THE COMPLEMENTARY EFFECT OF BIOSTIMULANTS AND NITROGEN FERTILIZER ON IMPROVING THE GROWTH AND PRODUCTIVITY OF SQUASH (CUCURBITA PEPO L.)

M.A. Taha Horticulture Dept., Fac. of Agric., Menoufia Univ., Shebin El- Kom, Egypt. Corresponding author email; <u>mohamed.taha@agr.menofia.edu.eg</u>

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ABSTRACT: Field research experiments were conducted during the two successive growing seasons of 2019/2020 and 2020 in the Experimental Farm, Faculty of Agriculture, Menoufia University to evaluate changes in growth, nutritional contents and yield of squash (Cucurbita pepo L.) under the foliar application of four biostimulant treatments *i.e.,* aqueous moringa leaf extract at 1:30 v/v (MLE) and potent microorganisms at 20 mL L^{-1} (EM1) as natural growth regulators each alone or tougher or in combinations with N at rates of 0, 75 and 100% of the recommended nitrogen fertilization rate. This research was coordinated in a Split plot design in three replications. The results showed that the addition of nitrogen at 100% led to a maximum significant increase in the growth chrematistics, that is, the number of leaves and the fresh and dry weight of leaves compared to the unfertilized control. The chlorophyll contents increase significantly with increasing N supply up to 75% with moringa leaf extract foliar application. The highest N, P and K contents were found in squash leaves treated with N at 100% of the recommended rate. The yield and its components of squash plants increased significantly with the increased of nitrogen supply up to 100% of the recommended rate. Combined application of EM1 and MLE resulted in a significant increase in growth traits and fruit yield compared to the control treatment.

Key words: N rates - EM1 - Moringa oleifera Leaf Extract - squash - quality

INTRODUCTION

Squash is а member of the Cucurbitaceae family and is one of the most frequently grown fruit vegetables around the world. Squash is classified as a high-level vegetable input, due to its high ability to produce a lot of biomass crop and its nutritional requirements in any case (Colla and Saccardo 2003). It is known that excessive nitrogen fertilization may increase plant activity and prolong the plant cycle, causing delayed harvest, nitrate accumulation in plant tissues and environmental pollution (Kant et al. 2011). Unfortunately, chemical fertilizers cause soil degradation and pollute the environment, despite their hiah costs. Recently, in modern agriculture and horticulture finding alternative and innate sources of biostimulants has been the main objective of interest for achieving future cultivation and improving crop yields (Abdalla 2013 and Calvo et al. 2014). Any prosperity in the farming system that leads to higher production must reduce the negative environmental impact of agriculture and enhance the sustainability of the system.

Application of plant extracts as stimulators/growth regulator (PGRs) is one of the approaches recently adopted in agricultural, particularly within a poor agricultural country striving to become economically and more socially advanced. During this endeavour, plant extracts enriched with bioactive compounds are used as growth stimulants due to the presence of allyl chemicals within the plant leaves such as Zeatin in moringa leaves, it has the potential along with other inorganic salts to promote growth expansion and the yield, similarly can alter the range of physiological and biochemical processes in plant cell metabolism (Yaronskaya et al. 2006, Tilly-Mándy et al. 2010 and Ashraf et al. 2016). One of all exogenous biostimulants plant is Effective Microorganisms (EM) which is a mixture of beneficial and effective microorganism which will be set by two methods of soil irrigation or foliar spraying. EM contains preferred types of microorganisms, including dominant groups of lactic acid bacteria, yeasts, smaller numbers of photosynthetic bacteria, actinomycetes and other types of organisms.

All of these are supposed to be mutually compatible with each other and are able to coexist in culture. EM plays a beneficial role in improving flower starter formation due to its effect on carbohydrates accumulation. It also has a stimulating effect on the biological process and amplification, protein and DNA synthesis and chlorophyll formation (Swelam, 2012 and Hamail et al. 2014). The importance of plant biostimulants consist of several bioactive compounds that may enhance various physiological processes, thus stimulating plant growth as well as productivity. on the other hand, reduce chemical fertilizers and increase nutrient utilization efficiency (Bulgari et al. 2015). Moringa leaf extract (MLE) is one of the ultimate plant biostimulants and can be used as a natural and alternative source of mineral 2010). nutrition (Phiri and Mbewe Recently, MLE has received a lot of regarding its beneficial attained applications and it contains cytokinins such as zeatin, antioxidants such as ascorbic acid, amino acids, flavonoid, carotenoids, vitamins A and C, phenolics, macronutrients as well as micronutrients (Rady et al. 2013, Latif and Mohamed 2016 and Gopalakrishnan et al. 2016).

Interestingly, not only the stimulant content of MLE, but also the balance between the contents of phytohormones, nutrients and antioxidants make it an excellent natural prebiotic of plants (Azra et al. 2013). The main hormone observed in MLE is zeatin and its intensity is almost thousands of times higher than that of the most studied plants to date (Rady et al. 2013). MLE has been used as a natural and eco-friendly biostimulant to improve growth and productivity traits of manv crops grown under natural conditions (Rehman et al. 2015, Ashraf et al. 2016, El zaawely et al. 2016 and Nasir et al. 2016) and even under stresses as well (Azra et al. 2013, Howladar 2014 and Latif and Mohamed 2016) and thus results in higher economic return. In addition, Ndubuaku et al. (2015) reported that plant height and number of leaves in cassava plants were increased mandatory for foliar application of MLE. In a comparative study by Aslam et al. (2016) observed that MLE application significantly increased the total chlorophyll content and soluble protein content in spinach leaves compared to growth regulators studied.

MATERIALS AND METHODS Field Experiment

Mielit hybrid squash seeds were used in this study. The seeds were sown on August 24 and 29 in 2019 and 2020 seasons, respectively in an open field on clay loam soil under surface irrigation system. The seeds were sown in rows with a distance of 40 cm between each hill and other. The rows were 4 meters in length and 70 cm in width. Each experimental plot area consists of four rows with a total area of 11.2 m². The physical and chemical analysis was performed by the Soil Testing Laboratory of the College of Agriculture according to standard procedures (Wilde et al., 1985) and the data obtained were presented in Table 1.

	Physical properties						Chemical properties						Bulk
Pra di	actical s stributi	ize on	Texture	рН	EC m.mhos	Organic matter%	Ca	ition me	eq/l	A	nion me	q/l	density
Sand%	Silt %	Clay%					Ca ⁺⁺	Mg **	Na **	Cľ	HCO ₃ ⁻	SO4	g/cm ³
31.89	23.93	41.29	Clay Ioam	7.5	0.42	1.73	0.75	0.56	0.30	4.35	0.60	02.00	1.55

Table (1) The main characteristics of the physical and chemical analysis of soil

Treatments and Experimental Design

This trial consisted of 12 treatments which were all related to three nitrogen fertilizer rates i.e., 0,75 and 100% and four biostimulants i.e., 0, EM1, MLE and EM1+MLE and their combination. The treatments were coordinated in a split plot design, with three replicates. Fertilizer N rates were set as the main plot while, biostimulants treatments were set as a sub plot factor.

NPK Fertilizer Application

A constant dose of calcium super phosphate (15.5% P₂O₅) was received for all experimental plots at 200 kg fed.⁻¹ during land planning. Potassium fertilizer in the form of potassium sulphate (48% K₂O) at 100 kg fed.⁻¹ was tied up in two equal doses, 3 and 6 weeks after sowing. Nitrogen fertilizer doses were used as 0, 75 and 100% of the recommended fertilizer rate. Nitrogen fertilizer was applied based on nitrogen equivalent i.e., 120 kg fed.⁻¹ as the recommended medium using mineral fertilizer from ammonium sulphate fertilizer (20.5% N). The amount of nitrogen fertilizer was divided into four equal parts during soil preparation and the remainder was added to each plot three weeks from sowing and repeated with 2 weeks intervals. Recommended agricultural management practices have been implemented for commercial production of squash (under the supervision of Egyptian Ministry of Agriculture).

Bio-Stimulants Application

Foliar applications including four biostimulants treatments i.e., foliar

application of water, EM1, MLE, and EM1 +MLE. EM1 (Effective microorganisms) at 20 ml L⁻¹. Moringa Leaf Extract (MLE) were prepared by harvesting fresh leaves and extracting them at full maturity stage. For extraction, the aqueous extract of moringa was prepared at a ratio of 1:30 (w/vol) by methods of (Phiri and Mbewe 2010), and the extracts were frozen overnight and pressed in a locally fabricated machine and purified twice by filtering through filter paper (Whatman No.1). After the refining process, the extract was centrifuged at 8000 × g for15 min to the diluted supernatant to obtain the extraction at a concentration of 3% for spraying the plant leaves (Azra et al., 2013) and its mixture plus control treatment (pure water). The spraying treatments started three weeks after the planting date and were repeated 3 times with an interval of 15 days during the growing seasons.

Evaluated Parameters

Plant sampling

In each experimental plot, three plants from the outer two rows were randomly selected for morphological characters and chemical composition, while the middle two rows were selected to determine the yield of fruits and its components.

Vegetative Characteristics Measured

Squash growth parameters data were obtained 1 week after the last foliar application of biostimulants *i.e.*, 65 days for measurements. Number of leaves and fresh weight of leaves as well as its dry weight which were oven dried at 70 °C until the weight stabilized were recorded.

Chlorophyll and Chemical Constitutes

Chlorophyll reading was determined in the fourth of the fully functional mature upper leaves, 1 week after the last foliar application of the biostimulant, using a portable chlorophyll meter (SPAD-502, Minolta Corporation, Ltd., Osaka, Japan). Twenty-one leaves were randomly measured per plot and averaged to a single SPAD value per treatment. Dry leaf samples were digested with concentrated sulfuric acid and H_2O_2 and analysed for N-P-K nutrients as described by Cottenie *et al.* (1982).

Yield and its components

There were eight picking that started at 44 days after planting and seven picking were carried out. Edible fruits were harvested from each sub-plot for eight times and the number of fruits/plants and total yield/plot were recorded by summarizing the successive harvest periods.

Fruit quality

It was determined in the middle of the harvest season using some fruits at random from each plot, as follows:

- 1- The physical characteristics of fruits, such as diameter of the fruit, a sample of five fruits in an edible stage was taken randomly from each sub plot in the second picking to determine the diameter,
- 2- The dry matter percentage of the fruits was determined as 100 g of each sub plot taken and dried at 70 °C to a constant weight and the average of dry weight was determined using standard methods as described in (AOAC, 1990),

3- The percentage of total soluble solids (TSS %) in edible fruit juice was estimated using a hand refractometer.

Statistical Analysis

All data obtained were statistically analysed by ANOVA using the COSTAT (2020) package. Duncan's multiple range test was performed at P = 0.05 on each of the significant variables that were measured.

RESULTS AND DISCUSSION

Plant Growth

The results presented in Tables (2,3 and 4) show the effect of the nitrogen fertilization levels and the implementation of biostimulants either alone or in their combinations effect on the vegetative growth characteristics of squash plants, expressed as the number of leaves and fresh and dry weight of leaves. In terms of fertilizer rates, different effects were observed as there was a prevailing trend on increasing growth traits with increasing fertilizer up to the highest level of 100% N. Plants that obtained 75% N combined with the biostimulants significantly increased growth characteristics with a clear superiority to 100% N relative to unfertilized observation. However, the dry weight of leaves for plants that obtained 100% nitrogen was almost twice that of unfertilized plants. The increase in vegetative characteristics with an increase in the fertilizer rate may be attributed to higher nitrogen supply and an increase in nitrogen uptake and its associated role in chlorophyll synthesis and thus photosynthesis and carbon dioxide assimilation (Jasso-Chaverria et al. 2005). Nitrogen has been described as dominating plant growth by being a major component of chlorophyll, protein and amino acids which plays an important role in photosynthesis activity

(Sumeet *et al.* 2009). In the same way, based on the high leaf biomass, the results can be attributed to the aforementioned increase in leaf number and can be attributed to the role of nitrogen in the formation of dry and fresh matter as well as various energy-rich compounds that regulates photosynthesis and plant production (Wu *et al.* 1998). Our findings coincide with (Pokluda *et al.*, 2018) those of previous squash studies which reported that plants treated with 0, 75, 100 and 150 kg N ha⁻¹ were different from the control group (0 N) about 21.0% on influencing on leaf number and biomass yield of 100 and 150 kg N ha⁻¹ was about 99% higher than that of the control.

Table (2): Effect of biostimulants, nitrogen rates and their interactions on the number of leaves/plants

Treatments		Seasons							
		2019	9/2020			2020/2021			
		N levels							
	0%	75%	100%	Mean	0%	75%	100%	Mean	
Control	18.00 ^a	20.33 ^a	21.33 ^a	19.89 ^B	18.33 ^a	20.67 ^a	25.00 ^a	21.33 ^B	
EM1	20.00 ^a	21.00 ^a	25.33 ^a	22.11 ^{AB}	20.67 ^a	21.33 ^ª	25.63 ^a	22.54 ^{AB}	
MLE	21.00 ^a	23.00 ^a	25.67 ^a	23.22 ^{AB}	22.00 ^a	25.00 ^a	26.43 ^a	24.48 ^{AB}	
EM1 + MLE	21.33 ^a	24.28 ^a	27.67 ^a	24.43 ^A	23.33 ^a	25.67 ^a	28.09 ^a	25.70 ^A	
Mean	20.08 ^B	22.15 ^A	25.00 ^A		21.08 ^B	23.17 ^A	26.29 ^A		

Table (3): Effect of biostimulants, nitrogen rates and their interactions on the fresh weight of leaves (g/plant)

Treatments	Seasons									
		/2021								
	N levels									
	0%	75%	100%	Mean	0%	75%	100%	Mean		
Control	638.85 ^a	864.95 ^a	1233.24 ^a	912.35 ^C	715.42 ^a	947.35 ^a	1352.57 ^a	1005.11 ^C		
EM1	1152.36 ^a	1218.64 ^a	1467.85 ^a	1279.62 ^B	1294.37 ^a	1342.58 ^ª	1549.34 ^ª	1395.43 ^B		
MLE	1234.58 ^a	1438.84 ^a	1589.37 ^a	1420.93 ^B	1329.48 ^a	1536.26 ^a	1684.07 ^a	1516.60 ^B		
EM1 + MLE	1476.54 ^a	1688.28 ^a	1843.33 ^a	1669.38 ^A	1514.21 ^a	1709.34 ^a	1987.94 ^a	1737.16 ^A		
Mean	1125.58 ^B	1302.68 ^{AB}	1533.45 [^]		1213.37 ^B	1383.88 ^{AB}	1643.48 ^{AB}			

Table (4): Effect of biostimulants, nitrogen rates and their interactions on the dry weight of leaves (g/plant)

Treatments		Seasons							
		2019	9/2020		2020/2021				
		N levels							
	0%	75%	100%	Mean	0%	75%	100%	Mean	
Control	46.33 ^a	70.27 ^a	94.42 ^a	70.34 ^B	49.56 ^a	73.48 ^a	102.33 ^a	75.12 ^c	
EM1	68.88 ^a	94.88 ^a	114.47 ^a	92.74 ^A	70.24 ^a	96.85 ^a	123.48 ^a	96.86 ^B	
MLE	66.62 ^a	90.45 ^ª	121.34 ^a	92.80 ^{AB}	69.52 ^a	94.48 ^a	130.45 ^a	98.15 ^{AB}	
EM1 + MLE	90.14 ^a	116.33 ^a	128.36 ^a	111.61 ^A	92.35 ^a	119.48 ^a	139.40 ^a	117.08 ^A	

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Mean	67.99 ^C	92.98 ^{BC}	114.56 [^]	70.42 ^C	96.07 ^{AB}	123.92 ^A	

As for the biostimulants, different effects were observed, the number of leaves and the fresh and dry weight of squash leaves were effectively improved by the application of the biostimulants either individually or in a combination manner relative to untreated treatment. These effects were significant and maximal with MLE application when combined with EM1. EM1contains selected types of microorganisms, including dominant groups of lactic acid bacteria, yeasts, a smaller number of photosynthetic bacteria, actinomycetes and various types of organisms. It is claimed that all these elements are mutually compatible with each other and able to coexist in culture. Also, it has a stimulatory effect on cell division and hypertrophy, protein and DNA synthesis and chlorophyll formation (Hamail et al., 2014).

In addition, moringa leaf extract is high in phytohormones, especially zeatin, gibberellins and ABA (Rady et al., 2013 and Rady and Mohamed, 2015). Moringa leaf extract is also rich in different classes of plant hormones, especially salicylates, including benzoic, cinnamic and salicylic acids. Moreover, moringa leaves are rich in essential amino acids, minerals and vitamins (A, B1, B2, B3, C, and E) as well as antioxidant compounds such as phenolics (Basra et al., 2011, Busani et al., 2011, Howladar, 2014 and El Sohaimy et al., 2015). The effect of size with combinations of Moringa leaf extract + Effective Microorganisms from the single agent may be attributed to the complementarity of each other and this integration could be suggested to increase the effect of the integrated application on growth characteristics. Therefore, these results can also be also attributed to the catalytic effect on photosynthetic pigments and mineral contents (Tables 5, 6, 7 and 8).

Chlorophyll Contents

The results presented in Table (5) show the effect of the levels of N fertilization and the implementation of biostimulants either alone or through combinations as well as the effect of the interactions on chlorophyll contents. Compared with the competent control, chlorophyll reading the increased significantly as the nitrogen supply increased by 100%. A large interaction of chlorophyll content that leads to more efficient use when both are at a satisfactory level (75 and 100 %N of the recommended rate) may be an indication that the nitrogen has been taken up by the plant and then used in cell multiplication, DNA synthesis and energy formation that acts as structural for photosynthesis chloroplast. It has been confirmed that nitrogen is one of the components of chlorophyll formation as described in (Lawlor, 2002). In contrast, it was observed that nitrogen deficiency concentration reduced the individual leaf area index and the total leaf area decreased liaht interception for photosynthesis (Cechin and Fumis, 2004).

Regarding the effect of biostimulants, in general, the results show that spraying squash plants with EM1 or MLE either alone or in combinations increase the chlorophyll content compared to the control. The most effective and significant values were recorded by EM1+ MLE foliar spray. The modified chlorophyll content and photosynthetic vigour of spinach and radish leaves were observed by Aslam et al. (2016) and Ashraf et al. (2016), respectively. The effect of the interactions between nitrogen fertilizers and the biostimulants on chlorophyll contents were consistent, indicating that both factors do not act individually. The superior combinations of treatments were 100%N of the recommended rate associated with the EM1 + MLE mixture compared with all possible combinations tested.

Table (5): Effect of biostimulants, nitrogen rates and their interactions on the chlorophyll content

Treatments		Seasons							
		2019	9/2020		2020/2021				
		N levels							
	0%	75%	100%	Mean	0%	75%	100%	Mean	
Control	39.00 ^ª	41.40 ^ª	41.84 ^ª	40.75 ^c	41.20 ^c	41.48 ^ª	45.90 ^ª	42.86 ^c	
EM1	41.14 ^c	43.40 ^c	47.36 [°]	43.97 ⁸	42.00 ^{cd}	45.04 [°]	48.90 ^c	45.31 ^B	
MLE	41.60 ^b	46.52 ^b	49.46 ^b	45.86 ^{AB}	44.06 ^b	49.00 ^b	51.80 ^{bc}	48.29 ^{AB}	
EM1 + MLE	44.10 ^a	47.40 ^a	51.00 ^a	47.50 [^]	45.20 ^a	50.80 ^a	54.00 ^a	50.00 ^A	
Mean	41.46 ^c	44.68 ^B	47.42 ^A		43.11 ^c	46.58 ^B	50.15 [^]		

Table (6) Effect of biostimulants, nitrogen rates and their interactions on the nitrogen %

Treatments		Seasons							
		201	9/2020		2020/2021				
		N levels							
	0%	75%	100%	Mean	0%	75%	100%	Mean	
Control	2.22 ^f	2.61 ^{с-е}	3.21 ^e	2.68 ^C	2.28 ^e	2.73 ^{c-d}	3.36 ^d	2.79 ^C	
EM1	2.68 ^e	2.86 ^{ef}	3.42 ^{a-d}	2.99 ^B	2.89 ^{ef}	2.94 ^d	3.52 [°]	3.12 ^B	
MLE	2.74 ^{e-f}	2.91 ^{de}	3.53 ^b	3.06 ^A	2.81 ^{ab}	2.98 ^b	3.64 ^b	3.14 ^{AB}	
EM1 + MLE	3.31 ^{a-d}	3.45 ^{c-e}	4.11 ^a	3.62 ^A	3.37 ^a	3.56 ^a	4.13 ^a	3.69 ^A	
Mean	2.74 ^C	2.96 ^B	3.57 ^A		2.84 ^C	3.05 ^B	3.66 ^A		

Treatments		Seasons							
		201	9/2020		2020/2021				
		N levels							
	0%	75%	100%	Mean	0%	75%	100%	Mean	
Control	0.39 ^d	0.43 ^d	0.46 ^c	0.43 ^c	0.40 ^d	0.44 ^d	0.47 ^c	0.44 ^c	
EM1	0.46 ^c	0.51 [°]	0.56 ^b	0.51 ^B	0.47 ^c	0.52 ^c	0.58 ^b	0.52 ^B	
MLE	0.49 ^{ab}	0.56 ^b	0.61 ^{ab}	0.55 ^B	0.49 ^b	0.58 ^b	0.63 ^b	0.57 ^B	
EM1 + MLE	0.50 ^a	0.63 ^a	0.70^a	0.61 ^A	0.50 ^a	0.64 ^a	0.70^a	0.61 ^A	
Mean	0.46 ^B	0.53 ^A	0.58 ^A		0.47 ^B	0.55 ^A	0.60 ^A		

Treatments	Seasons									
		201	9/2020		2020/2021					
		N levels								
	0%	75%	100%	Mean	0%	75%	100%	Mean		
Control	3.30 ^a	3.51 ^a	4.06 ^a	3.62 ^A	3.41 ^a	3.52 ^a	4.10 ^a	3.68 ^A		
EM1	3.76 ^a	3.79 ^a	4.15 ^a	3.90 ^A	3.77 ^a	3.81 ^a	4.16 ^a	3.91 [^]		
MLE	3.77 ^a	3.81 ^a	4.25 ^a	3.94 ^A	3.78 ^a	3.83 ^a	4.29 ^a	3.97 [^]		
EM1 + MLE	3.79 ^a	3.82 ^a	4.32 ^a	3.98 ^A	3.80 ^a	3.84 ^a	4.38 ^a	4.01 ^A		
Mean	3.66 ^B	3.73 ^A	4.20 ^A		3.69 ^B	3.75 [^]	4.23 ^A			

Nutrients Contents

Regardless of the biostimulant treatments, the results show that by increasing the nitrogen supply from 75 to 100% of the recommended rate, the N content becomes stronger and the differences between them are significant. Table (6) displays the response of squash to nitrogen fertilizer with the reaction of biostimulants. Regarding the effect of biostimulants, the results show that spraying squash plants with EM1 or MLE either alone or in their combinations increased the N content compared to untreated plants. These results can be assigned to MLE which is independent of N fertilization and foliar treatment can increase nitrogen accumulation in plant tissues. For the interactions between nitrogen modifiers and biostimulants, the results revealed that the single application of MLE in combination with 100%N were more efficient and gave higher nitrogen content than EM1 application, in both seasons.

The main and interaction effects of biostimulants and nitrogen fertilizer doses on leaf P% are given in Table (7). Differentiation by nitrogen concentrations indicated that the application of 100% N actually increased the leaf P% compared to the control treatment, in both seasons and the P% increased significantly and gradually with the permanent application of EM1 or MLE, indicating that both factors do not act separately. It was determined that the combined applications of EM1 + MLE associated with a supply of 100%N showed a higher P content. As for the K content, compared to the unfertilized treatment, a trend, of a significant increase in the K content with increasing the rate of N up to 100% but it is still statistically similar to other N rates (75%). Treatments with various biostimulants and their interactions with N modifiers had no significant effect on the K% of leaves, as shown in Table (8).

Furthermore, integrated treatments significantly excessed individual treatments. Among all the constituent treatments, the integrative EM1 + MLE + 100% N treatment was the best, with N% increased by 90 % and 77%, P% by 24% and 23% and K% by 26% and 33% in the 2019 and 2020 growing seasons, respectively. The enhancement in nutrient contents (N. P. K) by application of both EM1 and MLE can be attributed to the fact that these extracts are rich sources of mineral nutrients and hormones such as GAs, auxin and zeatintype cytokinin's that increase metabolic processes including nutrient absorption that increases the content of nutrients in plant tissues (Latif and Mohamed 2016 and Hamail et al., 2014).

Yield and Yield Components

Data of the effect of treatments used on yield and its components are presented in Tables (9 and 10). Yield and its components of squash varied widely under different levels of nitrogen or application of biostimulants. The results show that N fertilizer levels significantly (P < 0.05) affected the number of quash fruits per plant and the total yield/plot. effect of Regarding the nitrogen fertilization rates on yield and its components, the results indicate a significant increase with increasing nitrogen supply up to 100% of the recommended rate, in the two seasons compared to the control treatment. Plants that obtained 100% Ν of the recommended rate resulted in higher fruit vield and quality. However, there was a significant decrease in yield and its components when a minimum rate N was applied compared with the other reactions. The increase in fruit yield with the application of nitrogen levels may be due to the effect of nitrogen on

enhancement of vegetative growth and leaf area/plant creating a large surface available for photosynthesis. Nitrogen is an essential nutrient in the composition of plant dry matter and many energy-rich compounds that regulate photosynthesis and plant production; thus, our results are in a complete agreement with the research previously cited by Ng'etich *et al.* (2013) and Shafeek *et al.* (2016).

Table (9): Effect of biostimulants,	nitrogen rates and their interactions on the number of
fruits/plant.	

Treatments	Seasons									
	2019/2020				2020/2021					
		N levels								
	0%	0% 75% 100% Mean 0% 75% 100%								
Control	4.12 ^d	6.14 ^c	8.01 ^d	6.09 ^D	4.19 ^c	6.17 ^c	8.14 ^c	6.17 ^D		
EM1	4.35 [°]	6.64 ^b	8.38 ^{bc}	6.46 ^C	4.47 ^b	6.79 ^b	8.47 ^b	6.58 ^C		
MLE	5.13 ^b	7.68 ^{ab}	8.48 ^b	7.10 ^B	5.27 ^{ab}	7.78 ^{ab}	8.68 ^{ab}	7.24 ^B		
EM1 + MLE	5.29 ^a	7.89 ^a	9.04 ^a	7.41 [^]	5.37 ^a	7.97 ^a	9.18 ^a	7.51 ^A		
Mean	4.72 ^c	7.09 ^B	8.48 ^A		4.83 ^C	7.18 ^B	8.62 ^A			

Table (10): Effect of biostimulants, nitrogen rates and their interactions on the Total yield kg/plot.

Treatments	Seasons									
		2019/2020				2020/2021				
		N levels								
	0%	0% 75% 100% Mean 0% 75% 100% M								
Control	18.11 ^d	20.54 ^d	20.55 ^d	19.73 ^D	20.97 ^d	21.45 ^d	22.35 ^d	21.59 ^D		
EM1	23.48 ^c	24.99 ^{bc}	32.67 ^c	27.05 ^C	24.16 ^c	26.93 ^{bc}	33.73 [°]	28.27 ^C		
MLE	25.48 ^b	26.71 ^b	34.08 ^b	28.76 ^B	25.81 ^b	27.16 ^b	35.11 ^b	29.36 ^B		
EM1 + MLE	25.54 ^a	28.98 ^a	35.54 ^a	30.02 ^A	27.01 ^a	31.26 ^a	37.96 ^a	32.08 ^A		
Mean	23.15 ^C	25.31 ^B	30.71 ^A		24.49 ^C	26.70 ^B	32.29 ^A			

Regarding the biostimulants to treatments, squash yield was positively affected by using all. However, the throughout adjustment was the same: EM1+ MLE > MLE> EM1 > control. The significant increase in the total yield over resulting the control from these treatments was 52.2, 48.6 and 54.8, 36.0 and 37.1, 31.0 % during the two seasons, respectively. Improving the yield and its components by spraying EM1 (Effective microorganisms) can be proposed for its beneficial role of improving flower formation especially female flowers. Moreover, this effect can be attributed to the presence of five subfamilies of microorganisms: a) Lactic acid bacteria: these bacteria are distinguishing by their

sterilizing properties. They strong suppress harmful microorganisms and promote rapid decomposition of organic addition, they substances. In can eliminate the reproduction of the fungus fusarium, which is a harmful. b) Yeasts: make antimicrobial substances that are beneficial for plant growth. Their metabolites are food for other bacteria such as the lactic acid and actinomycete. Actinomycetes: C) these suppress harmful fungi and bacteria and can live simultaneously with photosynthetic bacteria. d) Photosynthetic bacteria: these bacteria play the fundamental role in the activity of EM. They synthesize beneficial substances from secretions of roots, organic matter and/or harmful

gases (e.g., hydrogen sulphide) by using sunlight and the heat of soil as sources of energy. They participate to a better use of sunlight or, in other words, better photosynthesis. The metabolites sophisticated by these micro-organisms are directly absorbed into plants. Along with, these bacteria excess the number of other bacteria and act as nitrogen binders. e) Fungi: that bring about leavening these breaks down the organic substances quickly. This suppresses smell and prevents damage that can caused by harmful insects (Xu, 2000) on sweet corn. This is in agreement with similar observations by Tong (1998) on watermelons, Hafiz and Mahmoud (2003) on squash and Habashi et al. (2007) on cucumber.

Additionally, this significant result probably attributed to moringa extract concentration of 3% which caused rising squash growth parameters, *i.e.*, in content and chlorophyll number of leaves/plant which maximized photosynthesis and increasing sink capacity through supply the photoassimilates from leaves and translocation to build high quality fruit and yield as substantive by Thomas and Howarth (2000), they illustrated that application of MLE extract whereas it is rich with zeatin like cytokinin and this may be induced cytokinin bio-synthesis turned maximum number in of photosynthetic active leaves and that is obvious from number and area of leaves per plant maintaining the chlorophylls in higher concentrations which be inverted on plant yield. Moringa leaves also, contain appropriate from amounts stimulant substances encouraged celldivision cell-enlargement, rate. strengthens plants, eventually produces more and higher yield (Fuglie, 2000). Also, Azooz et al. (2004) informed that moringa extract was capable to enhance the photosynthetic apparatus in treated plant, which leads to increase in plant productivity and fruit dry matter. Moringa leaf extract as well, contains plant growth hormone, called zeatin which has been reported to increase yields by 25 to 30% for nearly any crop as aforesaid by (Jason, 2013). Our results are a line with those found by Palada (1996) who pointed out that yield of tomato was significantly increased when treated by foliar application with moringa leaf extract. Caceres (1999) deduced that moringa leaf extract contains high level of elements and hormone which make it act as growth and yield enhancer. As well as, Culver et al. (2012) revealed that moringa extract applied every two weeks up to harvest significant increases in tomato yield and its components as well as fruit dry matter. Ozobia (2014) noted that moringa extract as foliar application was improved eggplant yield and physical characters of fruit. The same results were affirmed by many researchers as Muhammed et al. (2013) on onion and Bashir et al. (2014) on tomato, Oluwagbenga and Odeghe (2015) and Aluko (2016) on pepper.

Fruit quality

Concerning to the effect of the three levels of N fertilization and foliar application of bio-stimulants besides the interactions effect on fruits quality of squash plants, expressed as fruit diameter, TSS and dry matter %. Data in Tables (11, 12 and 13) show that all combinations treatments led to significant increases in fruit quality contrast to the control treatment (Water foliar application) and at the same time enhanced its components, i.e., fruit diameter, dry matter % as well as total soluble solids. Data also cleared that spraying squash plant with moringa leaf extract effective microorganisms + incorporated with 100% recommended nitrogen rate was sufficient to produce the superiority results of the previous parameters in the two growing seasons.

The prominence effect with the combinations of EM1+ MLE than the single factor may be allocated to the integrated of each other and such this integration could be suggested to increase the effect of the individual application upon growth characters.

Therefore, these results may be also attributed to the stimulatory effect upon photosynthetic pigments and minerals content.

Table (11): Effect of biostimulants, nitrogen rates and their interactions on the diameter of fruit.

Treatments	Season									
	2019/2020				2020/2021					
		N levels								
	0%	0% 75% 100% Mean 0% 75% 100% Mea								
Control	11.07 ^d	11.53 [°]	13.07 ^ª	11.89 ^D	11.33 ^ª	12.40 ^d	13.55 ^ª	12.43 ^D		
EM1	11.60 ^{bc}	12.72 [⊳]	13.14 ^c	12.49 ^c	11.69 [°]	13.30 ^{bc}	14.50 [°]	13.16 ^c		
MLE	12.83 ^b	13.09 ^{ab}	13.50 ^b	13.14 ⁸	12.92 ^b	14.29 ^b	16.74 ^b	14.65 ^B		
EM1 + MLE	13.56 ^a	13.59 ^a	14.96 ^a	14.04 ^A	13.77 ^a	15.35 ^a	17.32 ^a	15.48 ^A		
Mean	12.27 ^c	12.73 ^B	13.67 ^A		12.43 ^c	13.84 ⁸	15.53 [^]			

Treatments	Season								
		201	9/2020		2020/2021				
	N levels								
	0% 75% 100% Mean 0% 75% 100%								
Control	3.05 ^b	3.65 ^b	3.75 ^b	3.48 ^B	3.66 ^b	3.85 ^b	4.25 ^b	3.92 ^B	
EM1	3.65 ^a	4.05 ^a	4.15 ^a	3.95 ^A	3.87 ^a	4.14 ^a	4.20 ^a	4.07 ^A	
MLE	3.75 ^a	4.15 ^a	4.17 ^a	4.02 ^A	4.00 ^a	4.25 ^a	4.29 ^a	4.18 ^A	
EM1 + MLE	3.85 ^a	4.25 ^a	4.29 ^a	4.13 ^A	4.03 ^a	4.30 ^a	4.34 ^a	4.22 ^A	
Mean	3.58 ^B	4.03 ^A	4.09 ^A		3.89 ^c	4.14 ^B	4.27 ^A		

 Table (12): Effect of biostimulants, nitrogen rates and their interactions on TSS%.

Treatments	Season									
		2019	9/2020		2020/2021					
		N levels								
	0% 75% 100% Mean 0% 75% 100%									
Control	4.36 ^c	4.48 ^d	4.57 [°]	4.47 ^c	4.55 [°]	4.87 ^d	5.04 [°]	4.82 ^c		
EM1	4.40 ^b	4.61 ^{bc}	4.83 ^b	4.61 ^B	4.59 ^b	4.83 ^c	5.15 ^b	4.86 ^B		
MLE	4.63 ^a	4.70 ^b	5.24 ^a	4.86 ^B	4.82 ^a	4.89 ^b	5.26 ^a	4.99 ⁸		
EM1 + MLE	4.93 ^a	5.23 ^a	5.28 ^a	5.15 [^]	4.99 ^a	5.16 ^a	5.31 ^a	5.15 [^]		
Mean	4.58 ^c	4.76 ^B	4.98 ^A		4.74 ^c	4.94 ^B	5.19 [^]			

CONCLUSION

In all cases, based on the study, it can be concluded that nitrogen fertilizer at the recommended rate (100%) gave the preferred fruit yield and its components. The combinations of effective microorganisms plus treatment with moringa leaf extract had the best effect on the overall yield and quality. While, the single application of moringa leaf extract gave the highest number of fruits per plant.

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التأثير التكاملي للمنشطات الحيوية والتسميد النيتروجيني في تحسين النمو وانتاجية قرع الكوسة محمد عبد المحسن محمد طه

قسم البساتين – كلية الزراعة – جامعة المنوفية

الملخص العربى

تهدف الدراسة الحالية إلى إظهار الإختلافات فى النمو الخضري والمحتوي الكيماوي والإنتاجية لمحصول قرع الكوسة تحت تأثير الرش بالمخصبات الحيوية بمستخلص مائي لأوراق المورينجا بمعدل (1:30 حجم/حجم) والمخصب الحيوي EM1 بمعدل 20 مل/لتر كمنشطات نمو طبيعية بصورة فردية أو مخلوطهما مع مستويات مختلفة من المعدل الموصي به من التسميد الأزوتى بنسبه 0 ، 75 و 100 % خلال الموسمى الخريفي 2020/2019 ، 2021/2020. تم تصميم هذه التجربة بنظام القطع المنشقة في ثلاث مكررات . أظهرت النتائج أن إضافه النيتروجين بنسبه 100% من الجرعه الموصي بها (120 كجم /فدان) أدي إلى زيادة معنوية كبيرة في صفات النمو الخضري المختلفة والمحصول مقارنة بالكنترول. كما أوضحت النتائج ان إضافة مخلوط كل من المحصب الحيوي ومستخلص اوراق المورينجا مع مستويات التسميد الأزوتى أدت الى زيادة معنوية كليرة في صفات النمو الخضري المختلفة والمحصول مقارنة

أسماء السادة المحكمين

أ.د/ عبدالفتاح حسن سليم قسم النبات الزراعى – كلية الزراعة – جامعة المنوفية
 أ.د/ فتوح أبو اليزيد على فسم البساتين – كلية الزراعة – جامعة المنوفية