Original Research Article

Effect of GA_3, 2,4-D and NAA Including Plant Micro-Nutrients on Fruit Set and Yield of Mandarin in Nepal

Abstract

Mandarin (*Citrus reticulate* Blanco), is a widely grown important fruit in the globe including Nepal. The low productivity due to poor fruit set and retention is one of the pertinent issues of Nepal regarding this crop. The fruit set and growth are hormonal regulated metabolism that determine ultimate yield of Mandarin. In order to determine foliar spray of growth regulators including micro-plant nutrients; this study was conducted in Dhankuta, Nepal during 2019 and 2020. Eight treatments were evaluated; comprised of foliar sprays of three plant growth regulators viz. GA_3 @ 20 ppm; 2,4-D @ 15 ppm; and NAA @ 50 ppm; alone and their combinations including three micro-plant nutrients: B @ 0.4% + Zn @ 0.2% + Ca @ 1%. 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 results revealed that of total 445 to 632 flowers stand after petal-fall during post-bloom period, a range of 95.5 to 93.9% fruitlets were dropped during late March until late April among the treatments. An average of 5.1% flowers was set to fruitlets during this period. Compared with control, foliar sprays of GA_3 + NAA resulted as the best that increased the fruit set by 32.6%, having fruit set of 6.1% against 4.6% of the control treatment. With respective to fruit drops in four intervals of one and half months, two spray treatments: GA_3 + 2,4-D + micro-nutrients, and 2,4-D alone performed better among the treatments for reducing the fruit drop. Compared to control treatment, the lowest fruit drop percentage of 17.6, 5.4, 18.3 and 54.6%% occurred respectively in June, July, September and October interval periods at the foliar spray of GA_3 + 2,4-D + micro-nutrients. Moreover, the least fruit drop was recorded at GA_3 + 2,4-D + micro-nutrients spray (63.9%) compared to the control spray (77.6%) during May to June. In later periods, spraying of GA_3 + 2,4-D + micro-nutrients had the least fruit drop percentage during September and October, as recorded respectively 33.9 and 24.6%. The fruit set during all four periods, observed from June to pre-harvest stage, late October; ranged from 36.2 to 22.4% during 1st June; the highest at GA_3 + 2,4-D + micro-nutrients and the least at control. The highest fruit set occurred at GA_3 + 2,4-D + micro-nutrients that topped to the control by 61.6, 154.5, 211.1, and 193.3% respectively in four observed periods. Similarly, the spray treatment GA_3 + 2,4-D + micro-nutrients had the significantly highest the 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

Background

Mandarin (*Citrus reticulate* Blanco), is a high economical and widely grown fruit crop across the world including Nepal (USDA, 2023; Shrestha 2015; NFDC, 2021). The average productivity of mandarin is recorded to 10.8 t/ha for 2020/21 in Nepal (MoALD, 2022), which is much lower than its yield potential (FAOSTAT, 2022; Saxena et al., 2018). The fruit setting, abscission and growth determine ultimate yield (Thimann, 1977) that are hormonal controlled physiological processes (Talon et al., 1997), besides other agronomical and environmental factors being involved (Addicott, 1982; Sexton and Roberts,1982). In particular, gibberellin and auxin are associated with fruit set and growth (Domingo et al., 2007); gibberellin is responsible for the transition of ovary into fruit set, leading to fruit development (Talon et al., 1997; Ben-Cheikh et al., 1997) as it induces auxin synthesis to support growth and development (Wittwer et al., 1957; Sastry and Muir, 1963; Mapelli et al., 1978). Auxins are particularly involved in the development of reproductive structures, fruit set and growth (Cecchetti et al., 2008); as pollen grains contain sufficient auxins that are transferred to the ovary after pollination to support fruit growth (Chen and Zhao, 2008). It also involves in suppressing fruit abscission (Agustí et al., 2002).

In mandarin crop, gibberellic acid (GA3), napthylacetic acid (NAA), 2-4 dichlorophenoxy acetic acid (2,4-D) are usual hormones used o increase fruit set and yield in the citrus (Ullah et al., 2014; Mahdi et al., 2012; Berhow, 2000; Fagan, 1972). Dutta and Banik (2007) 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 and to increase fruit size in mandarin (Ullah et al., 2014; Kaska, 1989; Agustí et al., 2002; Chao, 2005; El-Otmani et al., 2000). However, hormonal application to the citrus trees is very scanty to the citrus sector in Nepal. So far, the response of the Nepalese mandarin cultivars to hormones on fruit retention, and yield is yet to be investigated. Owing to these contexts, this study conducted to investigate the effects of plant growth regulators on fruit set and yield of mandarin in Nepal.

2. Materials and Methods

The study was conducted during two consecutive crop seasons of 2019 and 2020 at the Chungmang Farm of Agricultural Research Station, Dhankuta, Nepal (26.22°N, 78.18°E) at an altitude of 1030 m. above sea level. The climate of the site is characterized as sub-tropical with warm summers, where the maximum temperature exceeded 35˚C in May and June. The winters are cool as minimum temperature reached as low as 2˚C in December and January; frost was not occured during the experiment period. Usually, the major precipitation as monsoon arrived in the second fortnight of June and lasted till September that the annual rainfall ranged between 650 to 751 mm. Drought occured afterward of monsoon rain. The site has low fertilite sandy soil with minimal organic matter, crop was managed under rainfed condition. The experiment was designed with eight treatments of foliar sprays, (1) GA_3 @ 20 ppm; (2) 2,4-D @ 15 ppm; (3) NAA @ 50 ppm; (4) GA_3 @ 20 ppm + 2,4-D @ 15 ppm; (5) GA_3 @ 20 ppm + NAA @ 50 ppm; (6) B @ 0.4% + Zn @ 0.2% + Ca @ 1%; (7) GA_3 20 ppm + 2,4-D 15 ppm + 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 spray solutions of the respective treatments were prepared in the Soil Laboratory of ARS, Pakhribas using standard procedures (Lide and Milne, 1994; Ahrens, 1994; Spencer, 1994). The experiment was laid out in the randomized complete block design (RCBD) with three replications. Two branches per experimental unit tree were selected for determining fruit set and drop; and fruit number and yield were measured from the whole tree. 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 (2nd March) until the end of fruit set (21st April) 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). Five similar sub-branches from two tagged sample branches of each experimental unit were randomly selected.

$$\textbf{Fruit set (\%)} = \frac{\text{No. of set fruitlets (after flowers dried up)}}{\text{No. of flowers (during full-bloom)}} \times 100$$

$$\textbf{Fruit retention (\%)} = \frac{\text{Total no. of retained}}{\text{Total no. of fruitlets}} \times 100$$

Total number of fruits and weight per tree were measured, 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; and Genstat® for Windows 16th edition were used for the ANOVA analysis. The Fisher’s Protected Least Significant Test (LSD) was used, when the test result was significant.

1. Results

1.1 Effects of foliar sprays of plant growth regulators including micro-plant nutrients on bloom fruit set

Both numbers of flowers as well as fruitlets set per branch in mandarin differed significantly, but fruitlet set% did not differ significantly among the foliar sprays (Table 1). The numbers of flowers stand after petal fall at post-bloom period were ranged from 445 to 632 flowers per branch. A heavy drop of flowers occurred from 95.5 to 93.9% during late March until late April. An average of 5.1% flowers was set to fruitlets during this period. Compared with control, foliar sprays of GA_3 + NAA, NAA and 2,4-D increased the fruit set by 32.6, 26.0 and 23.9% respectively that the highest fruit set observed at 6.1% of GA_3 + NAA against 4.6% of the control treatment. Thus, single spray of 20 ppm GA_3 in combination with 50 ppm NAA at mid-bloom period resembled significant for the fruit set in mandarin.

Table 1 Flowers and fruitlets set of mandarin as affected by gibberellins and auxins spray including micro-plant nutrients, 2019

Foliar sprays	Number per branch [a]		Fruitlets set%
	Flowers	Fruitlets	
GA_3	445^{b}	20^{b}	4.5
2,4-D	497^{b}	29^{ab}	5.7
NAA	494^{b}	29^{ab}	5.8
GA_3 + 2,4-D	465^{b}	22^{ab}	4.7
GA_3 + NAA	560^{ab}	36^{a}	6.1
B + Zn + Ca (micro-nutrients)	509^{ab}	25^{ab}	4.8
GA_3 + 2,4-D + micro-nutrients	538^{ab}	26^{ab}	4.7
No spray	632^{a}	27^{ab}	4.6
Mean	518	27	5.1
P-value	*	*	ns
LSD	129.5	14.2	2.3
CV%	21.4	46	38.6

[a] Indicates number of fully developed flowers and fruitlets set from 20th March to 24th April. Unlike letters within column indicate significant difference at 5% level. Single foliar sprays of GA_3, 2,4-D, and NAA, alone or their combinations including micro-nutrients was applied in 8th March during mid-bloom period.

1.2 Effects on fruit drop and set during different growing periods

The fruit drop after June during four subsequent one and half month periods varied significantly among the spray treatments (Table 2). The average fruit drop occurred heavily during earlier period in June and late July by 71.7 and 74.9% respectively, and then it reduced to 20.1% in subsequent periods. Two spray treatments: GA_3 + 2,4-D + micro-nutrients, and 2,4-D alone performed better among the treatments for reducing the fruit drop. Compared to control treatment, the lowest fruit drop percentage of 17.6, 5.4, 18.3 and 54.6%% occurred respectively in June, July, September and October interval periods at the foliar spray of GA_3 + 2,4-D + micro-nutrients. However, lower fruit drop also occurred at the foliar spray of 2,4-D, micro-nutrients GA_3 + NAA, and GA_3 + 2,4-D that were statistically at par with the GA_3 + 2,4-D + micro-nutrients. Moreover, the least fruit drop was recorded at GA_3 + 2,4-D + micro-nutrients spray (63.9%) compared to the control spray (77.6%) during May to June. In later periods, spraying of GA_3 + 2,4-D + micro-nutrients had the least fruit drop percentage during September and October, as recorded respectively 33.9 and 24.6%.

Table 2 Effect of PGR on the fruits drop of mandarin orange during 2019 & 2020

Foliar sprays [a]	Total fruits count in 13 May	Fruit drop %			
		12 June	29 July	10 Sep.	25 Oct.
GA_3	129^{ab}	74.6^{a}	75.4^{ab}	20.8^{b}	18.8^{bc}
2,4-D	152^{ab}	68.7^{ab}	71.7^{ab}	38.1^{ab}	30.9^{a}
NAA	139^{ab}	74.8^{a}	65.9^{b}	34.9^{ab}	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}	21.8^{b}	15.7^{bc}
Micro-nutrients (B + Zn + Ca)	110^{b}	69.9^{ab}	73.3^{ab}	41.9^{a}	23.1^{abc}
GA_3 + 2,4-D + micro-nutrients	130^{ab}	63.9^{b}	76.0^{ab}	33.9^{ab}	24.6^{ab}
Control	109^{b}	77.6^{a}	80.4^{a}	41.5^{a}	18.7^{bc}
Mean	132	71.7	74.9	33.7	20.1
P-value	*	*	*	*	*
LSD (5%)	64.3	10.12	13.3	17.3	10.5
CV%	59.8	17.3	21.9	63.4	64.5

[a]20 ppm GA_3, 15 ppm 2,4-D and 50 ppm NAA including 0.4% B, 0.2% Zn and 1% Ca were applied in 5 liters of spray solution per tree; Whole-branch counts made at fortnight intervals from May 13 to October 20; 3 single-tree replications per treatment; Unlike letters indicate significant differences by Duncan's multiple range test, 5% level.

Fruit set at 45 days intervals during crop periods due to the effects of foliar sprays of GA_3, 2,4-D and NAA including micro-nutrients, alone and their combinations in 2019 and 2020 are shown in Table 3. There were significant variations on fruit set during all four periods, observed from June to pre-harvest stage, late October. It ranged from 36.2 to 22.4% during 1st June; the highest at GA_3 + 2,4-D + micro-nutrients and the least at control. The fruit set occurred at GA_3 + 2,4-D + micro-nutrients, topped to control by 61.6, 154.5, 211.1, and 193.3% respectively in four observed periods. However, it was statistically at par with others spray treatments except GA_3 and NAA in 12th June, and control. The fruit set after 12th June drastically reduced to 6.6% in 29th July, 4.4% in 10th September and 3.6% in 25th October.

Table 3 Effect of PGR on the fruits dropping and retention of mandarin orange during 2019 and 2020

Spray treatments	Total fruits count in 13 May	Fruit set (%)			
		12 June	29 July	10 Sep.	25 Oct.
GA_3	129^{ab}	25.4^{b}	6.5^{ab}	5.4^{a}	4.7^{a}
2,4-D	152^{ab}	31.3^{ab}	8.6^{a}	5.2^{a}	3.7^{ab}
NAA	139^{ab}	25.2^{b}	7.0^{ab}	5.0^{ab}	4.5^{a}
GA_3 + 2,4-D	106^{b}	27.9^{ab}	6.8^{ab}	4.5^{ab}	3.8^{ab}
GA_3 + NAA	184^{a}	28.3^{ab}	3.9^{b}	2.7^{ab}	2.2^{ab}
Micro-nutrients (B + Zn + Ca)	110^{b}	30.1^{ab}	8.5^{a}	5.3^{a}	4.2^{a}
GA_3 + 2,4-D + micro-nutrients	130^{ab}	36.2^{a}	8.4^{a}	5.6^{a}	4.4^{a}
Control	109^{b}	22.4^{b}	3.3^{b}	1.8^{b}	1.5^{b}

Mean	132	28.3	6.6	4.4	3.6
P-value	*	*	*	*	*
LSD (5%)	64.3	10.1	4.3	3.1	2.7
CV%	59.8	43.9	80.9	87.5	93.5

[a]20 ppm GA_3, 15 ppm 2,4-D and 50 ppm NAA including 0.4% B, 0.2% Zn and 1% Ca were applied in 5 liters of spray solution per tree; Whole-branch counts made at fortnight intervals from May 13 to October 20; 3 single-tree replications per treatment; Unlike letters indicate significant differences by Duncan's multiple range test, 5% level.

2. 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 spray treatment; 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, and they were also statistically at par.

Table 4 Effect of PGR on the fruit number and yield, 2019 & 2020

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
P-value	***	***
LSD (5%)	103.4	9.92
CV%	15.7	22.5

3. Discussion

3.1 Effects of foliar sprays of GA_3, 2,4-D and NAA including plant micro-nutrients (B, Zn and Ca) on bloom fruit sets

Fruit setting, abscission and retention determine fruit yield of mandarin (Lima et al., 1980; Thimann, 1977)**;** and are associated with the regulation of hormonal, nutritional and environmental factors (Gillaspy et al., 1993). Most citrus species including mandarin have perfect flowers, self-incompatible (Krezdorn, 1970). Citrus species usually produce over 0.1 million flowers (Davies, 2002), but the percentage of fruit set to flower number is very small, ranging from 0.1 to 3% (Lovatt et al., 1984). Similarly, Lima (1983) reported to flowers and fruitlets dropping occurred during blooming period account over 97.5%. In Nepal, mandarin starts flowering from March-April, and fruit matures in the October onward (Subedi et al., 1994). In present study, 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. The 10 ppm

GA_3 applied between full-bloom and two-thirds petal-fall stage has resulted in a significant promotion of set and yields on tangelos and mandarins (Soost and Burnett, 1961; Krezdorn and Cohen, 1962; Rivero et al., 1968). Likewise, Gonzalez et al. (1994) reported the reduction of post-bloom abscission and subsequent increase of yield of Tangor as a result of gibberellic acid sprays during fruit set. Similar result of increased fruit set and yield by two-folds in Clementine mandarin by applying 20 ppm GA at the end of flowering was reported (Yesiloglu, 1988). Moreover, Nkansah et al. (2012) investigated 25 ppm GA and 25 ppm NAA, foliar sprayed at full bloom stage as the best results in terms of increasing fruit set, fruit retention, higher fruit numbers, fruit weight and yield. During post-bloom stage, growing fruitlets compete for metabolites and tend to abscise under shortage of carbohydrates. While gibberellins activate signal for the onset of ovary growth leading to fruit development (Talon et al., 1997). Similarly, endogenous hormones and their balance play a modulating role in the mobilization of nutrients to the developing organs (Browning, 1966).

3.2 Effects on fruit drop and sets during different growing periods

Fruit dropping was more severe during June to August that caused upto 92-100% of fruit loss (Pandey and Rana, 1993). In the present study, however, fruit drop was reported to 71.7 and 74.9% respectively in June and late July. But, Eti (1987) found June drop accounting 57.8 to 86.5% in Clementine mandarin. In later stage during October, only 20.1% fruit drop was reported in this study. Two spray treatments GA_3 + 2,4-D + micro-nutrients, and 2,4-D performed better among the treatments for reducing the fruit drop. Compared to control treatment, the lowest fruit drop of 17.6, 5.4, 18.3 and 54.6%% occurred respectively in June, July, September and October interval periods at foliar spray of GA_3 + 2,4-D + micro-nutrients. However, lower fruit drop also occurred at the foliar spray of 2,4-D; micro-nutrients; GA_3 + NAA; and GA_3 + 2,4-D that were statistically at par with the GA_3 + 2,4-D + micro-nutrients. Moreover, the least fruit drop was recorded at GA_3 + 2,4-D + micro-nutrients spray (63.9%) compared to the control spray (77.6%) during May to June. Fruit dropping was more severe during June to August that caused upto 92-100% of fruit loss (Pandey and Rana, 1993). In later periods, spraying of GA_3 + 2,4-D + micro-nutrients had the least fruit drop percentage during September and October, as recorded respectively 33.9 and 24.6%. Pre-harvest drop occurred due to adverse climate, and diseases and insect invasions. Some diseases such as brown rot, root rot, pink disease, black rot, stylar-end rot, twig die-back have been known to be associated with pre-harvest fruit drop.

3.3 Effects of foliar sprays on fruit yield characteristics

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. Similar results were reported by Babu et al. (1982) as foliar spray with 0.6% ZnSO4 and 20 ppm 2,4-D resulted in the increased fruit number and yield; and additionally, Yesiloglu (1988) also reported the increased fruit set and yield with two-folds by applying 20 ppm GA at the end of flowering. The reduction of postbloom abscission and subsequent increase of yield of Tangor as a result of gibberellic acid sprays during fruit set was also observed (Gonzalez et al., 1994). Likewise, Babu et al. (1982) found that sprayed with 0.6% ZnSO4 and 20 ppm 2,4-D have yielded more fruits. 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. Yesiloglu (1988) also reported that the fruit set and yield increased with two-folds in Clementine mandarins by applying 20 ppm GA at the end of flowering. Summer drop from early June until early August reduced the number of fruits per tree by as much as 17% (Lima et al., 1980). Likewise, Lima and Davies (1984) observed that single spray of 10 or 20 ppm 2,4-D, alone or in combination with 20 ppm GA at or within 9 weeks of midbloom, resulted in significant control of summer fruit drop.

Conclusion

The fruit set and growth are hormonal regulated metabolisms that determine ultimate yield of Mandarin. The effects of GA_3 + NAA foliar spray has the significantly highest fruit set during bloom;

having fruit set of 6.1% against 4.6% of the control treatment, which is 32.6% higher than the control treatment. Likewise, foliar applications of GA_3 + 2,4-D + micro-nutrients performed the best among the treatments for reducing the fruit drop during whole intervals of crop periods; compared to the control treatment, 17.6, 5.4, 18.3 and 54.6%% lower fruit drop occurred respectively in June, July, September and October interval periods. The fruit set ranged from 36.2 to 22.4% during 1st June; the highest at GA_3 + 2,4-D + micro-nutrients and the least at the control. Similarly, the same treatment had 61.6, 154.5, 211.1, and 193.3% higher fruit set than that of the control treatments, respectively in four intervals. The spray treatment GA_3 + 2,4-D + micro-nutrients had the significantly highest the 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. 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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