

Effect of mulching and nutrition on essential oil yield, quality and nutrient uptake in sweet basil (*Ocimum basilicum* L.)

Comment [J1]: *Ocimum basilicum*

ABSTRACT

A field experiment was conducted at College of Horticulture, Bengaluru to investigate the influence of different mulches and nutrition on essential oil yield, quality and nutrient uptake in sweet basil. The treatment combinations consisted of four different mulches viz., no mulch, black plastic, silver plastic and organic mulch (spent lemon grass) each with four different nutrient levels viz., 100 per cent RDF, combination of 75 per cent RDF with humic acid, 75 per cent RDF with microbial consortia and 75 per cent RDF, humic acid and microbial consortia. Use of black mulch recorded maximum essential oil content (1.13 %), yield (97.21 kg/ha), N (54.56 kg/ha), P (37.71 kg/ha) and K (57.85 kg/ha) uptake. Application of 75 per cent RDF with humic acid resulted in maximum essential oil content (1.21 %) whereas, 75 per cent RDF with humic acid and microbial consortia recorded maximum essential oil yield (79.03 kg/ha), P (24.82 kg/ha) and K (48.88 kg/ha) uptake. Maximum nitrogen uptake was found in application of 100 per cent RDF. Among interaction, maximum essential oil content (1.44 %) and yield (109.92 kg/ha) was noticed in combination of black mulch with 75 per cent RDF and humic acid. The highest methyl chavicol content (73.85%) was observed in plants grown with black mulch along with application of 75 per cent RDF and microbial consortia.

Keywords: *Humic acid, Methyl chavicol, Microbial consortia, Mulch, Sweet basil*

1. INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) is a significant annual herb in the lamiaceae family, which is grown for its leaf inflorescence. The genus *Ocimum* consists of numerous species and varieties, ranging from 50 to 150 (Runyoro et al., 2010)^[14]. It is commonly used as a culinary herb and spice, and its essential oil finds usage in flavouring food, perfumery, and the pharmaceutical industry. The composition of the essential oil varies depending on the chemotype, with high levels of linalool (up to 55%), methyl chavicol (up to 70%), methyl cinnamate, or eugenol (Grayer et al., 1996^[8]; Vieira and Simon, 2006^[20]). The unchecked use of weedicides and chemical fertilizers in agriculture is causing severe harm to soil health and reducing the quality of herbs produced. Therefore, it is crucial to cultivate basil that is free from weed

Comment [J2]: *Ocimum basilicum*

infestation and chemical residues, while also ensuring sustainable soil health by enhancing the physical, chemical, and biological properties of the soil (Sarrou et al., 2016)^[15]. Regions that prioritize heavy chemical use in farming have caused negative consequences for the environment, agriculture, and health. A potential remedy to decrease dependence on herbicides involves adopting mulch as an alternative approach. (Thakur et al., 2019)^[18]. Intensive farming practices, aimed at achieving higher yields, often involve the extensive use of agrochemicals that are both expensive and detrimental to the environment. However, the application of humic acid to soil can improve its physical and chemical properties, increase the availability of nutrients, promote chlorophyll synthesis, and enhance the permeability of plant membranes (Fortun, 1990)^[5]. Additionally, the use of biofertilizers can help to fix, solubilize, and mobilize major nutrients in the soil, making them readily available to plants (Gaur and Gaind, 1990)^[6]. These biofertilizers can also synthesize various phytohormones, such as auxins, gibberellins, and cytokinins, which can positively influence plant growth and development (Befrozfar et al., 2013)^[3].

2. MATERIAL AND METHODS

2.1 Site characterization

A field experiment was conducted during Rabi, 2018-2019 at the Department of Plantation, Spices, Medicinal and Aromatic crops, College of Horticulture, UHS Campus, GKVK, Bengaluru (Geographical coordinates 12°58'1 N, 77°35'1 E and 930 m MSL). The experimental site was a fairly levelled land with red sandy clay loam soil of fairly uniform fertility status and belonged to the order of alfisols.

2.2 Experimental design

The experiment was conducted in a factorial randomized complete block design (FRCBD) with sixteen treatment combinations replicated thrice as follows.

- T1: control - No mulch with 100% RDF (160:80:80 kg/ha)
- T2 – No mulch with 75% RDF + Humic acid @ 10 kg/ha
- T3 – No mulch with 75% RDF + Microbial Consortia @ 5 kg/ha
- T4 – No mulch with 75% RDF + Humic acid @ 10 kg/ha + Microbial Consortia @ 5 kg/ha
- T5 – Plastic mulch Black with 100% RDF
- T6 – Plastic mulch Black with 75% RDF + Humic acid @ 10 kg/ha
- T7 – Plastic mulch Black with 75% RDF + Microbial Consortia @ 5 kg/ha
- T8 – Plastic mulch Black with 75% RDF + Humic acid @ 10 kg/ha+ Microbial Consortia @ 5 kg/ha
- T9 – Plastic mulch Silver with 100% RDF
- T10 – Plastic mulch Silver with 75% RDF + Humic acid @ 10 kg/ha
- T11– Plastic mulch Silver with 75% RDF + Microbial Consortia @ 5 kg/ha
- T12– Plastic mulch Silver with 75% RDF + Humic acid @ 10 kg/ha+ Microbial Consortia @ 5 kg/ha
- T13– Organic mulch with 100% RDF
- T14– Organic mulch with 75% RDF + Humic acid @ 10 kg/ha
- T15– Organic mulch with 75% RDF + Microbial Consortia @ 5 kg/ha
- T16– Organic mulch with 75% RDF + Humic acid @ 10 kg/ha + Micro

2.3 Experimental details

Healthy seeds of sweet basil variety CIM-Saumya were procured from IIHR, Bengaluru and were mixed with sand in a 1:5 ratio before being sown in well-prepared raised beds. The beds were 4m long, 1m wide and 10 cm high, with seeds sown in rows spaced 5 cm apart. Germination of the seeds was observed in just 2 days, with 90 per cent of the seeds sprouting within eight days. To ensure optimal growth, organic manure (FYM) was applied 15 days prior to transplanting the seedlings in the main field. The recommended dose of fertilizers (RDF) at 160:80:80 kg/ha were applied in the form of straight fertilizers such as urea, single super phosphate and muriate of potash. The basal application involved applying 50 per cent N along with the full dose of P₂O₅ and K₂O, while the remaining 50 per cent nitrogen was top-dressed thirty days after transplanting.

The experimental plots were mulched using black and silver plastic of 50μ thickness, as well as organic mulch made from spent lemon grass at a rate of 3t/ha. Seedlings of forty days old were transplanted to the plots with a spacing of 60 cm between rows and 40 cm within rows. After transplanting, the plots were immediately irrigated, and regular irrigation was given daily for an hour through drip irrigation. Humic acid and microbial consortia were applied 15 days after transplanting at a rate of 10 kg/ha and 5kg/ha, respectively. Plots without mulch were kept free from weeds throughout the growing

Comment [J3]: Use normal degree sign. You can find it in insert

Comment [J4]: Use normal degree sign. You can find it in insert

Comment [J5]: Let there be conditency in your work. If you want to use colon (:), use colon for all and if you want to use hyphen (-), use it for all

Comment [J6]: At is more preferred to @...

Comment [J7]: Percent

Comment [J8]: Whats the N for?

Comment [J9]: P₂O₅

Comment [J10]: K₂O

Comment [J11]: 5 spent lemon grasses

period by weeding at regular intervals. The crop was harvested after 60 days of transplanting when it reached the 50 per cent flowering stage, as this stage contains the maximum percentage of essential oil.

2.4 Observations recorded

Observations were recorded at 30 days and 60 days after transplanting (harvest). Fresh and dry herbage yield were recorded for a plot size of 4.8 × 4.0 m and estimated yield per hectare was calculated. The herbage was dried in shade house. Essential oil was estimated using Clevenger's apparatus. Four hundred grams of the fresh herb consisted of both leaves and inflorescence was taken to estimate the essential oil content according to different treatments and expressed in percentage (%) on dry weight basis. The distillation time was standardized to one and a half hours.

The nitrogen, phosphorous and potassium uptake by plant was computed using following formula

$$\text{Nutrient uptake (kg/ha)} = \frac{\text{Dry matter yield (kg/ha)} \times \text{nutrient content (\%)}}{100}$$

2.5 Statistical analysis

The experimental data recorded on various biometric parameters collected during the study were subjected to statistical analysis by Fisher's method of analysis of variance (ANOVA) as outlined by Sundararaj et al. (1972)^[17]. Wherever the "F" test was significant for comparison of treatment means, LSD values were worked out at 0.05 probability level.

3. RESULTS AND DISCUSSION

3.1 Oil yield

Plants grown on black mulch exhibited the highest essential oil content (1.13%) and yield (97.21 kg/ha) on a dry weight basis, which was significantly different from other treatments. The combination of 75% recommended dose of fertilizers (RDF) with humic acid resulted in the maximum essential oil content (1.21%), and the combination of 75% RDF with humic acid and microbial consortia resulted in the maximum yield (79.03 kg/ha). The interaction between mulch and nutrition also had a significant effect, with the highest oil content (1.44%) observed in plants grown on black mulch with 75% RDF and humic acid (Table 1). Plants grown with black mulch may have had increased oil content due to enhanced essential oil biosynthetic pathways activated by photosynthetic light. Humic acid, which can improve enzymatic activity, membrane permeability, cell division and metabolism, may have also contributed to maximizing herbage yield. Studies by Abaas (2014)^[1] in *Calendula*, Giri et al. (2016)^[7] in sweet basil, Mendonca et al. (2013)^[13] in *Melissa officinalis*, and Sarrou et al. (2016)^[15] in sweet basil have reported similar findings. Humic acid can also improve nutrient uptake, soil physical properties, and synthesize semi-hormonal substances (Turkmen et al., 2005)^[19]. The highest essential oil yield observed may be attributed to both the maximum essential content and dry herbage yield. Humic acid application can increase yield and quality by providing nutrients to the plant, while simultaneously increasing the rate of photosynthesis and other plant physiological activities (Khoshghalba et al., 2017)^[12]. These results are consistent with the findings of Hendawy et al. (2015)^[10] in *Mentha piperita* and El-Ziat et al. (2017)^[4] in sweet basil.

3.2 Oil quality

Plants grown with black mulch and application of 75 per cent RDF and microbial consortia showed the highest content of methyl chavicol (73.85%) (Fig 1) while, the lowest was recorded in plants grown with silver mulch and 75 per cent RDF, humic acid and microbial consortia (29.67%). The highest content of linalool (56.90%) was observed in silver mulch with the application of 75 per cent RDF, humic acid, and microbial consortia, and the lowest was found in black mulch with the application of 75 per cent RDF and microbial consortia (13.73%). The observed high levels of methyl chavicol and linalool in plants could be explained by the activation of secondary metabolism, potentially due to the increased activity of enzymes involved in the biosynthesis of essential oils and improved metabolism. This variation in content among the treatments suggests that different combinations of mulch and nutrition may have differential effects on enzyme activity and metabolism. Similar findings have been reported by Sarrou et al. (2016)^[15] in sweet basil and Thakur et al. (2019)^[18] in *Rosa damascena*.

Comment [J12]: Record of observations

Comment [J13]: Use equation editor to properly insert your formula for proper arrangement to avoid lifting

Comment [J14]: Reference too old, update

Comment [J15]: *Melissa officinalis*

Comment [J16]: *Melissa officinalis*

3.3 Nutrient uptake

Influence of different mulch, nutrition and their combination had a substantial impact on uptake of nitrogen, phosphorous and potassium as depicted in Table 2.

3.3.1 Nitrogen uptake

Plants grown with black mulch had significantly higher nitrogen uptake (54.56 kg/ha) compared to all other mulches, with the lowest being in organic mulch (19.68 kg/ha). Plants treated with 100 per cent RDF had the highest nitrogen uptake (43.37 kg/ha), whereas the lowest was recorded in plants treated with a combination of 75 per cent RDF and humic acid (30.85 kg/ha). Among the interaction of factors, plants grown with black mulch and treated with 100 per cent RDF recorded the highest nitrogen uptake (70.18 kg/ha), which was significantly different from all other treatment combinations. The use of black mulch has resulted in higher nitrogen uptake by the plants, which could be due to factors such as increased mineralization under mulched conditions, reduced leaching, and optimal moisture levels that improve nutrient availability in the soil solution, ultimately leading to increased nutrient uptake by the plants. This finding is supported by previous studies conducted on sweet basil (Heidari et al., 2012)^[9], lettuce (Khazaie et al., 2013)^[11], thyme (Solberg and Dragland, 2014)^[16], and ashwagandha (Anuroopa et al., 2017)^[2], which have reported similar results.

3.3.2 Phosphorous uptake

In terms of phosphorus uptake, plants grown with black mulch recorded the highest uptake (37.71 kg/ha), which was significantly different from all other mulches, while the least was recorded in plants with organic mulch (8.39 kg/ha). Among nutrition, the combination of 75 per cent RDF, humic acid and microbial consortia resulted in the highest phosphorous uptake (24.82 kg/ha), which was similar to plants treated with 75 per cent RDF and microbial consortia (24.71 kg/ha). The least phosphorous uptake was found in plants treated with 75 per cent RDF and humic acid (15.89 kg/ha). Among the interaction of factors, black mulch with the application of 75 per cent RDF and microbial consortia resulted in the highest phosphorous uptake in soil (39.03 kg/ha). The use of mulch helps retain moisture in the soil and prevent nutrient loss, while applying humic acid improves the soil's nutrient-holding capacity. Additionally, microbial consortia, containing phosphorous solubilizing bacteria, aids in the solubilization of inorganic phosphorus and mineralization of organic phosphorus. This is due to the action of low molecular weight organic acids secreted by bacteria and the synthesis of various phosphatases, resulting in increased phosphorous content in the soil. These findings are consistent with those of other studies, such as Heidari et al. (2012)^[9] in sweet basil, Khazaie et al. (2013)^[11] in lettuce, Solberg and Dragland (2014)^[16] in thyme, and Anuroopa et al. (2017)^[2] in ashwagandha.

3.3.3 Potassium uptake

Plants grown with black mulch also recorded the highest potassium uptake (57.85 kg/ha), while the least was recorded in plants with organic mulch (30.64 kg/ha). The combination of 75 per cent RDF, humic acid and microbial consortia resulted in the highest potassium uptake (48.88 kg/ha). In contrast, the least was found in plants treated with 75 per cent RDF and humic acid (39.23 kg/ha). The interaction between mulch and nutrition did not vary significantly for potassium uptake. The increased potassium uptake in the plants might be attributed to the secretion of organic acids by potassium mobilizing bacteria, such as *Frateuria aurantia*, which enhances the mobilization of potassium in the soil. These beneficial soil microorganisms are rhizosphere-competent and can colonize various ecological niches in the rhizosphere, thereby facilitating the mobilization of a wider range of nutrients. This finding is consistent with the studies of Heidari et al. (2012)^[9] in sweet basil, Khazaie et al. (2013)^[11] in lettuce, Solberg and Dragland (2014)^[16] in thyme, and Anuroopa et al. (2017)^[2] in ashwagandha.

Comment [J17]: 75 percent. Ensure uniformity in writing your percent as percent and not as per cent

Table 1. Influence of mulching and nutrition on essential oil content and yield

| Treatment | Essential oil content (%) | Essential oil yield (kg/ha) |
|-------------------------------|---------------------------|-----------------------------|
| M ₀ | 1.13 | 67.21 |
| M ₁ | 1.13 | 97.21 |
| M ₂ | 1.10 | 78.34 |
| M ₃ | 0.94 | 45.24 |
| S.Em. ± | 0.03 | 3.34 |
| CD at 5% | 0.009 | 9.65 |
| N ₀ | 1.00 | 64.83 |
| N ₁ | 1.21 | 73.29 |
| N ₂ | 0.99 | 70.86 |
| N ₃ | 1.10 | 79.03 |
| S.Em. ± | 0.03 | 3.34 |
| CD at 5% | 0.009 | 9.65 |
| M ₀ N ₀ | 1.17 | 66.97 |
| M ₀ N ₁ | 1.30 | 73.50 |
| M ₀ N ₂ | 1.05 | 58.54 |
| M ₀ N ₃ | 0.98 | 69.81 |
| M ₁ N ₀ | 0.98 | 81.36 |
| M ₁ N ₁ | 1.44 | 109.92 |
| M ₁ N ₂ | 0.91 | 93.02 |
| M ₁ N ₃ | 1.20 | 104.56 |
| M ₂ N ₀ | 1.04 | 70.00 |
| M ₂ N ₁ | 1.09 | 71.65 |
| M ₂ N ₂ | 1.00 | 74.60 |
| M ₂ N ₃ | 1.27 | 97.13 |
| M ₃ N ₀ | 0.82 | 41.01 |
| M ₃ N ₁ | 0.99 | 38.08 |
| M ₃ N ₂ | 0.98 | 57.28 |
| M ₃ N ₃ | 0.96 | 44.60 |
| S.Em. ± | 0.006 | 6.68 |
| CD at 5% | 0.017 | NS |

Comment [J18]: ± S.E.M

Comment [J19]: ± S.E.M

Comment [J20]: ± S.E.M

Comment [J21]: Insert 'Key' at the based of the table to interpret the the meaning of what you have in your table in horizontal format

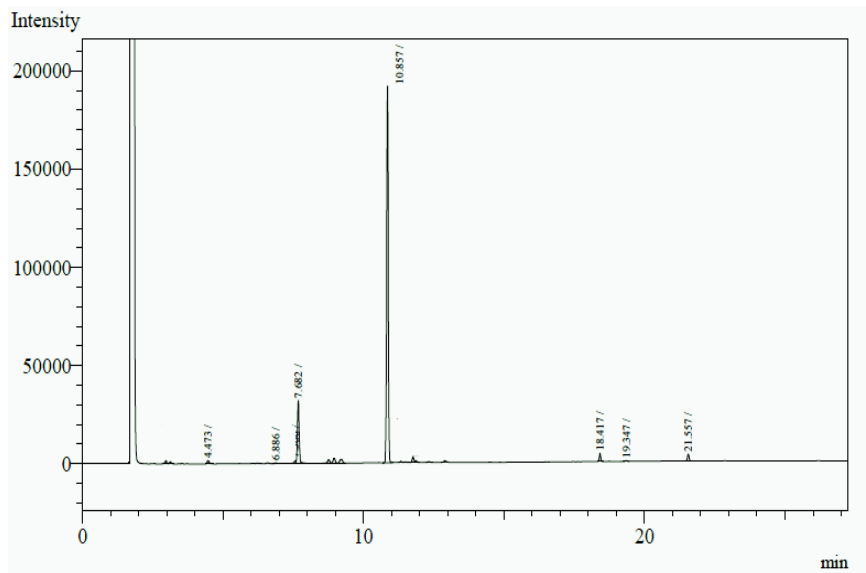


Fig 1. Influence of black mulch along with combination of 75 per cent RDF and humic acid on methyl chavicol and linalool content in essential oil of basil

Comment [J22]: Include the instrument you used in getting your graph as part of your caption and the analysis carried out (Note: you only stated the effect)

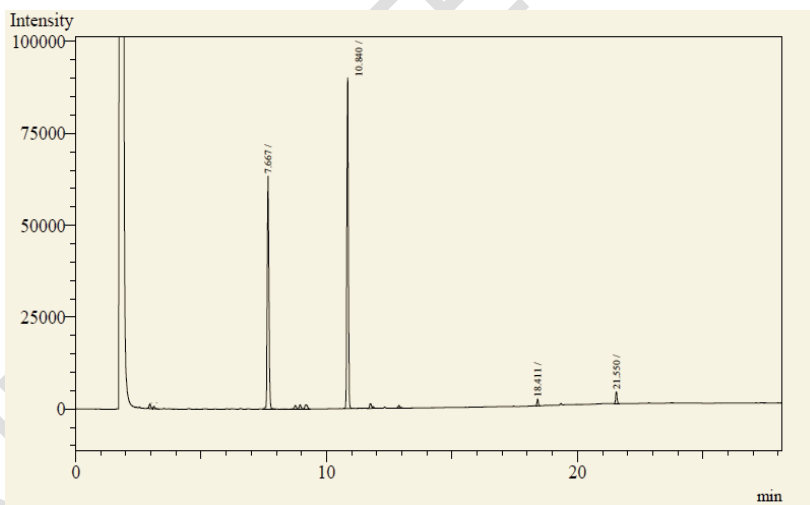


Fig 2. Influence of black mulch along with combination of 75 per cent RDF and humic acid on methyl chavicol and linalool content in essential oil of basil

Comment [J23]: Include the instrument you used in getting your graph as part of your caption and the analysis carried out (Note: you only stated the effect)

Table 2. Influence of mulching and nutrition on plant nutrient uptake

| Treatments | N (kg/ha) | P (kg/ha) | K (kg/ha) |
|-----------------------------------|------------------|------------------|------------------|
| M₀ | 37.34 | 12.79 | 38.17 |
| M₁ | 54.56 | 37.71 | 57.85 |
| M₂ | 40.14 | 27.73 | 47.66 |
| M₃ | 19.68 | 8.39 | 30.64 |
| S.Em. ± | 2.15 | 1.38 | 2.44 |
| CD at 5% | 6.23 | 4.00 | 7.04 |
| N₀ | 43.37 | 21.19 | 39.28 |
| N₁ | 30.85 | 15.89 | 39.23 |
| N₂ | 39.27 | 24.71 | 46.93 |
| N₃ | 38.24 | 24.82 | 48.88 |
| S.Em. ± | 2.16 | 1.39 | 2.44 |
| CD at 5% | 6.23 | 4.00 | 7.04 |
| M₀N₀ | 47.64 | 12.09 | 32.41 |
| M₀N₁ | 30.96 | 7.49 | 38.41 |
| M₀N₂ | 31.68 | 16.52 | 35.90 |
| M₀N₃ | 39.09 | 15.05 | 45.97 |
| M₁N₀ | 70.18 | 36.96 | 51.14 |
| M₁N₁ | 37.17 | 36.44 | 50.31 |
| M₁N₂ | 58.37 | 39.03 | 66.77 |
| M₁N₃ | 52.54 | 38.42 | 63.18 |
| M₂N₀ | 41.19 | 28.71 | 43.72 |
| M₂N₁ | 32.04 | 10.63 | 46.13 |
| M₂N₂ | 44.09 | 34.66 | 46.59 |
| M₂N₃ | 43.26 | 36.93 | 54.20 |
| M₃N₀ | 14.48 | 7.02 | 29.84 |
| M₃N₁ | 23.23 | 9.02 | 22.05 |
| M₃N₂ | 22.93 | 8.64 | 38.48 |
| M₃N₃ | 18.08 | 8.88 | 32.18 |
| S.Em. ± | 4.31 | 2.77 | 4.88 |
| CD at 5% | 12.45 | 8.00 | NS |

4. CONCLUSION

To summarize the findings, the use of black mulch has been shown to enhance essential oil content, essential oil yield, and nutrient uptake in plants. Meanwhile, supplementing with 75% RDF and humic acid led to the highest essential oil content, while adding microbial consortia resulted in the highest essential oil yield, phosphorous uptake, and potassium uptake. Providing 100% RDF resulted in the highest nitrogen uptake. When combined with black mulch, 75% RDF and humic acid yielded the highest essential oil content and essential oil yield. On the other hand, black mulch combined with 75% RDF and microbial consortia resulted in the highest methyl chavicol content, while silver mulch with 75% RDF, humic

Comment [J24]: Reconstruct this sentence

acid, and microbial consortia yielded the highest linalool content. Overall, the combination of black mulch, 75% RDF, and humic acid was the most effective in achieving maximum essential oil yield.

REFERENCES

1. Abaas IS. Effect of biological competition of weeds on growth and volatile oil yield of marigold (*Calendula officinalis* L.) as medicinal plant used in herbal medicine of Iraq. *Int J Pharm Pharm Sci*. 2014; 6(1): 217-219.
2. Anuroopa N, Bagyaraj DJ, Bagela A, Rao EV. Inoculation with selected microbial consortia not only enhances growth and yield of *Withaniasomnifera* but also reduces fertilizer application by 25% under field conditions. *Proc Indian Nat Sci Acad*. 2017; 83: 957-971.
3. Befrozfar MR, Habibi D, Asgharzadeh A, Sadeghishoe M, Tookaloo MR. Vermicompost, plant growth promoting bacteria and humic acid can affect the growth and essence of basil (*Ocimumbasilicum* L.). *Ann Biol Res*. 2013; 4(2): 8-12.
4. El- Ziat RA, Swaefy HM, Esmail EE. The response of red rubin basil plant to organic fertilizer and humic acid versus chemical fertilizers. *Middle East J Agric Res*. 2017; 6(4): 928-934.
5. Fortun A, Benayas J, Fortun C. The effects of fulvic and humic acids on soil aggregation: a micromorphological study. *J Soil Sci*. 1990; 41: 563-572.
6. Gaur AC, Gaind S. Role of phosphorus solubilizing microorganism in crop productivity and enriched organic manure. National seminar on organic farming org. by JNKVV Jabalpur and IGKVV, Raipur at college of Agriculture Indore. 20-29 sep. 1992: 134-142.
7. Giri P, Pandey ST, Royand S, Behera B. Weed dynamics, herbage and oil yield of sweet basil (*Ocimumbasilicum*) under various weed management practices. *J Crop Weed*. 2016;12(1):145-149.
8. Grayer RJ, Kite GC, Goldstone FJ, Bryan SE, Paton A, Putievsky E. Intraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimumbasilicum*. *Phytochem*. 1996;43: 1033-1039.
9. Heidari M, Golpayegani A. Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimumbasilicum* L.). *J Saudi Soc Agric Sci*. 2012; 11, 57-61.
10. Hendawy SF, Hussein MS, El-Gohary AE, Ibrahim ME. Effect of organic fertilization on growth, yield and oil content of *Mentha piperita* var. *citrata*. *Asian J Agric Res*, 2015; 9(5):237-248.
11. Khazaie HR, Rezaie EE, Bannayan M. Application times and concentration of humic acid impact on aboveground biomass and oil production of hyssop (*Hyssopus officinalis*). *J Med Plant Res*. 2013; 5(20): 5148-5154.
12. Khoshghalba H, Babaeib M, Najafabdic MY. How different concentrations of humic acid, zinc, nitrogen and boron influence quantitative and qualitative yield of German Chamomile (*Matricaria chamomilla* L.). *J App Environ Biol Sci*. 2017;7(11): 53-59.
13. Mendonca MC, Santos MF, Simoes RA, Silva-man R, Blank AF. Production and entomofauna associated to lemon balm (*Melissa officinalis* L.) cultivated with synthetic mulch. *Rev Bras PI Med*. 2013;8(1): 63-67.
14. Runyoro DO, Ngassapa K, Vagionas N, Alianni K, Graikou, Chinou I. Chemical composition and antimicrobial activity of the essential oils of four *Ocimum* species growing in Tanzania. *Indian J Agric Res*. 2010;44(1): 48-52.
15. Sarrou E, Chatzopoulou P, Koutsos TV, Katsiotis S. Herbage yield and essential oil composition of sweet basil (*Ocimumbasilicum* L.) under the influence of different mulching materials and fertilizers. *J Med Plants Stud*. 2016;4(1): 111-117.
16. Solberg SO, Dragland S. The effects of dark colored plastic, fiber and paper mulches on yield and aver-wintering of perennial herbs., *J Herbs Spices Med Plants*. 2014; 12(1): 13-23.
17. Sundararaj N, Nagaraju S, Venkataramu MN, Jagannath MK. Design and analysis of field experiments. University of Agricultural Sciences, Bangalore, India, 1972, pp.139.

Comment [J25]: *Ocimum basilicum*

Comment [J26]: *Ocimum basilicum*

Comment [J27]: *Ocimum basilicum*

Comment [J28]: *Ocimum basilicum*

Comment [J29]: *Ocimum basilicum*

18. Thakur M, Bhatt V, Kumar R. Effect of shade level and mulch type on growth, yield and essential oil composition of damask rose (*Rosa damascena* Mill.) under mid hill conditions of Western Himalayas. PLoS ONE. 2019; 14(4): 1-14.

19. Turkmen O, Demir S, Sensoy S, Dursun A. Effect of arbuscular mycorrhizal fungus and humic acid on the seedling development and nutrient content of pepper grown under saline soil conditions. J Biol Sci. 2005;5(5): 565-574.

20. Vieira FR, Simon JE. Chemical characterization of basil (*Ocimum* spp.) based on volatile oils. Flavour Fragr J. 2006; 21: 214-221.

Comment [J30]: *Ocimum*

Comment [J31]: Update some of your old refernces and change your page layout orientation to potrait

UNDER PEER REVIEW