Effect of foliar application of micronutrients on growth, fruit retention and yield parameters of acid lime (*Citrus aurantifolia* Swingle)

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_Cogent Food & Agriculture_ (2022), 8: 2112421
**Effect of foliar application of micronutrients on growth, fruit retention and yield parameters of acid lime (Citrus aurantifolia Swingle)**

Saurav Bastakoti\(^1\)*, Saurav Nepal\(^1\), Dipika Sharma\(^1\) and Arjun Kumar Shrestha\(^1\)

**Abstract:** The deficiency of micronutrients is widespread in Nepal, which results in poor growth, fruit drop and lower yield. June drop and pre-harvest drop reduce fruit retention and yield of Citrus. A study on the effects of micronutrients on June drop and pre-harvest drop of Citrus has not been conducted in Nepal and few in the global range. So, the effects of foliar application of micronutrients (Zn, B and Cu) were studied on plant growth, fruit retention and yield parameters of acid lime (Citrus aurantifolia Swingle) var. Sun Kagati-1. The findings revealed the significant effect of Zn alone or in combination with plant canopy spread under growth parameters. Zn and B alone or in combination significantly affected fruit retention and yield parameters of acid lime. Zn and/or B resulted in significant minimization of June drop and pre-harvest drop of fruits with a greater reduction in combination type treatments. Cu enhanced fruit weight and thereby led to greater yield. Treatment Zn (0.1%)+Cu (0.05%)+B (0.05%) was best on which fruit set was 26.14% higher as compared to control. Similarly, the June and pre-harvest drops were 18.52% and 5.18% lower in the treatment, respectively. Moreover, yield due to the treatment was 1.6 times greater than that of control as a result of higher fruit number and fruit weight. Thus, the application of Zn, B and Cu can be beneficial in obtaining a greater fruit set. Further, their role in the minimization of June and pre-harvest drop augments the fruit yield of acid lime.

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**ABOUT THE AUTHOR**

Mr Saurav Bastakoti is an agriculture graduate who passed bachelor's degree in agriculture from Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal. He is the author of a booklet titled “Integrated Nutrient Management in Citrus fruits”. He executed the research as the main researcher and writer. Mr Saurav Nepal and Ms Dipika Sharma also passed their bachelor's degree in agriculture from AFU and assisted in writing this research. Mr Arjun Kumar Shrestha, PhD is a Professor at the Department of Horticulture as well as Director at the Directorate of Research and Extension in AFU. He supervised this research and helped in research designing as well as writing. The essence of this research is its connection with the livelihood of Citrus farmers of Nawalparasi East district and its assistance to obtain greater income through improvement in yield.

**PUBLIC INTEREST STATEMENT**

Obtaining a greater and stable yield is pivotal for commercial Citrus cultivation, however poor plant growth and fruit drop can be challenging factors as this reduces yield. Various studies suggest that the problems can be ameliorated by the use of micronutrients since they are involved in various enzymatic processes that promote growth, increase fruit set and minimize fruit drop. Also, people of Nawalparasi East district are oblivion to micronutrient use and there is also a problem of fruit drop in Citrus orchards of the region. Thus, this study was conducted in acid lime to explore the effect of micronutrients on the minimization of fruit drop (June drop and pre-harvest drop) and impart cognizance of micronutrients’ use to obtain greater yield.
Subjects: Agriculture & Environmental Sciences; Crop Science; Horticulture

Keywords: zinc; boron; copper; Citrus; fertilization; fruit set; June drop

1. Introduction

Acid Lime (Citrus aurantifolia Swingle), one of the Citrus fruits, has been cultivated in about 67 districts of Nepal (MOALD, 2020) and is considered a high-value commodity with number one priority by Master Plan for Horticulture Development (Shrestha et al., 2012). It is cultivated from plains to high hills and east to west in Nepal. It is an important citrus fruit in Nawalparasi East district and is connected with the livelihood of farmers.

The productivity of Citrus largely depends on nutrition as it is a highly nutrient-responsive crop and the depletion of nutrients limits its productivity (Zoremaluang et al., 2019). Among the nutrients, though the requirement of micronutrients is less as compared to primary nutrients, it is equally important for plant metabolism, growth and development (Bhanukar et al., 2018). An adequate supply of micronutrients results in a good yield and quality of fruits (Babu & Yadav, 2005). However, their deficiency results in poor growth and lower yield. Deficiencies of micronutrients cause poor orchard establishment and drastic loss of yield with poor quality (Marschner, 2012). Unavailability, continuous uptake and limited or no supply cause deficiency of these nutrients. The most common deficiency in all Citrus-producing areas is zinc (Zn) deficiency, which is aggravated by a high level of phosphorus (P) fertilization (Boarretto et al., 2001). Also, high levels of P and heavy metals like copper (Cu) cause a deficiency of iron (Fe; Marschner, 2012). Boron (B) deficiency is also observed in Citrus orchards frequently (Yang et al., 2022). In soils high in organic matter, Cu deficiency is observed since it forms a complex with organic substances (Marschner, 2012). Deficiency or inadequate nutrition in a plant can be corrected by fertilization. However, most of the fertilizer applied in the soil may be lost due to leaching, run-off, denitrification and volatilization (Zoremaluang et al., 2019). B is easily leached from most soils and its deficiency occurs in a wide range of cropping systems (Marschner, 2012). Also, several studies suggest that increased environmental stress due to climate change limits the soil-derived nutrients as a result of higher incidence and duration of heat and drought (Ishfaq et al., 2022). These limitations by the interaction of different nutrients and losses in soil affect the productivity of crops and can be mitigated in the case of foliar fertilization. Foliar spray of micronutrients has an advantage over soil application due to its high effectiveness and rapid plant response (Bhanukar et al., 2018). Foliar application is 7–21 times more effective than soil application (Zaman et al., 2019) as nutrients are directly applied onto the leaves where their metabolism takes place and gives a quicker response due to easy access to nutrients (Harris & Puvanitha, 2018). In addition to it, Citrus is a deep-rooted fruit crop, so soil-applied micronutrients may have a less significant effect (Tariq et al., 2007). Sultana et al. (2016) reported a greater yield of tomato through foliar application of Zn and B as compared to soil application. Obreza et al. (2010) also found the foliar application of micronutrients to be superior to soil application. Under stressful conditions also, foliar fertilization increases crop yield by 15–19% (Ishfaq et al., 2022).

Micronutrients like zinc (Zn), boron (B) and copper (Cu) are involved in various enzymatic processes regulating plant growth and physiological processes in acid lime (Ilyas et al., 2015). Zinc is responsible for the activation of more than 150 enzymes and hormones such as growth hormones and respiration enzymes, also involved in synthesizing enzymes that manufacture the plant auxins, generating chlorophyll and photosynthesis (Mohammed et al., 2018). Zn plays a key role in the synthesis of tryptophan (Reetika et al., 2020), a precursor of auxin, which promotes plant growth and flowering (Mohammed et al., 2018). Zn affects fruit set through its role in flowering and pollination by generating the pollen tube (Marschner, 1995). B also promotes plant growth and increases pollen grain germination,
pollen tube elongation, fruit set percentage and finally the yield (Abd-Allah, 2006). Zn and B prevent abscission layer formation, which can be a helpful mechanism in reducing flower and fruit drops (Jat & Kacha, 2014; Smith & Johnson, 1969). Zn and B help to obtain greater yield by achieving good fruit retention through enhanced fruit set and lower fruit drop (Noor et al., 2019). Several studies have been conducted that suggest the use of Zn and B to enhance plant growth and yield. Noor et al. (2019) reported a lower June drop of sweet orange when supplied with Zn and B as compared to control. Rajkumar et al. (2014) also reported reduced fruit drop and enhanced fruit set, fruit retention, fruit number, fruit weight and yield of Guava with increased concentration of ZnSO₄ and H₂BO₃ from 0% to 1%. Cu helps in the stabilization of chlorophyll and influences photosynthesis (Ilyas et al., 2015). Also, Cu is indispensable for carbohydrate and nitrogen metabolism in Citrus (Stenico et al., 2009). Khurshid et al. (2008) reported increased fruit weight and yield per tree under foliar application of Cu in Orange trees as compared to untreated trees. Zoremtruangi et al. (2019) found an increment in fruit set, reduced fruit drop and greater yield with the application of Zn, B and Cu. Apart from yield, these micronutrients also play an important role in the quality parameters of Citrus fruits. Singh et al. (2018a) reported enhanced juice percentage, total soluble solids, ascorbic acid, reducing sugars and total sugar, while reduced peel thickness, number of seeds per fruit, non-reducing sugars and titratable acidity with the combined application of Zn, B and Cu.

There is a problem of nutrient management in Nawalparasi East district as stated by the officials of Prime Minister Agriculture Modernization Project (PMAMP)-Project Implementation Unit, Nawalparasi East. Farmers in the region are less aware of the micronutrient’s use. Limited or no supply of micronutrients has resulted in poor soil nutrition. In a study by Andersen (2007), severe deficiencies of micronutrients were reported in Nepal. Eighty to 90% of soil samples were deficient in B, 20–50% in Zn and 10–15% in molybdenum (Mo). Chhetri et al. (2017) also mentioned the widespread deficiency of micronutrients in Nepal. The deficiencies of micronutrients lead to poor plant growth, lower fruit set, excessive fruit drops, smaller fruit size, low quality of fruits and lower yield. The problems of fruit drop and lower yield existed in the region, which might be attributed to the deficiency of micronutrients. Like, Zn deficiency results in low flowering intensity, fruit set and yield, as well as enhances the problems of fruit drop (Srivastava & Singh, 2005). Similarly, B deficiency also results in premature shedding of fruits (Yang et al., 2022). Further, environmental stresses due to climate change aggravate flower and fruit drops because of impairment of hormonal balance (Sawicki et al., 2015) which ultimately impacts yield. Many studies suggest the use of micronutrients to obtain greater yield. Among the micronutrients, Zn, B and Cu are commonly available in the region. Though the effects of Cu nutrition in fruit set and drop have been studied a few, its significant effect on yield has been reported. However, Morschner (2012) mentioned that Cu deficiency affects fruit formation more strongly compared to vegetative growth. Effects of micronutrients have not been studied on acid lime (whose climatic condition differs from mandarin and sweet oranges in Nepal) in the country and also very few in other Citrus fruits. Further, the effect of micronutrients on the minimization of June drop and pre-harvest drop has been studied less. Therefore, it was hypothesized that the foliar application of Zn, B and Cu will help to improve fruit set and minimize fruit drop, thereby improving yield. Thus, the effect of foliar application of Zn, B and Cu was investigated on plant growth, fruit retention and yield parameters of acid lime.

2. Materials and methods

2.1. Research site

The research was conducted in a commercial acid lime orchard located at Madhyebindu-15, Ranitar, Nawalparasi East located within the coverage area of PMAMP Citrus Zone. The research site is situated at an elevation of 150 m above sea level. Its geographical coordinate is 27.59°N and 84.00°E. The research site is shown in Figure 1.
Figure 1. Map of Nepal showing research site.

Figure 2. Layout of research.
2.2. Soil analysis
A composite sample was derived by mixing the soil samples taken from three randomly selected spots 1 m away from the acid lime tree at the experimental site. The characteristics of the soil are mentioned in Table 1. A high level of P is reported which limits the availability of Zn and also high N and OM content limits the availability of Cu in soil (Marschner, 2012).

2.3. Selection of acid lime tree
Twenty-one uniform trees were selected randomly to carry out research, and each tree was randomly tagged according to the treatment.

2.4. Selection of branches
Five branches were selected randomly from all the sides of each selected tree and tagged with ribbon.

2.5. Experimental details
Design: Randomized Complete Block Design (RCBD) (Figure 2)

Replication (R): 3
Treatment (T): 7
Number of trees: 21
Age of tree: 4 years old
Spacing of trees: 3.5 m × 3.5 m
Number of branches: 5 per tree
Variety: Sun Kagati-1 (Grafted)

Each tree was taken as one experimental unit.

| Soil properties                      | Unit | Value  | Remarks           | Test method                                      |
|--------------------------------------|------|--------|-------------------|--------------------------------------------------|
| Texture                              | -    | -      | loam              | Mechanical analysis                              |
| pH                                   | -    | 5.8    | Moderately acidic | pH meter (1:2.5 soil water suspension)           |
| Organic matter (OM) content          | %    | 4.04   | High              | Walkley-Black method                             |
| Total Nitrogen (N)                   | %    | 0.20   | High              | Kjeldahl method                                  |
| Available Phosphorus (P$_2$O$_5$)    | Kg ha$^{-1}$ | 188.60 | Very high         | Modified Olsen's bicarbonate method              |
| Available Potassium (K$_2$O)         | Kg ha$^{-1}$ | 739.20 | Very high         | Flame photometer method                          |
| Zinc                                 | ppm  | 0.50   | Low               | Atomic Absorption Spectrophotometer              |
| Boron                                | ppm  | 0.38   | Low               |                                                  |
| Copper                               | ppm  | 0.65   | Medium            |                                                  |

Table 1. Soil characteristics of the research site

Bastakoti et al., Cogent Food & Agriculture (2022), 8: 2112421
https://doi.org/10.1080/23311932.2022.2112421
2.6. Treatment details
Micronutrient sources named Double chelated-double action Zinc (liquid formulation), copper sulphate and borax containing Zn (10%), Cu (25.47%) and B (14.6%), respectively, were used in the preparation of treatment solutions since they are commonly available in the region. These sources were procured from nearby Agro shops. Concentrations of these micronutrients were decided based on various studies (Bhayar & Ramdevputra, 2016; Bhanukar et al., 2018; Singh et al., 2018a; Tagad et al., 2018; Kamei et al., 2019; Reetika et al., 2020; Ruchal et al., 2020) and expressed as total elemental Zn, B and Cu content. Solution of Zn (0.1%), B (0.05%) and Cu (0.05%) was prepared by dissolving 10 ml of the liquid Zn formulation, 3.5 gm of borax and 2 gm of copper sulphate per litre of water, respectively. A sticker with the trade name Hybrid SB was used at the rate of 3 ml per litre treatment solution to prevent the solution from being washed off (Ruchal et al., 2020). Following are the treatments used in the experiment:

T1: Water (Control)
T2: Zn (0.1%)
T3: Cu (0.05%)
T4: B (0.05%)
T5: Zn (0.1%)+B (0.05%)
T6: Cu (0.05%)+B (0.05%)
T7: Zn (0.1%)+Cu (0.05%)+ B (0.05%)

Note: Each treatment was randomly allocated within each replication. All other cultivation practices were done as per farmer’s practice and were maintained the same for all the treatments.

2.7. Treatment application
Treatment solutions were sprayed on foliage using a battery-operated Knapsack sprayer until run-off condition. Treatments were applied twice; first at the full flowering stage on 6 March 2021, and the next when fruitlets were at the pea-sized stage on 27 April 2021. The spraying was done during the morning, i.e. from 7 AM (Ante Meridiem) (after dew had been removed from leaves) to 9 AM.

2.8. Observations recorded
2.8.1. Growth parameters
2.8.1.1. Stem and branch diameter. Diameters of stem and branch were measured at the time of harvest using Vernier calliper and expressed in centimetres (cm).

2.8.1.2. Plant height. Plant height was measured twice by using a measuring tape, twice; firstly, 24 hours before the first application and the next at the time of harvest. Height increment was calculated by using a formula given below (Bisen et al., 2020; Tagad et al., 2018). It was expressed in metres (m).

\[\text{Height Increment} = \text{plant height at harvest} - \text{plant height before}\]

2.8.1.3. Plant canopy spread. The canopy spread of the plant from east to west and north to south was measured at the time of harvest using a measuring tape (Arunadevi et al., 2019; Tagad et al., 2018). It was expressed in m.
2.8.2. Fruit retention parameters

2.8.2.1. Flowers per branch. The total number of flowers was counted manually before first spraying from the selected branches after full flowering and expressed as flowers per branch.

2.8.2.2. Fruit set per branch. The number of fruitlets in the selected branches was counted manually after the fruits had been set completely. Fruit set of each branch was obtained using a formula given below, and the average fruit set per branch for each treatment was thus calculated. The fruit set was expressed in the form of a percentage (Ruchal et al., 2020).

\[
\text{Fruit set (\%)} = \frac{\text{Total number of fruitlets in a branch}}{\text{Total number of flowers in the branch}} \times 100
\]

2.8.2.3. June drop per branch. Fruits that remained in each selected branch were counted manually at the end of June. Thus, June drop from selected branches of each tree was obtained using a formula given below and the average June drop per branch for each treatment was thus calculated. June drop per branch was expressed in the form of a percentage (Noor et al., 2019).

\[
\text{June drop (\%)} = \frac{\text{Number of fruit set in a branch} - \text{Fruit retained till June in the branch}}{\text{Number of Fruit set in the branch}} \times 100
\]

2.8.2.4. Pre-harvest drop per branch.

Fruits that remained during the time of harvest in each selected branch were counted manually. Thus, pre-harvest drop from selected branches of each tree was obtained using a formula given below and the average pre-harvest drop per branch for each treatment was thus calculated. Pre-harvest drop per branch was expressed in the form of a percentage.

\[
\text{Pre – harvest drop (\%)} = \frac{\text{Fruits retained till June in a branch} - \text{Final fruit number in the branch}}{\text{Fruit retained till June in the branch}} \times 100
\]

2.8.3. Yield parameter

2.8.3.1. Number of fruits. The total number of fruits present during harvesting was counted manually for each treatment and expressed as fruits per tree (Tagad et al., 2018).

2.8.3.2. Fruit weight. Matured fruits were placed in weighing balance to obtain 1 kg fruits from each treatment. The average weight of fruit for each treatment was calculated by using a formula given below and expressed in grams (gm).

\[
\text{Fruit weight (gm)} = \frac{1000}{\text{Number of fruits present in a kg}}
\]

2.8.3.3. Yield.

Yield was calculated by using a formula given below and expressed in kilogram (kg) per tree (Tagad et al., 2018).

\[
\text{Yield (kg/tree)} = \frac{\text{Number of fruits per tree} \times \text{Fruit weight in gm}}{1000}
\]

2.9. Data analysis

Data were recorded and entered treatment-wise in MS excel under three replications. Analysis was done using R studio software. The “Gvlma” package was used for testing the assumptions of ANOVA. ANOVA and DMR analysis were done at 5% level of significance using the package “agricolae”. DMR analysis was done to find out the significant differences among the treatments. Analyzed data were finally presented in the form of tables and graphs. LSD value was taken at 5% level of significance.
3. Results and discussion

3.1. Growth parameters
In growth parameters, significant effects were not found for stem diameter, branch diameter and plant height increment with the foliar application of Zn, B and Cu (Table 2). However, Zn (0.1%) alone or in combination increased plant canopy spread significantly (Table 2). A similar effect of Zn on plant spread was found by Bhanukar et al. (2018). Ram and Bose (2000) reported a significant effect of foliar application of ZnSO₄ (0.5%) on plant canopy spread as compared to control. Yadav et al. (2020) also reported the effect of Zn on plant spread increment of Kagzi lime as compared to water. The non-significant effect of all the treatments on stem diameter, branch diameter and plant height increment might be due to training and pruning practices, in winter, where farmers mostly cut down vertical branches from the top canopy, which might have directed reserves towards lateral growth favouring canopy spread. Further, the role of Zn in the active synthesis of tryptophan, a precursor of auxin, enhanced photosynthetic activity through chlorophyll synthesis and resulted in significant tissue growth and development (Rawat et al., 2010) as compared to control. This increased canopy spread. But, the findings of Zoremaluangi et al. (2019) revealed the significant effect of B (0.1%) and Cu (0.4%) also on both the canopy spread of Khasi mandarin; however, no effect was seen on plant height. The significant effect of B (0.052%) on the percentage increase in canopy spread and plant height of sweet orange was also reported by Bhanukar et al. (2018) which is in contrast to our findings. Though B and Cu increased canopy spread, their significant effect was not seen as compared to control which might be attributed to their utilization in reproductive success than vegetative growth and the involvement of macronutrients along with these nutrients to enhance growth among all the treatments (Marschner, 2012; Mousavi et al., 2012). Further research can be done to compare the growth of acid lime by the use of micronutrients during the vegetative stage and reproductive stage. The highest east-west spread (3.10 m) and north-south spread (3.25 m) were observed for treatment Zn (0.1%)+Cu (0.05%)+B (0.05%; Table 2) which is ascribed to the cumulative effect of three nutrients; however, the results were not significantly different from the results of Zn (0.1%)+B (0.05%). This suggests that micronutrients in combination work better than individual spray.

3.2. Fruit retention parameters
Significant variation was found for fruit retention parameters among treatments (Table 3). Zn (0.1%) and B (0.05%) alone or in combination treatment showed significant effects on fruit set, June drop and pre-harvest drop as compared to control (water spray). This finding conforms with Ruchal et al. (2020), Noor et al. (2019) and Yadav et al. (2020) also reported the effect of Zn and B on the fruit set of Kagzi lime as compared to water. Cu (0.05%) alone did not show a significant effect on fruit set and fruit drop. This is similar to the findings of Kumar et al. (2018) where Cu at a concentration of 0.06% (0.25% CuSO₄) did not show a significant effect on the fruit set of Kagzi lime as compared to control but, in contrast, reduced fruit drop. However, the fruit set was significantly affected at a greater Cu concentration (0.50% CuSO₄). Trees sprayed with a combination type of treatment showed greater effects on fruit retention parameters. The maximum final fruit set (64.38%) and minimum values of June drop (46.41%) and pre-harvest drop (9.16%) of acid lime were observed for treatment Zn (0.1%)+Cu (0.05%)+B (0.05%) solution, which was, however, not significantly different from Zn (0.1%)+B (0.05%; Table 3). Zoremaluangi et al. (2019) reported maximum fruit set and minimum fruit drop when treated with Zn (0.5%)+B (0.1%)+Cu (0.4%) in mandarin. The significant effects of Zn and B on fruit set might be due to their role in pollination and reducing flower drop. Zn might have influenced pollination through its effect on pollen tube generation (Marschner, 1995). Also, Zn helps in auxin synthesis, and Radović et al. (2016) reported the effect of auxin on pollen germination and pollen tube growth. In addition to it, auxin inhibits abscission and facilitates the ovary to remain attached to the shoot, resulting in lower flower and fruit drop (Jat & Kacha, 2014). Similarly, an increase in fruit set by B might also be associated with
| Treatments                      | Diameter         | Plant height          |        |           |          |          |
|--------------------------------|------------------|-----------------------|--------|----------|----------|----------|
|                                | Stem (cm)        | Branch (cm)           | Before (m) | Harvest (m) | Height Increment (m) | EW spread (m) | NS spread (m) |
| Control (water spray)          | 7.46             | 2.96                  | 2.42   | 2.79     | 0.37     | 2.28<sup>c</sup> | 2.43<sup>c</sup> |
| Zn (0.1%)                      | 7.85             | 3.35                  | 2.31   | 2.73     | 0.42     | 2.80<sup>ab</sup>| 2.92<sup>cd</sup> |
| Cu (0.05%)                     | 7.57             | 3.06                  | 2.30   | 2.66     | 0.36     | 2.50<sup>bc</sup> | 2.57<sup>bc</sup> |
| B (0.05%)                      | 7.90             | 3.21                  | 2.21   | 2.56     | 0.35     | 2.67<sup>bc</sup> | 2.74<sup>bc</sup> |
| Zn (0.1%)<sup>+</sup>B (0.05%) | 8.19             | 3.07                  | 2.32   | 2.72     | 0.40     | 2.95<sup>ab</sup> | 2.95<sup>ab</sup> |
| Cu (0.05%)<sup>+</sup>B (0.05%) | 7.82             | 3.06                  | 2.45   | 2.84     | 0.39     | 2.87<sup>ab</sup> | 2.70<sup>bc</sup> |
| Zn (0.1%)<sup>+</sup>Cu (0.05%) | 8.07             | 3.08                  | 2.35   | 2.78     | 0.43     | 3.10<sup>a</sup>  | 3.25<sup>a</sup>  |
| SEM (±)                        | 0.10             | 0.05                  |        |          | 0.01     | 0.07     | 0.07     |
| F test                         | NS               | NS                    |        |          | NS       | *        | *        |
| LSD (α = 0.05)                 | -                | -                     |        |          | -        | 0.42     | 0.39     |
| CV (%)                         | 5.61             | 7.41                  |        |          | 11.73    | 8.62     | 7.89     |
| Grand mean                     | 7.84             | 3.11                  |        |          | 0.39     | 2.74     | 2.79     |

Note: *, ** and *** denote significance at p = 0.05, p = 0.01 and p < 0.001 respectively, EW = East-West, NS = North-South, CV = Coefficient of Variation, SEm = Standard Error of Mean, LSD = Least Significant Difference, Treatment means within a column followed by the different letter(s) were significantly different at p = 0.05 level by Duncan Multiple Range Test.
enhanced pollination. B influences pollen tube growth by the formation of the boron-sorbitol complex (enhances absorption, translocation and metabolism of sugar in pollen) and synthesis of pectin material for the cell wall of the growing pollen tube (Negi et al., 2011) which enhances fruit set by improved pollination. B also increases pollen grain germination and pollen tube elongation (Abd-Allah, 2006). B is responsible for the increment and alteration of sugar composition in nectar (Smith & Johnson, 1969) which stimulates insect pollination and, hence, fruit set. Significant effect of Zn and B on minimization of June drop and pre-harvest drop might be associated with the prevention of abscission layer formation (Jat & Kacha, 2014; Smith & Johnson, 1969). The application of Zn and B is responsible for the formation and movement of carbohydrates from leaves to fruits that encourages the cellulose formation (Khan et al., 2015). This increases the strength of cell wall and reduces fruit drop. Though Cu (0.05%) increased fruit set and minimized June drop and pre-harvest drop than control, its significant effect was not seen which might be due to low concentrations since Kumar et al. (2018) reported that the application of 0.50% CuSO₄ (0.12% Cu) affected fruit set and drop significantly on Kagzi lime. However, Ilyas et al. (2015) reported 0.3% CuSO₄ to be toxic to photosynthetic attributes in mandarin. Hence, further study can be done with different levels of Cu to determine its effects on fruit set and drops as well as other characteristics.

### 3.3. Yield parameters

The number of fruits per tree, fruit weight and yield per tree significantly varied due to treatments (Table 4). Zn and B alone or in combination significantly affected all the studied yield parameters, while Cu showed its effects on fruit weight and yield only. Yadav et al. (2020) also reported the effect of Zn and B on the number of fruits per plant and fruit yield per plant of Kagzi lime as compared to water. Similarly, Razzaq et al. (2013) reported a significant increase in the yield of Kinnow mandarin with foliar application of Zn and B. The significant effects of Zn and B are associated with increased fruit number due to enhanced fruit set and minimized fruit drop (June and pre-harvest drop; Table 3) and further, their role in better photosynthesis and accumulation of starch in fruit increased fruit weight (Venu et al., 2014) which ultimately improved yield. B stimulates rapid mobilization of water and sugar in the fruit, which leads to the accumulation of dry matter within the fruit (Bhatt et al., 2012) and thus, increases the fruit weight. Also, Zn and B are involved in hormonal metabolism, increase in cell division and expansion of cell wall (Zoremtluang et al., 2019) which increased fruit weight. Cu

### Table 3. Effect of foliar application of Zn, B and Cu on fruit retention parameters of acid lime in Navalparasi East, Nepal, 2021

| Treatments                  | Final fruit set (%) | June drop (%)  | Pre-harvest drop (%) |
|-----------------------------|---------------------|----------------|----------------------|
| Control (water spray)       | 38.24              | 64.93          | 14.34                |
| Zn (0.1%)                   | 58.02              | 52.70          | 10.11                |
| Cu (0.05%)                  | 44.66              | 59.54          | 12.03                |
| B (0.05%)                   | 52.80              | 52.94          | 10.42                |
| Zn (0.1%)+B (0.05%)         | 64.87              | 64.14          | 9.16                 |
| Cu (0.05%)+B (0.05%)        | 53.00              | 53.00          | 9.16                 |
| Zn (0.1%)+Cu (0.05%)+B (0.05%) | 64.38              | 46.41          | 9.16                 |

SEm (±) 2.13  1.71  0.46

F test ***  *  *

LSD (α = 0.05) 2.81

CV (%) 14.4

Grand mean 10.99

Note: *, ** and *** denote significance at p = 0.05, p = 0.01 and p < 0.001 respectively, CV = Coefficient of Variation, SEm = Standard Error of Mean, LSD = Least Significant Difference, Treatment means within a column followed by the different letter(s) were significantly different at p = 0.05 level by Duncan Multiple Range Test.
Table 4. Effect of foliar application of Zn, B and Cu on yield parameters of acid lime in Nawalparasi East, Nepal, 2021

| Treatments                             | Fruit number (no./tree) | Fruit weight (gm) | Yield (kg/tree) |
|----------------------------------------|-------------------------|-------------------|-----------------|
| Control (water spray)                  | 490c                    | 27.69c            | 13.73c          |
| Zn (0.1%)                              | 695.33ab                | 35.43c            | 24.56c          |
| Cu (0.05%)                             | 598.33ac                | 31.77d            | 18.98e          |
| B (0.05%)                              | 691.09ac                | 33.50 cd          | 23.37 cd        |
| Zn (0.1%)+B (0.05%)                    | 739a                    | 41.86b            | 30.80c          |
| Cu (0.05%)+B (0.05%)                   | 656.67ad                | 33.82cd           | 22.20cd         |
| Zn (0.1%)+Cu (0.05%)+B (0.05%)         | 787.33a                 | 45.52a            | 35.70e          |

SEm (±) 25.37 1.32 1.59
F test ** *** ***
LSD (α = 0.05) 124.10 3.29 4.49
CV (%) 10.48 5.19 10.43
Grand mean 665.38 35.65 24.19

Note: *, ** and *** denote significance at p = 0.05, p = 0.01 and p < 0.001 respectively, CV = Coefficient of Variation, SEm = Standard Error of Mean, LSD = Least Significant Difference, Treatment means within a column followed by the different letter(s) were significantly different at p = 0.05 level by Duncan Multiple Range Test.

(0.05%) increased fruit weight and this might be due to its relation to chlorophyll stability and ultimately better photosynthesis (Ilyas et al., 2015). The effect of Cu on yield was due to its association with fruit weight (Table 4). The combination type of treatments resulted in greater yield parameters, which suggest the use of micronutrients in combination to obtain higher yield. The highest values were seen in treatment Zn (0.1%)+Cu (0.05%)+B (0.05%); Table 4). Zoremtluangi et al. (2019) also reported maximum fruits per tree and yield when treated with Zn (0.5%)+B (0.1%)+Cu (0.4%) in mandarin, while the maximum weight of fruit was reported in the case of Zn + B+ Mn. The combined effect of different levels of ZnSO₄, boric acid and CuSO₄ on the number of fruits per plant, fruit weight and yield were observed on sweet orange with maximum observations on treatment ZnSO₄ (0.5%)+ boric acid (0.7%) + CuSO₄ (0.7%); Singh et al., 2018b). Ilyas et al. (2015) also reported the improved photosynthetic attributes, number of fruits, fruit weight and fruit yield with the foliar application of Zn, B and Cu.

Besides yield, micronutrients like Zn, B and Cu are also found to improve the quality parameters of Citrus fruits. Various studies suggest the role of these nutrients in enhancing the juice percentage, total soluble solids, ascorbic acid, reducing sugars and total sugar as well as reducing peel thickness, number of seeds per fruit, non-reducing sugars and titratable acidity with the application of Zn, B and Cu (Singh et al., 2018a). However, these quality parameters are not discussed in this study due to limitations of resources and a major focus on fruit retention.

4. Conclusions
Zn and B supply is imperative to acid lime farming due to their role in increasing fruit set and minimizing June drop and pre-harvest drop, thereby leading to greater yield. Cu also helps in obtaining better yield. Though these nutrients have different roles within a plant, their combined application can enhance the production than that of individual spray. Among the treatments, Zn (0.1%)+Cu (0.05%)+B (0.05%) was the best since it resulted in greater plant canopy spread, fruit set and yield as well as minimized fruit drop significantly. Hence, it is recommended to spray Zn, B and Cu in combination to enhance fruit retention by improving fruit set and reducing June drop and pre-harvest drop of fruit. This in turn will help to obtain greater yield.
Acknowledgements
Mr Bishnu Prasad Sharma (Senior Agriculture Officer) and Mr Ravi Kiran Adhikari (Agriculture Officer) from Prime Minister Agriculture Modernization Project-Project Implementation Unit, Nawalparasi East are gratefully acknowledged for their immense help, inspiration, supervision and suggestion during the entire research period. We extend sincere commemoration to Mrs Yam Kumari Shrestha, Senior Horticulture Development Officer at National Centre for Fruit Development, Kirtipur, for time-to-time guidance and suggestions. We are also deeply indebted to numerous solicitous hands whose contribution has played a pivotal role.

Funding
The authors received no direct funding for this research.

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Disclosure statement
No potential conflict of interest was reported by the author(s).

Citation information
Cite this article as: Effect of foliar application of micronutrients on growth, fruit retention and yield parameters of acid lime (Citrus aurantiifolia Swingle), Saurav Bastakoti, Saurav Nepal, Dipika Sharma & Arjun Kumar Shrestha, Cogent Food & Agriculture (2022), 8: 2112421.

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