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Influence of brassinosteroids, jasmonic acid and chlorocholine chloride on yield and economics of tuberose cv. Prajwal

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Abstract
A study was carried out for two years at Horticultural Research Station, Kovvur aimed to know the economic viability of foliar spraying plant growth regulating chemicals in tuberose. Foliar spraying with 28-homobrassinolide (28 HBL), Chlorocholine chloride (CCC) and jasmonic acid (JA) was assessed each at three different concentrations along with water spray as control. The experiment was conducted in Randomized Block Design with three replications. Application of 28-homobrassinolide at 1.0 ppm promoted leaf area and early flowering. However, plants sprayed with CCC at 1500 ppm recorded highest floret (11.84 t ha^{-1}) and bulb (13.77 t ha^{-1}) yield with highest benefit-cost ratio of 3.29. Thus, it could be recommended to foliar spray 28 HBL at 1.0 ppm for early flowering and CCC at 1500 ppm for getting maximum returns per unit area.

Keywords: Tuberose, Prajwal, Jasmonic acid, CCC, 28 Homobrassinolide, economics

Introduction
Tuberose (*Polianthes tuberosa* L.) is one of the most important bulbous flower crops, known for its elegant and fragrant spikes. From economic point of view both floret and bulb production are important. Like any bulbous crops, flower and bulb production in tuberose is influenced greatly by management and environmental factors. One of the important management practices is the use of plant growth regulators for obtaining quality florets and bulbs. Further, to get positive results with growth regulators, time of application and concentration of growth regulating chemicals are of outmost importance. In view of this, an experiment was initiated to identify and standardize the dose of plant growth regulators to get higher economic benefit to the farmer in terms of floret and bulb yield per unit area.

Materials and Methods
An experiment was conducted with an objective to study the effect of plant growth regulating chemicals on floret, bulb yield and economics of tuberose cv. Prajwal. The present investigation was carried out at the Horticultural Research Station, Kovvur, DrYSRHU, Andhra Pradesh during 2015-16 and 2016-17. Healthy bulbs of tuberose cv. Prajwal were used as planting material. The experiment was conducted in randomized block design and replicated three times.

The experiment consisted of ten treatments viz. 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 (Chlorocholine chloride) 1000 ppm; T_8=CCC 1500 ppm; T_9=CCC 2000 ppm; T_{10}=Water spray (Control). Growth regulating chemicals were applied as foliar sprays at 30 and 60 days after sprouting. Recommended package of practices of DrYSRHU were followed during the period of experimentation.

Leaf area was measured at two months interval from 60 DAP (days after planting) to 240 DAP by destructive sampling of five plants at random in each treatment. The various observations on floral parameters were recorded from ten randomly selected plants in the net plot area. The data collected for all the characters studied were subjected to statistical analysis by adopting 'Analysis of Variance' (ANOVA) technique as suggested by Panse and Sukhatme (1967)[16]. Means that differed significantly were separated using the Least Significant Difference (LSD) test procedure at 5 % level of significance.

Results and Discussions

Growth parameters

Leaf area (dm^2) $clump^{-1}$

Table 1: Effect of different growth regulating chemicals on leaf area (dm^2) $clump^{-1}$ at different stages of growth in tuberose cv. Prajwal

Treatments	60 DAP	120 DAP	180 DAP	240 DAP
T_1:28 HBL 0.5 ppm	12.06	42.18	65.79	85.77
T_2:28 HBL 1.0 ppm	12.47	43.61	68.02	88.68
T_3:28 HBL 2.0 ppm	11.04	38.79	60.51	77.78
T_4:JA 50 µM	11.14	38.96	60.77	79.23
T_5:JA 100 µM	10.52	37.34	58.25	74.87
T_6:JA 200 µM	10.47	36.10	56.31	73.42
T_7:CCC 1000 ppm	10.73	37.53	58.54	77.41
T_8:CCC 1500 ppm	11.65	40.75	63.56	82.87
T_9:CCC 2000 ppm	11.55	40.39	63.01	82.14
T_{10}:Water spray (Control)	10.22	35.74	55.76	72.35
Mean	11.18	39.14	61.05	79.45
S.Em	-	0.19	0.29	0.38
C.D. at 5%	NS	0.55	0.85	1.13

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

The leaf area $clump^{-1}$ recorded at two months interval was significantly influenced by different growth regulating chemicals at all growth stages except at 60 DAP (Table 1). From 120 DAP, leaf area $clump^{-1}$ was significantly improved by all growth regulating chemicals over control except at high concentrations of JA. The mean leaf area $clump^{-1}$ was gradually increased from 39.14 dm^2 at 120 DAP to 79.45 dm^2 at 240 DAP. Maximum leaf area $clump^{-1}$ (43.61, 68.02 and 88.68 dm^2 at 120, 180 and 240 DAP respectively) was recorded with the foliar spray of 1.0 ppm 28 HBL. The results are corroborated with the findings of Padmalatha *et al.* (2013)[13] in gladiolus. Brassinolides (BL) stimulated leaf elongation (Chon *et al.*, 2000)[1] coupled with an increase in leaf number which might be the reason for enhanced leaf area. Ono *et al.* (2000)[12] also explained that enhanced leaf area by brassinosteroids (BR) might be due to significant increase in blade length, epidermal cells and chlorophyll parenchyma.

JA increased leaf area $clump^{-1}$ at lower concentration while at higher concentration there was not much increment in leaf area $clump^{-1}$ over control. At higher concentration, JA could not enhance leaf area significantly, as exogenously applied JA nullifies endogenous gibberellic acid level in the plant there by reduced the cell elongation in leaves and consequently the leaf area. Heinrich *et al.* (2013)[5] stated that the increased levels of JA antagonize the biosynthesis of gibberellic acid by repressing the transcription of the gibberellic acid biosynthetic genes.

CCC also increased leaf area $clump^{-1}$over control which could be attributed to the production of a greater number of leaves $clump^{-1}$ and leaf expansion by diversion of photo assimilates towards the leaves at the expense of internodal elongation of stem. Similar findings were reported by Zheng *et al.* (2012)[29] in oriental lily hybrids.

Leaf area index (LAI)

Table 2: Effect of different growth regulating chemicals on leaf area index at different stages of growth in tuberose cv. Prajwal

Treatments	60 DAP	120 DAP	180 DAP	240 DAP
T_1:28 HBL 0.5 ppm	1.340	4.686	7.310	9.530
T_2:28 HBL 1.0 ppm	1.385	4.845	7.558	9.853
T_3:28 HBL 2.0 ppm	1.227	4.310	6.723	8.642
T_4:JA 50 µM	1.237	4.329	6.753	8.804
T_5:JA 100 µM	1.169	4.149	6.472	8.319
T_6:JA 200 µM	1.163	4.011	6.257	8.157
T_7:CCC 1000 ppm	1.192	4.170	6.505	8.601
T_8:CCC 1500 ppm	1.294	4.527	7.063	9.207
T_9:CCC 2000 ppm	1.283	4.488	6.338	9.127
T_{10}:Water spray (Control)	1.135	3.971	6.195	8.038
Mean	1.243	4.349	6.717	8.828
S.Em	-	0.021	0.033	0.043
C.D. at 5%	NS	0.061	0.097	0.126

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

Leaf Area Index was also significantly differed among the treatments at all growth stages except at 60 DAP (Table 2). It was observed that the mean LAI $clump^{-1}$ increased from 1.243 at 60 DAP to 8.828 at 240 DAP. The highest mean LAI $clump^{-1}$ (4.845, 7.558 and 9.853 at 120, 180 and 240 DAP respectively) was recorded with the foliar spray of 1.0 ppm 28 HBL. This could be attributed to an increase in leaf area $clump^{-1}$ by increasing cell division and cell elongation activities in meristematic tissues. Similar results were reported by Sengupta *et al.* (2011) [21] in green gram. The enhanced leaf area index was also noticed over control with the application of CCC. Similarly, high LAI with CCC application was observed by Roopa (2012) [19] in onion. It might be due to an increase in leaf number and leaf expansion by stimulation of Rubisco activity (Pando and Srivastava, 1985) [15] and hill reaction activity.

Flower parameters

Table 3: Effect of different growth regulating chemicals on days to 50% flowering and spike characters in tuberose cv. Prajwal

Treatments	Days to 50% flowering (d)	No. of florets per spike	Floret weight per spike (g)	Length of spike (cm)
T_1:28 HBL 0.5 ppm	139.89	50.58	96.45	99.00
T_2:28 HBL 1.0 ppm	138.50	52.36	96.81	102.04
T_3:28 HBL 2.0 ppm	141.97	46.61	89.31	99.02
T_4:JA 50 µM	152.35	46.59	88.44	96.14
T_5:JA 100 µM	157.89	46.38	87.15	95.19
T_6:JA 200 µM	163.43	46.15	84.60	88.05
T_7:CCC 1000 ppm	145.43	49.77	95.38	90.43
T_8:CCC 1500 ppm	144.04	49.32	94.51	87.58
T_9:CCC 2000 ppm	143.33	47.51	91.04	86.63
T_{10}:Water spray (Control)	150.97	45.25	85.87	94.24
Mean	147.78	48.05	90.95	93.83
S.Em	0.86	0.22	0.45	0.52
C.D. at 5%	2.60	0.65	1.32	1.53

28 HBL: 28 Homobrassinolide; JA: Jasmonic acid; CCC: Chlorocholine chloride

Days to 50% flowering (d)

Significant differences were observed among different growth regulating chemicals for days to 50% flowering (Table 3). Days to 50% flowering ranged from 138.50 to 163.43. 28 HBL and CCC treated plants were flowered earlier as compared to the untreated plants (control). Among all treatments, plants treated with 28 HBL particularly at lower concentrations (0.5 ppm and 1.0 ppm) induces early flowering. Significantly, early flowering was noticed with 1.0 ppm 28 HBL (138.50) followed by 0.5 ppm 28 HBL (139.89). Similar results were obtained with the application of Brassinolides by Mogollon and Ojeda (2005) [10] in peace lily, Padmalatha *et al.* (2013) [13] in gladiolus and Vijayakumar *et al*. (2017) [26] in china aster. It was further confirmed from the studies of Li *et al*. (2010) [9] that nearly all BR-deficient and BR-insensitive mutants exhibit late flowering phenotype in *Arabidopsis*. Application of CCC also recorded early flowering as compared to control as reported by Singh *et al.* (2010) [24] in tuberose.

The flowering was delayed in plants treated with JA. The highest number of days (163.43) to 50% flowering was recorded with 200 µM JA. Diallo *et al.* (2014) [2], also reported that Methyl jasmonates delayed flowering in wheat. At molecular level, delayed flowering with JA was explained by Zhai *et al.* (2015) [28]. JA promotes the degradation of JAZ (JASMONATE-ZIM) domain family of transcriptional repressors of JA which in turn repress the expression of florigen gene FLOWERING LOCUS T (FT) thereby trigger the signaling cascades to delay flowering.

Number of florets spike^{-1}

Among the treatments, application of 28 HBL 1.0 ppm recorded significantly highest number of florets spike^{-1} (52.36) and lowest (45.25) was observed in control.

BR promoted vegetative growth of plants in terms of leaf area which enabled the leaves to produce more photosynthates. The increase in availability of more assimilates to shoot apex at the time of floral primordial initiation resulted in an increase in number of florets spike^{-1}. Padmalatha (2011) [14] also reported that BR increased the number of florets spike^{-1} in gladiolus.

JA also enhanced the number of florets spike^{-1} over control. Similar results were reported by Kumar *et al.* (2008)[8] in gladiolus and explained that jasmonates plays an important role in the formation of flowers which was evident that the presence of relatively high levels of jasmonates in the developing plant reproductive tissues.

CCC increased the number of florets spike^{-1} which might be due to its role in enhancement of the sink potential as reported by Khan *et al.* (2007) [7]. Similar results were reported by Gawai *et al.* (2014) [4] in tuberose and Sudhakar and Kumar (2012) [25] in gladiolus.

Floret weight spike^{-1} (g)

Significant differences were noticed among different growth regulating chemicals for floret weight spike^{-1}(Table 3). The highest floret weight spike^{-1} was observed with 28 HBL 1.0 ppm (96.81 g) followed by 28 HBL 0.5 ppm (96.45 g) and CCC 1000 ppm (95.38). Yu *et al.* (2004) [27] found that BR treated leaves had a higher quantum yield of PSII electron transport than water sprayed (control) plants which might have resulted in the production of more photosynthates that were available to enhance the floret weight in a spike. The results are in tune with the findings of Vijayakumar *et al*. (2017) [26] in China aster.

However, the lowest floret weight spike^{-1} was recorded with 200 µM JA (84.60 g) which was on par with control (85.87 g). Slight reduction in floret weight spike^{-1} with JA spray might be due to reduction in the supply of carbohydrates to shoot apex at the time of flowering on an account of its growth inhibiting action.

Length of spike (cm)

The spike length was differed significantly among the different levels of growth regulating chemicals (Table 3). The longest spike was observed with 28 HBL 1.0 ppm (102.04 cm) while shortest spike was observed at CCC 2000 ppm (86.63 cm) which was preceded by CCC 1500 ppm (87.58 cm) and JA 200 µM (88.05 cm).

JA and BR significantly increased the mean spike length over control except at higher concentration of JA. Similar results were obtained by Kumar *et al.* (2008) [8] in gladiolus. The increase in spike length with BL application could be attributed to the fact that BRs regulate the stem development through the expression of genes involved in cell elongation and wall extensibility (Zurek *et al.*, 1994 [30]; Horvath *et al.*, 2003 [6]). At higher concentration of JA, reduction in leaf area reduced supply of photo assimilates to the sink tissue ultimately resulted in dwarfing of spikes.

However, application of CCC reduced spike length due to the inhibition of cell division and elongation in sub-apical meristems and elongation of internodes in a spike by inhibiting, or blocking of gibberellins biosynthesis (Moore, 1989) [11]. Further, it was explained that CCC was associated

with reduced elongation of the internodes, rather than lowering the number of internodes (Shekoofa and Emam, 2008) [22]. Similar results were observed earlier in tuberose (Singh and Desai, 2013) [23].

Yield parameters

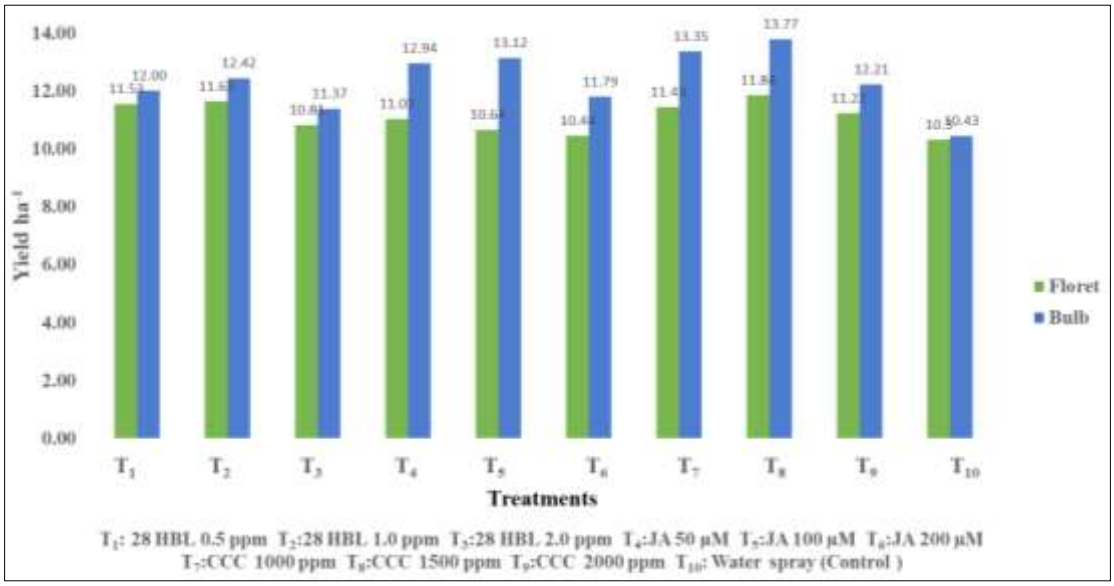

Fig 1: Effect of growth regulating chemicals on floret and bulb yield ha^{-1} in tuberose cv. Prajwal

Floret yield ha^{-1} (t)

The perusal of data (Fig. 1) on floret yield per ha^{-1} showed that foliar spray of CCC 1500 ppm recorded the maximum floret yield (11.84 t ha^{-1}). Application of CCC might have increased the rate of photosynthesis by increasing the chlorophyll content per unit area and the size of the mesophyll cells in the leaves. This might lead to more rapid exchange of CO_2 into mesophyll cells by virtue of their larger surface area (Dulizhao and Oosterhius, 2000) [3]. Increased production of spikes clump^{-1} (2.207) coupled with increased floret number (49.32) and floret weight (94.51g) spike^{-1} was indicative of positive influence on floret yield ha^{-1}. Similar results were reported by Sanap *et al.* (2000) [20] in tuberose.

In the present study BR also increased the floret yield ha^{-1} over control at all concentrations. Application of brassinolids also increased the floret yield ha^{-1} in China aster (Vijayakumar *et al.*, 2017) [26]. BR increased the initial activity of Rubisco enzyme, followed by a substantial increase in sucrose phosphate synthase, sucrose synthase, acid invertase activities thereby increased the contents of sucrose, soluble sugars, and starch (Yu *et al.*, 2004) [27]. Further, BR also enhanced the sucrose translocation to the apical sink (Petzold *et al.*, 1992) [18] and thus contributed to an enhanced the floret yield.

Bulb yield ha^{-1} (t)

The variations in bulb yield ha^{-1} as influenced by different growth regulating chemicals (Fig. 1) were found significant. Highest bulb yield ha^{-1} (13.77 t) was recorded with 1500 ppm CCC over control (10.43 t). Application of CCC regulate the source sink ratio by reducing the partitioning of carbohydrates to floral spike which was evident from the reduction in spike length and enhancement of bulb weight as compared to control. The results are in accordance with the findings of Patel *et al.* (2010) [17], Sudhakar and Kumar (2012) [25] in gladiolus.

Economics

Table 4: Benefit-cost ratio as influenced by bulb inducing growth regulators in tuberose cv. Prajwal

Treatment	Total cost (₹)	Returns (₹)		Gross returns (₹)	Net returns (₹)	Benefit-cost ratio
		Floret	Bulb			
T_1:28 HBL 0.5 ppm	537835	922400	720000	1642400	1104565	3.05
T_2:28 HBL 1.0 ppm	541010	930400	745200	1675600	1134590	3.10
T_3:28 HBL 2.0 ppm	539200	864800	682200	1547000	1007800	2.87
T_4:JA 50 µM	889367	881600	776400	1658000	768633	1.86
T_5:JA 100 µM	1244314	851200	787200	1638400	394086	1.32
T_6:JA 200 µM	1958688	835200	707400	1542600	-416088	0.79
T_7:CCC 1000 ppm	535260	914400	801000	1715400	1180140	3.20
T_8:CCC 1500 ppm	539740	947200	826200	1773400	1233660	3.29
T_9:CCC 2000 ppm	535380	897600	732600	1630200	1094820	3.04
T_{10}:Control (Water spray)	525620	824000	625800	1449800	924180	2.76

28 HBL: 28 Homobrassinolide; JA: Jasmonic acid; CCC: Chlorocholine chloride

Sale price of floret per kg Rs.80/- and bulb per kg Rs.60/- Lot of variation was observed in total cost of cultivation due to variation in the cost of growth regulating chemicals and harvesting costs (Table 4). Gross returns varied due to the differences in the floret and bulb yields due to the influence of growth regulating chemicals which resulted in a wide variation among the treatments for benefit – cost ratio. The highest gross returns, net returns and Benefit – cost ratio was recorded with CCC 1500 ppm (3.29) followed by CCC 1000 ppm (3.20).

Conclusion

Summarily it could be concluded that the application of 28 HBL at 1.0 ppm promoted early flowering. However, exogenous application of CCC at 1500 ppm resulted in a greater number of spikes and bulbs plant^{-1}. Thus, this simple intervention will yield maximum returns per unit area.

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