FOLIAR SUPPLEMENTATION OF COPPER BASED ZINC NANO FERTILIZER ON GROWTH AND FLOWERING OF LISIANTHUS

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In the name of Allah, The Most Gracious and The Most Merciful.
Most [All] praise is [due] to Allah, Lord of the worlds.
The Entirely Merciful, the Especially Merciful,
(Surah Fatiha 1:1-3)

DEDICATED TO
MY BELOVED PARENTS
All that I am, or hope to be, I owe to them

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CERTIFICATE

This is to certify that the thesis entitled ***"FOLIAR SUPPLEMENTATION OF COPPER BASED ZINC NANO FERTILIZER ON GROWTH AND FLOWERING OF LISIANTHUS"*** *submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE in HORTICULTURE****, embodies the result of a piece of authentic research work carried out by* ***RAISA ISLAM,*** *Registration No.* ***14-05854*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma to any other institute.*

I further certify that any help or source of information, received during the course of this investigation, has been duly acknowledged.

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- The authoress

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ABSTRACT

A field experiment was conducted in the Horticulture farm of Sher-e-Bangla Agricultural University, Dhaka during the period from October 2019 to April 2020 to evaluate the effect of foliar application of supplement copper based zinc nano fertilizer on three lisianthus varieties. Lisianthus varieties viz. V_1 (Purple Picotee), V_2 (Light Pink), V_3 (Super Magic Type Blue); and copper based zinc nano fertilizer application with different concentration in: N_0 (Control; No nano fertilizer application), N_1 (1ml/L), and N_2 (2ml/L) were used in this experiment arranged in Randomized Complete Block Design with three replications. Data on growth and flower yield attributes parameters were collected in which all the treatment showed significant variations. Among varieties, number of branches (4.9), stem length (55.3 cm), no. flower buds/stem (13.6), no. of flowers/plant (37.6), vase life (17.7 days) were observed in V_1 (Purple Picotee) which is an early variety among the three varieties whereas minimum in V_2 (Light Pink) is a late variety. Again, 2ml/L copper-based zinc nano fertilizer application influenced growth characteristics of plant, where tallest plant height (78.8 cm), maximum number of leaves (104.3), SPAD value (56.7), stem diameter (5.4 mm), stem length (56.8cm) were recorded. Furthermore, maximum flower yield per hectare (9.1million) were found in V_1N_2 and minimum (2.1 million) in V_2N_0. In view of overall performances, Purple Picotee lisianthus with the application of 2ml/L of copper based zinc nano fertilizer would be the potential for flower yield as well as quality.

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ABBREVIATIONS AND ACCRONYMS

AEZ = Agro-ecological Zone

Agric. = Agricultural

ANOVA = Analysis of Variance

BARI = Bangladesh Agricultural Research Institute

Biol. = Biology

CV = Coefficient Variance

DAT = Days after Transplanting

EPB = Export Promotion Bureau

et al. = And others

GDP = Gross Domestic Product

Hort. = Horticulture

J. = Journal

LSD = Least Significance Difference

mm = Millimeter

RCBD = Randomized Complete Blocked Design

Res. = Research

SAU = Sher-e-Bangla Agricultural University

Sci. = Science

SRDI = Soil Resource Development Institute

Technol. = Technology

UPOV = Union of Protection of Plant Varieties

Viz. = Namely

CHAPTER I

INTRODUCTION

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Introduction

Lisianthus (*Eustoma grandiflorum*, Bengali name: Nandini) is one of the top ten lucrative and expensive cut flowers in the world for its divergent color and an extended vase life. The flower belongs to the family Gentianaceae, is native to the prairie states of North America particularly the eastern slope of Rocky Mountains, USA where it is called Prairie gentian. Lisianthus comes from the Greek word "*lissos*", meaning smooth, and "*anthos*", which means flower.

It is a moderately cold-tolerant annual or biennial herbaceous ornamental species used as cut flower and pot flower due to its big and attractive rose like flowers with variable size and shape, along with long stalks and long duration in vases (up to 6 weeks) (Roh and Lawson, 1988; Uddin *et al.*, 2004; Shimizu and Ichimura, 2005; Yamada *et al.*, 2008; Mousavi *et al.*, 2012)

Although introduced into Europe from the USA in 1835 as a garden plant, it was not until the 1980's when it was introduced as a cut flower worldwide (Halevy and Kofranek, 1984). This species has been extensively hybridized over the last few decades to produce a host of series, each with its own color variants and stand-alone cultivars. Scientists have developed modern cultivars offer a wide range of color including purple, rose, pink, blue, yellow, light green, white and a variety of bicolor with variations in patterning single, semi-doubles or doubles and a range of seasonality.

It is a slow growing plant, requires almost 5 to 6 months from sowing to flowering (Uddin *et al.*, 2004). Reduction of the growth period and to promote the early flowering is one of the important object in case of lisianthus production.

Since lisianthus was introduced into Bangladesh almost a decade ago and its commercial cultivation was started in 2016, it is a new flower for Bangladesh.

The environmental condition in Bangladesh is suitable for commercial production of lisianthus due to temperature condition. As successful flower production requires optimal cultivation technology including proper fertilizer management, it needs to develop balanced fertilizer management for good quality flower. Because increase in crop productivity depends mostly on the type of fertilizers used to supplement the essential nutrients of plants. In most cases, macro nutrients containing fertilizers are applied in the soil while considering less importance to the micro nutrients. Micronutrients require in small quantities but these supplemental elements play a vital role in plant development (Mohsen *et al.* 2016). In soil the availability of micronutrients is subjected to factors like pH, soil organic matter (SOM), clay content, redox potential, biological activity and cation-exchange capacity (Fageria *et al.* 2002). Moreover, micronutrients are prone to leached down or get fixed in the soil when those are applied on the soil. For overcoming all these problems, generally trace elements are supplied by foliar application in which liquid fertilizers are directly sprayed onto leaves. As foliar application can reduce the time lag between application and is untaken by plant during the rapid growth phase.

In modern agricultural practices, smart technology like nano particle nutrients are used because of their high nutrient use efficiency in plants compared to conventional chemical fertilizers. Minimized nano scale (1–100 nm) nutrient particles are new generation of the synthetic fertilizers differing from the presence of the nutrients in the macro scale (Naderi and Danesh-Shahraki, 2013).

Deductively, nano-form micronutrients such as iron, manganese, zinc, copper, and iron are being carefully analyzed presently for applications in crops and plants as for their requirement in plant growth and development. Research has been found that uptake of iron, manganese, and copper in nano particle form may be more efficient method compared to soil application in conventional method where they get adsorbed on plant surface, soil particles and hence are less available to root system (Taiz and Zeiger, 2010). In recent years, many studies demonstrated the growth enhancement and better functioning of plants at an optimum concentration of nano fertilizer.

To evaluate the effect of foliar spray of nano fertilizer solution on growth and yield of lisianthus considering the importance of micronutrient like zinc and copper in flowering plant, a field experiment was carried out in the semi-arid highland region of Sher-e-Bangla Nagar, Dhaka, Bangladesh.

This chapter focuses on the effect of engineered micronutrient copper based zinc nanoparticles containing fertilizer on lisianthus plants.

By keeping the above information the present research was undertaken with the following objectives:

- To evaluate the performance of lisianthus varieties
- To optimize the copper based zinc nano fertilizer for lisianthus production
- To study the interaction effect of copper based zinc nano fertilizer on lisianthus varieties

CHAPTER II

REVIEW AND LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Lisianthus can meet the national and international market demands through its wide range of colors and export potentiality. With this view in mind, the present research work was conducted to find out the effect of varieties of lisianthus on copper-based zinc containing nano fertilizer which provides the micro nutrients for the plant. Some important research works related to lisianthus and nano fertilizer that have been conducted so far have been presented here according to year in a descending order.

Lisianthus related literature

Ahmad *et al*. (2017) conducted an experiment at the Horticulture Farm of Sher-e-Bangla Agricultural University during of April to October, 2016 to screen some lisianthus lines for production in Bangladesh. Fifteen lisianthus lines viz. L_1 (Nandini Moonlight), L_2 (Nandini Suvro), L_3 (Nandini Chandra), L_4 (Nandini Pink light), L_5 (Nandini Lemon Double), L_6 (Nandini Lemon Single), L_7 (Nandini Pink Cup), L_8 (Nandini Rose), L_9 (Nandini Royal Violet), L_{10} (Nandini Violet Single), L_{11} (Nandini Blue Vase), L_{12} (Nandini Ocean Violet), L_{13} (Nandini Purple Bell), L_{14} (Nandini Purple Picotee) and L_{15} (Nandini Lavender) were used in that experiment arranged in a Randomized Complete Block Design with three replications. Data on different growth and flowering parameters were taken to which all the lines showed significant variations. L_8 showed the lowest rosette % (4.7) whereas the maximum was observed in L_{12} (17.7). In most of the parameters, L_9 showed the best result (plant height-68.8 cm, no. of leaves- 51.7, flower head diameter- 7.3 cm, stem length- 53.5 cm and vase life- 20.7 days) giving it rank 1 on the basis of the total score although all the lines showed promise to be a quality cut flowers. So, this flower has the potential to be promising addition to the present flower market.

Uddin *et al*. (2015) conducted at the roof top net house, Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka, under 2a Biotech Lab, 2014 to October, 2014. Eight varieties of Nandini (*Eustoma grandiflorum*) namely, V_1 , Mickey Rose; V_2 , Pink Rose (single); V_3 , Pink Picotee; V_4 , Blue Rim; V_5 , Chandra; V_6 , Pink Rose (double); V_7 , Blue Bell and V_8 , Royal Violet were used. Growth and yield traits had significant variation among the varieties. The highest chlorophyll percentage and leaf area were observed in Chandra. Chandra produced the maximum number of flowers and also showed higher percentage of seed germination but the maximum amount of seeds were harvested with Pinky Rose (double).

A pot experiment was conducted for the first time in Bangladesh, at the Horticulture Farm, Sher-e-Bangla Agricultural University, Dhaka to screen out the adaptability of seven lisianthus cultivars namely Micky Rose, Pink Rose, Azuma No Yosooi, Purple Edge Glass, Piccolo Blue, Mellow Purple and Royal Violet for commercial cultivation in Bangladesh by Uddin *et al*. (2013). The experiment was conducted during November, 2010 to July, 2011. Significant differences among cultivars were noted for all the attributes. The highest number of flowers (16.0/plant) was produced by Piccolo Blue while the lowest from Pink Rose (7.0/plant). All the cultivars showed very good shelf life (12.0-25.0days) under normal conditions which is the characteristic features of lisianthus. All the seven lisianthus cultivars performed satisfactorily as ideal cut flowers.

Harbaugh *et al*., (2002) stated that lisianthus is emerging as an important cut flower in the United States while in European and Asian markets, it is already listed among the top ten cut flowers. Many new cultivars have been released in the United States within the last 5 years, but comparative performance trials of these cultivars have been lacking. That trial evaluated 47 cultivars of lisianthus representing series (cultivar groups) that were marketed in the United States in 1998. Evaluations were made for rosetting, plug performance, cut-flower characteristics (vegetative and flowering attributes) as well as postharvest longevity of cut flowers. Significant differences among cultivars were found for all of the attributes evaluated. 'Malibu Purple', 'Catalina Blue Blush', and 'Alice Pink' were selected as the best performers in the seedling (plug) stage since those had less than 5% rosettes, large leaves and vigorous root systems. Cultivars were placed in classes based on flower color, flower size, and number of petals (single or double flowers). Cultivars were ranked for each of the attributes and the total rank sum of all attributes (TRS) was used to select the best in class. Cultivars selected as best in class were 'Malibu Purple', 'Malibu Blue Blush', 'Alice Purple', 'Balboa Blue', 'Avila Blue Rim', 'Mellow Pink', 'Flamenco Wine Red', 'Flamenco Rose Rim', 'Alice Pink', 'Avila Rose', 'Echo Pink', 'Alice White' and 'Mariachi White'.

Copper related literature

Fouad *et al*. (2020) evaluated the response of rainfed wheat (*Triticum aestivum* L.) to foliar copper (Cu) application in correcting Cu deficiency in calcareous soils. Two native soil Cu contents were tested in successive growing seasons. The soil "1" contained 0.35 mg kg^{-1} of Cu (Diethylenetriamine Pentaacetic Acid extraction). It was evaluated during the 2016-17 season. The soil "2" contained 0.61 mg kg^{-1}. It was studied during the 2017-18 season. The rainfall amount was around 289 and 429 mm, respectively, for 2016-17 and 2017-18 seasons. For the soil "1", the Cu treatments were: control, 0.2, 0.4, 0.6, 0.8, and 1%. For the soil "2", the Cu tested levels were: control, 0.01, 0.03, 0.05, 0.1, and 0.2%. Cu was applied in the sulfate form at the early booting stage. The results showed that the response of grain yield to Cu foliar feeding was not related to the tested native soil Cu content. A significant grain yield got increased, due to Cu spray as was revealed during the rainfall season (429 mm) in soil "2". That increase was around 8% at 0.018% of Cu compared to control. However, Cu foliar application higher than 0.03% induced leaf damage. The Cu content of flag leaf and kernels showed a linear response to Cu supply. Flag leaf Cu content was around 5 mg kg^{-1} in control and exceeded 30 mg kg^{-1} at Cu application over than 0.03%.

Barriois *et al*. (2018) stated that exposure of some plants to high concentrations of heavy metals may increase anthocyanin concentration. That study determined the effects of Cu spraying on hibiscus (*Hibiscus sabdariffa* L.) leaves at various dosages and number of doses on anthocyanin content, physical and chemical characteristics, and calyces yield. For that purpose, hibiscus genotype *Reina Roja* was grown under rainfed conditions. During the vegetative stage of the plants, Cu was sprayed two, four or six times with 150, 300 and 450 mgL^{-1}. The results indicated that four and six sprayings with 150, 300 and 450 mg Cu L^{-1} reduced dry calyces yield. Two sprayings at either Cu dosage did not modify calyces yield. Added Cu increased significantly anthocyanin content and titratable acidity. Anthocyanin content increased the most (57 and 44%) when Cu was sprayed six times at 300 and 450 mgL^{-1}. The data suggested that two sprayings with 150 mg Cu L^{-1} could improve nutritional quality of hibiscus extracts without affecting dry calyces yield.

Seedling production in protected nursery is the basis of the São Paulo state citrus culture, and fertilization management is one of the main pitfalls in the process. Copper deficiency and excess in citrus seedlings have become a serious concern for seedling nursery owners. Based on these considerations, the study aimed at evaluating the effects of copper fertilization on citrus seedlings.

Tecchio *et al*. (2015) carried out an experiment at a commercial seedling nursery in Botucatu city/SP and consisted of 5 treatments- T_1: control, T_2: copper oxyfluoride (1.8 gL^{-1}), T_3: cupric oxide (500 gL^{-1}), T_4: copper chelate EDTA (0.04 mlL^{-1}) and T_5: copper sulphate (2.5gL^{-1}). After treatment allocation, monthly evaluations were performed for 5 months for mean plant height (cm), stem diameter (mm), above ground and root dry matter (g) and mean copper level in the leaves (mg kg^{-1}) of the rootstocks of the Rangpur lime (*Citrus limonia*, Osbeck) in the two first evaluations. The three following evaluations were performed in the "Valencia" orange cultivar (Citrus sinensis, Osbeck). The experiment was completely randomized with a split plot design, in which plots corresponded to treatments with copper fertilization, and subplots to months of evaluation. Mean values of copper were considered excessive, mostly in the products applied to the leaves.

Choudhury and Yusop (2004), was conducted a greenhouse experiment at the University Putra Malaysia to evaluate the effects of nitrogen (N) and copper (Cu) fertilizations on rice yield and fertilizer N efficiency using 15N tracer technique. Four rates of N (0, 60, 120 and 180 kg N ha^{-1} and three rates of Cu (0, 5 and 10 kg Cu ha^{-1}) were used in that study. Nitrogen was applied as 15N tracer technique. Four rates of N (0, 60, 120 and 180 kg N ha^{-1}) and three rates of Cu (0, 5 and 10 kg Cu ha^{-1}) were used in this study. Nitrogen was applied as 15N labelled urea. Grain yield increased significantly due to N fertilization up to 120 kg N ha $^{-1}$. Regression analysis indicated that grain yield response due to N fertilization that was quadratic in nature. Estimated N rate for the maximum yield was 158 kg N ha^{-1}. Copper application did not increase grain yield although the soil was deficient in Cu. The 15N atom excess percentage in both grain and straw, and fertilizer N uptake by rice plant increased gradually with increasing N rates. Recovery (%) of fertilizer N was around 40% irrespective of N and Cu rates. The non-significant effect of Cu might be due to higher Cu adsorption in the soil. Plant analysis indicated that copper content in the straw was below the critical deficiency level of 6 mg kg^{-1}. Those findings indicated that higher rate of Cu fertilizer (above 10 kg Cu ha^{-1}) may be useful in that soil to increase rice yield and fertilizer N efficiency if Cu was applied as basal. Alternately, Cu might be applied as foliar spray on standing crop to avoid Cu adsorption in the soil.

Dell (1981) found out that anther development and pollen sterility were followed in plants of wheat, oat, barley, sweetcorn, sunflower, petunia and subterraneum clover grown at a range of copper supplies. Copper-deficient plants had increased pollen sterility. Lignified wall thickenings got reduced or absent in the endothecia of anthers from Cu-deficient plants. Reduced seed set may resulted both from reduced pollen fertility or failure of the stomata to rupture due to decreased lignification of anther walls.

Zinc related literature

Saeed *at al*. (2013) stated that zinc being an activator of certain enzymes, regulates antioxidant activity; therefore, that could enhance the shelf life of cut flowers. The research was conducted on zinc (Zn) nutrition of gladiolus for two years (2010–2011) in the greenhouse. Graded levels of zinc, viz., 0, 2, 4, 6, 8 and 10 mg Zn kg^{-1} were applied in soil media. Results in both the experimental years revealed significant positive response to zinc application on growth and vase life attributes of gladiolus. Zinc at 6 mg kg^{-1} rendered the highest impact for increasing the leaf area, spike length, flower size, fresh and dry biomass weight. Less number of days to flowering and higher count of florets per spike was recorded with 8 mg Zn kg^{-1}. Chlorophyll and protein contents were highest at 6 mg Zn kg^{-1}; whereas Zn contents were highest with 10 mg Zn kg^{-1}. Vase quality parameters like percent florets opened, vase life and fresh weight change were greater with 8 mg Zn kg^{-1}, and the least membrane leakage was also ensured at that rate. Antioxidant enzymes, viz., SOD, CAT, POD and free radical scavenging activities in cut flowers remained at the highest level with 6 mg Zn kg^{-1}. This study concluded that Zn applied at 6–8 mg kg^{-1} imparted greater beneficial effects on growth, production, vase life quality and antioxidative activities in gladiolus cut flower, and further higher application rates render non-significant improvement.

Moreira *et al.* (2018) stated that Zinc (Zn) is an essential micronutrient for plant growth and development, and its deficiency in plants has been widely reported in many regions of the world. About 50% of soil used for agriculture contain a low level of plant-available Zn, which reduces yield of the plant.

Paul *et al*. (2016) conducted research to study the effects of foliar application of zinc on growth and yield of wheat (BARI gom-25) grown under water stress condition at the farm of the Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207 under the AEZ 28 (Madhupur Tract) on November, 2013 to March, 2014. The experiment was designed in randomized complete block for factor A (represented as irrigation), with factor B (represented as Zn application) a split plot on factor A with 16 treatments combination comprising 4 irrigation treatments regular irrigation, skipped irrigation at crown root initiation state, skipped irrigation at booting stage and skipped irrigation at heading and flowering stages of growth) and four application of zinc i.e. one as soil application (ZnSoil: 2 kg ha^{-1}) and three as foliar application (Zn_1: 0.02%, Zn_2:0.04% and Zn_3: 0.06% of zinc). Zinc Sulphate Monohydrate ($ZnSO_4.H_2O$) was used as a source of Zn. Water at crown root initiation stage had the most negative effect on growth and yield of wheat. The interaction effect of irrigation and foliar application of zinc significantly influenced the yield and yield components of wheat. The highest yield (3.57 t ha^{-1}) was recorded in the combined treatment Zn_2 that received skipping irrigation at flowering and heading stage along with 0.04% foliar application of zinc. Thus, foliar application of zinc played a major role on yield and yield components of wheat under water stress condition.

Farahat *et al*. (2007) conducted a pot experiment at National Research Center, Dokki, Cairo, Egypt, Research and Production Station, Nuberia. The aim of the work was to study the effects of foliar spray of ascorbic acid (0, 20, 40 ppm) and zinc (0, 20, 40 ppm) and their interactions on vegetative growth and some chemical constituents of *Cupressus sempervirens* plant. Most criteria of dry weight of the plant organs were significantly affected by application of the two factors used in the study. Foliar application and ascorbic acid 40ppm and zinc 40 ppm separately promoted all carotenoids, total soluble sugars, total phenols except zinc 20 ppm compared with control plants, whereas Zn at 20 and 40 ppm application had insignificant effects on total soluble phenols. Essential oil content was significantly increased by the application of the two factors which were used in the study. The highest recorded data were obtained in plants treated with Asc. 40 ppm+Zn 40 ppm and Asc. 40 ppm+Zn 20 ppm, those increased significantly all growth parameter, except day weight of shoots and fresh weight of roots compared to control plants. Chl (a) and Chl (b) were significantly increased by the two factors collectively under study. Whereas decreased carotenoids content. Total soluble phenols and oil percent content were significantly increased by the application of ascorbic acid and Zinc might be recommended for increased vegetative growth of cupressus plant for its source of wood for different uses and the increment of oil percent for medicinal uses.

Both copper and zinc related literature

Johura *et al*. (2021) experiment was carried out a pot experiment at the net house to study the effects of Zn and Cu on the growth and yield of tomato. There were six doses of fertilizers in the experiment, viz., T_0:Control, T_1: Recommended dose of fertilizer (N 160 P 50 K 100 S 20) kg/ha, T_2: 75% NPKS from inorganic fertilizer and 25% NPKS from cowdung, T_3: Recommended dose of fertilizer with Zn and Cu (N 160 P 50 K 100 S 20 +Zn 4 +Cu 4) kg/ha, T_4: Recommended dose of fertilizer with Cu (N 160 P 50 K 100 S 20 +Cu 4) kg/ha, T_5: Recommended dose of fertilizer with Zn (N 160 P 50 K 100 S 20 +Zn 4) kg/ha. Data were taken on growth, yield contributing characters, yield and the collected data were statistically analyzed for evaluation of the treatment effects. All the plant parameters got influenced significantly by the application of Zn and Cu with other chemical fertilizers. The tallest plant, the maximum number of leaves per plant, number of branches per plant, the maximum number of flowers cluster per plant number of flowers per plant were produced by recommended doses of fertilizers with Zn and Cu (N 160 P 50 K 100 S 20 +Zn 4 +Cu 4) kg/ha. The higher number of fruits per plant was observed in T_3 treatment. The zinc and copper of tomato significantly influenced on the yield of fruits per plant. The maximum yield of fruits per plant (347.60 g) was obtained from T_3 treatment but the minimum (183.73 g) was obtained from control treatment.

Dutta (2020) stated that abiotic stresses, predominantly salinity, drought, heat, cold and heavy metal stress leads to significant loss in agricultural productivity. Loss of farmable land triggered by the rising population further aggravated the scenario. It is estimated that 70% of loss in crop production was the result of exposure to abiotic stresses (Wild, 2003). Exogenous application of trace elements or micronutrients such as copper (Cu), Zinc (Zn) and iron (Fe) has been able to mitigate these environmental stresses and enhance crop yield by maintaining optimum soil nutrient quotient. Studies reported that those micronutrients are associated with vital physiological processes namely, photosynthesis, respiration, protein stabilization, carbohydrate metabolism, cell division, protein and nucleic acid synthesis etc. However, there is limited information regarding the relation between exogenous applications of those micronutrients with major stress tolerance mechanisms.

Wojtkowiak *et al*. (2014) studied the impact of mineral fertilization with or without multi-component fertilizers on the content of microelements in soil and spring triticale grains was investigated in field trials, in 2009-2011. The experiment was carried out on 8 fertilizing treatments, which included two varieties of spring triticale: Andrus and Milewo. The contents of available zinc and manganese were higher on plots cropped with the cultivar Andrus and nitrogen fertilization with urea or with urea and ammonium nitrate. It was also found that the contents of available manganese, zinc and iron in the analyzed soils was within the natural average range. The higher contents of manganese and zinc in grains was detected after the application of multi-component fertilizers. Nitrogen fertilization at a dose of 120 $kgha^{-1}$ together with Azofoska and Ekolist resulted in an increase in the iron content in cv. Andrus. The regression analysis between the content of the analyzed microelements in soil and in triticale grains revealed significant increase in the iron, manganese and zinc content in grains together with the increase in the contents of those elements in soil under cv. Milewo. With respect to the zinc content in soil and in grain from this variety, the coefficient of determination was the closest to the coefficient of a linear correlation (R_2=0.9105). It was shown that increases in the contents of microelements in soil were not always accompanied by an increase in the contents of those elements in spring triticale grains.

Hansch and Mendel (2009) described that micronutrients are involved in all metabolic and cellular functions. Plants differ in their need for micronutrients, and we will focus would be given there only on those elements generally accepted as essential for all higher plants: boron (B), chloride (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn). Several of those elements are redox-active that makes them essential as catalytically active cofactors in enzymes, others have enzyme-activating functions, and yet others fulfill a structural role in stabilizing proteins. In this review, focus was given on the major functions of mineral micronutrients, mostly in cases where those were shown as constituents of proteins, making a selection and highlighting some functions in more detail.

Foliar application related literature

Rakibuzzaman *et al.* (2019) accomplished an experiment at the horticultural farm, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh to evaluate the effects of foliar application of neem oil and natura one for brinjal production. The experiment was conducted with four treatments, viz. Control (T_0), Natura-one (T_1), Neem oil (T_2) and Neem oil + Natura one (T_3). The study was carried out to examine different characters like plant height, number of branches, infested branches, branch infestation (%), number of leaves/plant, chlorophyll percentage, number of flowers/plant, number of fruits/plant, infested fruits, fruit infestation (%), yield/plant (kg), yield/ha (ton) and yield increase (%) over control. Lower infested shoots and fruits (0.2 and 0.2 plant^{-1}, respectively) and percentage (11.9 and 16.9, respectively) were found in T3. The highest yield (57.3 t ha^{-1}) and increased yield percentage over control (13.47%) were also found in T_3 treatment. In view of the overall performances, foliar application of neem oil and natura one had potentiality to combat the insect damage as well increase yield.

Sonmez *et al.* (2017) studied the effects of both soil copper applications (SCuA) and foliar copper application (FCuAF) on micronutrient contents (copper, iron, manganese and zinc) of tomato plants. For that, Cu was applied as a factorial combination rates (0 [S_1], 1000 [S_2] and 2000 mg kg^{-1} [S_3], soil) and frequencies (no application [F_1], biweekly [F_2] and weekly [F_3], foliar). Two separate experiments were conducted to observe the effects of different Cu containing chemicals. Two fungicides, Gunner and Tenn-Cop 5E (containing Cu oxychloride and copper salts of fatty and rosin acids, respectively), were used for foliar copper applications. $CuSO_4 \cdot 5H_2O$ was used to provide to soil. In the experiment-I (Gunner, Cu oxychloride) and in the experiment II (Tenn-Cop 5E, copper salts of fatty and rosin acids), Cu and Mn contents of plant samples increased with increasing SCuA and FCuAF. Fe contents of leaf and fruit samples were affected by SCuA and decreased with increasing of SCuA. Root Fe content generally decreased with increasing of both SCuA and FCuAF. Leaf, fruit and root Zn contents were affected by both SCuA and FCuAF and Zn contents of plant samples decreased with increasing SCuA and FCuAF. As a result, both SCuA and FCuAF, especially aiming to control plant diseases, showed different effects on Cu, Fe, Mn and Zn contents of tomato plants. It was determined that, by taking into account the amount and frequency of applications, the Cu doses applied either to soil or leaf were too high. Thus, it would be useful to conduct more in detailed studies to determine Cu toxicity limits on tomato plants at different soil pH levels by gradually decreasing Cu doses.

Nano particle related literature

Saeed *et al*. (2020) described that he nano-technologies and nano-materials have drawn incredible consideration in recent years. Nano-particles are the particles having size ranging from 1 to 100 nm (1 nm is equivalent to 10^{-9} m). The nano-particles are usually categorized into different classes, and their classification is based on size, shape, material production, and dimension. They showed superior properties, i.e. enhanced reactivity, high BET surface area, sensitiveness and steadiness compared to their bulk materials. In that paper, different approaches of synthesizing nano-particles, including sol gel, chemical vapor deposition, and biosynthesis were talked over. In the treatment of wastewater, nano-particles offered a possibility for effective adsorption of contaminants organic as well as inorganic.

Al-juthery and Saadoun (2018) carried out an experiment at the fields of Al-Husseiniya District, Taliaa Township, Babylon Governorate, to study the response of the Jerusalem artichoke to foliar application of micro nutrients nano fertilizes of Nano Iron, Zinc, Copper, and Manganese foliar applied at 25, 50, 75 and 100 g nano fertilizer 100 L^{-1} water, and 1 kg naonfertilizers ha^{-1}(as recommended) dissolved in 400 liters of solution ha^{-1}. The experiment included single, di, tri and tetra combinations, as well as a tetra combination of a traditional source, in addition to control (distil water only). Growth parameters tested were chlorophyll SPAD, dry matter yield of vegetative, tubers yield, inulin yield and % of sucrose and ascorbic acid. Results indicated that nano-applied treatment (Cu+ Zn+ Fe+ Mn) was significantly higher followed by the triple, di and single spray combinations, in yield of fresh and dry tubers, vegetative and inulin yield giving 77.928, 19.906, 6.584 and 13. 235 Mg ha^{-1}, respectively, compared with traditional fertilizers (34.320, 6.284, 3.908, and 3.345 Mg ha^{-1}) and the control (22.655, 3.234, 3.390 and 1.201 Mg ha^{-1}), respectively. The highest % of sucrose and ascorbic acid (vitamin c) (61.13% and 8.1%) were with tetra nano (Cu +Zn +Fe+ Mn) than other treatments.

Tulasi *et al*. (2018) described that nanotechnology is a revolutionary change in science and has generate extensive opportunity in the field of biotechnology, medicine, pharmaceuticals, electronics and agriculture. Present situation in nanoscience is one in which there is the great potential for transforming agriculture and food production through efficient management of soil nutrients, pesticide, herbicide and water management. The development of nano materials could open up the novel thing in the discipline like agronomy in relation to maximization of crop production along with quality of the produce. Nano fertilizers in plant nutrition can play crucial role in resolving the problem of low nutrient use efficiency, soil residues and water pollution. Use of nano material is one of the innovative idea for enhancing nutrient use efficiency and helps for reduction in the environmental degradation. The use of nano fertilizers helps in encouraging plant growth, crop production and reduces the soil toxicity of the soil. Nano fertilizers are also helpful to moderate the negative effects, caused by the excessive use of fertilizers and reduce the frequency of application of fertilizer. In many part of the country, soil is deficient in major nutrients and at the sometime there is widespread multinutrient deficiency due to dominant cropping pattern. Negative nutrient balance and low fertilizer use efficiency is often associated with many part of the country. From single plant nutrient deficiency, at present country is experiencing multi- nutrient deficiency. The nano technology is miracle in plant nutrition system as it becoming important aspect in crop management practice. The advancement in modern techniques in fertilizer application system like nano technology will saves substantial amount of budgetary provisions. The fertilizer use efficiency can be improved drastically by avoiding and minimizing the precious nutrient via different ways and means due to nanotechnology in nutrient supplementation.

Al-juthery *et al.* (2018) were conducted an experiment in autumn of 2017 at the College of Agriculture, University of Qadisiyah, to investigate the effects of foliar application of SMP nan/o-fertilizes, SW seaweed ibanad multi and HP growth regulator hypertonic on growth and yield of potato. Treatments included 4 levels (25, 50, 75 and 100g nano fertilizer $100L^{-1}$ water foliar applied, 1kg nano fertilizers ha^{-1} (as recommended) dissolved in 400 liters of solution ha^{-1}. The experiment included single, di, tri combinations, in addition to control (water only). Growth and yield parameters tested were chlorophyll, dry matter yield of vegetative part, fresh tubers yield, biological yield, harvest index and % of starch, crude protein, ascorbic acid and water use efficiency (WUE). Results indicated that (SMP+SW+HP) applied treatment was significantly higher followed by the di and single spray combinations, in yield of fresh, dry tubers, vegetative yield and biological yield giving 32.76, 7.280, 2.194 and 10.110 Mg ha^{-1}) respectively, compared with the control (18.86, 3.626, 1.174, and 5.258 Mg ha-^{1}), respectively. The highest % of starch, crude protein and ascorbic acid (vitamin c) (16.74 %,9.2 and 1.463%) were with tri spry (SMP+SW+HP) than other treatments.

For sustainable production in crop, production nano particles may be effective tools in agriculture for better pest and nutrient management because those nano-materials having more penetration capacity, surface area and use efficiency which avoid residues in environment. Size below 100 nm nano-particles can use as fertilizer for efficient nutrient management which are more eco-friendly and reduce environment pollution. Hence, these agricultural useable nano-particle developed with the help of nanotechnology can be exploited in the value chain of entire agricultural production system (Morales-Díaz *et al*., 2017; Al-Juthery and Saadoun, 2018)

Abdel-Aziz *et al*. (2018) described that nano particle containing fertilizer are easily absorbed by the epidermis of leaves translocated to stems which facilitated the uptake of active molecules and enhanced growth and productivity of wheat. Nano fertilizer have large surface area and particle size less than the pore size of leaves of the plant which can increase penetration into the plant tissues from applied surface and improve uptake and nutrient use efficiency and uptake of the nutrients. (Dimkpa, *et al*., 2015 and Qureshi, *et al*., 2018).

Ali and Al-juthery (2017) stated that micronutrients can be considered as one of the main component for high and good quality agricultural products and human health. Many researchers and scientists around the world indicated that more than 3 billion person around the world are affected by shortage of micronutrients, especially Fe and Zn .This problem cannot be solved through food additives at developing poor countries and biofortification is the only answer through naturally enriched agricultural products with micronutrients. Micronutrients can limit food quality and quantity in spite of the low amounts required by crops compared to macronutrients. Micronutrient are available in different formulas and structures as mineral, synthetic chelated and organic fertilizers and can be applied either to soil or as foliar or mixed of both. However, fertilizers use efficiency (FUE) or nutrient recovery by plants is still 5%. Recently the use of nanotechnology for micronutrients in agricultural production are being adopted and hoped this technology will have a vital part in solving the low recovery but the issue is at its outsets and still in the need of further understanding, investigations and financial support.

Morales-Díaz *et al*. (2017) stated that agriculture stands to be benefited from nanotechnology in areas such as combating pests and pathogens, regulating growth and quality of crops, and developing intelligent materials and nanosensors. The objective of that paper was to provide an overview of the uses of nanomaterials (NMs) and nanoparticles (NPs) in plant nutrition, highlighting their advantages and potential uses, but also reviewing their possible environmental destination and effects on ecosystems and consumers. NPs and NMs have been shown to be an attractive alternative for the manufactures of nanofertilizers (NFs), which are more effective and efficient than traditional fertilizers. Because of their impact on crop nutritional quality and stress tolerance in plants, the application of NFs is increasing. However, there are virtually no study on the potential environmental impacts of NPs and NMs when used in agriculture. Such studies are necessary because NPs and NMs can be transferred to ecosystems by various pathways where those can cause toxicity to organisms, affecting the biodiversity and abundance of these ecosystems, and may ultimately even be transferred to consumers.

Dimkpa (2014) stated that the expansion of nanotechnology raised concerns about the consequences of nano materials in plants. There, the effects of nanoparticles (NPs; 100–500 mg/kg) on processes related to micronutrient accumulation were evaluated in bean (*Phaseolus vulgaris*) exposed to CuO NPs, a mixture of CuO and ZnO (CuO:ZnO) NPs, and in CuO NP-exposed plants colonized by a root bacterium, *Pseudomonas chlororaphis* O_6 (PcO_6) in a sand matrix for 7 days. Depending on exposure levels, the inhibition of growth by CuO NPs was more apparent in roots (10% - 66%) than shoots (9% - 25%). In contrast, CuO:ZnO NPs or root colonization with PcO_6 partially mitigated growth inhibition. At 500 mg/kg exposure, CuO NPs increased soluble Cu in the growth matrix by 23 fold, relative to the control, while CuO:ZnO NPs increased soluble Cu (26 fold), Zn (127 fold) and Ca (4.5 fold), but reduced levels of Fe (0.8 fold) and Mn (0.75 fold). Shoot accumulations of Cu (3.8 fold) and Na (1 fold) increased, while those of Fe (0.4 fold), Mn (0.2 fold), Zn (0.5 fold) and Ca (0.5 fold) got reduced with CuO NP (500 mg/kg) exposure. CuO:ZnO NPs also increased shoot Cu, Zn and Na levels, while decreasing that of Fe, Mn, Ca and Mg. Root colonization got reduced shoot uptake of Cu and Na, 15% and 24%, respectively. CuO NPs inhibited ferric reductase (up to 49%) but stimulated cupric (up to 273%) reductase activity; while CuO:ZnO NPs or root colonization by PcO6 altered levels of ferric, but not copper reductase activity, relative to CuO NPs. Cu ions at the level released from the NPs did not duplicate these effects. Those findings demonstrated that in addition to the apparent phytotoxic effects of NPs, NP exposure also had subtle impacts on secondary processes such as metal nutrition.

Norman and Hongda (2013) stated that nano scale science and nanotechnologies are envisioned to have the potential to revolutionize agriculture and food systems.

Gutierrez *et al*. (2011) stated that at the nano-scales the matter presents altered properties which are novel and very different from those observed at the macroscopic level. The change in properties is due to the reduced molecular size and also because of changed interactions between molecules. The properties and possibilities of nanotechnology, which have great interest in agricultural revolution, are highly reactive, enhanced bioavailability and bioactivity, adherence effects and surface effects of nanoparticles as well.

Nano particle on lisianthus related literature:

Seydmohammadi *et al*. (2020) conducted a research in greenhouse condition by spraying various concentrations of nano ZnO (3, 6 and 9 mgL^{-1}) and nano $CaCO_3$ (250, 500 and 750 mgL^{-1}) every 20 days. The research was aimed to investigate the effects of foliar application of nano ZnO and nano $CaCO_3$ on growth and flowering of lisianthus (*Eustoma grandiflorum* cv. Mariachi Blue). According to the research, foliar spraying of nano ZnO (6 mgL^{-1}) on lisianthus increased the number of leaf and lateral branches, leaf chlorophyll content and petal anthocyanin content. Nano ZnO spray also increased number of flowers. The experiments indicated that the plants sprayed with 500 mgL^{-1} nano $CaCO_3$, entered the flowering stage earlier and flowered about 15 days earlier than the control plants, while foliar spraying of nano ZnO delayed the flowering time where foliar spraying during the growth period with nano $CaCO_3$ (500 mgL^{-1}) increased the number of flowers per plant. Also, the number of flowers was 56.3% higher than the control treatment. An increase in the plant size was observed with the use of nano $CaCO_3$. The highest flower diameter, plant height and leaf length in lisianthus were obtained by foliar spraying of nano $CaCO_3$. The research paper showed that calcium carbonate and zinc oxide nano fertilizers had significant effects on growth characteristics and flowering quality of lisianthus.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

A field experiment was conducted at the Horticulture Farm, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh from November 2019 to April 2020 to determine the effects of foliar application of copper based zinc fertilizer on three varities of lisianthus. This chapter contains a brief description of the experimental site, climatic condition and soil, materials used for the experiment, treatment and design of the experiment, production methodology, intercultural operations, data collection procedure and statistical analysis of the experiment which are presented under the following headings.

3.1 Experimental site

The study was conducted at the Horticulture Farm, Sher-e-Bangla Agricultural University, Dhaka. The experimental location was 23074^0N latitude and 90035^0E longitude and at an elevation of 8.2m from the sea level (Anon. 1989).

3.2 Climatic condition

The experimental site was located in the subtropical monsoon climatic zone in Dhaka, set aparted by heavy rainfall during the months from April to September (Kharif season) and scanty of rainfall during the rest of the year (Rabi season). Plenty of sunshine and moderately low temperature prevails during October to March (Rabi season), which is generally preferred for flower production in Bangladesh. As lisianthus is a non-photosensitive crop, the experiment was not bounded by any timeframe of the year.

3.3 Characteristics of the soil

The experimental soil belongs to the Madhupur Tract under AEZ No. 28 (UNDP-FAO, 1988). The land was medium high and the soil series was Tejgaon. The soil characteristics of experimental plot were analyzed at the

SRDI, Soil Testing Laboratory, Khamarbari, Dhaka and the experiment field primarily had a pH of 6.5.

3.4 Experimental materials

3.4.1 Planting materials

Lisianthus seeds were collected from Takii Seed, Japan. In October, 2019, the seeds were sown in 102 hole plug trays filled with growth medium and placed in lisianthus plant factory which is used as the growth chamber for germination and subsequent growth of the seedlings. Required care for proper development of seedlings were taken and 60 days old seedlings (with 4 pair true leaves) were taken for transplanting in the field.

3.4.2 Land preparation

The soil was brought to fine tilth with cross plowing using power tiller. Then the area was divided into plots of 3m X 1m according to the layout of the experiment (Figure.1). BARI recommended manures and fertilizer doses for marigold used was follows -

- Cowdung- 7 ton/ha
- Urea- 225 kg/ha
- TSP- 200 kg/ha
- MoP- 150 kg/ha

All these were applied to the plot soil during the time of final land preparation.

3.4.3 Treatments and layout of the experiment

The double factorial experiment was laid out in the Randomized Complete Blocked Design (RCBD) with three replications (Figure 1) and 30 cm distance from row to row and 20 cm distance from plant to plant was maintained in each replications.

The treatments of the double factorial experiment were as follows:

Factor A:

V_1-Purple Picotee; V_2-Light Pink & V_3-Super Magic Type Blue

Factor B:

Copper based zinc nano particles were applied on this experiment are given below:

N_0 - (No nano fertilizer application)

N_1 – 1 ml/L nano fertilizer application

N_2 – 2 ml/L nano fertilizer application

3.5 Application of nano fertilizer

Nano particles containing liquid fertilizers were used to this experiment. First application was done 20 DAT and further application was done 40, 60 DAT in corresponding manner. Three blocks were assigned for three lisianthus varieties and replicated three times in each treatment and in control conditions.

The treatment combinations were: V_1N_0, V_2N_0, V_3N_0, V_1N_1, V_2N_1, V_3N_1, V_1N_2, V_2N_2, V_3N_2.

3.6 Intercultural operations

3.6.1 Irrigation

During seedling germination, growth and development, mist irrigation was provided using a hand sprayer to keep the growth medium moist. After transplanting of the seedlings in the main field, over-head irrigation was provided through a pipe as and when necessary.

3.6.2 Weeding

Weeding was done as and when necessary.

3.6.3 Disease and pest control

To prevent fungal infection, Dithane M-45 was sprayed 3 times at 15 days interval along with Pyrithrum @ 1.5ml/L to prevent insect attack. At the vegetative stage, Furadan 5G @ 3 g/L was also applied to protect from soil nematode and clybio was applied @2ml/L on each plot for providing beneficial microorganism to the soil.

3.6.4 Staking

Staking was provided to the plants using bamboo sticks for the support.

3.7 Parameters studied

I. Growth parameters

- Plant height (cm)
- No. of leaves per plant
- No. of branches per plant
- Stem diameter (mm)
- Stem length (cm)

II. Physiological parameter

- SPAD value

III. Yield parameters

- Days to flower bud initiation
- Days to full bloom
- Number of flower buds/stem
- Number of flowers/stem
- Number of flowers/plant
- Flower head diameter (cm)

IV.Qualitative parameter`

- Vase life (days)

V. Color measurement using CIELab (L* a* b* c* and h_{ab})

3.8 Data collection for the experiment

3.8.1 Plant height

Plant height was measured using a graduated measuring scale at 30, 45,60,75,90 days after transplantation (DAT) of the plant and recorded in centimeter (cm).

3.8.2 Number of leaves per plant

Total no. of leaves was determined by counting all the leaves from the base to the tip of the plant at 30, 45,60,75,90 days after transplantation (DAT) of the plant.

3.8.3 Number of branches per plant

No. of branches was measured by counting the branches containing flowers and flower buds at maturity stage.

3.8.4 SPAD value

Chlorophyll content or SPAD value was measured using a portable chlorophyll meter (SPAD-502, Minolta, Japan). The procedure of this measurement was non-destructive. Data were collected from three randomly selected leaves taking three data from each leaves and the mean was derived (Plate 2. a).

3.8.5 Days to flower bud initiation

Days to flower bud initiation was determined by counting from the days of transplanting to the appearance of the first flower bud on the plant.

3.8.6 Days to full bloom

Days to full bloom was determined by counting from the day of appearance of the bud to the complete opening of the flower.

3.8.7 Number of flower buds/stem

Number of flower buds per stem was counted up to blooming of the first flower and the mean value was calculated.

3.8.8 Number of flowers/stem

Number of flower per stem was counted just before harvesting the stem and mean value was calculated.

3.8.9 Number of flowers/plant

No. of flower/plant was counted just before harvesting and the mean value was calculated.

3.8.10 Flower head diameter

Flower head diameter was measured using Digital caliper-515 (DC-515) in millimeter (mm). The data was then converted to centimeter (Plate 2.c).

3.8.11 Stem diameter

Stem diameter was measured using Digital caliper-515 (DC-515) in millimeter (mm). Mean value was derived from the collected data (Plate 2.d).

3.8.12 Stem length

Stem length was measured using a measuring scale from each of the flowering ones. The measurement was done from the first internode from the soil and recorded in centimeter (cm) (Harbaugh *et al*., 2000)

3.8.13 Vase life

Three stems were selected at random from each treatment, cut to almost 25 cm, and placed in a 500 ml conical flask containing tap water. Stems having one flower about to bloom and one or two unopened flower-bud were selected. Stem ends were cut and tap water was changed in every alternate days. The number of days till the wilting of flowers and buds were recorded to determine the vase life of lisianthus.

3.9 Petal color measurement

Colorimetric measurement of the three lisianthus varieties was done using IWAVE WF32 precision colorimeter (Shenzhen Wave) following L* (lightness), a* and b* (two Cartesian coordinates) including C* and hab (Chroma & hue angle) based on CIELab scale with standard observer 100 and standard illumination D65 (CIE, 1986; McGuire, 1992) (Plate 2.f.). Beams effective axes were at 45±20 from the normal of the specimen surface in illuminated petals. Metric chroma, C* and hue angel, hab were calculated as C*= (a*2+b*2)0.5 and hab= tang-1 (b*/a*) (Gonnet, 1998). The minimum for L* is zero which represents black. The a* and b* axes have no specific numerical limits. Positive a* is red and negative a* is green. Positive b* is yellow and positive b* is blue. The individual petals were separated and were placed under the measurement port for color measurement. In case of Purple Picotee the distinguishing color portion of the petals were arranged under the measuring port of the colorimeter and the test was conducted.

3.10 Statistical analysis

The data recorded for different parameters were statistically analyzed using Statistix-10 scientific analysis software to find out the significance of variation among the treatments and treatment means were compared by the Least Significant Difference (LSD) test at 5% level of probability.

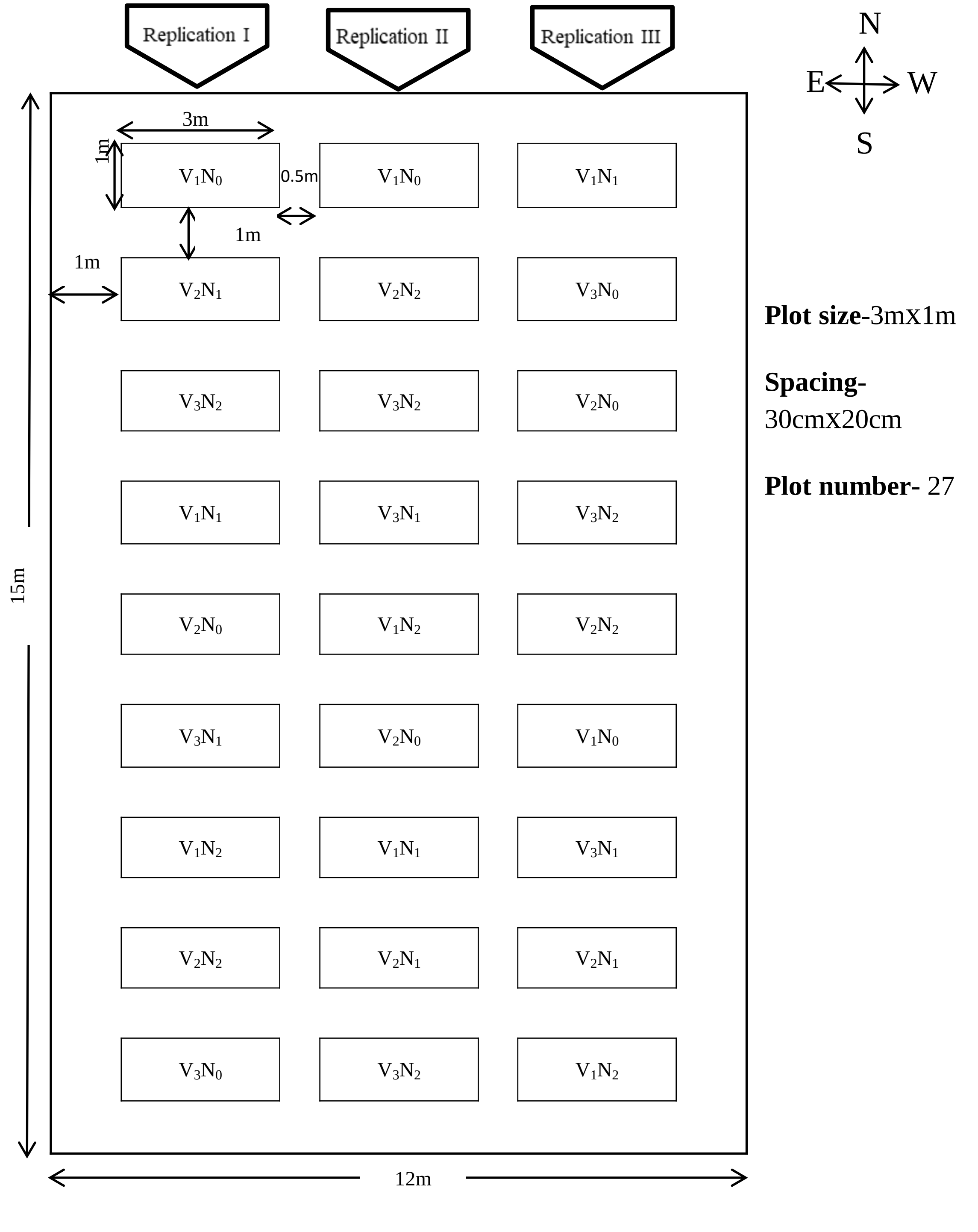


Figure 1. Layout of the experiment

(a)

(b)

(c)

(d)

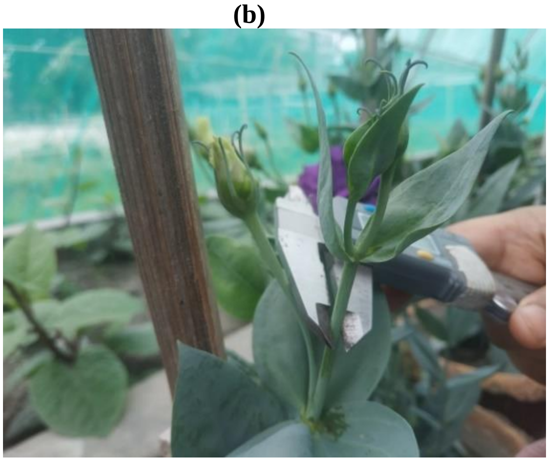
(e)

(f)

Plate 1. Pictorial presentation of the experiment

(a) Seedling of lisianthus; **(b)** Transplanting of seedling in the field; **(c)** Intercultural operation; **(d)** Nano fertilizer solution; **(e)** First application of nano fertilizer at 20 DAT; **(f)** Second application of nano fertilizer at 40 DAT.

(a) **(b)**



(c) **(d)**

(e) **(f)**

Plate 2. Pictorial presentation on data collection

(a) Measurement of chlorophyll percentage using SPAD; **(b)** Leaf and stem number count; **(c)** Measurement of flower diameter using Digital Caliper -515(DC-515);**(d)** Measurement of stem diameter using Digital Caliper -515(DC-515; **(e)** Measurement of stem height using meter scale in cm; **(f)** CIELab color coordinate measurement of flower petals using IWAVE WF32 precision colorimeter (Shenzhen Wave).

CHAPTER IV

RESULT AND DISCUSSION

Plate 3. Pictorial presentation of Lisianthus varieties under different treatments

Here, V_1: Purple Picotee; V_2: Light pink and V_3: Super magic type blue and N_0: No nano fertilizer application; N_1: 1ml/L nano fertilizer application; N_2: 2ml/L nano fertilizer application

CHAPTER V

SUMMARY AND CONCLUTION

CHAPTER V

SUMMARY AND CONCLUSIONS

5.1 Summary

One of the vital goals of lisianthus as a cut flower producer is to reduce the growth period and increase the quality of the flower. The current research was aimed to investigate the effects of foliar application of copper based zinc nano fertilizer on the growth and flowering of three lisianthus (*Eustoma grandiflorum*) varieties.

In order to study the effects of nano fertilizer and the performance of lisianthus varieties, this research was conducted to inspect the growth and yield responses of lisianthus varieties to copper based zinc nano fertilizer at the Horticultural Farm, Sher-e-Bangla Agricultural University, Dhaka during December 2019 to April 2020. The two factorial experiment included lisianthus varieties viz. V_1 (Purple Picotee), V_2 (Light Pink), V_3 (Super Magic Type Blue), and copper based zinc nano fertilizer application viz. N_0 - (Control, no nano fertilizer application), N_1-(1ml/L nano fertilizer), N_2-(2ml/L nano fertilizer).

Significant variations were observed in case of varieties as well as copper-based zinc nano fertilizer application on all the parameters which were as follows –

The highest plant height was found from V_1 (71.8 cm) and from N_2 (78.8 cm) whereas the shortest from V_2 (61.3 cm) and from N_0 (54.4 cm) at 90 days after transplanting. In case of the treatment combinations, the tallest plant (83.4 cm) was found in V_1N_2 while the shortest plant (48.2 cm) was found in V_2N_0 at 90 days after transplanting.

The maximum number of leaves (94.8) was found in V_3 and the minimum (81.6) in V_2 at 90 DAT. The maximum number of leaves in case of nano fertilizer (104.3) was found in N_2 while the minimum from N_0 (72.2) with 90 days after transplanting. In case of combined effects, the maximum number of leaves (110.0) was found in V_3N_2 which was statistically identical with V_1N_2 (105.0) whereas the minimum (64.0) in V_2N_0 with 90 DAT.

The maximum number of branches (4.9) was found in V_1 and minimum (4.1) in V_2 which was statistically identical with V_3 (4.4). In case of nano fertilizer treatment, the maximum number of branches (5.8) was found in N_2 while the minimum (3.4) in N_0. In case of combined effect of lisianthus varieties and copper based zinc nano fertilizer, the maximum number of branches (6.3) was found in V_1N_2 which was statistically identical (6.0) with V_3N_2 but the minimum (3.3) in V_2N_0 and V_3N_0 which was also statistically identical (3.7) with V_1N_0 where those gave statistically similar result (4.0) in V_2N_1 and $V_3N_{1,}$ respectively.

The highest SPAD value (56.0) was observed from V_3 whereas the least (42.7) from V_2. Considering the nano fertilizer treatment, the maximum SPAD value (56.7) was found with N_2 while the minimum (41.0) was in N_0. In case of the combination treatment, the maximum SPAD value (63.7) was found in V_3N_2 and the minimum (34.3) from V_2N_0.

Considering the variety, V_1 (Purple Picotee) needed least number of days (79.2) for bud initiation and while the utmost number of days (86.6) were required by V_2 (Light Pink). Concerning nano fertilizer treatment, V_2N_0 required maximum days (91.0) while minimum days (73.0) was needed in V_3N_2

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The maximum days required for full bloom (21.4) was found in V_2 and N_0 (22.6) whereas the minimum (19.2) in V_1 which was statistically identical (20.0) with V_3 and N_0 (18.1). In case of combination treatments, the maximum days needed for full bloom (23.7) was in V_2N_0 and the minimum (16.7) in V_1N_2 which was statistically similar with in V_3N_2 (18.0).

Considering the variety, the highest number of flower buds per stem (13.6) was in V_1 while the shortest number (10.2) was in V_2. In case of copper based zinc nano fertilizer application, the maximum number of flower buds per stem (14.2) was observed under N_2 and the minimum (9.4) in N_0. In the combined effects, the maximum number (16.3) was found in V_1T_2 while the minimum (7.7) in V_2N_0.

The maximum number of flowers per stem was recorded in V_1 (7.3) and N_2 (7.9) and the smallest in V_2 (4.7) and N_0 (4.4). The combination treatments indicated that the maximum number of flowers (9.0) was found in V_1N_2 and the minimum (3.0) from V_1N_0.

The maximum number of flowers per plant (37.6) was observed in V_1 but the minimum (23.3) in V_2. Considering nano fertilizer application, the maximum number of flowers (44.2) was found in N_2 and the minimum (18.0) in N_0. In case of combined effects, the maximum number of flowers per plant (54.3) was found in V_1N_2 and the minimum (12.7) in V_2N_0.

Considering the variety, the maximum flower yield per hectare (6.3 million) was found in V_1 while the minimum yield (3.9 million) in V_2. In case of nano fertilizer application, the maximum yield per hectare (7.4 million) was observed under N_2 and the minimum (3.0 million) in N_0. In combined effects, the maximum yield per hectare (9.1 million) was found in V_1N_2 whereas the minimum (2.1 million) in V_2N_0.

In case of variety, the utmost flower head diameter (5.3 cm) was observed in V_3 but the minimum (4.2 cm) in V_2. Considering nano fertilizer application, the maximum head diameter in flower (5.3 cm) was found in N_2 while the minimum (4.0 cm) in N_0. In case of combined effects, the maximum flower head diameter (6.3 cm) was found in V_3N_2 whereas the minimum flower head diameter (3.8 cm) was found in V_2N_0 which was statistically identical with (3.8) in V_1N_0 and statistically similar with (4.2 and 4.3 cm, respectively) in V_2N_1 and V_3N_0.

The maximum stem diameter was recorded in V_3 (5.5 mm) and N_2 (5.4 mm) but the smallest in V_2 and V_1 were statistically identical (4.3 and 4.5 mm, respectively) and N_0 (4.0 mm). The combination treatments indicated that the maximum stem diameter (6.1 mm) was found from V_3N_2 and the minimum (3.6 mm) in V_2N_0 which was statistically identical (3.7 mm) with V_1N_0.

In case of stem length of, variety V_1 (55.3 cm) and N_2 (56.8 cm) had the highest but V_2 (39.8 cm) and N_0 (41.2 cm) had the lowest values. In combined effects, the maximum yield per hectare (64.0 cm) was found in V_1N_2 while the minimum (31.0 cm) in V_2N_0.

Significant variation was observed regarding the vase life. The longest vase life (17.7 days) was found from V_1 while the least (15.4 days) from V_2 and similar was found (16.0 days) from V_3. In case of nano fertilizer application, the maximum vase life (18.0 days) was obtained from N_2 whereas the minimum (14.6 days) from N_0. In case of combined effect, the maximum vase life (19.7 days) was found in V_1N_2 but the minimum (14.0 days) was found in V_2N_0 which was statistically identical with V_3N_0. The minimum vase life was statistically similar with V_1N_0 and V_2N_1 (15.3 days).

To sum up, it can be articulated that the variety V_1 had great attributes and N_2 (2ml/L) application of copper based zinc nano fertilizer and combination treatment V_1N_2 were best for growth, yield and flower quality attributes of lisianhus.

5.2 Conclusions

In respect of the above results, it can be concluded that lisianthus varieties showed significant variation to copper based zinc nano fertilizer. According to the result, the variety, V_1 (Purple picotee) had the tallest plant height, number of leaves, number of branches, number of flower buds/stem, number of flowers/stem, number of flowers/plant, flower yield per hectare, flower head diameter, stem length and long vase life too. On the other hand, 2ml/L application nano fertilizer was excellent among the nano fertilizer treatment applied in terms of all parameters. Regarding combination studies, it can be easily stated that nano fertilizer had significantly positivel effects with all the yield attributes. Besides the combination, variety, V_1 treated with 2ml/L with copper based zinc nano fertilizer performed as the best combination for growth, yield and flowering quality attributes of lisianthus flower.

5.3 Recommendations

Based on the findings of the research, these recommendations can be made:

1. Variety V_3 (Purple Picotee) could be recommended for production in farmers field.

2. Use of supplemental nano fertilizer can stimulate the plant growth to increase yield.

5.4 Suggestions

From the findings, in the future the following research regarding nano fertilizer in lisianthus may be suggested:

1. Further experiments can be done on the frequency and doses of copper based zinc nano fertilizer application.

2. Research can be designed to investigate the role of these nano fertilizers in the physiological processes of lisianthus and other crops too.

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APPENDICES

APPENDICES

Appendix I. Analysis of variance for plant height at different days after transplanting of lisianthus						
Source of Variation	**Degrees of freedom**	**Mean square for plant height (cm)**				
		30 DAT	**45 DAT**	**60 DAT**	**75 DAT**	**90 DAT**
Factor A (Lisianthus varieties)	2	14.063*	66.034*	98.374*	134.535*	252.888*
Factor B (Nano fertilizer doses)	2	156.780 *	436.053*	646.975*	990.507*	1348.48*
Interaction (A×B)	4	1.435*	0.444*	3.353*	5.207*	3.41056*
Error	16	0.315	0.335	0.203	0.246	0.27125
***: Significant at 0.05 level of probability**						

Appendix II. Analysis of variance for the number of leaves per plant at different days after transplanting of lisianthus						
Source of variation	**Degrees of freedom**	**Mean square for number of leaves**				
		30 DAT	**45 DAT**	**60 DAT**	**75 DAT**	**90 DAT**
Factor A (Lisianthus varieties)	2	46.704*	116.778*	201.81*	961.33*	394.93*
Factor B (Nano fertilizer doses)	2	250.926*	724.111*	1206.26*	1510.11*	2320.48*
Interaction (A×B)	4	0.481*	1.222*	3.70*	12.78*	4.43*
Error	16	4.134	9.111	8.40	6.15	9.45
***Significant at 0.05 level of probability**						

Appendix III. Analysis of variance for the data of SPAD value, Days to flower bud initiation and Days to full bloom of lisianthus				
Source of variation	**Degrees of freedom**	**Mean square of**		
		SPAD value	**Days to bud initiation**	**Days to bloom**
Factor A (Lisianthus varieties)	2	423.523*	123.370*	11.4444*
Factor B (Nano fertilizer doses)	2	610.151*	258.370*	44.7778*
Interaction (A×B)	4	3.889*	4.093*	0.3889*
Error	16	4.071	2.954	0.8194
***Significant at 0.05 level of probability**				

Appendix IV. Analysis of variance for the data of branch number per plant, stem length and stem diameter of lisianthus				
Source of variation	**Degrees of freedom**	**Mean square of**		
		Number of branches /plant	**Stem length (cm)**	**Stem diameter (mm)**
Factor A (Lisianthus varieties)	2	1.1481*	580.454*	3.86855*
Factor B (Nano fertilizer doses)	2	11.2593*	548.640*	4.16005*
Interaction (A×B)	4	0.3148*	4.786*	0.02264*
Error	16	0.3843	1.879	0.05699
***Significant at 0.05 level of probability**				

Appendix V. Analysis of variance for the data of no. of flower buds/stem, no. of flowers /stem and no. of flowers /plant of lisianthus				
Source of variation	**Degrees of freedom**	**Mean Square of**		
		Number of buds/stem	**Number of flowers/stem**	**Number of flowers /plant**
Factor A (Lisianthus varieties)	2	20.3333*	16.5926*	458.81*
Factor B (Nano fertilizer doses)	2	51.4444*	26.7037*	1576.15*
Interaction (A×B)	4	0.7778*	0.0370*	22.48*
Error	16	1.3611	0.3287	2.66
***Significant at 0.05 level of probability**				

Appendix VI. Analysis of variance for the data of flower yield/hectare (million), flower head diameter (mm) and vase life (days) of lisianthus				
Source of variation	**Degrees of freedom**	**Mean Square of**		
		Flower yield/hectare (million)	**Flower head diameter (mm)**	**Vase life (days)**
Factor A (Lisianthus varieties)	2	12.7449*	2.92593*	12.0370*
Factor B (Nano fertilizer doses)	2	43.7819*	3.70037*	26.9259*
Interaction (A×B)	4	0.6245*	0.37926*	0.5926*
Error	16	0.0739	0.09218	0.9398
***Significant at 0.05 level of probability**				