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Influence of concentration and mode of application of different growth regulators on dendrobium hybrid *Thongchai Gold*

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ABSTRACT

An experiment was conducted to determine the influence of concentration and mode of application of different growth regulators on growth and yield of Dendrobium hybrid "Thongchai Gold" in subtropical cool climate zone. Various concentrations of GA_3 and BA were applied as foliar spray and drenching mode. GA_3 100 ppm in combination with BA 100 ppm followed by GA_3 200 ppm increased the plant height (31.63 cm), number of shoots per plant (5.17), cane girth (1.71 cm), number of leaves per cane (8.21), internodal length (3.19 cm), spike girth (0.45 cm), number of flowers per spike (12.63) and number of spikes per cane (2.80). The spike length (29.38 cm) was noticed highest in GA_3 50 ppm and rachis length (19.66 cm) was observed highest in GA_3 200 ppm. Growth regulators and mode of applications also influenced nutrient content (NPK) of plants. Among the mode of applications, media drenching with the different growth regulator treatment was found to be best for the Dendrobium orchids.

Keywords: Drenching, foliar spray, growth regulators, yield

Dendrobiums are tropical orchids requiring warm, humid and shady conditions and the second largest genus of the orchid family with more than 1500 species. Dendrobium production has steadily increased with the influx of new farms being developed and with established farms that have increased in acreage. In India commercially grown in the states of Kerala, Tamil Nadu, Karnataka and Maharashtra, these orchids are easy to cultivate and produce abundant, colourful flowers. Plant growth regulators have been used successfully to modify flowering of many orchid species. Apical dominance and auxins suppress flowering of Aranda (Goh, 1985). The response of BA depends on the hybrids and successfully induced flowering in Aranthera, Aranda, Holtumara, Mokara, Dendrobium Lousiae 'Dark', Phalaenopsis, Dendrobium 'Nodoka', and Oncidium (Goh, 1985; Hew and Clifford,1993). Gibberellins transformed the normal vegetative shoot apex to a terminal inflorescence of Aranda 'Deborah' and induced flowering of *Bletilla striata*, Cymbidium and Cattleya hybrids (Goh *et al*., 1982; Goh, 1985). Addition of GA_3 with cytokinins enhanced the flowering effect and reduced flower deformity of Phalaenopsis and Dendrobium (Chen *et al*., 1997; Sakai *et al*., 2000). Injection of 100 mm of BA and 10mm GA_3 into Dendrobium 'Jaquelyn Thomas Uniwai Princess' pseudobulbs promoted off season flowering and increased the flower size and number of inflorescences per stem in Hawaii (Sakai *et al*., 2000).

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Application of cytokinin also induced flowering in Dendrobium. Earlier evidence showed that some Dendrobium species can be induced to flower by either cytokinin treatment or following an expose to low temperature treatments. More over flowering of Dendrobium depends on genetic makeup of species, hybrids and climate condition of particular area where it is available. Application of exogenous growth regulators to hybrids for enhancement of production is limited. In commercial orchid cut flower production, the production should have coincidence with market demand. Induction of flower and seasonality of flowering are the two major factors that govern pricing and marketability of orchid cut flowers. Keeping the above condition in view, the present study was undertaken to study the influence of different growth regulators (BA and GA_3) at different concentration and mode of application on Dendrobium hybrid "Thongchai Gold" for flower production under sub tropical cool climate of Sikkim.

MATERIALS AND METHODS

An experiment was conducted at National Research Centre for Orchids, Pakyong, Sikkim during the period of 2008-2012. Tissue cultured plants of uniform size of Dendrobium hybrid "Thongchai Gold" were grown in partially modified green house. Plants were grown in 15 cm perforated pot with a growing medium of broken bricks (2 to 3cm size) and coconut husk (1.5 to 3cm size) at a ratio of 1:1 Initial two years plants were grown for acclimatization with the sub tropical cool climate where average maximum air temperature ranged between 25^0C to 30^0C from April to September. The air temperature goes down slowly from end of October to February. During winter season minimum air temperature were recorded between 5^0C to 10^0C. New shoots of plant generally came up in the month of May to September and growth seized in winter month. Seven growth regulator treatments and two mode of application such as foliar spray and media drenching were followed at morning hours only. Plants were sprayed and drenched thoroughly with growth regulators of the consequent treatments such as distilled water as control, GA_3 50 ppm, GA_3 100ppm, GA_3 200 ppm, BA 50 ppm, BA 100 ppm and GA_3 100 ppm +BA 100 ppm. BA and GA_3 were dissolved in 95% ethanol at the concentrations required for each treatments and distilled water was added to make the final concentration of 10% ethanol. In case of drenching, complete saturation of growing medium with growth regulator solutions, excess solutions that passed through the pot holes were collected in tray and reused several times. In combine application, BA 100 ppm spray or drench was given 10 days after GA_3 spray or drenching of solution. Foliar application and drenching of chemicals were given at twice a year during the months of April and July (2011 and 2012). The water soluble fertilizer (NPK 20:20:20) was applied @ 1g/l as foliar spray at weekly intervals initially for two years and NPK (10:20:20) @1g/l from third year onward for boosting vegetative and flowering growth respectively.

The observations on plant height (cm), number of shoots per plant, cane girth (cm), number of leaves per cane, inter-nodal length (cm), spike length (cm), rachis length (cm), spike girth (cm), number of flowers per spike and number of spikes per cane were recorded periodically at regular intervals. The experiment was carried out in a factorial completely randomized design and replicated thrice. Total N was determined by micro Kjeldahl method (Bremner, 1965), total P was determined by Vanodomolybdo-phosphoric yellow colour method (Jackson, 1973) and total K was determined by flame photometer method (Jackson, 1973). The pooled data were subjected to statistical analysis by using the software SAS, following the user's guide SAS/STAT (1990).

RESULTS AND DISCUSSION

The growth of Dendrobium hybrid “Thongchai Gold” was significantly influenced by different concentrations of growth regulators and mode of application (Table 1).The plant height was recorded highest in the treatment combination of GA_3 100 ppm + BA 100 ppm (31.63 cm) and among the mode of applications, drenching (29.44 cm) with the above growth regulator treatments was found to be best and in case of interaction, significant influence was noticed. According to Karaguzel *et al*. (2004) soil drenches were effective as a method of plant growth regulator application compared to foliar sprays. Flowering of Dendrobium is directly correlated with the cane or pseudobulb growth and development. So, the effect of the different concentrations of plant growth regulators and mode of applications on number of leaves per cane, cane girth, number of canes and intermodal length of the cane were studied. Application of GA_3 100 ppm + BA 100 ppm consistently increased the shoots production (5.17) and followed by GA_3 200 ppm (4.93). Among the mode of applications, there was no significant difference noticed, however significant influence was recorded in case of interaction of growth regulator and mode of applications. Application of BA 100 ppm in combination with GA_3 100 ppm and BA 100 ppm alone increased the girth of the cane (1.71 and 1.60 cm respectively) and among the mode of applications, drenching recorded the highest cane girth (1.51 cm) as compared to foliar spraying. The interaction effect shows the significant difference between the mode of application and growth regulators. The treatment combination of GA_3 100 ppm + BA 100 ppm increased the number of leaves per cane (8.21) followed by GA_3 200 ppm (8.00) and among the mode, drenching of growth regulators (7.38). Application of GA_3 200 ppm improved internodal length (3.22cm). The significant influence was recorded in case of interaction of growth regulator and mode of applications with the above growth regulator combinations. Reduction of intermodal length was observed when BA was applied alone. However, the detrimental effect was reduced and inter nodal length was evident highest in plants which received GA_3 100 ppm in combination with BA 100 ppm followed by GA_3 200 ppm alone. Application of BA treatments promoted vegetative growth instead of flowering and this effect was alleviated by the addition of GA_3. In Phalaenopsis cv. 'Leda', flower primordial elongation promoted by GA_3 is fully prevented by BA treatments (Chen *et al*., 1997).

The perusal of table 2 shows that application of GA_3 50 ppm followed by GA_3 100 ppm + BA 100 ppm increased the spike length (29.38 cm and 28.81 cm respectively) and there is no significant influence was observed among the mode of applications, however, remarkable influence was recorded in case of interaction effect between the growth regulators and mode of applications. Sugar may be a component of the floral stimulus and its production in the apical meristem could be enhanced by GA_3 treatment. Chen *et al*. (1994) stated that the sucrose syntheses may be relatively important in the degradation of sucrose in GA_3 treated plants. The findings with GA_3 would seem to support nutrient diversion as a mode of action by which exogenously applied GA_3 could promote flowering in Phalaenopsis (Sachs *et al*., 1979). Our findings with GA_3 would seem to support the evidence by many workers (Chen *et al*., 1994; Chen *et al*., 1997; Sakai *et al*., 2000; Matsumoto, 2006). GA_3 stimulate the transport of assimilate through the phloem, or increase sucrose uptake, or act on metabolism of sucrose and its metabolic products (Morris and Arthur, 1984). Gibberellins are biochemically described as tetra cyclic diterpene acids and are associated with flowering induction in several species. When exogenously applied, these plant growth regulators lead to petal growth and flowering induction in long-day plants under conditions of short days. Philodendron cv. Black Cardinal plants were induced to flower under non-inductive conditions through application of GA_3 at 125, 250, 500 and 1,000 mg L^{-1}, increasing flowering percentage and inflorescence number per plant with increasing concentrations (Chen *et al.,* 2003). In flower cultivation, other species have their flowering induced by GA_3 applied via pulverization, such as *Dieffenbachia* (Henny, 1980), *Zantedeschia* (Corr and Widmer, 1987) and *Anthurium* (Henny and Hamilton, 1992**).** Chen *et al.* (1997) reported that flowering can be induced in *Phalaenopsis* cv. Leda with the application of GA_3 under conditions of high temperatures, non-inductive to flowering.

Table 1: Influence of concentration and mode of application of growth regulators on growth of Dendrobium hybrid *Thongchai gold*

Growth regulators	Plant height (cm)			Number of shoots plant^{-1}			Cane girth (cm)			Number of leaves cane^{-1}			Internodal length (cm)		
	S	**D**	**Mean**	**S**	**D**	**Mean**	**S**	**D**	**Mean**	**S**	**D**	**Mean**	**S**	**D**	**Mean**
Control	24.67	25.35	25.01	3.26	3.54	3.4	1.13	1.25	1.19	5.48	6.50	5.99	2.50	2.50	2.50
GA_3 50 ppm	28.20	29.07	28.64	3.76	3.94	3.85	1.33	1.37	1.35	6.49	6.63	6.56	2.59	2.88	2.74
GA_3 100ppm	28.70	30.60	29.65	4.45	4.11	4.28	1.38	1.45	1.42	6.86	6.85	6.85	2.94	3.09	3.02
GA_3 200 ppm	30.81	31.37	31.09	4.85	5.01	4.93	1.45	1.57	1.51	7.67	8.33	8.00	3.18	3.26	3.22
BA 50 ppm	25.80	27.59	26.70	4.10	4.29	4.20	1.40	1.48	1.44	6.79	7.19	6.99	2.67	2.83	2.75
BA 100 ppm	28.18	29.75	28.97	4.38	4.58	4.48	1.54	1.66	1.60	7.05	7.72	7.39	2.87	2.99	2.93
GA_3 100 ppm +BA 100 ppm	30.90	32.36	31.63	4.96	5.38	5.17	1.63	1.79	1.71	7.99	8.43	8.21	3.20	3.17	3.19
Mean	28.18	29.44		4.25	4.41		1.41	1.51		6.90	7.38		2.85	2.96	
SEm(±)															
S	**0.46**			**0.20**			**0.05**			**0.19**			**0.07**		
D	**0.40**			**0.14**			**0.04**			**0.15**			**0.05**		
S×D	**0.61**			**0.30**			**0.08**			**0.25**			**0.11**		
LSD(0.05)															
S	**1.30**			**0.57**			**0.15**			**0.54**			**0.21**		
D	**1.13**			**NS**			**0.10**			**0.41**			**NS**		
S×D	**1.72**			**0.83**			**0.22**			**0.72**			**0.30**		

Table 2: Influence of concentration and mode of application of growth regulators on flowering parameters of Dendrobium hybrid *Thongchai gold*

Growth regulators	Spike length (cm)			Rachis length (cm)			Spike girth (cm)			Number of flowers/spike			Number of spikes $cane^{-1}$		
	S	**D**	**Mean**	**S**	**D**	**Mean**	**S**	**D**	**Mean**	**S**	**D**	**Mean**	**S**	**D**	**Mean**
Control	22.24	22.89	22.57	15.26	15.43	15.35	0.36	0.38	0.37	9.06	9.24	9.15	1.33	1.58	1.45
GA_3 50 ppm	29.04	29.71	29.38	16.80	16.71	16.76	0.40	0.40	0.40	9.90	10.34	10.12	1.63	1.74	1.69
GA_3 100ppm	27.79	28.43	28.11	18.05	18.19	18.12	0.42	0.42	0.42	10.68	11.74	11.21	1.86	2.10	1.98
GA_3 200 ppm	28.62	28.80	28.71	19.25	20.06	19.66	0.43	0.44	0.44	12.20	12.99	12.60	2.21	2.52	2.37
BA 50 ppm	28.55	26.45	27.50	15.53	16.71	16.12	0.40	0.42	0.41	10.43	10.78	10.61	1.60	1.28	1.44
BA 100 ppm	25.79	27.46	26.63	16.44	18.08	17.26	0.42	0.43	0.43	11.15	12.34	11.75	1.52	1.24	1.38
GA_3 100 ppm +BA 100 ppm	28.01	29.61	28.81	18.64	19.99	19.32	0.44	0.46	0.45	12.03	13.23	12.63	2.54	3.05	2.80
Mean	27.16	27.76		17.14	17.88		0.41	0.42		10.80	11.52		1.94	2.24	
SEm(±)															
S	**0.53**			**0.51**			**0.01**			**0.28**			**0.11**		
D	**0.44**			**0.35**			**0.00**			**0.23**			**0.08**		
S×D	**0.72**			**0.73**			**0.01**			**0.37**			**0.14**		
LSD(0.05)															
S	**1.48**			**1.45**			**0.0159**			**0.80**			**0.30**		
D	**NS**			**NS**			**0.0124**			**0.65**			**0.23**		
S×D	**2.04**			**2.06**			**0.0214**			**1.06**			**0.39**		

Rachis length (19.66 cm) was recorded highest with the application of GA_3 200 ppm followed by GA_3 100 ppm in combination with BA 100 ppm (19.32 cm) and the mode of applications also failed to show significant difference. Application of GA_3 100 ppm in combination with BA 100 ppm resulted in highest spike girth of 0.45 cm and it was on par with GA_3 200 ppm (0.44 cm). Among the mode of applications media drenching (0.42 cm) was found to be best and significant influence was recorded in case of interaction of growth regulator and mode of applications. Number of flowers per spike was recorded highest with the application of GA_3 100 ppm in combination with BA 100 ppm (12.63) followed by GA_3 200 ppm (12.60) and among the mode of applications media drenching (11.52) was found to be best. The significant difference was noticed between the interaction of different concentrations of growth regulators and mode of applications. Flowering is a process that plant changes from vegetative to reproductive phase, from leaf to flower (Boonyakiat, 2003; Taiz and Zeiger, 2006). This process is controlled by hormonal level (Ruamrungsri, 2004) and environmental condition (Hew and Yong, 1997). Application of GA_3 100 ppm in combination with BA 100 ppm increased the number of spikes per cane (2.80). BA application alone produced deformed spikes which adversely affected the appearance of the spike. However, this detrimental effect of BA was reduced when GA_3 was applied along with BA. The results are in line with the findings of Rajeevan and Swapna (2003). The results obtained by Higuchi and Sakai (1977) were partly in line with the results of present study. They observed reduced number of florets in Dendrobium with the application of BAP at 4000 mg/l but productivity was found to increase. According to Goh and Yang (1978) application of GA_3 when combined with BA improved flowering in Dendrobium hybrid 'Lady Hochoy'. In *Dendrobium* 'Louisae', GA_3 alone had any effect on floral bud initiation, while GA_3 at 0.001M+BA 0.01M, floral buds were appeared earlier than BA alone, these floral buds were better development and reached 100% of flower (Goh, 1979).

We observed deformed flower buds from plant treated with BA or GA_3. Applications of GA_3 (Chen *et al*., 1997) or BA (Sakai *et al.,* 2000) treatment to increase flowering often results in deformed flowers and usually require the combination of both for normal flower development. According to Blanchard and Runkle (2008) plants treated with BA_1, BA_2, or BA+GA within each treatment had flowers with abnormal floral structures (for example, additional carpels, stamens, petals, or sepals); however, there were no trends observed among treatments. In Phalaenopsis 'Alice Girl,' BA1 applied 6 weeks after transfer to 23^{0}C caused several plants to develop "crooked" inflorescences (for example, acute bend at 1 node)**.** Kim *et al*. (2000) noted that 5–13% of Doritaenopsis plants treated with 400 mg L^{-1} BA had malformed inflorescences at emergence and 3–7% of plants had blasted flower buds. In our study, application of BA on some orchid clones significantly reduced the width of open flowers by 1.2 cm or less. The decrease in flower number per inflorescence could be caused by competition among developing inflorescences for available assimilates. Additional research is required to elucidate the specific effects of BA on inflorescence development of these orchids.

Treatment of Phalaenopsis flowering shoots with GA_3 at warmer temperatures increases levels of the active GA_1 required for the promotion of flower development to the same extent as levels found in flowering shoots grown at cool temperatures (Su *et al.,* 2001). We compared the percentage of plant with visible inflorescences in foliar spray and drenching of plant with growth regulators. It was observed that 15% plant produced inflorescence in control while 46% in the plant sprayed with BA 100 ppm GA_3 100 ppm in 1st September followed by GA_3 100 ppm. In all cases inflorescences increased as days progressed. The maximum inflorescence was recorded up to 15th November and decreased slowly thereafter (Fig.1). Over all; GA_3 100 ppm enhanced the emergence of inflorescence. Same pattern of inflorescences development was observed in case of drenching, however maximum percentage of plants with inflorescence was recorded on 30th November and thereafter it decreased slowly. Like spraying, plants drenched with BA 100 ppm + GA_3 100 ppm produced more number (97%) of inflorescence as compared to other treatments (Fig.2). The monthly production behavior was also noted during the experimentation period. It was recorded that the spraying of growth regulators influenced flower production for the period of September to December(Fig.3a,3b,3c,3d).There was an increasing trend of production at lower level of GA_3 application, however, the production fall at higher concentration (GA_3 200pm). The average production due to spraying of BA marginally increased at 100 ppm concentration.

The combine effect of GA 100ppm + BA 100ppm had improved the production more than 1.5 fold over control i.e. average 10.1 spike /pot/years in control and 16.55 in case of combined application of growth regulator. Same type of variation was observed in case of drenching of growth regulator (Fig.4a, 4b, 4c, 4d). The outstanding feature we observed that the production in drenched plant was increased more than the spraying irrespectively of growth regulator. The average production was around 2.5 fold more in combine application of GA_3 100ppm + BA100ppm as compare to control. The lower production was caused in spraying invariably due to the plant of CAM in nature. During extreme winter the production was fallen drastically for which data was not presented. The cause is that most of the hybrids of Dendrobium are warm loving plant. Further, it might be in action of growth regulator during lower temperature. Matsumoto (2006) reported that application of BA treatments promoted vegetative growth instead of flowering and this effect was alleviated by addition of GA_3 in Miltoniopsis orchid hybrids Bert Field 'Eleen' and Rouge 'Akatsuka' which confirm our findings. In an experiment, Sakai *et al*. (2000) injected different concentration of BA and GA_3 and recorded 100mM of BA and 10mM GA_3 into the Dendrobium Jaquelyn Thomas 'Uniwai Princess' pseudobulb increased the flower size and number of inflorescence.

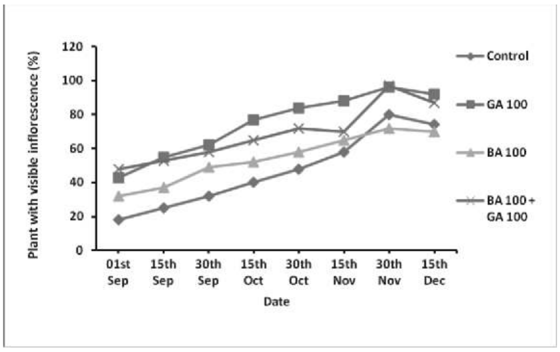

Fig. 1: Percentage of plants with visible inflorescences as influenced by spraying of growth regulators

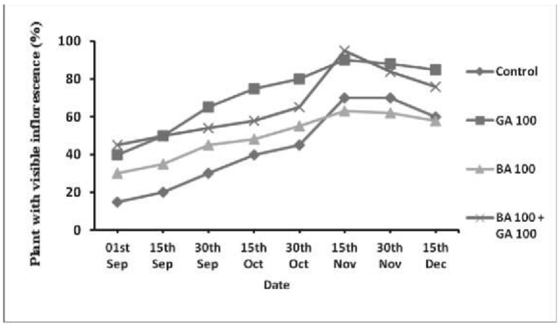

Fig. 2: Percentage of plants with visible inflorescences as influenced by drenching of growth regulators

The table 3 depicts that the NPK content in leaves of Dendrobium hybrid Thongchai Gold was significantly influenced by the different concentrations of growth regulators, mode of applications and the interaction of both. Application of GA_3 100 ppm in combination with BA 100 ppm increased the N (2.99), P (0.36) and K (3.35) content of leaves. Further, the mode of application as well as interaction with growth regulators also influenced the nutrients content. This might be due to increased nutrient content which would have contributed to the higher relative growth rate of the plants. Increased nutrient status in different plant parts are due to accumulation of carbohydrates, which may take place gradually with the advancement of crop growth (Patil and Janawade, 1999).

For the foregoing discussion it can be concluded that the spike production, length of spike, rachis length and intermodal length are the commercially important characters in orchids. Considering these factors GA_3 100 ppm combined with BA 100 ppm and GA_3 200 ppm alone can be suggested as an ideal growth regulator combination for Dendrobium orchids at Sikkim condition. Our result shows that combination of both the growth regulators can be recommended to enhance the productivity without adversely affecting the quality of the spikes, however; further investigation is required for time and period of application.

Table 3: Influence of concentration and mode of application growth regulators on NPK content of Dendrobium hybrid *Thongchai gold*

Growth regulators	Nitrogen			Phosphorus			Potassium		
	S	**D**	**Mean**	**S**	**D**	**Mean**	**S**	**D**	**Mean**
Control	2.73	2.78	2.76	0.26	0.27	0.27	3.07	3.10	3.09
GA_3 50 ppm	2.80	2.87	2.84	0.29	0.29	0.29	3.11	3.15	3.13
GA_3 100ppm	2.83	3.02	2.93	0.30	0.31	0.31	3.13	3.20	3.17
GA_3 200 ppm	2.90	3.08	2.99	0.31	0.33	0.32	3.17	3.25	3.21
BA 50 ppm	2.67	2.83	2.75	0.30	0.31	0.31	3.20	3.26	3.23
BA 100 ppm	2.78	2.90	2.75	0.32	0.32	0.32	3.23	3.30	3.27
GA_3 100 ppm +BA 100 ppm	2.87	3.10	2.99	0.35	0.36	0.36	3.30	3.40	3.35
Mean	2.80	2.94		0.34	0.31		3.17	3.24	
SEm(±)									
S	**0.05**			**0.01**			**0.03**		
D	**0.03**			**0.01**			**0.02**		
S×D	**0.07**			**0.01**			**0.04**		
LSD(0.05)									
S	**0.16**			**0.02**			**0.08**		
D	**0.09**			**0.02**			**0.06**		
S×D	**0.19**			**0.02**			**0.11**		

Note: S- Spraying, D- Drenching

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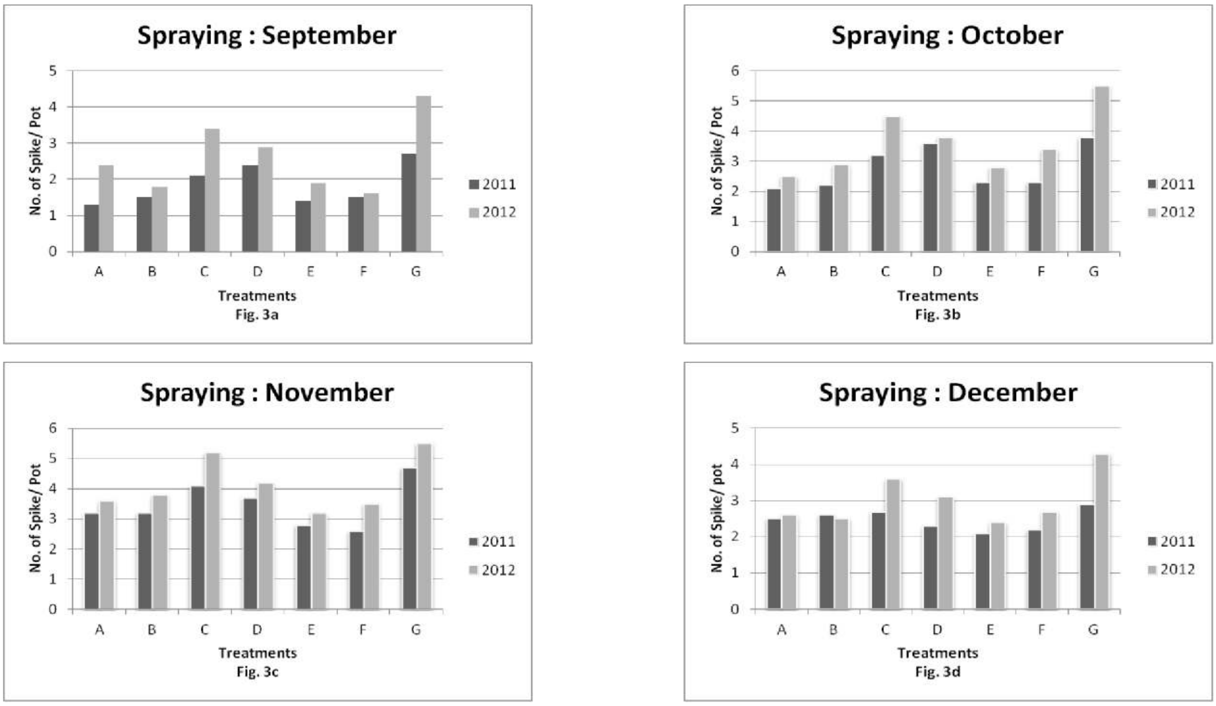

Fig. 3a, 3b, 3c, 3d showing the monthly production of spike^{-1} pot by spraying of growth regulators. A-Control, B-GA_3 50ppm, C- Ga_3 + 100ppm, D- Ga_3 + 200ppm, E-BA + 50ppm, F-BA + 100ppm, G-GA_3 + 100ppm BA100ppm.

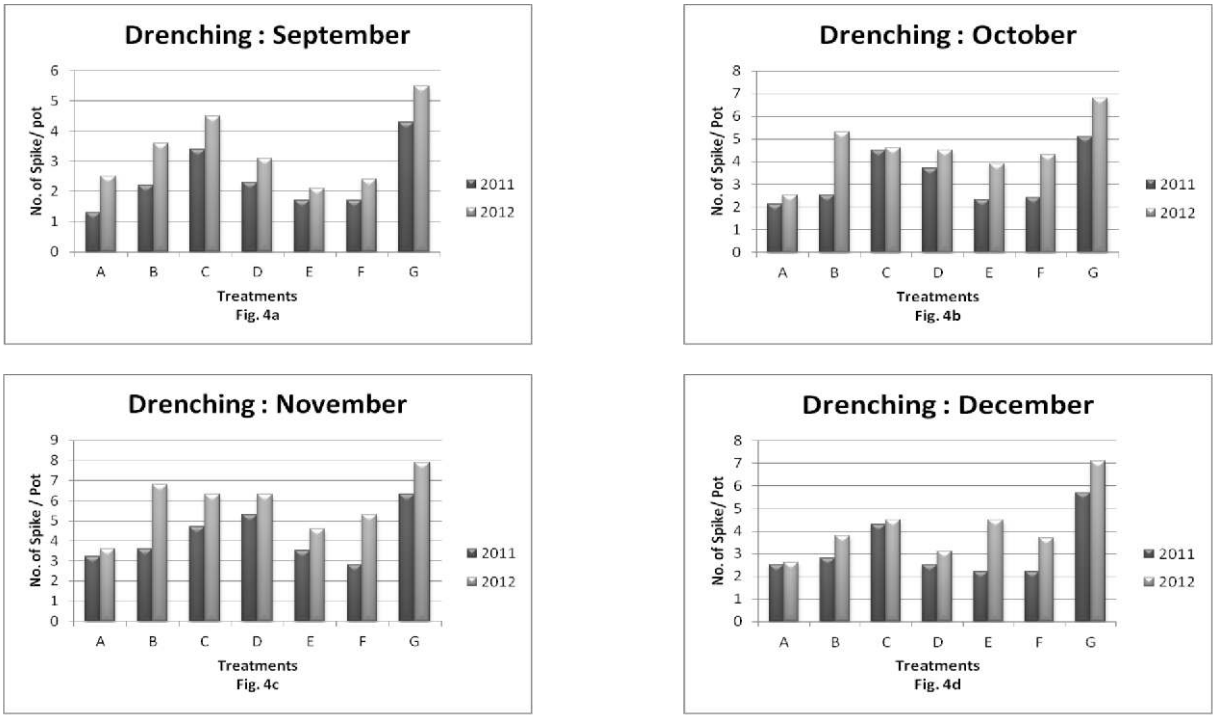

Fig. 4a, 4b, 4c, 4d showing the monthly production of spike^{-1} pot by drenching of growth regulators. A-Control, B-GA_3-50ppm, C- Ga_3 + 100ppm, D- Ga_3 + 200ppm, E-BA + 50ppm, F-BA + 100ppm, G-GA_3 + 100ppm BA100ppm.

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