RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
54 (2) 389-396, 2022
http://doi.org/10.54910/sabrao2022.54.2.14
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

# MARINE ALGAE EXTRACTS, AND NANO FERTILIZER WITH ZINC AND COPPER EFFECTS ON GROWTH, AND MACRO- AND MICRONUTRIENTS COMPOSITION OF APPLE TREES

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## SUMMARY

Bioactive compound marine algae extracts (MAE), nano-zinc (nZn) and nano-copper (nCu) fertilizer effects on growth traits, and macro– and micronutrient composition in apple trees were studied. The recent study was carried out in 2021 at the laboratories of the Department of Horticulture and Landscape Gardening, Faculty of Agriculture, University of Kufa, Kufa, Iraq, and Department of Horticulture, College of Agriculture, AL-Qasim Green University, Babylon, Iraq. In the conducted experiment, the treatments comprise the foliar application of marine algae extracts with two concentrations at 0.5 and 1 ml $L^{-1}$, nZn at 1 and 2 g $L^{-1}$, nCu at 1 and 2 g $L^{-1}$, and a control in a randomized complete block design with three replications. The results showed that apple trees treated with marine algae extracts were superior in enhancing the shoot length, diameter, leaves per tree, leaf area, leaf dry matter, and percentages of nitrogen (N), phosphorus (P), potassium (K), and copper (Cu). The other two treatments (nZn and nCu) also showed highest values for some characteristics and moderate values for other traits that excelled the control treatment. Zinc percentage was significant in apple tree leaves when treated with two concentrations of nZn (1, 2 g $L^{-1}$) reaching 1.89% and 1.97%, respectively. The highest Cu percentages were recorded in the apple tree leaves treated with both concentrations of nCu (1, 2 g $L^{-1}$) reaching 3.66% and 3.78%, respectively. The potassium percentage was significantly high in apple tree leaves for all the treatments. The control treatment was recorded with the lowest values in all the characteristics.

**Keywords:** Apple trees, marine algae extracts, nano-zinc, nano-copper, growth, macro– and micronutrients composition

**Key findings:** Marine algae extracts (MAE) were found promising in increasing the shoot length, diameter, leaves per tree, leaf area, leaf dry matter, and percentages of macro-nutrients, i.e., N, P, K, and Cu, followed by nano-zinc and nano-copper in apple trees.

Communicating Editor: Dr. Samrin Gul
Manuscript received: February 18, 2022; Accepted: April 23, 2022.


**To cite this manuscript:** Kareem KhA, Alojany ZOO, Al-Janabi ASA (2022). Marine algae extracts, and nano fertilizer with zinc and copper effects on growth, and macro- and micronutrients composition of apple trees. *SABRAO J. Breed. Genet.* 54(2): 389-396. http://doi.org/10.54910/sabrao2022.54.2.14

## INTRODUCTION

The old apple (*Malus domestica* L. Borkh) is a perennial plant with a long-lasting juvenile (incapable of flowering) stage characterized by vegetative growth and the apple plant's incapacity to respond to flower-inducing signals (Wu and Poethig, 2006). Under natural conditions, the vegetative stage can span five to ten, or even 12 years (Hanke *et al*., 2007). The vegetative transition marks the end of the juvenile stage. In adulthood, the plants become capable to respond to flower-inducing signals. Once apple trees reached adulthood, they blossom once or twice a year. Only a tiny percentage of shoot meristems develop flower buds terminally on fruiting spurs, and laterally on long shoots, in contrast to annual plants with the remaining meristems still being vegetative (Bangerth, 2009; Upadyshev, 2022).

Minerals have a direct and indirect impact on fruit yield and quality (Spinelli *et al*., 2009). In agricultural systems, the efficient use of fertilizers is essential for increasing the yield in apple fruit and other crop plants (Dong *et al*., 2005; Motyleva *et al*., 2021). Synchronizing fertilizer administration to crop needs is difficult because of the inability of plant leaves to fully absorb nutrients, foliar application of fertilizers has become a common way of giving nutrients to plants (Weinbaum, 1988). The beneficial effects of seaweed extract application were due to various components that may function and show recital at various concentrations, although the mechanism of action is yet unknown (Fornes *et al*., 2002). Marine algae extracts (MAE) is a new bio-fertilizer including macro– and micro-elements, as well as, containing certain growth regulators, polyamines, and vitamins, which can be used to enhance the vegetative development, yield, nutritional status, and fruit quality in olive trees, vineyards, and various crop plants (Abd-El-Migeed *et al*., 2004).

The influence of these extracts on cell metabolism via the activation of antioxidant molecules, may boost-up plant development and stress tolerance (Cardozo *et al*., 2007; AL-Hadrawi and Al-Janabi, 2020; Mazhar *et al*., 2021). The elements like nitrogen, phosphorus, zinc, copper, boron, and potash play a vital physiological role in stimulating and promoting plant growth and development (Vanitha *et al*., 2016; Zubkova *et al*., 2022). The low application rates, uniform fertilizer distribution, and quick responses to applied foliar fertilization are advantageous. Micronutrients such as Fe (iron), Zn, Mn (manganese), Cu, and B (boron) are not only obligatory but also crucial for plant growth and productivity in tropical and sub-tropical fruit crops (Yadav and Solanki, 2015; Mazhar *et al*., 2021).

Zinc (Zn) is a critical element for plants, and Zn shortage is common in many crops (Marschner, 2012; Ojeda-Barrios *et al*., 2014). Zinc is required for the activation of a large number of enzymes, including dehydrogenases, aldolases, isomerases, transphosphorylases, and RNA and DNA polymerases, as well as, tryptophan synthesis, cell division, membrane structure maintenance, and photosynthesis. It also serves as a regulatory cofactor for protein synthesis in higher plants (Marschner, 2012; Al-Janabi and Alhasnawi, 2021). Copper also plays an important role in plant biological processes by activating many enzymes, including polyphenol oxidase, oxidizing enzymes, as well as, the photosynthesis electron transfer process. The investigations have shown that the chloroplasts contain roughly 70% of the total copper in the leaves, confirming its significance in the photosynthetic process. Further, copper has long been used in combination with other materials to treat fungal illnesses (Zhang and Schmidt, 2000).

The majority of Iraqi soils have a high amount of calcium carbonate, and basically respond to many vital processes. When macro– and micronutrients are introduced to the soil directly, a portion of them, especially the micronutrients, may be exposed to loss, fixation, and sedimentation, resulting in the plant not to benefit from them. For this reason, the researchers began looking for alternatives to use them as a foliar spray in olive trees and various other orchards (Abd-El-Migeed *et al*., 2004). Therefore, the recent study aimed to determine the effects of foliar spraying of MAE and nano-fertilizers of zinc and copper on the vegetative growth characteristics of apple trees.

## MATERIALS AND METHODS

### Plant material and procedure

The study was conducted in 2021 at the laboratories of the Department of Horticulture and Landscape Gardening, Faculty of Agriculture, University of Kufa, Kufa, Iraq, and Department of Horticulture, College of Agriculture, AL-Qasim Green University, Babylon, Iraq. Two year old apple trees were cultivated in polyethylene bags containing 5 kg of soil in a private nursery in Najaf, Iraq. The

**Table 1.** Physical and chemical properties of the experimental soil.

| No. | Properties | Values |
|---|---|---|
| 1 | Sand % | 41.2 |
| 2 | Clay % | 38.9 |
| 3 | Silt % | 19.9 |
| 4 | Texture | Sandy clay |
| 5 | F.C. % | 18.44 |
| 6 | pH | 7.88 |
| 7 | EC (dS/m) | 0.78 |

soil characteristics are provided in Table 1. The apple trees were kept in the experimental nurseries until 1 June 2021. The experiment was laid out in a randomized complete block design (RCBD) with three treatments having two concentrations each, i.e., MAE at 0.5 and 1 ml $L^{-1}$, nZn at 1 and 2 g $L^{-1}$, and nCu at 1 and 2 g $L^{-1}$, and the control (without spray) replicated three times. The apple plants were sprayed twice, i.e., on 15 March and 15 April 2021, and five apple trees were kept in each experimental unit. As a result, the whole experiment comprised a total of 135 trees. The data were statistically examined, and a comparison was done using Duncan's multiple range test with a probability of 5%. All of the data were tabulated and statistically analyzed using the Genestate software Ver.12.

**Data recorded**

The data on the following plant growth parameters were recorded on 1 August 2021. The shoot length (cm) was measured using a tape measure. The stem diameter (mm) was measured with a vernier caliper at the shoot length of 5 cm above the ground surface. The leaves per tree were counted in three trees chosen at random from each treatment and then averaged. The average leaf area (cm²) of six to 10 leaves from the apex of trees under treatment was evaluated using a gravimetric approach base on the relationship between dry weight and area and compared with a known area (Reisinauer, 1978). Following the leaf dry weight, a disc (0.302 $cm^2$) from each leaf was taken and weighed. Until weight fixing, the leaves were oven-dried at 70 °C. In calculating the leaf dry matter (%) and leaf area, the following equations were used:

Leaf dry matter (%) = (leaf dry weight/leaf fresh weight) ×100

Leaf area = (LDW × AD) / DWD

Where,
LDW = leaf dry weight (g)
AD = area of the disc taken from the leaves (0.302 cm²)
DWD = dry weight of discs taken from the leaves (g).

**Total chlorophyll**

The reading of 20.20 A645 + 8.02 A663: A663 and A645 was obtained from the spectrophotometer at 663 and 645 nanometer, respectively, after using an acetone alcohol for extraction of chlorophyll. Total chlorophyll = 20.20 × A645 + 8.02 × A663 (Arnon, 1949).

**Macro and micronutrients**

Apple leaf samples were prepared and the concentrations of N, P, and K were determined using the Kjeldahl process, spectrophotometry, and photometry of flame emissions. The Zn and Cu contents were measured using atomic absorption spectrophotometry, as described by Chapman and Pratt (1961).

## RESULTS

**Length increment**

Results revealed that apple trees treated with two different concentrations of MAE (0.5, 1 ml $L^{-1}$) had a substantial increase in shoot length, reaching 52.30 cm and 59.41 cm, respectively (Table 2). The MAE were also found at par with two concentrations of nZn (1, 2 g $L^{-1}$), and nCu (1, 2 g $L^{-1}$) with values of 39.13 cm and 40.43 cm, and 36.13 cm and 39.12 cm, respectively. The control treatment did not result in a substantial increase in shoot length and obtained a length of only 22.20 cm.

**Stem diameter**

In comparison to other treatments, nCu with 2 g $L^{-1}$ concentration resulted in a significant

**Table 2.** Effects of marine algae extracts, nano-zinc, and nano-copper fertilizers on the shoot length and stem diameter in apple trees.

| Treatments | | Length increment (cm) | Stem diameter (mm) |
|---|---|---|---|
| Control | | 22.2 c | 1.52 c |
| MAE | 0.5 ml $L^{-1}$ | 52.30 a | 7.13 b |
| | 1 ml $L^{-1}$ | 59.41 a | 8.01 b |
| nZn | 1 g $L^{-1}$ | 39.13 b | 7.50 b |
| | 2 g $L^{-1}$ | 40.43 b | 7.73 b |
| nCu | 1 g $L^{-1}$ | 36.13 b | 7.59 b |
| | 2 g $L^{-1}$ | 39.12 b | 9.03 a |

**Table 3.** Effects of marine algae extracts, nano-zinc, and nano-copper fertilizers on the leaves per tree and leaf area in apple trees.

| Treatments | | Leaves $trees^{-1}$ | Leaf area ($cm^2$) |
|---|---|---|---|
| Control | | 41.2 c | 44.5 d |
| MAE | 0.5 ml $L^{-1}$ | 79.2 a | 72.01 a |
| | 1 ml $L^{-1}$ | 91.7 a | 74.20 a |
| nZn | 1 g $L^{-1}$ | 63.0 b | 66.31 b |
| | 2 g $L^{-1}$ | 60.7 b | 68.23 b |
| NCu | 1 g $L^{-1}$ | 72.4 b | 56.73 c |
| | 2 g $L^{-1}$ | 71.8 b | 60.83 c |

increase in tree diameter (9.03 mm), followed by MAE with 1 ml $L^{-1}$ treatment (8.01 mm), nZn with 1 g $L^{-1}$ (7.73 mm), and nCu with 1 g $L^{-1}$ (7.59 mm) (Table 2). The control treatment showed the least diameter increase in apple trees (1.52 mm).

**Leaves per trees**

Apple trees treated with 0.5 and 1 ml $L^{-1}$ of MAE produced maximum and at par leaves per tree (79.2 and 91.7, respectively) (Table 3). The above concentrations were followed by two concentrations of nZn and nCu fertilizers ranging from 60.7 to 72.4. The control treatment produced only 41.2 leaves per tree which was the least value compared with all other treatments.

**Leaf area**

The foliar application of MAE (0.5, 1 ml $L^{-1}$) achieved a leaf area of 72.01 and 74.01 $cm^2$ and had a substantial stimulatory effect on leaf area parameters in apple trees (Table 3). The two concentrations of nZn (1, 2 g $L^{-1}$) follow with at par leaf area of 66.31 and 68.23 $cm^2$, respectively. The leaf area (56.73 and 60.83 $cm^2$) attained by the trees treated with 1 and 2 g $L^{-1}$ of nCu, respectively, were moderate, whereas the control treatment was recorded with the least leaf area (44.5 $cm^2$).

**Leaf dry matter**

Foliar application of both concentrations of MAE (0.5, 1 ml $L^{-1}$), nZn (1, 2 g $L^{-1}$), and nCu (1, 2 g $L^{-1}$), produced the maximum and same leaf dry matter ranging from 50.21% to 57.99% (Table 4). That range of leaf dry matter was significantly greater than the control treatment (29.10%).

**Total chlorophyll**

The highest and same total chlorophyll contents were recorded in the apple trees treated with foliar application of nCu (1, 2 g $L^{-1}$) which produced 0.676 and 0.788 mg $g^{-1}$ of chlorophyll, respectively (Table 4). These leading treatments were followed by the application of MAE and nZn with the same values of total chlorophyll, i.e., 0.553, 0.591, 0.533, and 0.511 mg $g^{-1}$, respectively. The lowest total chlorophyll content was found in the control treatment of apple trees (0.340 mg $g^{-1}$).

**Macro– and micronutrients in the leaves of apple trees**

*Nitrogen*

In foliar application of MAE (0.5, 1 ml $L^{-1}$), the highest and same percentage of nitrogen

**Table 4.** Effects of marine algae extracts, nano-zinc, and nano-copper fertilizers on the leaf dry matter and total chlorophyll content in apple trees.

| Treatments | | Percentage of leaf dry matter (%) | Total chlorophyll (mg/g F.W) |
|---|---|---|---|
| Control | | 29.10 b | 0.340 c |
| MAE | 0.5 ml $L^{-1}$ | 50.21 a | 0.553 b |
| | 1 ml $L^{-1}$ | 54.12 a | 0.591 b |
| nZn | 1 g $L^{-1}$ | 56.68 a | 0.533 b |
| | 2 g $L^{-1}$ | 57.03 a | 0.511 b |
| nCu | 1 g $L^{-1}$ | 57.29 a | 0.676 a |
| | 2 g $L^{-1}$ | 57.99 a | 0.788 a |

**Table 5.** Effects of marine algae extracts, nano-zinc, and nano-copper fertilizers on the concentration of N, P, K, Zn, and Cu % in apple trees.

| Treatments | | N (%) | P (%) | K (%) | Zn (%) | Cu (%) |
|---|---|---|---|---|---|---|
| Control | | 1.376 c | 0.133 c | 0.213 b | 1.01 c | 2.01 b |
| MAE | 0.5 ml $L^{-1}$ | 1.978 a | 0.333 a | 0.640 a | 1.13 b | 3.31 a |
| | 1 ml $L^{-1}$ | 1.993 a | 0.243 b | 0.661 a | 1.11 b | 3.40 a |
| nZn | 1 g $L^{-1}$ | 1.783 b | 0.301 a | 0.703 a | 1.89 a | 2.10 b |
| | 2 g $L^{-1}$ | 1.791 b | 0.371 a | 0.761 a | 1.97 a | 2.22 b |
| nCu | 1 g $L^{-1}$ | 1.820 b | 0.339 a | 0.849 a | 1.82 ab | 3.66 a |
| | 2 g $L^{-1}$ | 1.891 ab | 0.322 a | 0.868 a | 1.80 ab | 3.76 a |

concentration, i.e., 1.978% and 1.993%, respectively, were observed in the apple tree leaves (Table 5). These leading treatments were also found at par with the nCu application (2 g $L^{-1}$) having a nitrogen content at 1.891% in the apple tree leaves. Both concentrations of nZn (1, 2 g $L^{-1}$) and one dose of nCu (1 g $L^{-1}$) application followed the above treatments with the same values of nitrogen content ranging from 1.782% to 1.820%. The control treatment (having no foliar spray of bioactive compounds and nano-fertilizers) showed the least content of nitrogen in the tree leaves at 1.376%.

*Phosphorus*

The bioactive compound MAE, nZn, and nCu fertilizers foliar application, and the control showed non-significant effects for phosphorus (%) in the leaves of apple trees (Table 5). Numerically the bioactive compounds showed enhanced content of phosphorus ranging from 0.243% to 0.371% as compared with the control (0.133%).

*Potassium*

In the case of potassium content in the leaves of apple trees, the foliar application of bioactive compound (MAE) and nano-fertilizers of zinc and copper showed increased values ranging from 0.640% to 0.868% (Table 5). The nCu application treatments were leading, followed by nZn fertilizer and MAE for potassium content. The control treatment revealed the least potassium content (0.213%).

*Zinc*

The nZn fertilizer applications (1, 2 g.$L^{-1}$) exhibited significantly the highest and same percentage of zinc content 1.89% and 1.97%, respectively, in the leaves of apple trees (Table 5). These leading zinc treatments were also found comparable with nCu fertilizer concentrations in zinc content (1.82% and 1.80%), followed by bioactive compound MAE (1.13% and 1.11%). The lowest value of zinc content (1.01%) appeared in the control treatment of the apple tree leaves.

*Copper*

In the case of copper content, the highest and at par values were recorded in the leaves of apple trees with the application of nCu fertilizer (3.66% and 3.76%) and bioactive compound MAE (3.31% and 3.40%), followed by nZn fertilizer (2.10% and 2.22%) (Table 5). The apple tree leaves in the control treatment expressed the lowest composition of copper (2.01%).

## DISCUSSION

By comparing the foliar application of bioactive compounds and nano-zinc and nano-copper fertilizers, the results showed that marine algae extracts have the best performance in improving the growth and quality parameters of apple trees. The findings correspond with those of Turan and Kose (2004). Past studies revealed that in apple leaves (Mac-an-tsaoir and Archer, 2008, 2010), in grapevines (Abd-El-Moniem and Abd-Allah, 2008), the rising content percentages of N, P, and K were determined, and increased dry leaf weight was observed in *Vitis vinifera* plants (Mancuso *et al*., 2006). The vital role of bioactive compound algal extracts was recorded in promoting cell division and enhancing organic product production, resulting in glucose and protein accumulation in the leaves of 12- year-old Anna Apple trees (Mansour *et al*., 2006). These past findings also revealed that algae extracts treatment was very successful in boosting shoot length, leaf area, total leaf carbohydrates, and leaf mineral content.

Plant growth regulators, organic osmolytes (e.g., betaines), amino acids, mineral nutrients, vitamins, and vitamin precursors are all known to be found in seaweeds (Berlyn and Russo, 1990). Seaweed contains kahydrin, alginic acid, and betaines, which work together to improve the formulation's efficacy (Verieri *et al*., 2006). Micronutrients like zinc and copper, as well as, macronutrients like potash, play an important role in enhancing plant growth and productivity. Despite considering the micronutrient requirements, zinc and copper are essential and equally important for plant growth and productivity in rice (Das, 2014) and tropical and subtropical fruit crops (Yadav and Solanki, 2015).

Nano-particulate Zn properties (size, unique surface area, and reactivity) influence the solubility, diffusion, and accessibility of Zn to plants, and nano-material can be exploited to generate new Zn fertilizers for balanced crop nutrition (Subramanian *et al*., 2008, 2015; Subramanian and Rahale, 2009, 2012; Subramanian and Tarafdar, 2011; Mosanna and Khalilvand, 2015; Al-Janabi and Alhasnawi, 2021). Zinc is a necessary component of many enzymes, including transphosphorylases, dehydrogenases, isomerases, aldolases, DNA and RNA polymerases, as well as, being involved in cell division, tryptophan synthesis, membrane structure maintenance, and photosynthesis, and further, acting as a regulatory co-factor in protein synthesis in higher plants (Marschner, 2012).

The copper effects increased enzymatic processes and leaf area, resulting in increased photosynthesis, leading to an increase in leaf manufactured material, then, increasing growth and improving yield traits in fig fruits (Al-Qawami *et al*., 2002), apricot trees (Metep and Hasan, 2020), and pomegranate and mulberry trees (Al-Janabi *et al*., 2021). In the recent study, the addition of nano-copper and nano-zinc fertilizers application also raised the chlorophyll content in the leaves and leaf dry weight, which helped boost the plant's physiological activities. The use of nano-fertilizers was vital in the representation processes of carbohydrate and protein in pears (Gobara, 1998) and orange fruits (Hasan *et al*., 2019.

## CONCLUSIONS

Algae extracts were found promising to enhance the shoot length, diameter, leaves per tree, leaf area, leaf dry matter, and percentages of macronutrients, i.e., nitrogen (N), phosphorus (P), potassium (K), and copper (Cu) in apple trees. The other two treatments (nZn and nCu) also gave comparable high values for some characteristics, and moderate values for other traits, besting the control treatment. The foliar application of bioactive compounds and nano-zinc and nano-copper fertilizers showed that marine algae extracts are best performers to improve the growth and quality parameters of apple trees.

## ACKNOWLEDGMENTS

We are grateful to all workers in the laboratories of the Department of Horticulture and Landscape Gardening, Faculty of Agriculture, University of Kufa, Kufa, Iraq, and Department of Horticulture, College of Agriculture, AL-Qasim Green University, Babylon, Iraq.

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