



## Effect of foliar application of GA<sub>3</sub>, ethrel and copper sulphate on flowering behaviour and sex ratio of *Jatropha curcas* L.

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**Abstract:** Effect of various plant growth substances viz., GA<sub>3</sub> (25, 50, 100 ppm), ethrel (1500, 2000, 2500 ppm), copper sulphate (0.1, 0.2, 0.5%) as foliar spray treatment applied during January to October was studied at 15 days intervals. The flowering behaviour and sex-expression of *Jatropha curcas* was investigated during randomized block design (RBD) at the College Farm, Navsari. Looking to the results, it was noticed that GA<sub>3</sub> 50 ppm resulted in increased number of inflorescence per plant (31.25), number of flower per inflorescence (76.87), number of (male 94.75/female 4.01) flowers, flower sex ratio (24.22) in *J. curcas*. The Male: Female flower ratio was the lowest at 14<sup>th</sup> spray stage under majority of treatments indicating increased number of female flowers which is ultimately reflected by increased fruit and seed yield of *J. curcas*.

**Keywords:** Flowering and male/female ratio, *J. curcas*, Plant growth regulators

### INTRODUCTION

*Jatropha curcas* is a perennial deciduous shrub belonging to the family Euphorbiaceae, which probably originated in Central America and is widely distributed in the tropics and subtropics (Makkar and Becker, 2009). *Jatropha* seed content is about 30-40% oil, which is an ideal feedstock for producing biodiesel (Sunil *et al.*, 2008). At present, however, seed yield of *Jatropha* is poor and insufficient for the biodiesel industry (Sanderson, 2009; Divakara *et al.*, 2010). As a cross-pollinated shrub, *Jatropha* is monoecious and produces male and female flowers in the same inflorescence (Liu *et al.*, 2008). Normally, female flowers initiate at the centre of inflorescences and are surrounded by a group of male flowers (Jongschaap *et al.*, 2007). Occasionally bisexual (hermaphrodite) flowers occur (Dehgan and Webster, 1979). Each *Jatropha* inflorescence is composed of 100-300 flowers and yields approximately 10 or more ovoid fruits (Kumar and Sharma, 2008). One of the most likely reasons for poor yield is that *Jatropha* has few female flowers resulting from a very low female-to-male flower ratio, which, depending on the genotype, is about 1:29-1:13 (Raju and Ezradanam, 2002; Tewari *et al.*, 2007). Thus, increasing the number of female flowers seems critical for the improvement of *Jatropha* seed yield. Studies of exogenous applications of various plant growth regulators (PGRs) and analysis of endogenous phytohormones showed that PGRs play important roles in floral development (Krizek and Fletcher, 2005; Irish, 2009; Santner *et al.*, 2009). GA<sub>3</sub> application has been shown to increase inflorescence meristem activity and promote floral initiation in

several species (Kiba and Sakakibara, 2010). In the present investigation, we studied the application of plant growth regulators such as GA<sub>3</sub>, ethrel and copper sulphate their role in enhancing the flowering variation in male: female flower ratio *J. curcas* L.

### MATERIALS AND METHODS

**Study site:** The experimental site is located at 20°-95' North latitude and 75°-90' East longitude at an altitude of 10 meters above mean sea level. The experiment site *i.e.* Forestry farm, Navsari Agricultural University, Navsari (Gujarat, India) is located three kilometers away in the west from Navsari and 12 kilometers away in the east from Arabian seashore, the historical place 'Dandi'.

**Climate and weather:** The climate of South Gujarat is typically tropical characterized by fairly hot summer, moderately cold winter and warm humid monsoon. Generally monsoon in this region commences in the second week of June and retreats by the end of September. Pre-monsoon rains in the last week of May or in the first week of June are not uncommon. Most of the precipitation is received from South West monsoon, concentrating in the months of July and August. Average annual rainfall of this region is about 1431 mm. The winter season sets usually towards the end of October. The lowest temperature of the season is recorded either in December or January (10 to 23.8 °C) and hence these two months are the coldest months of the season. From February onwards, the temperature starts rising and reaches the maximum in the month of May. Thus, May is the hottest month of the summer season.

**Preparation of solution of growth substances:** Gibberellic acid (GA) at three different concentrations of 25 ppm, 50 ppm and 100 ppm were used. Solutions were prepared by dissolving GA in small volume of isopropyl alcohol and final volume was made up with demineralized water after adjusting pH to 7.5–7.8. A few drops of surfactant were added to the solution. Plants sprayed with demineralized water containing only surfactant were considered as control. The selected time of spray was early morning hours and spraying was initiated from the time foliar bud emerged. Each inflorescence received three sprays of equal volume of solution at an interval of 15 days (Makwana *et al.*, 2010). Ethrel at three different concentrations 1500, 2000 and 2500 ppm were applied. Solution prepared taken Ethrel 0.15, 0.20 and 0.25 ml was measured individually and final volume was made 1 liter by adding distilled water. Ethrel used as 40 percent aqueous solution (Joshi *et al.*, 2011). Copper sulphate 1, 2 and 5 g was weighed on electronic balance and diluted by distilled water to make up to 1 litre of the solution and made 0.1, 0.2 and 0.5 ppm stock (Hirayama and Alonso, 2000).

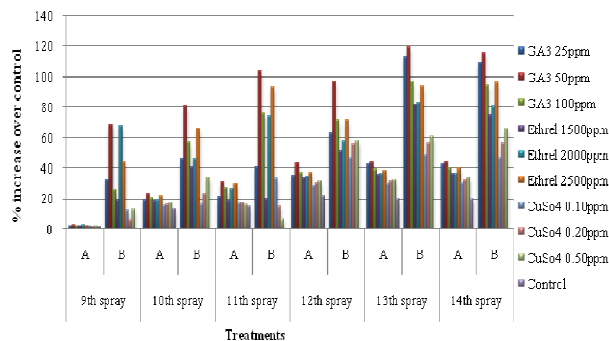
**Method adopted for foliar spray:** The foliar application of respective treatment, were applied using a hand sprayer (Ganesh Pvt. Ltd). First spray was applied at leaf less stage and afterwards at every 15 days intervals during the morning hours. Both the surface of leaves and apical meristem were fully moistened. Each inflorescence received three sprays of equal volume of

solution at an interval of 15 days. Ten plants were taken per treatment. Total number of flowers and sex ratio were calculated 1 week after the last spray whereas fruit yield was measured one month after the last spray. Test and control plants were tagged with appropriate labels to follow flower development till about one and half months.

**Statistical analysis:** The data obtained from field trials during the course of the present investigation were analysed statistically by using the random block design (RBD) with the standard mean error (SEM) and critical difference (CD) was evaluated at 5% level of significance. All the experiments were carried out taking three replicates for each treatment (Panse and Sukhatme, 1967).

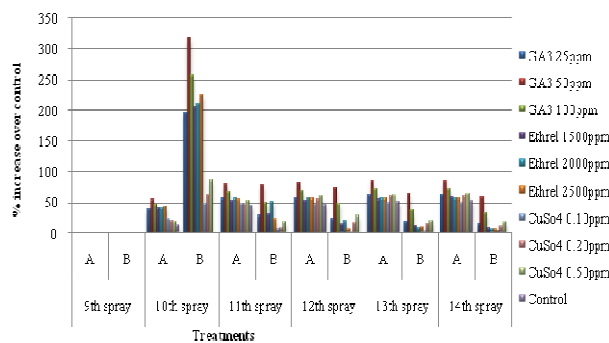
**RESULTS AND DISCUSSION**

Exogenous GA has been shown to promote the switch from vegetative growth to flowering in a variety of plants. Most species in which applied GA can induce flowering are long-day or cold-requiring plants, and many of these normally grow as rosettes under noninductive conditions (Zeevaert., 1991). Love *et al.* (2009) demonstrated that ethylene was an endogenous regulator of meristem growth in *Pouulus*. Both applied and ectopically produced ethylene stimulated xylem growth by means of cambial cell division. It has been shown by Arteca and Arteca, (2007) that different plant parts of *Arabidopsis thaliana* varied in copper sulfate (CuSO<sub>4</sub>) induced ethylene production, inflores-



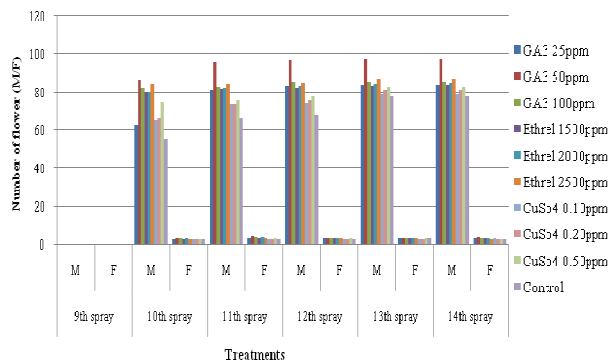
A: Number of inflorescence per plant; B: % Increase over control

**Fig. 1.** Effect of plant growth substances on number of inflorescence per plant in *J. curcas* L.

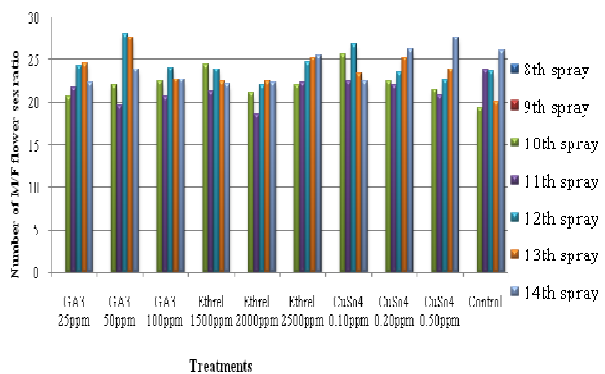


A: Number of flower per inflorescence; B: % Increase over control

**Fig. 2.** Effect of plant growth substances on number of flower per inflorescence in *J. curcas* L.



**Fig. 3.** Effect of plant growth substances on number of flower (M/F) per plant in *J. curcas* L.



**Fig. 4.** Effect of plant growth substances on number of (M/F) flower sex ratio of *J. curcas* L.

cences showed the greatest induction, while all other plant parts tested produced significantly less.

**Number of inflorescence per plant:** In all of the foliar application of PGRs influences on number of inflorescence per plant was positive up to 13<sup>th</sup> spray stage. However, after 14<sup>th</sup> spray no significant effect on number of inflorescence per plant was discernible though statistically the number was more in each treatment. Application of GA<sub>3</sub> (50, 25 and 100 ppm) and ethrel (2500 ppm) induced plant cell division and increased number of inflorescence per plant as compared to other exogenous application. (Fig. 1) At this stage maximum percent increase over control was seen in GA<sub>3</sub> 50 ppm (119.06%) which was followed by GA<sub>3</sub> 25 ppm (112.92%) and GA<sub>3</sub> 100 ppm (97.28%) respectively. The maximum number of inflorescence per plant found during 9<sup>th</sup> to 10<sup>th</sup> spray of GA<sub>3</sub> 50ppm with value (2.99 to 23.66%) followed by ethrel 2500 ppm (21.78%), GA<sub>3</sub> 100 ppm (20.53%), GA<sub>3</sub> 25 ppm (19.11%), ethrel 2000 ppm (19.11%) and least in control (13.11%). Maximum percentage increase over control seen in GA<sub>3</sub> 50ppm with (119.06%) during 13<sup>th</sup> spray followed by GA<sub>3</sub> 25 ppm (112.92%), GA<sub>3</sub> 100 ppm (97.28%), ethrel 2500 ppm (93.91%) and least in CuSO<sub>4</sub> 0.10 ppm (48.08%). ethrel 2500 ppm was next to GA<sub>3</sub> 100 ppm with a value of (93.91%) and number of inflorescence per plant from 9<sup>th</sup> spray day to 14<sup>th</sup> spray days found maximum in GA<sub>3</sub> 50 ppm (31.25 and 97.39%) which followed by ethrel 2500 ppm (28.19 and 77.49%) and GA<sub>3</sub> 100 (27.55 and 70.18%) respectively. Similar work GA at 50 and 100 ppm resulted in an increase in total number of flowers by 15% and 42 % respectively reported by Makwana *et al.* (2010) with different concentration of GA<sub>3</sub> (10, 100 and 1000 ppm) in *J. curcas*. (Tewari, 2007) also reported similar work in inflorescence of *J. curcas*. The increase in the number of inflorescence in treated plants compared to control plant may be due to the synergistic effect of Gibberellins within the cell. This was in agreement with the study of (Trusov and Bottella, 2006) in pineapple and in ridge guard (Hilli *et al.*, 2008).

**Number of flower per inflorescence:** It was observed that increasing number of spray increased total number of flower per inflorescence up to 13<sup>th</sup> application. The effect of exogenous foliar application exhibited most pronounced and significant effect at 10<sup>th</sup> spray stage on T<sub>2</sub> (GA<sub>3</sub> 50 ppm) with (318.76%) consecutive values of percent increase over control followed by T<sub>3</sub> GA<sub>3</sub> 100 ppm (257.24%) and T<sub>6</sub> (Ethrel 2500ppm) with (225.61%) respectively. Maximum number of flowers per inflorescence found in GA<sub>3</sub> 50 ppm (79.57%) followed by GA<sub>3</sub> 100 ppm (67.00%), GA<sub>3</sub> 25 ppm (56.84%), ethrel 2000 ppm (56.11%) and least in control (44.44%) during 10<sup>th</sup> to 11<sup>th</sup> spray of experiment.

However, number of flower per inflorescence significantly higher in GA<sub>3</sub> 50 ppm (76.87%) and percentage increase control (118.70%) which followed

by GA<sub>3</sub> 100 (64.93%). The favourable concentration seems to be GA<sub>3</sub> 50 ppm, but higher concentration *i.e.* 100 ppm was significant difference for maximum number of flowers/inflorescence (Fig. 2). Makwana and Robin (2013) reported that GA (100 and 1000 ppm) and Ethrel (15 and 25 ppm) result in an increase in total number of flowers per inflorescence because the levels of GA and auxins were monitored in these plants. Amongst other biochemical changes brought about by GA and Ethrel application was a pronounced change in endogenous level of Auxin and GA during the initial stages of inflorescence development. However, it has also been reported that spray of GA<sub>3</sub> 50 ppm, Ethrel 500 ppm induces the flower inflorescence in Ridge guard (Hilli *et al.*, 2008).

**Types of flower (male/female) per plant:** The number of female flowers significantly increased by application of GA<sub>3</sub> 50ppm after 10<sup>th</sup> and 11<sup>th</sup> application. The female flowers ranged between 3.92 to 4.92 in GA<sub>3</sub>. This was followed by ethrel 2000 ppm with (3.82 to 4.44), GA<sub>3</sub> 100 ppm with (3.67 to 4.01) and least in control (2.91 to 2.79) (Fig. 3). Also male flower increased by GA<sub>3</sub> and Ethrel application at 13<sup>th</sup> and 14<sup>th</sup> spray stages. Along with observed that the number of male flowers higher than female flower. The number of male flowers is maximum in GA<sub>3</sub> 50 ppm with 10<sup>th</sup> (86.22) and 11<sup>th</sup> (96.23) spray after that gradually increase number of male flowers followed by Ethrel 25000 ppm (84.35) in 10<sup>th</sup> spray, GA<sub>3</sub> 100 ppm (82.43) and least in control (55.33). However, numerically maximum numbers of male/female flowers were recorded from GA<sub>3</sub> 50 ppm (94.75/4.01) treated plant after fourteenth applications. It might be due to increase level of gibberellins in plants might have increase in flowering through rapid cell elongation and cell division process. This leads to greater accumulation of carbohydrates owing to more photosynthesis, which result in to increase of flowering. Wijaya *et al.* (2009) reported that the total average number of female flowers per inflorescence increased in *J. curcas* produces flowers in racemose inflorescences with dichasial cyme pattern. The flowers are unisexual. The average number of male flowers per inflorescence varied from 78 to 291, with a mean of  $159.9 \pm 37.51$  while the number of female flowers per inflorescence varied from 2 to 20, with a mean of  $7.9 \pm 2.82$ . However, in rainy season phenological clock of *J. curcas* like dry forests appears to set during the interphase of winter and summer enabling the community to take full advantage of the rainy season for productivity and recruitment of seeds, while the period of food availability to pollinators and seed predators varies in high number of female flowers in *Jatropha* accessions (Ghosh and Singh, 2008).

**Number of male/female flower sex ratio:** This effect was clearly reflected in male:female ratio (Fig. 4). Foliar application of different treatments significantly impacted the ratio from 10<sup>th</sup> to 14<sup>th</sup> application among all the treatments CuSO<sub>4</sub> 0.10 ppm (25.64) exhibited an increase where as all other treatments exhibited a reduction followed by Ethrel 1500 ppm (24.37), GA<sub>3</sub> 100 ppm (22.46) and least in control (19.30) in 10<sup>th</sup>

spray after that 12<sup>th</sup> and 13<sup>th</sup> spray found maximum male:female sex ratio in GA<sub>3</sub> 50 ppm with (28.06 and 27.58) but after 14<sup>th</sup> spray found maximum male/female sex ratio in CuSO<sub>4</sub> 0.20 and 0.50 ppm with (26.20 and 27.66). Similar work has been reported by Chaudhary *et al.* (2006) in that the application of plant growth regulators is beneficial for overall development of the plant and increasing the yield of the *J. curcas* plants. It is suggested that higher concentration of Ethrel (i.e. 150 ppm) was found to promote morpho-physiological development, increase female flower development leading to feminization, decrease in male-female ratio, resulting in an improved yield of the plants expressed as an increase in both fruit and seed yield of *J. curcas*. Raju and Ezradanam, (2002) and Tewari, *et al.* (2007) reported that low female-to-male flower ratio, which, depending on the genotype, is about 1:29–1:13 in *J. curcas*. We observed that the ratio of male to female flowers we observed ranged from 13:1 to 26:1. These results are similar to those obtained by (Tewari, 2007 and Divakara *et al.*, 2010) and (Chang-wei *et al.*, 2007) who observed in their report that this ratio varies and changes drastically from 13:1 to 108:1 with the fall in temperature. The total average numbers of female (eight) and male (one hundred and sixty) flowers is comparable to the result in *J. curcas*.

## Conclusion

From the foregoing it was concluded that exogenous application of GA<sub>3</sub>, ethrel as well as Copper sulphate by way of foliar spray were effective in improving the yield of *J. curcas* L. However, the most pronounced effects were seen in GA<sub>3</sub> 50 ppm followed by Ethrel 2000 ppm and GA<sub>3</sub> 100 ppm. The effect was mediated via increased number of inflorescence, increased number of flower per inflorescence, increased number of male as well as female flowers, ultimately leading to increased fruit and seed yield of *J. curcas*.

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