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Variation in yield components and bioactive contents of black cumin seeds (*Nigella sativa* L.) with application of animal manures in ultisol lowland of Bengkulu

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ABSTRACT

Black cumin (*Nigella sativa* L) is known as herbal medicine that grows in subtropical regions and spreads from Asia to Europe. Plant parts that are used are seeds that contain bioactive compounds such as thymoquinone and thymol. Information about the growth and production from Indonesia is still limited. Therefore it is necessary to develop efforts to cultivate black cumin in Indonesia, especially in sub-optimal land as initial information in the field of plant adaptation studies. This study aimed to determine the effect of manure on yield components and bioactive content of black cumin in the ultisol lowland of Bengkulu. The study was conducted at Dehasen Bengkulu University research station at an altitude below 100 m above sea level. The accessions of India, Syria, and Kuwait were used at the main plot, whereas media for growing viz cow manure, chicken manure, and without manure as a subplot. The study was conducted in February 2019 using the Split Plot Design with three replications. Observations were made on the components of yields and bioactive levels of seeds. The results showed that the application of chicken manure increased the components of the yield of black cumin seed by parameter number of capsules per plant, number of seeds per capsule, and number of seeds per plant and tended to reduce the weight of 1000 seeds. An increasing level of thymoquinone in the cultivation of black cumin in ultisol lowland of Bengkulu was obtained in the cow manure application. The increased levels of seed thymoquinone were accompanied by decreased levels of thymol for all accession used.

INTRODUCTION

Black cumin (*Nigella sativa* L.) is an annual aromatic plant with medicinal properties and has been widely used in various countries such as India, Syria, and Kuwait (Hamid, 2012). The seeds are a source of potassium, calcium, sodium, and other substances that play a role in health (Abas-Ali et al., 2012). This plant is cultivated in the Mediterranean region and develops in various regions, including India and Pakistan (Abdolrahimi, Mehdikhani, Hasanzadeh-Gorttappe, 2012). In Pakistan, this plant grows with a plant height of 74.6-90.5 cm, seed production per plant 8.75 - 9.22 g and the weight of 1000 seeds 2.23-2.80 g (Rabbani, Ghafoor, & Masood, 2011). Plant capsules are relatively large, have 3-7 follicles, each capsule containing many seeds. Average seed lengths range from 1-5 mm, black or dark gray with a rather rough surface, the inside of the seeds are white and oily (Benkaci-Ali, Baliouamer, Meklati, & Chemat, 2007).

Most of the biochemical and medicinal properties of this plant are caused by the content of thymoquinone in its seeds and other compounds such as thymohydroquinone, dithymoquinon, and thymol. Thymoquinone is a bioactive compound from the terpenoid group, one of the most abundant compounds found in essential oils of black cumin seeds (Lewinsohn et al., 2012). Thymol is a compound synthesized by several accessions as an alternative pathway to carvacrol or as an additional pathway for the mechanism of thymoquinone biosynthesis (Wajs, Bonikowski, & Kalemba, 2008). Black cumin has been used as a cure for various diseases associated with respiratory and digestive disorders, kidney and liver function, immunity and circulation of blood flow, rheumatism and diseases associated with swelling (Malhatora, 2006).

In its origin country, black cumin grows in the highlands, low-temperature range below 20 ^{o}C, with low rainfall and alkaline soils. Black cumin grows in Jordan at 530-880 m above sea level (m asl) with an average temperature of 6.9-17.4 ^{o}C and rainfall 319.2-462.5 mm per year (Talafih, Haddad, Hatar, & Kharallah, 2007). In Turkey black cumin grows at the texture of high clay soils having, low salt content, low organic matter, low nitrogen, and phosphate content, high pH (7.8), low rainfall (349.4-424.1 mm per year) and low temperatures (between 9-10 ^{o}C) (Tuncturk, Tuncturk, & Ciftci, 2012). Black cumin grows in Iran at 1,209 m asl with an average temperature of 14 ^{o}C and rainfall of 140 mm per year (Khoulenjani & Salamati, 2011).

In Indonesia, black cumin research revealed that it could grow in the highlands of Indonesia (1,315 m asl) with a temperature range of 15.48-26.26 ^{o}C. Their research concluded that black cumin produced 363.05 kg per ha of seeds and 625 mg per kg of thymoquinone levels'. Whereas in the middle and lowlands (550 and 350 m asl) at temperature range 22.47-29.83 ^{o}C and 22.73-31.73 ^{o}C, black cumin cannot grow and not even germinate (Ridwan, Ghulamadi, & Kurniawati, 2014). Beside, Herlina, Aziz, Kurniawati, and Faridah (2017) informed that black cumin accessions of India and Kuwait could grow and produce in the lowlands (220 meters above sea level).

Data from Badan Pusat Statistik (2013) showed that black cumin imports in 2013 of the US $ 244,076. As an effort to reduce imports, it is necessary to develop black cumin cultivation in Indonesia. One of which is utilizing sub-optimal land in the lowlands, including ultisol. Ultisol land is one of acid mineral land with an area of up to 45,794,000 ha or about 25% of Indonesia's total land area (Prasetyo & Suriadikarta, 2006). To develop black cumin cultivation technology in ultisol land, it is necessary to conduct the study on the adaptation mechanism of several black cumin accessions as a basis for developing black cumin cultivation technology in ultisol lowland of Bengkulu.

Ultisol is acidic land with low organic matter content. Efforts are needed to improve nutrient availability and soil quality. Manure is a product that contains many elements. It improves soil fertility and increases the availability of nutrients and consequently increasing growth, yield, and chemical composition of the crop (El-Sharkawy & Abdel-Razzak 2010). Animal manure application improves the soil structure and soil moisture content, provides the plant with essential nutrients, increases growth, number of umbrella per plant and biological yield, and finally led to increasing seed yield (Ahmadian, Tavassoli, & Amiri, 2011). This research aimed to obtain effect of animal manures in ultisol lowland of Bengkulu. This research was also carried out to determine the performances of yield components, thymoquinone, and thymol levels of black cumin'

MATERIAL AND METHODS

Plant material and experimental conditions

Seeds as plant material were harvested from the experiment conducted from February to June 2019 at Dehasen Bengkulu University research station (below 100 m asl). This experiment used the Split Plot Design with three levels of accession (Syria, India, and Kuwait) as the main plot and three levels of manure application (cow manure, chicken manure, and without manure) subplot with three replications. The black cumin seeds from the country of origin, i.e., Syria, India, and Kuwait were planted in polybags (a diameter of 20 cm and a height of 20 cm) filled with the mixture of soil and manure according to treatment (1:1) (v/v) and agricultural lime (CaCO3) 2 ton/ha as the plant growth media. Seeds were hydro primed for 12 hours and continued with GA3 10-5 M primed for 1 hour before planting. After the draining process, three seeds were planted in a polybag. An "ultraviolet" plastics were used as shading for covering the plants from the sun hit. Urea and SP-36 in dose given at 120 kg N/ha and 157 kg P_2O_5 per ha at five weeks after planting.

The parameters recorded were yield component consisting of the number of capsules per plant, number of seeds per capsule, number of seeds per plant, the weight of 1000 seeds, thymoquinone, and thymol content. Content of thymoquinone and thymol content was analyzed at the Laboratory of Biochemical and Laboratory of Chemical, Department of Food and Technology, Faculty of Agriculture Technology, Bogor Agricultural University, Indonesia. Supporting data collected from sites location were maximum, minimum, daily air temperature, and relative humidity using thermohygrometer. The reference standard of thymoquinone and thymol was purchased from Sigma Chemical Company (Sigma, MO, USA). All the solvents and chemicals used for extraction and analysis were of high purity and quality.

Measurement of thymoquinone and thymol content

Measurement was done following the standard protocol described by Al-Saleh, Billedo, and El-Doush (2006). Grounded black cumin seed sample of 0.01 g was extracted with 1 mL methanol, vortexed for 1 min, and sonicated for 20 min. After that, it was left overnight in constant rotamix, vortexed for 1 min and centrifuged for 25 min at 1400 rpm. The supernatant was aspirated, and an aliquot of 20 mL was injected into high-performance liquid chromatography with UV detector at a wavelength of 275 nm. The mobile phase used was methanol: water (75:25) at 1.0 mL/min flow rate. Calibration curves of peak area versus the concentration 20, 40, 80, and 160 mg/mL for thymoquinone and 2, 4, 8, and 16 mg/mL for thymol were constructed.

Statistical analysis

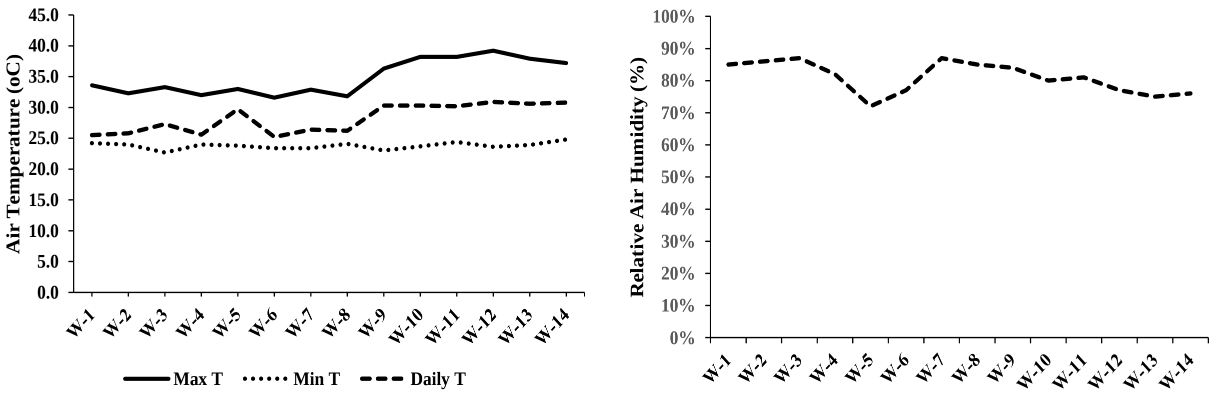
Results were analyzed by one-way analysis of variance to identify significant differences between the groups, and compare means test analyzed by the Duncan Multiple Range Test at 5% level.

RESULTS AND DISCUSSION

Differences in air temperature (maximum, minimum, and daily) environments produce various responses in crop growth and production, represented by different morphological, physiological, and biochemical responses. According to Sakata and Higashitani (2008), plant sensitivity and tolerance to the environment conditions depend on the character of each species and the genotype of the plant. Air temperature in the experimental plot of ultisol lowland of Bengkulu during the planting in February to June 2019 relatively fluctuated, especially the daily temperature in the range of 25.2 to 32.0ºC. The minimum temperature range from 22.7 to 25.5ºC, the maximum temperature range from 31.6ºC to 39.4ºC, and the air humidity range from 65% to 89% (Figure 1).

Plants have complex reactions in the face of growing environmental stress. When stress is increasing, plants make adjustments through physiological and biochemical processes through changes in the structure of complex proteins in chloroplasts and decreasing enzyme activity (Ahmad, Diwan, & Abrol, 2010), then followed by changes in plant architecture and morphology (Hua 2009), a short growth period (Barnabás, Jäger, Feher 2008). Flowering and reproduction periods are the most sensitive to stress, which can directly impact crop productivity (Thakur, Kumar, Malik, Berger, & Nayyar, 2010).

The growing environment of black cumin plants in ultisol lowland of Bengkulu has a temperature (daily temperature more than 25 oC) higher than the temperature of origin land which is below 20oC, and also higher than the temperature in the lowlands research location conducted by Herlina et al. (2017).



Remarks: W-1 = First week, W-2 = Second week, etc

Figure 1. Maximum, Minimum, Daily Air Temperature and Relative Air Humidity Fluctuations of black cumin Growing Environment in Ultisol Lowland of Bengkulu

Table 1. The Effect of Manure on the Number of Capsules per Plant and Seeds per Capsule

Kinds of Manure	Number of Capsules per Plant			Number of Seeds per Capsule		
	India	Syria	Kuwait	India	Syria	Kuwait
Cow Manure	5,8 a	5,1 a	4,7 a	28,3 a	34,3 a	22,7 a
Chicken Manure	6,3 a	5,6 a	5,1 a	38,7 a	33,8 a	29,3 a
Without Manure (Control)	4,2 b	3,9 b	4,4 a	30,9 a	12,9 b	26,4 a

Means in the same column followed by the same letters are not significantly different at 5% by DMRT.

Table 2. The Effect of Manure on the Number of Seeds per Plant and Weight of 1000 Seeds

Kinds of Manure	Number of Seeds per Plant			Weight of 1000 Seeds (g)		
	India	Syria	Kuwait	India	Syria	Kuwait
Cow Manure	163,7 ab	176,6 a	108,5 a	1,5 b	1,6 b	1,8 a
Chicken Manure	246,9 a	188,3 a	148,7 a	1,5 b	1,6 b	1,8 a
Without Manure (Control)	134,6 b	49,6 b	117,2 a	1,7 a	1,8 a	1,8 a

Means in the same column followed by the same letters are not significantly different at 5% by DMRT.

The analysis of manure used in this study gave results of 0.63% N, 0.20% P, 0.37% K, 12.68% C for chicken manure, and 0.98% N, 0.16% P, 0.43% K, 14.62% C for cow manure. The application of manure given has significant influence ($P < 5\%$) on the yield components of black cumin. All yield components consisting of many capsules per plant, number of seeds per capsule, number of seeds per plant, and weight of 1000 of Syrian accession were significantly affected ($P < 5\%$) by the application of manure, but not for the number of seeds per capsule and number of seeds per plant of Indian accessions. However, for Kuwait's accession, the role of manure application did not significantly affect ($P > 5\%$) for all observed yield components. The role of manure application has increased the highest yield component in the number of capsules per plant of Indian accessions by 50.0% compared to without manure application. The variable number of seeds per plant of Syrian accession by 279% compared to without manure application. Meanwhile, the manure application reduced the weight of 1000 Indian accession seeds by 10.6% and Syrian accession by 6.5% (Tables 1 and 2).

The effect of the interaction of manure application and black cumin accession is presented in Table 3. Data in Table 3 showed that the application of chicken manure on Indian accessions gave the highest value on all parameters of the observed production components, except the weight of 1000 grains. The application of chicken manure increases the number of capsules Indian accession by 9.5% compared to cow manure, and by 50% compared to control. Herlina et al. (2017) informed that Indian accessions planted in the lowlands (220 m asl) produced 10-12 capsules per plant, 30-43 seeds per capsule, 296 - 543 seeds per plant, and 1.6-2.2 g weights 1000 seeds. Kuwait accession produced an average of 12 - 13 capsules per plant, 44 – 46 seeds per capsule, 535 – 560 seeds per plant, and 1.5 - 2.8 g weights 1000 seeds. Compared with the research of Herlina et al. (2017), all yield components observed in this study still gave low results. This condition is thought to be related to the growing environmental conditions of research, which have higher average daily temperatures.

Table 3. The Effect of Manure and Accession on Yield Components

The Kinds of Manure	Accession	Number of Capsule per Plant	Number of Seeds per capsule	Number of Seeds per Plant	Weight of 1000 seeds (g)
Cow Manure	India	5,8 b	28,3 bc	163,7 bc	1,49 f
	Syria	5,1 cd	34,3 ab	176,6 b	1,55 de
	Kuwait	4,7 de	22,7 c	108,5 de	1,81 ab
Chicken Manure	India	6,3 a	38,7 a	246,9 a	1,52 ef
	Syria	5,6 bc	33,8 b	188,3 b	1,58 d
	Kuwait	5,1 cd	29,3 bc	148,7 c	1,79 b
Without Manure (Control)	India	4,2 ef	30,9 abc	134,6 d	1,70 c
	Syria	3,9 f	12,9 d	49,6 f	1,69 c
	Kuwait	4,4 e	26,4 bc	117,2 cd	1,84 a

Means in the same column followed by the same letters are not significantly different at 5% by DMRT.

The results of this study showed that all yield components observed provide application manure better than control, except for the parameter weight of 1000 seeds. Adimihardja, Juarsah, and Kurnia (2000) informed that the application of cow, goat, and chicken manure in ultisol soils significantly increases C-organic of soil and production of soybean and corn. In this study, application both manures increased the production of black cumin seeds. The same results were delivered by Hadi, Ghanepasand, Noormohamadi, and Darzi (2014), which informed that manure application successfully manipulates the growth of black cumin, resulting in beneficial changes in yield and yield components. This result was related to manure's character in improving the physical, biological, and chemical characteristics of the soil. Furthermore, the improvement of soil quality has a positive effect on plant growth and production. Also, Raihan (2000) stated that manure could function as a supplier of soil nutrients and increase water retention. Increased groundwater availability will accelerate the decomposition of organic matter, which will produce organic acids and ultimately increase the availability of several types of nutrients.

Although the data obtained are not consistent, the results of this study inform that all yield components observed provide the application of chicken manure relatively better than cow manure application. The application of chicken manure has an advantage in the speed of nutrient supply and improving soil structure. Other than that, Suastika, Sutriadi, and Kasno (2005) informed that the application of chicken manure on oxisols soil increases corn production compared to the application of cow manure. The positive effect of chicken manure would create favorable soil physical and chemical conditions, which favorably affect the solubility and availability of nutrients and increase the nutrient uptake. Some nutrients are essential for plant growth and development involved in photosynthesis functions, DNA synthesis, protein formation, and respiration (Diacono, Rubino, & Montemurro, 2013).

This study also inferred that manure application also plays a role in reducing soil stress levels. According to Wahid, Gelani, Ashraf, &

Foolad (2007), environmental stress conditions allow a decrease in sink and source activity, causing a decrease in the quality of plant growth. This causes an increase in the efficiency of source distribution from leaves, stems, and other plant parts as a potential strategy to improve seed filling under stress conditions. Data in this study showed that the parameter weight of 1000 seeds for each accession on control treatment is higher than manure application treatment. This is thought to be related to the efforts of the plants in responding to the higher environmental stress in the treatment without the application of manure and proves that plants in the control treatment have higher stress levels. The quality of plant growth, especially on yield components in the treatment without manure, was lower, but the seed size increased (Table 3).

Plants have very complex reactions in the face of a different growing environment. As an introduction crop, black cumin, which has been planted in ultisol lowland of Bengkulu, will face very different growing environmental conditions. When it grows, the plant adapts through physiological and biochemical processes with changes in the structure of complex proteins in chloroplasts and decreases enzyme activity (Ahmad et al., 2010). The level and production of bioactive compounds produced through biochemical processes will also be directly affected by the growing environment.

Tabel 4. The Effect of Manure on Thymoquinone (THQ) and Thymol (THY) Content

The Kinds of Manure	THQ Content (µg/g seed)			THY Content (µg/g seed)		
	India	Syria	Kuwait	India	Syria	Kuwait
Cow Manure	634,07 a	342,44 a	362,15 a	19,87 b	19,72 b	24,72 a
Chicken Manure	200,77 b	168,19 b	185,60 b	38,64 a	25,40 b	32,68 a
Without Manure (Control)	178,14 b	173,00 b	187,23 b	46,53 a	34,27 a	30,07 a

Means in the same column followed by the same letters are not significantly different at 5% by DMRT.

Tabel 5. The Effect of Manure and Accession on Thymoquinone (THQ) and Thymol (THY) Content

The Kinds of Manure	Accession	THQ Content (µg/g seed)	THY Content (µg/g seed)
Cow Manure	India	634,07 a	19,87 f
	Syria	342,44 b	19,72 f
	Kuwait	362,15 b	24,72 ef
Chicken Manure	India	200,77 c	38,64 b
	Syria	168,19 c	25,40 de
	Kuwait	185,60 c	32,68 c
Without Manure (Control)	India	178,14 c	46,53 a
	Syria	173,00 c	34,27 bc
	Kuwait	187,23 c	30,07 cd

Means in the same column followed by the same letters are not significantly different at 5% by DMRT.

black cumin seeds contain very diverse bioactive ingredients, and the main ones are thymoquinone (THQ; 2-isopropyl-5-methyl-1.4-benzoquinone), which functions as antioxidants, and thymol (THY; 2-isopropyl-5 -methyl phenol) which also functions as an antioxidant (Hadad & Salam, 2012). The results showed that the application of cow manure increased the level of thymoquinone. Meanwhile, the chicken manure application did not affect the thymoquinone levels of all accession used in this study (Table 4). According to Hidayati, Benito, Kurnani, Marlina, and Harlia (2011), the available phosphorus content is related to the substrate's nitrogen content. Higher nitrogen levels caused an increase in the activity of phosphorus decomposing microorganisms. The higher nitrogen content of cow manure used in this study was considered to influence the availability of phosphorus compounds needed for the formation of thymoquinone black cumin seeds. All of the thymoquinone produced in this study have very low values compared to the research of Herlina et al. (2017), especially for India and Kuwait accessions planted in the lowlands with a range of thymoquinone content is 1,600-2,500 µg per g seeds.

The data in Table 4 shows that all accessions used in this study produced thymol compounds in an amount that was relatively close to the research results of Herlina et al. (2017). At that research, thymol levels for India and Kuwait's accession in the lowlands in the range 16.18 - 50.81 µg per g seeds. This study's results showed a tendency that increased levels of thymoquinone would reduce the thymol level of each accession. The highest level of thymol in each accession was produced in the control treatment, although statistically, it was not significantly different from the chicken manure treatment.

The interaction of the application of manure and accession treatments gave the best results of thymoquinone levels for the combination of cow manure applications in Indian accessions with a value of 85.16% higher than Syrian accessions, and 75.08% compared to Kuwait accessions. Meanwhile, the highest value of the thymol content was produced in a combination of control treatments with Indian accessions (Table 5). An increase in thymoquinone levels is followed by a decrease in thymol levels in this study is thought to be related to the biosynthetic pathway of the two metabolite compounds. Thymoquinone and thymol are secondary metabolite products formed from geranyl diphosphate, which are connected to y-terpinene and then aromatized into p-cymene and followed by carvacrol hydroxylation and thymo-hydroquinone, thymoquinone oxidation, while thymol hydroxylation as an alternative biosynthetic pathway (Botnick et al. 2012). Wajs et al. (2008) stated that thymol was a compound synthesized by several accessions as an alternative pathway to carvacrol.

CONCLUSION AND RECOMMENDATIONS

The application of chicken manure increases the production component of black cumin seeds through the parameter number of capsules per plant, number of seeds per capsule, and number of seeds per plant and tends to reduce the weight of 1000 seeds. Increasing the level of thymoquinone in black cumin cultivation in Bengkulu's acid mineral land can be done by adding cow manure. Increased seed thymoquinone levels were accompanied by decreased levels of thymol, which is thought to be related to the biosynthetic pathway of the two metabolite compounds' with the type of manures or genetic traits of accessions. It is also suspected that a form of the mechanism of plant adaptation in response to environmental stress. It is suggested to experiment with the same technology package on middle and high altitudes in Bengkulu's acid mineral land for further research.

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