



Responsiveness of Cauliflower (*Brassica oleracea* var. *botrytis* L.) to micronutrients

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Abstract

Cauliflower (*Brassica oleracea* L. var. *botrytis*) a cool season vegetable which belongs to the family Cruciferae or Brassicaceae. It is grown in winter season. It is originated from Europe and developed from broccoli. It is a vegetable crop grown for its edible white and tender curd developed from short floral parts. It is very sensitive to low temperature and high heat. The aim of this study is to determine the effect of foliar application of boron, iron and molybdenum on cauliflower growth, quality and yield. The study was carried out in Vegetable Research Area of Institute of Horticultural Sciences, University of Agriculture Faisalabad in 2019-2020. Seedlings of 30 days were raised then transplanted to field in Randomized Complete Block Design (RCBD) using three replicates. Different doses of micronutrients were applied as foliar spray like boron (0.2%, 0.5%), iron (0.2%, 0.5%) and molybdenum (0.1%, 0.3%) on cauliflower. The data for different vegetative and biochemical parameters including plant height (cm), number of leaves plant⁻¹, number of days taken from transplantation to curd formation, days to curd initiation to maturity, foliage fresh weight (g), foliage dry weight (g), curd weight (g), curd area (cm²), leaf area (cm²), curd color, vitamin C (mg/g), chlorophyll content (spad meter), total soluble salts (Brix°), yield per plant (kg) and yield per hectare (ton) was recorded and statistically analyzed. It can be concluded that among all the treatments T₇ (Mo: 0.1% + Fe: 0.5% + B: 0.5%) showed the best result for all parameters.

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Introduction

Cauliflower (*Brassica oleracea* L. var. *botrytis*) a cool season vegetable which belongs to the family Cruciferae or Brassicaceae. It is grown in winter season. It is originated from Europe and developed from broccoli (Anonymous, 2017). It is a vegetable crop grown for its edible white curd which is developed by short floral parts. Cauliflower is very sensitive to lowest and highest temperature but it grows best in cool area (Sani *et al.*, 2018).

Cauliflower (*Brassica oleracea* L. var. *botrytis*) is grown all over the world but mostly in Bangladesh. Recently in 2016 it was reported that China and India globally producing 25.2 MT of cauliflower while the United States, Spain, Mexico and Italy are secondary producers which are generating 0.4 to 1.3 MT productions annually (FAOSTAT, 2017). Among all these countries, Pakistan is 10th producer of cauliflower, generating 209010 tonnes production by 11420 hectare cultivated area annually (Anjum *et al.*, 2016). Cauliflower is a very tasty crop which is a source of different vitamins including thiamine, riboflavin, nicotinic acid and minerals like magnesium and calcium and a good amount of proteins as well (Yadav *et al.*, 2014). Most of the crops need macro and micronutrients in specific amounts to grow well and to produce maximum production as well. The micronutrients are very important as macronutrients. Plant needs micronutrients in small amount that are required to produce good growth, quality and yield of the crop. Their trace amount can efficiently increase the overall crop production (Yadav *et al.*, 2018).

The micronutrients that are needed to plants in trace amount are boron, copper, manganese, iron, zinc, molybdenum and chlorides which can be available to plant by soil, fertilizers or by other sources of these nutrients (Dubey *et al.*, 2015). The micronutrients act as catalyst to improve the different biochemical reactions in the plant that triggered the plant growth and production (Karthick *et al.*, 2018). However, deficiency of trace elements can induce different physiological disorders which leads towards the reduction in plants growth and ultimately in quality and production of crops (Sharma and Kumar, 2016).

Boron primarily improves the calcium metabolism and its solubility and also assists in uptake of nitrogen by plants (Pandav *et al.*, 2016). Another microelement, the molybdenum is required to plants for nitrates assimilation and the atmospheric nitrogen fixation, it is also involve in processes of sulphur metabolism and proteins synthesis. Its trace amount positively affects the formation of carotenoids and also helps in translocation of iron element in plants (Das, 2018).

Iron, the essential trace element needed by plants for their proper functioning. It plays a vital role in chlorophyll synthesis and assists in uptake of other essential elements (Pandev *et al.*, 2016). However, the micronutrients play vital role in better growth and development of crops. The production and quality of crops is decreasing, therefore micronutrient applications to maintain health of the soil, crop production and quality as well are the immense needs of the era. Micronutrients are helpful in improving growth, yield, fruit setting, post-harvest life of crops and resistance development against stresses.

Therefore, the present research was conducted to examine the effect of different doses of micronutrients like boron (B), molybdenum (Mo) and iron (Fe) on cauliflower growth, quality and yield.

Materials and methods

The Study was conducted at Vegetable Research Area, Institute of Horticultural Sciences, University of Agriculture Faisalabad during the year 2019-2020. Cauliflower “Shumaila” hybrid seeds were obtained from Al- Rai seed store, Dajkot Road Faisalabad. Seedlings were raised and then transplanted after 30 days of sowing to field.

The different levels of boron, iron and molybdenum and their combinations with different concentrations were applied as foliar at 30, 45 and 60 days after transplantation of seedling. Transplantation of seedlings was done on ridges. Different concentrations and combinations of boron, iron and molybdenum were prepared at the rate of T₀ = Control, T₁ = Mo: 0.1%, T₂ = Mo: 0.3%, T₃ = Fe: 0.5%, T₄ = Fe: 0.2%, T₅ = B: 0.2%, T₆ = B: 0.5%, T₇ = Mo: 0.1%

+ Fe: 0.5% + B: 0.5% and T₈- Mo: 0.3% + Fe: 0.2% + B: 0.2% and applied at 30, 45 and 60 days after transplantation of seedlings. Recommended dose of fertilizer and irrigation were given when required.

Plot size

Row to row distance was 70cm and plant to plant was 30cm. The net plot size was Net plot size = 3.04m × 18.29 m = 55.60m²

Methods

Direct sowing of seeds was done on ridges. Different concentrations and combinations of boron, iron and molybdenum were prepared and applied at 30, 45 and 60 days after transplantation of seedlings. Recommended dose of fertilizer and irrigation was given when required.

Fertilizer Application

Recommended fertilizer application of nitrogen, phosphorus and potash were applied accordingly. One third of NPK was applied at sowing while remaining NPK was applied throughout the season as needed by the crop.

Plant height (cm)

Height of the plant was calculated with a measuring tape. The height was recorded by placing the measuring tape at the soil surface by the base of the plant and then measured height of the plant up to the tallest growing point on randomly selected plant; their average was calculated and analyzed statistically.

Number of leaves plant⁻¹

The number of leaves was counted on randomly selected plants at the time of head harvest. Their average was calculated and analyzed statistically.

Number of days taken from transplantation to curd formation

Numbers of days taken to curd formation after transplantation were counted on randomly selected plants. The estimation of the date of the curd appearance was done by removing or repelling leaves with care which has covered the just appearing heads then their average was calculated and analyzed statistically.

Days to curd initiation to maturity

Numbers of days taken to maturity from curd initiation were counted on randomly selected plants. For this purpose days were counted from curd initiation to maturity then their average was calculated and analyzed statistically.

Foliage fresh weight (g)

Foliage fresh weight of the plant was calculated after harvesting by digital weight balance in grams (gm) from randomly selected plants then their average was calculated and analyzed statistically.

Foliage dry weight (g)

Fresh leaves were harvested and dried. Dry weight of the foliage was calculated after 72 hours drying the foliage in an oven at 60 °C. Dry weight was calculated by digital weight balance then their average was calculated and analyzed statistically.

Curd weight (g)

Fresh curds were harvested and then weighed on a balance in grams (g) from randomly selected then their average was calculated and analyzed statistically.

Curd area (cm²)

Head area measured in centimeters with the help of measuring tape and their average was calculated and analyzed statistically.

Chlorophyll content (Spad meter)

Chlorophyll contents were determined by the chlorophyll meter.

Leaf area (cm²)

For this purpose leaves were taken randomly selected plants from each treatment. Leaf area was calculated with the help of leaf area meter (C1-203 Leaser leaf area meter) and average was computed.

Curd color

Color of mature curd was determined with the Horticulture Color Chart.

Vitamin C (mg/100g)

For the analysis of ascorbic acid, fully ready cauliflower were taken each form each replication separately and washed properly. Ascorbic acid content

of each replication was determined according to a method by Ruck (1961). 10mL cauliflower juice was taken in 100mL volumetric flask and volume was made up to the mark by 0.4% oxalic acid solution and filtered. Then 5mL filtered solution was taken and titrated against 2,6-dichlorophenolindophenol dye, until light pink end point. Ascorbic acid was calculated by using following formula.

Preparation of dye

Dye was prepared by adding 42 mg NaHCO₃ and 52 mg, 2, 6- dichlorophenolindophenol dye in 200mL a volumetric flask. Volume was made up to the mark by distilled water, filtered and always freshly prepared dye was used. Ascorbic acid (mg/100ml juice) = $1 \times R_1 \times V \times 100 \div R \times W \times V_1$

Where,

R =mL of dye used to titrate against 2.5mL (1ml standard ascorbic acid + 1.5mL 0.4% oxalic acid) of reference solution (standard reading)

R =mL of dye used to titrate against V₁ of aliquot (sample reading)

V =Volume of aliquot made by 0.4% oxalic acid

V₁ =mL of juice taken for titration

W =mL of juice taken

Total soluble solids (Brix°)

TSS of juice was measured by using digital refractometer (ATAGO, RS-5000, Atago, Japan). The calibration of instrument was done with deionized water before and after taken the reading and cleaning with tissue paper as well. Single drop fresh juice is enough, was placed on clean prism of the instrument and reading was recorded directly in unit brix.

Yield hectare⁻¹

Yield hectare⁻¹ was calculated by taking the yield from 1 m² areas of each treatment and then by multiplying with 10000m².

Results and discussions

Foliar application of micronutrients exhibited positive impact on cauliflower plant height. Among the all treatments, T₇ (Mo: 0.1% + Fe: 0.5% + B: 0.5%) showed the maximum plant height 32.11cm as

compared to T₀ (Control) treatment that gave 23.33cm plant height. While other treatments showed intermediate results regarding to plant height of cauliflower. Vegetative growth is also triggered by microelement iron that is a component of ferredoxin that is an electron transporting protein. Iron is affiliated with chloroplast and assist in photosynthetic process reported by Hazra *et al.* 1987. Similar findings were reported by Thakur *et al.*, 1991 that application of boron enhanced the plant height of cauliflower and our finding is in accordance with their findings.

Foliar application of micronutrients exhibited highly significant influence on number of leaves per plant. The treatment T₇ (Mo: 0.1% + Fe: 0.5% + B: 0.5%) produced highest number of leaves per plant whereas the lowest number of leaves per plant were observed by T₀ (control) treatment.

The remaining treatments T₂ (Mo: 0.3%), T₅ (B: 0.2%), T₄ (Fe: 0.2%), T₈ (Mo: 0.3% + Fe: 0.2% + B: 0.2%), T₁ (0.1% Mo), T₃ (Fe: 0.5%) and T₆ (B: 0.5%) showed the gradual increase in number leaves per plant from T₂ towards T₆ treatment. The application of boron increased the number of leaves per plant in cauliflower investigated by Thakur *et al.* 1991. Number of leaves and plant height is increased by the accelerating effect of molybdenum on cauliflower vegetative growth that enhanced the photosynthetic activities reported by Sharma, 2002.

Among all treatments, T₇ (Mo: 0.1% + Fe: 0.5% + B: 0.5%) taken least number of days to produce curd (62.217 days) whereas the control treatment T₀ taken highest number of days to produce the curd of Cauliflower. All other treatments like T₆ (B: 0.5%), T₃ (Fe: 0.5%), T₁ (0.1% Mo), T₈ (Mo: 0.3% + Fe: 0.2% + B: 0.2%), T₄ (Fe: 0.2%), T₅ (B: 0.2%) and T₂ (Mo: 0.3%) showed gradual decrease in number of days taken from plantation to curd formation from T₆ towards T₂. It is noticed that as the micronutrients dose is increased the days required to start curd initiation are decreased. This can be due to more absorption of micronutrients in balanced proportion which improved the physiological activities of plant and triggered the synthesis of endogenous growth

hormones for early formation of curd. Our findings are in accordance with the findings of Kumar and Choudhary, 2002.

The treatment T₇ (Mo: 0.1% + Fe: 0.5% + B: 0.5%) taken least number of days to mature the curd of plant as compared to the treatment T₀. All other treatments like T₆ (B: 0.5%), T₃ (Fe: 0.5%), T₁ (0.1% Mo), T₈ (Mo: 0.3% + Fe: 0.2% + B: 0.2%), T₄ (Fe: 0.2%), T₅ (B: 0.2%) and T₂ (Mo: 0.3%) showed gradual decrease in number of days taken from curd initiation to maturity from T₆ towards T₂ treatment. Foliar application of combined dose of micronutrients revealed reduction in the period required for curd initiation and curd maturity. This earliness might be due to enhancement of physiological activities of plant and fast translocation of photosynthetic products towards the curd of Cauliflower Lashkari *et al.*, 2007.

The treatment T₇ (Mo: 0.1% + Fe: 0.5% + B: 0.5%) depicted maximum foliage fresh weight (1144.0 gm) as compared to control treatment T₀ (644.3 gm) that showed minimum foliage fresh weight. The remaining treatments T₂ (Mo: 0.3%), T₅ (B: 0.2%), T₄ (Fe: 0.2%), T₈ (Mo: 0.3% + Fe: 0.2% + B: 0.2%), T₁ (0.1% Mo), T₃ (Fe: 0.5%) and T₆ (B: 0.5%) showed the gradual increase in foliage fresh weight from T₂ towards T₆ treatment. Kumar *et al.*, 2012 reported that combined effect of foliar application of micronutrients positively influenced the biomass production of plant.

Among all treatments, foliage dry weight significantly increased in treatment T₇ (Mo: 0.1% + Fe: 0.5% + B: 0.5%) as compared to control T₀ treatment. Other treatments including T₂ (Mo: 0.3%), T₅ (B: 0.2%), T₄ (Fe: 0.2%), T₈ (Mo: 0.3% + Fe: 0.2% + B: 0.2%), T₁ (0.1% Mo), T₃ (Fe: 0.5%) and T₆ (B: 0.5%) showed the gradual increase in foliage dry weight from T₂ towards T₆ treatment. Thakur *et al.*, 1991 reported that foliar application of boron enhanced the dry mass of cauliflower. This is due to the combined effect of boron and molybdenum. Translocation of carbohydrates is enhanced by boron application and photosynthesis and metabolic processes are enhanced by molybdenum.

The treatment T₇ (Mo: 0.1% + Fe: 0.5% + B: 0.5%) depicted maximum curd weight (1416.3gm) as compared to control treatment T₀ (1028.7gm) that showed minimum curd weight. The remaining treatments T₂ (Mo: 0.3%), T₅ (B: 0.2%), T₄ (Fe: 0.2%), T₈ (Mo: 0.3% + Fe: 0.2% + B: 0.2%), T₁ (0.1% Mo), T₃ (Fe: 0.5%) and T₆ (B: 0.5%) showed the gradual increase in curd weight from T₂ towards T₆ treatment. Kannan *et al.*, 2018 reported that foliar application of micronutrients enhanced the curd production of cauliflower. Furthermore, maximum production of curd is obtained by combined dose of micronutrients reported by Singh, 2003.

Foliar application of micronutrients exhibited positive impact on cauliflower curd area. Among the all treatments, T₇ (Mo: 0.1% + Fe: 0.5% + B: 0.5%) showed the maximum curd area 16.66cm as compared to T₀ (Control) treatment that gave 11.22cm plant height. While other treatments showed gradual increment in results regarding to curd area of Cauliflower. It is observed that big curd is produced by foliar application of combined dose of micronutrients that enhanced the carbohydrate synthesis and its translocation towards the curd of plant which ultimately helped in production of wide curd. Similar results were reported by Kotur *et al.*, 1998; Singh, 1976; Kumar and Choudhary, 2002; Prasad and Yadav, 2006. Curd color of cauliflower was greatly influenced by micronutrients foliar application. Various variations were observed.

Among all the treatments, T₈, T₂ and T₀ showed cream color of curd whereas the remaining all treatments produced white color of curd. Chlorophyll contents of cauliflower significantly influenced by foliar application of micronutrients. The treatment T₇ (Mo: 0.1% + Fe: 0.5% + B: 0.5%) depicted maximum chlorophyll contents (106.11nm) as compared to control treatment T₀ (33.44nm) that showed minimum chlorophyll contents. The remaining treatments T₂ (Mo: 0.3%), T₅ (B: 0.2%), T₄ (Fe: 0.2%), T₈ (Mo: 0.3% + Fe: 0.2% + B: 0.2%), T₁ (0.1% Mo), T₃ (Fe: 0.5%) and T₆ (B: 0.5%) showed the gradual increase in chlorophyll contents from T₂ towards T₆ treatment. Boron application enhanced the growth

attributes due to increment in metabolic and photosynthetic activities which are ultimately enhanced cell division and elongation (Hatwar *et al.*, 2003). Furthermore, application of boron assist the synthesis of tryptophan that is precursor of indole acetic acid which

is required for plant biomass accumulation and growth. The micronutrients iron as a component of ferredoxin and electron transport is linked to chloroplast. Acceleration of photosynthetic activity is needed for vegetative growth of plant (Patil *et al.*, 2008).

Table 1. Vegetative attributes of cauliflower influenced by foliar spray of micronutrients (B, Fe and Mo).

Treatment	Plant height (cm)	No. of leaves per plant	number of days taken from plantation to curd formation	Number of days taken from curd initiation to curd maturity	foliage fresh weight (g)	foliage dry weight (g)	curd area (cm ²)
T ₀	23.33 ^F	9.33 ^F	71.55 ^A	18.22 ^A	644.33 ^I	45.06 ^H	10.778 ^D
T ₁	29.10 ^C	13.77 ^{BCD}	64.22 ^D	15.33 ^{DE}	942 ^D	88.77 ^C	12.889 ^{BC}
T ₂	25.00 ^E	11.22 ^E	69.10 ^B	17.66 ^A	742 ^H	65.38 ^G	11.222 ^D
T ₃	30.55 ^B	14.29 ^{ABC}	63.22 ^E	14.99 ^{DE}	1015 ^C	90.00 ^C	13.333 ^{ABC}
T ₄	27.11 ^D	13.14 ^D	66.44 ^C	16.22 ^{BC}	832 ^F	72.99 ^E	12.000 ^{CD}
T ₅	26.11 ^{DE}	12.11 ^E	68.55 ^B	16.77 ^B	777.33 ^G	69.19 ^F	11.333 ^D
T ₆	31.44 ^{AB}	14.51 ^{AB}	62.21 ^F	14.66 ^{EF}	1033.66 ^B	98.62 ^B	13.778 ^{AB}
T ₇	32.11 ^A	14.920 ^A	60.44 ^G	14.11 ^F	1144 ^A	108.076 ^A	14.556 ^A
T ₈	28.66 ^C	13.327 ^{CD}	64.88 ^D	15.66 ^{CD}	868 ^E	75.88 ^D	12.111 ^{CD}

Table 2. Vegetative and biochemical attributes of cauliflower influenced by foliar spray of micronutrients (B, Fe and Mo).

Treatment	Curd color	Leaf area (cm ²)	chlorophyll contents (Spad meter)	Vitamin C (mg/g)	Total Soluble Salts (Brix ^o)	Yield per plot (Kg)	yield per hectare (tons)
T ₀	Cream	10.77 ^D	33.44 ^I	42.22 ^G	5.22 ^C	9.66 ^H	145.29 ^H
T ₁	White	12.88 ^{BC}	84.55 ^D	57.44 ^{BC}	6.44 ^{AB}	14.52 ^C	208.70 ^C
T ₂	Cream	11.22 ^D	43.77 ^H	45.66 ^F	5.55 ^{BC}	12.18 ^G	184.62 ^G
T ₃	White	13.33 ^{ABC}	90.33 ^C	59.66 ^B	6.44 ^{AB}	14.66 ^C	220.22 ^B
T ₄	White	12.00 ^{CD}	66.11 ^F	50.66 ^D	6.11 ^{ABC}	13.12 ^E	196.13 ^E
T ₅	White	11.33 ^D	51.33 ^G	48.11 ^E	5.77 ^{ABC}	12.68 ^F	189.73 ^F
T ₆	White	13.77 ^{AB}	98.77 ^B	63.44 ^A	6.77 ^A	15.65 ^B	221.80 ^B
T ₇	White	14.55 ^A	106.11 ^A	64.88 ^A	6.88 ^A	17.09 ^A	266.33 ^A
T ₈	Cream	12.11 ^{CD}	98.77 ^E	55.55 ^C	6.33 ^{ABC}	13.82 ^D	200.46 ^D

The treatment T₇ (Mo: 0.1% + Fe: 0.5% + B: 0.5%) depicted maximum leaf area (14.55cm²) as compared to control treatment T₀ (10.77cm²) that showed minimum leaf area. The remaining treatments T₂ (Mo: 0.3%), T₅ (B: 0.2%), T₄ (Fe: 0.2%), T₈ (Mo: 0.3% + Fe: 0.2% + B: 0.2%), T₁ (0.1% Mo), T₃ (Fe: 0.5%) and T₆ (B: 0.5%) showed the gradual increase in leaf area from T₂ towards T₆ treatment. Vegetative growth is triggered by the combined dose of boron and molybdenum in Cauliflower. Leaf length and leaf area are increased reported by Cheng 1984; Singh 2003 and Partha *et al.*, 2006. Furthermore, our findings are supported by Balyan *et al.* 2004.

The treatment T₇ (Mo: 0.1% + Fe: 0.5% + B: 0.5%) produced highest number of leaves per plant whereas the lowest Vitamin C contents (mg/g) observed by T₀

(Control) treatment. The remaining treatments T₂ (Mo: 0.3%), T₅ (B: 0.2%), T₄ (Fe: 0.2%), T₈ (Mo: 0.3% + Fe: 0.2% + B: 0.2%), T₁ (0.1% Mo), T₃ (Fe: 0.5%) and T₆ (B: 0.5%) showed the gradual increase Vitamin C (mg/g) contents from T₂ towards T₆ treatment. Foliar application of boron and combined dose positively influenced the ascorbic acid content reported by Kumar *et al.* 2010.

The treatment T₇ (Mo: 0.1% + Fe: 0.5% + B: 0.5%) produced highest number of leaves per plant whereas the lowest total soluble salts observed by T₀ (Control) treatment. The remaining treatments T₂ (Mo: 0.3%), T₅ (B: 0.2%), T₄ (Fe: 0.2%), T₈ (Mo: 0.3% + Fe: 0.2% + B: 0.2%), T₁ (0.1% Mo), T₃ (Fe: 0.5%) and T₆ (B: 0.5%) showed the gradual increase in total soluble salts from T₂ towards T₆ treatment. Foliar feeding of

combined dose of micronutrients boron and iron at the rate of 5% enhanced the TSS contents in cauliflower recorded by Lashkari *et al.* (2008).

Among the all treatments, T₇ (Mo: 0.1% + Fe: 0.5% + B: 0.5%) showed the maximum yield per plot 17.09kg as compared to T₀ (control) treatment that gave 9.66kg yield per plot. While other treatments showed intermediate results regarding to yield per plot of cauliflower. Among the all treatments, T₇ (Mo: 0.1% + Fe: 0.5% + B: 0.5%) showed the maximum yield per hectare 266.33 tons as compared to T₀ (control) treatment that gave 245.29 tons yield per hectare. While other treatments showed intermediate results regarding to yield per plot of cauliflower. Improvement of yield production resulted by the foliar feeding of combined dose of micronutrients is due to its accelerating effect on photosynthetic and metabolic activities that ultimately increase the metabolites the are required for division of cells and their elongation, reported by Hatwar *et al.* 2003. Molybdenum positively enhanced the yield character due to its stimulatory influence that enhance the photosynthetic capacity and metabolic pool needed for the production of saccharides, investigated by Mohamed el-Sayed Ahmed *et al.* 2011. In addition, Dhakal *et al.* 2009 documented that curd yield is increased due to the synergetic interaction influence between phosphorus and boron element. Our findings are in accordance of with their findings.

Conclusion

It can be concluded that among all the treatments T₇ (Mo: 0.1% + Fe: 0.5% + B: 0.5%) showed the best result regarding to all the parameters including plant height (cm), number of leaves plant⁻¹, number of days taken from transplantation to curd formation, days to curd initiation to maturity, foliage fresh weight (g), foliage dry weight (gm), curd weight (g), curd area (cm²), leaf area (cm²), curd color, vitamin C (mg/g), chlorophyll content (Spad meter), total soluble salts (Brix), yield per plant (Kg) and yield per hectare (ton). However, it is inferred that foliar application of micronutrients showed significant impact on vegetative and biochemical attributes of cauliflower. In addition, the micronutrients, boron, iron and

molybdenum at the rate of 0.5% and 0.1% proved best dose and combination of effective performance.

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