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Influence of zinc and copper on morphological characters for improving herbage yield of vegetatively propagated *Bacopa monnieri* (L.)

S Tamta, A Kumar, R Kumar, SC Shankhdhar and D Shankhdhar

Abstract
Micronutrient affect growth, development, flowering and plant yield, as they alter many basic physiological processes, including photosynthesis and respiration. Foliar application is a promising agronomic strategy as it involves direct adsorption and loading of nutrients from leaf surface to phloem in comparatively far less quantity than soil applications. Cu and Zn are essential trace nutrients taking part in redox reactions, structural configuration of several enzymes and nucleic acid metabolism. Present investigation entails the evaluation of most suitable treatment of zinc sulphate and copper sulphate to improve growth and yield of *Bacopa*. However, the effects of the combinations of these micronutrients were significant and the growth response to these micronutrients was clearly dependent upon their concentrations. It is concluded that all the growth parameters mostly pronounced at T1 (1ppm $CuSO_4$) and T5 (2.5ppm $ZnSO_4$) alone and T11 (1ppm $CuSO_4$+2.5ppm $ZnSO_4$) in combination when compared to untreated plants during Monsoon and Winter season (2019-2020). These treatments result in maximum number of upright shoots per m^2, length of upright shoots (cm), number of leaves per m^2 that leads to improved herbage yield (gm^{-2}). However, foliar treatments before 30 days of harvesting were found appropriate for rapid proliferation of foliage that give improved herbage yield of *Bacopa* cultivation.

Keywords: *Bacopa*, zinc, copper, herbage yield

Introduction

Bacopa monnieri L. commonly referred as “Brahmi” belongs to family Scrophulariaceae, a perennial and creeping herb that found in wet, damp, and marshy areas and forms a dense mat of vegetation on soil surface. The whole herb is a major source of a raw material for the production of drugs and herbal formulations. The best characterized phytochemicals in *Bacopa monnieri* are dammarane-type triterpenoid saponins known as bacosides, with jujubogenin or pseudo-jujubogenin moieties as aglycone units. It has high medicinal value and is second in the list of most essential Indian medicinal plants [1-3]. Therefore, the cultivation of *Bacopa monnieri* needs special attention. Balanced supply of essential nutrients is one of the most important factors in improving crop quality and increasing yields. Micronutrients (Zn, Cu, B, Fe and Mn) are as essential as macronutrients (N, P and K) but required in minute status for the normal growth of plant. Zinc (Zn) is required for the catalytic activity of different metabolic enzymes, including dehydrogenases, aldolases, isomerases, transphosphorylases, RNA and DNA polymerases, and it is also involved in the synthesis of tryptophan, cell division, maintenance of membrane structure and potential, and photosynthesis, and acts as a regulatory cofactor in protein synthesis [4]**.** However, copper (Cu) is required for physiological and biological functions, protein trafficking, antioxidative activity and hormone signalling of plants [5]. Combined effect of two nutrients may be synergistic or antagonistic for example, Zn strongly depresses Cu absorption, Cu competitively inhibits Zn absorption and Cu nutrition affects the redistribution of Zn within plants [6]**.** Scarce information is available in the literature regarding the effect of the Zn and Cu sole/combine with *Bacopa* yield. This means that in-depth research needs to be performed to determine the appropriate concentration (sole/ combined) for a growth of plants. For getting desirable therapeutic benefits of this medicinally important plant and its continuous depletion in wild, commercial cultivation, an urgent need for quick assessment of various essential nutrients is must as these are the elicitors for growth and also helps in enhancing the metabolites in plants. Hence, during investigation we were tried to explore effects of different levels of zinc and copper either alone or in combinations on *Bacopa* herbage yield grown during monsoon and winter season.

Material and Methods

The experiment was conducted in the department of Plant Physiology, College of basic sciences and humanities, GBPUA&T, Pantnagar, (Uttarakhand)during the monsoon and winter seasons of 2019-2020.The climate of the site retains humid subtropical with hot and dry summers and cool winters. Winter season extend from November to March while the monsoon sets during 2nd and 3rd week of June and continues till the end September. Temperature is highest in the months of May-June and lowest in the month of December-Jan. The experimental field (10 x 5 m^2) was prepared with 75 X 50cm^2 plots and between each replication, an irrigation channel of 25 cm was made. Three stolons of 5-7 cm were planted 25 cm spacing in each plot in the month of July as monsoon season crop and harvested in Oct and Nov. Regenerates were allowed to grow further for winter season and harvested in March and April. The first irrigation was applied just after planting following with subsequent irrigations were provided as and when the soil show dryness. Manual weeding was done to check the weed flora. The foliar treatments were imposed after complete establishment of crop. *Bacopa* crop is ready to harvest in three and four months i.e., 90-120 days. Hence, the first and second foliar application was applied before 30 days of harvest. Freshly prepared solution of the Copper sulphate ($CuSO_4.H_20$) and Zinc sulphate ($ZnSO_4.H_20$) alone and in combinations of both were given as treatments [T0:(CONTROL); T1: (0.5ppm $CuSO_4$); T2: (1ppm $CuSO_4$), T3: (1.25ppm $ZnSO_4$); T4: (2ppm $CuSO_4$); T5: (2.5ppm $ZnSO_4$);T6: (5ppm $ZnSO_4$);T7: (0.5ppm $CuSO_4$+1.25ppm $ZnSO_4$); T8: (0.5ppm $CuSO_4$+2.5ppm $ZnSO_4$); T9: (0.5ppm $CuSO_4$+5ppm $ZnSO_4$); T10: (1ppm $CuSO_4$+1.25ppm $ZnSO_4$);T11: (1ppm $CuSO_4$+2.5ppm $ZnSO_4$); T12: (1ppm $CuSO_4$+5ppm $ZnSO_4$); T13: (2ppm $CuSO_4$+1.25ppm $ZnSO_4$); T14: (2ppm $CuSO_4$+2.5ppm $ZnSO_4$); T15:(2ppm $CuSO_4$+5ppm $ZnSO_4$)].

Sample collection

The plant samples were collected at thirty days interval after each spray for the measurement of various morphological growth parameters (number of shoots, shoot length, number of leaves and herbage yield). A 20 cm^2 quadrant was used for collecting the samples for measuring the yield from each plot and finally expressed in per meter square.

Number of upright shoots per meter square

At the time of harvest, 20 cm^2 quadrant is placed over well-established foliage surface and upright shoots were counted. The total number of upright shoots were finally calculated in per m^2

Upright shoot length (cm)

Three longest upright shoots from each replication were selected randomly and measured with the help of cm scale and expressed in cm.

Number of leaves per meter square

One of the longest upright shoot was selected and the leaves were counted. The counted number of leaves multiplied with the total number of shoots (measured above); this helps in calculating total number of leaves that later expressed in per m^2.

Herbage yield

Before sample collection flooding was done for easy harvesting and the whole crop was carefully uprooted with the help of khurpi/Trowel from 20 cm^2 quadrant from each replication. The fresh plant material was washed out properly with tap water to remove soil particle. Fresh herbage yield was measured with the help of weighing balance and then the sample was dried in oven until the dried herb yield become constant. The parameter recorded and expressed in gram per meter square.

Statistical Analysis

Experiment was performed in completely randomized block design **(**CRBD) with three replications for each treatment. ANOVA of collected data was calculated by using statistic 10 software. Means were compared with least significant difference (LSD) at 5% probability level ($P \leq 0.05$).

Results and Discussion

The succulent stem cuttings planted in the field readily rooted and produced a number of branches/shoots. Under the hot and humid conditions of monsoon month, a rapid proliferation of branches was observed and the entire soil surface became covered with a network of foliage several cm thick. At the onset of winter, the crop entered into senescence which continued until the onset of spring in Feb-March. The crop renewed growth after it. The proliferation of multiple shoots and number of leaves was higher in monsoon season crop whereas in winter crop showed less foliage growth that affect the whole yield. But after micronutrient exposure, winter season crop gives a better herbage yield over untreated plant. It was concluded from the present experiment that the growth parameters were significantly increased by moderate level of zinc and copper when applied individually though the higher doses gave reduced growth but not exhibit decreased values in comparison to control.

Number of upright shoots per m^2

The graphical presentation of data (Fig.1) indicated that number of shoots per m^2 increased significantly in comparison of control after each spraying of different level of Zn and Cu alone/combine. After first foliar spray the number of shoots per m^2 was significantly increased due to application of T8and T14 showed similar mean value (86.67 per m^2) during Monsoon crop while in winter crop, highly significant values (78.33 per m^2) when *Bacopa* treated with T5. Whereas the minimum number of shoots appeared in the control (53.33 and 43.33 per m^2) of each season harvest. Among the different treatments, second foliar spray of T5 showed significant elevation that reached to (93.33 per m^2) in Monsoon crop. It can be seen that the number of upright shoots showed highest mean value (78.33 per m^2) at T5 and T11 during winter crop. While the minimum number of shoots appeared in the control (56.67 and 45.00 per m^2) in both season harvest. It is found that 2.5ppm $ZnSO_4$ alone and if combine with 0.5ppm, 1ppm and 2ppm $CuSO_4$ given in treatments (T8, T11 and T14) showed the increment in number of shoots per meter square. Zinc overcome the negative effect of individually applied Cu (T4) and recorded better foliage proliferation.

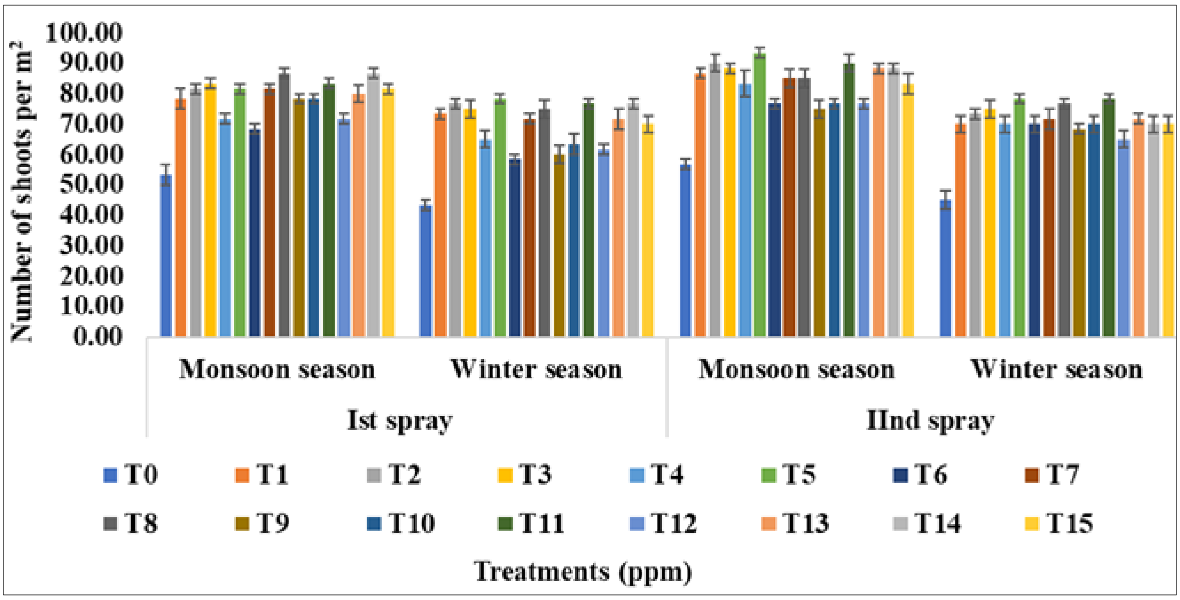

Fig 1: Effect of Zn and Cu on the Number of shoots per m² during monsoon and winter season crop (2019-2020). Data are mean of three replications ± standard deviation. Vertical bars are the standard error of mean (n=3).

Length of upright shoot (Cm)

The graphical representation of data (Fig.2) showed enhanced length of shoots exposed to different level of Zn and Cu alone/combine in comparison to control. This has been recorded that after first foliar spray, T2application significantly affect the length of shoot with maximum average value (22.67cm) over control (14 cm) during monsoon season. The data regarding winter season T5, T11 and T13 respectively, exerted a positive effect on length of shoot having similar maximum value (13.83 cm) in *Bacopa* plant over control (9.33 cm). Among the treatments, during monsoon season T11 showed increased shoot length (23.17cm) after second foliar spray as compared to control (14.67 cm). During winter season, T11 recorded the maximum length of shoot (18.33 cm) followed by T5 (18.17 cm) in comparison to control (11.33 cm).

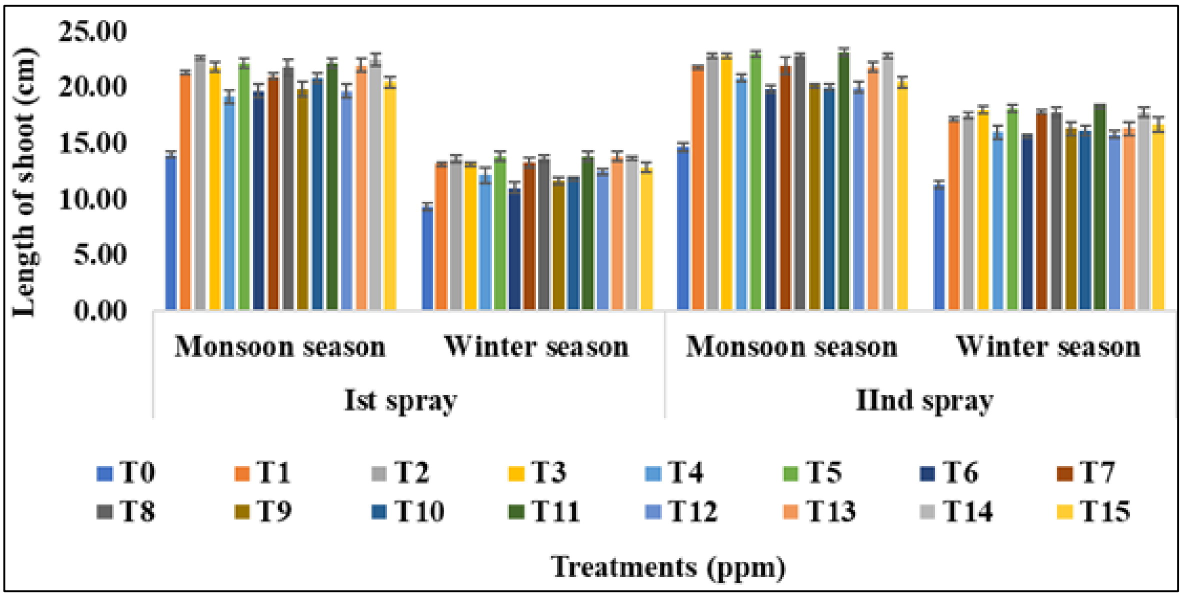

Fig 2: Effect of Zn and Cu on the Length of shoot (cm) during monsoon and winter season crop (2019-2020). Data are mean of three replications ± standard deviation. Vertical bars are the standard error of mean (n=3).

Number of leaves per m^2

During monsoon season, the maximum number of leaves (7,083.33 per m^2) was recorded at T3 when compared with control (3550 per m^2) after first spray represented data in (Fig 3). In winter season T8 showed high significance in number of leaves (5366.67 per m^2) which is statistically at par with T5 (5,358.33 per m^2) over control (1875per m^2). After second foliar spray, maximum number of leaves was observed in T5 (6,991.67 per m^2) followed by T2 (6,758.33per m^2), T8 (6,508.33 per m^2), T2 (6,508.33 per m^2) and T3 (6483.33 per m^2) respectively, at monsoon harvest. An increase in leaf numbers appeared in T5 (5875 per m^2) followed by T11 (5741.67 per m^2) treatments during winter harvest. However, minimum number of leaves was obtained in control (3875 and 2391.67per m^2) after monsoon and winter season harvest. It seems that the combination of Cu and Zn caused more positive effect when Zn applied in higher dose than Cu given in T8 and T11 treatment.

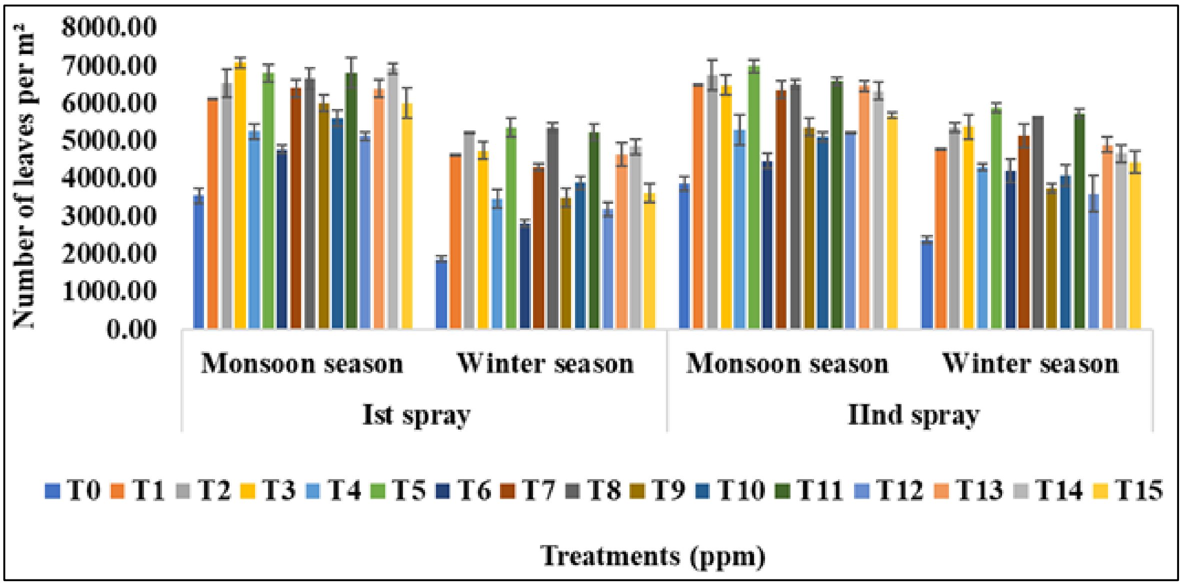

Fig 3: Effect of Zn and Cu treatment on the Number of leaves per m² during monsoon and winter season crop (2019-2020). Data are mean of three replications ± standard deviation. Vertical bars are the standard error of mean (n=3)

Herbage yield (gm^{-2})

In the present investigation all the above growth components were found significant under different concentrations and combinations of Zn and Cu when compared to untreated plants that ultimately result in higher herbage yield. Result presented in (Fig.4.), at monsoon harvest, plants treated with T5 showed significant higher mean value (210.38 gm^{-2}) of fresh herbage yield after first spray when compared to control (149.02 gm^{-2}). While the observation of winter crop, noted closely similar maximum values (152.33 gm^{-2}), (151.42 gm^{-2}) with T8 and T5over control (110.82 gm^{-2}). However, after second spray T5 registered best in fresh herbage yield (210.02 gm^{-2}) in comparison to control (146.82 gm^{-2}) during monsoon season. Among the treatments, T2 (164.23 g m^{-2}) followed by T5 (163.28 gm^{-2}) revealed higher average values of fresh herbage yield when compared with control (110.07 gm^{-2}) during winter season harvest. Data given (Fig.5), the dry herbage yield was significantly increased at T5 having maximum average values (22.43 gm^{-2}) after first spray at monsoon harvest over control (11.95 gm^{-2}). It was noticed that during winter season maximum increment (17.93 gm^{-2}) in dry herbage yield was recorded at T2 followed by T5 concentrations that reached (17.28 gm^{-2}) with comparison to control (12.25 gm^{-2}). Among the second foliar treatments, the dry herbage yield was significantly increased at T5 having maximum average values (23.25 gm^{-2}) in Monsoon crop. During winter season, dry herbage yield to be increased by (18.70 gm^{-2}) with T2 treatment. Minimum yield (16.22 gm^{-2}) and (11.87 gm^{-2}) showed in untreated plant during both seasons.

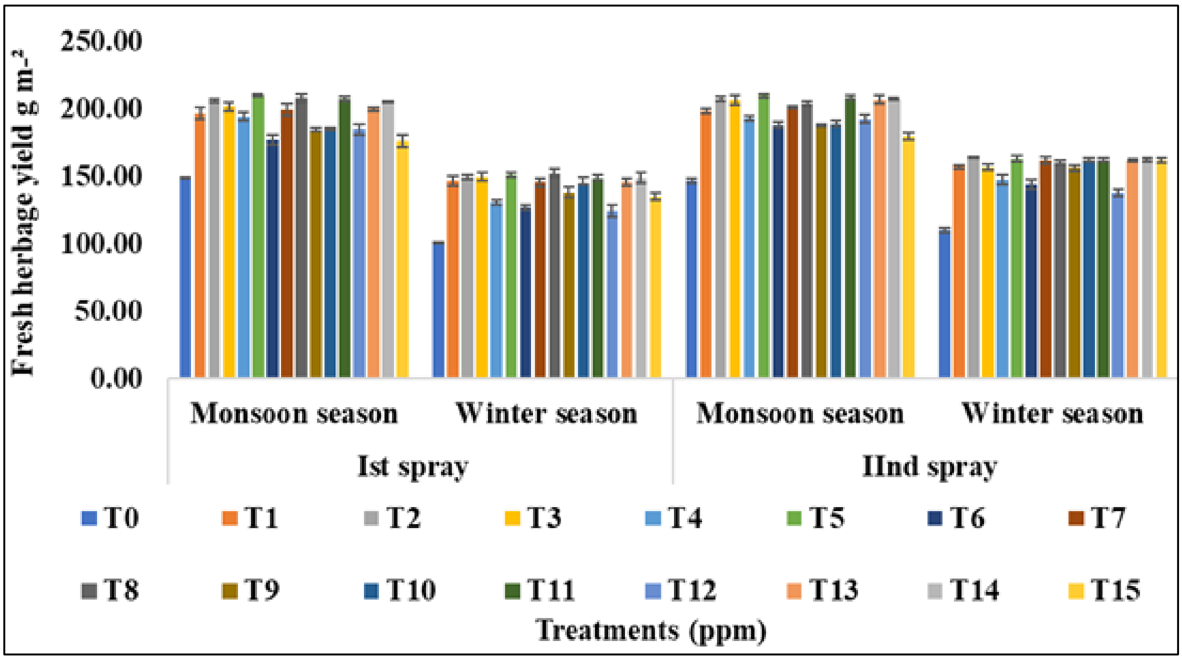

Fig 4: Effect of Zn and Cu treatment on the Fresh herbage yield g m^{-2} during monsoon and winter season crop (2019-2020). Data are mean of three replications ± standard deviation. Vertical bars are the standard error of mean (n=3).

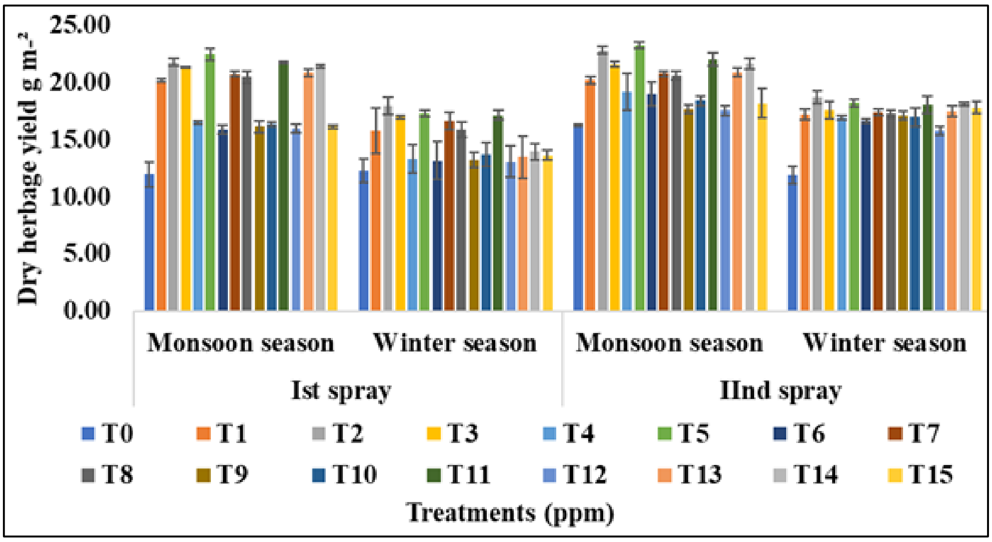

Fig 5: Effect of Zn and Cu treatment on the Dry herbage yield g m^{-2} during monsoon and winter season crop (2019-2020). Data are mean of three replications ± standard deviation. Vertical bars are the standard error of mean (n=3).

Foliar application enhanced the uptake of nutrients through aerial parts of plant and improves overall growth of plant. This may be the reason for high number of leaves and a greater number of shoots and improved shoot length that leads to higher biomass production. Zn role on protein synthesis, cytochrome function, carbohydrate, nucleic acid and lipid metabolism Radić *et al.* (2010) [7] and Mousavi *et al.* (2012) [8]. Cu is also an essential element and involved in photosynthesis, respiration, oxidative stress protection and cell wall synthesis (H€ansch and Mendel (2009) [9] and Broadley *et al.* (2012) [10]. According to our observations highest number of shoots, number of leaves and shoot length were mostly obtained with T2, T5 and T11 that ultimately improved herbage yield. A good foliage surface could be related to the improved physiology of plants like photosynthesis and enhanced nutrient uptake that give higher crop yield. Plant elongation may be due to increased cell division and cell elongation is an important morphological parameter, mainly for fresh produces. Earlier, attempt also related with our experiment, varying concentration of micronutrient *in vitro* observed number of adventitious shoots, fresh weight and dry weight *of Bacopa monnieri* were obtained in the medium with 0.12 mM Zn concentration Naik *et al.* (2015) [11]. The present study draws support from Chowdhary *et al.* (2016) [12], *in vitro* regenerated multiple shoot cultures of *Bacopa monnieri* in which$CuSO_4$ at varying concentrations noted mean number of shoots was maximum in the treatment of Cu over control. Furthermore, our findings match by previous research, reporting growth parameters of gladiolus, maximum number of leaves/hill (12.58) was observed in the treatment combination Zn 0.4% + Cu 0.2% followed by Zn 0.4% + Cu0.4%, Zn 0.2% + Cu 0.2% and Zn 0.2% + Cu 0.4% which was significant to the other treatment. Control plant (treated with distilled water) recorded minimum number of leaves/hill (9.92) followed by Cu 0.4%, Cu 0.2%, Zn 0.4% and Zn0.2% Singh *et al.* (2015)[13].Similar observation has also been made by Singh *et al*. (2012)[14] who carried out an experiment on gladiolus and reported that all the studied growth parameters were like height of plant, length of leaves, maximum number of leaves and width of leaves, fresh weight and dry weight were enhanced significantly by addition of the combination of zinc and copper**.** Although micronutrients are essential for plant's growth and development, their concentrations should be applied in plant need ranges because adverse effects may identified in higher concentrations. In our investigation number of shoots, leaves and shoot length of *Bacopa* plants were maximum between 1.25-2.5ppm $ZnSO_4$and 0.5-1ppmof $CuSO_4$. But showed a lower value when there was a further increase in Cu and Zn level (2ppm $CuSO_4$and 5ppm $ZnSO_4$). The low value due to excess of copper and zinc that might reduction in cell division, toxic effect on photosynthesis, respiration and protein synthesis. Earlier it was reported, the higher concentration of copper (3.2 µM) leads to 1.97 and 1.78 times reduced fresh (12.5 g plant^{-1}) and dry biomass (1.32 g plant^{-1}), respectively in *Centella asiatica* Prasad *et al.* (2018) [15]. Similar observations in *Sesuvium portulacastrum* indicated that root and shoot length, number of leaves, total leaf area and dry weight of root and shoot increased at low levels (copper, 100-200 mg kg^{-1} and zinc, 100-300 mg kg^{-1}) and decreased at high levels (copper, 300-600 mg kg^{-1} and zinc, 400-600 mg kg^{-1}) Kalaikandhan *et al.* (2014) [16]. The decrease in biomass in excess of micronutrient might be due to low protein formation, resulting in the inhibition of photosynthesis, as well as hampering carbohydrate translocation Manivasagaperumal *et al.*, (2011) [17]. But supplementation of micronutrient in combination helps in ameliorated the adverse effects of high level of Cu and Zn. In present experiment, Zn and Cu when applied in combination givenT8, T9, T11, T12, T13, T14 and T15 treatments showed highest values of growth parameters when compared with high level of single T4 and T6 treatments. These findings are in agreement with the result of Abbasifar *et al.*, (2020) [18] who carried out an experiment on Basil and reported that all the studied growth parameters were increased significantly on foliar application of zinc and copper nanoparticles (NPs) sole/combine. The positive effect of zinc fertilizer on auxin biosynthesis that can stimulate cell division and better absorption of minerals and thus increase the plant growth Cakmak (2000) [19], El-Tohamy and El-Greadly (2007) [20]. The increase in growth parameters of the plants as a result of using copper is due to the role of this element in the formation of regulatory proteins as well as in mitochondrial respiration and hormone signalling Singh *et al.*, (2017) [21]. Thus, micronutrients help in the biosynthesis of photo-assimilates and increase in various plant metabolites responsible for cell division and elongation resulting in an increased plant growth characteristic. An increase might be due to the active involvement of these elements in chlorophyll synthesis, cell division, meristematic activity of the tissue, and expansion of cells. The results from the present studies also indicate that temperature and humidity may also affect Brahmi's growth. Phrompittayarat, *et al.* (2011) [22], Ganjewala *et al.* (2001) [23] and Mathur *et al.* (2000) [24] reported that the lowest growth of Brahmi was found in winter harvest which is in agreement to our findings.

Conclusion

Data obtained from the present study on the various morphological parameters of *Bacopa monnieri* such as number of shoots, shoot length, number of leaves and herb yield showed positive response to foliarly applied Cu and Zn in both the seasons. Though the growth of new shoots and leaves was slow during winter crop but proliferate better in comparison to control when Zn and Cu as T1 (1ppm $CuSO_4$), T5 (2.5ppm $ZnSO_4$) and T11 (1ppm $CuSO_4$+2.5ppm $ZnSO_4$) were applied prior to one month of harvest. Moreover, further studies of micronutrients are required under field conditions in order to acquire the secondary metabolites production and better herbage yield in *Bacopa* and other herbal crops.

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