

The Potentiality Biotic- Elicitation with Chitosan or Vitamin C to Achieve Integrated and Sustainable Development for Sage *Salvia Officiealis* Under Sustainable Agriculture Systems

Tarek Elsayed S. A., El Sayed S. A.

Radioisotopes Department, Nuclear, Research Center, Egyptian Atomic Energy Authority, Cairo, Egypt

Email address:

dr.tarekelsayed64@gmail.com (Tarek E. S. A)

To cite this article:

Tarek Elsayed S. A., El Sayed S. A. The Potentiality Biotic- Elicitation with Chitosan or Vitamin C to Achieve Integrated and Sustainable Development for Sage *Salvia Officiealis* Under Sustainable Agriculture Systems. *Journal of Plant Sciences*. Vol. 9, No. 4, 2021, pp. 151-162. doi: 10.11648/j.jps.20210904.14

Received: July 22, 2021; Accepted: August 7, 2021; Published: August 24, 2021

Abstract: Sage (*Salvia Officiealis*) an ornamental and medicinal plant, is cultured in sustainable agriculture system world wide especially in Mediterranean region. It has long used and well conducted in traditional and official medicine pharmaceutical, food, cosmetic and perfume industries because in its diverse biologically active compounds. Therefore, field experiment was conducted for two subsequent seasons (2019 and 2020) in factorial split – plot design for design for three replications. The main plot; elicitors; chitosan (CH), vitamin C (VC) and non- elicitor (NE). Whereas, the sub- main plot; (NPK) as chemical fertilizer and bio- organic fertilizer, humic acid (HA), moringa dry leaves extract (ML). The statistical analysis of variance for the recorded analysis of variance for the recorded data revealed that multi- repeating elicitation with (CH), (VC) along (ML), (HA), (NPK) achieve highly significantly positive impacts on biomass leaves yield, g. /m², essential oil components and antioxidant activity of sage, in which (CH) excel at (VC) along (ML) excel at (VC) along (ML) excel at (HA) excel at (NPK) at both two seasons. These results support the potent CH, VC to achieve integrated sustainable development of sage under biofertilizers (ML), (HA) that excel at chemical fertilizer (NPK) without accreditation on agrochemical microbiocides and / or insecticides.

Keywords: Elicitation, Chitosan, Vitamin C, Aromatic and Medicinal Plant, Biofertilizers

1. Introduction

Sage, *Salvia officinal*, is an aromatic medicinal plant belong to the family Lamiaceae, is a perennial woody shrub native to Mediterranean area and is cultivated all over the world often used culinary preparations and folk medicine for various health condition [1] So, is the source of a vast variety of bioactive compounds valuable for pharmaceutical and food [2] Aerial parts of *S. officinalis* in treatment many diseases such as diabetes, cancer, hot flushes, obesity, diarrhea as well as for regulation of cholesterol level and for memory improvement [3-4] So, is used as natural remedy in treating and curing arterial hypertension, bowel, stomach and spinal cord disorder, respiratory tract, inflammation, physical and mental fatigue, nervousness, skin ulceration,

caught, bronchitis, dental abscess and cellulitis ([5]. Single extracts possess very strong antioxidant and antineurodegenerative properties [6]. Also, so is an effective alternative agent to reduce the severity of psychological premenstrual symptoms (PMS) [7]. (So) leaves contain a diversity of bioactive secondary metabolites (BSMs) [8-11], it has proved among other positive biological effect it has exceptional antioxidant, antimicrobial activity [12]; on a wide range. SMS, Such as essential oil and its terpenes and phenolics, flavonoids, flavonols are the reasons for bioactive health effects [9-13].

SMS, has potential importance on human health [14-16] and play a major role in adaptation of plant to the changing environmental over coming biotic and abiotic stresses [17-21].

Sage essential oil (SEEO), is an (1), effective alternative agent to reduce the severity of psychological and physical

symptom of the premenstrual syndromes PMS [7]. (2), Showed in vivo chemopreventive properties against skin cancer and two human lung cancer, cell line significantly inhibition [22]. (3), The different molar ratio of compounds contained the may exert significant on their biological efficacy and their components demonstrated antimicrobial, antifungal, and food preservative [23, 24]. (4), can be used be used as alternative to synthetic fungicides, promising for use in integrated pest management [25]. (5), anticancer activity and its principal constituents against hormone – dependent tumor cells [26]. EO, has been development of alternative strategies to reduce bactericides, virocidic, pesticides as well as elicitation to upraise biomass production and quality [27]. Consequently, renewed interest has been placed on discovery and use of natural bioactive resources in medicinal and aromatic plants to control disease ([28, 29]. Bioactive secondary metabolites (BSMs) phenolics, flavonoids, flavonols, phytoalexin, essential oil, quonone which has beneficial properties and pharmaceutical function [30, 31] were very low and mainly depends of physiological and environmental stage of plant [32-34]. Also, play a major role in the adaptation of plants to the changes environment and overcoming biotic and abiotic stresses [17, 20]. Elicitation, also qualitative improvement BSMs, essential oil which has microbiocides, pesticides characteristics [35, 36]. Exceedingly, improving the health promoting qualities. EO can play as potential source of natural biocide for inexpensive and environmental friendly disease control method [37-42]. Also, Eos effective and safe natural as biopesticides for crop protection [43; 38; 44]. It can be considered a potential innovative technological strategy in plant protection and biological control and co-friendly alternative to chemical microbiocides and insecticides [45].

Elicitors, application can be used to increase SMs production in the plants and to enhance its qualitative values for fresh produce enriched food, or as a raw ingredient for feed/ food and pharmaceutical products [46]. The elicitation technique is one of the strategies employed in the cultivation of the medicinal plants to increase the content of BSMs [47]. Elicitors are defined as natural or synthetic (biotic, or abiotic) substances that, when applied to plants in small concentrations, initiate or increase the synthetic BSMs [48]. Biotic and abiotic elicitation is a reliable application invitro and in vivo to improve biomass and BSMs production and quality [49-56]. Elicitation, stimulating BSMs production and quality and accumulation in leaves through enhancing the transcription of biosynthetic genes involved in BSMs biosynthesis pathway ([57]. The potential of elicitors to induce systemic resistance (ISR) prior to infection leading SM phytoalexin in [58-60, 35]. Successful practical application of initiating sMs, phytoalexins PAs formation and accumulation by elicitors confirmed the validity and practicability for ISR as biological control and crop protection [17, 35, 58] against microbial and pests. Therefore, elicitation can be considered a potential strategy in plant protection and biological control [21].

Chitosan (CH) is a natural biopolymer modified from chitins which act as a potential biostimulant and elicitor in agriculture. It is non-toxic, biodegradable and biocompatible which favors potentially broad application, it enhanced the physiological response and mitigate the adverse effect of abiotic stresses through stress transduction pathway via secondary messenger (s). Chitosan treatment stimulate photosynthetic rate, stomatal closure through ABA synthesis; enhances antioxidant enzymes via nitric oxide and hydrogen peroxide signaling pathways and induces production of organic acids, sugars, amino acids, and other metabolites which are required for osmotic adjustments, stress signaling, and energy metabolism under stress [61]. It has widely applied in the field of agriculture environment, pharmaceutical, medicinal and industrial food processing [62-65] CH, effects on growth yield attributes and physiological activities [62, 66-73]. improved quantitative production and enhancing tolerance of crop plants to biotic and abiotic stresses [63] exhibited strong antifungal [74], antibacterial [62, 75], nematocidal [76], virucidal [77, 78], and bio-insecticide [79] for horticultural crops. Also, vitamin C (ascorbic acid) can be used as biotic elicitor to enhance [80].

Since the beginning of green revolution the agriculture has changed by excessive use agrochemicals, fertilizers, microbiocides, [81] in order to increase production. These agro chemicals has been in discriminately used not only in grains and horticulture but also for medicinal and aromatic plants [34]. Agrochemicals impact the environment preventing sustainable development [82-84]. Several studies indicated and association between the increase use of these agrochemicals and health – related problems [85, 4, 87]. Medicinal and aromatic plants are compatible with organic cultivation practices, also have a tendency for producers and consumers. In sustainable agriculture and organic farming systems. The application of bio- organic fertilizers to increase soil fertility are considered as alternative methods for chemical fertilizers. Organic agriculture (OA) has been growth in recent years and the pharmaceutical manufacture of organic medicinal and aromatic plants has strongly increased during the past decade. Therefore biofertilizers to improve plant growth is the key factor in organic culture under organic farming condition, may be provide positive impact on plant growth and productivity [88-90]. Solitary bio- fertilizers affect the crop yield of medicinal and aromatic plants, also affected quality and quantity of EO [91-94]. The application of biofertilizers [95, 96] capable to improving plant growth yield production and quality [97] and reducing problems associated with the use of chemical fertilizers [98, 99].

Many reports have shown the positive impact of biofertilizers on plant growth and productivity [88-90, 100].

Moringa, *Moringa oleifera*, (MO), Is called Miracle vegetable because it is both medicinal and a functional food can be promoted among farmers as a possible supplement or substitute inorganic fertilizer.

Every parts of (MO), Been consumed by human and used

for nutrient, green manure, biopesticide, [101]. Mo one of the such alternative being investigated to ascertain its effect on growth and yield of crops and thus can be promoted among farmer as a possible supplement or substitute to inorganic fertilizers. (MO) leaves are rich in protein (28%) and contain reasonable amounts of amino acids [102] and high amount of mineral's nutrients [103] autstaning of vitamin A, B and C, contain Ca, K, [104] (MO) Leaves have been reported to be valuable source of both macro and micro- nutrients, rich source of B- carotene, Protein, Vitamin C calcium and potassium and acts as good source of natural antioxidants [13, 105]. (MO) essential elements; Mg, Ca, Na, K range 10-12 to 10.99 mg/Kg, 2.6 to 5.64 mg / K., 4.3 to 5.2 mg/Kg, 1.26 to 1.77 mg/ Kg, respectively. Among the heavy metals concentrations of Cu fall in the range 0.81-1.44 mg/Kg while that of zinc fall in the range of 0.37 mg/Kg both lying below toxic level. The level toxic metals (Cd, Cr, Pb and Hg were not detected). The results of this study indicate that the concentration of the entire essential and heavy metals are below the range of WHO/ FAO limits [106]. It is concluded that (MO) leaf extracts can be recommended to be used effectively by farmers as bio-organic fertilizer for various crops due to its high productivity, high nutritive value antioxidant effect, easy preparation, low cost and environmental nature [101]. (M/O) could be easily applied as a natural fungicide against fungal pathogens of mainly important plants [107, 35]. (MO) leaves extract accelerated growth of young plants, strengthened plants, improved resistance to pests and diseases, increased leaf duration, increased number of roots, produced more and larger fruits and generally increased yield by 20-35% [11, 108, 109].

(MO) promoting growth and suppressed plant diseased Accelerate plant growth and induce ISR against pathogen and decrease its development [110]. (ME) Eco- friendly fungicide for plant diseases control [107]. (MO) Achieved improvement (promoting) plant growth and suppressed plant diseased. Accelerated plant growth and induce ISR against pathogen growth and decrease development in line with [110].

(MO), as bio-organic fertilizer [101] can be recommended to be used as for various crops due to its high productivity, high nutritive value antioxidant easy preparation low cost and environmental nature [101] MO) green manure, bio pesticide [101] one of such alternative being investigated to ascertain its effect on growth and yield of crops and thus can be promoted among farmers as possible sublement or substitute to inorganic ferlizer [11], (MO) leave, have insecticidal potential [111] in medicinal plants.

The application of humic acid (HA) the in vivo cultivation of medicinal plants opens up the opportunity for the development of organic fertilizer for agricultural systems aiming at good quality raw material without pesticides microbiocides, pesticides with increased concentration of SMs biologically and pharmacologically interesting [112-114]. Application of (HA) in medicinal plant in vivo can contribute to increase of the biosynthesis of SMs metabolites and to the activity of bioactive substances from different classes, such as

flavonoids, coumarins, total phenols, total flavonoids [36]. (HA), increased growth development and organogenesis of the plant in the field, influencing on differentiation of the vegetative tissues in the production of flowers [115], increased fresh or dry biomass with EO content [116]. (HA) enhancing growth, yield phytochemical components [116] (HA) increase of the biosynthesis of BSMs [73].

On the fath of that has been mentioned hereinbefore, the ultimate of the recent reteaches in this area; has been development of alternative control strategies to reduce depending on thynthetic microbiocides, pesticides as well as elicitation to upraise biomass, SMS production and quality and enhancing health benefits compounds. Also, to provide evidence of the usefulness of strategies aiming to limit agrochemical, as well as the potential of elicitation, in sustainable plant protection for agriculture since plant protection strategies are often insufficient and the application of chemical – based pesticides, microbiocides has negative effect on animal, human and the environment. Novel greener tools could present efficient alternative for management pests and plant diseases using promising strategies, the use of elicitation. therefore, the present study has been conducted to evaluate the potentiality of biotic elicitation to achieve integrated and sustainable development for sage. *Salvia officinal* is under sustainable agriculture systems.

2. Material and Methods

2.1. Practical Field Experimental Designe

Two subsequent seasons field experimental trial 2019 and 2020, were designed as factorial split plot designee based randomized complete block designee with 3 replications. The main plote 3 elicitors, non- elicitor (NE), Chitosan (CH) 300 ppm, vitamin C (VC) 300 ppm, whereas, sub- main plot were three fertilizers, NPK 15: 15: 15, 209/m² (and 30 ml/L from solution of 5% from 5% from each 5% Fe, Zn, Mg, Cu were added) as traditional fertilizer, Humic acid (HA) 5 g/m², and moringa dry leaves extract (ML), 5 g/m² as bio fertilizers.

Therefor, 3 elicitors (NE, CH, VC) entracted with 3 fertilizers (NPK, ML, HA) formed 9 application treatments (T1-9).

Sage seeds were sown 1st march for both seasons, 2019, 2020 (as one season vegetative crop) in plots 3.5 x 2m 5 Rows / Plot and 70, 40 Cm enter and intra spacing.

2.2. Elicitor and Fertilizer Application

The resultant one month old plants at both two seasons (2019, 2020) were foliarly sprayed monthly with (NE), (CH) and (VC) and fertigated with (NPK), (HA) and (MLE), up tile one month before harvesting at 1st Ougust (at full flowering).

2.3. Biomass and Bioactive Compound Yield Production and Quality

2.3.1. Biomass Yield

Aerial parts, especial leaves were harvested at 1st Ougust

2019 and 2020 then were air dried for determination dry biomass yield, Kg/m².

2.3.2. *Quli-quantitative Bioactive Secondary Metabolites (BSMs) Assay*

Extraction procedure:

The samples of Ssga powder 915 g.) were placed in the filter cartridge in a classical soxhlet apparatus and apparatus and extracted with 150 ml of an apparatus and extracted with 150 ml of an appropriate solvent for 3 h. for this extraction, two solvents were used, ethanol (100%) and ethyl acetate (100%). The samples of saga extracts were stored in glass vials wit Teflon sealed at 20±0.5°C in the absence of light.

Total penolic content (TPC) assay:

TPC was assayed by folin – ciocalau clorimetric method [117] Methanolic extracts (0.1 ml) was mixed with 2.5 ml. distilled water followed by the addition of 1 ml (2N) folen – cicaltea ragnet. Then 0.5 ml 20% Na₂CO₃ was added after 5 min and mixed well the color was developed after 5 min and mixed well the color was developed after 3 min in the dark at 24°C and the absorbance was measured at 760 nm by vesible spectrophotometer. The absorbance was calibrated using a standard surve wit gallic acid and were expressed as mg of gallic acid equivalent per gram dry weight of leaves.

Total flavonoid content (TFC) assay:

TFC was determined calorimetrically using the method described [118] the methanol leaves extract standard (0.02 ml) were mixed wit 1-475 ml distilled water. Ten 0.075 ml 5% NaNO₃ solution was added. After 5 min, the absorption was measured at 510 nm using spectrophotometer the absorbance were expressed as mg. of quercetin equivalents per gram dry leaves weight.

Total flavonols assay:

Total flavonols content (TFL) were estimated as rutin equivalents (RE), expressed as mg ruting/g callus extract [119]. The rutin calibration curve was prepared by mixing 2 mL of 0.5-0.015 mg mL⁻¹ rutin ethanolic solutions with 2 mL (20 gL⁻¹) AlCl₃ and 6 mL (50 gL⁻¹) of sodium acetate. The absorbance of reaction mixture was read at 440 nm after 2.5 h at 20°C. the same procedure was carried out wit 2 mL of callus extract (10 gL⁻¹) instead of rutin solution.

Antioxidant activity (AA):

The DPPH radical scavenging activity was tested by the method of [120]. Briefly, various treatments under investigation,. DPPH solution was also prepared by dissolving 6.0 mg. of DPPH in 100 ml methanol then, 1.0 ml of from treatment was added into the test tube containg 2.0 ml of DPPH solution control was prepared by adding 1.0 of methanol to 2 ml of DPPH solution. The mixture was shaken vigorously and was left to stand in the dark for 30 min. the absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The scavenging activity of DPPH radical was calculated using the following equation: Scavenging activity (%)=(1-(A sample at 517 nm)/(A control at 517 nm))×100

Essential oil% (EO%):

EO was determined according to Masong (2005). RM by

continuous extraction (Soxilet) 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 til to the farther analysis.

Essential oil yield, g./m² (EoYg./m²):

Egyg/m² were determined by multiplying dry leaves yield, g./m² with EO%.

Essential oil contents:

Compositions of EO were determined by GC- FID and GC- MS analyses they were achieved on an Agillary Technologies 7890GC equipped wit FID and mass spectrophotometer detctors using a HP – 5 MS% capillary column (30.00m X 0.25, 0.25 µm film thicknesses). The carrier gas was belium at a flow of 0.8 ml/ min. initial column temperature was 60°C/min. the splite ratio was 40: 1. The injector temperature was set at 300°C. the acquisition range was 50-550 m/Z in electron impact (E1) mode using an ionization voltage of 70 ev. The assential oils were diluted 1:100 in n – hexan, then 0.1 µL were injected into GC systems.

Identification of EO components:

Identification of the components were performed on the bases of retention index (R1), determined wit reference of the homologous series of n- alkonos, C2-C30, under identical experimental conditions, comparing with the mass spectra library search (NIST and wiley), and wit the mass spectra literature date. The relative amounts of individual components were calculated based on CG peak area (FID response).

Statistical analysis:

The data sets were firstly tested for normality by the Anderson and Darling normality tests using a statistical analysis system (SAS) (SAS 2003). Also, in both two subsequent seasons (2019 and 2020) there interaction was not significant, therefore. The pooled mean values of 2 year for all the traits were subjected to statistical analysis of variance was done for all traits. A least significant difference (LSD) test was used for mean comparison of treatment at 1% level.

3. Results and Discussion

Biomass, Essential Oil Yield Production

3.1. Biomass, Dry Leaves Yield, g/m² (DLY, g²)

Malti- repeating biotic elicitation wit (CH) or (VC) performed significant as as% control (NE NPK), as the following: 25, 20, 15% for T6 (CH.MO), T5 (CH.HA), T4 (CH.NPK), respectively, 21, 17, 10% for T9 (VC.MO), T8 (VC.HA), T7 ((VC.NPK), 18, 11, 0% for T3 (NE. NPK) T2 (NE.NPK), T1 Zero control= 929.3 g/m² as represented Table 1 and Figure 1.

In dispit, there is no pest and microbial diseases incidence in the field experiment in both two seasons (2019, 2020) without using any agrochemical pesticide and / or microbioside.

Many reports, that subborted, back up our results, declared positive impacts on yield productivity for elicitation and /or

biofertilizer that excel traditional chemical fertilizers [71, 36, 94, 80, 72, 88, 90, 89, 100, 96, 67, 98, 99].

Also, MO [108]. And HA [116] exhibited positive impacts on yield productivity as biofertilizers.

Table 1. *S. officinalis* Dry leaves yield, g/m² essential oil% (EO%), essential yield, g/m² (EOY, g/m²) in response to elicitation with non- elicitor (NE), chitosan (CH), vitamin C (VC) under fertilizer; (NPK) humic acid (HA) and moringa dry leaves extracts (ML) at two seasons 2019 and 2020.

Application Treatment	Dry leaves Yield, g. /m ²			Essential oil%			Essential oil yield, g. /m ²		
	2019	2020	Pooled mean	2019	2020	Pooled mean	2019	2020	Pooled mean
T1- (NE / NPK)	927.4	931.2	929.3(0)	2.35	2.33	2.34(0)	2117.3	2175.9	2176.6(0)
T2- (NE / HA)	1028.4	1025.7	1031.5(11)	2.63	2.61	2.62 (12)	2704.7	2701.9	2703.3(24)
T3-(NE / ML)	1093.3	1090.9	1096.6 (18)	2.75	2.72	2.74 (17)	3006.6	3003.8	3005.2(38)
T4- (CH / NPK)	1065.5	1068.3	1066.9(15)	3.10	3.12	3.11 (32)	3303.1	3305.3	3304.2(51)
T5- (CH / HA)	1111.8	1109.9	1110.8(20)	3.17	3.15	3.16 (35)	3524.4	3530.7	3524.1(62)
T6-(CH / ML)	1158.1	1162.7	1160.4(25)	3.31	3.33	3.32 (42)	3833.3	3834.9	3834.1(76)
T7-(VC / NPK)	1019.2	1015.9	1017.5(10)	2.94	2.91	2.93 (25)	2996.4	2995.8	2996.1(38)
T8- (VC / HA)	1084.0	1081.7	1082.8(17)	3.06	3.07	3.06 (30)	3317.0	3318.5	3313.5(52)
T9- (VC / ML)	1121.1	1122.3	1121.7(21)	3.20	3.22	3.21(37)	3587.5	3586.9	3587.2(65)
LSD1%			13.9			0.03			7.2

Values between parenthesis were percent of control (NE / NPK).

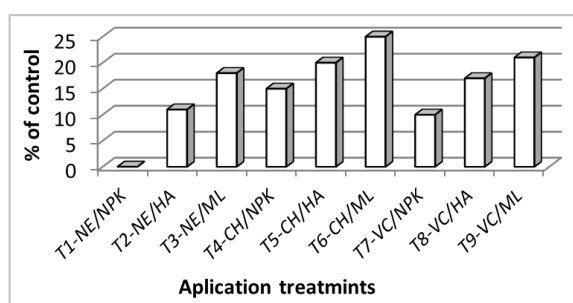


Figure 1. Dry leaves yield, g/m² for sage elicited by BH, VC under NPK, HA, ML.

3.2. Essential Oil Percentage (EO%)

Multi – repeating elicitation with CH or VC resulted significant positive impact on EO% in dry leave yield expressed as percent over that of control T1 (NE/ NPK) = 2.34% along ML > HA > (NPK) > (NENPK), as the follong; 42, 35, 32% T6 (CH/ML), T5 (CH/HA), T4, (CH/NPK), respectively 37, 30, 25% T9 (VC/ML), T8 (VC/ HA), T7 (VC/NPK) respectively excel, 17, 12, 0% T3 (NE/ML), T2 (NE / HA), T1 (NE/ NPK) Zero control =2.34% as represented Table 1 and Figure 2.

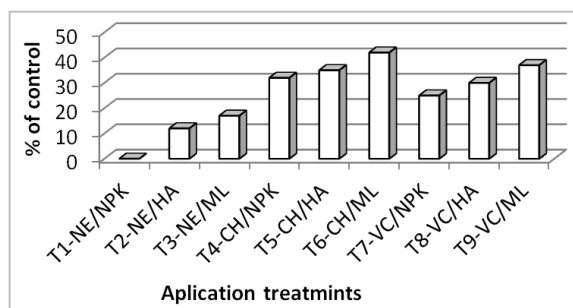


Figure 2. Essential oil percentage for sage elicited by BH, VC Under NPK, HA, ML.

3.3. Essential Oil Yield, g/m² (EOY, g-m²)

Multi repeating CH or VC elicitation along ML, HA,

NPK evoked significant appraise EOY, g/m² as percent over control (T₁ NE/ NPK = 2176.6 g/m²) that has been listed in table 1 and representing Figure 3 as the following; 76, 62, 51% T6 (CH/ML), T5 (CH/ HA), T4 (CH/ NPK), respectively;

65, 52, 38% T9 (VC/ML) T8 (VC/HA), T7 (VC/NPK) respectively;

38, 24, 0% T3 (NE/ML), T2 (NE/HA), T1 (NE /NPK) Zero control = 217.6 g/m², respectively Table 1 Figure 3.

These results were compatible with that has been declared by [80, 69, 93, 39, 94, 72].

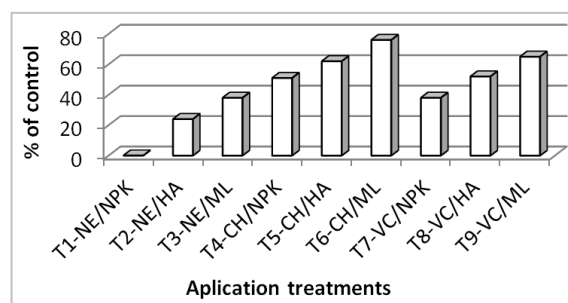


Figure 3. Essential oil yield, g/m² for sage elicited by BH, VC Under NPK, HA, ML.

4. Bioactive Secondary Production and Quality

Multi- repeating CH or VC elicitation integrated with biofertilizers ML, HA and traditional NPK agrochemical fertilizer evoked significant inhancement expressed as percent of control T1 (NE/NPK) for the following traits.

4.1. Total Phenolics Contents as mg GA/g. Dry Leaves

96, 82, 75% T6 (CH/ML), T5 (CH/HA), T4 (CH/ NPK), respectively;

91, 78, 63% T9 (VC/ ML), T8 (VC/HA), T7 (VC/NPK), respectively;

30, 25, 0% T3 (NE/ML,) T2 (CH/ HA), T1 (NE/ NPK) Zero

Control = 126.3 mg GA / g dryleaves), Table 2 Figure 4.

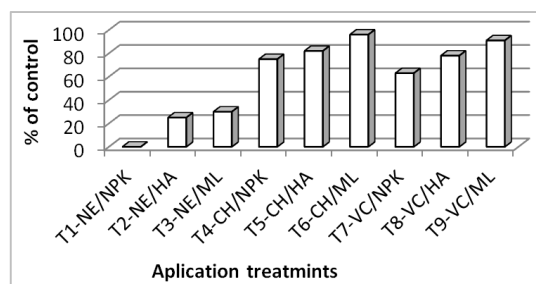


Figure 4. Total phenolic, mgGA/g dry leaves for sage elicited by BH, VC under NPK, HA, ML.

4.2. Total Flavonoids Content, as mg Ru/ g.dry Leaves (TFNC, mg Ru/g.DL)

85, 73, 69% T6 (CH/ML), T5 (CH/HA), T4 (CH/NPK) respectively;

67, 63, 53% T9 (VC/ML), T8 (VC/HA), T7 (VC/NPK) respectively;

35, 25, 0% T3 (NE/ML), T2 (NE/HA), T1 (NE/NPK) Zero control = 215.0 mg /gDL) aslisted Table 2 represented Figure 5.

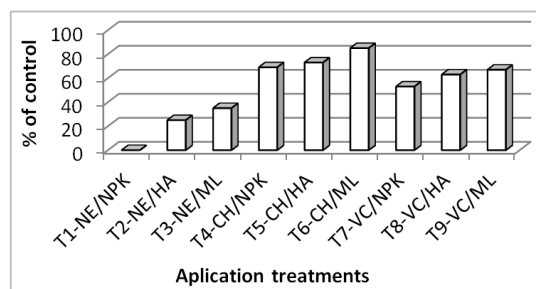


Figure 5. Total flavonoids, mg/g.dry leaves for sage elicited by BH, VC under NPK, HA, ML.

4.3. Total Flavonols Content, as mg Ru/ g.dry Leaves (TFNC, mg Ru/g.DL)

74, 54 52% T6 (CH/ML), T5 (CH/HA), T4 (CH/NPK) respectively excel;

62, 50, 45% T9 (VC/ML), T8 (VC/HA), T7 (VC/NPK) respectively excel;

29, 23, 0% T3 (NE/ML), T2 (NE/HA), T1 (NE/NPK) Zero control = 21.9 mg Ru/gDL);

As listed table 2 represented Figure 6.

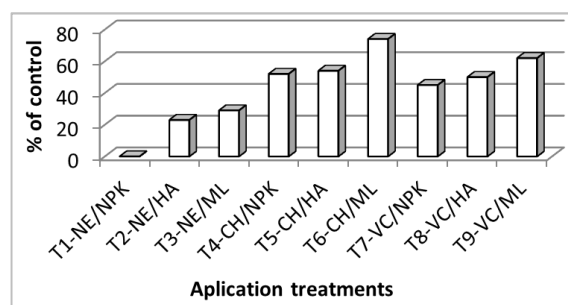


Figure 6. Total flavonols, mg Ru/g.dry leaves for sage elicited by BH, VC under NPK, HA, ML.

The quantitative and qualitative BsMs has been extensively reported due to elicitation application [49, 35, 73]. Also, moringa and humic acid, chitosan vitamin C enhanced BSMs [121, 122].

These BSMs, improving the health promoting quality [41, 120] (2) enhancing tolerance of plants to biotic and biotic stresses [63], (3) improved resistance to bests and diseases ([109, 108]; Co- friendly to control plant disease and pest [107, 111], (4) have to protect and biological control diseases and bests [21, 35].

Table 2. *S. officinalis* total phenolics flavonoids, flavonoLs and antioxidant activity elicited by chitosan (CH) and vitamin c(VC) aunder NPK, Humic acid (HA) and morenga dry leaves extract (ML). at two seasons, 2019 and 2020.

Application Treatment	Total phenolics, mg.GA/g dray leaves			Total flavonoids, as mg QA / g. Ca			Total flavonoLs, as mg RU / g. dray leaves			Antioxidant activity%		
	2019	2020	Pooled mean	2019	2020	Pooled mean	2019	2020	Pooled mean	2019	2019	Pooled mean
T1- (NE / NPK)	126.6	125.9	126.3 (0)	215.2	213.9	215.0 (0)	21.6	22.2	21.9 (0)	62	63	62.0 (0)
T2- (NE / HA)	158.3	159.7	159.0 (25)	269.0	271.2	270.0 (25)	27.0	26.7	26.9 (23)	65	66	65.5 (5)
T3- (NE / ML)	164.6	165.3	165.0 (30)	290.5	289.5	290.0 (35)	28.5	27.9	28.2 (29)	68	67	67.5 (8)
T4-(CH / NPK)	221.6	220.9	221.3 (75)	363.7	364.8	364.3 (69)	33.5	32.8	33.2 (52)	78	77	77.5 (24)
T5-(CH / HA)	229.2	230.1	229.7 (82)	370.2	371.6	370.9 (73)	34.8	32.5	337 (54)	81	80	58.5 (29)
T6- (CH / ML)	248.1	247.5	247.8 (96)	398.1	397.5	397.8 (85)	38.4	37.9	38.2 (74)	85	86	85.5 (38)
T7-(VC / NPK)	206.4	205.9	206.2 (63)	329.3	330.1	329.7 (53)	31.8	31.6	31.7 (45)	69	70	69.5 (11)
T8-(VC / HA)	224.1	225.4	224.8 (78)	350.8	349.3	350.0 (63)	33.5	32.8	32.8 (50)	73	74	73.5 (18)
T9- (VC / ML)	241.8	240.6	241.2 (91)	359.4	358.9	359.2 (67)	35.6	35.1	35.4 (62)	78	79	78.5 (26)
LSD1%			3.2			2.6			0.4			1.4

Values between parenthesis were percent of control (NE / NPK).

4.4. Essential Oil Constituents

The major oil constituent of SEO were carophyllene (31.50%), β penine (15.50%) and α - penene (5.20%). CH or VC along ML, HA, NPK actuated significant positive

impacts for total percent of SOEO expressed as percent of control (NE/NPK) = 68.97%) table 3 and represented Figure 7 as the following:

34, 30, 26% T6 (CH/ML), T5 (CH/HA), T4 (CH/NPK) respectively;

31, 25, 24% T9 (VC/ML), T8 (VC/ HA), T7 (VC/ NPK) respectively;

27, 22, 0% T3 (NE/ML), T2 (NE/ HA), T1 (NE/NPK). Respectively;

Table 3. *S. officinalis* essential oil components in response of elicitation with chitosan (CH) and vitamin C under (NPK), humic acid (HA) and Moringa dry leaves extract for pooled mean of two seasons 2019 and 2020.

Application Treatment	Carophyllene	B.pen-ine	∞ pen- ine	Camph- ine	Cis-oci-mine	Tran-oci mine	Germa-crene	Camp-nore	Total%
T1- (NE / NPK)	31.50(0)	15.50(0)	5.20 (0)	13.7(0)	2.80(0)	2.20(0)	6.30(0)	4.18(0)	68.97(0)
T2- (NE /HA)	33.39(6)	15.81(2)	5.25(1)	14.11(3)	2.83(1)	2.22(1)	6.43(2)	4.18(2)	84.22(22)
T3-(NE /ML)	35.60(13)	16.12(4)	5.30(2)	14.25(4)	2.88(3)	2.27(3)	6.56(4)	4.26(4)	87.23(27)
T4- (CH /NPK)	34.64(10)	16.28(5)	5.36(3)	14.66(7)	2.86(2)	2.24(2)	6.62(5)	4.31(5)	86.98(26)
T5- (CH /HA)	36.23(15)	17.05(10)	5.41(4)	15.07(10)	2.88(3)	2.27(3)	6.80(8)	4.43(8)	90.14(30)
T6-(CH / ML)	37.80(20)	17.36(12)	5.46(5)	15.34 (12)	2.94(5)	2.31(5)	6.93(10)	4.51(10)	92.65(34)
T7-(VC / NPK)	34.02(8)	15.97(3)	5.30(2)	14.39 (5)	2.83(1)	2.22(1)	6.49(3)	4.22(3)	85.44(24)
T8- (VC / HA)	35.28(12)	16.28(5)	5.36(3)	14.80 (8)	2.86(2)	2.24(2)	6.68(6)	4.35(6)	85.85(25)
T9- (VC / ML)	37.17(18)	16.74(8)	5.41(4)	14.93 (9)	2.91(4)	2.29(4)	6.74(7)	4.39(7)	90.58(31)
LSD1%									0.72

Values between parenthesis were percent of control (NE / NPK).

4.5. Antioxidant Activity (AA)

CH or VC biotic elicitor interacted with ML, HA, NPK resulted significant enhancement for AA represented as percent of control (NE/ NPK= 62.0%) as the following;

38, 29, 24% T6 (CH/ ML), T5 (CH/ HA), T4 (CH/ NPK), respectively, excel;

26, 18, 11% T9 (VC/ML), T8 (VC/ HA), T7 (VC/NPK);

8, 5, 0% T3 (NE/ ML), T2 (NE/ HA), T1 (NE/ NPK) Zero Control= 62.0, as represented Table 2 and Figure 7.

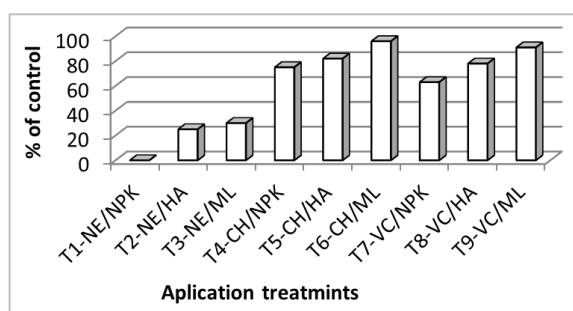


Figure 7. Antioxidant activity for sage elicited by BH, VC Under NPK, HA, ML.

In dispute, there is no pests and microbial diseases incidence in the field experiment in both two seasons (2019-2020) without using any agrochemical pesticide and/ or microbiocide which in consequence of biotic elicitors (CH), VC integrated with bio fertilizers; ML, HA and NPK chemical traditional fertilizer under investigation. Since these elicitors and bio fertilizers trigger plants to induce systemic resistance ((ISR) by regulating the expression of genes involved for production and accumulation of BSMs which overcoming biotic and biological stresses protect and biological control against pests and microbial diseases [116, 72]. Exceedingly, chitosan and moringa proved microbiocide and insecticide [65] which spur plant defense system inducing the immune system lead to promot yield and

SE, as β SM has been quantitative and qualitative improved through elicitation [41, 120, 73, 72, 43].

Also, Eo have microbiocidal and insecticidal potential to protect and biological control [43, 44, 123, 35].

inhancing health benefits [67] which conformed our results.

5. Conclusion

Overall results revealed that multi- repeating elicitation with biotic elicitors, CH or VC under biofertilizes, ML, HA and NPK agrochemical fertilizer could be considered as a reliable technological significant scale up significantly biomass, bioactive secondary metabolit yield production and quality and inhancing health promoting benefits without accreditation on agrochemical microbiocides and or insecticides then, this strategy supported strong evidence its potentiality to achieve entegrated and sustainable development *S. officinalis* under biofertilizer that excel agrochemical fertilizer agriculture system.

References

- [1] Lopresti, A. L. (2017). Salvia (sage): a review of its potential cognitive- enhancing and protective effects. *Drugs in R&D*, 17 (1), 53-44.
- [2] Salamatin, A. A.; Khaliullina, A. S.; Khaziev, R. Sh.(2020). Extraction of aromatic abietane diterpenoids from salvia officinalis leaves by petroleum ether: data resolution analysis *Industrial crops and prouducts*; 143: 111909.
- [3] Martina Jakovljevic Stela Jokic Maja Molnar, Midhat Jasic Jurislav Babic Huska Jukic and Ines Banjari.(2019) Bioactive profile of various *Salvia Officiealis* L. preparations plants, 8, 55; doi: 10.3390/plants803005.
- [4] Sena, T. R. R. DE; et al. (1996). Hearing care and quality of life among workers exposed to pesticides. *Ciência & Saúde Coletiva*, v. 18, n. 6, p. 1753–1761.
- [5] Bouaziz, M.; Yangui, T.; Sayadi, S.; Dhoub, A. (2009). Disinfectant properties of essential oils from *Salvia Officiealis* L. cultivated in Tunisia. *Food Chem. Toxicol.* 47, 2755–2760.

- [6] Cilerdzic, J.; Aradski, A. A.; Stajic, M.; Vukojevic, J.; (2019). Duletic-Lausevic, S. Do Ganoderma Lucidum and *Salvia Officinalis* extract exhibit synergistic antioxidant and antineurodegenerative effects Journal of food Measurement and Characterization; 13 (4): 3357-3365.
- [7] Abdnezahd, R.; Simbar, M.; Sheikhan, Z.; Mojab, F.; Nasiri, M. (2019). *Salvia Officinalis* reduces the severity of the premenstrual syndrome Complementary Medicine Research; 26 (1): 39-46.
- [8] Durling, N. E., Catchpole, O. J., Grey, J. B., Webby R. F., Mitchell, K. A., Foo, L. Y., Perry, N. B., (2007). Extraction of phenolic and essential oil from dried sage (*Salvia Officinalis*) using ethanol -water mixtures. Food chem. 101, 1417-1424.
- [9] Lu, Y., Yeap Foo L., (2002). Polyphenolic of salvia—a review. phytochemistry 59, 117-140.
- [10] Miura, K., Kikuzaki, H., Nakatani, N., (2002). Antioxidant activity of chemical components from sages (*Salvia Officinalis* L.) and thyme (*Thymus vulgaris* L.) measured by the oil stability index method J. Agric. Food chem. 50, 1845-1851.
- [11] Perry, N. B., Anderson, R. E., Brennan, N. J., Douglas, M. H., Heaney, A., McGimpsey, J. A., Smallfield, B. M., (1999). Essential oils from dalmatian sage (*Salvia Officinalis* L.) Variations among individuals, plant parts, seasons, and sites, J. Agric. Food Chem 47.
- [12] Pavic, V.; Jakovljevic, M.; Molnar, M.; Jokic, S. (2019). Extraction of carosic acid and carnosol from sage (*Salvia Officinalis* L.) Leaves by supercritical fluid extraction and their antioxidant and antibacterial activity Plants; 8 (1): 16.
- [13] Pedro Henrique Gornri: Ana Claudia; Jonathan Fogaca (2019). Albuquerque Silva. Plant elicitation with salicylic acid increases Bioactive compounds content and antioxidant activity in the infusion of *Achillea millefolium* L. Bioscience Jornal Uberlandia, v, 35, n 1, p 289-295.
- [14] Wu, Q. D. and H. D. VanEtten. (2004): Introduction of plant and fungal genes into Pea (*Pisum sativum* L.) hair roots reduces their ability to produce pisatin and affects their response to a fungal Pathogen. Molecular plant Microbe Interaction; 17 (7): 798-804.
- [15] Gao MingBo; Zhang, W.; and R. ChengJiang. (2011): Significantly improved taxuyunnanine C production in cell suspension culture of *Taxus chinensis* by process intensification of repeated elicitation, sucrose feeding, and in situ adsorption. World Journal of Microbiology and Biotechnology; 27 (10): 2271-2279.
- [16] Wang–YaNing; Wei–YaHui; Hao–HaoYong; Ji–JingYuan (2007): Advances in the research of resveratrol. Acta Botanica Boreali Occidentalia Sinica; 27 (4): 852-857.
- [17] Jansen, M. A. K.; Hectors, K. O'Brien, N. M.; Guisez, Y. and G. Potters. (2008): Plant stress and human health: do human consumers benefit from UV-B acclimated crops Plant Science; 175 (4): 449-458.
- [18] Edreva, A.; Velikova, V.; Tsonev, T.; Dagnon, S.; Gurel, A.; Aktas, L. and E. Gesheva. (2008): Stress protective role of secondary metabolites: diversity of functions and mechanisms. General and Applied Plant Physiology, 341 (1/2): 67-78.
- [19] Treutter, D. (2005): Significance of flavonoids in plant resistance and enhancement of their biosynthesis. Plant Biology; 7 (6): 581-591.
- [20] Marpelli, S.; Brambilla, I. M.; Radyukina, N. L.; Ivanov, Yu. V.; Kartashov, A. V.; Reggiani, R. and V. V. Kuznetsov. (2008): Free and bound polyamines changes in different plants as a consequence of UV – B light irradiation. General and Applied Plant Physiology; 34 (1/2): 55- 66.
- [21] Ferreira, T. P. deS.; Veloso, R. A.; Santos, G. R. dos; Santos, L. P. dos; Ferriet, T. P. de S.; Barros, A. M.; Possi, R. D.; Aguiar, R. W. de S. (2018). Enzymatic activity and elicitor of phytoalexins of lippia sidoides Cham and endophytic fungi. African journal of Biotechnology 17 (15): 521-530.
- [22] Ersilia Alexa, Renata Maria Sumalan, Corina Danciu, Diana Obistoiu, Monica Negrea, Mariana-Atena Poiana, Cristian Rus, (2018), Isidora Radulov, Georgeta Pop and Cristina Dehelean Synergistic Antifungal, Allelopathic and Anti-Proliferative Potential of *Salvia Officinalis* L., and *Thymus vulgaris* L. Essential OilsMolecules 23, 185.
- [23] Sonker, N., Pandey, A. K. and Singh, P. (2015). Efficiency of *Artemisia nilagirica* (Clarke) Pamp essential oils as mycotoxicant against post harvest mycobiota of tablegrapes. J.Sci.FoodAgric. 95, 1932–1939.
- [24] Noha Khalil, Mostafa Fekry, Mokhtar Bishr, Soheir El-Zalabanib, Osama Salamaa. (2018). Foliar spraying of salicylic acid induced accumulation of phenolics, increased radical scavenging activity and modified the composition of the essential oil of water stressed *Thymus vulgaris* Plant Physiology and Biochemistry 123, 65–74 L.
- [25] Alcalá-Orzco, M.; Caballero-Gallardo, K.; Stashenko, E. E.; Olivero-Verbel, J. (2019). Repellent and fumigant action of the essential oils from *Elettaria cardamomum* (L.) Maton, *Salvia officinalis* (L.) Linnaeus, and *Lippia origanoides* (V.) Kunth against *Trilbolium castaneum* and *Uloides dermestoides* Journal of Essential Oil-Bearing Plants; 22 (2): 18-30.
- [26] Privitera, G.; Luca, T.; Castorina, S.; Passanisi, R.; Ruberto, G.; Napoli, E. (2019). Anticancer activity of *Salvia Officinalis* essential oil and its principal constituents against hormone-dependent tumor cells Asian Pacific Journal of Tropical Biomedicine; 9 (1): 24-28.
- [27] Misra, N., Misra, R., Mariam, A., Yusuf, K., Yusuf, L., (2014). Salicylic acid alters antioxidant and phenolics metabolism in *Catharanthus roseus* grown under salinity stress. Afr. J. Tradit., Compl. Altern. Med. 11, 118–125.
- [28] Sharifi-Rad J, Salehi B, Stojanović-Radić ZZ, Fokou PVT, Sharifi-Rad M, Mahady GB, et al. (2017). Medicinal plants used in the treatment of tuberculosisethnobotanical and ethnopharmacological approaches. Biotechnol Adv. S 0734–9750 (17) 30077–0.
- [29] Elansary HO, Szopa A, Kubica P, Ekiert H, Ali HM, Elshikh MS, et al. (2018). Bioactivities of traditional medicinal plants in Alexandria. Evid-Based Complementary Altern Med.: 1463579.
- [30] Tettey, C. O.; Ocloo, A.; Nagajyothi, P. C. N.; Lee, K. D. (2014). An in vitro analysis of antiproliferative and antimicrobial activities of solvent fractions of *Taraxacum officinale* (Dandelion) leaf. Journal of Applied Pharmaceutical Science; 4 (3): 41-45. 24.

- [31] Ovadje P, Chatterjee S, Griffin C, Tran C, Hamm C, Pandey S. (2011). Selective induction of apoptosis through activation of caspase-8 in human leukemia cells (Jurkat) by dandelion root extract. *J. Ethnopharmacol.* 133: 86-91.
- [32] Dixon, R. A., (2001). Natural products and plant disease resistance. *Nature* 411, 843–847.
- [33] Oksman-Caldentey, K. M., Inze, D., (2004). Plant cell factories in the post-genomic era: new No table of figures entries found. ways to produce designer secondary metabolites. *Trends in Plant Science* 99.
- [34] Pereira, L. A., & Raimunda, A. D. S. (2016). The intensive use of pesticides—The new face of the agrarian question. *Okara: Geografia em Debate*, 10, 185-194.
- [35] Mohamedy, R. S. R.; Mohamed, S. K., (2018). Effect of Moringa oleifera seed oil, root and leaf extracts on growth of major pathogenic fungi of tomato, green bean and potato in vitro. *International Journal of Agricultural Technology*, 14 (2): 505-520.
- [36] Tarek El- Sayed, S. A. 2Bosila, H. A. and 1El- Sayed, S. A., (2020). Appraising elicitation potency by electric current incorporated with chitosan and Nano- selenium to broost qualitative essential oil yield production in thyme. *Asian Journal of Science and Technology* Vol. 11, Issue, 01, pp. 10712-10721.
- [37] Somayeh Ghahari, Heshmatollah Alinezhad, Ghorban All Nematzadeh (2018). Phytochemical Antioxidant and Biological Activities of the Essential Oil of Astragalus alopecurus pall. Fruits from northern iran. *Journal of Essential oil Bearing plants*.
- [38] Lopez-Reyes, J. G.; Spadaro, D.; Gullino, M. L.; Garibaldi, A. (2010) Efficacy of plant essential oils on postharvest control of rot caused by fungi on four cultivars of apples in vivo. *Flavour Fragr. J.* 25, 171–177.
- [39] Plaza, P.; Torres, R.; Usall, J.; Lamarca, N.; Vinas, I. (2004). Evaluation of the potential of commercial post-harvest application of essential oils to control citrus decay. *J. Hortic. Sci. Biotechnol.* 79, 935–940.
- [40] Sciences, B.; Ziedan, E. H. E.; Farrag, E. S. H. (2008). Fumigation of peach fruits with essential oils to control postharvest decay. *Res. J. Agric. Biol. Sci.* 4, 512–519.
- [41] Sarkhosh, A.; Vargas, A. I.; Schaffer, B.; Palmateer, A. J.; Lopez, P.; Soleymani, A.; Farzaneh, M. (2017). Postharvest management of anthracnose in avocado (*Persea americana* Mill.) fruit with plant-extracted oils. *Food Packag. Shelf Life*, 12, 16–22.
- [42] Šegvić Klarić, M.; Kosalec, I.; Mastelić, J.; Piecková, E.; Pepeljnjak, S. (2007). Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against moulds from damp dwellings. *Lett. Appl. Microbiol.* 44, 36–42.
- [43] Houda Banani, Leone Olivieri, Karin Santoro, Angelo Garibaldi, Maria Lodovica Gullino and Davide Spadaro Thyme and Savory. (2018). Essential Oil Efficacy and Induction of Resistance against *Botrytis cinerea* through Priming of Defense Responses in Apple Foods 2018, 7, 11.
- [44] Servili, A.; Feliziani, E.; Romanazzi, G. (2017). Exposure to volatiles of essential oils alone or under hypobaric treatment to control postharvest gray mold of table grapes. *Postharvest Biol. Technol.* 133, 36–40.
- [45] Kaskin, N. and B. Kunter. (2009): The effects of callus age, UV irradiation and incubation time on trans resveratrol production in grapevine callus cultures. *Tarim – Billileri - Dergisi*; 15 (1) 9-13.
- [46] Poulev, A. et al. (2003). Elicitation, a new Window into plant Chemodiversity and Phytochemical drug discovery. *J. Med chem., united states*, V. 46, p. 2542-2547, may.
- [47] Perez, M. G. F. et al. (2014). Effect of chemical elicitors on peppermint (*Mentha piperita*) plants and their impact on the metabolite profile and antioxidant capacity of resulting infusion. *Food chem, Barking*, V. 156, p. 273-278, aug.
- [48] Namil, s. et al. (2014). Effects of UV-B radiation on total phenolic, flavonoid and hypericin contents in *Hypericum retusm Aucher* grown under n vitro conditions. *Nat Prod Res, England*, V. 28, n. 24, p. 2286-2292, aug.
- [49] Michał Świeca.(2016). elicitation and treatment with precursors of phenolics synthesis improve low-molecular antioxidants and antioxidant capacity of buckwheat sprouts *Acta Sci. Pol. Technol. Aliment.*, 15 (1), 17–28. DOI: 10.17306/J.AFS.1.2.
- [50] Kiran Sharma, Rasheeduz Zafar.(2016). Optimization of methyl jasmonate and-cyclodextrin for enhanced duction of taraxerol and taraxasterol in (*Taraxacum officinale* Weber) cultures *Plant Physiology and Biochemistry* 103, 24e30.
- [51] Nieves Baenas, Cristina Garcia-Viguera and Diego A. Moreno. (2014). Elicitation: A Tool for Enriching the Bioactive Composition of Foods *Molecules*. 19, 13541-13563.
- [52] Zhou ML. Bai DQ. Tang Y. Zhu xm Shao JR (2012). Genetic diversity off our new species related to southwestern Sichuan buckwheats as revealed by karyotype ISSR and allozyme characterization *plant Evol.* 298: 751-759.
- [53] Tarek Elsayed S Ahamed, and El- Sayed, S. A. (2018). Verification and Validation of Dandelion (*Taraxacum officinal*) Seeds- Gamma Irradiated under Elicitation with Nano- and Micro- Zinc for Potential Optimization Biomass and enhancing Phenolics, Flavonoids and Antioxidant Activity. *volum 3, Issue 10*.
- [54] Tarek Elsayed Sayed Ahamed (2019). Bioprospecting Elicitation with Gamma Irradiation Combine with Chitosan to Enhance, Yield Production, Bioactive Secondary Metabolites and Antioxidant Activity for Saffron *Journal of Plant Sciences* 7 (6): 137-143.
- [55] Agrawal N, Sharma S (2013): Appraisal of Garden Cress (*Lepidiumsativum*L.) and Product Development As An All Pervasive And Nutrition Worthy Food *Food Stuff Annals. Food Science and Technology* Volume 14, Issue 1.
- [56] Rawat, J. M.; Balwant Rawat; Susmita Mishra. (2014). Effect of elicitation on picrotin and picrotoxinin oroduction from in vitro products of kurrooa *African journal of Biotechnology*; 13 (51): 4612-4619.
- [57] Geunen, S.; and J. M. C. Geunen:.(2013). Influence of photoperiodism on the spatio- temporal accumulation of steviol glycosides in *Stevia rebaudaiana* (Berton). *Plant, Science*; 198: 72-82, 2013.
- [58] Adam, A.; Jourdan, E.; Pgena, M.; Duby, F.; Domes, J. and P. Thonart. (2005): Resistance induced in cucumber and tomato by a non-pathogenic *Pseudomonas putida* strain-Parasitical; 6 (2/4): 13-22.

- [59] Hussain S, Fareed, S Ansari, S, Rahman, A., Ahmad, I. Z., saeed, M., 2012. Current approaches toward production of secondary plant metabolites. J. pharm. Bioallied Sci. 4, 10-20.
- [60] Aardr Kachroo; Vincelli, P.; Pradeep Kachroo,(2017). Signalis mechanisms underlying resilience responses what have we learned, and how is it applied. Phytopathology; 107 (12): 1452-1461.
- [61] Akash Hidangmayum; Padmanabh Dwivedi; Deepmala Katiyar; Akhouri Hematranjan. (2019). Application of chitosan on plant responses with special reference to abiotic stress physiology and Molecular Biology of plants; 25 (2): 313-326.
- [62] Sathiyabama, M.; Akila, G.; Einstein Charles, R. (2014). Chitosan- induced defence responses in tomato plants against early blight disease caused by *Alternaria solani* (Ellis and Martin) Sorauer. Arch. Phytopathol. Plant Prot. 47, 1777–1787.
- [63] Valletta, A., De Angelis, G., Badiali, C., Brasili, E., Miccheli, A., Di Cocco, M. E., Pasqua, G., (2016). Acetic acid acts as an elicitor exerting a chitosan-like effect on xanthone biosynthesis in *Hypericum perforatum* L. root cultures. Plant Cell Rep. 35 (5), 1009e1020.
- [64] Katiyar D, Hematranjan A, Singh B (2015). Chitosan as promising natural compound to enhance potential physiological responses in plant: a review. Indian J plant physiol 20 (1): 1-9.
- [65] Zheng Fangliang; Zheng WenWen; LiLiMei; Pan SiMing; Liu MeiChen; Zhang WeiWei; Liu HongSheng; Zhu ChunYu. (2017). Chitosan controls postharvest decay and elicits response in kiwifruit Food and Bioprocess Technology; 10 (11): 1937-1945.
- [66] Zagzog, O. A.; Gad, M. M.; Hafez, N. K. (2017). Effect of Nano-chitosan on Vegetative Growth, Fruiting and Resistance of Malformation of Mango. Trends Hortic. Res. 6, 673–681.
- [67] El-Ghaouth, A., Arul, J., Grenier, J., Benhamou, N., Asselin, A., Belanger, R., (1994). Effect of chitosan on cucumber plants: suppression of *Pythium aphidodermatum* and induction of defense reactions. Phytopathology 84, 313–320.
- [68] Romanazzi, G., Nigro, F., Ippolito, A., Di Venere, D., Salerno, M., (2002). Effects of pre- and postharvest chitosan treatments to control storage grey table grapes. J. Food Sci. 67, 1862–1867.
- [69] Aziz, A., Trotel-Aziz, P., Dhuicq, L., Jeandet, P., Couderchet, M., Vernet, G., (2006). Chitosan oligomers and copper sulfate induce grapevine defense reactions and resistance to gray mold and downy mildew. Phytopathology 96, 1188–1194.
- [70] Trotel-Aziz, P., Couderchet, M., Vernet, G., Aziz, A., (2006). Chitosan stimulates defense reactions in grapevine leaves and inhibits development of *Botrytis cinerea*. Eur. J. Plant Pathol. 114, 405–413.
- [71] Ruiz-García, Y., Gómez-Plaza, E., (2013). Elicitors: a tool for improving fruit phenolic content. Agriculture 3, 33–52.
- [72] Tarek El- Sayed, S. A and El- Sayed, S. A. (2021). Potential Assessment Multi-Repeating abiotic / biotic Motivation coincide Biofertilizers to Optimize Black Cumin (*Nigella sativa* L.) Seed Yield Production and Quality Agricultural Sciences, 2021, 12, 69-83.
- [73] Tarek Elsayed S Ahamed, and El- Sayed, S. A., (2020). Verifying potential of *Moringa oleifera* Extract Application as Bio- Fertilizer for Basil Plants (*Ocimum basilicum* L.) Elicited with Gamma Irradiation and / or Nano- Zinc Oxide to Ameliorate Biomass Quantity and Quality Asian Journal of Science and Technology Vol. 11, Issue, 04, pp. 10888-10897.
- [74] Ben-Shalom, N.; Fallik, E. (2003). Further suppression of *Botrytis cinerea* disease in cucumber seedlings by chitosan-copper complex as compared with chitosan alone. Phytoparasitica 2003, 31, 99–102.
- [75] Algam, S.; Xie, G.; Li, B.; Yu, S.; Su, T.; Larsen, J. (2010). Effects of *Paenibacillus* strains and chitosan on plant growth promotion and control of *Ralstonia* wilt in tomato. J. Plant Pathol. 92, 593–600.
- [76] Escudero, N.; Lopez-Moya, F.; Ghahremani, Z.; Zavala-Gonzalez, E. A.; Alaguero-Cordovilla, A.; Ros-Ibañez, C.; Lacasa, A.; Sorribas, F. J.; Lopez-Llorca, L. V. (2017). Chitosan increases tomato root colonization by *Pochonia chlamydosporia* and their combination reduces root-knot nematode damage. Front. Plant Sci. 8.
- [77] Bondok, A. (2015). Response of Tomato Plants to Salicylic Acid and Chitosan under Infection with Tomato mosaic virus. Am.-Eur. J. Agric. Environ. Sci. 15, 1520–1529.
- [78] Firmansyah, D. (2017). Use of Chitosan and Plant Growth Promoting Rhizobacteria to Control Squash Mosaic Virus on Cucumber Plants. Asian J. Plant Pathol. 11, 148–155.
- [79] Li, Y.; Qin, Y.; Liu, S.; Xing, R.; Yu, H.; Li, K.; Li, P. (2016). Preparation, Characterization, and Insecticidal Activity of Avermectin-Grafted-Carboxymethyl Chitosan. BioMed Res. Int.
- [80] Nasiri, Y.; Zandi, H.; Morshedloo, M. R. (2018). Effect of Salicylic and ascorbic acid on essential oil content and composition of dragonhead (*Dracocephalum moldavica* L.) under organic farming Journal of essential Oil-Bearing Plants; 21 (2): 362-373.
- [81] Lazzari, F. M., & Souza, A. S. (2017). Green Revolution: Impacts on Traditional Knowledge (pp. 1-16). 4o Congresso Internacional de Direito e Contemporaneidade.
- [82] Bombardi, L. M. (2019). Geografia do uso de agrotóxicos no Brasil e conexões com a União Europeia. 2. ed. São Paulo: FFLCH – USP.
- [83] Nasralla Neto, E.; et al. (2014). Health surveillance and agribusiness: the impact of pesticides on health and the environment. Danger ahead! Ciência & Saúde Coletiva, v. 19, n. 12, p. 4709–4718.
- [84] Rattner, H.; Franco Netto, G (2009). Environment, health and sustainable development. Ciência & Saúde Coletiva, v. 14, n. 6, p. 1965–1971.
- [85] Pignati, W. A. et al. (2017). Distribuição espacial do uso de agrotóxicos no Brasil: uma ferramenta para a Vigilância em Saúde. Ciência & Saúde Coletiva, v. 22, n. 10, p. 3281–3293.
- [86] Abreu, P. H. B. DE; Alonzo, H. G. A. (2014). Rural work and health risks: a review into de “safe use” of pesticides in Brazil. Ciência & Saúde Coletiva, v. 19, n. 10, p. 4197–4208, Out.
- [87] El-Serafy, R. S., (2015). Effect of Silicon and Calcium on Productivity and Flower Quality of Carnation. Ph.D. Thesis. Fac. Agric. Tanta Univ., Egypt. El-Serafy, R. S., 2018. Growth and productivity of roselle (*Hibiscus sabdariffa* L.) as affected by yeast and humic acid. Sci. J. Flowers Ornamental Plants 5 (2), 195–203.

- [88] Caradonia, F., Battaglia, V., Righi, L., Pascali, G., La Torre, A., (2018). Plant biostimulant regulatory framework: prospects in Europe and current situation at international level. *J. Plant Growth Regul.* 38 (2), 438–448. *basilicum L. plants. Alex. J. Agric. Res.* 60 (1), 1–16.
- [89] El-Serafy, R. S., (2019). Silica nanoparticles enhance sphysio-biochemical characters and postharvest quality of Rosa hybrid L. cut flowers. *J. Hortic. Res.* 27 (1), 47–54.
- [90] Rashmi, K. R., Earanna, N., Vasundhara, M., (2008). Influence of biofertilizers on growth, biomass and biochemical constituents of *Ocimum gratissimum L.* *Biomed* 3 (2), 123–130.
- [91] Azzaz, N. A., Hassan, E. A., Hamad, E. H., (2009). The chemical constituent and vegetative and yielding characteristics of fennel plants treated with organic and bio-fertilizer instead of minera fertilizer. *Aust. J. Basic Appl. Sci.* 3 (2), 579–587.
- [92] El-Naggar, A. H. M., Hassan, M. R. A., Shaban, E. H., Mohamed, M. E. A., (2015). Effect of organic and biofertilizers on growth, oil yield and chemical composition of the essential oil of *Ocimum basilicum L. plants. Alex. J. Agric. Res.* 60 (1), 1–16.
- [93] Yousefzadeh, s., s. a. m. Modarres sanavy, m. Govahi, o. s. Khatamian oskooie, (2015). Effect of organic and chemical fertilizer on soil characteristics and essential oil yield in dragonhead. *Journal of Plant Nutrition* 38 (12), 1862-1876.
- [94] Sakhubai H. (2016). Tummaratti, Laxminarayana Hegde. Effect of Bio – Fertilizers on Growth, Yield and Quality of Buckwheat (*Fagopyrum esculentum* Moench). *Environment & Ecology* 34 (3B): 1258-1261.
- [95] Khalil Karimzadeh Asl · Mehrnaz Hatami (2019). Application of zeolite and bacterial fertilizers modulates physiological performance and essential oil production in dragonhead under different irrigation regimes *Acta Physiologiae Plantarum* 41: 17.
- [96] Adekiya, A. O.; Agbede, T. M.; Aboyeji, C. M.; Dunsin, O.; Ugbe, j. o. (2019). Green manures and NPK fertilizer effect on soil properties, growth, yield mineral and vitamin C composition of okra (*Abelmoschus esculentus* (L.) Moench). *Journal of the Saudi of Agricultural Sciences*; 18 (2) 218-223.
- [97] Yadav, K. K., (2019). Smritkana Sarkar. Biofertilizers, impact on soil fertility and crop productivity under sustainable agriculture. *Environment and Ecology*; 37 (1): 89 – 93.
- [98] Kamlesh Kumar Yadav, (2019). Smritkana Sarkar Biofertilizers, Impact on Soil Fertility and Crop Productivity under Sustainable Agriculture *Environment and Ecology* 37 (1): 89–93, January–March.
- [99] Xu Feng, Yongqing Xu, Dan Liu, Lina Peng, Jiamin Dong, Shukuan Yao, Yanzhong Feng, Zhe Feng, Fenglan Li, (2020). Baozhong Hu. Effects of organic cultivation pattern on Tomato production plant growth characteristics quality disease resistanc and soil physical and chemical properties. *Acta Sci Pol. Hortorum Cultus*, 19 (1) 71–84.
- [100] Mona M. Abdalla (2013). The potential of *Moringa oleifera* extract as a biostimulant in enhancing the growth, biochemical and hormonal contents in rocket (*Eruca vesicaria* subsp. *Sativa*) plants. Vol. 5 (3), pp. 42-49.
- [101] Melesse A., and K. Berihm. (2013). Chemical and mineral compositions of pods of *Moringa stenopetala* and *Moringa oleifera* cultivated in the lowland of Gamogfa Zone,. *Journal of Environmental Occupational Science* 2: 33-38.
- [102] Aslam M, Anwar F, Nadeem R, Rashid U, Kazi TG, Nadeem M, (2005). Mineral Composition of *Moringa oleifera* leaves and pods from Different Regions of Punjab, Pakistan *Asian J plant Sci.*
- [103] Manzoor, M., F, Anwar, T, Iqbal, and M. Bhanger. (2007). Physico-chemical characterization of *Moringa concanensis* seeds and seed oil. *Journal of American Oil Chemistry Society* 64: 413-19.
- [104] Suaib Luqman, Suchita Srivastava, Ritesh Kumar, Anil Kumar Maurya, and Debabrata Chanda. (2012). Experimental Assessment of *Moringa olifera* and Fruit for Its Antistress, Antioxidant, and Scavenging Potential Using In Vitro and In Vivo Assays. Evidence- Based Complementary and Alternative Medicine Volum Article ID 519084, 12 pages.
- [105] Tsegaye Mega Kaba; Kyusse Gudishe Goroya. (2109). Determination of concentration of some essential and heavy metals in roots of *Moringa stenopetala* using Flame Atomic Absorption Spectroscopy. *Journal of Medicinal plants Reserch*; 13 (4): 89-95.
- [106] Zaker, M. (2016). Natural plant products as Eco-friendly Fungicides for plant Diseases control- A Review. *The Agriculturists* 14: 134-141.
- [107] Nouman W, Siddiqui MT, Basra SMA (2011). *Moringa oleifera* leaf extract: An innovative priming tool for rangeland grass. *Turk. J. Agric. For.* TUBITAK 35: 1-11.
- [108] Marwad, A. R. M. A. (2018). Using *Moringa oleifera* extract as biostimulant enhancing the growth, yield and nutrients accumulation of pea plants. *Journal of plant Nutrition*; 41 (4): 425-431.
- [109] Hasan, M.; Bae HanHong. (2017). An overview of stress-induced resveratrol synthesis in grapes: perspectives for resveratrol-enriched grape products. *Molecules*; 2017, 22 (2): 294.
- [110] Kazam Ali; Amna Khan; Muhammad Sagheer; Hanit, C, M.,; Astif, H, M.,; Yasir, T, A.,; Atti Wasaya. (2019). Biological activities of medicinal plants against the population of *trigoderma granarh*. *Zeitschrift rur Arznei-& Gerwurzplflanzen*; 2019, 24 (1): 38-44.
- [111] Canellas. L., P., Olivares F, L., Aguiar, N. O., Jones D. L., Nebbioso, A., Mazzei, P., Piccolo, A (2015) Humic and fulvic acid as biostimulants in horticulture. *Acta Sci. pol hortorum Culus* 196, 15 – 27.
- [112] Halpern, M., Bar-Tal, A., Ofek, M., Minz, D., Muller, T., & Yermiyahu, U. (2015). The Use of Biostimulants for Enhancing Nutrient Uptake. *Advances in Agronomy*, 13 0, 141-174.
- [113] Olivares, F. L. (2017). Plant growth promoting bacterium and humic substances: Crop promotion and mechanisms of action. *Chemical and Biological Technologies in Agriculture*, 4, 1.
- [114] Costa, V. C. O. (2008). Chemical composition and modulation of bacterium l drug resistance of the essential oil from the leaves of *Rollinia leptopetala* R. E. Fries. *Revista Brasileira de Farmacognosia*, 18, 245-248.

- [115] Zaheer Ahmad, Qudrat Ullah Khan, Abdul Qadoos, Muhammad Jamil Khan, Abida Saleem and Zarina Bibi. (2020). Humic acid, an effective amendment used for amelioration of Phosphatic fertilizer and enhancing maize yield Pure Appl. Biol., 9 (1): 750-759, March.
- [116] Cicco, N., M. T. Lanorte, M. Paraggio, M. Viggiano, and V. Lattanzio. (2009). A reproducible, rapid and inexpensive Folin-Ciocalteum method in determining phenolics of plant methanol extracts. Microchem. J. 91: 107-110.
- [117] Wu, C. H., Y. H. Dewir, E. J. Hahn, and K. Y. Paek. (2006). Optimization of culturing conditions for the production of biomass and phenolics from adventitious roots of *Echinacea angustifolia*. J. Plant Biol. 49: 193-199.
- [118] Akkol EK, Goger F, Kossar M, Baser KHC (2008) Phenolic composition and biological activities of *Salvia halophila* and *Salvia virgata* from Turkey, Food Chem 108 P 942-649.
- [119] Chan EWC, Lim YY, Omar M (2007). Antioxidant and antibacterial activity of leaves of *Etlingera species* (Zingiberaceae) in Peninsular Malaysia. Food Chem., 104 (4): 1586-1593.
- [120] Said-Al Ahl, H., Gendy, A. G., & El, E. A. O. (2016). Humic acid and indole acetic acid affect yield and essential oil of dill grown under two different locations in Egypt. International Journal of Pharmacy and Pharmaceutical Sciences, 8, 146-157.
- [121] Hendawy, S. F., Hussein, M. S., El-Gohary, A. E., & Ibrahim, M. E. (2015). Effect of Foliar Organic Fertilization on the Growth, Yield and Oil Content of *Mentha Piperita* Var. *Citrata*. Asian Journal of Agricultural Research, 9, 237-248.
- [122] Freitas, T. F. S. de; Stout, M. J.; Sant Ana, J. Effects of exogenous methyl jasmonate and salicylic acid on rice resistance to *Oebalus pugnax*. Pest Management Science; 2019. 75 (3): 744-752.