

Effects of GA₃, 2,4-D and NAA Including Plant Micro-Nutrients on Fruit Set and Yield of Mandarin in Dhankuta District of Nepal

Abstract

This study was conducted with an objective of determining the foliar sprays of plant growth regulators including plant micro-nutrients, suitable for the higher fruit set and yield of mandarin in the specific agro-climate and cultivars of Nepal. The experiments were carried in Dhankuta, Nepal during 2019 and 2020. Eight treatments were evaluated; comprised of foliar sprays of three plant growth regulators, viz. GA₃ @ 20ppm; 2,4-D @ 15ppm; and NAA @ 50ppm; alone and their combinations including three plant micro-nutrients: B @ 0.4% + Zn @ 0.2% + Ca @ 1%. The foliar sprays were applied five times, first started at full-bloom, second at two months after the first spray; and then every six weeks intervals till 25th September. An average of 5.1% flowers was set to fruitlets after petal fall during post-bloom. Similarly, the highest fruit set occurred at GA₃ + 2,4-D + micro-nutrients that topped to the control by 61.6, 154.5, 211.1, and 193.3% respectively in June, July, September and October interval periods. In addition, the spray treatment of GA₃ + 2,4-D + micro-nutrients had the significantly highest number of fruits (995 nos) and total fruit weight (64.2 kg) per tree that exceeded to the control by 201.5 and 175.5% respectively.

Keywords Fruitlets; Fruit set; plant growth regulators; plant micro-nutrients; mandarin

1. INTRODUCTION

Mandarin (*Citrus reticulata* Blanco) is an economically important fruit crop in the world, including Nepal [1, 2, 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 (GA₃) 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 naphthylacetic 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 nutrients; particularly nitrogen (N), potassium (K), 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]. Nitrogen is especially associated with photosynthesis and plant growth that causes fruit set and yield [18, 19]; K is vital for regulating CO₂ uptake, thus enhancing photosynthesis; and activation of biochemical enzymes, thus influencing flowering and fruiting. Moreover, 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, GA₃, 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 GA₃ 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]. 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 maintained 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) GA₃ @ 20ppm; (2) 2,4-D @ 15ppm; (3) NAA @ 50ppm; (4) GA₃ @ 20ppm + 2,4-D @ 15ppm; (5) GA₃ @ 20ppm + NAA @ 50ppm; (6) B @ 0.4% + Zn @ 0.2% + Ca @ 1%; (7) GA₃ 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 [28, 29]. The PGR: GA₃ (C₁₉H₂₂O₆) (CAS 77-06-5); [2,4-D](#) (C₈H₆Cl₂O₃) (CAS 2707-72-9); and NAA (C₁₂H₁₀O₂) (CAS 86-87-3); and plant micro-nutrients: zinc sulfate (ZnSO₄.7H₂O) (Zn 34.65%); sodium tetraborate or borax (Na₂B₄O₇ 10H₂O) (B 11.11%); and calcium chloride (CaCl₂), (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 drop and retention percentages were measured from the same branches by counting manually on fortnightly basis (formula given below).

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

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

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 [30].

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 GA₃ + 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 GA₃ + NAA, NAA and 2,4-D foliar sprays. Thus, it shows that foliar applications of GA₃ + NAA, NAA and 2,4-D at spring flush or pre-flowering period resembled significant to improve the bloom fruit set in mandarin.

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 GA₃ @ 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 in 8th March during mid-bloom period; total number of fully developed flowers in 20th March, and fruitlets set in 1st May were evaluated; and unlike letters within column indicate significant differences ($P \leq 0.05$).

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 GA₃ + 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 at 12th June, 29th July, 10th September and 25th October periods. However, lower fruit drop also occurred at the foliar sprays of GA₃ + 2,4-D that were statistically at par with the GA₃ + 2,4-D + micro-nutrients. During the first two periods, average fruit drop occurred heavily that recorded of 71.7 and 74.9% respectively in 12th June and 29th July; while it reduced to 33.7 and 20.1% in later periods. In conclusion, two foliar sprays of GA₃ + 2,4-D + micro-nutrients, and 2,4-D performed better among the treatments for reducing the fruit drop.

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 13 th May	Fruit drop %			
		12 th June	29 th July	10 th Sep.	25 th 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 GA₃ @ 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 ($P \leq 0.05$).

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 GA₃ + 2,4-D + micro-nutrients (36.2%), closely followed by GA₃ + 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, GA₃ + 2,4-D, GA₃ + NAA, micro-nutrients, and GA₃ + 2,4-D + micro-nutrients. It eventually shows the fruit set occurred at GA₃ + 2,4-D + micro-nutrients, topped to the control by 61.6%. The average final fruit set reduced to 3.6% in 25th October, while GA₃ + 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 13 th May	Fruit set (%)	
		12 th June	25 th 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 GA₃ @ 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 ($P \leq 0.05$).

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 GA₃ + 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.

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 GA₃ @ 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 ($P \leq 0.05$).

3. DISCUSSION

Fruit setting, abscission and retention determine fruit yield of mandarin [31, 32]; and are associated with the regulation of hormonal, nutritional and environmental factors [33, 34]. Citrus species usually produce over 0.1 million flowers [35], but the percentage of fruit set to flower number is very small, ranging from 0.1 to 3% [36, 37]. Similarly, El-Rahman et al. [38] 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 GA₃ + 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. [39] 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 [40]; 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 in 12th June and 29th July (Table 2). But, Eti [41] found June drop at 57.8 to 86.5% in Clementine mandarin. In later stage in 25th October, only 20.1% fruit drop was reported in this study. The foliar sprays of GA₃ + 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 in 12th June, 29th July, 10th September and 25th October interval periods at foliar spray of GA₃ + 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% [32]. Likewise, Lima and Davies [50] also observed that single spray of 10 or 20ppm 2,4-D, alone or in combination with 20ppm GA₃ 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 [42]; hence leaf photosynthesis appears to be crucial in developing fruit set [43]; as a result, plant nutrients are the limiting factors. These findings suggest that better effects of PGR including plant nutrients including nitrogen, potassium including boron, zinc and calcium are possibly due to the increased photosynthesis rate, eventually resulting in higher fruit set. Similarly, Embleton et al. [44] 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, 45], and calcium as main component of cell wall, increases strength cellular membrane, resulting in reduced fruit drop [46]. 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 [47].

In the present study, the spray treatment; GA₃ + 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. [48] as foliar spray with 0.6% ZnSO₄ and 20ppm 2,4-D resulted in the increased fruit number and yield; and additionally, Yesiloglu [49] 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 [39]. 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.

4 CONCLUSION

The fruit set and growth are hormonal regulated metabolisms that determine ultimate yield of Mandarin. The effects of GA₃ + NAA foliar spray has the significantly highest fruit set during bloom. Likewise, foliar applications of GA₃ + 2,4-D + micro-nutrients performed the best among the treatments for reducing the fruit drop during whole intervals of crop periods. The spray treatment GA₃ + 2,4-D + micro-nutrients had the significantly highest the number of fruits and total fruit weight. To sum up, combined foliar application of gibberellic acid, 2,4-D and micro-nutrients was resulted as the best for improving the fruit set, retention and yield of mandarin.

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COMPETING INTERESTS

Authors have declared that no competing interest exists.

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