EFFECTIVENESS OF NITROGEN SPRAY ON GROWTH ATTRIBUTES AND OIL CONTENTS OF TULSI PLANT

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خلاصہ

تلسی ایک خوبصورتی اور ادویات میں استعمال کیا جانے والا پودا ہے۔ بے شمار فوائد کے باوجود اسکی کاشت پاکستان میں بہت کم ہے۔ اسکی ترقی کے لیے زرعی یونیورسٹی فیصل آباد میں نائٹروجن کی مختلف مقدار کا استعمال کر کے تلسی تجربہ کیا گیا۔ نتائج کی پڑتال سے علم ہوا کہ نائٹروجن کا استعمال تلسی کے لیے مفید ہے۔ نائٹروجن کی سپرے کے نتیجے میں تلسی کی بڑھوتری اور تیل کی مقدار میں خاطر خواہ اظافہ ہوا۔

Abstract

Tulsi is an important ornamental plant and also used for different medicinal purposes. Cultivation of Tulsi is not much common in Pakistan although it has many health benefits and source of valuable products. A pot experiment was conducted at University of Agriculture Faisalabad to identify the nitrogen (N) requirement of Tulsi for enhanced growth and oil contents. Data for different parameters was taken with equal days interval and analysis of that recorded data was done by using HSD test at 5% probability level. Data analysis showed that higher N application produced maximum leaves plant^{-1} (10.667), root length (10.933 cm), shoot length (11.067 cm), plant height (22.000 cm) plant fresh weight (1.386), plant dry weight (0.9000), chlorophyll contents (16.800), leaf area (9.966), leaf area index (4.9800) and oil contents (0.7567). In conclusion, all the levels of N spray produced significant results when compared to controlled pot. It is clear from the data that Tulsi plant needs higher amount of N to produce more growth and oil contents.

Keywords: Tulsi, *Ocimumtenuifloru m*L., valuable products, health benefits, chlorophyll contents, leaf area, oil content.

Introduction

Tulsi (*Ocimumtenuifloru m*L.) is an aromatic and perennial plant belonging to family Lamiaceae. Two type of Tulsi are found within cultivation: (i) Sri Tulsi has green leaves (ii) Krishna Tulsi has purple leaves. It is used as medicinal plant since 4000-5000 B.C and first used as herbal medicine by China (Hosseinzadeh *et al*., 2015). In Pakistan, it is wild and cultivated in Azad Jammu and Kashmir ascending to 1800m in Himalayas and down to tropical areas as a medicinal plant. It is cultivated in kitchen gardens for culinary purposes. These plants required a wide variety of soils and climate conditions like long days and high temperature for the best growth and high yield of essential oils. The plant extract can be used as botanical pesticide due to their allopathic and ayurvadic properties (Bhavya *et al*., 2018).

It has insecticidal activity (Kulkarni and Adavirao, 2018) and repellent action against mosquitos (Bhavya *et al*., 2018) and has a good source of nectar in its flowers for honeybees (Yamani *et al*., 2016). It also has antifungal activity against Asperigillus specie (Hosseinzadeh *et al*., 2015). Tulsi seedling was inoculated with *Bacillus lehensis* and my corrhizal fungi which increased the fluoride tolerance level of herb (Nurzynska-Wierdak *et al*., 2011b). The plant is considered as an adaptogen, balancing different process in the body. The crude extract is used to alleviate potential of various kind of stress (Winston, 2019). It is used for diabetes treatment by reducing the blood glucose levels and cholesterol levels. It is also used to prevent headaches, stomach complaints, cough, cold, heart problems, malaria, fever, inflammation and also used in many other kinds of poisonous diseases (Rahman *et al*., 2014). Both normal and diabetic animals have lipid-lowering action by using of Tulsi plant (Rahimi, 2015).

Extract of Tulsi is used to eliminate bacterial contamination of raw drinking water which is directly obtained from the water sources. In Ayurvedic eye drop preparations, the extract of Tulsi leaves is mixed in

triphala to cure for glaucoma, chute, chronic conjunctivitis and many other sore eye diseases. In the traditional use of medicine, it is used as antidote for insect bite, dog bite and scorpion bite (Hosseinzadeh *et al*., 2015). It is used in herbal cosmetics preparation due to its anti-microbial activity. The stored grains are mixed with dried leaves of Tulsi to repel insects (Kashif and Ullah, 2013). The plant comprises Vitamins (A and C) and certain other minerals like calcium, iron and many phytochemicals. The chemical structure of Tulsi is very complex that comprises of many nutrients and some other biological complexes (Monga *et al*., 2017).

Tulsi plants producing some secondary metabolites that are very effective against some blood sucking arthropods like mosquitos (Ojewumi *et al*., 2018).The plants produced polyphenolic compounds during their development for different reasons like defense in contrast to insects, microbes and herbivores (Ma *et al*., 2015).Nitrogen is basic nutrient that build up many organic compounds like amino acid, peptides, protein, enzyme and nucleic acid, affecting growth, yield and composition of plants (Nurzynska-Wierdak *et al*., 2012). Nitrogen application as a fertilizer is strongly affected to increase weight of leaves, effects on content of essential oil, yield and content of chloroplast dyes (Daneshian *et al*., 2009). Nitrogen application commonly enhances oil yield in aromatic plants by increasing biomass yield per unit of plant. The contents of fertilizers are also related with climate conditions and cultivation treatments including irrigation and harvest methods. It is resulted that the mixture of organic and inorganic nitrogen fertilizers significantlyoil content and also altered the chemical structure of oil.

The effect of urea fertilization on photosynthetic parameters enhanced the metabolism of nitrogen, carboxylation performance and addition of β-asarone (Shetty *et al*., 2019). The application of Urea enhanced the height of plants, length and width of their leave blades but not significantly affect the plant diameter and number of sprout branching (Nurzynska-Wierdakand Borowski *et al*., 2011a). The combination of cowdung and NPK has great effect on the soil quality, growth and outcomes of Tulsi (Hossain *et al*., 2015). The nitrogen fertilization has affected significantly on overall phenolic components concentration and antioxidant properties of basil cultivars (Ma *et al*., 2015). Chemical arrangement of essential oil is affected by organic and inorganic mixture of nitrogen.

Novelty of this research includes the promotion of Tulsi cultivation in Pakistan with determined optimal dose of N fertilizer for better production. Objectives of the research also include,

- The evaluation the effeteness of nitrogen levels on Tulsi
- Theincrement of essential oil content in the leaves

Materials and Methods

Experiment

Pot experiment was executed at Faisalabad using local variety of tulsi. Seeds were taken from Ayub Agriculture Research Institute, Faisalabad. Pots were filled with equal amount of sand and fifteen seeds were sown in each pot and replicated thrice. Hoagland nutrient solution (1/2 strength) was used to nourish all the plants and the pots were irrigated in alternate days to avoid drying. Plants were thinned after leaves emergence to maintain plantspot^{-1}.

Treatments

Different levels of N (50 kg ha^{-1}, 100 kg ha^{-1} and150 kg ha^{-1}) were used as treatments and these were applied as foliar spray. Urea fertilizer containing 46% N was used as source of N. Foliar spray was applied in splits, first after one month of plant emergence while the second after two months of plant emergence. All treatments were used along with a controlled pot receiving no urea application but receiving all other nutrients and irrigations.

Observations

Data for No. of leaves per plant, root length, shoot length, plant height, plant fresh weight, plant dry weight, root fresh weight, shoot fresh weight, chlorophyll contents, leaf area, leaf area index and oil contents were recorded during the experiment.

Procedures to Record Observations

After seventy days of growth, the first seedling was uprooted and washed up with tap water to take out the extra sand particles. For each replication, total number of leaves per plant was physically calculated. The root length of a randomly selected plant was measured from the base to tip of root using measuring tape. The shoot length of a randomly selected plant was measured from the top of plant to base. The average was taken for each replication. At first data, the plant was uprooted from the pots and rinsed with clean water to eliminate the extra sand particles. After that plants were wrapped with the aid of filter paper which absorb extra amount of water from root, shoot and leaves. For the fresh weight, these plants were put on the digital balance for calculation. Then average weight was observed of fresh plants. After the measurement of the fresh weight of plants, the

plants were wrapped in bags and put them in the oven for drying at 72^0C for 24 hours. After drying, the dried plants were placed on digital balance and weighed. The average dry weight was calculated of each replication. The plant leaves were cleaned with the help of tissue paper to calculate the chlorophyll content. The leaf was placed inside the chlorophyll meter (CCM-200 plus Bio-scientific USA) for calculating the chlorophyll content (Parry *et al.*, 2014). Three readings were taken from each treatment and averaged. Leaf area was observed by the multiplication of length and width of leaves.

Leaf area= length of leaf × width of leaf

After that the average of leaf area of all plants was calculated.

Leaf area index was calculated by following formula proposed by Williams (1946):

$$LAI = \frac{Total\ leaf\ area\ of\ plant}{Ground\ area\ occupied\ by\ plant}$$

Leaf area ratio was measures using formula proposed by Radford (1967).

$$LAR = \frac{Leaf\ area\ of\ plant}{Dry\ weight\ of\ plant}$$

The fixed oil contents were extracted from the plant through the Soxhlet extractor,and the essential oil was extracted by the hydro-distillation methods. In the Soxhlet extractor, dried form of leaves is used. In hydro-distillation method both dried and fresh leaves were used.Data recorded was analyzed statistically at 5% probability of LSD test (Steel *et al*., 1997).

Results and Discussion

Number of Leaves

Leaves per plant are important in plant growth and health as these are indicator of photosynthesis and, diseases, nutritional disorders and other stress conditions encountered by plants. Number of leaves in Tulsi were significantly enhanced with N application as foliar spray at higher rates. Data recorded is reported in Table 1. The maximum mean value for number of leaves (10.667) was detected in the treatment where 150 kg/ha N spray was applied. However, number of leaves produced by the treatment receiving 100 kg spray of N ha^{-1} was statistically at par with 150 kg N spray ha^{-1}. The lowest value for leaves $plant^{-1}$ (6.666) was observed in control. While the treatment 50 kg N spray ha^{-1} produced leaves, which were at par with control receiving none of the treatments. Exogenous application of N spray brought positive impacts on the growth, chemical and essential oil components of holy basil plants. Leave $plant^{-1}$ were increased significantly because N played its role in cell formation an increased the total leaves on a plant. N also played its role in increasing the chlorophyll contents of leaves which resulted in enhanced photosynthesis rate. Increased photosynthesis improved the source sink relation which helped plants to gain more weight over time. Ability of N to form new cells also helped the plants to increase their height as the increasing N concentration escalated the plant height in all treatments.

Root Length (cm)

Length and spreading of root are pivotal in crop nutrition and biomass accumulation as these are directly related to nutrient uptake and plant development. Longer roots explore more soil and take up the nutrients for plants from soils with high efficiency. These are also valuable in dry seasons for the uptake of water from deep water table. Root length was increased significantly with N spray at higher rates. Table 1 expresses data observed from different treatments for root length. Significantly, the maximum value for root length (10.933 cm) was recorded from treatment receiving 150 kg/ha N spray.While the root length produced by 100 kg N/ha was at par with 150 kg N/ha. Whereas the lowest value for root length (7.766 cm) was observed in control receiving no N spray. However, root length from 50 kg N ha^{-1} was statistically at par with control.

Table 1. Growth and chemical parameters of Tulsi plant influenced by different levels of foliar applied nitrogen.

Values sharing different letter are statistically significant at 5% probability of HSD test

Treatment	Leaves plant^{-1}	Root length (cm)	Shoot length (cm)	Plant height (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Plant fresh weight (g)	Plant dry weight (g)	Chlorophyll contents	Leaf area	Leaf area index	Leaf area ratio	Oil contents
Control	6.6667 c	7.7667 c	7.4667 c	15.233 c	0.2200 c	0.4267 c	0.9000 c	0.1867 c	13.500 c	7.0767 c	3.5333 c	37.947 a	0.5367 c
50 kg N ha^{-1}	8.0000 bc	8.8333 bc	8.5333 bc	17.233 bc	0.3033 bc	0.5200 bc	1.0867 bc	0.2333 bc	14.700 bc	8.1433 bc	4.0700 bc	35.007 ab	0.6233 bc
100 kg N ha^{-1}	9.3333 b	9.8333 ab	9.5333 ab	19.367 ab	0.3400 ab	0.6167 ab	1.2067 ab	0.2567 ab	15.700 ab	9.1367 ab	4.5633 ab	33.147 bc	0.6800 ab
150 kg N ha^{-1}	10.667 a	10.933 a	11.067 a	22.000 a	0.4133 a	0.7067 a	1.3867 a	0.9000 a	16.800 a	9.9667 a	4.9800 a	30.293 c	0.7567 a

Shoot Length (cm)

Shoot length is indicator of good plant nutrition and photosynthetic rate. It is also important because larger plants will definitely have more leaves and hence more photosynthesis which is the most significant process in plants to make their own food. Larger shoots help plants to utilize more sunlight, air and space. Shoot length also respond positively to foliar applied N. There was a significant increase in shoot length of Tulsi in response to higher N application as foliar spray. Data recorded is demonstrated in Table 1 which shows that significantly, the highest shoot length (11.067 cm) was reported from treatment with highest N concentration (150 kg/ha). While the lowest shoot length was conceived in control treatment (without N spray).However, the shoot length produced by 100 kg N/ha was at par with 150 kg N/ha and the shoot length produced by 50 kg N/ha was at par with values from control. Application of N increased the formation of new cells which elongated the shoot of plants.

Plant Height (cm)

There was an escalation in plant height with foliar application of N. Significant increasing trend in plant height found with increasing concentration of N in spray form. Significantly, the maximum value for plant height (22.00 cm) was recorded in the treatment where 150 kg N/ha was used as foliar spray. However, plant height reported from treatment with 100 kg N/ha was statistically at par with treatment having 150 N/ha. Whereas the least plant height (15.23 cm) was recorded in controlled pot. Values recorded for plant height from treatment with 50 kg N/ha was at par with control. Data concerning plant height in response to N spray is presented in table 1.The plant height has significant results with the increasing amount of nitrogen application which occurred in the medium status of the soil nitrogen. Similarly, the positive effect of nitrogen has been reported by different authors (Moro *et al*., 2015).

Root Fresh Weight (g)

Increase in root weight is helpful in exploiting more soils for nutrient uptake and plant anchor in soil. Root gained more biomass in response to foliar spray of N. Data recorded for root fresh weight is illustrated in table 1. The maximum root weight (0.4133 g) was noted from treatment where 150 kg N/ha was sprayed. However, the minimum root weight (0.2200 g) was observed from control. Value for root weight recorded from treatment with 100 kg N ha^{-1} was at par with treatment receiving 150 kg N ha^{-1}, while the treatment having 50 kg N ha^{-1} produced root fresh weight which was non-significant with comparison to control (without any treatment).

Shoot Fresh Weight (g)

Data recorded for shoot fresh weight is given in Table 1. Data recorded showed that the greatest shoot weight (0.7066 g) was observed from highest N (150 kg/ha) applied as foliar spray. Whereas the lowest value for shoot weight (0.4267 g) was obtained from control.Data analyzed for root weight from the treatment with 100 kg N ha^{-1} was statistically at par with the treatment receiving 150 kg N ha^{-1}, while the treatment having 50 kg N ha^{-1} produced root fresh weight which was statistically at par with control (without any treatment). Previous studies have demonstrated that basil plants require relatively low nitrogen fertilization to provide good growth (Ma *et al*., 2015). But other studies demonstrated increased yield response of basil with the increasing amount of nitrogen fertilization rates (Sifola and Barbieri, 2006). But these nitrogen fertilization rates can be affected with different soil organic matter, soil organic nitrogen and its mineralization rates in different soil types.

Plant Fresh and Dry Weight (g)

Plant fresh and dry weight was positively improved with application of N spray. Maximum plant fresh weight (1.3867 g) was noted with N spray (150 kg/ha) in comparison to control which received no treatment. The lowest plant fresh weight (0.9000 g) was also reported from same treatment. However, the treatment receiving 100 kg N ha^{-1} produced plant fresh weight which was statistically at par with 150 kg N ha^{-1}. The observed data is presented in table 1. Plant dry weight was also influenced in similar way and similar treatment with 150 kg N ha^{-1} produced maximum plant dry weight. The value of plant dry weight observed from treatment 100 kg N ha^{-1} was at par with 150 kg N ha^{-1}.The lowest plant dry weight (0.1867 g) was recordedfrom control. However, the treatment 50 kg N ha^{-1} was statistically at par with control (no treatment applied).The final yield of fresh and dry herbs was affected by the application of N (Radusiene *et al*., 2019). The nitrogen fertilization generally increased the oil content in the medicinal and aromatic plants by increasing the amount of biomass yield per unit land area, leaf area development and photosynthetic rate (Daneshian *et al*., 2009).

Chlorophyll Contents

Chlorophyll contents occupy great importance in photosynthesis. Chlorophyll contents were influenced positively with N spray. Data recorded for chlorophyll contents is provided in table 1. Significantly, the highest chlorophyll contents (16.800) were reported from 150 kg/ha N spray. While the lowest chlorophyll contents (13.500) were attributed from control receiving no treatment. Value from chlorophyll contents obtained fromtreatment with 100 kg N ha^{-1} was statistically at par with treatment having 150 kg N ha^{-1}.Whereas treatment with 50 kg N ha^{-1} produced results at par with those observed form control. The chlorophyll content and essential oil content were significantly increased with the increasing amount of nitrogen continuously (Wasaya *et al*., 2017).

Leaf Area and Leaf Area Index

Data recorded for both parameters is presented in the table 1. Leaf area of each leaf form plants was significantly influenced by N spray. Significantly, the maximum leaf area (9.966) was observed from 150 kg N/ha, while the lowest leaf area (7.076) was conceived from control. However, data recorded from treatments with 100 kg N ha^{-1} and 50kg N ha^{-1} showed that these treatments were statistically at par with treatments 150 kg N ha^{-1} and control, respectively.Similarly, the highest vale for leaf area index (4.98) was observed from 150 kg N/ha, while the lowest value (3.53) was noted from control. In addition, values recorded form 100 kg N ha^{-1} and 50 kg N ha^{-1} were statistically at par with 150 kg N ha^{-1} and control, respectively. Leaf area index was also positively influenced with N spray because N assisted the plants in increasing the leaf size. Nitrogen has a significant positive impact on the plant height, fresh and dry weight of roots and shoots and oil (Razaq *et al*., 2017).

Leaf Area Ratio

Leaf area ratio showed a negative relation with foliar applied N. Maximum leaf area ratio (37.94) was recorded in control while, the minimum leaf area ratio (7567) was noticed from treatment with highest N concentration (150 kg N/ha). The treatment of 50 kg N ha^{-1} produced results at par with control and the treatment with 100 kg N ha^{-1} was at par with 150 kg N ha^{-1}. Data recorded for leaf area ratio is presented in table 1.

Oil Contents

Oil contents were measured which showed positive relation with N sprayed. Oil contents showed an increasing trend to increase in N application. Data recorded for oil contents is given in table 1. Significantly, the maximum oil contents (0.7567) were calculated form pots treated with 150 kg N/ha. While the lowest oil contents (0.5367) were observed from control. The treatment with N 100 kg/ha was statistically at par with treatment conceiving 150 kg N ha^{-1}. While the treatment with 50 kg N/ha was at par with control. It was revealed that the content of basil oil was increased with nutrient management. The application of nitrogen enhanced the yield above ground mass, leaf essential oil content and oil yield (Singh *et al*., 2016). The enhancement of essential oil yield due to nitrogen application does not only increase in the leaf weight but also the leaves essential oil concentration (Singh *et al*., 2016). Nitrogen application also affected the antioxidant activity of the basil which was treated with the highest amount of nitrogen levels (Ma *et al*., 2015).

Conclusion

Foliar applied N plays a pivotal role in increasing the growth, chemical composition and oil contents of Tulsi plant. Increase in N levelas foliar spray produced supreme results in comparison to control. There is direct relation of N application with chlorophyll and oil contents of Tulsi plant.

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References

Bhavya, M.L., Chandu, A.G.S. and Devi, S.S (2018). *Ocimumtenuiflorum* oil, a potential insecticide against rice weevil with anti-acetylcholinesterase activity. Ind. Crops Prod. (126):434-439.

Daneshian, A., Gurbuz, B., Cosge, B. and Ipek, A (2009). Chemical components of essential oils from basil (*Ocimumbasilicum* L.) grown at different nitrogen levels. Int. J.Natu.Engi. Sci.(3): 9-11.

Hossain, M.E., Haque, A.N.A., Rahman, K.M., Rahman, M.A. and Kari, M.R (2015). Combined effect of fertilizer and manures on growth and yield of Tulsi (*Ocimum sanctum*) and pudina (*Mentha arvensis*) in Rabi season at Bangladeshi Agriculture farming areas of mymensing district. Ban. J. Environ. Sci. 28: 85-88.

Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A. and Armand, R (2015). The application of medicinal plants in traditional and modern medicine: a review of Thymus vulgaris. Int. J. Clini. Med. (9):635.

Kashif, M. and Ullah, S (2013). Chemical composition and minerals analysis of*Hippophaerhamnoides, Azadirachta indica, Punica granatu* and *Ocimum sanctum* leaves. World J. DairyFood Sci. (8): 67-73.

Kulkarni, K.V. and Adavirao, B.V (2018). A review on: Indian traditional shrub Tulsi (Ocimum sanctum): the unique medicinal plant. J. Med. Plants Stud. *6*(2):106-110.

Ma, D., Sun, D., Li, Y., Wang, C., Xie, Y. and Guo, T (2015). Effect of nitrogen fertilisation and irrigation on phenolic content, phenolic acid composition, and antioxidant activity of winter wheat grain. J. Sci. Food Agric. *95*(5):1039-1046.

Monga, S., Dhanwal, P., Kumar, R., Kumar, A. and Chhokar, V (2017). Pharmacological and physico-chemical properties of Tulsi (Ocimumgratissimum L.): An updated review. The Pharma Inno. *6*(4):181.

Moro, B.M., Nuhu, I.R., Ato, E. and Naathanial, B (2015). Effect of nitrogen rates on the growth and yield of three rice (Oryza sativa L.) varieties in rain-fed lowland in the forest agro-ecological zone of Ghana. Int.J.Agric. Sci. *5*(7):878-885.

Nurzynska-Wierdak, R. and Borowski, B (2011a). Dynamics of sweet basil (*Ocimumbasilicum* L.) growth affected by cultivar and foliar feeding with nitrogen. Acta Sci. Polo.Horto. Cultus.10(3): 307-317.

Nurzynska-Wierdak, R., Rozek, E. and Borowski, B (2011b). Response of different basil cultivars to nitrogen and potassium fertilization: total and mineral nitrogen content in herb. Acta Sci. Polo.Horto. Cultus. 10(4): 217-232.

Nurzynska-Wierdak, R., Rozek, E., Dzida, K. and Borowski, B (2012). Growth response to nitrogen and potassium fertilization of common basil (*Ocimumbasilicum* L.) plants. Acta Sci. Polo.Horto. Cultus. 11(2): 275-288.

Ojewumi, M.E., Adeyemi, A.O. and Ojewumi, E.O (2018). Oil extract from local leaves-an alternative to synthetic mosquito repellants. Pharmacophore 9(2):1-6.

Parry, C., Blonquist, J. and Bugbee, B (2014). In situ measurement of leaf chlorophyll concentration: Analysis of the optical;/absolute relationship. Plant Cell Environ. 37:2508-2520.

Radford, P.J (1967). Growth analysis formulae-their use and abuse. Crop Sci. 7:171-175.

Radusiene, J., Marksa, M., Ivanauskas, L., Jakstas, V., Çalişkan, O., Kurt, D., Odabaş, M.S. and Çirak, C (2019). Effect of nitrogen on herb production, secondary metabolites and antioxidant activities of Hypericum pruinatum under nitrogen application. Ind. Crops Prod. (139):2-9.

Rahimi, M (2015). A review: anti diabetic medicinal plants used for diabetes mellitus. Bull. Environ.Pharmacol. Life Sci. *4*(2):163-180.

Rahman, K.M., Sattar, M.A. and Rahman, G.M.M (2014). Effect of Fertilizer and Manures on Growth and Yield of Tulsi and Pudina Medicinal Plant. J. Environ. Sci.Natu. Res.7(2): 13-16.

Razaq, M., Zhang, P. and Shen, H.L (2017). Influence of nitrogen and phosphorous on the growth and root morphology of Acer mono. *PloS one 12*(2):1-13.

Shetty, P., Acharya, C. and Veeresh, N (2019). Effect of Urea Fertilizer on the Biochemical Characteristics of Soil. Int. J. App. Sci.Biotechnol. 7(4):414-420.

Sifola, M.I. and Barbieri, G. (2006). Growth, yield and essential oil content of three cultivars of basil grown under different levels of nitrogen in the field. Scientia Horticulturae.108(4): 408-413.

Singh, M., Khan, M.M.A. and Naeem, M. (2016). Effect of nitrogen on growth, nutrient assimilation, essential oil content, yield and quality attributes in Zingiber officinale Rosc. J. Saudi Soc. Agric. Sci. 15(2):171-178.

Steel, R.G.D., Thorrie, J.H., Dicky, D. A. (1997). Principles and procedures of statics, a biometrical approach, 3rd ed. McGras Hill Book Co., Singapore.

Wasaya, A., Tahir, M., Ali, H., Hussain, M., Yasir, T.A., Sher, A. and Ijaz, M. (2017). Influence of varying tillage systems and nitrogen application on crop allometry, chlorophyll contents, biomass production and net returns of maize (Zea mays L.). Soil Till. Res. 170:18-26.

Williams, R. F. (1946).The physiologyofplantgrowthwithspecialrelationreferencetotheconceptofnet assimilation rate. Ann. Bot. 10:41-72.

Winston, D. (2019). Adaptogens: herbs for strength, stamina, and stress relief. Simon and Schuster.

Yamani, H.A., Pang, E.C., Mantri, N. and Deighton, M. A. (2016). Antimicrobial activity of Tulsi (*Ocimumtenuiflorum*) essential oil and their major constituents against three species of bacteria. Front. Microbiol. (7):681