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EXOGENOUS APPLICATIONS OF PLANT GROWTH REGULATORS INFLUENCE THE REPRODUCTIVE GROWTH OF *CITRUS SINENSIS* OSBECK CV. BLOOD RED

A.S. KHAN[1]*, T. SHAHEEN[2], A.U. MALIK[1], I.A. RAJWANA[3], S. AHMAD[1] AND I. AHMAD[1]

[1]*Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan*
[2]*Department of Bioinformatics and Biotechnology, GC University, Faisalabad, Pakistan*
[3]*Department of Horticulture, University College of Agriculture, Bahauddin Zakariya, University, Multan, Pakistan*
[*]*Corresponding author: ahmad_khan157@yahoo.com*

Abstract

To study the influence of exogenous applications of plant growth regulators on the reproductive behaviour of low bearing sweet orange (*Citrus sinensis* Osbeck) trees, three separate experiments were conducted on twelve years old 'Blood Red' Sweet orange trees budded on Rough Lemon (*Citrus jambheri* L.) root stock. In the first experiment, trees were sprayed with 20 mg L^{-1} 2, 4-D and GA_3 alone or in combination at mid bloom (MB) stage, whilst in the second and third experiments 20 mg L^{-1} 2, 4-D and GA_3 alone or in combination were sprayed at MB + 6 weeks after MB, and at MB + 22 and 28 weeks after MB stages, respectively. A single tree was selected as an experimental unit and each treatment was replicated four times. Data regarding the flowering intensity, flower drop, fruit set, fruit drop and fruit harvest percentages (%) were collected and analyzed statistically. In all experiments exogenous application of 20 mg L^{-1} 2, 4-D and GA_3 alone or in combination to Blood Red sweet orange trees reduced the flower drop % and increased the fruit set % as compared to untreated trees. Application 2, 4-D and GA_3 alone or in combination at MB did not affect the fruit drop % and fruit harvest % in contrast to untreated trees. The trees sprayed with 20 mg L^{-1} GA_3 alone or in combination with 2, 4-D at MB + 22 and 28 weeks after MB exhibited highest reduction in the fruit drop % compared to control trees. In conclusions application GA_3 (20 mg L^{-1}) alone or in combination of 2, 4-D (20 mg L^{-1}) at MB + 22 and 28 weeks after MB can be used effectively to increase the fruit set and reduce the fruit drop in 'Blood Red' sweet oranges.

Introduction

Citrus is one of the leading fruit crops of the tropical and subtropical world with respect to its area and production. At present about 106 MMT citrus fruit is being produced in the world (Anon., 2010). Among different citrus species grown in the world sweet orange (*Citrus* sinensis Osbeck) is dominated in the world Citriculture due to its wide range of varieties, distribution and high nutritive value. More than 60 percent world citrus production is contributed by the sweet orange. Currently Brazil, USA and China are leading sweet orange producing countries with 24, 10 and 3 MMT production respectively (Anon., 2010).

Like in the world citrus also holds number one position in area (192 thousand hectare) as well as production (2.5 MMT) in Pakistan (Anon., 2010). Unfortunately, in Pakistan from last four to five decade sweet orange cultivation has been declined because of low production (Malik *et al*., 1993). Erratic bearing and low productively of sweet oranges in Pakistan and particularly in Punjab has replaced the sweet orange with Kinnow mandarin (Malik *et al*., 1993; Saleem *et al*., 2008a; Saleem *et al*., 2008b; Saleem *et al*., 2008c).

The low productivity of sweet oranges has been found to be associated with various causes such as adverse climatic conditions (Davies & Albrigo, 1994), malnutrition (Alva *et al*., 2006; Ashraf *et al*., 2012), poor insect pest and diseases management (Chen *et al*., 2006; Rab *et al*., 2012) and hormonal imbalance (Agustí *et al*., 2006). Sweet orange trees often produce profuse flowers and consequently a substantial number of flowers and fruits shed off as a way of reducing heavy fruit load (Modise *et al*., 2009), followed by series of drops during the period of fruit growth and development (Anthony *et al*., 1999). However, the degree of low production in sweet orange varies from area to area and from grove to grove within the same locality. Many studies have been devoted to investigate the physiological, genetic and climatic factors associated with this problem (Alva *et al*., 2006; Chen *et al*., 2006; Davies & Albrigo, 1994).

The use of 2,4-dichlorophenoxyacetic acid (2,4-D) and gibberellic acid (GA_3) has become a wide spread practice in the citrus producing centuries of the world to control fruit drop at various stages during fruit growth and development. The primary use of these plant growth regulators is for controlling fruit drops, however, the increase in fruit size, delay in fruit maturity and management of physiological disorders have also been reported with their applications (Anthony *et al*., 1999). Exogenous applications of these growth regulators has been tested on different citrus species (Anthony *et al*., 1999; Nawaz *et al*., 2008) alone or in combinations (Saleem *et al*., 2008b; Saleem *et al*., 2008c) either at full bloom or at preharvest stage. At present, very little is known about the effects of combine applications of 2, 4-D and GA_3 at different stages of fruit growth and development on the management of reproductive growth of sweet oranges.

Keeping in view the tremendous utility of sweet oranges for fresh use, making concentrate, juices and other industrial uses both at local market and for export, it is necessary to overcome low yielding problem in sweet orange. The revival of sweet orange production through overcoming the problem of heavy fruit drop will extremely be beneficial for running local industry and the export of the products like concentrate and juices for foreign exchange earnings. Hence the present studies were planned to investigate the effects of exogenous applications of 2, 4-D and GA_3 alone or in combination at various stages of fruit growth and development on the reproductive behaviour of sweet orange (*Citrus sinenesis* Osbeck.) cultivar Blood Red.

Materials and Methods

Plant materials: The experiments were carried out on thirteen year old sweet orange (*Citrus sinensis* Osbeck) cv. Blood Red budded on rough lemon (*Citrus jambheri* Lush.) rootstock at Experimental Fruit Garden Sq. No. 9 , Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Forty eight trees uniform in size, grown under similar agro-climatic conditions, received similar cultural practices, planted with square system of layout at 20 ft distances were selected for the studies. The uniformity of the experimental block was ensure by conducting soil fertility analysis at different depths from four sites of the experimental orchard as reported earlier by Saleem *et al*., (2008b).

Treatments: In the Experiment-I the sweet orange trees were sprayed with 20 mg L^{-1} 2,4-D and GA_3 along or in combination at mid bloom stage. The experimental design was randomized complete block design with single tree representing an experimental unit and replicated four times. In the Experiment-II trees were sprayed with 20 mg L^{-1} 2,4-D and GA_3 along or in combination at mid bloom stage and 6 weeks after mid bloom, whilst in Experiment-III, the experimental trees were sprayed with 20 mg L^{-1} 2,4-D and GA_3 along or in combination at mid bloom stage followed by at 20 and 28 weeks after mid bloom stages. The aqueous solution of 20 mg L^{-1} 2,4-D or GA_3 were prepared as reported earlier by Saleem *et al*., (2007) and were sprayed on the whole trees to run off.

Reproductive growth: To study the effects of experimental treatments on the reproductive growth, four branches each of 4 cm diameter were selected at four sides of the trees. The data regarding reproductive behaviour including total numbers of flowers (buds + flowers), flower drop, fruit set, fruit drop, fruit harvest were collected and analysed statistically. Total numbers of flowers (including total buds as well as flowers) were counted on tagged branches and average was calculated. Total drop between the period of full bloom and initial fruit set was taken as flower drop and percentage was calculated on the basis of total number of flowers per twig. After fruit setting, total number of set fruit was counted and average fruit set percentage was also calculated on the basis of total flowers produced. Fruit drop from the tagged braches was calculated periodically and fruit drop percentage was determined separately for initial fruit drop, June drop and pre harvest drop. The fruit drop from fruit setting to Mid May was considered as Initial fruit drop, from mid May to end July as June drop and from August to harvest as preharvest drop. Periodic fruit drop at different intervals was calculated on the basis of fruit set, while total fruit drop was calculated at the time of total fruit count at harvest. Finally total numbers of fruit harvested during first week of November from each tagged branch were also counted to determine the fruit harvest percentage.

Statistical analysis: The data from all the tree experiments were subjected to analysis of variance (ANOVA) using MSTAT-C statistical software (Freed, 1994) using RCBD separately. The effects of various treatments were accessed within the ANOVA for various parameters and least significant differences (LSD) were calculated by using the F test ($p\leq0.05$). All assumptions of the analysis were checked to ensure the validity of the statistical analysis. There were four treatments replicated four times and one tree was taken as a treatment unit.

Results and Discussion

Total no of flowers and total no of flowers dropped: The average numbers of flowers produced by the control trees were higher as compared to trees treated 2,4-D alone and lower in contrast to trees treated with GA_3 alone or combined application of with 2,4-D and GA_3 (Fig. 1A). However, the differences in the number of flowers produced are more likely linked with the inherent potential of each experimental tree irrespective of the treatments. In the experimental sweet orange trees flower bud initiation and differentiation process have already been completed before the applications of experimental treatments. The total number of flower drop was maximum in trees treated with 2,4-D and GA_3 applications alone at MB while it was minimum in the treatment of GA_3 and 2,4-D application at MB + 20 and 28 weeks after MB as compared to control (Fig. 1B). All other treatments showed significantly higher flower drop in contrast to the control. The results indicated that both growth regulators are involved in the regulation of flower drop as both act as thinning agent and 2,4-D also used as an abscission agent (Iglesias *et al*., 2007). Application of these growth regulators also showed the less numbers of flower drop when applied at MB + 20 and 28 weeks after MB application as compared to other treatments. These plant growth regulators have property to stimulate the abscission in the flowers which cause heavy flower drop. As observed by the Martinez *et al.,* (2004) that flowering of Hernandina was reduced by 25% and that of Orogrande by 60% when GA_3 was applied at 20-50 mg L^{-1} as a foliar spray (6 L $tree^{-1}$) to the whole citrus trees. Gibberellin treatment is a common agricultural practice that is currently used to inhibit flowering in citrus trees. Applications of GA_3 during citrus bud development have been widely shown to inhibit flower production (Guardiola *et al*., 1982), leading to a greater ratio of terminal flowers in leafy shoots and consequently a higher fruit development (Iglesias *et al*., 2007). These results also shown by the Takahara *et al*., (2001) who used the ethychlozate and GA_3 for flower induction in citrus trees but GA_3 showed the inhibitory effect. Gibberellins are thought to be pivotal effectors responsible for the ovary-fruit transition (Ben-Cheikh *et al*., 1997). Gibberellins activate cell division and cell enlargement processes in vegetative organs (Talon *et al*., 1991) and therefore are generally associated with the initiation of growth (Talon & Zeevaart, 1992).

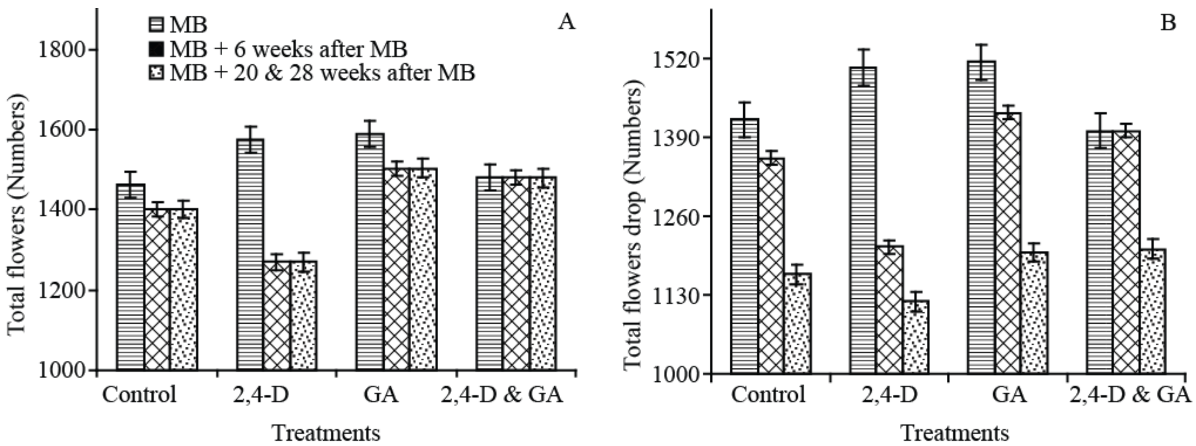

Fig. 1. Effects of foliar applications 20 mg L^{-1} 2, 4-D and GA_3 alone or in combination at mid bloom (MB) or at MB + 6 weeks after MB or at MB + 20 & 28 weeks after MB on total number of flower produced (A) and total flower drop (B) of Blood Red sweet orange. Vertical bars represent ± SEM. n = 4 replicates.

Flower drop (%): There was slight significant difference in the flower drop (%) among all the treatments at all stages but the control trees showed significantly higher flower drop (%) which means that both growth regulators have the ability to retain the flowers when applied alone or in combination (Fig. 2). Generally, gibberellins are mostly found to reduce the flower production and to get the maximum and high quality yield by reducing the tree crop load. It acts as a thinner agent, but sometimes it also showed the ability to retain flowers as earlier have been reported by the (Iglesias *et al*., 2007). While 2,4-D has the ability to delay or stimulate the abscission. Reproductive processes in citrus are strongly affected by plant growth regulators indicating that the regulatory mechanism controlling fruit set and abscission of ovaries are controlled by critical hormonal component (Talon & Zeevaart, 1992). Overall, these studies suggest that a complex set of hormonal interactions occur during fruit development. Thus, GAs and cytokinins are generally considered to be positive regulators of fruit growth while auxins have been reported to act as stimulators of growth and also as abscission agents. According to previous reports most commercially important citrus cultivars bloom prolifically producing as many as 100,000-200,000 flowers on a mature tree. However, less than 1-2% of these flowers produce harvestable fruit (Lee, 2003). In citrus, major flower drop occurs from early flowering stages until 2 or 3 weeks after full bloom. Ovule fertilization usually plays a major role in subsequent retention and 2.5 to 3 months after full bloom a major fruit let drop often occurs due to carbohydrate and hormonal competition (Guardiola *et al*., 1982; Sanz *et al*., 1987).

Fruit set (%): Data pertaining to fruit set % showed that foliar 2,4-D and GA_3 alone or in combination significantly improved the fruit set % as compared to the control (Fig. 3). The application of 2,4-D and GA_3 alone or in combination showed significantly high fruit set % at all three stages of application as compared to control which shows that both growth regulators have the ability to produce more fruit as compared to the untreated plants. It is generally accepted that GAs are involved in set and development of citrus fruits. The support for this hypothesis comes from several studies reporting that exogenous application of GA_3 considerably improves parthenocarpic fruit set and growth of self incompatible genotypes such as Clementine that in the absence of cross-pollination show negligible parthenocarpic fruit set (Soost & Burnett, 1961). Previous study of El-Sese (2005) confirms that the number of fruits per tree and the total yield (kg) increased with GA_3 treatments compared with the control. The involvement of hormones in fruit setting has been reported by many worker but the exogenously applied hormones like GA_3 and 2, 4-D had inconsistent effect on fruit setting as Davies & Albrigo (1994) reported that GA_3 application @ 30 mg L^{-1} did not improve fruit set of most of the citrus cultivars. Saleem *et al.,* (2008a) also showed that final fruit set was significantly affected by GA_3 treatments individually as well as in combination with auxins with maximum fruit set of 32.3% in 45 mg L^{-1} GA_3 treated citrus trees compared with control.

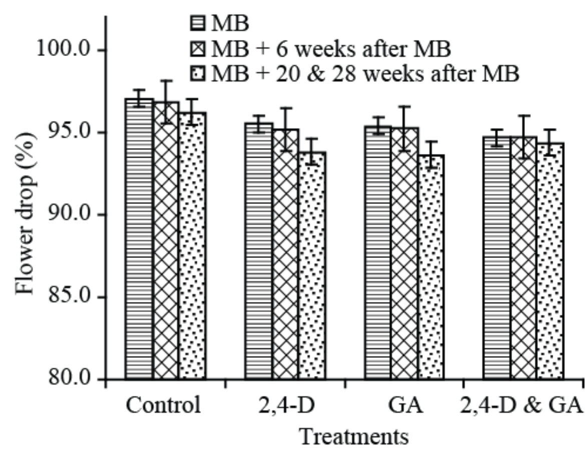

Fig. 2. Effects of foliar applications 20 mg L^{-1} 2, 4-D and GA_3 alone or in combination at mid bloom (MB) or at MB + 6 weeks after MB or at MB + 20 & 28 weeks after MB on flower drop percentage of Blood Red sweet orange. Vertical bars represent ± SEM. n = 4 replicates.

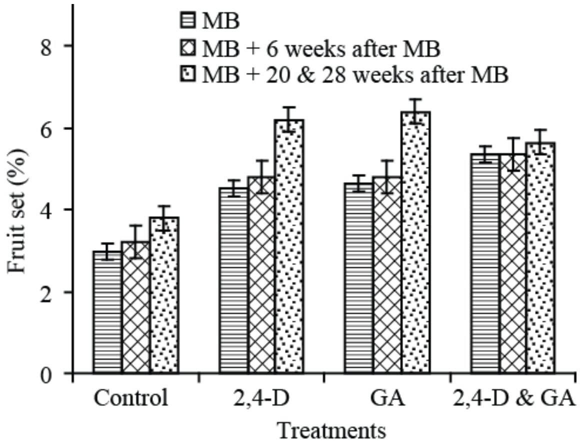

Fig. 3. Effects of foliar applications 20 mg L^{-1} 2, 4-D and GA_3 alone or in combination at mid bloom (MB) or at MB + 6 weeks after MB or at MB + 20 & 28 weeks after MB on fruit set percentage of Blood Red sweet orange. Vertical bars represent ± SEM. n = 4 replicates.

Fruit drop (%): The results clearly indicate that the treated trees exhibited significantly more initial fruit drop (%) at all stages of application as compared to control (Fig. 4A). The GA_3 and 2,4-D are used for abscission to control the overloading of fruit for better quality as reported by Iglesias, *et al.,* (2007). The role of 2,4-D in abscission is quite complicated and depends upon many external and internal factors. It has been observed that application of 2,4-D soon after fruit set results in acceleration of abscission (Lee, 2003). One of the current hypotheses on the hormonal regulation of abscission suggests that the balance between specific plant growth regulators at the abscission zone controls cell separation processes and eventually fruit drop (Brown, 1997). In citrus organs, the effect of the auxin/ ethylene balance, for example, has been largely associated with the abscission of fruit, leaves and flowers. Thus, it has been suggested that auxin levels must fall below a certain threshold in the citrus abscission zone before ethylene can stimulate abscission (Goren, 1993). The June drop (%) was significantly higher in the control trees at all stages as compared to other treatments at all stages. Among different growth regulator applications minimum June drop was exhibited by trees treated with 2, 4-D or GA_3 or combination 2, 4-D and $GA_3$of at MB + 6 weeks after MB stage (Fig. 4B). Growth regulator especially GAs are correlated with the ABA which is the main abscission causing factor. A decrease in GA increases the ABA level, which indicate that both plant growth substances are fundamental factors controlling to initial fruit set and June drop with other essential components (Iglesias *et al*., 2007). The similar results have also been reported by Saleem *et al*., (2008a) that GA_3 and 2,4-D were involved in reducing the June drop but they found non-significant difference among both treatments. The preharvest drop (%) was also significantly higher in the control in contrast to the trees sprayed with 2, 4-D and GA_3 either alone or in combinations (Fig. 4C). The lowest preharvest drop was observed in the trees treated with 2, 4-D alone or in combination of GA_3 at MB + 20 and 28 weeks after MB stage. On an average control trees exhibited 2.3 folds higher preharvest drop as compared to treated trees. Similarly, 78% reduction in fruit drop has been reported earlier by application of combination of GA_3 and 2,4-D in citrus (El-Otmani, 1992). Agusti *et al.,* (2006) had also the same results that 15 mg L^{-1} 2,4-D was found to reduce the abscission by 50-75%. At the end of phase II or at the onset of phase III of fruit growth, synthetic auxins are commercially used to prevent or delay eventual preharvest fruit drop in citrus (Agustí *et al*., 2002).

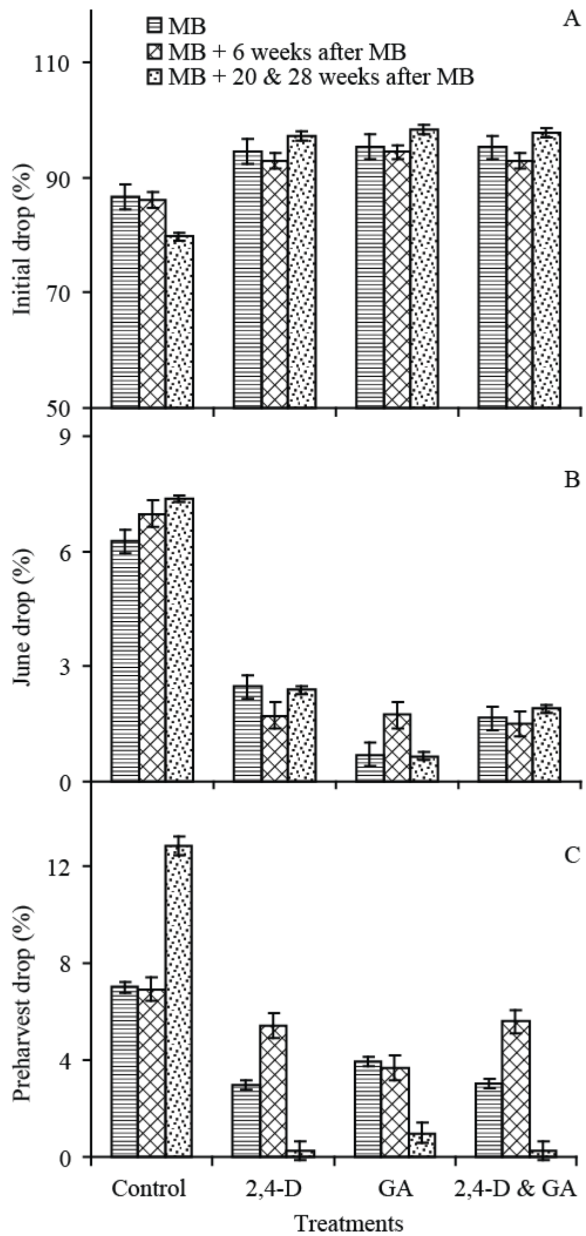

Fig. 4. Effects of foliar applications 20 mg L^{-1} 2, 4-D and GA_3 alone or in combination at mid bloom (MB) or at MB + 6 weeks after MB or at MB + 20 & 28 weeks after MB on initial drop (A), June drop (B), and preharvest drop (C) of Blood Red sweet orange. Vertical bars represent ± SEM. n = 4 replicates.

Final fruit drop and fruit harvest (%): The final fruit drop (%) was significantly reduced when 2, 4-D and GA_3 were applied either alone or in combination at MB + 20 and 28 week after MB stage (Fig. 5A). Highest final fruit drop was observed in control trees as compared to 2, 4-D and GA_3 treated trees. The cause of this drop is probably related to competition among fruit lets for carbohydrates, water and hormones (Lima and Davies, 1984). It has also been reported that exogenous application of GAs reduces the fruit drop of non-pollinated ovaries in seeded citrus varieties (Iglesias *et al*., 2007). Our results also in line with the previous results of findings of Saraswathi *et al*., (2003) who reported that spraying of 2,4-D (20 ppm) or GA_3 (20 ppm) at the green mature stage of mandarin fruits helped to increase the fruit retention percentage in Kinnow contrast to untreated fruit. The foliar application of 2, 4-D and GA_3 either alone or in combination at MB + 20 and 28 week after MB stage significantly increased the final fruit harvest % (Fig. 5B), which shows that 2,4-D and GA_3 are helpful in getting more fruit at harvesting. Previous studies have also found the sane results that the percentage of harvested fruits was highest with a combined application of 2,4-D and GA_3 or a single application of GA_3 mandarin and sweet oranges (Chen *et al*., 2006; Saleem *et al*., 2008b; Saraswathi *et al*., 2003).

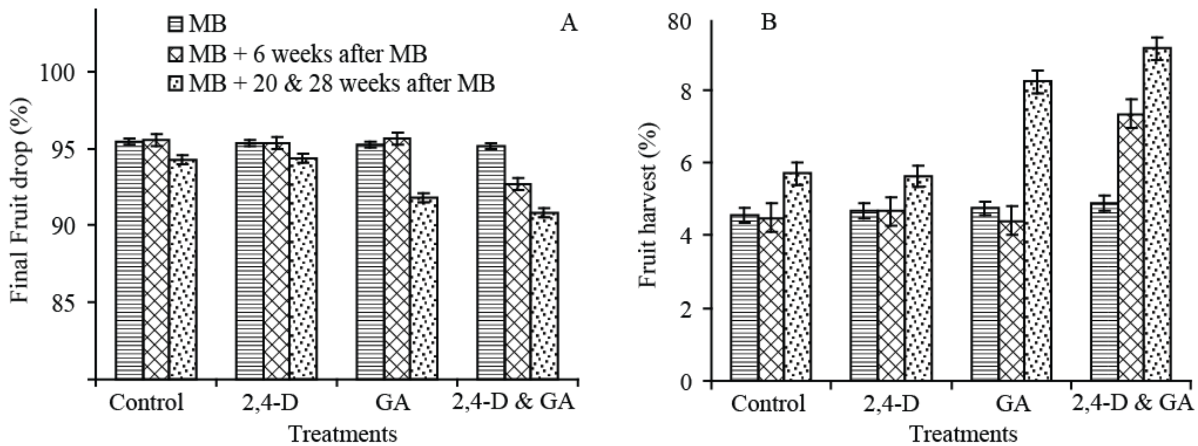

Fig. 5. Effects of foliar applications 20 mg L^{-1} 2, 4-D and GA_3 alone or in combination at mid bloom (MB) or at MB + 6 weeks after MB or at MB + 20 & 28 weeks after MB on final fruit drop (A) and fruit harvest (B) of Blood Red sweet orange. Vertical bars represent ± SEM. n = 4 replicates.

Conclusions

In conclusion, exogenous application of GA_3 (20 mg L^{-1}) alone or in combination of 2, 4-D (20 mg L^{-1}) at MB + 22 and 28 weeks after full bloom can be used effectively to increase the fruit set and reduce the fruit drop in 'Blood Red' sweet oranges.

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