Integrated effect of nitrogen and sulphur levels on productive traits and quality of black cumin *(Nigella Sativa* L.)

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Abstract

Nitrogen (N) and sulfur (S) play a crucial role in improving the grain yield and oil quality of oil crops. An experiment entitled "Integrated effect of nitrogen and sulphur levels on productive traits and quality of black cumin (*Nigella sativa* L.)" was conducted at Agricultural Research Farm, The University of Haripur during November to May (R*abi* season), 2020-21. The treatments consisted of four levels of nitrogen viz., 0, 20, 40 and 60 kgha^{-1} and sulphur viz. 0, 3, 6, 9 and 12 kgha^{-1}. The experiment was laid out in a randomized complete block design (two factor factorial) replicated thrice. Nitrogen and sulphur levels singly as well as in combination had significant effect on most of the characters studied. Significant effect of nitrogen levels, sulphur levels as well as their interaction were observed for Plant height (cm), capsules plant^{-1}, seeds capsule^{-1}, 1000 grain weight (g), biological yield (kgha^{-1}), grain yield (kgha^{-1}), nitrogen use efficiency and protein content (%). Application of nitrogen @ 40 kgha^{-1} coupled with sulphur at the rate of 12kgha^{-1} showed promising results regarding production and quality of black cumin. As this study was only conducted using nitrogen and sulphur, it is advisable to conduct an experiment with various essential plant nutrients along with growth regulators to check their synergistic effects for more reliable and acceptable recommendations in future.

Keywords: Nitrogen, black cumin, sulphur, Nitrogen use efficiency

INTRODUCTION

Black cumin (*Nigella sativa* L.) also known as Kalonji is one of the most widely examined plant possessing naturally occurring compounds with anti-cancer potential (Mehmood *et al*., 2021a).

Black cumin seeds have long been used as both a spice and a medicinal. The seeds have a bitter flavour, and even a tiny amount of entire seed causes a sense of tightness in the throat (Shrivastava *et al*., 2011). The medical potential of black cumin is enormous, and many researchers have praised its distinctive, diverse, and potent pharmacological properties. The herb's therapeutic value has been widely documented (Shahzad *et al*., 2020). Its pharmacological action as anti-tumor, anti-diabetic (Mathur *et al*., 2011), cardio protective (Ebru *et al*., 2008), gastro protective, anti-asthmatic (Tayman *et al*., 2013), anti-inflammatory (Chehl *et al*., 2009), neuro-protective (Akhtar *et al*., 2013), anticonvulsant (Sousa *et al*., 2011), anxiolytic (Perveen *et al*., 2014), antioxidant, antibacterial (Chaieb *et al*., 2011) and antifungal (Suthar *et al*., 2012), activities were immensely appreciated. Its seed contains 30% fixed oil and 0.3 to 0.4% essential oil (Hadi *et al*., 2016). Black cumin oil is rich in unsaturated fatty acid content and can be expected to offer considerable health benefits on consumption (Mehmood *et al*., 2021b). The most potent and fruitful medicinal properties lie in its volatile components (El-Tahir and Bakeet, 2006).

The major producing countries of black cumin are India, Sri Lanka, Afghanistan, Pakistan, Bangladesh, Turkey and Ethiopia (Giridhar *et al*., 2017). The production of black cumin in Pakistan has not been reported on commercial scale but it is widely used for various purposes in the country. With increase in demand for medicinal plants, black cumin is a potential specie for crop diversification and it can also reduce the risk of crop failure from which economic condition of smaller land holdings could also be improved (Iqbal *et al.*, 2010). The major crops grown in Haripur are wheat, maize and peas thus offering limited choice of seed spice crops for the farmers (Ayub *et al*., 2021). In such a scenario, among the several suitable seed spice crops, black cumin demands special attention due to its export value as well as increasing demand as medicinal spice (Mehmood *et al*., 2018).

Apart from season, yield is dependent to a large extent on soil nutrient status and external application of nutrients (Ayub *et al.,* 2020). Macro nutrients unavailability to the crops due to the calcareous nature of the soil of Pakistan is very common, particularly nitrogen (Khan *et al.,* 2020, Shah *et al.,* 2007). Nitrogen is a vital plant nutrient and is involved in enzymatic reactions, protein synthesis and is a main constituent of nucleic and amino acids but it is the most deficient nutrient (94%) in Pakistani soils (Mehmood *et al*., 2018). Soil of Haripur is generally medium to low in nitrogen content (Mehmood *et al*., 2020). Application of fertilizers, especially nitrogen had a considerable effect not only on quantity but also on the quality of the seed in many crops including Kalonji (Tuncturk *et al*., 2012).

Sulfur (S), in addition to nitrogen has an important position among secondary macro nutrients (Shah *et al*., 2020). When soil S is deficient, maximum yield of the crops cannot be exploited besides the availability of all necessary nutrients (Hasan *et al*, 2020). Sulfur is an essential nutrient necessary for plant nourishment and improving the oil contents of seeds and quality of the crop (Hammad *et al*., 2011). Combined application of S with N is affecting the physiological and biochemical processes of crops and thus requires intensive studies to find out their interaction for good and quality crop production (Rasheed *et al*., 2004). Nitrogen application to the corn along with S, improves not only grain yield and yield components, but also protein and oil of the crop (Saracoglu *et al*., 2011). Given the neophytic character of the crop in Haripur, it was planned to take up a systematic study titled "Integrated effect of nitrogen and sulphur levels on productive traits and quality of black cumin (*Nigella sativa* L.)" to find out the critical factors influencing the production.

MATERIALS AND METHODS

Soil samples were collected at random from 0 to 15 cm depth and a composite soil sample was prepared. These samples were used for the determination of various physio chemical tests by following the standard protocols. The results of the soil analysis presented in table 01 indicated that the experimental soil belonged to the textural class of silty clay loam, slightly alkaline in reaction.

Table. 1 Soil physiochemical analysis of experimental site at Agricultural Research Farm, University of Haripur

Soil texture	pH value	EC	N (%)	P (ppm)	K (ppm)	Organic matter	Ca+	Mg+	Hco_3	Cl
Silty Clay loam	7.85	1.19	0.064	8.71	74	0.55	5.25	0.75	5.8	0.12

EXPERIMENTAL DESIGN:

A randomized complete block design was used to replicate the treatments three times. Plot size was 6×2 m^2. Nitrogen and sulphur were applied according to the experimental protocol. Throughout the trial, all agronomic procedures were kept consistent and typical for all treatments. The experimental plots were ploughed thrice 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 and sub plots as per the layout of experiment. Black cumin variety NARC-1-Kalonji was used in the experiment. The seeds were sown on start of November. After 25 days of seedling emergence, the seedlings were thinned to maintain required spacing.

FERTILIZER MANAGEMENT:

The recommended dose of potassium and phosphorous @ 20-20 $kgha^{-1}$ were applied respectively, while the remaining treatments were as:

Factor A: Nitrogen levels

N1= 0 $kgha^{-1}$

N2= 20 $kgha^{-1}$

N3= 40 $kgha^{-1}$

N4 = 60 $kgha^{-1}$

Factor B: Sulphur levels

S1= 0 $kgha^{-1}$

S2 = 3 $kgha^{-1}$

S3 = 6 $kgha^{-1}$

S4 = 9 $kgha^{-1}$

S5 = 12 $kgha^{-1}$

PLANT PROTECTION MEASURES:

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. When the capsules got brown and dried, it was time to harvest the crop. The gathered plants were sun-dried for three to four days before being threshed with sticks. Following that, the seed was winnowed and cleaned for recording yield related data.

OBSERVATIONS ON THE CROP

Plant height (cm): Plant height was measured when the selected plants for data collection from each plot had flowered.

Branches $plant^{-1}$: At harvest, the branches of 10 randomly chosen plants were counted. The average was determined and reported as branches $plant^{-1}$.

Capsules plant^{-1}: The average was calculated by counting capsule plant^{-1} of 10 randomly chosen plants from each plot.

Number of seeds capsule^{-1}: Number of seeds capsule^{-1} of 10 random capsules from each subplot were counted and average was worked out.

1000-seed weight (g): 1000-seed weight was measured after taking hand sample of 1000 seeds from each treatment.

Biological yield (kgha^{-1}): The weight of the complete plant except roots w-as measured after harvesting. It was expressed as kg.

Seed yield (kgha^{-1}): Weight of clean seeds was recorded in grams plot^{-1}. The data was then converted into kgha^{-1}.

Nitrogen use efficiency: Nitrogen efficiency indices were calculated using the Equation:

Nitrogen use efficiency = G_t/N_f

Where, G_t is the total grain yield (kg) and N_f is the nitrogen fertilizer used (kg).

Protein content (%): Protein concentration in wheat grain was determined from the % nitrogen determined in the grain by Kjeldahl method (Bremner, 1996). 0.5g sample was added in digestion mixture of approximately 1g. Then added 7 ml H_2SO_4 digested and titrated with 0.1 normal HCl.

$$\text{Nitrogen} = \frac{\text{Normality of acid x 0.014 x100 x ml of HCl used for titration}}{\text{Weight of sample}}$$

Protein (%) = % N x Factor

Nitrogen (%) x 6.25

Statistical Analysis:

Analysis of variance was performed to confirm variability of data and validity of results using computer based software Statistix 8.1. The differences amongst treatments were separated using least significance difference test (LSD) at 0.05 probability level (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Plant height (cm)

Nitrogen dose had significant effect on plant height of black cumin. Sulphur application showed non-significant results regarding plant height while their interaction showed significant results (figure 1). Plant height (94.6 cm) responded linearly with N levels in experimental units applied with 40 kg N ha^{-1}, compared with control (75.6 cm). Figure 1 indicated that nitrogen @ 40 kg ha^{-1} and sulphur @ 12 $kgha^{-1}$ responded with a steeper trend of increase in plant height as compared to nitrogen @ 20 $kgha^{-1}$ and 60 $kgha^{-1}$. Nitrogen not only enhanced days to tasseling and silking but also increased plant height. The most probable reason for this could be that nitrogen improved leaf chlorophyll content and ultimately photo assimilate synthesis through the process of photosynthesis (Mehmood *et al*., 2018), which resulted in the high vegetative growth of the crop (Asim *et al.,* 2012). It might be due to nitrogen's positive influence on plant metabolism, which affects crop physiological processes and increases plant height. Walsh *et al*., (2020) showed that increased nitrogen treatment rates resulted in taller plants due to increased residual nitrogen absorption. Sulphur being a qualitative nutrient, has no role in vegetative growth of the plant and therefore showed no significant effect on days to tasseling, silking and plant height (Jeet *et al*., 2012). However, it has been observed that sulfur along with nitrogen significantly affected phenology, physiology and yield components of maize (Ali *et al.,* 2013).

Branches $plant^{-1}$

Branches $plant^{-1}$ recorded at 50% flowering was significantly influenced by nitrogen levels, sulphur levels and their interaction during the study. Figure 2 showed that nitrogen application significantly increased number of branches $plant^{-1}$ of black cumin with all the levels of sulphur. Statistically higher results were observed with application of nitrogen @ 40 $kgha^{-1}$ along with sulphur @ 12 $kgha^{-1}$. Lowest branches $plant^{-1}$ were observed in control plots. For instance, Mabl *et al*. (2015) observed that branches $plant^{-1}$ increased with increase in nitrogen levels. Likewise, Shilpi *et al*. (2012) reported that the rise in branches $plant^{-1}$ might be the result of increased nitrogen use efficiency.

Capsules $plant^{-1}$

Capsules $plant^{-1}$ plays a major role in the yield of the field crops because capsules are the major physiological portion of the plant, which determines the magnitude of the yield potential of the crop. Analysis of variance showed capsules plant^{-1} of black cumin were affected significantly from nitrogen dose, sulphur dose as well as their interaction. Maximum capsules plant^{-1} was observed with nitrogen application @ 40 kgha^{-1} followed by 20 kgha^{-1} and 60 kgha^{-1} whereas, minimum capsules plant^{-1} was observed in control plots. Regarding sulphur application levels, highest number of capsules were observed in 12 kg ha $^{-1}$ and lowest number of capsules were recorded in control (figure 3). Concerning interaction, capsules plant^{-1} significantly improved with application of nitrogen and sulphur at a certain limit. After which number of capsules plant^{-1} gradually decreases. It might be due to nitrogen's positive influence on plant metabolism, which affects the crop's physiological processes and increases production. Optimal nitrogen administration, according to several researchers, can result in more spikes/capsules. Bhat *et al.,* (2008) and Rana and Choudhary, (2008) both remarked on the conformity of the current investigation's findings (2006). Improved sulphur availability, which in turn boosted plant metabolism and photosynthetic activity, leading in higher development, might explain the rise in growth metrics under sulphur fertilizer. Gangwal *et al*, (2011) and Verma *et al.* (2012) also observed similar results. The significant function of sulphur in energy transformation, enzyme activation, and carbohydrate metabolism might explain the rise in capsules plant-1. Sulphur in an acceptable and appropriate proportion also aids in the beginning of floral primordia for its reproductive component, which in turn influences yield characteristics. These results corroborate the findings of Dongarkar *et al*., (2005) and Jat and Mehra (2007).

Number of seeds capsule^{-1}

Figure 4 depicting number of seeds capsules^{-1} of black cumin as effected by nitrogen levels, sulphur levels as well as their interaction. Highest seeds capsules^{-1} (52.53) were recorded with the application of nitrogen @ 40 kgha^{-1}, followed by (39.66) 20 kgha^{-1}. least (23.13) seeds capsules^{-1} were recorded in control treatment plots. Regarding sulphur application, highest seeds capsules^{-1} of black cumin (38.5) were recorded with higher sulphur application (12 kg ha^{-1}), whereas, least seeds capsules^{-1} (32) were recorded in control plots. Concerning the interaction, maximum seeds capsules^{-1} of black cumin (56) were observed in crop applied with nitrogen @ 40 kgha^{-1} along with sulphur application of 12 kgha^{-1}. In plant nutrition, nitrogen is the most important necessary nutrient, present in essential organic molecules, including proteins, amino acids, ribosomes, cytochrome, certain vitamins and chlorophyll. Atta, (2003) found that number of seeds/plant were increased by raising N up to optimum level. Bassiem & Anton (1998) observed that yield components were increased by escalating N doses. Raising N fertilization improved plant height, seeds capsule^{-1} and seed yield/ha, according to Fayed et al., (2000). Sulphur (S) is a nutrient that oil crops want, and it plays an important role in plant metabolism (Shelke *et al*., 2014). Sulphur is a component of three important amino acids present in plants, notably cysteine and methionine, which are required for protein synthesis. It is known as the "master nutrient" in the development of oil seeds. The addition of 12 kg S to the NPK substantially enhanced sesame yield (Kundu *et al*., 2010). Sarkar and Banik (2002) reported that Sulphur application increased seed yield as well as oil quality of sesame.

1000 seed weight

1000 seed weight of black cumin was significantly influenced by nitrogen dose, sulphur dose as well as their interaction during the study (figure 5). Highest 1000 seed weight was observed with nitrogen @ 40 kgha^{-1}, followed by 60 kgha^{-1}, whereas, lowest 1000 seed weight was observed in control treatments. Regarding sulphur application, highest 1000 seed weight was observed with sulphur application @ 12 kgha^{-1}. Lowering the sulphur dose resulted in lower 1000 seed weight of black cumin. Regarding interaction, highest 1000 seed weight was observed with nitrogen @ 40 kgha^{-1} along with sulphur dose of 12 kgha^{-1}. Lower dose of both fertilizers resulted in lower 1000 seed weight. Hocking and Stapper (2001) discovered that N fertilizer had no impact on 1000-seed weights, but Ahmad *et al*., (2011) and Kutcher *et al*., (2005) discovered that 1000-seed weight was lowered as the rate of N application increased. Another possibility might be related to late maturity induced by N fertilization, which, according to some authors, resulted in poor seed filling and a higher proportion of green seeds, however this was not seen in our study. Seed weight differences were connected to a short interval between anthesis and maturity, according to Cheema et al. (2001), and during that time, the supply of assimilates to the pods plays a critical role in the seed's growth.

Biological Yield

Figure 6 depicting biological yield of black cumin as affected from nitrogen and sulphur dose. Analysis of variance results reviled that nitrogen, sulphur as well as their interaction had significantly affected biological yield of black cumin. Highest biological yield was observed with application of higher dose of nitrogen, followed by 40 kgha^{-1}, whereas control plots showed lowest biological yield. Regarding sulphur application, maximum biological yield recorded with sulphur @ 12 kgha^{-1}. Lowering the sulphur rate resulted in lower biological yield as minimum biological yield was observed in control. Concerning the interaction, highest biological yield was obtained with higher fertilizer rate i-e 60 kg ha^{-1} nitrogen along with 12kg ha^{-1} sulphur. With gradual decrease in nitrogen and sulphur doses resulted in lower biological yield. Nitrogen levels had a significant effect on biological yield of black cumin and it has been observed that increasing N levels enhanced biological yield up to a certain level. The probable reason for this could be that nitrogen enhanced plant height, number of branches and ultimately increased the biomass of the plant (Hammad *et al.,* 2011: Kandil, 2013; Mehmood et al., 2021). Sulfur levels also had significant effect on biological yield, likewise other yield and yield components. Rasheed *et al.* (2004) reported that sulfur increased biological yield of the plants significantly.

Grain yield

In present study, significant variation was found in seed yield (Kg ha^{-1}) at different levels of nitrogen, sulphur, as well as their interaction. Highest grain yield was observed with application of nitrogen @ of 40 Kgha^{-1}, followed by 60 Kgha^{-1}, whereas, lowest grain yield was observed in control treatments. Regarding sulphur application, highest grain yield was recorded with application of sulphur @ 6 Kgha^{-1}, followed by statistically similar results were observed in with sulphur @ 9 Kgha^{-1} and 12 Kgha^{-1}. Concerning the interaction, highest grain yield was recorded in plots applied with 40 Kgha^{-1} nitrogen along with 6 Kgha^{-1} sulphur fertilizer (figure 7). The increase might be attributed to greater N availability, which would result in faster photosynthesis and hence higher carbohydrate production. Similar findings were also reported by Sokólski *et al*., (2020). Ahmad *et al*., (2011) and Yin *et al*., (2015) both found that increasing sulphur levels increased yield in onions and oilseed rape, respectively. It indicates that fertilizer application increased output, implying that the soil was nitrogen and sulphur deficient, resulting in large yield variations. Plants cultivated without nitrogen or sulphur consistently generated the lowest yield.

Nitrogen use efficiency

Figure 8 depicting nitrogen use efficiency of black cumin crop as affected by various nitrogen and sulphur dose. Analysis of variance reviled that nitrogen levels, sulphur levels as well as their interaction had significantly affected nitrogen use efficiency of black cumin. Highest NUE was observed with nitrogen @ 20 kgha^{-1}, followed by (19.54) nitrogen dose of 40 kgha^{-1}, whereas, higher nitrogen dose showed lower nitrogen use efficiency. Concerning sulphur application, maximum nitrogen use efficiency was observed with sulphur @ of 9 kgha^{-1}. Concerning the interaction, maximum nitrogen use efficiency was recorded in crop applied with nitrogen at the rate of 20kgha^{-1} along with sulphur at the rate of 9 kgha^{-1}. Increasing nitrogen dose will result in lowering nitrogen use efficiency. Nitrogen use efficiency may differ when a production factor is changed, for example, preceding crop, tillage system or water availability (Buchen *et al.,* 2017). Similarly, when N fertilizer rates are raised, NUE decreases (Duncan et al., 2018), because N becomes less limiting at high rates. Grain output will rise when N fertilizer is given to an agricultural system where N availability is restricting crop development, but a leveling off may occur if a cause other than N is limiting, such as a nutrient deficit. When the deficient nutrient is provided, this limitation may be overcome, resulting in a shift in NUE (Singh *et al*., 2018).

Sulfur (S) is another important nutrient for plants. S deficits in crops can be caused by a variety of factors, including low organic matter content in soils, soil erosion, and excessive nutrient removal by crops (De-Borja-Reis *et al*., 2021). S addition enhanced biomass and grain production in wheat, according to Carciochi et al., (2020), who reported a favorable interaction between N and S, which was represented in a higher NUE. Other research has looked into the N S interaction in wheat, but only at the grain yield level. S is a necessary component of enzymes involved in N metabolism, such as nitrate reductase and nitrite reductase (Swamy *et al*., 2005), its absence might result in a reduction in N uptake. The buildup of nitrates in S-deficient plants has been reported in certain studies (Salvagiotti *et al.*, 2009: Kopriva *et al*., 2009).

Protein content:

Protein content of black cumin was significantly affected by nitrogen and sulphur levels as well as their interaction during the study. Highest protein content was observed with nitrogen @ 40 kgha^{-1}, followed by nitrogen @ 60 kgha^{-1}, whereas, lowest protein content was observed in control. Regarding sulphur application, highest protein content was recorded with sulphur application @ 12 kgha^{-1}. Concerning the interaction, highest protein content was observed with optimum nitrogen application along with higher levels of sulphur. Statistically similar results regarding higher protein content was observed in 40kgha^{-1} and 9kgha^{-1} sulphur and 12kgha^{-1} sulphur. Increasing N and S levels increased the protein content positively (figure 9). The increase in protein content under influence of S addition seems to be due to increased S content in seed, which has a significant role in overall biosynthesis of proteins. Findings of Singh and Meena (2003) in mustard also provided support to findings of the present investigation. Under 80 kg N ha^{-1}, the low protein content was due to more N availability, which increased proteins substances in the seed. Under high N supply, a large portion of photosynthesis may have been diverted to protein formation, leaving a potential deficiency of carbohydrates, which would be degraded to 'acetyl co-enzyme A' for fatty acid synthesis. Singh and Meena (2003) reported similar findings.

Conclusion and Recommendation:

From this study, it can be concluded that application of nitrogen at the rate of 40 kg ha^{-1} along with sulphur at the rate of 12kg ha^{-1} produced higher seed yield, more protein content in seeds under agro climatic conditions of Haripur. Hence it is recommended to apply nitrogen at the rate of 40kgha^{-1} along with sulphur at the rate of 12kg ha^{-1} to black cumin crop.

Figure 1. Nitrogen and sulphur levels effect on plant height (cm) of black cumin

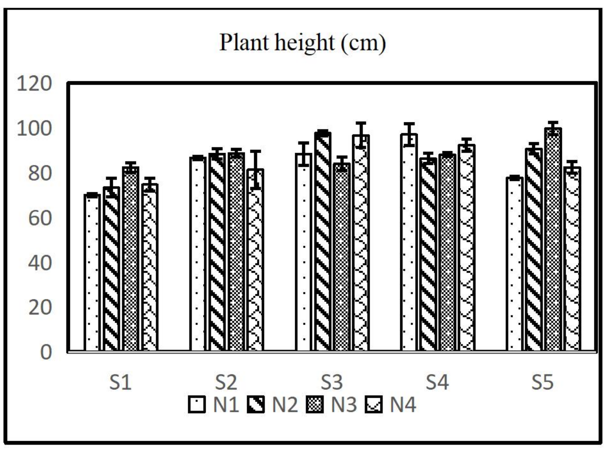


Figure 2. Nitrogen and sulphur levels effect on days to number of branches $plant^{-1}$ of black cumin

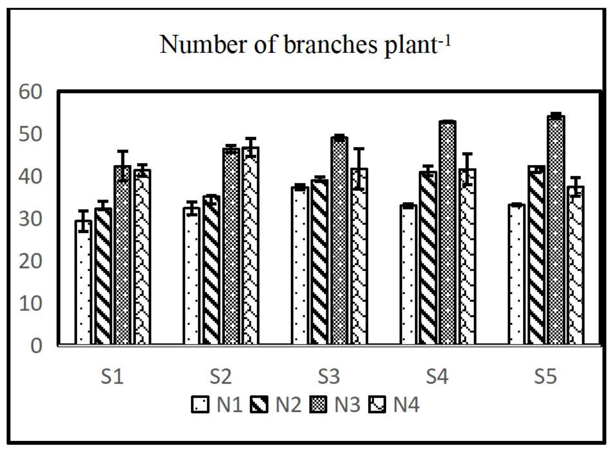
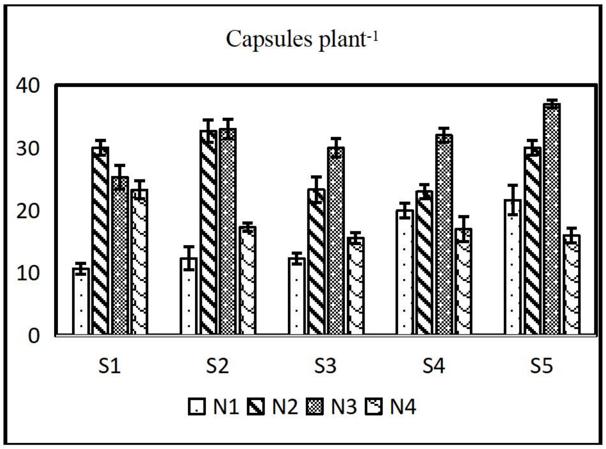


Figure 3. Nitrogen and sulphur levels effect on days to number of capsules $plant^{-1}$ of black cumin

Figure 4. Nitrogen and sulphur levels effect on number of seeds $capsules^{-1}$ of black cumin



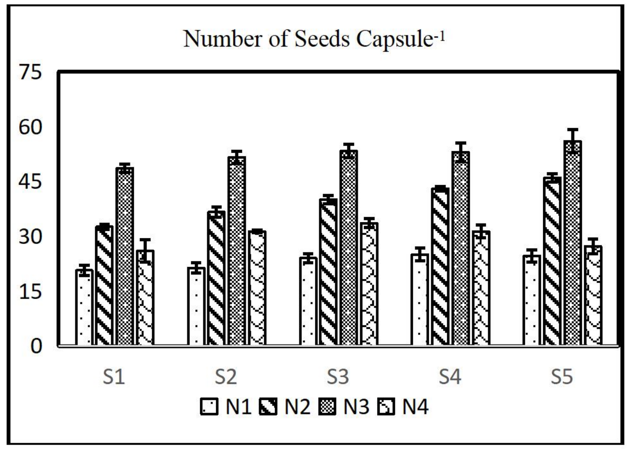
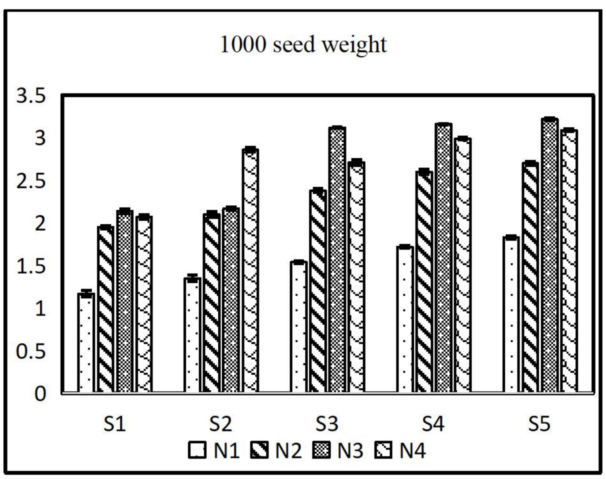


Figure 5. Nitrogen and sulphur levels effect on 1000 seed weight (g) of black cumin

Figure 6: Nitrogen and sulphur levels effect on biological yield of black cumin



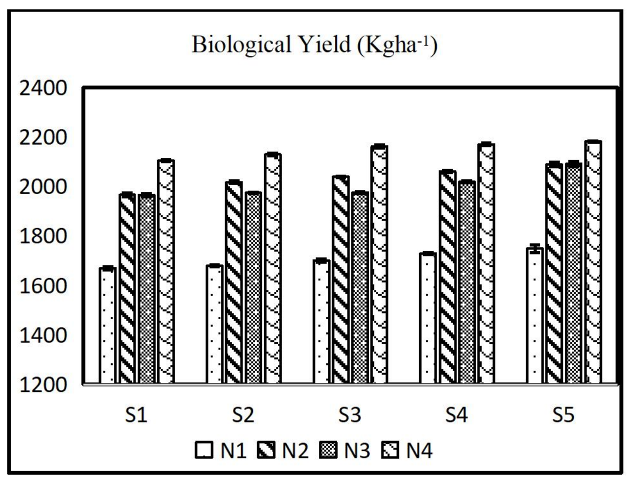
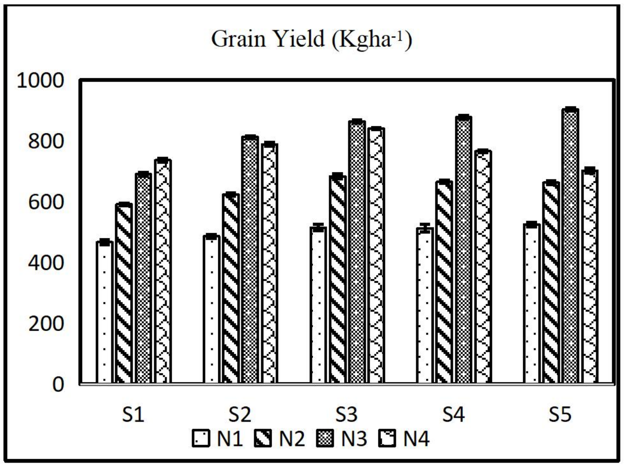


Figure 7. Nitrogen and sulphur levels effect on grain yield (Kg ha^{-1}) of black cumin

Figure 8. Nitrogen and sulphur levels effect on nitrogen use efficiency of black cumin



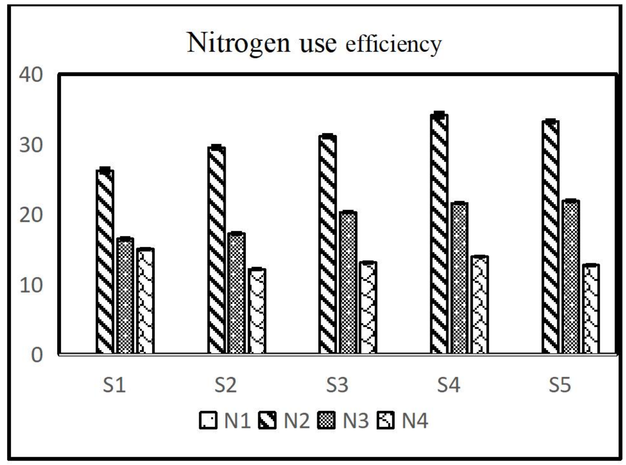
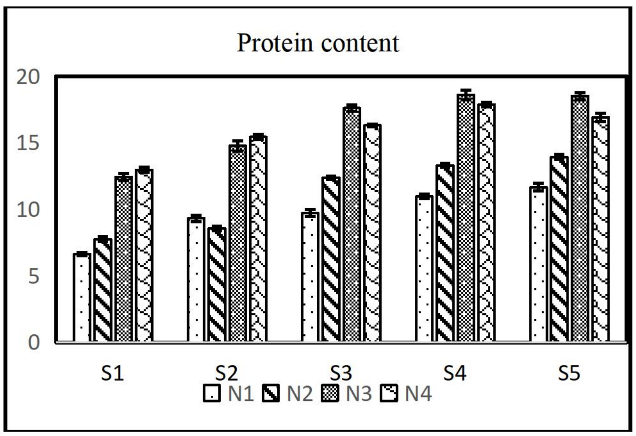


Figure 9. Nitrogen and sulphur levels effect on protein content of black cumin



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