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Reducing Severity of Late Blight (*Phytophthora infestans*) and Improving Potato (*Solanum tuberosum* L.) Tuber Yield with Pre-Harvest Application of Calcium Nutrients

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Abstract: The efficiency of pre-harvest application of calcium chloride alone, calcium nitrate alone, and combined application of calcium chloride and calcium nitrate (1:1) was evaluated in reducing the severity of *P. infestans* and improving potato tuber yield. Pot experiment was conducted in randomized complete block design with four replications. The treatments consisted of combination of two potato varieties (Shenkola and Gera) and three types of calcium nutrients (calcium chloride alone, calcium nitrate alone, and calcium chloride mixed with calcium nitrate), each at three levels (5, 10, and 15 g per liter per plant) and the control treatment (0 g of calcium nutrients). In comparison to the control treatment, the application of calcium nutrients significantly decreased the severity of late blight disease and improved potato tuber yield. The effect of calcium nutrients on the severity of late blight disease and potato tuber yield differed among the two potato varieties. The maximum severity reduction (60%) was noticed in the Gera potato variety with the application of calcium chloride mixed with calcium nitrate (1:1), supplied at 15 g per plant. However, the highest average tuber yield was obtained with the application of calcium nitrate at 15 g per plant, and average tuber yield was increased by 77% in both potato varieties. Hence, foliar application of either calcium nitrate alone or calcium nitrate mixed with calcium chloride was found to be more efficient than the application of calcium chloride alone. This result suggests that the nitrate ion present in the calcium nitrate may make a difference in terms of reducing the severity of late blight disease and improving potato tuber yield. The lowered severity of late blight disease and the increased tuber yield in potato plants sprayed with calcium nutrients may be because of the higher accumulation of calcium in the plant tissue.

Keywords: *Phytophthora infestans*; tuber yield; calcium nutrients

1. Introduction

Late blight disease, caused by *Phytophthora infestans*, is a major problem in potatoes worldwide [1]. Similarly, in Ethiopia, the damaging impact of *P. infestans* is increasingly becoming a serious problem in potato production and the disease is threat to food security. In Ethiopia, potato yield loss caused by *P. infestans* ranges between 29–100%, depending on variety used [2].

Different sources of inoculum can be responsible for the outbreak of *P. infestans*. Latently infected seed tubers, infected tubers (cull piles and volunteer potatoes), and closely related weed hosts are a primary source of inoculum. Late blight disease can be airborne [3], soil-borne, and seed-borne [4], and latently infected seed tubers are the most important source of primary inoculums leading to epidemic disease development. In Ethiopia, potato seed tubers are commonly produced through field multiplication of vegetatively produced seed tubers. However, field multiplication of vegetatively propagated seeds tubers leads to high late blight pressure and the production of poor-quality seed tubers [5].

A number of studies have investigated various methods to control late blight disease [6,7] including the genetic basis of resistance in host plants [8] and the application of chemicals. Indeed, the problem of *P. infestans* is further complicated with the breakdown of resistance in promising cultivars [9], and chemical control could negatively impact the soil environment and public health [10], and has resulted fungicide resistance development. Therefore, practically applicable mechanisms and sustainable methods that control late blight disease to an economically safe level are critically important for the future.

Optimizing the nutritional status of a crop with calcium nutrients could be a feasible way to decrease different diseases to an economically safe level [11]. For instance, calcium nutrients suppressing the severity of *Erwinia carotovora* subsp. *atroseptica* of potato [12] and sweet cherries [13], stem rot of soybean [14], brown rot of peach [15], early blight of potato [16], and late blight of potato and tomato [16,17] has been reported. Similarly, Subhani et al. [18] stated that higher calcium content in potato foliage resulted in more resistance of the plant against infection with late blight. Furthermore, the application of calcium can also reduce the input of fungicides [19], decrease potato tuber internal defects, and increase yield, storage life, tuber weight, tuber size and quality [20,21]. So far, in Ethiopia, the application of calcium nutrients in potato production is not recognized and information about calcium application rate is lacking. In potato production, calcium nutrients can be applied in the form of calcium chloride [16] or calcium nitrate [21]. This study was designed to determine the effect of calcium chloride, calcium nitrate, and the combination of calcium chloride mixed with calcium nitrate (1:1) on the severity of late blight disease and potato tuber yield, and to assess the extent to which the application of calcium nutrients reduces the severity of late blight disease and improves potato tuber yield. Hence, the output of this study can be used to amend calcium nutrients in potato nutrition, specifically in Ethiopia, where the use of calcium nutrients is less recognized.

2. Materials and Methods

Pot experiments were conducted at Arsi University, Assela, Ethiopia. The study was carried out from June to October 2016, under natural occurrences of late blight disease.

2.1. Plant Materials

Seed tubers of two potato varieties (Gera and Shenkola) were obtained from Adet Agricultural Research Center, West Gojam, Ethiopia, and used as plant material. Seed tubers were selected for uniformity of tuber size and number of buds per tuber. A single seed tuber (40–45 g) was planted at the center of each pot in the experimental unit.

2.2. Growth Substrate

A mixture of arable soil, sand, and compost (2:1:1 ratio by volume) was used as a growth substrate. The growth substrate was mixed efficiently and then filled into bottom-sealed, 10-liters pots to a total weight of 8 kg per pot. At the beginning of the experiment, the pots were watered with 2 liters of water until saturation. Later on, the water supply was carried out together with Ca nutrients. There was no application of fungicides, and all agronomic practices and cultural managements were kept uniform.

2.3. Experimental Design and Treatments

The study was conducted in a randomized complete block design (RCBD) with four replications. The treatments consisted of a combination of two potato varieties and three types of calcium nutrients: $CaCl_2$ alone, $CaNO_3$ alone, and $CaCl_2$ mixed with $CaNO_3$ (1:1), each at three levels (5, 10, and 15 g per liter per plant) and the control treatment (0 g of Ca nutrients). Totally, 20 treatments and 80 experimental units were included in the study. Calcium nutrients were applied through foliar application starting at one month after planting with 10-days intervals, until the plants were two months old. The control plants were sprayed with tap water alone.

2.4. Data Collection

2.4.1. Severity of Late Blight Disease

Severity of late blight disease was assessed with one week interval, starting at two months after planting until three months old. Disease severity was assessed by estimating leaf area infected and damaged by the disease. For the accurate estimation of the damaged leaf area, the diseased leaf was photographed as soon as the first necrotic damage was observed. Total areas of green color and chlorotic lesion area were measured with an estimation method for disease severity using RGB imagery [22] from Image J, a Java-based image processing tool (https://imagej.nih.gov/ij/). The damaged leaf area from Image J analyses was then used to evaluate the severity of late blight.

Hence, the percentage of infected leaf area for calcium-treated plants and control plants (grown without calcium application) was calculated based on the results obtained from Image J, a Java-based image processing tool. In this regard, late blight disease severity was determined according to Andrivon et al. [23] using a 1–10 scoring scale where: 1 = No lesions, 2 = a few circles, 3 = up to 5%, 4 = 5.1–10%, 5 = 10.1–25%, 6 = 25.1–50%, 7 = 50.1–75%, 8 = 75.1–85%, 9 = 85.1–95% and 10 = 95.1–100% leaf area covered with late blight symptom. The percentage of disease severity reduction was calculated as: $100\ (1 - \times/y)$, where x and y are the disease severity index for pre-harvest calcium-treated and control plants, respectively.

2.4.2. Tuber Yield

Total tuber yield was measured through destructive measurements, at 90 days after sowing. Tubers were harvested by washing the root system under running water. Total harvested yield (g) was measured and followed by the sorting of the diseased and poor-quality tubers. Healthy tubers with no defects were considered as marketable yield (g). Changes in yield due to the application of calcium nutrients were calculated from differences between the treatments.

2.4.3. Plant Height, Number of Branches, and Number of Tubers

At three months after planting, plant height was measured from the main stem. The measurement was carried out using a ruler (cm), starting from the soil surface (basal end of stem) to the top of the plant (shoot apex). The number of branches was counted at the same day of measuring plant height. The number of tubers was counted after harvesting stems and collecting tubers.

2.4.4. Tuber Tissue Analysis for the Determination of Calcium Content

The determination of calcium in the tuber tissue was carried out for each treatment within each replication. Tuber sample was randomly obtained; the sample was then oven-dried at 80 °C for 72 h and grinded into powder form. The powdered sample for each treatment (0.5 g) was dissolved in 20 mL of nitric acid. Deionized water (150 mL) was added to the dissolved sample and the solution was analyzed for the determination of calcium content through atomic absorption on a spectrophotometer using methodology described by Hamdi et al. [21].

2.5. Data Analysis

Statistical evaluations of the results were carried out using Genstat, 15th edition. The effect of calcium nutrients was considered as significant if $p < 0.05$. Significant mean comparison was conducted using the Duncan test ($\alpha = 0.05$). Simple linear regression analysis ($\alpha = 0.05$) was performed to determine the relationship between tuber calcium content and late blight disease severity index, as well as between tuber calcium content and tuber yield.

3. Results

3.1. Severity of Late Blight (P. infestans)

The experiment was conducted at Arsi University, where potato is repeatedly produced each year, and under natural occurrences of the disease. The pre-harvest application of calcium nutrients significantly ($p < 0.05$) affected the severity of late blight disease. In comparison to the control plants, potato plants supplied with calcium nutrients showed reduced severity of late blight both in the Shenkola and Gera potato varieties. In the Shenkola potato variety, the effects of $CaNO_3$ at all applied levels did not differ significantly and the severity of late blight reduction varied between 15–25% (Figure 1A). In the Gera potato variety, the application of $CaNO_3$ showed a higher reduction of severity of late blight at 10 g (50%) and 15 g per plant (45%) (Figure 1B). Except for the Gera potato variety sprayed with 5 g of $CaCl_2$, all levels of $CaCl_2$ also showed significant reduction of late blight severity both in the Shenkola and Gera potato varieties. However, the application of $CaCl_2$ at 15 g per pot showed the highest reduction in the severity of late blight both in the Shenkola (25%) and Gera (40%) potato varieties. Interestingly, the combined application of $CaNO_3$ and $CaCl_2$ at all levels significantly reduced the severity of late blight disease in both potato varieties. In the Shenkola potato variety, the effect of the combined application of $CaNO_3$ and $CaCl_2$ at 10 g and 15 g per plant did not differ significantly, and the severity of late blight disease was reduced by 25%. However, in the Gera variety, the application of $CaNO_3$ mixed with $CaCl_2$ showed the maximum severity reduction percentage (60%) at 15 g per plant.

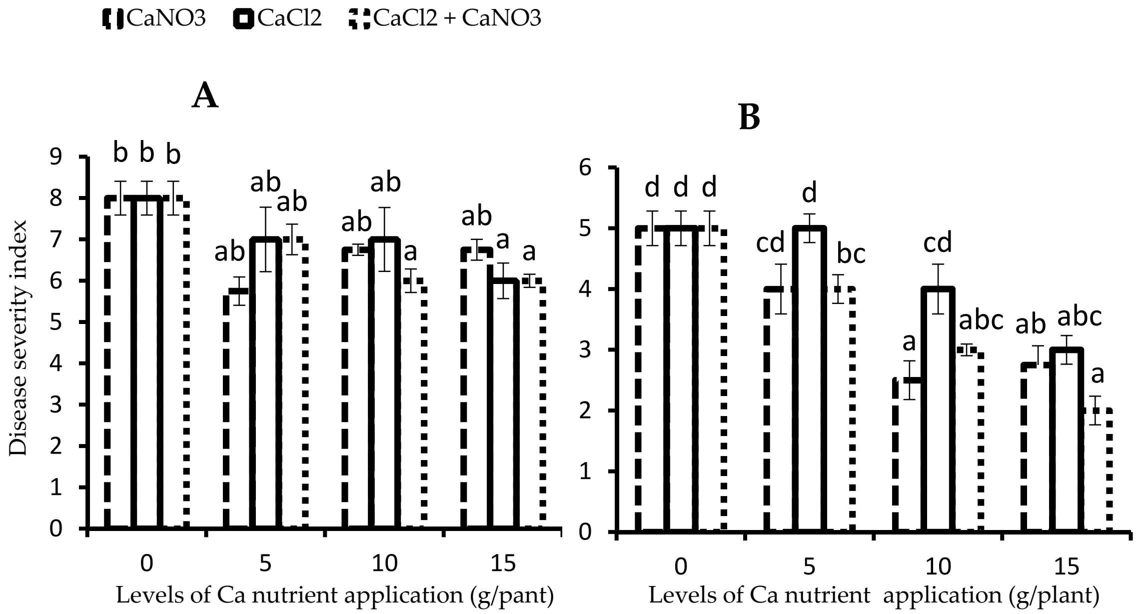

Figure 1. Severity of late blight disease in the Shenkola (**A**) and Gera (**B**) potato varieties supplied with $CaNO_3$ alone, $CaCl_2$ alone, and $CaNO_3$ mixed with $CaCl_2$ (1:1); each at 0 (control), 5, 10, and 15 g per plant. Error bar represents ±SE from four replicates. Different small letters indicate significant difference between treatments ($p < 0.05$).

3.2. Tuber Yield

The interaction between potato varieties and calcium nutrients was found to be significant ($p < 0.05$) for average tuber yield. In comparison to the control treatment, the application of $CaNO_3$ alone and the combined application of $CaCl_2$ and $CaNO_3$ (1:1) at all levels applied significantly increased potato tuber yield both in the Shenkola and Gera potato varieties. However, the highest average tuber yield was obtained with the application of $CaNO_3$ at 15 g per plant, and average tuber yield was increased by 77% in both potato varieties. Similarly, the increased average tuber yield in potato plants supplied with the combination of $CaCl_2$ and $CaNO_3$ ranged from 32–44% and 37–60% in the Shenkola and Gera potato varieties, respectively. The application of $CaCl_2$ slightly improved tuber yield in the Shenkola potato variety alone, and the maximum yield increment (25%) was obtained at 15 g per plant. In contrast, tuber yield was not significantly increased in the Gera potato variety supplied with all levels of $CaCl_2$ alone (Figure 2).

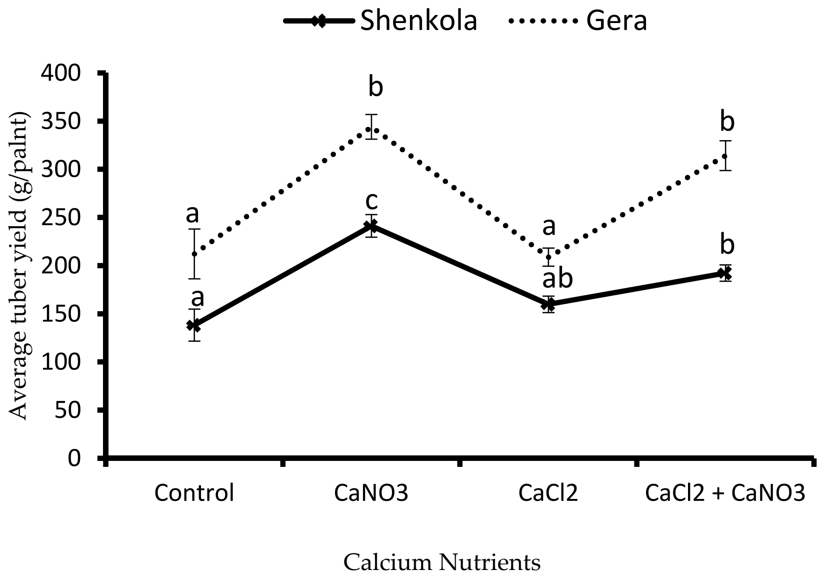

Figure 2. Average tuber yield of two potato varieties (Shenkola and Gera) for potato plants grown without calcium nutrient supply (control) and potato plants supplied with $CaNO_3$ alone, $CaCl_2$ alone, and $CaNO_3$ mixed with $CaCl_2$. Error bar represents ±SE from $n = 4$ for control plants and $n = 12$ for potato plants supplied with calcium nutrients. Different letters indicate significant difference between calcium nutrients ($p < 0.05$).

3.3. Plant Height

A significant interaction between calcium nutrients and potato variety was observed for plant height ($p < 0.05$). In the Shenkola potato variety, a significantly higher plant height was obtained with the combined application of $CaNO_3$ and $CaCl_2$ at 15 g per plant (45%) (Figure 3A). In the Gera potato variety, the higher plant height was obtained with the application of $CaNO_3$ alone at 5 and 10 g per plant. Indeed, in the Shenkola potato variety, the application of $CaCl_2$ at all applied levels slightly increased the plant height. However, in the Gera potato variety, plant height was significantly decreased due to the application of calcium chloride (Figure 3B).

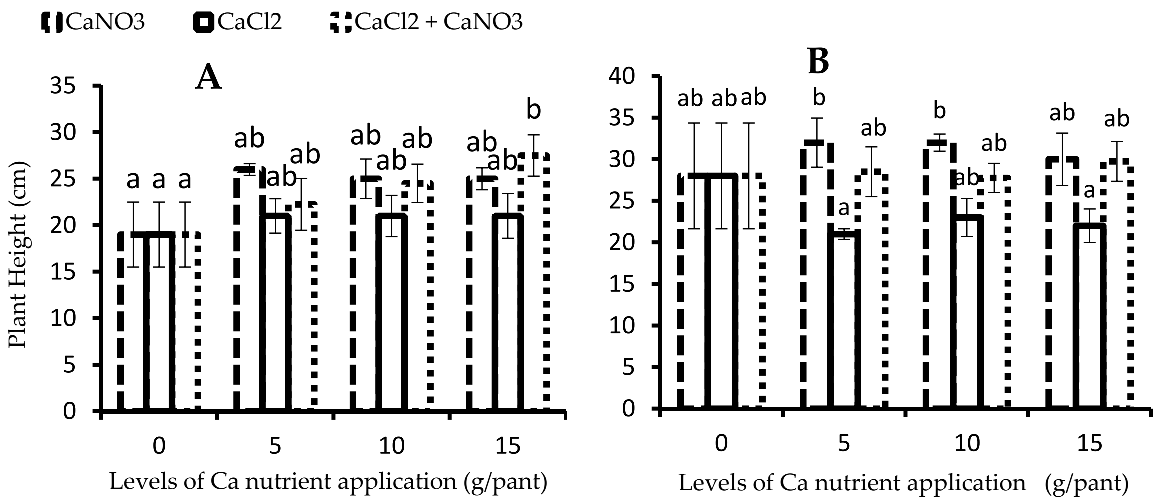

Figure 3. Plant height of the Shenkola (**A**) and Gera (**B**) potato varieties supplied with $CaNO_3$ alone, $CaCl_2$ alone, and $CaNO_3$ mixed with $CaCl_2$, provided at 0 (control), 5, 10, and 15 g per plant. Error bar represents ±SE from four replicates. Different small letters indicate significant difference between treatments ($p < 0.05$).

3.4. Number of Branches and Number of Tubers

Both number of branches and number of tubers were not significantly ($p > 0.05$) affected by the application of calcium nutrients (Table 1). Indeed, the total number of branches can be determined by the variety and total number of buds per seed tuber.

Table 1. Number of tubers and number of branches of two potato varieties (Shenkola and Gera) supplied with $CaNO_3$ alone, $CaCl_2$ alone, and $CaNO_3$ mixed with $CaCl_2$ (1:1), as well as their respective control plants.

Potato Varieties	Ca Nutrient Type (Treatment Label)	Ca Nutrient Levels (g/plant)	Numbers of Tubers	Numbers of Branches
Shenkola	Control	0	16	16
	$CaNO_3$	5 10 15	13 17 17	13 17 17
	$CaCl_2$	5 10 15	14 17 17	14 17 17
	$CaNO_3 + CaCl_2$	5 10 15	15 14 17	15 14 17
Gera	Control	0	15	15
	$CaNO_3$	5 10 15	17 16 13	17 16 13
	$CaCl_2$	5 10 15	15 14 15	15 14 15
	$CaNO_3 + CaCl_2$	5 10 15	16 17 13	16 17 13
p value ($\alpha = 0.05$)			0.984	0.707

3.5. Correlation Study

Linear regression analysis showed that the late blight disease severity index was negatively correlated with tuber calcium content. The reduced severity of late blight disease was significantly ($p < 0.05$) associated with a higher accumulation of tuber calcium content. In the Shenkola potato variety, the negative correlation between disease severity index and tuber calcium content ($R^2 = 0.207$) showed that the severity of late blight disease decreased by 0.5% for the increased trend in calcium tuber content by 1 mg per 100 g of dry weight (Figure 4A). Interestingly, in the Gera potato variety, a strong negative correlation was observed between late blight disease severity index and tuber calcium content ($R^2 = 0.696$), suggesting that for every unit increase in tuber calcium content, the severity of late blight disease decreased by 1.8% (Figure 4B).

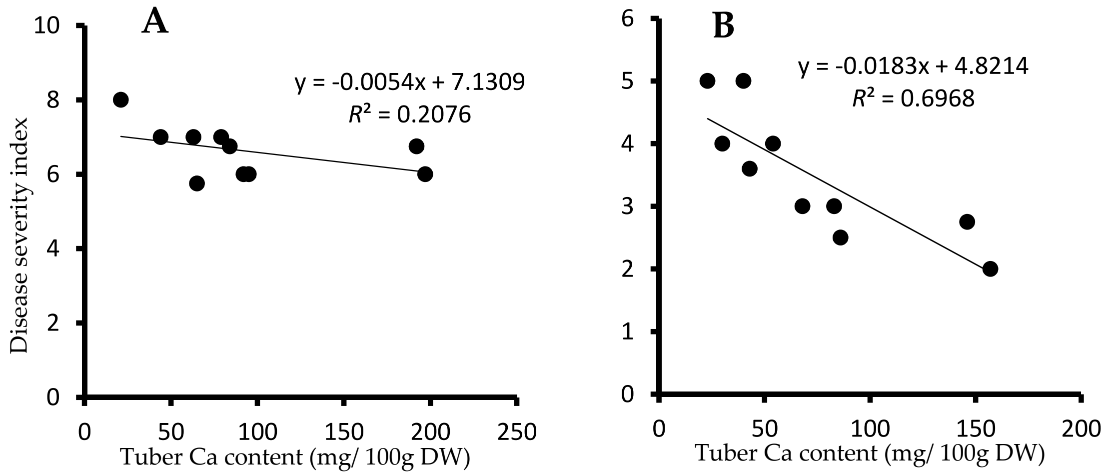

Figure 4. Correlation between late blight disease severity index and tuber calcium content in the Shenkola (**A**) and Gera (**B**) potato varieties.

Tuber yield was positively and significantly correlated with tuber calcium content ($p < 0.05$) in both potato varieties. In the Shenkola potato variety, a reasonable positive correlation ($R^2 = 0.280$) between tuber yield and tuber calcium content indicated that the additional accumulation of tuber calcium content (1 mg/100 g DW) increased average tuber yield by 0.361 g per plant (Figure 5A). In the Gera potato variety ($R^2 = 0.392$), the higher coefficient of regression showed that, for every increased tuber calcium content (1 mg/100 g DW), average tuber yield was increased by 0.931 g per plant (Figure 5B).

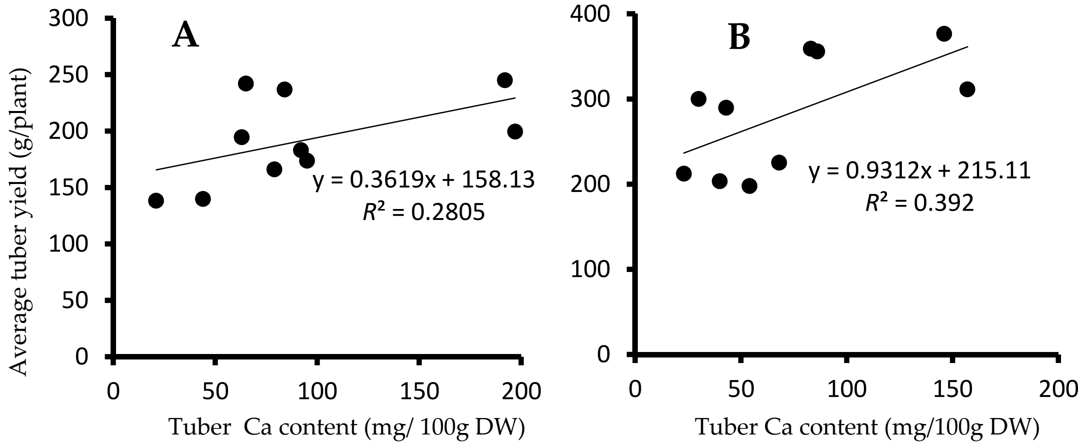

Figure 5. Correlation between average tuber yield per plant and tuber calcium content in the Shenkola (**A**) and Gera (**B**) potato varieties.

4. Discussions

4.1. Severity of Late Blight Disease and Potato Tuber Yield

Optimization of the nutritional status of potato with calcium nutrients has been reported to reduce different diseases to an economically safe level as well as improve tuber yield and quality [16,20,21,24]. In the current study, the efficiency of the pre-harvest application of calcium chloride alone, calcium nitrate alone, and the combined application of calcium chloride and calcium nitrate was evaluated in reducing the severity of *P. infestans* and improving potato tuber yield of Shenkola and Gera potato varieties.

It was noticed that pre-harvest applications of calcium nutrients reduced the severity of late blight both in the Shenkola (Figure 1A) and Gera (Figure 1B) potato varieties. Similarly, the application of calcium chloride decreasing the severity of late blight in potato and tomato plants [16], the application of calcium in the form of phosphite compound (CaPhi) decreasing late blight in potato seed tuber and foliage [17], and the higher leaf calcium content resulting in less infection with late blight disease [18] has been reported. The reduced severity of late blight disease in potato plants supplied with calcium nutrients may be because the calcium application increases tuber calcium content and lowers disease development. This can be explained by the negative correlation between tuber calcium content and disease severity index (Figure 4A,B). The impact of calcium in reducing the severity of late blight disease is generally attributed to the effect of calcium on host cell wall firmness and the activity of defense enzymes. According to Tobias et al., [25], the pre-harvest application of calcium nutrient increases cellular calcium status, enhances cell wall firmness, and prevents microorganisms from entering into the cell by passing the pectin. In addition, it was reported that the application of calcium nutrients increases the activity of defense enzymes, chitinase, and β-1, 3-glucanase. Consequently, the overexpression of chitinase and β-1, 3-glucanase increases plant resistance against infection of different fungal diseases [16,17]. This is due to the fact that chitinase and β-1, 3-glucanase is able to hydrolyze fungal cell wall component chitin and β-1, 3-glucan, respectively.

The current study also proved that the application of calcium nitrate alone or the combined application of calcium nitrate and calcium chloride significantly increased tuber yield both in the Shenkola and Gera potato varieties (Figure 2). This finding is in agreement with Ozgen and Palta [26], suggesting that the application of calcium either from calcium chloride or calcium nitrate during bulking increased tuber weight. Furthermore, the application of calcium nutrients increasing potato tuber marketable yield, storage life, tuber weight, tuber size and quality has been reported [20,21]. The increased tuber yield in potato plants supplied with calcium nutrients may be because the higher calcium accumulation in the tuber tissue enhances tuberization, as a positive correlation was found between tuber yield and tuber calcium content (Figure 5A,B). The higher slope of equation for the correlation between tuber yield and tuber calcium content in the Gera (0.931) and Shenkola (0.361) potato varieties, respectively, showed that the rate of increase in tuber weight is largely determined by the rate of accumulation of calcium in the tuber tissue. Similarly, El-Beltagy et al., [27] suggested that potato tuber yield increases with increasing calcium nutrients to the medium levels. In fact, calcium concentration influences tuber formation through the alteration of biochemical processes, such as changing the hormonal balance at the stolon tip. In contrast to the current result, Hirschi [28] reported that tuber weight/yield is not affected by the application of calcium nutrients, and this disagreement might be because of differences in potato variety response and differences in calcium nutrient application rates and methods.

Generally, in reducing the severity of late blight disease and improving potato tuber yield, the combined application of calcium nitrate with calcium chloride or the application of calcium nitrate alone was found to be more efficient than the application of calcium chloride alone. This result suggests that the nitrate ion present in the calcium nitrate may make a difference in terms of reducing the severity of late blight disease and improving potato tuber yield.

4.2. Plant Height, Number of Branches, and Number of Tubers

Abdur and Ihsan-ul [29] reported that the application of calcium chloride increases plant height in tomato plants. Similarly, the current result proved that potato plants supplied with calcium nutrients showed a higher plant height (Figure 3). The application of calcium nitrate alone or calcium nitrate mixed with calcium chloride was more efficient to increase plant height. This showed that the nitrate ion present in calcium nitrate enhances vegetative growth, as nitrogen plays a role in increasing plant height in potatoes [30].

This study also suggested that the application of calcium nutrients did not significantly increase the number of branches per plant (Table 1). Similarly, Abdur and Ihsan-ul [29] reported that foliar application of calcium chloride did not significantly affect the number of branches in tomato plants. This could be due to the fact that calcium has the impact of apical dominance, which inhibits the growth of lateral branches [31].

Moreover, the number of potato tubers was not significantly affected by the application of calcium nutrients (Table 1). In contrast to this result, Ozgen and Palta [26] reported that the total number of tubers per plant was significantly affected by the application of calcium, and provided evidence suggesting that the application of calcium to the soil can alter tuberization and reduce the number of tubers produced per plant. This disagreement may be due to the variation of the rate of application, time of application, and method of application among the studies. According to Mihovilovich et al., [32], potato tuber initiation occurs starting at 20 to 30 days after emergence, depending on the variety. In the current study, calcium nutrients were applied starting at one month after planting, with 10-days intervals until plants reached two months of age, which might be after tuberization had started. The reason for the non-significant effect of calcium nutrients on the number of tubers might be because the application of calcium during tuberization may not be useful to improve the number of tubers. Furthermore, the exact biochemical and molecular mechanism of calcium regulating potato tuberization is still unclear.

5. Conclusions

The pre-harvest application of either calcium nitrate alone or the combined application of calcium nitrate together with calcium chloride reduces the severity of late blight disease and improves tuber yield. Hence, foliar application of either calcium nitrate alone or calcium nitrate mixed with calcium chloride was found to be more efficient than the application of calcium chloride alone. This result suggests that the nitrate ion present in calcium nitrate may make a difference in terms of reducing the severity of late blight disease and improving potato tuber yield. Hence, the application of calcium nitrate or calcium chloride mixed with calcium nitrate (at 15 g per plant) as a source of calcium nutrient can be used as an alternative method for the management of late blight disease as well as to improve potato tuber yield. The lower severity of late blight disease and the increased yield in potato plants sprayed with calcium nutrients may be because of the higher accumulation of calcium in the plant tissue.

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Conflicts of Interest: The authors declare no conflicts of interest.

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