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Effects of Plant Growth Regulators Including Micro-nutrients on Fruit Set and Yield of Mandarin in Dhankuta District of Nepal

Amar Bahadur Pun [a*], Arjun Kumar Shrestha [a], Kalyani Mishra Tripathi [a] and Dilli Ram Baral [a]

[a] *Department of Horticulture, Faculty of Agriculture, Agriculture and Forestry University, Rampur, Chitwan, Nepal.*

Authors' contributions

This work was carried out in collaboration among all authors. Author ABP developed the study theme and carried out the experiment, including data analysis and preparing manuscript of the paper. Author AKS principally guided the experiment design, data interpretation and writing. Authors KMT and DRB supervised the experiment and guided paper writing. All authors read and approved the final manuscript.

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ABSTRACT

The study evaluated foliar applications of three plant growth regulators, viz. GA_3 @ 20ppm; 2,4-D @ 15ppm; and NAA @ 50ppm; alone and their combinations including three plant micro-nutrients, viz. B @ 0.4% + Zn @ 0.2% + Ca @ 1% in order to determine the best foliar spray of growth hormones and plant nutrients for the increased fruit set and yield of mandarin. The foliar sprays were applied five times, started from full-bloom until late September (pre-harvest stage). The experiments were conducted in Dhankuta, Nepal during 2019 and 2020. The highest bloom fruit set of 6.1% was recorded at the foliar application of GA_3 20ppm + NAA @ 50ppm, compared to the control treatment

**Corresponding author: Email: amarppun@gmail.com;*

of 4.6%. Likewise, there were significant effects of plant growth regulations and micro-nutrients applications on the fruit drops across the fruit growing periods. The foliar application of GA_3 + 2,4-D + micronutrients resulted in the lowest fruit drop during the months of June (63.9%), July (65.9%), and September (20.8%), as opposed to the control treatment's fruit drops of 77.6, 80.4, and 41.5%. In addition, the fruit drops occurred at the foliar sprays of GA_3 + 2,4-D were statistically at par with the GA_3 + 2,4-D + micro-nutrients. Also, the GA3 + 2,4-D + micronutrient spray treatment had the largest number of fruits (995 nos.) and total fruit weight (64.2 kg) per tree, which were considerably higher than the control by 201.5 and 175.5%, respectively. To sum up, a combined foliar application of gibberellic acid, 2,4-D and micro-nutrients is recommended for improving the fruit set, retention and yield of mandarin.

Keywords: Fruitlets; fruit set; plant growth regulators; plant micro-nutrients; mandarin.

1. INTRODUCTION

Mandarin (*Citrus reticulate* Blanco) is an economically important fruit crop in the world, including Nepal [1-3]. The average productivity of mandarin is recorded to 10.8 t/ha for 2020/21 in Nepal [4], which is much lower than its yield potential [5,6]. The fruit set, abscission and growth that determine ultimate fruit yield [7]; are hormones-regulated physiological processes [8], besides other agronomical and environmental factors being involved [9]. In particular, gibberellic acid (GA_3) and auxin are associated with fruit set and growth [8] as gibberellin is responsible for the transition of ovary into fruit set, leading to fruit development [10,11] since it induces auxin synthesis to support growth and development [12]. The napthylacetic acid (NAA) and 2,4-dichlorophenoxy acetic acid (2,4-D) as the auxins are particularly involved in the development of reproductive structures, fruit set and growth [13]; as pollen grains contain sufficient auxins that are transferred to the ovary after pollination to support fruit growth [14]. It also involves in suppressing fruit abscission [15]. Likewise, plant micro-nutrients; particularly calcium (Ca), zinc (Zn) and boron (B) are most significant to the fruit set, growth and yield of mandarin [16]. Moreover, there are several reports of the fruit drop due to B, Zn including Ca deficiency in mandarin [17]. In addition, B as associated with the reproductive development, is linked to pollination and fertilization of flowers, causing fruit set and growth [20]. Likewise, Zn is an essential component of many enzymes, required for protein and carbohydrate biosynthesis [21]; thus, it is vital for new growth of leaves, flower buds, and fruit growth. Similarly, Ca is an important constituent of cell walls, involving in cell membrane integrity [22]; besides an important role in the pollen germination and growth [23]. In mandarin crop, GA_3, NAA, and 2,4-D are the usual hormones used for improving the fruit set and yield in the citrus [24,25]. Dutta and Banik [26] observed that foliar applications of GA_3 and NAA significantly increased fruit size and weight, including improving fruit retention and quality. Similarly, NAA and 2,4-D are used to control pre-harvest fruit drop in mandarin [25,27]. Additionally, the synergistic effect of the foliar application of Boric acid @0.2% + ZnSO4 #0.5% with gibberellins was achieved for the increased fruit set and fruit yield in sweet orange [28]. However, studies on the response of Nepalese mandarin cultivars to PGR as well as plant nutrients on fruit retention, and yield is very scanty. Owing to these contexts, this study conducted to investigate the effects of PGR as well as plant nutrients on the improvement of fruit set and yield of mandarin in Nepal.

2. MATERIALS AND METHODS

The study was conducted at the Chungmang Farm of Agricultural Research Station (ARS), Dhankuta, Nepal (26.22°N, 78.18°E) at an altitude of 1030 m above sea level during 2019 and 2020. The climate is characterized as sub-tropical with warm summers, where the maximum temperature exceeded 35˚C in May and June; the winter minimum temperature reached as low as 2˚C in December and January during the experiment period. Usually, the major precipitation as monsoon arrived in the second fortnight of June, with the annual rainfall ranging between 650 to 751 mm. The site has sandy texture with minimal organic matter (1.7%). The local variety of mandarin of 42 years aged seedlings trees planted in the farm on the hexagonal planting system with 6 m apart were used. As much as possible, healthy trees of uniform vigor, size and fruit bearing level were selected and maintaied under the uniform husbandry in rainfed condition. The trees were fertilized with 250:400:300 g NPK and 60 kg FYM tree^{-1} once in a year after fruit harvest in February.

The experiment was designed with eight treatments of foliar sprays: (1) GA_3 @ 20ppm; (2) 2,4-D @ 15ppm; (3) NAA @ 50ppm; (4) GA_3 @ 20ppm + 2,4-D @ 15ppm; (5) GA_3 @ 20ppm + NAA @ 50ppm; (6) B @ 0.4% + Zn @ 0.2% + Ca @ 1%; (7) GA_3 20ppm + 2,4-D 15ppm + B @ 0.4% + Zn @ 0.2% + Ca @ 1%; and (8) control, water spray. The foliar sprays were applied five times, first started from full-bloom, second after two months; and then every six weeks intervals till 25th September. The whole trees were sprayed with four liters solution per tree thoroughly to a point of run-off to ensure of complete drenching. The spray solutions were prepared in the Soil Laboratory of ARS, Dhankuta, Pakhribas, using standard procedures [29,30]. The PGR: GA_3 ($C_{19}H_{22}O_6$) (CAS 77-06-5); 2,4-D ($C_8H_6Cl_2O_3$) (CAS 2707-72-9); and NAA ($C_{12}H_{10}O_{2)}$ (CAS 86-87-3); and plant micro-nutrients: zinc sulfate ($ZnSO_4.7H_2O$) (Zn 34.65%); sodium tetraborate or borax ($Na_2 B_4 O_7$ $1OH_2O$) (B 11.11%); and calcium chloride ($CaCl_2$), (Ca 35.38%) were procured from the BTC, a chemist laboratory for preparing the spray solutions. The experiment was laid out in the randomized complete block design (RCBD) with three replications as single-tree representing one replication. Two branches from the opposite sides of middle vertical part of the experimental tree were selected randomly for determining fruit set and retention. Fruit set was evaluated through counting of fully-grown flowers number, and their set to fruitlets in each two sample branches from the full-bloom period (20th March) until the end of fruit set (1st May) at 7 days interval. Likewise, June as well as pre-harvest fruit droop and retention percentages were measured from the same branches by counting manually on fortnightly basis (formula given below).

$$\text{Bloom fruit set (\%)} = \frac{\text{Total fruitlets set}}{\text{Total blooms}} \times 100$$

$$\frac{\text{June}}{\text{pre}} - \text{harvest fruit drop (\%)} = \frac{\text{Total fruits dropped}}{\text{Total initial fruits count}} \times 100$$

Moreover, the total fruit number and yield per tree were measured from the whole tree at the time of commercial harvest (9th December 2019 and 15th December 2020), and the data were transformed based on the number of fruiting branches of each tree. The ADEL-R (Analysis and design of experiments with R for Windows). Version 2.0 was used for the ANOVA analysis; and the treatment mean comparison for significance difference was carried out at 0.05 probability level [31].

3. RESULTS

3.1 Post-bloom Fruit Set

The fruitlets set during post-bloom was investigated as flower initiation started from late January; and flowering from early March and lasted till early May; while early fruit set and abscission taking place during this period in the condition of the experimental site. The fruitlets set occurred after petal fall at post-bloom period were ranged from 4.5 to 6.1%, however, these were non-significantly different among the foliar spray treatments (Table 1). The highest fruit set was recorded at the foliar application of GA_3 + NAA (6.1%), closely followed by NAA spray (5.8%) and 2,4-D spray (5.7%), but the control treatment recorded the lowest flower set (4.6%). While comparing to the control treatment, the fruit sets were increased by 32.6, 26.0 and 23.9% respectively at GA_3 + NAA, NAA and 2,4-D foliar sprays. Thus, it shows that foliar applications of GA_3 + NAA, NAA and 2,4-D at spring flush or pre-flowering period resembled significant to improve the bloom fruit set in mandarin.

3.2 June and Pre-harvest Fruit Drops

The foliar applications of PGR and micro-nutrients had a significant difference on the fruit drops occurred at 45-days interval periods during four stages from June to October (Table 2). The least fruit drops were recorded at the foliar sprays of GA_3 + 2,4-D + micro-nutrients across the four intervals, except 25th October period. When compared to the control spray, it had lower fruit drops by 17.6, 18.0, 49.8 and 23.9%, respectively on 12th June, 29th July, 10th September and 25th October periods. However, lower fruit drop also occurred at the foliar sprays of GA_3 + 2,4-D that were statistically at par with the GA_3 + 2,4-D + micro-nutrients. During the first two periods, average fruit drop occurred heavily that recorded of 71.7 and 74.9% respectively on 12th June and 29th July; while it reduced to 33.7 and 20.1% in later periods. In conclusion, two foliar sprays of GA_3 + 2,4-D + micro-nutrients, and 2,4-D performed better among the treatments for reducing the fruit drop.

Table 1. Effects of foliar sprays of PGR including plant micro-nutrients on the post-bloom fruit set

Foliar spray treatments	No. of flowers	Fruitlets set%
GA_3	445^{b}	4.5
2,4-D	497^{b}	5.7
NAA	494^{b}	5.8
GA_3 + 2,4-D	465^{b}	4.7
GA_3 + NAA	560^{ab}	6.1
B + Zn + Ca (micro-nutrients)	509^{ab}	4.8
GA_3 + 2,4-D + micro-nutrients	538^{ab}	4.7
No spray	632^{a}	4.6
Mean	518	5.1
F-test	*	Ns
LSD (0.05)	129.5	2.3

Single foliar sprays of GA_3 @ 20ppm, 2,4-D @ 15ppm, and NAA @ 50ppm, alone or their combinations including micro-nutrients (B @ 0.4% + Zn @ 0.2% + Ca @ 1%) were applied on 8th March during mid-bloom period; total number of fully developed flowers on 20th March, and fruitlets set on 1st May were evaluated; and unlike letters within column indicate significant differences (P≤0.05)

Table 2. Effects of foliar sprays of PGR including plant micro-nutrients on the fruits drop during different periods

Foliar spray treatments	Total fruits count in 13th May	Fruit drop %			
		12th June	29th July	10th Sep.	25th Oct.
GA_3	129^{ab}	74.6^{a}	75.4^{ab}	33.9^{ab}	18.8^{bc}
2,4-D	152^{ab}	74.8^{a}	71.7^{ab}	38.1^{ab}	30.9^{a}
NAA	139^{ab}	68.7^{ab}	73.3^{ab}	21.8^{b}	14.0^{c}
GA_3 + 2,4-D	106^{b}	72.1^{ab}	73.2^{ab}	36.4^{ab}	15.4^{bc}
GA_3 + NAA	184^{a}	71.7^{ab}	83.0^{a}	34.9^{ab}	15.7^{bc}
B + Zn + Ca	110^{b}	69.9^{ab}	76.0^{ab}	41.9^{a}	23.1^{abc}
GA_3 + 2,4-D + micro-nutrients	130^{ab}	63.9^{b}	65.9^{b}	20.8^{b}	18.7^{bc}
Control	109^{b}	77.6^{a}	80.4^{a}	41.5^{a}	24.6^{ab}
Mean	132	71.7	74.9	33.7	20.1
F-test	*	*	*	*	*
LSD (0.05)	64.3	10.12	13.3	17.3	10.5

Five times foliar sprays of GA_3 @ 20ppm, 2,4-D @ 15ppm and NAA @ 50ppm, including plant micro-nutrients (B @ 0.4%, Zn @ 0.2% and Ca @ 1%); alone and their combinations respective to the treatments were carried out during different crop periods with 5 liters solution per tree; whole-branch counts of fruit retained and drop were made at fortnight intervals from 13th May to 25th October; data were observed from the two sample branches and mean of 3 single-tree replications per treatment; and unlike letters indicate significant differences (P≤0.05)

There was significant effect of foliar application on fruit set during June as well as pre-harvest periods (Table 3). In June, the highest fruit set occurred at GA_3 + 2,4-D + micro-nutrients (36.2%), closely followed by GA_3 + 2,4-D (31.3%), while the least fruit set was recorded at the control spray (22.4%). However, fruit sets were significantly at par among the 2,4-D, GA_3 + 2,4-D, GA_3 + NAA, micro-nutrients, and GA_3 + 2,4-D + micro-nutrients. It eventually shows the fruit set occurred at GA_3 + 2,4-D + micro-nutrients, topped to the control by 61.6%. The average final fruit set reduced to 3.6% on 25th October, while GA_3 + 2,4-D + micro-nutrients contributed to the highest fruit set of 4.7% compared to the least fruit set of 1.5% recorded at the control treatments. However, the fruit sets occurred at other treatments, except that of the control, were statistically at par.

Table 3. Effects of foliar sprays of PGR including plant micro-nutrients on the fruits set

Foliar spray treatments	Total fruits count in 13th May	Fruit set (%)	
		12th June	25th Oct.
GA_3	129[ab]	25.4[b]	4.5[a]
2,4-D	152[ab]	27.9[ab]	3.7[ab]
NAA	139[ab]	25.2[b]	4.4[a]
GA_3 + 2,4-D	106[b]	31.3[ab]	3.8[ab]
GA_3 + NAA	184[a]	28.3[ab]	2.2[ab]
B + Zn + Ca (micro-nutrients)	110[b]	30.1[ab]	4.2[a]
GA_3 + 2,4-D + micro-nutrients	130[ab]	36.2[a]	4.7[a]
Control	109[b]	22.4[b]	1.5[b]
Mean	132	28.3	3.6
F-test	*	*	*
LSD (0.05)	64.3	10.1	2.7

Five times foliar sprays of GA_3 @ 20ppm, 2,4-D @ 15ppm and NAA @ 50ppm, including plant micro-nutrients (B @ 0.4%, Zn @ 0.2% and Ca @ 1%); alone and their combinations respective to the treatments were carried out during different crop periods with 5 liters solution per tree; whole-branch counts of fruit retained and drop were made at fortnight intervals from 13th May to 25th October; data were observed from the two sample branches and mean of 3 single-tree replications per treatment; and unlike letters indicate significant differences (P≤0.05).

Table 4. Effects of foliar sprays of PGR including plant micro-nutrients on fruit numbers and yield

Foliar spray treatments	Fruit yield characteristics	
	Number per tree	Total weight per tree (kg)
GA_3	514[c]	33.62[c]
2,4-D	727[b]	52.97[b]
NAA	365[d]	27.65[cd]
GA_3 + 2,4-D	412[cd]	28.11[cd]
GA_3 + NAA	405[d]	22.04[d]
B + Zn + Ca (micro-nutrients)	717[b]	46.22[b]
GA_3 + 2,4-D + micro-nutrients	995[a]	64.20[a]
Control	330[d]	23.30[d]
Mean	558	37.3
F-test	***	***
LSD (0.05)	103.4	9.92

Five times foliar sprays of GA_3 @ 20ppm, 2,4-D @ 15ppm and NAA @ 50ppm, including plant micro-nutrients (B @ 0.4%, Zn @ 0.2% and Ca @ 1%); alone and their combinations respective to the treatments were carried out during different crop periods with 5 liters solution per tree; number of fruits and total weight per tree were measured during commercial harvest in 2nd week of December; data were means of 3 single-tree replications per treatment; and unlike letters indicate significant differences (P≤0.05)

3.3 Effects of Foliar Sprays on Fruit Yield Characteristics

The number of fruits and total fruit weight per tree were significantly differed among the spray treatments (Table 4). The foliar spray of GA_3 + 2,4-D + micro-nutrients had the significantly highest results for the number of fruits (995 nos) and corresponding total fruit weight (64.2 kg) per tree that exceeded to the control by 201.5 and 175.5% respectively. The intermediate results for both fruit numbers and weight were observed at 2,4-D and micro-nutrients, which were also statistically at par.

4. DISCUSSION

Fruit setting, abscission and retention determine fruit yield of mandarin [32,33]; and are associated with the regulation of hormonal, nutritional and environmental factors [34,35]. Citrus species usually produce over 0.1 million flowers [36], but the percentage of fruit set to flower number is very small, ranging from 0.1 to 3% [37,38]. Similarly, El-Rahman et al. [39] reported to fruitlets dropping occurred during blooming period in Navel orange accounted over 97.5%. In present study, as flowering started from early March that the numbers of flowers stand after petal fall at post-bloom period were ranged from 445 to 632 flowers per branch, while the highest fruit set of 6.1% occurred at GA_3 + NAA foliar spray in contrast to 4.6% of the control treatment (Table 1). Similar findings of Ullah et al. [25] showed that foliar sprayed of 25ppm GA and 25ppm NAA at full bloom stage resulted in a significant increasing of fruit set, fruit retention, higher fruit numbers, fruit weight and yield of mandarin. Likewise, Gonzalez et al. [40] reported the reduction of post-bloom abscission and subsequent increase of yield of Tangor as a result of gibberellic acid sprays during fruit set. This is probably associated with the increased of growth hormone, gibberellins, since it activated for the onset of ovary growth leading to fruit development [41]; and it also played a modulating role in the mobilization of nutrients to the developing organs [7].

In the present study, fruit drop was reported to 71.7 and 74.9% respectively on 12th June and 29th July (Table 2). But, Eti [42] found June drop at 57.8 to 86.5% in Clementine mandarin. In later stage on 25th October, only 20.1% fruit drop was reported in this study. The foliar sprays of GA_3 + 2,4-D + micro-nutrients, and 2,4-D performed better among the treatments for reducing the fruit drop. The results revealed that 17.6, 5.4, 18.3 and 54.6%% lower fruit drop were achieved, respectively on 12th June, 29th July, 10th September and 25th October interval periods at foliar spray of GA_3 + 2,4-D + micro-nutrients compared to the control spray (Table 2). Summer drop from early June until early August reduced the number of fruits per tree by as much as 17% [33]. Likewise, Lima and Davies [43] also observed that single spray of 10 or 20ppm 2,4-D, alone or in combination with 20ppm GA_3 within 9 weeks of mid-bloom, resulted in significant control of summer fruit drop.

Specifically, fruit set during developing fruitlets largely depends on photo-assimilates [44]; hence leaf photosynthesis appears to be crucial in developing fruit set [45]; as a result, plant nutrients are the limiting factors. These findings suggest that better effects of PGR including plant micro-nutrients including boron, zinc and calcium are possibly due to the increased nitrogen metabolism, photosynthesis rate, and reproductive growth, eventually resulting in higher fruit set. Similarly, Embleton et al. [46] showed that during fruit developing stage, developing ovaries and fruitlets need nitrogen for protein biosynthesis that its deficiency leads to an intense fruitlet abscission. The effects of ZnSO4 on reducing fruit drop as it involves in protein biosynthesis [22,47], and calcium as main component of cell wall, increases strength cellular membrane, resulting in reduced fruit drop [48]. The effect of boron in the treatments for the higher fruit retention was possibly associated with its role in reproductive growth and fruit set as deficiency causes flowers and fruitlets drop since it has structural role in cell wall as its deficiency significantly affects reproductive development including pollen viability, pollen infertility, reduced flower production, premature flowering and fruit down fall [49].

In the present study, the spray treatment; GA_3 + 2,4-D + micro-nutrients had the significantly highest results for number of fruits (995 nos) and corresponding total fruit weight (64.2 kg) per tree that exceeded to the control by 201.5 and 175.5% respectively (Table 4). Similar results were reported by Babu et al. [50] as foliar spray with 0.6% ZnSO4 and 20ppm 2,4-D resulted in the increased fruit number and yield; and additionally, Yesiloglu [51] also reported that the fruit set and yield increased with two-folds in Clementine mandarins by applying 20ppm GA at the end of flowering. The reduction of post-bloom abscission and subsequent increase of yield of Tangor as a result of gibberellic acid sprays during fruit set was also observed [40]. In the present study, the intermediate results for both fruit numbers and weight were observed at 2,4-D and micro-nutrients and were also statistically at par.

5. CONCLUSION

Initial fruits set and their subsequent growth is very crucial for the higher yield of mandarin; and are hormonal and plant nutritional regulated physiological processes. In this study, the foliar application of GA_3 @ 20ppm + NAA @ 50ppm during mid-bloom period performed the best for the highest bloom fruit set. Likewise, a combined foliar application of GA_3 @ 20ppm + 2,4-D @ 15ppm with micro-nutrients had the significant effect on reducing the fruit drop, and subsequent higher fruit yield of local cultivar of Mandarin in Dhankuta, Nepal. To sum up, a combined foliar application of plant growth regulators with plant micro-nutrients had significant effect on the improvement of fruit set, fruit drop control and subsequent fruit yield of mandarin.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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