greatly enhance the potential of the reproductive sink (Juli and Jose, 1987; Atkins and Pigeaire, 1993; Ouzounidou and Ilias, 2005) to attract more photoassimilates, thereby having a direct effect on number of capsules and hence seed yield. Additionally, KIN is also effective in preventing flower/pod abortion (Nagel, 2001), which determines the number of mature capsules at harvest and hence overall yield. Such results corroborate the findings of Mousa *et al*. (2001).

**Seed protein content and protein yield plant$^{-1}$:** Results indicated that seed protein content increased with increasing levels of N fertilization and 80 kg N ha$^{-1}$ proved most stimulatory. These results are expected, as N is vital in basic protein structure, as well as an active constituent of RNA and DNA, which are essential for protein synthesis (Marschner, 1996). A high N rate increased the seed protein content and protein yield plant$^{-1}$ (Table 1). Soluble proteins were increased by increased N supply under favourable growth condition (Sugiyama *et al*., 1984). Greef (1994) reported that high values of protein fraction were found in photosynthetically active leaf tissue. This is true especially under condition of favourable N supply. These results suggested that the high N rate increased amino acid synthesis in the leaves and stimulates the accumulation of protein in the seeds rather than oil. A similar finding in cotton was reported by Patil *et al*. (1997).

Seed protein content and protein yields plant$^{-1}$ were increased in plants treated with $GA_3$ or KIN compared with control (Table 1). The plausible explanation may be that, either of the hormone encourages the conversion of amino acid into protein (Martin and Northcote, 1982). Such findings were confirmed by those of Basalah and Mohammad (1999) and Borse and Dhumal (2001).

**Seed oil content and yield plant$^{-1}$:** Seed oil content was found to decrease with increasing levels of basal N (Table 1), which may be because the sink became more voluminous due to application of fertilizer, resulting in dilution with growth. Also, it is known that available N stimulates the accumulation of protein in the seed rather than oil (Sawan *et al*., 2001). The increased seed protein content (Table 1) further supports the results obtained. However, due to enhanced production of seeds, the oil yield plant$^{-1}$ was so spectacular that it out balanced the lowered oil content, which is an obvious commercial advantage for the seeds. Similar results were obtained by Pandrangi *et al*. (1992).

On the contrary, application of either of the growth regulators resulted in increase in the seed oil content significantly above that of the control (Table 1). Oil biosynthesis is an integration of several metabolic pathways, which require association of several metabolic steps, including continuous production of pre-cursors and also their transport and translocation to the active sites of synthesis. Any disruption in normal metabolic pathways affects the sequence of steps in oil biosynthesis. Thus a plant may adjust its metabolic pathway in response to various factors, including nutrient

Table 1: Effect of N rate and foliar application of phytohormones on capsule number, seed yield, seed oil, seed protein and oil and protein yields

| Treatments | | No. of capsule (plant$^{-1}$)[1] | Seed yield (g plant$^{-1}$)[1] | Seed oil (%)[2] | Oil yield (g plant$^{-1}$)[2] | Seed protein (%)[2] | Protein yield (g plant$^{-1}$)[2] |
|---|---|---|---|---|---|---|---|
| N rate (kg ha$^{-1}$) | | | | | | | |
| Control | 0 | 15.01 | 1.21 | 37.38 | 0.452 | 19.78 | 0.239 |
| | 40 | 19.10 | 1.44 | 36.86 | 0.549 | 22.01 | 0.328 |
| | 60 | 21.95 | 1.90 | 36.67 | 0.697 | 23.40 | 0.445 |
| | 80 | 26.03 | 2.26 | 36.53 | 0.826 | 25.55 | 0.577 |
| | 100 | 25.10 | 2.31 | 36.43 | 0.852 | 26.01 | 0.600 |
| LSD[3] at 5% | | 2.04 | 0.21 | NS[4] | 0.120 | 1.15 | 0.101 |
| Phytohormones (M) | | | | | | | |
| Control | 0 | 14.85 | 1.09 | 36.36 | 0.408 | 20.25 | 0.221 |
| $GA_3$ | $10^{-5}$ | 20.65 | 1.72 | 37.46 | 0.634 | 21.30 | 0.367 |
| KIN | $10^{-5}$ | 18.45 | 1.56 | 37.06 | 0.567 | 21.11 | 0.329 |
| LSD at 5% | | 1.15 | 0.14 | 0.06 | 0.051 | 0.18 | 0.029 |

[1]Combined statistical analysis for the two seasons, [2]Mean data from a fold replicate comosite for the two seasons, [3]LSD: Least Significant Difference, [4]NS: Non Significant

Table 2: Effect of N rate and foliar application of phytohormones on seed oil properties[1]

| Treatments | | Acid value | Iodine value | Saponification value |
|---|---|---|---|---|
| N rate (kg ha$^{-1}$) | | | | |
| Control | 0 | 37.66 | 115.20 | 203.24 |
| | 40 | 36.19 | 112.21 | 206.84 |
| | 60 | 35.00 | 116.18 | 208.19 |
| | 80 | 35.14 | 115.01 | 209.78 |
| | 100 | 35.01 | 114.01 | 210.00 |
| LSD[2] at 5% | | NS[3] | NS | NS |
| Phytohormones (M) | | | | |
| Control | 0 | 35.56 | 118.21 | 201.37 |
| $GA_3$ | $10^{-5}$ | 35.10 | 116.25 | 199.42 |
| KIN | $10^{-5}$ | 33.09 | 113.24 | 198.24 |
| LSD % | | NS | NS | NS |

[1]Mean data from a four replicate composite for the two seasons, [2]LSD: Least Significant Difference, [3]NS: Non Significant

and phytohormone application (Singh *et al*., 1999). Therefore, our results can be accounted for through the preferential synthesis of oil rather protein induced by the hormone application (Sawan *et al*., 1988). This, coupled with increased seed yield, boosted the overall oil yield plant$^{-1}$ as observed herein. The positive influence of phytohormones on oil yield have also been reported by other researchers on *Nigella* and other medicinal crop plants (Farooqi *et al*., 1996; Mousa *et al*., 2001; Hashmi, 2003).

**Seed oil properties:** The acid and iodine values were found decreased, but saponification value showed perceptible increase (albeit non-significant), with increasing N rates (Table 2). Similar results were obtained by the application of phytohormones ($GA_3$ or KIN). These findings are in agreement with those of El-Halawany (1979) and Sawan *et al.* (1982). In this context, it may be stated that low acid and iodine values are considered good for oil quality, storage and hydrogenation and high saponification value contributes towards digestibility (Downey and Rimmer, 1993).

Over-all results of our investigation indicate that addition of N at 80 kg ha$^{-1}$ and spraying of *Nigella* plants with plant growth regulators (especially $GA_3$) have the most beneficial effects and may be judiciously exploited for its large scale commercial cultivation.

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