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EFFECT OF FOLIAR APPLICATION OF GA_3, ETHREL AND COPPER SULPHATE ON FLOWERING BEHAVIOUR AND YIELD OF *JATROPHA CURCAS* L.

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ABSTRACT
Effect of various plant growth substances viz., GA_3 (25, 50,100ppm), Ethrel (1500, 2000, 2500ppm), 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, sex-expression and yield of *Jatropha curcas* L was investigated during in Randomized Block Design (RBD) at the College Farm, Navsari. Looking to the results, it was noticed that GA_3 50ppm 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), number of fruits per inflorescence (3.71), number of fruits per plant (88.0) and fruit(453.33) as well as seed (355.55) yield per plants in plant treated with GA_3 50ppm as compared to control between 9th to 14th spray. The Male: Female flower ratio was the lowest at 14th spray stage under majority of treatments indicating increased number of female flowers which is ultimately reflected by increased fruit and seed yield. Ethrel 2000 ppm and $GA_3$100ppm followed GA_3 50ppm in its similar effects.

INTRODUCTION

Jatropha curcas (hereafter refer as Jatropha) 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 center 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_3 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_3, Ethrel and Copper Sulphate their role in enhancing the flowering variation in male:female flower ratio *Jatropha curcas* L.

MATERIALS AND METHODS

Study site

The experimental site is located at 20º-95' North latitude and 75º-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 25ppm, 50ppm 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 15days (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 per cent 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. Fruit yield was observed for 6 and 7 months at an interval of 15days. After each treatment, dried seeds were collected and weighed.

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 (Zeevaart, 1991). Love *et al*. (2009) demonstrated that ethylene was an endogenous regulator of meristem growth in *Poulus*. Both applied and ectopically produced ethylene stimulated xylem growth by means of cambial cell division. It has been shown that different plant parts varied in copper sulfate ($CuSO_4$) induced ethylene production, inflorescences showed the greatest induction, while all other plant parts tested produced significantly less (Arteca and Arteca, 2007).

Number of inflorescence per plant

In all of the foliar application of PGRs influences on number of inflorescence per plant was positive up to 13th spray stage. However, after 14th spray no significant effect on number of inflorescence per plant was discernible though statistically the number was more in each treatment. Application of GA_3 (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_3 50 ppm (119.06%) which was followed by GA_3 25 ppm (112.92%) and GA_3 100 ppm (97.28%) respectively. The maximum number of inflorescence per plant found during 9th to 10th spray of GA_3 50 ppm with value (2.99 to 23.66%) followed by Ethrel 2500 ppm (21.78%), GA_3 100ppm (20.53%), GA325ppm (19.11%), Ethrel 2000ppm (19.11%) and least in control (13.11%). Maximum percentage increase over control seen in GA_3 50ppm with (119.06%) during 13th spray followed by GA_3 25 ppm(112.92%),GA_3 100 ppm

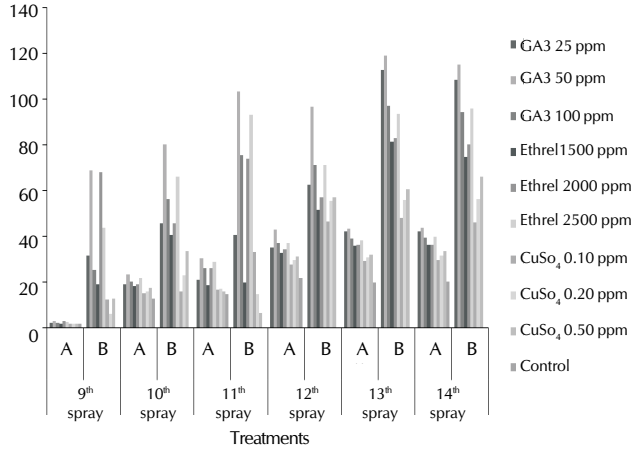
Table 1: Effect of plant growth substances on fruit yield and seed yield per plant in *J. curcas*.

Treatment	Fruit yield (g)	% increase over control	Seed yield (g)	% increase overcontrol
T_1GA_3 25ppm	286.66	39.45	216.66	21.87
T_2GA_3 50ppm	453.33	120.54	355.55	100.00
T_3GA_3 100ppm	331.11	61.08	271.11	52.50
T_4Ethrel 1500ppm	271.11	31.89	207.78	16.88
T_5Ethrel 2000ppm	350.11	70.32	310.78	78.75
T_6Ethrel 2500ppm	296.66	44.32	340.78	35.44
T_7CuSo_4 0.10ppm	244.44	18.92	214.44	20.63
T_8CuSo_4 0.20ppm	242.88	18.16	208.89	17.50
T_9CuSo_4 0.50ppm	241.99	17.72	201.11	13.12
T_{10} Control	205.55	39.45	177.77	21.87
S.Em. +	13.23		20.28	
C.D. at 5%	39.30		60.26	
C.V. %	7.83		13.12	

(97.28%), Ethrel 2500 ppm (93.91%) and least in $CuSo_4$ 0.10 ppm (48.08%). Ethrel 2500 ppm was next to GA_3 100 ppm with a value of (93.91%) and number of inflorescence per plant from 9th spray day to 14th spray days found maximum in GA_3 50ppm (31.25 and 97.39%) which followed by Ethrel 2500ppm (28.19 and 77.49%) and GA_3 100 (27.55 and 70.18%) respectively. Similar work reported by Makwana *et al*. (2010) with different concentration of GA_3 (10, 100 and 1000 ppm) in *Jatropha curcas*. Teweri, (2007) also reported similar work in inflorescence of *Jatropha 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 Botella (2006) in pineapple and in ridge guard (Hilli *et al*., 2008).

Number of flower per inflorescence

Fig. 3 showed that increasing number of spray increased total number of flower per inflorescence up to 13th application. The effect of exogenous foliar application exhibited most pronounced and significant effect at 10th spray stage on T_2 (GA_3 50 ppm) with (318.76%) consecutive values of percent increase over control followed by T_3 GA_3 100 ppm (257.24%) and T_6 (Ethrel 2500ppm) with (225.61%) respectively. Maximum number of flowers per inflorescence found in GA_3 50 ppm (79.57%) followed by GA_3 100 ppm (67.00%), GA_3 25 ppm (56.84%), Ethrel 2000 ppm (56.11%) and least in control (44.44%) during 10th to 11th spray of experiment. However, number of flower per inflorescence significantly higher in GA_3 50 ppm (76.87%) and percentage increase control (118.70%) which followed by GA_3 100 (64.93%). The favourable concentration seems to be GA_3 50 ppm, but higher concentration *i*.e. 100 ppm was significant difference for maximum number of flowers/inflorescence. The beneficial effect of GA_3 at particular concentration could be attributed to an increased rate of photosynthetic activity thus, resulting in the cell elongation and rapid cell division on the growing portion (Arora and Pratap, 1988). However, also reported that spray of GA_3 50 ppm, Ethrel 500 ppm in ridge guard induce the flower inflorescence Hilli *et al*. (2008).



A - No. of inflorescence per plant, B - % increase over control

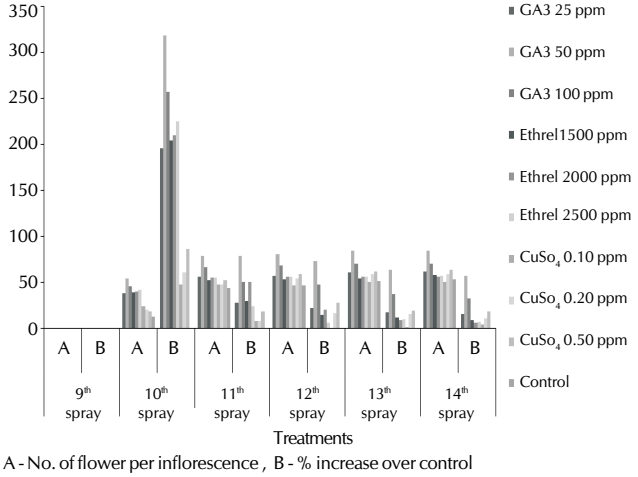
Figure 1: Effect of plant growth substance on number inflorescence per plant in *Jatropha curcas* L.

Types of flower (male/female) per plant

The number of female flowers significantly increased by application of GA_3 50ppm after 10th and 11th application. The female flowers ranged between 3.92 to 4.92 in GA_3. This was followed by Ethrel 2000ppm with (3.82 to 4.44), GA_3 100ppm with (3.67 to 4.01) and least in control (2.91 to 2.79) (Fig. 3). Also male flower increased by GA_3 and Ethrel application at 13th and 14th spray stages. Along with observed that the number of male flowers higher than female flower. The number of male flowers is maximum in GA_3 50 ppm with 10th (86.22) and 11th (96.23) spray after that gradually increase number of male flowers followed by Ethrel 25000 ppm (84.35) in 10th spray, GA_3 100 ppm (82.43) and least in control (55.33). However, numerically maximum numbers of male/female flowers were recorded from GA_3 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* L. However, rainy season favours 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. 3a). Foliar application of different treatments significantly impacted the ratio from 10th to 14th application among all the treatments $CuSO_4$ 0.10 ppm (25.64) exhibited an increase where as all other treatments exhibited a reduction followed by Ethrel 1500 ppm (24.37),GA_3 100 ppm (22.46) and least in control (19.30) in 10th spray after that 12th and 13th spray found maximum male:female sex ratio in GA_3 50 ppm with (28.06 and 27.58) but after 14th spray found maximum male/female sex ratio in $CuSO_4$ 0.20 and 0.50 ppm with (26.20 and 27.66).Similar work reported by (Chaudhary *et al*., 2006) auxin treated plants this increase in yield might be due to the switching of the floral primordial towards development of female flowers, fertilization



A - No. of flower per inflorescence , B - % increase over control

Figure 2: Effect of plant growth substance on number of flower per inflor escence in *Jatropha curcas* L.

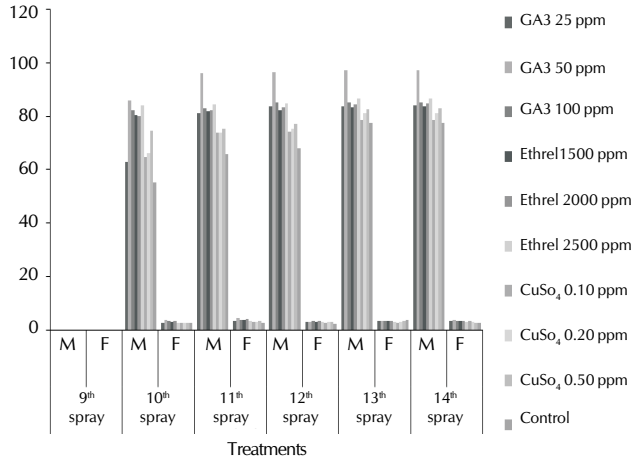


Figure 3: Effect of plant growth substance on number of flower (M/F) per plant in *Jatropha curcas* L.

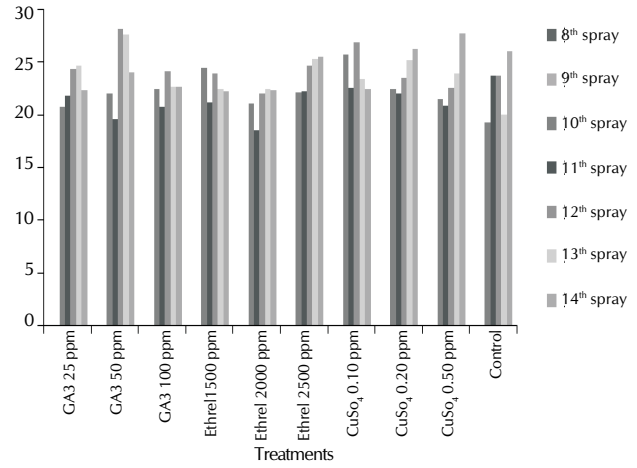


Figure 3a: Effect of plant growth substance on number of M/F flower sex ratio *Jatropha curcas* L.

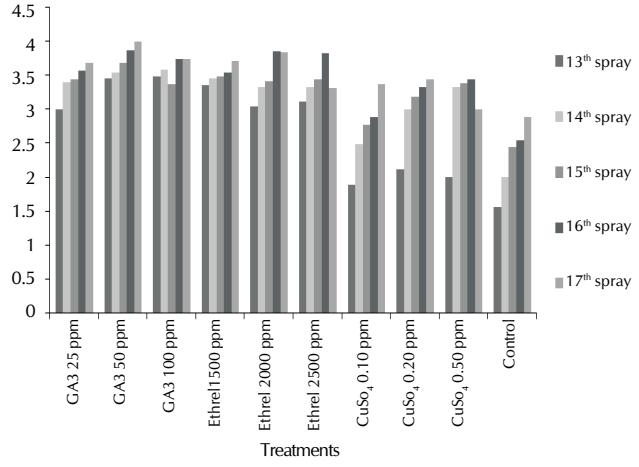


Figure 4: Effect of plant growth substance on number of fruit per inflorescences per plant of *Jatropha curcas* L.

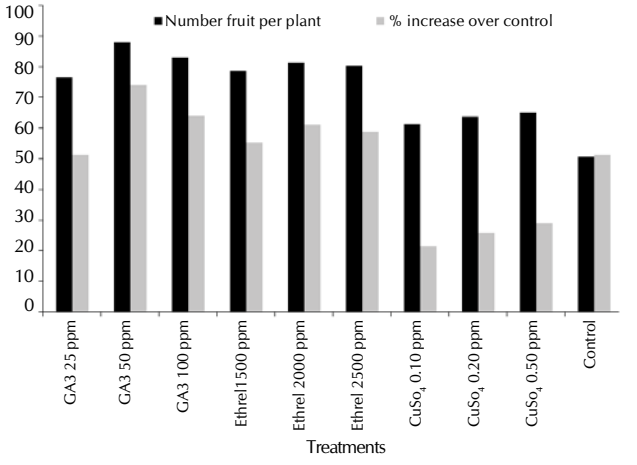


Figure 5: Effect of plant growth substance on number of fruit per plant of *Jatropha curcas* L.

of more female flowers in *Jatropha curcas*. (Raju and Ezradanam, 2002; Tewari *et al*. 2007) reported that low female-to-male flower ratio, which, depending on the genotype, is about 1:29–1:13 in *Jatropha curcas*. The ratio of male to female flowers we observed ranges from 13:1 to 26:1. These results are similar to those obtained by (Teweri, 2007 and Divakara *et al*., 2010) 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 obtained by Chang-wei *et al.,* 2007).

Number of fruits per inflorescence

After initiation of male and female flowers produce fruits with spray of different foliar application. It was recorded maximum in GA_3 50 ppm followed by GA_3 100 ppm, Ethrel 1500 ppm, Ethrel 2000 ppm, Ethrel 2500 ppm and least in control. Number of fruits per inflorescences per plant found maximum in initial stage of fruiting (13th spray) in GA_3 100 ppm with 3.48 followed by GA_3 50 ppm with (3.46) and least in control (1.55). After that 15th to 17th spray of foliar application found maximum in GA_3 50 ppm with (3.68 to 4.00) followed by GA_3 100 ppm with 3.75 and 3.75 (Fig 4). Application of growth regulators (GA_3 - 1000ppm) increases male/female flower ratio ultimately increases the production of fruits in *Jatropha curcas* (Makwan *et al.* 2010). Since, GA regulates the development of flowers by activation of LFY and AP1 genes. GA activates the floral meristem LFY signal which up regulates AP1 promoter, responsible for flowering (Jack, 2004).

Number fruits per plant

The statistical analysis of data reveals that the number of fruit per plant at harvest was significantly influenced by different treatments. The maximum number of fruit per plant was recorded under treatment GA_3 50 ppm being (88.00) that was at par with GA_3 25 ppm (76.60,) GA_3 100 ppm(83.20), in Ethrel 1500 ppm(78.60) and Ethrel 2000 ppm (81.53) while the minimum number of fruit per plant was found under control being (50.60). Application of all three concentration of $CuSO_4$ (0.10, 0.20 and 0.50 ppm) did not affect the number of fruit per plant and remained statistically equal to control. The data on percent increase over control reveals that all the treatment under study was effective in increasing the number of fruits. Among all the treatments GA_3 50 ppm ranked 1st where as

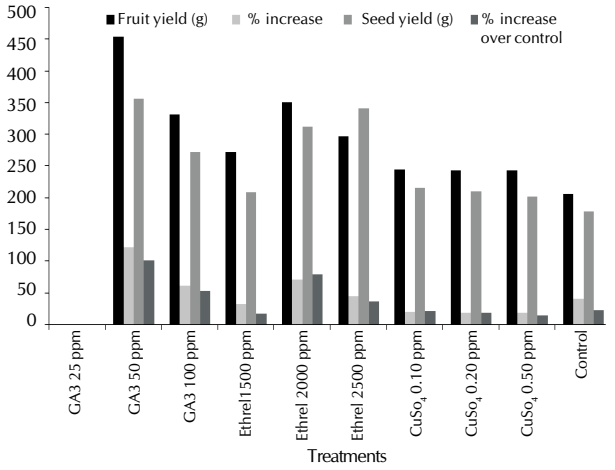


Figure 6: Effect of plant growth substance on fruit and Seed yield per plant of *Jatropha curcas* L.

(GA_3 100 ppm), (Ethrel 2000 ppm) and (Ethrel 2500 ppm) followed in their effects with respective values of percent increase over control of 73.91%, 64.03, 61.12% and 58.75% (Fig 5). However, application of Ethrel 150 ppm recorded maximum fruit yield per plant as compared to other treatments (Joshi *et al.,* 2011). These results were in conformity with Singh and Choudhury (1988) for bottlegourd, Arora and Pratap (1988) for pumpkin.

Fruit and seed yield per plant

The data pertaining to fruit and seed yield per plant (Table 1) indicates that application of different treatment significantly increased fruit and seed yield per plant. Application GA_3 50 ppm recorded significantly maximum fruit yield (453.33 g), which was followed by Ethrel 2000 ppm and GA_3 100 ppm respectively with percent increase over control values of 120.54%, 70.32% and 61.08% for the fruit yield. Similarly, the same three treatments *viz*. GA_3 50 ppm was followed by Ethrel 2000 ppm and GA_3 100 ppm were significantly effective in improving the seed yield over control. A considerable increase in fruit yield per plant and seed yield was reported compared with control (Joshi *et al.,* 2011). Also Ghosh *et al*. (2010) found an unexpected 5 to 11 fold increase in Jatropha fruit and seed yield in the year by applying a biosynthesis inhibitor of the plant hormone gibberellins. Rao *et al*. (2008) argued that the low number of female flowers is one of the factors causing low yield of *Jatropha curcas*. 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 and increase male and female flower ratio ultimately increase the fruit production.

From the foregoing it can be concluded that exogenous application of GA_3, Ethrel as well as Copper sulphate by way of foliar spray were effective in improving the yield of *Jatropha curcas* L. However, the most pronounced effects were seen in GA_3 50 ppm followed by Ethrel 2000ppm and GA_3 100 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.

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