Research Article

Effect of Foliar Spray of Bulb Inducing Chemicals on Tuberose Cv. Prajwal

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Abstract

Tuberose is one of the popular fragrant flower crops commercially grown in India for both loose and cut flower. Non availability of quality bulbs is the main constraint in tuberose cultivation. To enhance the bulb production in tuberose cv. Prajwal, an experiment was conducted at Horticultural Research Station, Kovvur, under the aegis of DrYSRHU, Andhra Pradesh, India during the years 2015-16 and 2016-17 with 10 treatments replicated thrice in Randomized Block Design. The treatments comprise foliar application of 28 Homobrassinolide (28 HBL), Jasmonic acid (JA) and Cycocel (CCC) at three different concentrations with water spray as control. Among the different treatments, plants sprayed with CCC@1500 ppm recorded highest bulb yield of 13.77 t ha^{-1} with propagation coefficient of 835.94%. However, highest bulblet yield of 2.44 t ha^{-1} was registered in plants sprayed with JA@100 µM.

Keywords: Tuberose, Jasmonic acid, CCC, 28 Homobrassinolide, bulb production

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Introduction

Tuberose (*Polianthes tuberosa* L.) is one of the most important fragrant flower crops commercially cultivated in India. It is native to Mexico and placed under the family *Asparagaceae*. It occupies a very special and prime position among the ornamental bulbs for its elegant long spikes and fragrant florets. It is also grown in pots, beds and borders for garden decoration. In the recent past, the area under tuberose is expanding in faster pace due to its ease in cultivation, great demand in the market for cut flowers, loose flowers and also for the extraction of high valued natural flower oil. Non availability of quality planting material one of the impediments for its area expansion in non-traditional areas. Enhanced production of high-quality propagules is therefore, necessary to meet the growing demand of tuberose farmers. One of the strategies to improve the production of bulbs is application of plant growth regulating chemicals, which has a prominent role in bulb or tuber production. Plant growth retardant *i.e.,* CCC improved the propagation coefficient in tulip [1]. Novel plant growth regulators like brassinosteroids and jasmonic acid (JA) also enhanced the bulb or corm production in various crops. Exogenously applied jasmonates induce or promoted bulb formation in narcissus [2]. Foliar sprays of jasmonic acid and brassinosteroids were found to increase the number of corms, cormels per unit area and propagation coefficient in gladiolus [3]. Hence, a study was conducted to determine the effect of foliar spray of bulb inducing chemicals on growth and bulb production in tuberose cv. Prajwal.

Materials and Methods

A field experiment was conducted at Horticultural Research Station, Kovvur, DrYSR Horticultural University, Andhra Pradesh, India for two consecutive years 2015-16 and 2016-17. The site of experiment is located at $17^0$00’ N latitude, $81^0$43’ E longitude and 15.66 m above mean sea level which receives annual rainfall of 110 cm from Southwest monsoon (June to September), Northeast monsoon (October to November) and through summer showers. The experiment was carried out on black alluvial soil with a pH of 7.6, Electrical conductivity 0.42 dSm^{-1}, organic carbon 0.48%, available nitrogen 188.6 kg ha^{-1}, available phosphorus 20.5 kg ha^{-1} and available potassium 543.9 kg ha^{-1}. Bulbs of single petaled tuberose cultivar Prajwal procured from Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore were used as planting material. This cultivar is being grown in major tuberose producing states of India for loose flower purpose.

The experiment was executed with 10 treatments replicated thrice in Randomised Block Design. Treatments comprises of foliar spray of three plant growth regulating chemicals at three different concentrations along with water spray as control. The treatments were T_1: 28-homobrassinolide (28 HBL) @0.5 ppm; T_2: 28-homobrassinolide @1.0 ppm; T_3: 28-homobrassinolide @2.0 ppm; T_4: Jasmonic Acid (JA) @50 µM; T_5: Jasmonic Acid @100 µM; T_6:

Jasmonic Acid @200 µM; T_7: CCC (Cycocel) @1000 ppm; T_8: CCC @1500 ppm; T_9: CCC @2000 ppm; T_{10}: Water Spray (Control). As per the treatments, freshly prepared growth regulating chemicals mixed with commercial surfactant (0.05%) was used for foliar sprays at 30 and 60 days after sprouting. During the field preparation, well decomposed farmyard manure at 25 t ha^{-1} was incorporated into all experimental plots uniformly. Nitrogen, phosphorus and potassium were applied @ 200:200:200 kg ha^{-1} in the form of urea, single super phosphate and muriate of potash respectively. Entire dose of phosphorus and potassium was applied as basal and nitrogen was applied in three split doses at 30, 60 and 90 days after planting. The bulbs were treated with 0.2 per cent carbendazim 50% WP for 30 minutes and shade dried immediately. Treated bulbs were planted at a spacing of 30 cm x 30 cm at 5 - 6 cm depth. Field was irrigated immediately on the next day of planting. Irrigations were given at an interval of one to two weeks depending on the soil moisture condition and irrigation was stopped one month prior to digging of bulbs.

From ten randomly tagged plants in each treatment and in each replication leaf number was recorded at 60 days interval from 60 DAP (days after planting) to 240 DAP. Total chlorophyll content was recorded with the help of a digital chlorophyll meter SPAD-502 plus (KONICA MINOLTA) from five tagged plants in each treatment after final spray of growth regulating chemicals at three different positions of the leaves and mean was worked out and expressed in SPAD units. The mother bulb, bulb and bulblet parameters were recorded at 240 days after planting by uprooting the clumps. A total of ten representative clumps were sampled for recording observations on weight, number and diameter of mother bulb, bulbs and bulblets. Propagation coefficient was calculated as the ratio of total weight of mother bulb, bulb, bulblets obtained and weight of the bulb planted, multiplied by 100 and expressed in percentage [4]. The data were subjected to statistical analysis by the method of analysis of variance [5]. The differences among treatment means were tested using the critical differences (C.D) at 5 per cent level of probability [6].

Results and Discussions

Number of leaves clump^{-1}

Number of leaves clump^{-1} significantly differed among the treatments at all stages of growth except at 60 DAP (**Table 1**). It was observed that the mean number of leaves clump^{-1} increased from 16.29 at 60 DAP to 94.85 at 240 DAP. The highest mean number of leaves clump^{-1} (42.77, 91.90 and 105.67 at 120, 180 and 240 DAP respectively) was recorded with the application of 1.0 ppm 28 HBL. The lowest number of leaves clump^{-1} (35.06, 75.33 and 86.62 at 120, 180 and 240 DAP respectively) was recorded in control which was on par with 200 µM JA (35.41, 76.08 and 87.48 at 120, 180 and 240 DAP respectively).

Table 1 Effect of different growth regulating chemicals on number of leaves clump^{-1} in tuberose cv. Prajwal

Treatments	Number of leaves clump^{-1}			
	60 DAP	120 DAP	180 DAP	240 DAP
T_1: 28 HBL 0.5 ppm	17.55	41.37	88.89	102.21
T_2:28 HBL 1.0 ppm	18.15	42.77	91.90	105.67
T_3:28 HBL 2.0 ppm	16.14	38.05	81.75	94.00
T_4:JA 50 µM	16.21	38.21	82.11	94.41
T_5:JA 100 µM	15.54	36.62	78.69	89.21
T_6:JA 200 µM	15.02	35.41	76.08	87.48
T_7:CCC 1000 ppm	15.62	36.81	79.09	92.24
T_8:CCC 1500 ppm	16.96	39.97	85.87	98.74
T_9:CCC 2000 ppm	16.81	39.62	85.12	97.88
T_{10}:Water spray (Control)	14.88	35.06	75.33	86.62
Mean	16.29	38.39	82.48	94.85
S.Em	-	0.18	0.39	0.45
C.D. at 5%	NS	0.54	1.15	1.33

28 HBL: 28 Homobrassinolide; JA: Jasmonic acid; CCC: Cycocel; DAP: Days after planting

At early stages of growth, the differences were not significant due to the application of single spray of growth regulating chemical and initial slow growth. From 120 DAP, application of 28 HBL at 1.0 ppm produced maximum number of leaves over rest of the treatments at all stages of crop growth which could be attributed to its significant positive effect on cell division and cell elongation. The increase in number of leaves clump^{-1} could be also attributed to growth promoting activity of BRs (Brassinosteroids). Similar results were reported by [7] in gladiolus.

The number of leaves clump^{-1} was significantly more with BR and JA sprays at 120, 180 and 240 DAP at all concentrations over control except at higher concentration of JA. Similar results with BR and JA were reported by [3] in gladiolus. With increase in concentration of JA, number of leaves clump^{-1} were reduced and on par with control. These results are in accordance with the findings of [2] in *Narcissus triandrus* under *in vitro* conditions.

The number of leaves clump^{-1} produced in CCC treatments was intermediary between BR and JA. The increment in number of leaves clump^{-1} with CCC treatment over control could be attributed to inhibition of cell elongation and diversion of food materials towards the lateral buds and helps in the production of more number of leaves clump^{-1}. Similar results were reported in tuberose [8] and tulip [1].

Total chlorophyll content after final spray (SPAD units)

The differences in total chlorophyll content among different growth regulating chemicals were found significant (**Figure 1**). Total chlorophyll content ranged from 54.05 SPAD (JA 200 μM) to 65.78 SPAD (28 HBL1.0 ppm). The highest total chlorophyll content was recorded in the plants sprayed with 1.0 ppm 28 HBL whereas the lowest was recorded in the plants sprayed at 200 μM JA (54.05 SPAD) and it was on par with 100 μM JA (55.13 SPAD).

All the growth regulating chemicals recorded the highest total chlorophyll content over control except JA at high concentrations. The highest total chlorophyll values with 28 HBL were also recorded in china aster [9]. Increased chlorophyll content due to CCC was reported in iris [10] and in oriental lily hybrids [11]. CCC block the conversion of geranyl geranyl pyrophosphate (GGPP) to entkaurene (GA precursor) [12]. GGPP is diterpene precursor substance, involved in the synthesis of a variety of substances, including carotenoids and chlorophyll. Due to the hampered conversion from GGPP to entkaurene, GGPP may be more converted to diterpenes, and this may be the reason that CCC increase chlorophyll content [13]. The application JA at higher concentration appeared to have inhibited the synthesis of chlorophyll which might be due to down-regulation of the expression of genes involved in chlorophyll constitution [14].

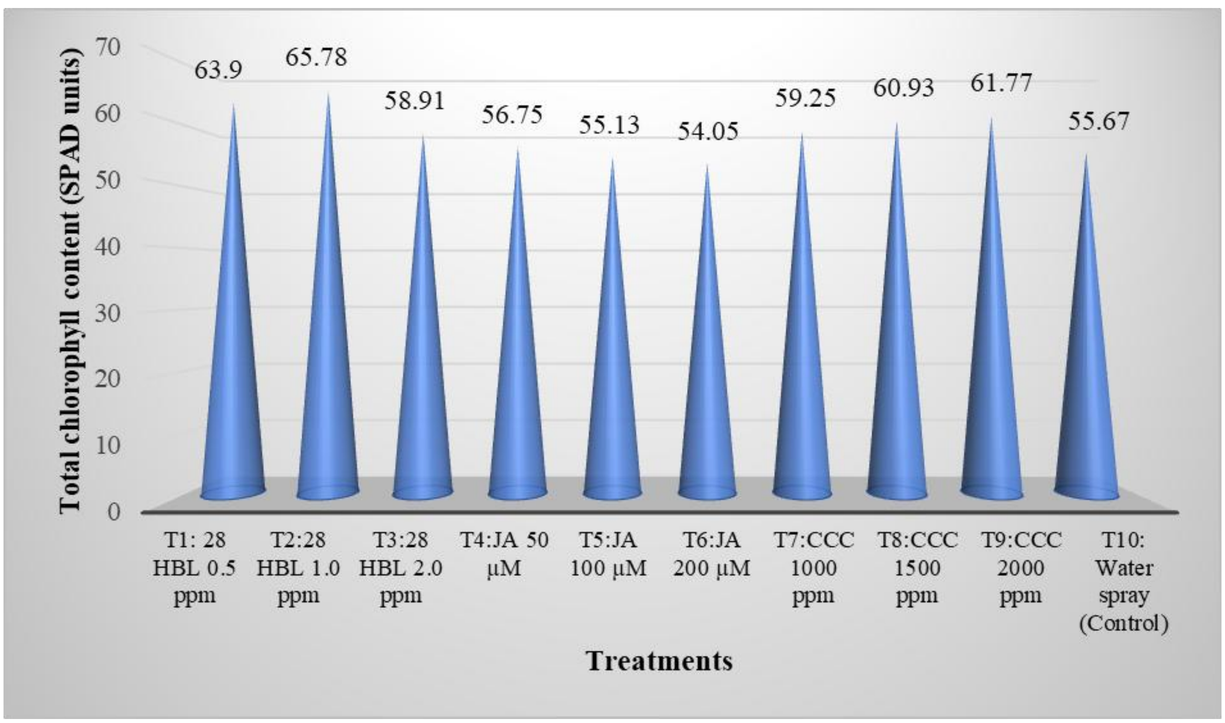

Figure 1 Effect of different plant growth regulating chemicals on total chlorophyll content (SPAD units) in tuberose cv. Prajwal

Bulb Parameters

It is evident from the data (**Table 2**) that the application of different growth regulating chemicals was significantly influenced all the bulb parameters.

Weight of mother bulb (g)

Among the treatments, 28 HBL 1.0 ppm recorded significantly highest mother bulb weight (118.61 g) which was superior to rest of the treatments while the lowest mother bulb weight (89.86 g) was recorded in control. Among different levels of 28 HBL, 1.0 ppm proved to be optimum for mother bulb weight. The highest mother bulb weight was recorded with 28 HBL over the rest of plant growth regulators could be attributed to the fact that 28 HBL was associated with an enhanced level of DNA, soluble protein and various carbohydrate fractions in the plant [15]. Further, BRs treatment might have resulted in an increase in assimilate transportation from source to sink and their ultimate conversion into final reserved food material in the mother bulb there by enhanced its weight.

JA also improved the mother bulb weight next to BR. JA mediated induction of VSP (Vegetative storage proteins) synthesis under conditions of high sugar accumulation thus creating an effective sink for carbon, nitrogen and releases phosphate group from sugar phosphate pools for further carbon fixation [16].

Table 2 Effect of different growth regulating chemicals on mother bulb and bulb parameters in tuberose cv. Prajwal

Treatments	Weight of mother bulb (g)	Diameter of mother bulb (cm)	Number of bulbs $clump^{-1}$	Weight of bulbs $clump^{-1}$ (g)	Average diameter of bulb (cm)
T_1: 28 HBL 0.5 ppm	115.02	6.45	8.43	145.12	2.56
T_2:28 HBL 1.0 ppm	118.61	6.67	8.73	146.89	2.79
T_3:28 HBL 2.0 ppm	105.13	5.82	7.95	134.54	2.56
T_4:JA 50 µM	111.42	6.22	9.09	153.06	2.91
T_5:JA 100 µM	112.99	6.16	9.02	151.83	2.89
T_6:JA 200 µM	101.54	5.60	8.29	139.48	2.65
T_7:CCC 1000 ppm	103.34	5.71	9.39	158.00	3.01
T_8:CCC 1500 ppm	106.93	5.94	9.68	162.93	3.10
T_9:CCC 2000 ppm	97.94	5.88	8.58	144.42	2.75
T_{10}:Water spray (Control)	89.86	5.49	7.33	123.44	2.35
Mean	106.28	5.99	8.65	145.97	2.76
S.Em	0.91	0.04	0.07	1.22	0.024
C.D. at 5%	2.75	0.12	0.22	3.67	0.073

28 HBL: 28 Homobrassinolide; JA: Jasmonic acid; CCC: Cycocel; DAP: Days after planting

Diameter of mother bulb (cm)

Application of 28 HBL 1.0 ppm registered significantly highest mother bulb diameter (6.67 cm) whereas the lowest was recorded in control (5.49 cm) which was on par with 200 µM JA (5.60 cm). The increase in mother bulb diameter could be attributed to stimulation of cell division by BRs and accumulation of reserve food material in the bulbs [17].

Number of bulbs $clump^{-1}$

Application of CCC at 1500 ppm produced significantly highest number of bulbs $clump^{-1}$ (9.68) over the rest of the treatments. The lowest number of bulbs $clump^{-1}$ was recorded in control (7.33). Bulb number is primarily determined by the process of bulb initiation. All CCC treatments had a stimulatory effect on formation of new bulbs compared to the control. The above results are in conformity with those reported in iris [10], in tulip [1] and in tuberose [18]. This could be ascribed to the fact that, CCC could act directly on the root meristem and increase the cytokinin production [19] and its transport to the shoots, thus lead to the production of an increased number of lateral shoots by counteracting or eliminating the apical dominance which ultimately results in a greater number of bulbs $clump^{-1}$. Treatment with JA was found to increase the mean rate of bulb production over control. Similar results were also obtained in gladiolus [3]. JA promoted *in vitro* bulb formation in shoot cultures of *Narcissus* suggesting that its presence is positively influenced the formation and enlargement of bulbs in *Narcissus* plants [2].

Weight of bulbs $clump^{-1}$ (g)

Application of CCC at 1500 ppm enhanced the bulb weight $clump^{-1}$ significantly (162.93 g) over the rest of the treatments. The lowest bulb weight $clump^{-1}$ was observed in control (123.44 g). The results are in agreement with those reported in gladiolus [20], [21] and in tulip [1]. Bulb weight $clump^{-1}$ is dependent on the process of bulb growth. Number of leaves $clump^{-1}$ and total chlorophyll content in leaves were significantly influenced the degree of bulb growth. With an increase in the assimilate synthesis and supply to the growing sink organs which in turn enhanced the bulb weight $clump^{-1}$. CCC could also improve nutrient uptake from soil, promote water balance and increase protein synthesis in growing organs [22] thereby improve yield.

JA significantly enhanced the bulb development even at very low concentrations (1-10 μM) under *in vitro* conditions [23] and suggested that JA might play an important role in the formation and development of storage organs in plants such as bulbs.

BRs known to delay the leaf senescence and enhance the chlorophyll content which might have favoured the bulbing process. BRs increased the ATPase enzyme activity leading to proton extrusion [24], there by activating cell wall-loosening enzymes. Turgor pressure then drives cell expansion as new cell wall and membrane material would have been synthesized and secreted promoting growth.

Diameter of bulb (cm)

CCC 1500 ppm produced the largest sized bulbs (3.10 cm) over the rest of the chemical sprays whereas, control registered the smallest sized bulbs (2.35 cm). The present results are in agreement with the findings of [20] in gladiolus. Application of CCC increased the sucrose export simultaneously to plant parts below the fed leaves (roots and bulbs) without affecting assimilate transport to the florets [25] and thereby CCC improved the bulb size. Enhanced bulb size was recorded with BRs in *Dioscorea alata* under *invitro* conditions [26]. JA also enhanced the bulb size in the present study which was in accordance with the findings of [27] in transgenic potato.

Propagation coefficient (%)

The propagation coefficient reveals the multiplication rate of bulbs and bulblets produced by a plant. The propagation coefficient values exhibited significant variations among different growth regulating chemicals (**Figure 2**). The average propagation coefficient values ranged from 662.68% to 835.94%. CCC at 1500 ppm recorded significantly the maximum propagation coefficient (835.94%) followed by 50 μM JA (825.31%), 100 μM JA (824.16%) and 1.0 ppm 28 HBL (821.36%) over rest of the treatments. Minimum propagation coefficient value was recorded in control (662.68%).

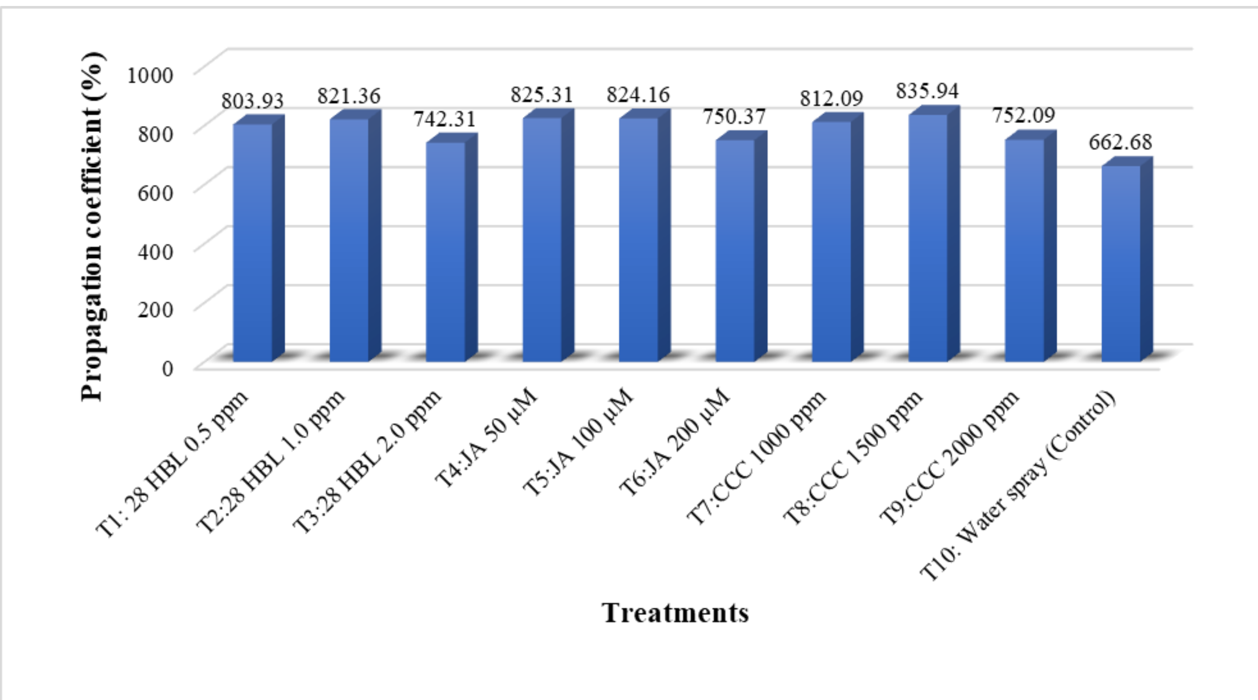

Figure 2 Effect of different growth regulating chemicals on propagation coefficient in tuberose cv. Prajwal

The growth regulator treatments significantly improved the propagation coefficient over control. Among all the plant growth regulating chemicals, CCC application significantly improved the propagation coefficient. Higher biosynthesis of chlorophyll and photosynthesis resulted in higher sugar accumulation due to CCC [28]. Increased number of leaves and total chlorophyll content in leaves resulted in more assimilation of food material which might have diverted towards bulbs production and thereby improved the propagation coefficient over control. These results are in tune with the findings of [20] in gladiolus and [1] in tulip.

JA and BRs also significantly increased propagation coefficient in tuberose. Similar results were obtained in gladiolus [3]. JA and methyl jasmonates exert tuber inducing effect by eliciting the expansion of cells in potato tubers under *in vitro* conditions [29]. The JA induced cell expansion was attributable to both an increase in osmotic pressure due to accumulation of sucrose and changes in cell wall architecture that appears to affect the extensibility of the wall. Moreover, the synthesis of cellulose might be indispensable for JA induced cell expansion. The tuberization and expansion of cells induced by JA always involve reorientation of cortical microtubules (MTs), suggesting that JA controls the direction of cell expansion by changing the arrangement of MTs. In the present study, BRs also enhanced propagation coefficient. Higher propagation coefficient with 28 HBL 1.0 ppm might be due to production of heavier mother bulbs coupled with moderate bulb and bulblet weight as compared to other treatments.

Yield

The variations in mother bulb, bulb and bulblet yield ha^{-1} as influenced by different growth regulating chemicals (**Figure 3**) were found significant.

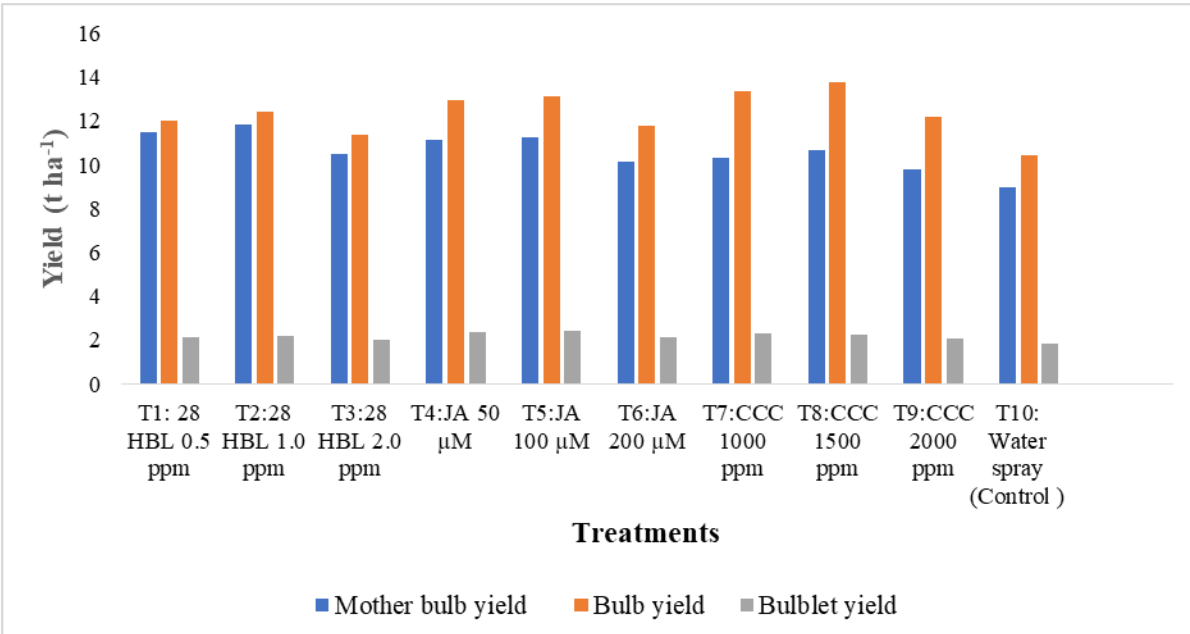

Figure 3 Effect of different growth regulating chemicals on mother bulb, bulb and bulblet yield ha^{-1} in tuberose cv. Prajwal

Mother bulb yield (t ha^{-1})

The mother bulb yield ranged from 8.97 t ha^{-1} to 11.84 t ha^{-1}. Mother bulb yield recorded with 28 HBL 1.0 ppm (11.84 t ha^{-1}) was superior to the rest of the treatments. Control treatment was found to be significantly inferior to all other treatments with respect to mother bulb yield (8.97 t ha^{-1}). Similarly, application of synthetic brassinolide increased the bulb yield in the onion cv. Alice [30]. JA also improved mother bulb yield next to BR. This was because of the fact that, jasmonates were potent inducers of vegetative storage protein gene expression [31] and hence played a pivotal role in enhancement of mother bulb yield.

Bulb yield (t ha^{-1})

Among various treatments, bulb yield ha^{-1} ranged from 10.43 t to 13.77 t. The highest bulb yield ha^{-1} (13.77 t) was recorded with 1500 ppm CCC while the lowest bulb yield ha^{-1} (10.43 t) was recorded in control. The results are in accordance with the findings of [10] in iris and [21] in gladiolus. Further, increase in chlorophyll content in leaves together with reduction of stem length could improve transportation of photoassimilates into bulbs in *Lilium* Oriental hybrids [11]. As the concentration of CCC increases, the bulb yield was increased up to 1500 ppm and later slightly reduced at 2000 ppm in tuberose cv. Prajwal.

Increased bulb production with BRs over control was associated with increased rate of photosynthesis and direct translocation of photosynthates to the sink (bulb). Similar results were reported in potato [32].

JA also increased bulb production in tuberose. Application of methyl jasmonate enhanced the endogenous salicylic acid content in the leaf sheath and promoted bulbing in garlic [33]. Further, JA played an important role in formation and enlargement of corms in *Gladiolus hybridus* by isolating three genes involved in the jasmonic acid biosynthesis from the *in vitro* grown corms [34].

Bulblet yield (t ha^{-1})

The bulblet yield ha^{-1} was found to exhibit significant variations among different growth regulating chemicals. Bulblet yield was ranged from 1.86 t ha^{-1} to 2.44 t ha^{-1}. The mean bulblet weight was 2.18 t ha^{-1}. Among the treatments, 100 µM JA recorded significantly highest bulblet weight (2.44 t ha^{-1}) which was superior to rest of the treatments. The lowest bulblet weight (1.86 t ha^{-1}) was recorded in control. Similarly, maximum number of small cormels with JA 10 ppm recorded in gladiolus cv. Shabnum [3]. More number of average bulbs per basal plate were also obtained on medium supplemented with JA in garlic cv. Ptujski Jesenski [35]. Enhanced tuber yield was found in transgenic potato plants with JMT gene encoding jasmonic acid carboxyl methyl transferase gene [27].

Conclusion

Foliar spray of either JA or CCC or BRs was found to be highly effective in enhancement of total bulb (mother bulbs. marketable bulbs and bulblets) production in the decreasing order over water spray. However, foliar application of CCC 1500 ppm was recommended to get highest marketable bulb yield in tuberose cv. Prajwal.

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