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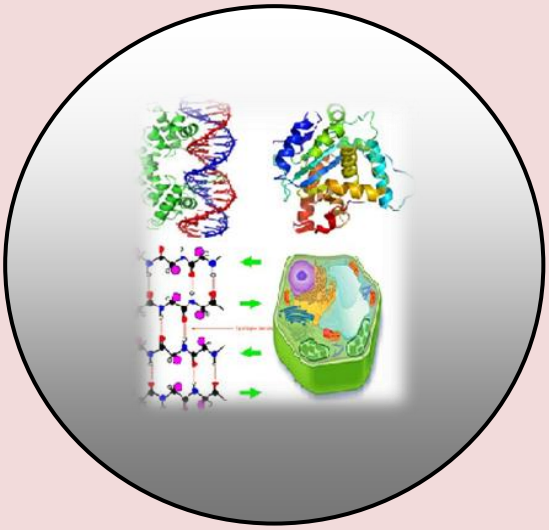

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Growth, Physio-biochemical and Yield Responses of two Menthol Mint Cultivars to Eight Leaf-applied Plant Growth Regulators

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ABSTRACT

Plant growth regulators (PGRs) modulate growth and development of plants differentially. This ability of PGRs was tested for Mentha arvensis L. (menthol mint) which is an important medicinal and aromatic plant. Thus, a complete factorial randomised pot experiment was conducted in a net house of the Department of Botany, Aligarh Muslim University, Aligarh, India. Three healthy and uniform looking suckers of cultivar Kosi or Kushal per pot were transplanted. The pots were filled with a mixture of soil and farmyard manure (4:1). The plants were sprayed thrice with 10^{-6}M solution each of eight structurally different PGRs, namely benzyl aminopurine (BAP), gibberellic acid (GA_3), indole acetic acid (IAA), indole butyric acid (IBA), kinetin (Kn), methyl jasmonate (MJ), salicylic acid (SA) and triacontanol (Tria).There was an additional water-sprayed treatment. First spray was applied at 60, second at 75 and third at 90 days after transplanting (DAT). Sampling was done at 100 and harvesting at 120 DAT. The spray of SA proved best particularly for herbage yield, essential oil (EO) content, EO yield per plant and menthol content. Cultivar Kushal performed better than Kosi particularly for menthol content and menthol yield per plant. The interaction treatment effect was noted to be significant for herbage yield per plant, with interactions SA× Kushal equalled by SA× Kosi giving maximum value.

Keywords: Menthol mint; plant growth regulators; growth; photosynthesis; antioxidant enzymes; essential oil and menthol.

INTRODUCTION

The medicinal and aromatic plants are important as their products are used in medicines, industries and as pharmaceutical products. Menthol mint, is an important MAP cultivated across globe for the extraction of useful products. In view of its increasing demand in various industries, the traditional cultural practices are not adequate. PGRs may play an important role for improving the performance of this crop. Moreover, the performance of its cultivars under local conditions has not been reported yet. Keeping these points in view, the present pot experiment was planned and executed on two cultivars of menthol mint.

MATERIALS AND METHODS

This pot experiment was performed during summer season. Three suckers of cultivar Kosi or Kushal per pot were transplanted to earthen pots (25× 25 cm) containing 5 Kg mixture of sandy loam soil and

farmyard manure in the ration 4:1. The soil mixture had available nitrogen (N), phosphorous (P) and potassium (K) at 97.3 mg N, 20.38 mg P_2O_5 and 179.80 mg K_2O kg^{-1} soil. A uniform recommended basal dose of N (as urea), P (as single superphosphate) and K (as muriate of potash) at 100 kg N + 50 kg P_2O_5 + 50 kg K_2O ha^{-1}, ($N_{100}P_{50}K_{50}$) equivalent to 44.6 mg N + 22.3 mg P_2O_5 and 22.3 mg K_2O kg^{-1} soil was applied (Gupta et al., 2004; Khanuja et al., 2006). Half of the N and full of P and K were applied at the time of transplanting and the remaining N was top-dressed in two splits, the first at 35 DAT and the second at 70 DAT. The plants were sprayed thrice with BAP, GA_3, IAA, IBA, Kn, MJ, SA or Tria at 10^{-6}M concentration. There was an addition water-sprayed treatment. The first, second and third spray were applied at 60, 75 and 90 DAT respectively. The required amount of IAA, IBA, Kn and BAP was dissolved in 10 mL 1N sodium hydroxide separately and the final volume was made 100mL with double distilled water (DDW). Similarly, the required quantity of GA_3 and MJ was dissolved in 10 mL ethanol separately and the final volume was maintained up to 100mL with DDW. The required quantity of SA and Tria was dissolved in 10 mL DDW separately and the final volume was maintained up to 100mL with DDW. The design of the experiment was factorial randomized. Each treatment was replicated five times. Watering of plants was done as and when required. The standard cultural practices were adopted for the crop husbandry. The sampling was done at 100 DAT and harvesting at 120 DAT.

Determination of growth biomarkers

One plant from each pot was sampled. The plant samples were washed with running tap water. After surface drying plant samples with blotting sheets, the growth biomarkers were determined. Leaf number per plant was noted manually. The leaf area was noted by a leaf area meter (ADC Bioscientific, Hoddesdon, Herts, UK). Leaf area per plant was calculated by multiplying the leaf number per plant and area per leaf. After cutting roots, the fresh weight per plant was obtained with the help of an electronic balance (Verbal 100 Super, Varanasi, Balance Works, Varanasi, India). After taking fresh weight, the samples were dried in a hot-air oven running at 80° C for 48 h and then their dry weight was determined on per plant basis.

Physio-biochemical parameters

The chlorophyll content was determined by using a Single Photoelectric Analysing Diode (SPAD) chlorophyll meter (SPAD-502; Konica Minolta Sensing Inc., Japan). Net photosynthetic rate (P_N), stomatal conductance (g_s), internal carbon dioxide (CO_2) concentration (C_i) and transpiration rate (E) were measured in fully expanded leaves of the plants at the sampling stage by using a portable photosynthesis system (LI-COR 6400, LI-COR, Lincoln, NE, USA). The activity of carbonic anhydrase (CA) was determined by the protocol of Dwivedi and Randhawa (1974). The activity of catalase (CAT) was estimated by measuring the disappearance of hydrogen peroxide (H_2O_2) as per the method of Aebi (1984). The peroxidise (POX) activity was determined by the method of Chance and Maehly (1956). The procedure of Kono (1978) was used for determining the activity of superoxide dismutase (SOD). The proline content was measured in freshly collected leaf samples by following the method of Bates et al. (1973). The leaf N content was estimated according to the method of Lindner (1944). The protocol of Fiske and Subba Row (1925) was used to estimate the total P content. The K content was determined by a flame-photometer (Model, ELICO, Cl22D, India) adopting the method of Hald (1946).

Yield parameters

At harvest, plant samples were washed with running tap water. After surface drying with blotting paper, the roots were cut and weight of the shoot per plant (herbage yield) was obtained. The essential oil (EO) content of menthol mint plants was extracted by using a Clevenger apparatus. The content of the EO was calculated by the following formula:

$$\text{Essential oil content (\%)} = \frac{\text{Oil extract (mL)}}{\text{Weight of leaf (g)}} \times 100$$

The EO yield per plant was computed on the basis of EO percentage and herbage yield per plant.

$$\text{Essential oil yield per plant (mL)} = \frac{\text{Essential oil content (\%)} \times \text{ Herbage yield per plant (g)}}{100}$$

The menthol content of EO was estimated using a gas liquid chromatography apparatus (Nucon 5700, New Delhi, India). The details are given in Khanam and Mohammad (2018). Menthol yield per plant was computed on the basis of its percentage and oil yield per plant.

$$\text{Menthol yield per plant (mL)} = \frac{\text{Menthol content (\%)} \times \text{ Oil yield per plant (g)}}{100}$$

Statistical analysis

Data were statistically analysed using SPSS, 17.0 for windows (SPSS, Chicago, IL, USA). Analysis of variance (ANOVA) was performed on the data. All the data are the mean of five replicates (n = 5) and vertical bars show standard error (± SE). Values of bars with the same letter are not significantly different, $P < 0.05$ Duncan's multiple range test.

RESULTS

The effect of spray treatments and their interactions with cultivars as also cultivar differences were found to be significant, except spray treatments on menthol yield per plant and interaction effect on leaf area per plant, enzyme activities, contents of proline, N and P, content and yield of EO and content and yield of menthol in EO, with cultivars not differing for leaf number, area per leaf, fresh and dry weight, P_N, gs, enzyme activities, contents of proline and nutrients, herbage yield, content and yield of EO.

The foliar application of SA proved best for leaf number, Kn as also IBA for area per leaf and GA_3 for leaf area per plant. The spray of SA gave maximum values for fresh and dry weight per plant. The leaf-applied SA improved leaf number by 23.81%, leaf area per plant by 3.56%, fresh weight per plant by 84.2% and dry weight per plant 12.6% over the water sprayed treatment.

The foliar spray of SA gave maximum values for SPAD, activities of CA, CAT, POX and SOD as also contents of proline, N, P and K, however spray of GA_3 registered highest values for P_N, gs and Ci. Tria application resulted in the maximum value for E. The application of SA improved SPAD value by 26.95%, P_N by 41.63%, gs by 3.35%, E by 33.17%, Ci by 24.69 %, CA activity by 37.89%, CAT activity by 7.59%, POX activity by 43.92%, SOD activity by 24.51%, proline content by 68.17%, N content by 84.29%, P content by 53.13% and K content by 176.03% over the water sprayed treatments.

The foliar application of SA gave maximum values for herbage yield per plant, EO content, EO yield per plant and menthol content of EO. The spray treatment of SA increased herbage yield per plant by 78.38%, EO content by 62.96%, EO yield per plant by 205.26% menthol content by 8.25% over the water sprayed treatment.

Cultivar Kushal gave higher values for leaf area per plant and dry weight per plant than Kosi. Cultivar Kushal registered 1.28 and 14.05% higher values for leaf area per plant and dry weight per plant respectively than Kosi.

Cultivar Kushal was better than Kosi for SPAD value and E. Cultivar Kushal exhibited 12.37% and 39.82% higher value for SPAD and E respectively than Kosi.

Cultivar Kushal proved superior to Kosi for menthol content and menthol yield per plant. Kushal exhibited 6.22 and 30.00% higher values for menthol content and menthol yield per plant respectively than Kosi.

Interactions SA × Kosi, Kn × Kushal, SA × Kushal and MJ × Kushal (also SA × Kushal) gave highest values for leaf number per plant, area per leaf, fresh weight per plant, and dry weight per plant respectively. Interaction SA × Kushal enhanced leaf number per plant by 9.61%, fresh weight per plant by 86.65% and dry weight per plant by 24.32% over Water × Kushal.

Interactions SA × Kushal gave the highest value for SPAD, GA_3 × Kushal for P_N, Gs and Ci and Tria × Kushal for E. Interaction SA × Kushal registered 31.20, 65.95, 1.90, 69.65, 24.06 and 167.46% higher value for SPAD, P_N, Gs, E, Ci and K content respectively than Water × Kushal.

Interactions SA × Kushal equalled by SA × Kosi gave highest values for herbage yield per plant. Interaction treatment SA × Kushal gave 67.79% higher value for herbage yield per plant than Water × Kushal.

DISCUSSION

The favourable effect of the eight structurally different leaf-applied PGRs on growth markers of two menthol mint cultivars is not beyond to understand. The amelioration in growth characters due to the spray of PGRs can be attributed to the fact that PGRs increases among others membrane permeability (Filek et al., 2004), cell division and enlargement (Taiz et al., 2015), hence higher values for growth parameters of PGRs treated plants. These results are in conformity with those of Naeem et al. (2011) on menthol mint, Khan et al. (2014) on lemongrass, Shukla et al. (1992) on Artemisia and Khanam and Mohammad (2017, 2018) on peppermint.

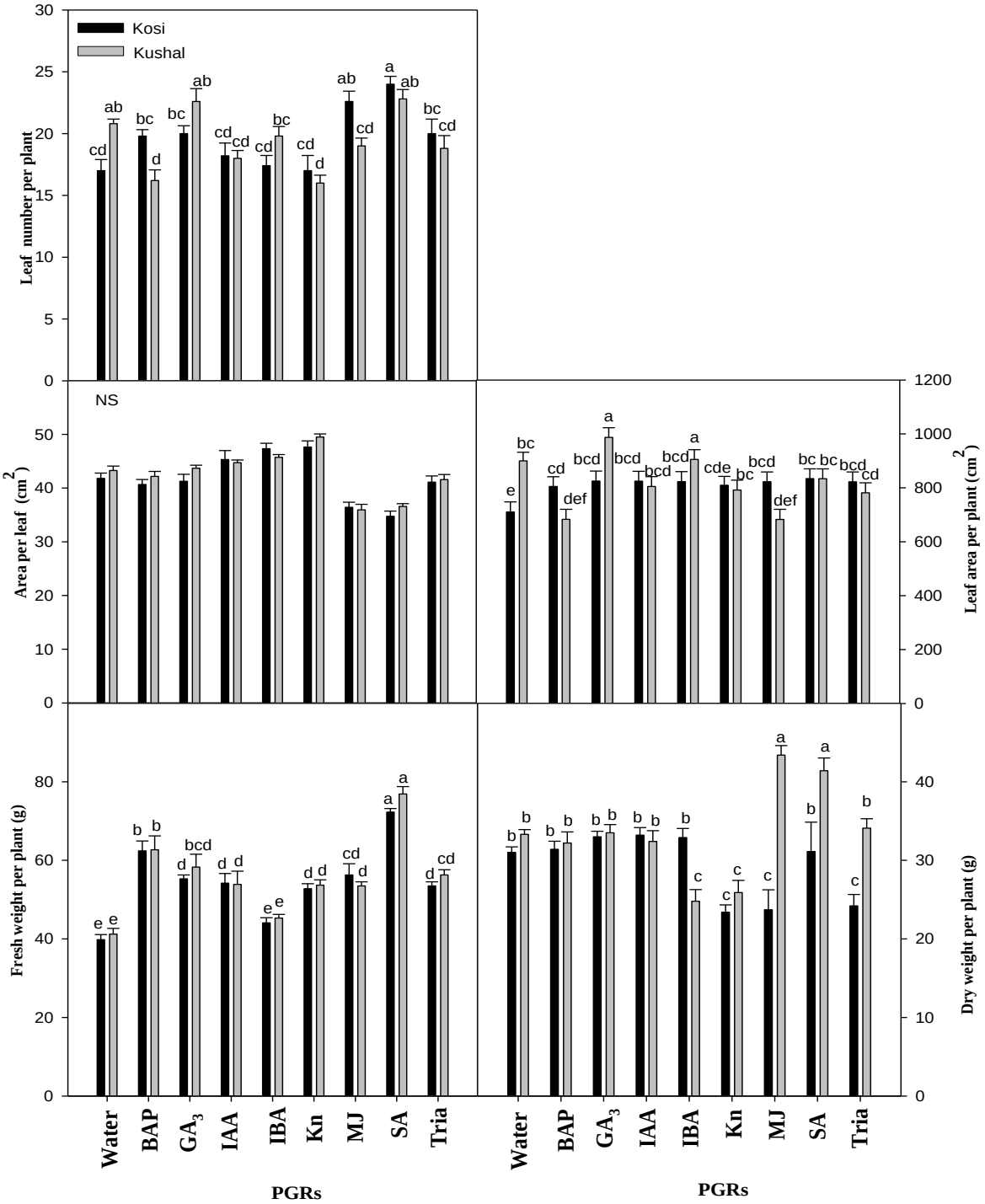

Figure 1. Effect of plant growth regulators on growth biomarkers of two menthol mint cultivars.

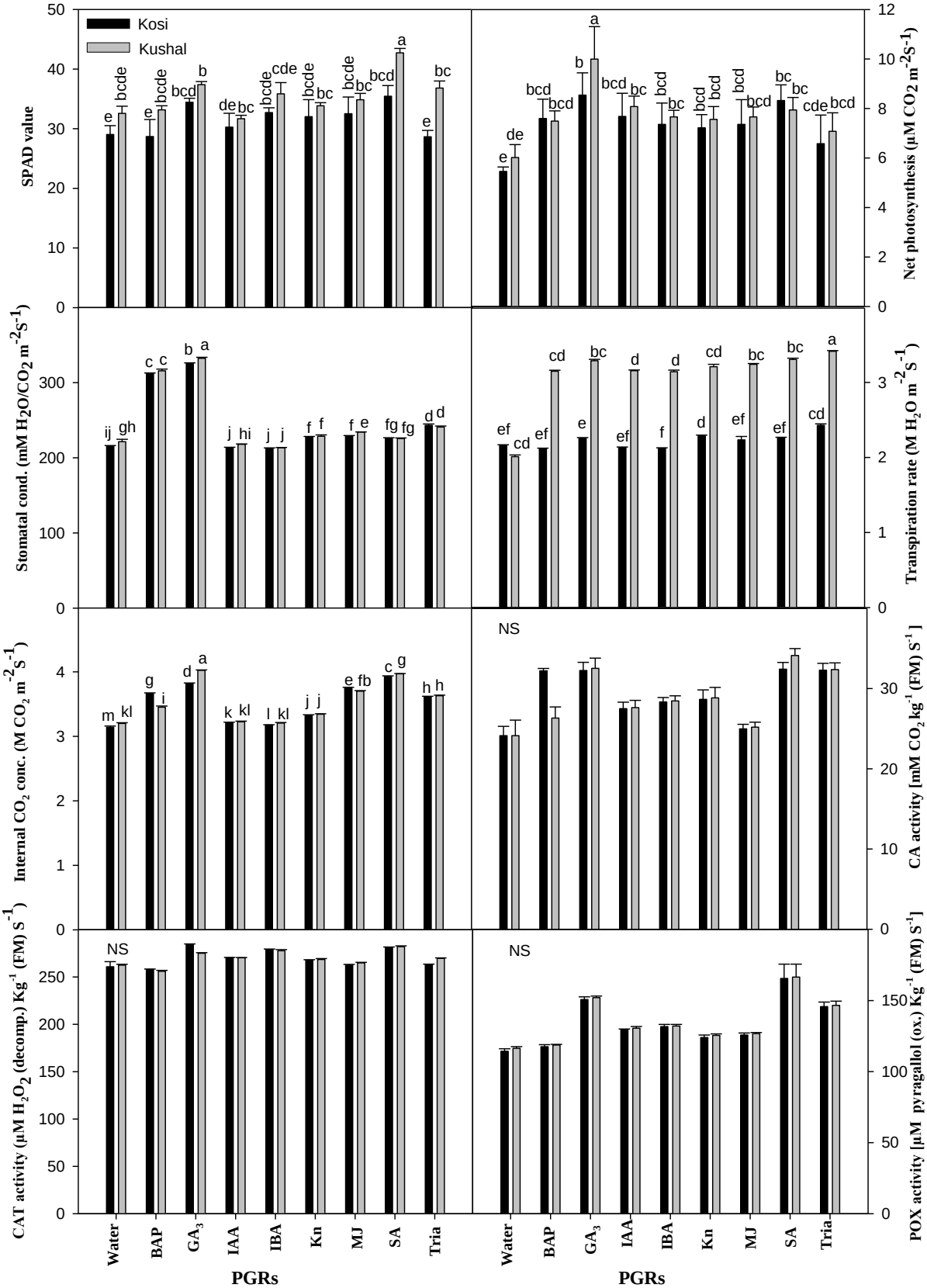

Fig 2. Effect of plant growth regulators on physio-biochemical attributes of two menthol mint cultivars.

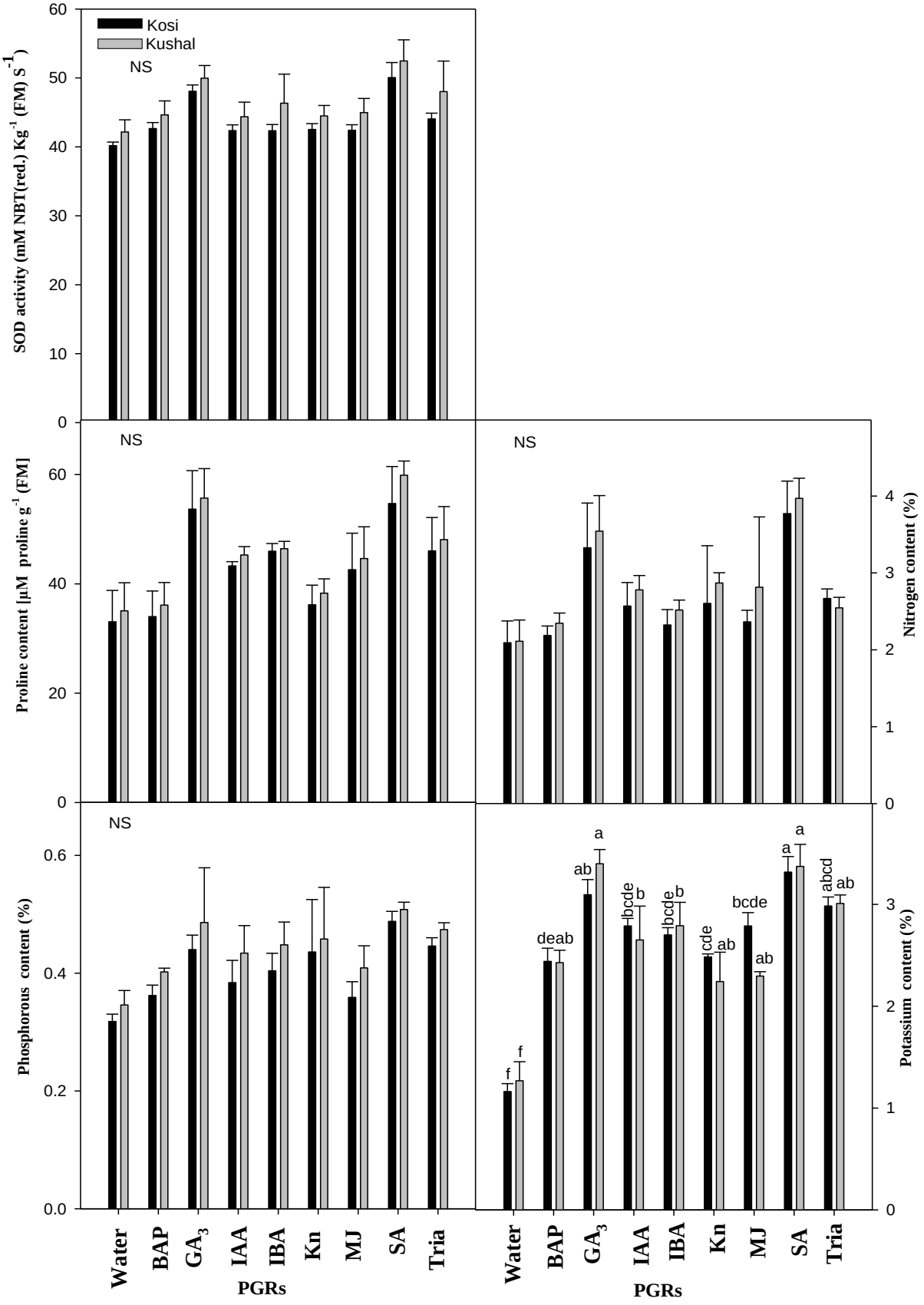

Figure 3. Effect of plant growth regulators on physio-biochemical attributes of two menthol mint cultivars.

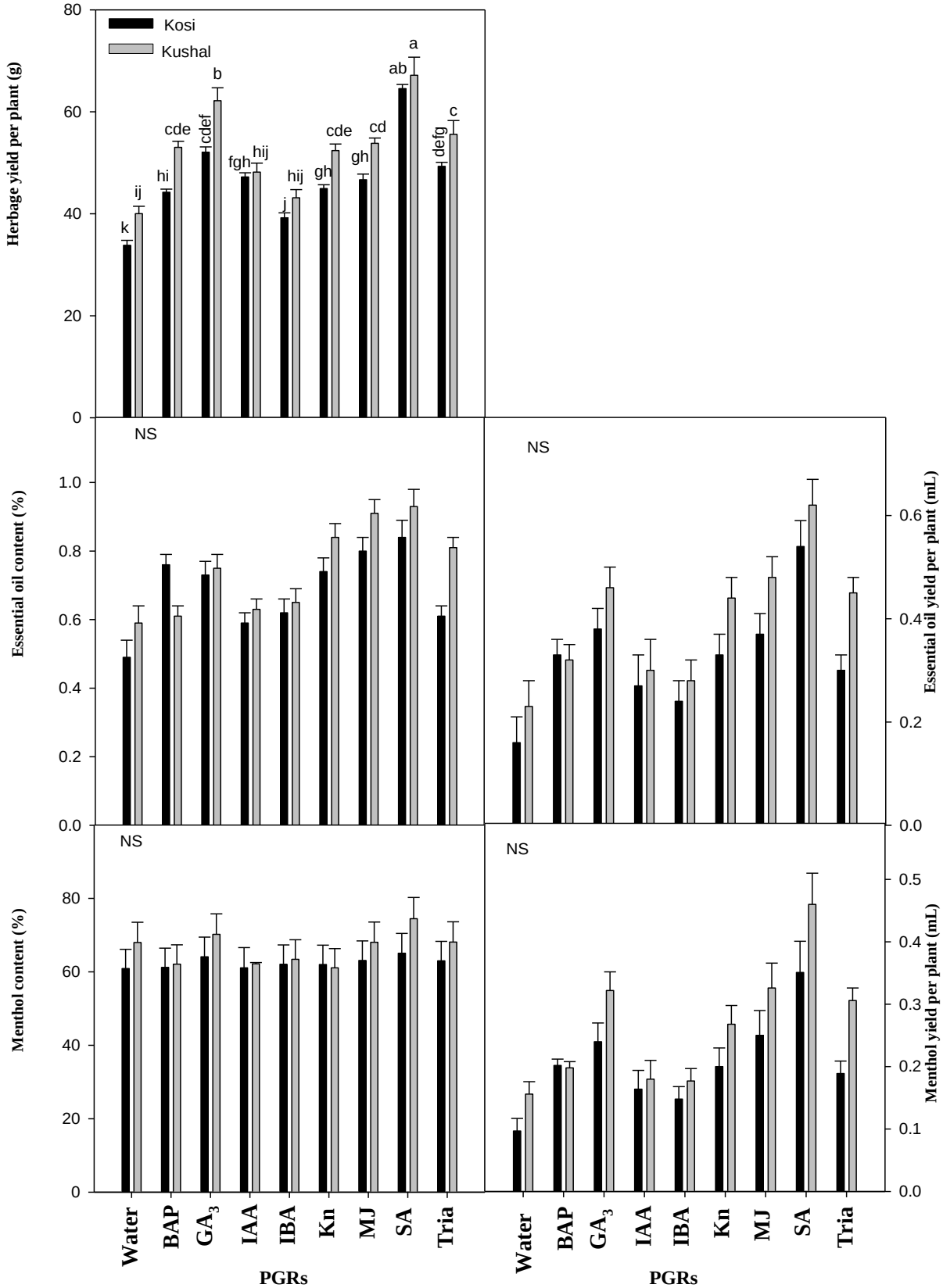

Figure 4. Effect of plant growth regulators on yield attributes of two menthol mint cultivars.

The ameliorative effect of PGRs on photosynthetic traits (Fig. 2) is self explanatory. The application of PGRs enhanced chlorophyll content, CA activity and E, which would have increased raw materials (CO_2 and H_2O) for photosynthesis, hence higher values for Ci, gs and finally P_N. These results corroborate with the findings of Aftab et al. (2010) on Artemisia, Naeem et al. (2011) on menthol mint and Khanam and Mohammad (2018) on peppermint. The up-regulation of antioxidant enzyme (CAT, POX and SOD) and non-enzymatic antioxidant (proline) on subjecting menthol mint plants to leaf-applied PGRs over the water-sprayed treatment (Figs 2 and 3) is not far to seek. The spray application of PGRs would have produced RNAs responsible for expressing genes which would have led to the synthesis of proteins including antioxidant enzymes as also proline synthesizing enzymes in comparatively greater quantity, hence higher values for activities of these enzymes and proline content in PGR treated plants. Jaleel et al. (2010) and Khanam and Mohammad (2018) working on periwinkle and peppermint respectively also reported higher values of these enzymes on treating plants with PGRs. The PGR-mediated improvement in leaf nutrient contents (Fig. 3) may be attributed to the enhance permeability resulted from the spray of regulators. Our findings on the improvement in leaf nutrients due to spray of PGRs are in accordance with the results of Aftab et al. (2010) on Artemisia, Naeem et al., (2011) on menthol mint, Ghazijahani et al. (2014) on sweet basil and Khanam and Mohammad (2018) on peppermint.

The positive effect of PGRs on herbage yield, EO content and yield and menthol content (Fig. 4) may be due to the improvement in overall plant growth metabolism of treated plants. These results are in accordance with the results of Khanam and Mohammad (2017, 2018) and Ahmad et al. (2019) on peppermint. The surpassing effect of SA spray on growth performance, photosynthetic characters, antioxidant enzymes and non-enzymatic antioxidant (proline), leaf nutrient contents and yield and quality characters over other PGRs (Figs.1-4) can be traced to its specific roles among others in maintaining of constant supply of auxins and gibberellins (Tomaszewski and Thimman, 1966), prevention of oxidation of auxin (Schneider and Wightman, 1974), inhibition of biosynthesis of ethylene (Leslie and Romani, 1986) and involvement in signal transduction pathways (Rivas-San Vicente and Plasencia, 2011). The superiority of SA over other PGRs has also been reported by Khanam and Mohammad (2017, 2018).

The better performance of cultivar Kushal over Kosi particularly for leaf area per plant, dry weight per plant, chlorophyll content, POX activity and menthol content studied at 100 and 120 DAT (Figs. 1-4) can be ascribed to the difference in their genetic constitution. These results broadly corroborated with the findings of Khanam (2018) on peppermint.

CONCLUSION

The foliar applications of PGRs improve the overall growth and development of menthol mint cultivars. Among the various PGR foliar treatments, spray of SA particularly on cultivar Kushal proved to be best as far as its performance is concerned.

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