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Influence of elicitors in enhancing the fixed oil content and yield of black cumin (*Nigella Sativa* L.)

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Abstract
Black cumin seeds contained substantial amount of fixed oil and it is used for different cases of medicines and food. The use of elicitors may be one of the best possible ways to achieve spectacular progress in increasing fixed oil content and yield in black cumin. Elicitors are known to trigger the synthesis of various phytochemical compounds in many horticulture crops including black cumin. An investigation was carried out with foliar spraying of different elicitors to know their role in altering the fixed oil content and yield in black cumin during *Rabi* seasons of 2018-19 and 2019-20. The results of the study inferred that, application of paclobutrazol at 50 ppm recorded the maximum fixed oil content of 38.74 %, followed by chitosan at 100 ppm (37.75 %) when the data was averaged over two years of experimentation. Whereas, the application of salicylic acid at 50 ppm resulted in maximum fixed oil yield of 471.07 kg per hectare because of higher seed yield, although the fixed oil content was comparatively low than plants treated with paclobutrazol at 50 ppm having highest fixed oil content.

Keywords: Black cumin (*Nigella sativa* L.), fixed oil, elicitors, paclobutrazol, salicylic acid

Introduction

India is admired as “Home of Spices” and is the largest producer, consumer and exporter of spices. Among the 109 spices listed by the International Organization for Standardization (ISO), India produces 63 spices. According to National Research Centre on Seed Spices (NRCSS) out of 63 spices, 20 of them are seed spices. Seed spices are annual herbs, whose dried seeds or fruits are used as spice. Seed spices are the important export oriented commodities which play a significant role in our national economy. They are primarily used for flavouring, seasoning and imparting aroma to variety of food items and beverages. Besides their importance in food industry, the seed spices have medicinal properties and thus, used by various pharmaceutical and cosmetic industries. Seed spices are high value, low volume crops and most remunerative commodities of arid and semi-arid regions of India. Coriander, Fenugreek, Fennel and Cumin are the major seed spices grown in India, apart from which, NRCSS has identified seven new crops *viz*., Black cumin (*Nigella sativa*), Black Mustard (*Brassica nigra*), Poppy seeds (*Papaver somniferum*), Sesame (*Sesamum indicum*), Parsley (*Petroselinum crispum*), Black caraway (*Carum bulbocastanum*) and Sweet fennel/European fennel *(Foeniculum vulgare* Miller ssp. *Capilaceum* var*dulce*) as future seed spices.

Black cumin (*Nigella sativa* L.) is an annual spicy herb, belongs to the *Ranunculaceae* family and native to Southern Europe, North Africa and Southwest Asia. It is commonly called as *Kalonji* in Hindi and black cumin, black seed, fennel flower in English and *Krishna jeerige* in Kannada (Datta *et al.*, 2012) [2]. It is a cool season crop, grows best at temperature ranging from 15 ^{0}C – 25 ^{0}C and can tolerate 5 ^{0}C – 30 ^{0}C with an annual rainfall of 400-500 mm (Kant *et al*., 2010) [8]. It is a good source of nutritionally essential components. The seeds have been used as medicine by various cultures and civilizations to treat and prevent a number of diseases. It has been used since thousands of years as a spice and food preservative as well as a protective and curative remedy for several disorders. Its great value as medicine is mentioned in various religious and ancient literatures which dates back to more than 2000 years. It is an important drug in the traditional Indian system of medicine like *Unani* and *Ayurveda*.

Like most herbs, the composition of black cumin varies with geographic distribution, time of harvest and agronomic practices. The dried seeds of black cumin are the commercial products used in food. Seeds contain 0.40 to 0.50 % essential oil, which has great demand in the pharmaceutical, food and perfumery industry (Datta *et al.*, 2012) [2]. Scientific investigations have depicted its composition of oils, proteins, carbohydrates, fibers, ashes, moisturizers *etc*.

The seed also contains good amount of vitamins and minerals like Cu, P, Zn and Fe. The fixed oil (36-38 %) contains linoleic (50-60%), oleic (20-23.4%), palmitic (12.5%), dihomolinoleic (10%) and eicosadienoic (3%) acids as well as arachidonic, stearic and myristic acid, betasitosterol, cycloeucalenol, cycloartenol, sterol esters and sterol glucosides along with other minor lipid constituents such as methylnonadeca-15, 17-dienoate, pentylhexadec-12-enoate, and pentylpentadec-11-enoate. Its health enhancing potential has been attributed to the active ingredients that are mainly concentrated in fixed or essential oil (Ramadan, 2007) [9]. Black cumin fixed oil is lipid fraction containing fatty acids, fat-soluble vitamins and small amounts of volatile constituents, whereas its essential oil comprises of only volatiles (Gholamnezhad *et al.*, 2016) [6]. With this brief background, an investigation was carried out with an objective to know the role of elicitors in altering the fixed oil content and yield in black cumin.

Material and methods

The study was conducted during two *rabi* seasons from November 2018 to March 2019 and October 2019 to February 2020 at Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Horticulture, University of Horticultural Sciences campus, Gandhi Krishi Vignana Kendra post, Bengaluru. The experimental field is located at an altitude of 930 m above MSL at $12^0 58^1$ North Latitude and $77^0 35^1$ East Longitude lying in the Eastern Dry Zone (zone-5) of Karnataka. The experiment was laid out in a randomized complete block design with 12 treatments and 3 replications and the seeds of black cumin variety Ajmer Nigella - 1 were procured from National research center on seed spices, Tabiji, Ajmer, Rajasthan and sown on 26^{th} November, 2018 during first season and 20^{th} October, 2019 during second season. The plots were watered immediately after sowing at alternate days till germination, thereafter the irrigation was given through drip irrigation system with 16 mm inline laterals with drippers of discharge 2 liters per hour spaced at 30 cm apart and laid in alternate rows.

Thinning was done at 30 days after sowing. The plots were kept free from weeds by regular hand weeding at three intervals *i.e.*, 30, 45 and 60 days after sowing. Elicitors were sprayed at 50 days after sowing *viz*., Salicylic acid - 50 ppm, Chitosan - 100 ppm, dry yeast - 5g/l, Potassium silicate - 200 ppm, NAA - 25 ppm, Kinetin - 25 ppm, Humic acid - 500 ppm, PGPR - 5000 ppm, Ancymidol - 50 ppm and Paclobutrazol - 50 ppm as foliar spray and pinching was done at 50 days after sowing, since the elicitors were sprayed at 50 DAS. Five plants in each treatment and in each replication were selected randomly and tagged for recording observations for plant characters and yield attributes. Damping off and collar rot diseases were observed at 30 and 40 DAS. Control measures are taken by drenching Capton at 1.5 g/l and Copper oxy chloride at 3 g/l at 30 and 40 DAS respectively. Harvesting was started based on the maturity of capsules, and the plants were cut back to the ground level by using secateurs and were tied into small bundles and stacked in threshing yard for drying with frequent turnings till moisture level was reduced.

Estimation of fixed oil content (%)

The estimation of fixed oil content was done at biofuel laboratory, Department of Forestry & Environmental Sciences, University of Agricultural Sciences, GKVK, Bangalore by using soxhlet apparatus. Petroleum ether was used as a solvent. Twenty grams of black cumin seeds were ground and powder was placed in thimbles. Thimbles were fitted to a jar, so that, it should come in contact with the solvent and placed in soxhlet extractor. About 110 ml of solvent was taken in each flask. The temperature was maintained at 150°C. About 3h 25 min was required to extract the oil from the powder and solvent was recovered for further extraction process. Then, the extracted sample was kept in oven to evaporate the solvent for 2 hour at 110°C and placed in desiccators to remove the moisture if any. The weight of sample was recorded and calculated by using the following formula,

$$\text{Fixed oil content (\%)} = \frac{W_2 - W_1}{W} \text{ X } 100$$

Where, W_1= Initial weight of the jar (g), W_2= Final weight of the jar (g),

W = Weight of the sample (g).

The fixed oil estimation was done using replicated pooled samples and hence the data was not subjected to statistical analysis. Keeping the fixed oil content as constant, the fixed oil yield was calculated by using the following formula

$$\text{Fixed oil yield (kg ha}^{-1}\text{)} = \frac{\text{Fixed oil content (\%) x seed yield (kg ha}^{-1}\text{)}}{100}$$

Statistical Analysis

The data collected during the experimentation was analysed by randomized completely block design (RCBD). Statistical analysis was performed using Web Agri Stat Package (WASP) Version 2 (Jangam and Thali, 2010) [7]. The level of Significance used in 'F' was p=0.05. Critical difference values were calculated whenever F-test was found significant.

Results and Discussion

Fixed oil content (%)

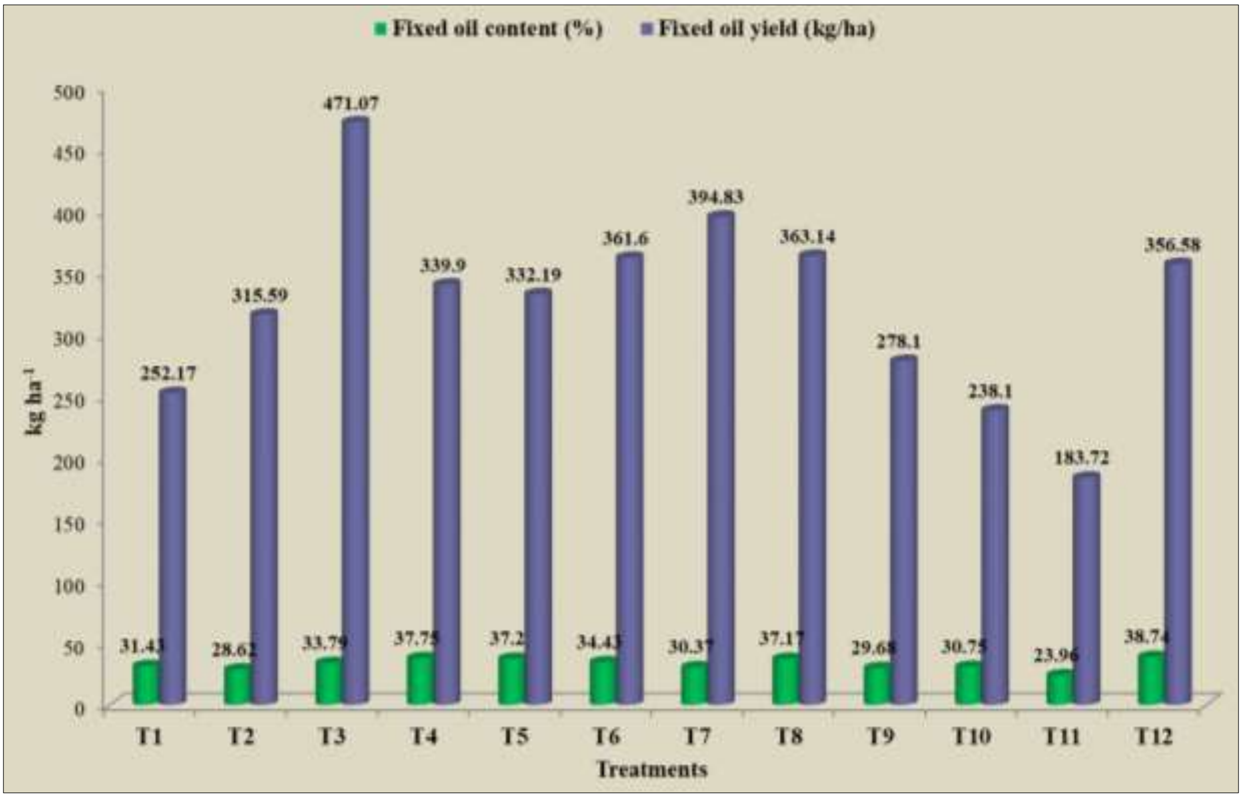
Black cumin seeds contained substantial amount of fixed oil. The data on the fixed oil content of black cumin seeds as influenced by Elicitors application are presented in Table 1 and Figure 1.

Application of elicitors caused for variation in fixed oil content of black cumin seeds and showed positive impact on the said parameter. During 2018-19, 2019-20 and in the pooled data of two years, the seeds obtained from plants applied with paclobutrazol at 50 ppm recorded the maximum fixed oil content (36.12, 41.36 and 38.74 % respectively), followed by chitosan at 100 ppm (35.80, 39.71 and 37.75 % respectively), dry yeast 5 g/l (35.00, 39.41 and 37.20 % respectively) and kinetin at 25 ppm (34.93, 39.41 and 37.17 % respectively). However, the minimum fixed oil content (21.85, 26.08 and 23.96 % respectively) was registered in seeds obtained from plants applied with ancymidol at 50 ppm, followed by plants pinched at 50 days after sowing (24.86, 32.38 and 28.62 % respectively) and humic acid at 500 ppm (26.22, 33.14 and 29.68 % respectively) during 2018-19, 2019-20 and in the pooled data of two years. The increase in fixed oil content may be due to role of these elicitors in activating acetyl-CoA, which is responsible for the synthesis of fatty oils in plastids and elicitors might also have helped in increasing the triacylglycerols (TAG's), which are essentially base of all fatty oils (Sidorov and

Table 1: Influence of elicitors on fixed oil content and yield in black cumin (*Nigella sativa* L.)

Treatments	Fixed oil content (%)			Fixed oil yield (kg ha^{-1})		
	2018-19	2019-20	Pooled	2018-19	2019-20	Pooled
T_1 – Control	27.20	35.67	31.43	261.30^{cde}	243.03^{cd}	252.17^{def}
T_2 - Pinching at 50 days after sowing	24.86	32.38	28.62	284.07^{bcde}	347.11^{abc}	315.59^{bcde}
T_3 - Salicylic acid 50 ppm	32.13	35.45	33.79	473.49^{a}	468.65^{a}	471.07^{a}
T_4 - Chitosan 100 ppm	35.80	39.71	37.75	355.37^{bc}	324.43^{abcd}	339.90^{bcde}
T_5 - Dry yeast 5000 ppm	35.00	39.41	37.20	336.23^{bcd}	328.15^{abc}	332.19^{bcde}
T_6 - Potassium silicate 200 ppm	33.53	35.33	34.43	349.05^{bcd}	374.14^{abc}	361.60^{bc}
T_7 - NAA 25 ppm	27.00	33.75	30.37	366.21^{b}	423.45^{ab}	394.83^{ab}
T_8 - Kinetin 25 ppm	34.93	39.41	37.17	350.58^{bc}	375.71^{abc}	363.14^{bc}
T_9 - Humic acid 500 ppm	26.22	33.14	29.68	256.17^{cde}	300.03^{bcd}	278.10^{cdef}
T_{10} - PGPR 5000 ppm	27.11	34.40	30.75	250.32^{de}	225.89^{cd}	238.10^{ef}
T_{11} - Ancymidol 50 ppm	21.85	26.08	23.96	199.49^{e}	167.96^{d}	183.72^{f}
T_{12} - Paclobutrazol 50 ppm	36.12	41.36	38.74	354.70^{bc}	358.45^{abc}	356.58^{bcd}
F - test	NA	NA	NA	**	**	**
S. Em ±	NA	NA	NA	33.89	54.54	36.67
CD at 5%	NA	NA	NA	99.40	159.97	107.55

****** Significant at 1% Means of the same category followed by different letters are significantly different as per DMRT
NA - Not analyzed (The fixed oil estimation was done using replicated pooled samples and hence the data was not subjected to statistical analysis)



T_1 - Untreated control	T_5 - Dry yeast 5000 ppm	T_9 - Humic acid 500 ppm
T_2 - Pinching at 50 DAS	T_6 - Potassium silicate 200 ppm	T_{10} - PGPR 5000 ppm
T_3 - Salicylic acid 50 ppm	T_7 - NAA 25 ppm	T_{11} - Ancymidol 50 ppm
T_4 - Chitosan 100 ppm	T_8 - Kinetin 25 ppm	T_{12} - Paclobutrazol 50 ppm

Fig 1: Influence of elicitors on fixed oil content and yield in black cumin (*Nigella sativa* L.) over two years of investigation

Tsydendambaev 2014) [10]. Supporting evidences in these aspects are available from the results of El-Gamal and Ahmed (2016) [4], who reported maximum fixed oil content with application of chitosan at 60 ppm in coriander. Likewise, Gendy *et al*. (2015) [5] in fenugreek and El-Din and Hendawy (2010) [3] in *Borago officinalis* also reported similar pattern of results which are in line with our findings.

Fixed oil yield (kg ha^{-1})

The data with respect to fixed oil yield in black cumin plants as affected by various elicitors is presented in Table 1 and Figure 1. The application of elicitors resulted in significant improvement in fixed oil yield during 2018-19, 2019-20 and when the data was averaged over two years. In the year 2018-19, significant differences in fixed oil yield were noted due to the application of various elicitors. Plants applied with salicylic acid at 50 ppm recorded maximum fixed oil yield (473.49 kg ha^{-1}) and significantly superior over all other treatments. Whereas, the minimum fixed oil yield (199.49 kg ha^{-1}) was obtained from the plants treated with ancymidol at 50 ppm, followed by PGPR at 5000 ppm (250.32 kg ha^{-1}), which were *on par* with each other during 2018-19. During

2019-20, the same trend prevailed, wherein, the application of salicylic acid at 50 ppm resulted in the highest fixed oil yield of 468.65 kg ha^{-1}) followed by NAA at 25 ppm (423.45 kg ha^{-1}), kinetin at 25 ppm (375.71kg ha^{-1}), potassium silicate at 200 ppm (374.14 kg ha^{-1}), paclobutrazol at 50 ppm (358.45 kg ha^{-1}), pinching at 50 DAS (347.11 kg ha^{-1}), dry yeast 5g/l (328.15 kg ha-1) and chitosan at 100 ppm (324.43 kg ha^{-1}) which were *on par* with each other. In contrast, the lowest fixed oil yield (167.96kg ha^{-1}) was recorded in the plants applied with ancymidol at 50 ppm (T_{11}), followed by T_{10} (225.89 kg ha^{-1}), T_1 (243.03 kg ha^{-1}) and T_9 (300.03 kg ha^{-1}), which were *on par* with each other during 2019-20.

The pooled data presented in Table 1 clearly reveals that, the impact of elicitors on fixed oil yield was significant with the application of salicylic acid at 50 ppm recording highest fixed oil yield of 471.07 kg ha^{-1}, which was significantly superior over all other treatments tried during the course of this investigation, except NAA at 25 ppm (394.83 kg ha^{-1}), which were *at par*. Application of ancymidol at 50 ppm (T_{11}) was inferior resulting in minimum fixed oil yield of 183.72 kg ha^{-1}. The maximum fixed oil yield from salicylic acid treated plants is purely due to maximum seed yield, although the fixed oil content was comparatively lower than plants treated with paclobutrazol, having highest fixed oil content. The increase in fixed oil yield is also due to elicitors, which play an important role in the synthesis of secondary metabolites, proteins and fatty acids. The results of the present study are directly in line with previous findings of Arpitha (2019) [1]. She reported that, the foliar application of salicylic acid at 100 ppm resulted in maximum fixed yield of 340 kg ha^{-1} because of higher fixed oil content with 33.57 per cent under Bangalore conditions.

Conclusion

From the above results it can be inferred that, application of elicitors caused for variation in fixed oil content of black cumin seeds and showed positive impact. The seeds obtained from plants applied with paclobutrazol at 50 ppm recorded the maximum fixed oil content, followed by chitosan at 100 ppm. But, the minimum fixed oil content was registered in seeds obtained from plants applied with ancymidol at 50 ppm during 2018-19, 2019-20 and in the pooled data of two years. However, the fixed oil yield varied significantly due to the application of elicitors. Salicylic acid sprayed at 50 ppm resulted in maximum fixed oil yield of 471.07 kg per hectare. While, lowest fixed oil yield was obtained from plants treated with ancymidol at 50 ppm (183.72 kg ha^{-1}) when the data was averaged over two years.

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References

1. Arpitha HS. Studies on varietal performance and effect of elicitors on growth, yield and quality of black cumin (*Nigella sativa* L.). M.Sc. (Hort.) Thesis, Univ. Hort. Sci., Bagalkot (India) 2019.
2. Datta AK, Saha A, Bhttacharya A, Mandal A, Paul R, Sengupta S *et al*. Black cumin (*Nigella sativa* L.) - A review. Journal Plant Development Sciences 2012;4(1):1-43.
3. El-Din AAE, Hendawy SF. Effect of dry yeast and compost tea on growth and oil content of *Borago officinalis* plant. Res J Agric and Biological Sci 2010;6(4):424-430.
4. El-Gamal, SMA, Ahmed HMI. Optimization coriander production for fruit and essential oil B: yield improvement by chitosan and salicylic acid foliar application. J Pl Production Mansoura Univ 2016;7(12):1481-1488.
5. Gendy ASH, Soliman AH, Mohammed NS. Response of growth, productivity and oil composition of fenugreek plants to foliar application of complete fertilizer, dry yeast and L-tryptophan under sandy soil conditions. Current Sci Int 2015;4(4):736-749.
6. Gholamnezhad Z, Havakhah S, Boskabady HM., Preclinical and clinical effects of *Nigella sativa* and its constituent, thymoquinone, A review. J Ethnopharmacology 2016;190:372-386.
7. Jangam AK, Thali P. WASP 2.0, ICAR Research Complex, Goa (India) 2010.
8. Kant K, Anwer MM, Meena SR, Meena RS. Advance production technology of nigella, 1st Edition, Ajmer 2010.
9. Ramadan MF. Nutritional value, functional properties and nutraceuticals applications of black cumin (*Nigella sativa* L.) an overview. Int J Food Sci Technol 2007;42:1208-1218.
10. Sidorov RA, Tsydendambaev VD. Biosynthesis of fatty oils in higher plants. Russian J Pl physiol 2014;61(1):1-18.