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Effect of Nano Calcite Foliar Fertilizer on the Growth and Yield of Rice (*Oryza sativa*)

K.H.C.H. Kumara[1], D.L. Wathugala[2*], R.F. Hafeel[3] and H.K.M.S. Kumarasinghe[2]

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ABSTRACT

Purpose: *Around the world nano calcite (NC) is used as an environmental friendly foliar fertilizer to enhance yield of many crops. In Sri Lankan context this fertilizer has not yet been tested for crops. Therefore, this study examined the effect of foliar application of NC on yield, yield components, seed quality and insect resistivity of rice and the interaction effect of NC foliar fertilizer with different rates of soil added fertilizer.*

Research Method: *Field experiments were conducted in 2015/2016 Maha and 2016 Yala seasons at the Regional Rice Research Institute, Ambalanthota, Sri Lanka. The rice variety used for the study was At 362. Four levels of nano calcite (0, 50, 100 and 150ppm) with 3 levels of soil added fertilizers (recommended fertilizer mixture, 25% over and above the recommendation) were tested. Data was collected at 30, 60 and 90 days after transplanting and at the harvesting.*

Findings: *It was found that the application of nano calcite foliar fertilizer has positive effects on growth, yield, seed quality and insect resistivity. Hundred ppm nano calcite treatment with recommended soil added fertilizer could increase the final yield approximately about 1 ton/ha. Twenty five percent reduction or increment of soil added fertilizer than the recommended level with the application of nano calcite foliar fertilizer did not showed any significant yield variation. However, higher concentrations of nano calcite greatly reduce the pest damages to the crop.*

Originality/ value: *Results of the experiment revealed that nano calcite foliar application can increase rice yield to a certain extent. However, further experiments need to be done to ensure the sustainability of the application.*

Keywords: *calcium carbonate, nano fertilizer, pest tolerance, yield increment, rice*

INTRODUCTION

Along with magnesium and sulfur, calcium is one of the three secondary nutrients essential for crop growth and development. These secondary nutrients are essential for healthy growth of the plant but in rather small quantities. The primary roles of the calcium within the plants are nutrient transportation into and within the plant, important constituent of cell walls, facilitating metabolic activities by neutralizing cell acids, increasing plant resistance to outside attacks and allowing more erect stems (White and Broadley, 2003). Plant roots absorb calcium from the soil solution and from the exchange complex in the form of Ca^{2+} ions. Although calcium is found in many natural minerals like calcite in soil, calcium disorders are often observed in crop plants. This happens due to various reasons. On one hand calcium in soil minerals is found in relatively insoluble forms such as $CaCO_3$ and on the other hand a low pH of the soil substrate

[1] *Faculty of Graduate Studies, University of Ruhuna, Sri Lanka*
[2*] *Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka*
[3] *Rice Research Station, Ambalantota, Sri Lanka*
wgdlakmini@crop.rh.ac.lk
https://orcid.org/0000-0003-1276-8060

affects the high concentration of cations such as Mg^{+2}, NH^{4+}, Fe^{+2} and Al^{+3} decreasing the calcium uptake by plants due to antagonistic effects. Besides, organic acids form chelate complexes with Ca^{2+} ions which could be insoluble as well (Läuchli and Grattan, 2012).

In modern days, foliar fertilization is increasingly adopted to alleviate nutrient deficiencies. Beside, particular interest has also been gained on nano fertilizers in plant nourishment, since they are environmentally safer and benign. Nano materials have high specific surface area due to its nanometric scale, which increases the availability to the plant and grater dissolution in water (Remya *et al*., 2010).

Several nano fertilizers produced from calcite are available in the market. Bio fertilizer HerbaGreen (Acting Bud Company, Serbia) produced through nanotechnology (tribo mechanical activation) is one such calcite fertilizer. Natural calcite consists several ingredients such as $CaCO_3$, MgO, K_2O and Na_2O in various constitutions. This technology does not interfere with the initial composition of the mineral. These nano particles enter into the plant cells through stomata opening because the granularity of the nanocalcite is far smaller than that of the stomata (Kara and Sabir, 2010). Inside the leaf this particle breaks down into CO_2 and CaO which are immediately available for plant (Chen *et al*., 2004). It is a basic physiological phenomenon that higher CO_2 concentration inside the plant cells reduces water need of the plant by limited evaporation because the atmosphere inside the plant is saturated with CO_2 causing the stomata to close. On the other hand, rubisco can easily bind with CO_2 and induces photosynthetic activity of the plant (Bowes, 1991). Meanwhile higher CO_2 concentrations inside the plant reduce photorespiration which is competitive process with photosynthesis, which consumes a lot of energy and water and reducing the photosynthesis by 20-30%. Also $Ca(OH)_2$ can be produced as a result of CaO combining with water then which can be caused for proper pH balancing of the cytoplasm enhancing metabolic activities (Dumancic, 2010).

The positive effects of nano calcite was observed by several authors for crops like lettuce (Ugrinović *et al*., 2011), maize and fodder sorghum (Raguposan *et al*., 2014), potatoes (Trawczyński, 2013), sugar beet (Artyszak *et al*., 2014, 2016), wheat and corn (Prifti and Maci, 2017), grape vine (Kara and Sabir, 2010), peanut (Liu *et al*., 2005) tomato and tankan (*Citrus tankan* Hayata) (Hua *et al*., 2015). It has been reported that the spraying nano calcite has increased the total crop biomass, crop productivity, crop growth, vigor, crop product quality and shortens vegetative period, improves resistance against pest and diseases and abiotic stresses, longers storage life, organoleptic qualities and colouration in those crops.

Therefore, around the world nano calcite is being used intensively as an environmentally friendly alternative for some synthetic fertilizers. But in Sri Lankan context, it has not yet been experimentally analyzed the effect of foliar application of nano calcite on yield, yield component and quality of rice (*Oriza sataiva*). An experiment conducted by Dimitrovski *et al.,* (2006) at Republic of Masadonia, to evaluate the effect of foliar application of nano calcite on some morphological and productive properties of rice variety *San Andrea* observed yield increment, but it was not significantly differ from control treatment. However these results might be changed under totally different environmental, soil and agronomic conditions. Therefore, the main objective of this research was to investigate the growth, yield and quality responses of rice to the foliar application of different concentrations of nano calcite. In addition the effectiveness of nano calcite for the protection from insect pests was also investigated.

MATERIALS AND METHODS

The experiment was conducted in 2015/2016 *Maha* (September to March) and 2016 *Yala*

(May to August) seasons at the Regional Rice Research Station, Ambalanthota (6^{0}130'N and 81^{0}. 032'S) which is situated in the low country dry zone of Sri Lanka. *Oryza sativa* variety At 362 and HerbaGreen product of nano calcite (Acting Bud, Serbia) was used for the experiment. According to the specifications of the company, HerbaGreen is a 100% natural product which consists $CaCO_3$, SiO_2, $MgCO_3$, Fe_2O_3, K_2O, Na_2O, S, P_2O_5 and MnO, 65.5, 17, 3.6, 3.4, 0.7, 0.5, 0.4, 0.2 and 0.1% respectively.

Fourteen days old rice seedlings were used for the field establishment. Two seedlings were established per hole with 15×15 cm spacing in field plots. The plot size was $18m^2$. Four concentrations of nano calcite (0, 50, 100,150ppm) was sprayed 20, 40 and 60 days after transplanting as a foliar fertilizer using a hand-pump pressure spray systems. All concentrations were prepared using tap water and water alone was used as the control. Two liters of each concentration was applied per plot.

Soil fertilizer was added to the plots according to the recommendations of Department of Agriculture, Sri Lanka. Triple Super Phosphate (55kg ha^{-1}) was applied as the basal dressing and 50, 75, 65 and 35kg ha^{-1} of urea was added at 2,4,6 and 7 weeks after transplanting, respectively. Muriate of Potash was added in the 4^{th} and 6^{th} weeks after planting with the rate of 25 and 35kg/ha respectively. Apart from that, two other soil added fertilizer levels (25% below the recommendation and 25% above the recommendation) were also tested to get an idea whether low level of soil fertilizer with nano calcium foliar fertilizer can give higher or similar yield compared to the recommended level of soil added fertilizer. Higher level of soil added fertilizer than the recommended level was also included to understand the effect of nano calcite foliar fertilizer with higher dose of soil added fertilizer on rice plant growth and yield.

All other management practices were identical for all treatments. The experiments followed the split plot design with 3 replications. Rice plant growth (plant height, number of leaves and tillers per plant, time taken for 50% flowering, leaf area, flag leaf area, shoot and root dry weight and chlorophyll content) and yield parameters (number of panicles per plant, number of filled grains per panicle and final yield) insect resistivity (number of insect damaged plants and number of insects per unit area) and grain quality parameters (1000 grain weight and grain length and width) were taken at 30, 60 and 90 days after transplanting and at the harvesting. Randomly selected three plants from each replicate were used for data collection. Flying insects were collected by using a one feet quadrate net. It was randomly thrown and the area was covered by the net. Insect attacks were also considered to identify insect resistance. In order to analyze significant differences, one factor analysis of variance with the level of significance α=0.05 was carried out. Statistical analysis was performed using STAR package developed by International Rice Research Institute for agricultural research (based on r programme) using LSD procedure.

RESULTS AND DISCUSSION

According to the results observed, plant height (Table 01), number of tillers per plant (Table 02), final yield (Figure 01), panicle length (Figure 02) and leaf area showed interaction effects of foliar and soil added fertilizer treatments. However, other growth and yield parameters measured (Table 03, Figure 03 & 04) showed only effect on application of different levels of nano calcite.

According to the Tables 01& 02, significant variations in plant height and number of tillers could be observed with the increase of nano calcite concentration under all soil added fertilizer levels. Nano calcite level 100ppm gave the highest plant height and number of tillers per plant, but most of the time the values were not significantly different with 150ppm concentration. It is interesting to mention that all the time, both plant height and number of tillers per plant has increased with the increase of nano calcite concentration up to 100ppm in all soil added fertilizer levels. Further increase has shown irregular trends. However, an experiment conducted by Dimitrovski *et al.*, (2006) to evaluate the effect of foliar application of Herbagreen on some morphological and productive properties of rice variety *San Andrea* did not show any significant difference for plant height and number of tillers per plant.

Table 01: Interaction effect of nano calcite foliar fertilizer levels and soil added fertilizer levels for plant height (cm)

NC Levels (ppm)	30 days DAT			60 days DAT			90 days DAT		
	-25%	R	+25%	-25%	R	+25%	-25%	R	+25%
2015/2016 *Maha* Season									
0	67.96^{c}	41.45^{c}	61.91^{d}	88.75^{c}	91.68^{c}	88.78^{c}	95.45^{b}	86.56^{d}	92.76^{c}
50	71.55^{b}	69.45^{b}	67.34^{c}	93.34^{b}	90.66^{c}	93.34^{b}	97.56^{b}	95.61^{b}	96.95^{b}
100	74.56^{a}	73.32^{a}	69.93^{b}	94.37^{b}	95.90^{a}	94.70^{a}	103.34^{a}	98.17^{a}	99.55^{a}
150	74.93^{a}	72.33^{a}	74.73^{a}	99.05^{a}	93.28^{b}	94.65^{a}	100.56^{a}	89.98^{c}	102.34^{a}
2016 *Yala* Season									
0	65.06^{c}	61.56^{d}	63.33^{c}	90.03^{c}	91.46^{bc}	88.94^{c}	93.23^{c}	84.22^{c}	92.33^{b}
50	72.15^{b}	67.35^{c}	71.09^{b}	94.15^{b}	90.16^{c}	93.34^{b}	96.75^{b}	86.38^{b}	91.34^{bc}
100	75.12^{a}	76.67^{a}	73.12^{a}	98.30^{a}	96.42^{a}	97.58^{a}	101.23^{a}	90.06^{a}	95.88^{a}
150	71.99^{b}	72.54^{b}	71.34^{b}	97.68^{a}	92.28^{b}	98.23^{a}	100.09^{a}	89.53^{a}	89.98^{c}

Soil added fertilizer levels (column groups).

Note:Mean values with the same letter are not significantly different within 30, 60 and 90days after transplanting (DAT), α =0.05, Nano Calcite(NC), soil added fertilizer level 25% below the recommended level(-25%), recommended fertilizer level (R), soil added fertilizer level 25% over the recommended level(+25%).

Table 02: Interaction effect of nano calcite foliar fertilizer levels and soil fertilizer levels for number of tillers per plant

NC Levels (ppm)	30 days DAT			60 days DAT			90 days DAT		
	-25%	R	+25%	-25%	R	+25%	-25%	R	+25%
2015/2016 *Maha* Season									
0	5.67^{b}	7.74^{c}	5.62^{b}	6.66^{c}	9.00^{ab}	9.00^{b}	6.55^{c}	5.49^{c}	6.78^{b}
50	5.44^{b}	8.38^{b}	5.89^{b}	9.00^{b}	8.66^{bc}	10.0^{ab}	8.76^{b}	6.22^{bc}	10.11^{a}
100	7.33^{a}	8.73^{a}	7.22^{a}	7.33^{a}	8.73^{a}	7.22^{a}	7.33^{a}	8.73^{a}	7.22^{a}
150	6.77^{a}	8.33^{b}	6.76^{a}	6.66^{c}	9.00^{ab}	9.00^{b}	7.77^{bc}	8.00^{a}	6.78^{b}
2016 *Yala* Season									
0	5.53^{b}	7.50^{c}	5.17^{c}	7.66^{d}	10.0^{b}	8.66^{cd}	6.37^{c}	5.44^{c}	7.45^{c}
50	6.45^{a}	8.13^{b}	6.03^{b}	10.33^{c}	9.33^{b}	12.00^{b}	8.43^{a}	5.19^{c}	11.12^{b}
100	6.78^{a}	8.59^{a}	6.23^{b}	14.67^{a}	13.00^{a}	13.66^{a}	6.91^{b}	7.29^{b}	13.23^{a}
150	6.55^{a}	7.81b^{c}	7.32^{a}	12.66^{b}	13.00^{a}	9.33^{c}	7.55^{b}	8.5^{a}	11.02^{b}

Soil added fertilizer levels (column groups).

Note: Mean values with the same letter are not significantly different within 30, 60 and 90 days after transplanting (DAT), α =0.05, Nano Calcite(NC), soil added fertilizer level 25% below the recommended level (-25%), recommended fertilizer level (R), soil added fertilizer level 25% over the recommended level(+25%).

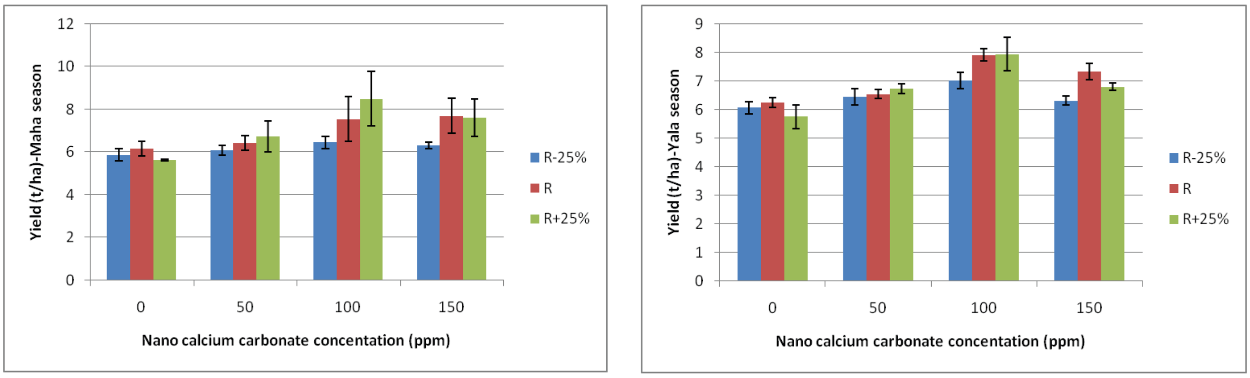

Figure 01: **Variation of grain yield in *Yala* and *Maha* seasons treated with different levels of nano calcite foliar and soil added fertilizers.**

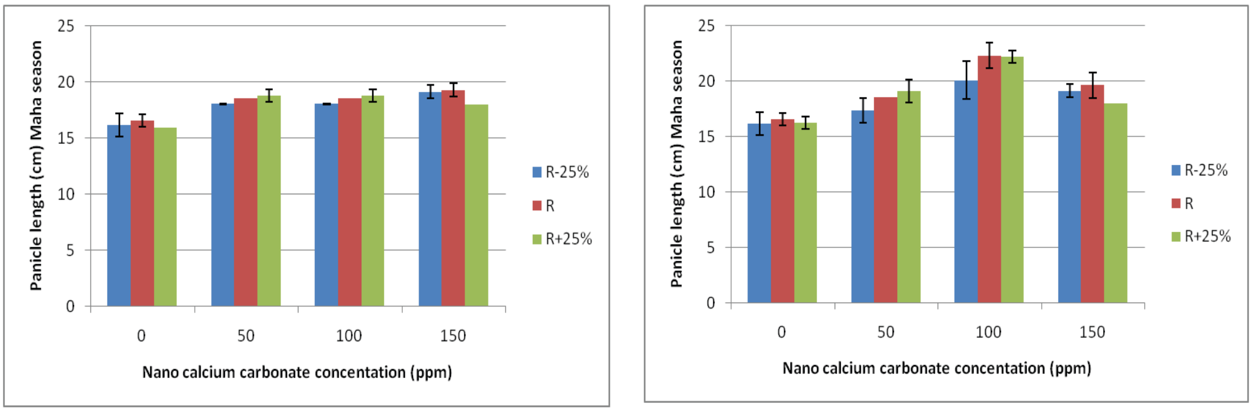

Figure 02: **Variation of panicle length in *Yala* and *Maha* seasons treated with different levels of nano calcite foliar and soil added fertilizers.**

As can be seen in Figure 01 and 02, statistically different highest yields and panicle lengths were determined in 100ppm and 150ppm nano calcite treatments with recommended and 25% over the recommended soil fertilizer levels. These data imply that rice yield can be increased with the application of 100ppm nano calcite foliar fertilizer with recommended level of soil fertilizer. According to the product specifications calcite foliar fertilizer can be immediately taken up by the plant, and decompose into CaO and CO_2. Hence, available CO_2 for photosynthesis increase significantly (Chithrani *et al*., 2015). Increased chlorophyll content (Figure 03) could also have facilitated the higher photosynthetic rate. Therefore, it can be assumed that the application of foliar nano-calcite positively influences the final yield. The study conducted by Dimitrovski *et al.,* (2006) have also observed higher yield in Herbagreen treated plants but the increment did not significantly differ with other treatments. Nonetheless, a number of studies have shown positive effect of these fertilizers on the yield of various crops. Artyszak *et al.,* (2014) observed higher root, leaves, biological and technological yield of sugar beet (var. Danuśka KWS) with the application of marine calcite (HerbaGreen basis). Chithrani *et al.,* (2015) and Raguposan *et al.,* (2014) received higher amount of fodder yield with the application of nano calcite for sorghum.

Effect of the treatments on grain length and width were slightly different among treatments (Table 03). All nano calcite treatments led to increment of grain quality parameters measured in the study. Nonetheless, the highest values of grain quality parameters were obtained from 150ppm nano calcite treatment in *Maha* season and 100ppm and 150ppm treatments in *Yala* season. Although it is not comparable, Sabir *et al.,* (2014) observed the enhanced berry length of grape with the application of nano calcite fertilizers. Similarly, a study conducted for 'Thompson seed-less' grapes, with foliar and/or soil $CaCl_2$ treatments also resulted in significantly large size berries than control (Bonomelli and Ruiz, 2010). Sawan *et al.,* (1997) also reported that the foliar application of calcium on Egyptian cotton can increase seed and fiber yield of cotton and also improvement of fiber properties. None of a literature reported about the grain quality characters of rice with the application of nano calcite foliar fertilizer.

In this experiment leaf chlorophyll content only gave a significant difference for nano calcite treatments (Figure 03). Always highest chlorophyll contents were given by 100ppm nano foliar fertilizer treatment (Figure 03). Several other authors have also reported positive results in chlorophyll contents for number of dicots and monocots. As an example, Chithrani *et al.,* (2015) observed significant differences for total chlorophyll content of sorghum (*Sorghum bicolor* L. Moench) compared to the control. Further Sabir *et al.,* (2014) reported significant increase of leaf chlorophyll content with nano calcite treatments of "Narince" grape cultivated on alkali soil.

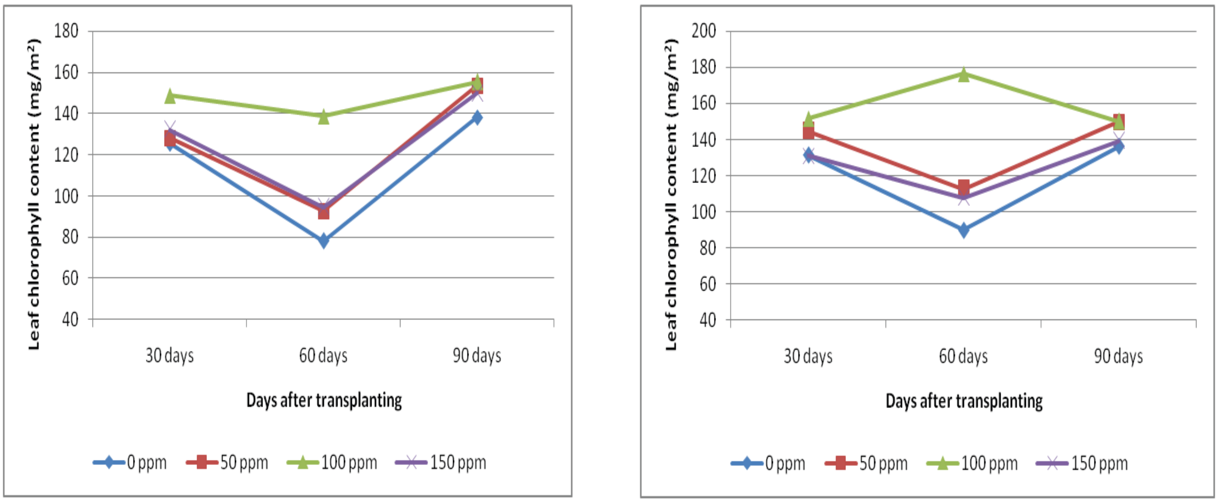

Figure 03: Variation of leaf chlorophyll content in *Yala* and *Maha* seasons treated with different levels of nano calcite fertilizer. Each data point represents the mean of data points obtained from nine replicates. Chlorophyll content was measured using standard colour chart. Each colour has a number and assigned SPAD (Single Photon Valance Diods) chlorophyll meter value.

Table 03: Effect of different concentrations of nano calcite on some growth and yield traits of rice collected at harvesting.

	Nano calcite concentration (ppm)			
	0	50	100	150
Maha Season (2015/2016)				
No. of panicles /plant	5.33^{b}	6.39^{a}	9.17^{a}	5.33^{a}
No. of Filled grains /panicle	120.00^{c}	126.00^{b}	137.00^{a}	120.00^{a}
1000grain weight (g)	24.76^{c}	25.47^{b}	26.32^{a}	24.76^{ab}
Grain length without husk (mm)	5.83^{c}	6.13^{b}	6.12^{b}	5.83^{a}
Grain width without husk (mm)	1.59^{c}	1.69^{b}	1.69^{b}	1.59^{a}
Yala Season (2016)				
No. of panicles /plant	6.11^{c}	7.22^{b}	8.72^{a}	7.61^{b}
No. of Filled grains /panicle	127.0^{b}	128.00^{b}	132.00^{a}	131.00^{a}
1000grain weight (g)	25.85^{c}	26.29^{b}	26.42^{a}	26.34^{b}
Grain length without husk (mm)	6.05^{c}	6.14^{b}	6.19^{a}	6.19^{a}
Grain width without husk (mm)	1.61^{c}	1.64^{b}	1.68^{a}	1.66^{ab}

Different letters in each column indicate significant difference at $p\leq0.05$

Figure 04 illustrates the time taken for 50% flowering was slightly triggered by nano calcite. As an example 3-5 days early flowering occurred than those of untreated plants but the effects are not statistically significant. Although it is not comparable, early flowering was reported by Sabir *et al.,* (2014) with effect of nano calcite application for graphs.

Insect damages such as leaf mite, yellow stem borer, gold midge and leaf folder damage were drastically reduced with the increase of nano calcite concentrations. Significantly lowest pest attacks were observed in 150ppm level (Table 04). Several studies have been proved that Ca and Si can strengthen plant against pest and diseases (Sacała, 2009). Ca and Si are the main minerals consist in Herbagreen. Hua *et al.,* (2015) have also reported that the application of nano calcium carbonate can increase plant protection against insect pests. They have also mentioned that nano calcium carbonate is a good alternative for chemical pesticides as it is a safe, human and environmentally friendly material.

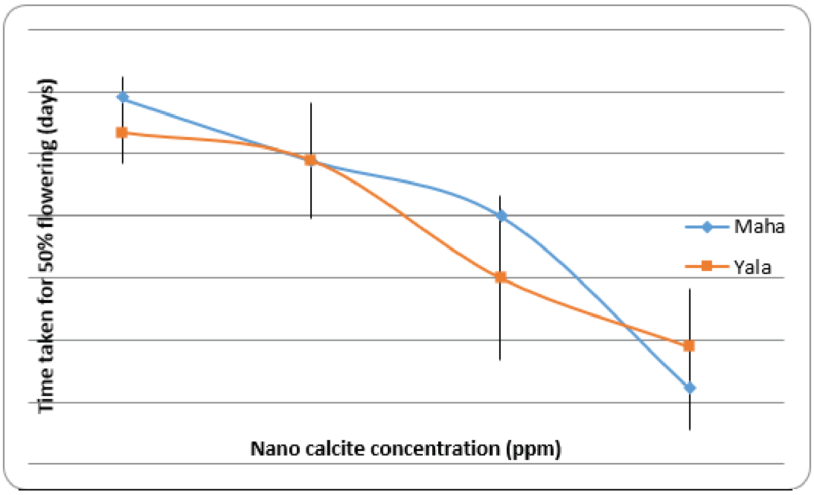

Figure 04: Variation of time taken for 50% flowering in *Yala* and *Maha* seasons treated with different levels of nano calcite fertilizer. Each data point represents the mean of data points obtained from nine replicates.

Table 04: Effect of different concentrations of nano calcite on presence of insect pests

	Nano calcite concentration (ppm)			
	0	50	100	150
Maha Season (2015/2016)				
Leaf folder damage/m^2	21.35^a	21.41^b	21.45^b	1.78^c
Gall midge count//m^2	42.12^a	22.91^b	21.47^b	5.39^c
Leaf mite count/cm^2	12.22^a	10.22^b	8.67^b	6.89^c
Yellow stem borer damage/ m^2	53.96^a	42.78^b	22.00^c	15.11^d
Yala Season (2016)				
Leaf folder damage/m^2	21.30^a	20.45^b	21.34^b	16.32^c
Gall midge count//m^2	22.23^a	22.63^a	21.54^a	16.35^b
Leaf mite count/cm^2	9.67^a	9.42^a	6.73^b	5.20^c
Yellow stem borer damage/ m^2	42.84^a	23.07^b	22.51^b	19.48^c

Different letters in each column indicate significant difference at $p \leq 0.05$

Natural calcite can be applied to the soil as a soil fertilizer. However, many studied indicated that the availability of many nutrients is limited due to high carbonate content in the soil (Sabir *et al*., 2010). Therefore, application of calcite as a foliar fertilizer will be more beneficial. However, concentrations and amounts of application of Ca fertilizer should be optimized to prevent some disadvantages. Jakobsen (1993) explained that, excessive application of K fertilizer decreases Ca and Mg uptake by plants and vice versa, indicating that the application of more calcium may cause K deficiency. Spraying fertilizers on leaves than direct application to the soil is a popular method as it does not depend much on various soil and plant characters.

In the present study, nano calcite foliar fertilizer consisted several other micro nutrients except Ca such as Fe, Si, Mg and Mn. These micro nutrients also play essential roles in plant physiology. For example, Fe is a co-factor for approximately 140 enzymes that catalyze unique biochemical reactions. Therefore, Fe fills many essential roles in plant growth and development, including chlorophyll synthesis, thylakoid synthesis and chloroplast development (Miller *et al*., 1995).

Silicon fertilizer application is also a widely used practiced in the world to increase crop production. The favourable effects of Si on many plant species growth and development was reported in many studies. The main role that Si plays is reduction of impacts on plants from abiotic and biotic stresses such as drought, disease, and insect stresses (Sacała, 2009). Tripathi *et al.,* (2012) reported that, silicon fertilization of the rice resulted in the increase of the fresh weight of the seedlings' aboveground parts and root length compared to control (without silicon fertilization). The authors also noticed the higher shoot and root dry matter of rice plants. Crusciol *et al.,* (2009) observed in an experiment for potato that silicon fertilization affected in the significant increase in dry weight of potato tubers per plant and the reduction of stems lodging. Therefore, not only harmonizing of photosynthetic process by CaO, other micro nutrients may also have contributed to the positive effects on rice growth, yield, seed quality and insect resistivity achieved in the present study.

CONCLUSIONS

Results of this study revealed some positive effects of use of environmentally safe nano-caicite foliar fertilizer for rice var. At 362. Fifty percent flowering dates of treated plants were few days earlier than those of control and significantly higher yield were observed in treated plants compare to untreated control. Among the different concentrations, the maximum performances were found at 100ppm nano calcite concentration with recommended level of soil added fertilizer. Therefore, it can be concluded that the application of nano calcite foliar fertilizer for rice would be benefited to enhance yield, grain quality and insect resistance. However, further studies are required under different environmental and soil conditions with different varieties and concentrations of nano calcite for the sustainability of yield increment. The ecological effects of the long term application of nano calcites to the paddy fields such as effect on soil microbial populations need to be investigated. Comparison of effect of nano calcite foliar fertilizer with the foliar application of NPK+ micro nutrient will also be important to acquire a comprehensible image of foliar fertilization of rice.

Data statement policy

The data sets generating during and/or analyzed during the current study are available in the Master of Crop Production Technology dissertation produced by Mr. KHCH Kumara and are available from the corresponding author on reasonable request.

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