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## RESEARCH ARTICLE

### VERIFYING POTENTIAL OF *MORINGAOLEIFERA* EXTRACT APPLICATION AS BIO- FERTILIZER FOR BASIL PLANTS ELICITED WITH GAMMA IRRADIATION AND / OR NANO- ZINC OXIDE TO AMELIORATE BIOMASS QUANTITY AND QUALITY

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#### ABSTRACT

Medicinal plants are in exhaust table source of bioactive secondary metabolites since, the side effects of chemical drugs and the human tendency to make greater use of natural products in order to keep their health as well as problems of modern medicinal system caused more attention of human to medicinal plant. Therefore the present investigation offers an efficient Co- friendly approach to enhance biomass and bioactive secondary metabolites production and quality for sweet Basil, medicinal and aromatic plant. Basil seeds were elicited with exposure to gamma ray doses 0,25,50,150 Gy (G1-4) as physical elicitor, then were planted in field experiment at complete randomized block design for three replicates. plants were ,sprayed solitary fertilized by Moringa leaves aqueous extract (MLE) 0.5%, and NPK 0.5% as. Plants at 5,8,10 month aged were elucidated, foliarly sprayed, with nano – zinc oxide concentration 0,30,50, 80ppb (Z<sub>1-4</sub>) .statistical analysis of variance for recording data on growth, biomass and bioactive secondary metabolites production revealed that, G<sub>2,3,4</sub> and Z<sub>2,3,4</sub> performed positive significant impact on recorded data under MLE that exceeded significantly under NPK, aside Z<sub>2-4</sub> excel G<sub>2-4</sub>. Whereas G integrated with Z achieved synergistic significant positive impact at which GZ integration under MLE excel significantly under NPK application. therefore, the overall results manifest strong field evidence that G,Z, GZ under investigation, could be considered as oriental technological tool reliant at field application ameliorate biomass and bioactive secondary metabolites production and of quality under MLE biofertilize which exceeded NPK application.

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## INTRODUCTION

Sweet Basil (*Ocimum basilicum* L) widely distributed through the warm regions of the world as herbaceous plant with extraordinary medicinal, aromatic and culinary properties (1-4). Basil has a high market demand and commercially important use in culinary application, food flavorings, preservatives, and cosmetics, pharmaceutical and industries (5). The medicinal, culinary and aromatic value depending on its bioactive phytochemical constituents included; alkaloids, flavonoids, phenolics, terpenoids, and essential oil (6-10). Leaves, stems, roots, flowers and seeds have a plethora of biological and pharmacological activities including; antioxidant, anti- inflammatory anti- allergic, immunomodulatory, anti- coagulant, anti- microbial, anti- stress, anti- ulcer, wound healing, anti- cataract, analgesic, antipyretic, anti- hypertensive, anti- diabetic, anti- fertility, central nervous system depressant, cardio protective, gastro protective, hepatoprotective, Reno protective, radio protective, chemo preventive and anti- cancer properties (11-23).

Basil essential oil responsible for characteristic aroma and biological activities, can be used in diverse applications in pharmacological, food, perfume and cosmetic industries. Also, it have the potential to be used as food preservative for cereals, grains, pulses, fruits and vegetables. Further, the use of essential oil with special emphasis on its antibacterial, bactericidal, antifungal and food preservative properties because its consist of a variety of active constituents. These various properties of Basil offer the possibility of using natural, ecofriendly, cost effective, renewable and easily biodegradable antimicrobials for food commodity preservation in the near future (23-28). The dependency on the use of inorganic fertilizer as source of plant nutrients by farmers and their cost is farther associated with land and soil degradation and environmental pollution (29). Thus there is continuous need to search for alternative safe natural sources of plant nutrients. FAO considered that organic agriculture is an effective mitigation strategy to climate change and can build robust soils that adopt better to weather extremes associated with climate changes. It is observed that innovative crops

cultivated under organic system present better quality and similar yields as with those cultivated under conventional system, and in some cases, even higher. Talking all these into account, organic agriculture could also be characterized as innovative and not only as traditional (30). Today, using symbiotic microorganisms with plants as the biofertilizers for providing nutrients is considered (31-34). *Moringaoleifera* L(MO) is cultivated all over the world as it is commonly used as nutritional and medicinal plant (35-36). It is called "Miracle vegetable" because it is both a medicinal and functional food has been used in human and animal nutrition. The young leaves and green pods are common vegetables in various parts of the world (37). Every part of this highly esteemed tree has long been consumed by human and used for various animal forage, fertilizer, foliar nutrient, green manure, biopesticide, water purification, lubrication oil) manufacture of perfume and hair care products (38). Different parts of this plant contain or profile of important minerals, proteins, vitamins, B. carotene, amino acids and various phenolics and provide a rich and rare combination of zeatin with several flavonoid pigments (39-41). So it is a good source of natural anti-oxidants (42). MO tree ranged height from 5 to 10m., it is found wild and cultivated in many countries of the tropics and subtropics (35). It is considered as one of the world's most useful trees, as almost every part of the tree has an impressive effect of food medication and industrial purposes (43-44). Moringa is one of such alternatives being investigated to ascertain its effect on growth and yield of crops and thus can be promoted among farmers as a possible supplement or substitute to inorganic fertilizers (29). Further, several researches have indicated that MO (Family Moringaceae) is a highly valued plant with multipurpose effects (44-47, 41).

It has been extensively investigated that pre-sowing seeds subjected to low gamma irradiation doses may be potentialize growth biochemical and dry biomass production as well as prefill of bioactive secondary metabolite (48-50). Also, elicitation with biotic, abiotic, or physical elicitor (51) represented a useful biotechnological tool to improve the production of secondary metabolites (52-54). Exceedingly since, nano-technology can represent a solution to increase the value of agriculture products and reducing environmental problems. The effect of nano-particles on plants can be bicaenifl (55-58). Nano-Particle, recently used in plants or plant extracts are very attractive and co-Friendly alternative to chemical and physiological methods (59-64). The production of SMs is very low (less than 1% dry weight) and mainly depends of the physiological and developmental stage of plant (65-66). Several biotechnological strategies have been applied to enhance the productivity of desirable SMs from cell, organ and plant (67-68). Elicitation with abiotic and biotic elicitor is one of few strategies that commercial application in the improvement of bioactive secondary metabolites production from plants as well as cell and organ cultures (69-74). Nowadays agriculture needs crop sustainability and the organic cropping system has emerged as an interesting alternative approach with respect to conventional one. On the other hand, the current unfavorable yield gap between organic cropping system has emerged as an interesting alternative approach with respect to conditional one. On the other hand, the current unfavorable yield gap between organic and conventional systems reduces the organic and conventional systems value. Considering the above side according to recent trend and future prospects of various

strategies to direct higher than the average biomass and bioactive secondary metabolites productivity in medicinal and aromatic plants are highlighted. Therefore, field experiment was conducted to evaluate MLE as biofertilizer compared with chemical NPK fertilizer for Basil plants elicited with gamma irradiation and nano-Zinc oxide as elicitors.

## MATERIALS AND METHODS

**Gamma irradiation:** Pre-Sowing sweet Basil, *ocimumbasilicm* L., (SB) seeds (8% moisture) were subjected to gamma irradiation doses 0,25,50,75Gy ( $G_{1-4}$ ) emitted from Co-60 gamma ray source at 1.5KGy/h dose rate.

**Execute field experiment:** At 20 March, 2017, gamma irradiated SB seeds were sown in trays contained soil sand, beat mixed (1:1:1 ratio V/V) subsequently established in greenhouse. Seedlings 4 weeks old were transplanted to field experiment in complete randomized block design for 3 replicates in plots 3x7m, consist 6 rows 60cm apart and 25 cm interspacing, i.e 140 plant plot (6.7 plant /m<sup>2</sup>) and to give target plant population 28140 plant per feedan or 67000 plant / ha. Brackish shallow well water 900ppm were used for irrigation though surface drip irrigation system.

**Fertilization: Nano-zinc oxide application:** Plants 2 month old were multi-repeating elucidated by foliar spray with nano-zinc-Oxide (20nm) at 0, 30, 50, 80 ppb. concentration, every month i.e. 2 time/ each cut.

**Biometric field quantitative traits:** The field experiment was harvested for 3 harvests (every 3 months from transplanting), 5 representative plants / cut for Growth traits, plant height, cm (PH) and fresh herbage weight / plant, g(FHY/P). the means for these 5 plant that were subjected for statistical analysis. Whereas, biomass as fresh herb yield / plot were dried up till 8% humidity the weighted as dry herb yield / plant that converted to dry herb yield /m<sup>2</sup>/ Cut. Total DHY, g/ m<sup>2</sup> for 3 cuts that were subjected to ANOVA statistical analyses as biomass yield production.

### Bioactive secondary metabolites (BSMs)

#### Essential oil % (EO%)

**EO%:** EO was determined according to Masong (75). by continuous extraction (Soxhlet) with acetone. The volatile oil solution obtained is evaporated under reduced pressure, in rotatory evaporator. The oil was weighted and stored in amber colored bottles at 20°C till the further analysis.

**EOY, g/m<sup>2</sup> (Eoy,g/m<sup>2</sup>):** EOY, g/m<sup>2</sup> were determined by multiplying dry herb yield, g/m<sup>2</sup> with EO%.

**EO content:** A GC with a FID detector was used to determine essential oil fraction in the dry basil herb samples. Separation was performed using H-P-5 (Cross linked 5% ME siloxane, 15m x 0.53mm x 1.5µm film) column at helium flow rate 2ml/min, injection temperature program 60°C, 40°C µp to 220°C, 2min at 220°C. Portions of 2µl. of an essential oil solution in hexane were injected onto the analytical column.

**Phenolic compounds:** Dry herb sample (2g) were extracted with ethanol (20ml) overnight in a shaker at room temperature

followed filtration through watman No. 1 filter paper the residues were re- extracted under the same conditions, the combined filtrates were evaporated in rotatory evaporation below 40°C. the obtained extracts after evaporation were weighted to determine the extract yield and stored until further use.

**Total phenolic content (TPC):** TPC was analyzed by folin-Ciocalteu colorimetric method (76). Methanolic extracts (0.1ml) were mixed with 2.5ml distilled water, followed by the addition of 0.1ml (2N) folin – Ciocalteu reagent. Then, 0.5ml 20% Na<sub>2</sub>CO<sub>3</sub> was added after 5min and mixed well. The color was developed after 30min in the dark at 24°C and the absorbance was measured at 760nm by visible spectrophotometer. The absorbance was calibrated using a standard using a standard curve with gallic acid and were expressed as mg of gallic acid equivalent per gram dry weight of leaves.

**Total flavonoid content (TFC):** TFC was determined calorimetrically using the method described by (77). The methanolic herb extract and standard (0.25ml) were mixed with 1.475ml distilled water. Then, 0.075ml. 5% NaNO solution was added. After 5min, the absorbance was measured at 510nm using spectrophotometer. The absorbance were expressed as mg of catechin equivalents per gram dry weight.

**Identification of flavonoids :** UHPLC, was used to separate and identify the flavonoids. The chromatographic system condition were set as follows; mobile phase, 0.03M orthophosphoric acid (A) and methanol HPLC grade (B); detector, UV280-360nm, C<sub>18</sub> Column (5.0µm, 4.6mm inner diameter [ID] X250mm); column oven temperature, 35°C; and flow rate, 1.0ml/min, Gradient elution was performed as follows; 0-10min in, 10% B; 10-15min in, 50% B; 15-20min; 100%B; and finally 5min for washing. Standards of, quercetin, kaempferol, rutin were used.

**Statistical Analysis:** All data were analyzed as a completely randomized design with three replicates. Means between treatments were separated by analysis of variance (ANOVA) and then statistically significant differences were assessed by calculated least significant difference (LSD) at 1% level. Plants were fertilized by foliar spray with two sole, fertilizers; NPK 15: 15: 15 (200Kg/ ha) and *Moringaoleifera* L aqueous leaves extract (MLE), 0.5%. Moleaves were purchased from herbal market (Cairo, Egypt), were powdered and passed through sieve No, 20, Aqueous extract 100gm. with 600ml. of water in a soxhlet extractor for 18-20h. the extract was concentrated to dryness. Under reduced pressure and controlled temperature (40-50°C). the extract was dissolved with water to get 0.5% concentration which has been used as bio- fertilizer.

## RESULTS

### Growth parameters

**Plant height, Cm (PH):** Mean of 5 representative, plant height, cm (PH) at 1-3 cut as well as its pooled for MLE were significantly increased by 3% over NPK application G<sub>2,3,4</sub> also increase 7,5,3% of control under NPK at that 8, 6, 4% of control under MLE application respectively

(Table<sub>1</sub>). Z<sub>2,3,4</sub> also achieved significantly scale up (PH) to 8,6,4% of control at NPK aside, 9,7,5% over control for MLE, respectively. Concerning, G<sub>2,4</sub> integrated with Z<sub>2,4</sub> actuated synergistic significant increase (PH) ranged up to, 11-18% over control for MLE and 9-16% over control for NPK. Exceedingly G<sub>2</sub>Z<sub>4</sub> achieved the best highest 16, 18% for NPK and MLE respectively (Table<sub>2</sub>Figure<sub>1</sub>).

**Fresh herb yield, g/ plant (FHY/P):** At such 1-3 cut, the mean (FHY/P) as well as its pooled mean. significantly by 2% for MLE than for NPK application at control level (table 1) G<sub>2,4</sub> acted significant increment (FHY/P) 6,3,2% control for NPK and 8, 5, 4% control for MLE. While Z<sub>2,3,4</sub> actuated 4,5,8% control for NPK and 6,7,10% control for MLE application, respectively. At that, G<sub>2,4</sub> integrated with Z<sub>2,4</sub>. Impacted synergistic increment (FHY/P) ranged 11-21% over control for NPK and 15-23% for MLE application. Further G<sub>2</sub>Z<sub>4</sub> represented the best application 21,23% for NPK and MLE, respectively (Table 1).

### Biomass production

**Dry herb yield / m<sup>2</sup>: (DHY, g/m<sup>2</sup>):** DHY g/ m<sup>2</sup>, 456.9 at NPK application (as standard control) whereas MLE application was 470.69. exceeded significantly 3% over NPK application (Table 2). G<sub>2,4</sub> actuated significant appraise up to 8, 5, 4% over control at MLE aside. 7,4,3% over control at NPK application. Z<sub>2,4</sub> achieved significant increase (DHY, g/m<sup>2</sup>) by 6,7,10 % over control at MLE and 5,6, 9% over control at MLE, respectively (Table 2). G<sub>2,4</sub> integrated with Z<sub>2,4</sub> resulted in significant synergistic positive impact for (DHY, g/m<sup>2</sup>) ranged 14-24% over control at MLE and at 12-22% over control at NPK application. G<sub>2</sub>Z<sub>4</sub>, Showed the best high DHY, g/m<sup>2</sup> as 24, 22% of control at MLE and NPK application, respectively (Table<sub>2</sub>Figure<sub>1</sub>).

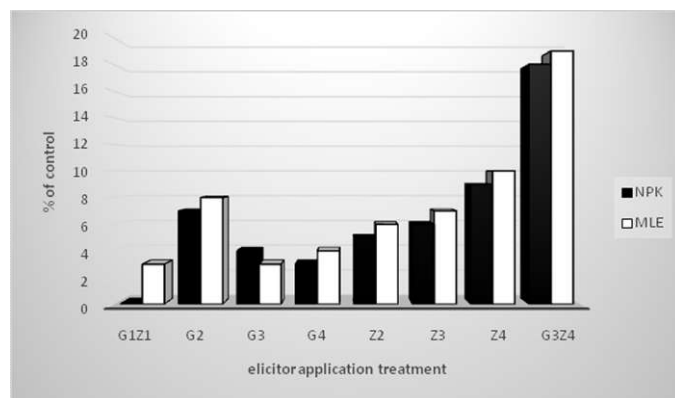


Figure 1. Dry herb yield g/m<sup>2</sup>

### Bioactive secondary metabolite (BSMs):

#### Essential oil (EO)

**Essential oil percent (EO %):** EO %, 0.411 for NPK (as standard control) increased significantly for 0.428 as 5% over NPK control (Table<sub>2</sub>). G<sub>2,4</sub> performed significant increase EO% by 6, 4, 3% over control at NPK application, respectively that exceeded to 8, 6, 5% over control, respectively at MLE application, also, Z<sub>2,4</sub>, increased significantly EO% by 7, 9, 10% of control at NPK that exceeded to 9, 11, 12% over control at MLE application, respectively.

**Table 1. Gamma irradiation and/ or nano. Zinc Impacted as elicitors on plant height, Cm and fresh herb yield/ plant, g. for Baselpantsfertilized by NPK, MLE**

Elicitor	Plant heigh,cm								Fresh herb yield, g / plant							
	1 <sup>st</sup> cut		2 <sup>nd</sup> cut		3 <sup>rd</sup> cut		Pooled <sub>3</sub> cuts		1 <sup>st</sup> cut		2 <sup>ND</sup> cut		3 <sup>rd</sup> . cut		Pooled <sub>3</sub> cuts	
	NPK	MLE	NPK	MLE	NPK	MLE	NPK	MLE	NPK	MLE	NPK	MLE	NPK	MLE	NPK	MLE
G <sub>1</sub> Z <sub>1</sub> Control	41.6(100)	42.8 (3)	45.3 (100)	46.7 (3)	53.7(100)	55.3 (3)	140.6(100)	144.8(3)	341.1(100)	348.0(2)	375.2(100)	382.8(2)	420.7(100)	429.2(2)	1137.0(100)	1160.0(2)
G <sub>2</sub>	45.1(7)	45.6(8)	485 (7)	48.6 (8)	57.2 (7)	58.0 (8)	150.4(7)	151.8(8)	361.6(6)	368.4(8)	397.7(6)	405.2(8)	445.9(6)	454.3(8)	1205.0(6)	1227.9(8)
G <sub>3</sub>	44.3(5)	44.7 (6)	47.2(5)	47.7(6)	56.1(5)	56.6 (6)	147.6(5)	149.0(6)	351.3(3)	358.1(5)	386.5(3)	394.0(5)	433.3(3)	441.7(5)	1171.1(3)	1193.8(5)
G <sub>4</sub>	43.5(3)	44.5 (4)	46.7 (3)	47.7(4)	55.3(3)	55.5(4)	144.8(3)	164.2(4)	348.0(2)	354.8(4)	382.7(2)	390.2(4)	429.1(2)	437.5(4)	1159.8(2)	1182.5(4)
Z <sub>2</sub>	54.6(8)	46.0(9)	48.9 (8)	49.4 (9)	57.7 (8)	58.2 (9)	151.8(8)	153.3(9)	354.8(4)	361.6(6)	390.2(4)	397.7(6)	437.5(4)	445.9(6)	1182.5(4)	1205.2(6)
Z <sub>3</sub>	44.7 (6)	45.1(7)	47.7(6)	48.1 (7)	56.6 (6)	57.2 (7)	149.0(6)	150.4(7)	358.1(5)	365.0(7)	394.0(5)	401.5(7)	441.7(5)	450.1(7)	1193.8(5)	1216.6(7)
Z <sub>4</sub>	43.9 (4)	44.3(5)	46.8(4)	47.2(5)	55.5 (4)	56.1(5)	146.2(4)	147.6(5)	368.4(8)	375.2(10)	405.2(8)	412.7(10)	454.4(8)	462.8(10)	1228.0(8)	1250.7(10)
G <sub>2</sub> Z <sub>2</sub>	47.3 (12)	48.5(15)	50.4 (12)	51.8 (15)	59.8 (12)	61.4 (15)	157.5(12)	161.7(15)	392.3(15)	399.1(17)	431.5(15)	439.0(17)	433.8(15)	492.2(17)	1307.6(15)	1330.3(17)
G <sub>2</sub> Z <sub>3</sub>	48.1(14)	48.9 (16)	51.3 (14)	52.2 (16)	60.9 (14)	62.0 (16)	160.3(14)	163.1(16)	402.5(18)	409.3(20)	442.8(18)	450.3(20)	496.4(18)	504.8(20)	1341.7(18)	1364.4(20)
G <sub>2</sub> Z <sub>4</sub>	48.9(16)	49.8 (18)	52.2(16)	53.1(18)	62.0 (16)	63.4(18)	1361(16)	165.9(18)	412.8(21)	419.6(23)	454.0(21)	461.5(23)	509.0(21)	517.4(23)	1375.8(21)	1398.5(23)
G <sub>3</sub> Z <sub>2</sub>	64.4(10)	47.3(12)	49.5 (10)	50.4(12)	58.8(10)	59.8 (12)	145.7(10)	157.5(12)	385.4(13)	388.8(14)	424.0(13)	427.8(14)	475.4(13)	479.6(14)	1284.8(13)	1296.2(14)
G <sub>3</sub> Z <sub>3</sub>	46.8(11)	47.7(13)	50.0 (11)	50.8 (13)	59.3 (11)	60.4(13)	156.1(11)	158.9(13)	395.7(16)	402.5(18)	453.2(16)	442.8(18)	488.0(16)	496.4(18)	1318.9(16)	1341.7(18)
G <sub>3</sub> Z <sub>4</sub>	47.7(13)	48.9 (16)	50.8(13)	52.2(16)	60.4(13)	62.0 (16)	158.9(13)	163.1(16)	405.9(19)	409.3(20)	446.5(19)	450.3(20)	500.6(19)	504.8(20)	1353.0(19)	1364.2(20)
G <sub>4</sub> Z <sub>2</sub>	46.4 (10)	468(11)	49.5 (10)	50.0(11)	58.8 (10)	59.3 (11)	154.7(10)	156.1(11)	378.6(11)	385.4(13)	416.5(11)	424.0(13)	467.0(11)	457.4(13)	1262.1(11)	1284.8(13)
G <sub>4</sub> Z <sub>3</sub>	46.0 (9)	47.3(12)	49.1(9)	50.4(12)	58.2 (9)	59.8(12)	153.3(9)	157.5(12)	388.8(14)	399.1(17)	427.8(14)	439.0(17)	479.6(14)	492.2(17)	1296.2(14)	1330.3(17)
G <sub>4</sub> Z <sub>4</sub>	46.2(11)	43.9 (14)	49.3(11)	64.8(14)	58.6 (11)	55.5 (14)	154.1(11)	146.2(14)	382.0(12)	392.3(15)	420.2(12)	431.5(15)	471.2(12)	483.8(15)	1273.4(12)	1307.6(15)
LSD%	0.2		0.3		0.4		0.7		1.5		2.3		2.7		5.3	

G<sub>1-4</sub>were: 0,25,50,75Gy, respectively.Z<sub>1-4</sub>were: 0,30,50,80ppb, respectively.

Values between parenthesis were percent over control.

**Table 2. Gamma irradiation and / or Nano-zinc impacted dry herb Biomass,g/m<sup>2</sup>,essential oil% (EO%) and essential oil yield, g/m<sup>2</sup> for Basel Plants fertilized by Moringaleaves extract and NPK**

Elicitor	DHB,g/m <sup>2</sup>		EO%		EOY,g/M <sup>2</sup>	
	NPK	MLE	NPK	MLE	NPK	MLE
G <sub>1</sub> Z <sub>1</sub> 0 control	456.9 (100)	470.6 (3.0)	0.411 (100)	0.428 (4.0)	187.8 (100)	201.4 (7.0)
G <sub>2</sub>	488.9 (7.0)	493.5 (8.0)	0.436 (6.0)	0.444 (8.0)	213.2 (14.0)	219.2 (17.0)
G <sub>3</sub>	475.2 (4.0)	479.8 (5.0) 475.2 (4.0)	0.428 (4.0)	0.436 (6.0)	203.4 (9.0)	209.2 (11.0)
G <sub>4</sub>	470.2 (3.0)	484.3 (6.0)	0.423 (3.0)	0.432 (5.0)	199.1 (6.0)	205.3 (9.0)
Z <sub>2</sub>	479.8 (5.0)	488.9 (7.0)	0.440 (7.0)	0.448 (9.0)	211.1 (13.0)	216.9 (16.0)
Z <sub>3</sub>	484.3 (6.0)	502.0 (9.0)	0.448 (9.0)	0.456 (11.0)	216.9 (16.0)	222.9 (19.0)
Z <sub>4</sub>	498.0 (9.0)	530.0 (16.0)	0.452 (10.0)	0.460 (12.0)	225.1 (19.0)	321.1 (23.0)
G <sub>2</sub> Z <sub>2</sub>	525.4 (15.0)	548.3 (20.0)	0.468 (14.0)	0.477 (16.0)	245.9 (30.0)	252.8 (34.0)
G <sub>2</sub> Z <sub>3</sub>	543.7 (19.0)	566.6 (24.0)	0.477 (16.0)	0.481 (17.0)	259.4 (38.0)	263.8 (40.0)
G <sub>2</sub> Z <sub>4</sub>	557 (22.0)	575.4 (26.0)	0.489 (19.0)	0.506 (23.0)	272.6 (45.0)	286.7 (52.0)
G <sub>3</sub> Z <sub>2</sub>	520.9 (14.0) 534.6 (17.0)	525.4 (15.0) 539.2 (18.0)	0.461 (12.0)	0.473 (15.0)	240.2 (27.0)	248.5 (32.0)
G <sub>3</sub> Z <sub>3</sub>	539.2 (18)	543.7 (19.0)	0.473 (15.0)	0.477 (16.0)	252.9 (34.0)	457.2 (36.0)
G <sub>3</sub> Z <sub>4</sub>	511.7 (12.0)	520.9 (14.0)	0.481 (17.0)	0.485 (18.0)	259.4 (38.0)	263.7 (40.0)
G <sub>4</sub> Z <sub>2</sub>	516.3 (13.0)	530.2 (16.0)	0.465 (17.0)	0.469 (14.0)	237.9 (26.0)	244.3 (30.0)
G <sub>4</sub> Z <sub>3</sub>	530.0 (16.0)	534.6 (17.0)	0.369 (15.0)	0.473 (17.0)	242.2 (33.0)	250.7 (36.0)
G <sub>4</sub> Z <sub>4</sub>	511.7 (12.0)	520.9 (14.0)	0.465 (17.0)	0.469 (14.0)	237.9 (26.0)	244.3 (30.0)
LSD%	1.3		0.003		1.5	

G<sub>1-4</sub> were: 0,25,50,75Gy, respectively.Z<sub>1-4</sub>were: 0,30,50,80ppb, respectively.

Values between parent his were percent over control.

Integrated G<sub>2-4</sub> with Z<sub>2-4</sub> actuated synergistic significant increments ranged 12-19% for NPK exceeded to 14-23% for MLE application Further G<sub>2</sub>Z<sub>4</sub>. showed excel range 19, 23 for NPK and MLE, respectively (Table 2, Figure 2).

**Essential yield, g/m<sup>2</sup> (EOY, g/m<sup>2</sup>):** EOY g/m<sup>2</sup>. 187.8 for NPK application, increased significantly up to 201.4 due to MLE

application which represented 7% over that NPK (Table 2). G<sub>2-4</sub> resulted significant increase EO% 14, 9, 6% of control at NPK application and 17, 11, 9% of control at MLE application, respectively while Z<sub>2-4</sub> also led to significant increase 13, 16, 19% over control at NPK application and 16, 19, 23% over control for NPK and MLE, respectively (Table 2). G<sub>2-4</sub> integrated with Z<sub>2-4</sub> acted significant synergistic positive

impacts (EOY, g/m<sup>2</sup>) ranged 26-45% over control at NPK and 30-52% over control at MLE application, exceedingly, G<sub>2</sub>Z<sub>4</sub> showed the best application treatment 45, 52% of control for NPK and MLE, respectively (Table 2, Figure 3).

**Essential oil contents (EOC):** The main chemical components for Basil EO were, methyl- chavicol%, linolool. %eugenol % geraniol% Linalyl% Total content significance- positive impacted with MLE>NPK application MLE acted

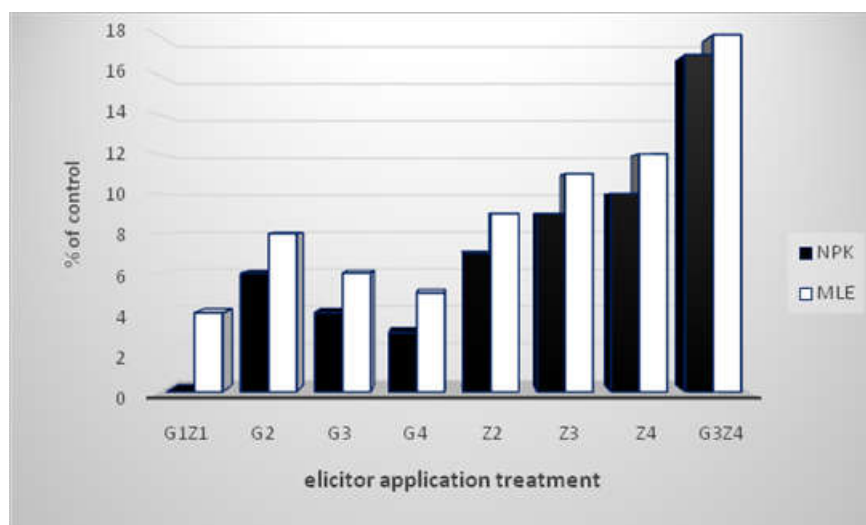
**Table 3. Gamma irradiation and / or Nano-zinc as elicitor actuated essential oil content on Basil plants fertilized with NPK and MLE**

Elicitor	% EO content											
	Methyl-chavicol%		Linalool%		Eugenol%		Geraniol%		Total content		Linalyl%	
	NPK	MLE	NPK	MLE	NPK	MLE	NPK	MLE	NPK	MLE	NPK	MLE
G <sub>1</sub> Z <sub>1</sub> 0 control	22.031 (100)	22.692 (3.0)	12.075 (100)	12.437 (3.0)	9.254 (100)	9.624 (4.0)	0.126 (100)	0.134 (6.0)	0.652 (100)	0.691 (6.0)	44.148 (100)	45.578 (3.0)
G <sub>2</sub>	23.133 (5.0)	24.508 (8.0)	12.678 (5.0)	13.432 (8.0)	9.532 (3.0)	10.298 (7.0)	0.129 (2.0)	0.148 (8.0)	0.678 (4.0)	0.754 (9.0)	46.150 (4.6)	49.140 (11.0)
G <sub>3</sub>	22.912 (4.0)	24.280 (7.0)	12.558 (4.0)	13.308 (7.0)	9.439 (2.0)	10.202 (6.0)	0.130 (3.0)	0.144 (7.0)	0.672 (3.0)	0.746 (8.0)	45.711 (3.6)	48.670 (10.0)
G <sub>4</sub>	22.692 (3.3)	24.054 (6.0)	12.438 (3.0)	13.184 (6.0)	9.346 (1.0)	10.106 (5.0)	0.127 (1.0)	0.142 (6.0)	0.665 (2.0)	0.740 (7.0)	45.268 (2.6)	47.628 (8.0)
Z <sub>2</sub>	23.133 (5.0)	24.620 (8.5)	12.714 (5.3)	13.432 (8.0)	9.365 (1.2)	10.394 (8.0)	0.130 (3.2)	0.145 (8.2)	0.667 (4.2)	0.749 (8.3)	46.009 (4.0)	49.340 (12.0)
Z <sub>3</sub>	23.352 (6.0)	24.734 (9.0)	12.779 (6.0)	13.557 (9.0)	9.550 (3.2)	10.316 (7.2)	0.131 (4.0)	0.146 (9.0)	0.674 (3.5)	0.755 (9.3)	46.486 (5.0)	49.508 (12.0)
Z <sub>4</sub>	23.574 (7.0)	24.692 (10.0)	13.041 (8.0)	14.915 (11.0)	9.624 (4.0)	10.134 (5.3)	0.133 (5.0)	0.149 (11.0)	0.684 (5.0)	0.767 (11.0)	47.056 (7.0)	50.567 (14.6)
G <sub>2</sub> Z <sub>2</sub>	23.352 (6.5)	24.258 (9.6)	12.859 (6.5)	13.606 (9.4)	9.578 (3.5)	10.586 (10.0)	0.132 (4.4)	0.146 (29.3)	0.688 (5.0)	0.760 (10.0)	46.609 (6.0)	49.356 (12.0)
G <sub>2</sub> Z <sub>3</sub>	23.463 (7.7)	25.974 (10.5)	12.920 (7.0)	13.706 (10.2)	9.716 (5.7)	10.490 (9.0)	0.133 (5.5)	0.147 (10.0)	0.692 (6.0)	0.774 (12.0)	64.924 (6.3)	50.191 (14.0)
G <sub>2</sub> Z <sub>4</sub>	24.014 (9.0)	25.415 (12.0)	13.405 (11.0)	14.178 (14.0)	9.902 (7.0)	10.414 (8.2)	0.134 (6.0)	0.150 (12.0)	0.697 (7.0)	0.780 (13.0)	48.151 (9.0)	50.937 (16.0)
G <sub>3</sub> Z <sub>2</sub>	24.675 (12.0)	26.096 (15.0)	13.766 (14.0)	14.552 (17.0)	9.856 (6.5)	10.616 (10.3)	0.138 (9.0)	0.154 (15.0)	0.710 (9.0)	0.794 (15.0)	49.145 (11.4)	52.212 (16.0)
G <sub>3</sub> Z <sub>3</sub>	24.234 (10.0)	25.710 (13.3)	13.282 (10.0)	14.054 (13.0)	9.670 (4.5)	10.682 (11.0)	0.136 (8.0)	0.153 (14.0)	0.700 (7.3)	0.782 (13.2)	48.022 (9.0)	51.381 (16.0)
G <sub>3</sub> Z <sub>4</sub>	24.014 (9.0)	25.415 (12.0)	13.405 (11.0)	14.178 (14.0)	9.902 (7.0)	10.414 (8.2)	0.134 (6.0)	0.150 (12.0)	0.697 (7.0)	0.780 (13.0)	48.151 (9.0)	50.937 (16.0)
G <sub>4</sub> Z <sub>2</sub>	23.706 (7.6)	25.029 (10.3)	12.968 (7.4)	13.742 (10.5)	9.588 (3.6)	10.827 (12.5)	0.132 (4.7)	0.148 (10.4)	0.682 (4.5)	0.764 (10.5)	49.145 (11.4)	52.212 (18.3)
G <sub>4</sub> Z <sub>3</sub>	24.344 (10.5)	25.800 (13.7)	13.282 (10.0)	14.078 (13.2)	9.688 (4.7)	10.682 (11.0)	0.134 (7.0)	0.151 (13.0)	0.701 (7.5)	0.784 (13.5)	47.076 (6.7)	50.510 (4.4)
G <sub>4</sub> Z <sub>4</sub>	24.454 (11.0)	25.982 (14.5)	13.404 (11.0)	14.178 (14)	9.948 (7.5)	10.422 (8.3)	0.138 (9.5)	0.154 (15.0)	0.705 (8.0)	0.789 (14.5)	48.149 (9.1)	51.495 (16.7)
LSD												0.015

G<sub>1-4</sub> were: 0, 25, 50, 75 Gy, respectively.

Z<sub>1-4</sub> were: 0, 30, 50, 80 ppb, respectively.

Values between parent his were percent over control.



**Figure 2. Essential oil %**

sum, 5 main EO content by significant 3% increment over that NPK application (Table 2). Sole G, Z performed significant positive impact for sum 5 main EOcontent aside Z> G. whereas, Gintegrated with Z resulted in Synergistic significant positive impact in sum 5 main content of EOup to 11.4, 18.3% for G<sub>2</sub>Z<sub>4</sub> at NPK,MLE, respectively (Table 2).

**Total phenolic content (TPC):** Table (4), represented that TPC, mg Gallic acid equivalent content (TPC, mgGA/g.&.w), showed significant increase up to 3% under MLE over NPK application G<sub>2,3,4</sub> actuated significant increase 7.6, 5% of control at MLE and 5.4,3%of control at UPK application, respectively.

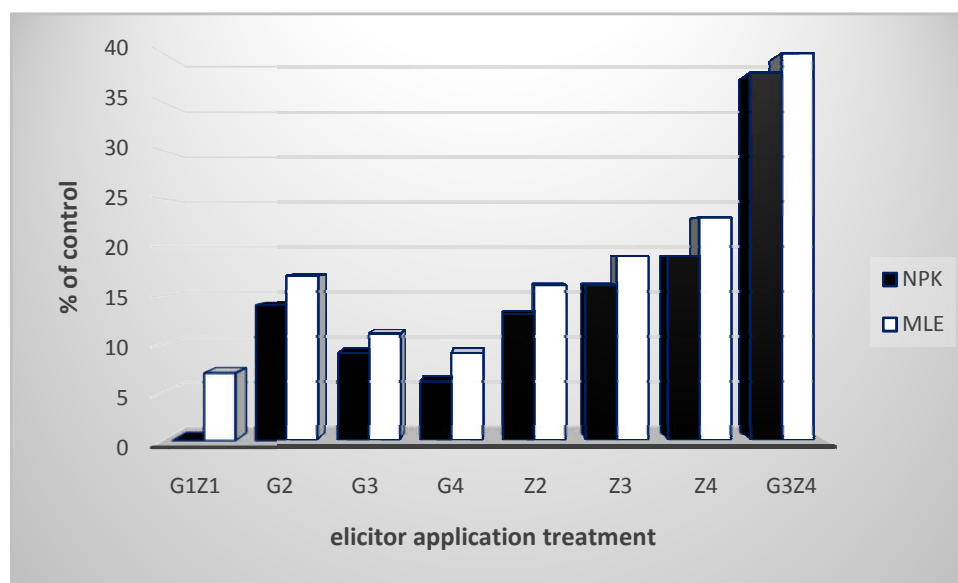
**Table 4. Elicitation With gamma irradiation, and / or Nano-zinc as elicitors impacts on Secondary Metabolites for Basil plants fertilized with NPK and MEL**

Elicitor	TPC (mgGAE/g.d.w)		TFOC (mgCE/gd.w)		Quercetin (mgGAE/g.d.w)		Kaempferol (mgGAE/g.d.w)		Rutin (mgGAE/g.d.w)	
	NPK	MLE	NPK	MLE	NPK	MLE	NPK	MLE	NPK	MLE
G <sub>1</sub> Z <sub>1</sub> 0 control	48.25 (100)	52.11 (8)	22.32 (100)	23.44 (5)	2.68 (100)	2.76 (3)	1.73 (100)	1.80 (4)	1.16 (100)	1.25 (8)
G <sub>2</sub>	50.66 (5)	51.63 (7)	23.66 (6)	24.10 (8)	2.86 (7)	2.89 (8)	1.88 (9)	1.90 (10)	1.25 (8)	1.30 (12)
G <sub>3</sub>	50.18 (4)	51.14 (6)	23.21 (4)	23.66 (6)	2.82 (5)	2.84 (6)	1.85 (7)	1.82 (5)	1.21 (4)	1.25 (8)
G <sub>4</sub>	49.601 (3)	50.66 (5)	22.98 (3)	23.44 (5)	2.78 (4)	2.82 (5)	1.82 (5)	1.85 (7)	1.19 (3)	1.24 (7)
Z <sub>2</sub>	52.11 (8)	52.59 (9)	24.99 (12)	25.44 (14)	2.84 (6)	2.86 (7)	1.87 (8)	1.92 (11)	1.27 (10)	1.28 (11)
Z <sub>3</sub>	53.08 (10)	54.04 (12)	25.67 (15)	26.12 (17)	2.92 (9)	2.95 (10)	1.88 (9)	1.90 (10)	1.30 (12)	1.35 (14)
Z <sub>4</sub>	53.56 (11)	54.52 (13)	26.78 (20)	27.23 (22)	2.97 (11)	3.00 (12)	1.90 (10)	1.94 (12)	1.34 (15)	1.38 (19)
G <sub>2</sub> Z <sub>2</sub>	57.9 (20)	58.38 (21)	27.90 (25)	28.79 (29)	3.05 (14)	3.11 (16)	2.07 (20)	2.16 (25)	1.45 (25)	1.49 (29)
G <sub>2</sub> Z <sub>3</sub>	58.86 (22)	59.35 (23)	28.35 (27)	29.01 (30)	3.10 (16)	3.14 (17)	2.25 (30)	2.34 (35)	1.56 (35)	1.56 (42)
G <sub>2</sub> Z <sub>4</sub>	60.32 (25)	61.67 (28)	30.13 (35)	30.58 (37)	3.18 (19)	3.29 (23)	2.46 (42)	2.51 (45)	1.69 (46)	1.72 (48)
G <sub>3</sub> Z <sub>2</sub>	55.00 (14)	55.97 (16)	27.23 (22)	27.67 (24)	3.00 (12)	3.08 (15)	1.99 (15)	2.11 (22)	1.39 (20)	1.44 (24)
G <sub>3</sub> Z <sub>3</sub>	56.45 (17)	57.42 (19)	28.12 (26)	28.57 (28)	3.11 (16)	3.14 (17)	2.18 (26)	2.22 (28)	1.43 (23)	1.74 (27)
G <sub>3</sub> Z <sub>4</sub>	57.9 (20)	58.86 (22)	29.24 (31)	29.68 (33)	3.16 (18)	3.19 (19)	2.28 (32)	2.38 (38)	1.54 (33)	1.56 (35)
G <sub>4</sub> Z <sub>2</sub>	55.48 (15)	56.45 (17)	27.00 (21)	27.45 (23)	3.03 (13)	3.05 (14)	1.97 (14)	2.06 (19)	1.454 (25)	1.48 (28)
G <sub>4</sub> Z <sub>3</sub>	55.97 (16)	56.93 (18)	27.90 (25)	28.34 (27)	3.08 (15)	3.11 (16)	2.11 (22)	2.16 (25)	1.47 (27)	1.50 (29)
G <sub>4</sub> Z <sub>4</sub>	56.93 (18)	57.90 (20)	28.79 (29)	29.01 (30)	3.14 (17)	3.16 (18)	2.19 (29)	2.23 (29)	1.51 (30)	1.53 (32)
LSD 1 %		0.2		0.13		0.04		0.02		0.03

G<sub>1-4</sub>were: 0,25,50,75Gy, respectively.

Z<sub>1-4</sub>were: 0,30,50,80ppb, respectively

Values between parent his were percent over control.



**Figure 3. Essential oil yield, g/m<sup>2</sup>**

Also, Z<sub>2,3,4</sub> invoked significant increase 9, 12, 13% of control at MLE and 8, 10, 11% of control at NPK, respectively. Whilst G<sub>2-4</sub> integrated with Z<sub>2-4</sub> exhibited synergistic significant positive impact ranged 16-28, 12-25% of control for MLE and NPK, respectively aside G<sub>2</sub>Z<sub>4</sub> excite the best integrated application (Figure 4).

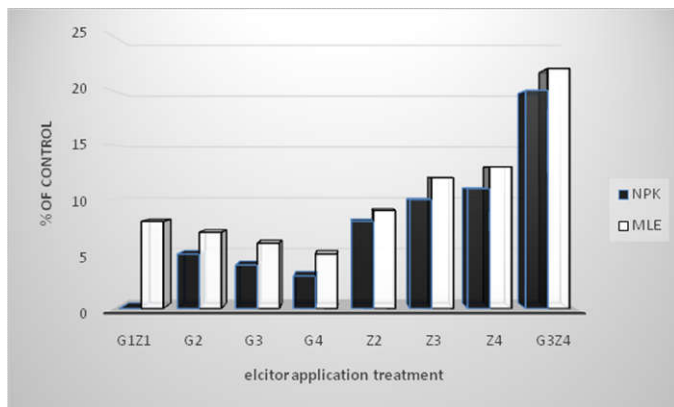


Figure 4. Total phenolic content, mg Gallic acid .equivalent/g.d.w

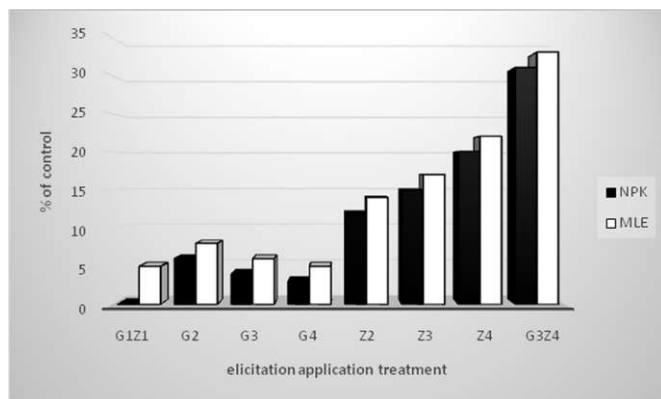


Figure 5. Total flavonoids content, mg catechin equivalents/g.d.w

**Flavonoids identification (FI):** Quercetin, kaempferol and rutin, that were identified from flavonoids, were increased 3,4,8% at MLE over that of NPK application. at zero control whereas, G<sub>2,3,4</sub> achieved significant increase (8, 6,5%), (10,5,7%), (12,8,7%), of control at MLE and (7,5,4%), (9,7,5%), (8,4,3%) of control at NPK for quercetin, kaempferol and rutin, respectively. At that, Z<sub>2,3,4</sub> also resulted in significant increase (7,10,12%), (11, 10, 12%), (11,14,19%) of control at MLE and (6,9,11%) (8,9,10%), (10,12, 15%) of control at NPK application, respectively (Table 4 Figure 4). Concerning G<sub>2-4</sub> integrated with Z<sub>2-4</sub> achieved synergistic significant positive impact ranged (14-23%), (19-45%), (24-48%) of control at ML E application whereas at NPK were (13-19%), (14-42%), (20-46%) of control for quercetin, kaempferol and rutin, respectively. Exceedingly, G<sub>2</sub>Z<sub>4</sub> exhibited the best integrated application (23,45,48%) of control at MLE and (19,42,46%) of control at NPK, respectively (Table 4). respectively.

## DISCUSSION

At G<sub>1</sub>, Z<sub>1</sub>, MLE resulted significant positive impact as percent of NPK for growth (3PH, 2FH, g/P), Biomass (3DHy, g/m<sup>2</sup>), SMs (4EO%, (7EOY, g/m<sup>2</sup>), chavicol, 3% linolool. % euggenol, 6% geraniol 6% linalyl 3% Total continentiutin). Therefore:

MLE potent meliorate SB biomass production and quality as biofertilizer and organic elicitor. These findings were in line and confirmed recently (78-82). Elicitation with sole, (G) or (Z) invoked significant positive impact growth biomass SMs production and quality under MLE over NPK. whereas, G integrated with Z achieved synergistic significant positive impact aside under MLE exceeded significantly under NPK. These findings owing to Nano- particles, abiotic and biotic elicitors can have beneficial effects on morphological physiological characteristics that led to enhance herb morphology physiological characteristics that led to enhance herb biomass and BSMs production and quality (83-86,51). At that elicitors promoted growth and accelerated SMs accumulation by regulating the expression of the metabolite biosynthesis related genes (87,88,82).

## Conclusion

Overall, the results emphasis to recommend solitary- elicitation with low gamma irradiation dose (G) or nano- zinc oxide (Z) under Moringa leaves extract (MLE) as biofertilizer or conventional chemical (NPK) fertilizer; significantly promoted growth, appraising biomass, bioactive secondary metabolites production and quality for Basil (*Ocimum basilicum*). Whereas, Integrated (GZ) achieved synergetic significant responses, aside (G), (Z), (GZ) under (MLE) exceeded significantly (NPK). Therefore, highlight biofertilizer (MLE) that could be substitute conventional chemical fertilizer incorporated with reliable elicitation with G, Z, or GZ, in attaining appraisal biomass yield and main health- promoting secondary metabolites.

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