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

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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 biomess leaves yield, g. /m^2, 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 Lamilaceae, 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 pharaceutical and food [2] Aerial parts of *S. oficinalis* in treatment many diseases such as diabetes, cancer, hot flushes, obesity, diarrhea as well as for regulation of colestrol level and for memory improvement [3-4] So, is used as natural remedy in treating and curing arterial hypertension, bowel, stomac and spinal cord disorder, respiratory tract, inflammation, physical and mental fatigue, nervousness, skin ulceration, caugh, brunchities, dental absence 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 parementrual 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 rang. SMS, Such as essential oil and its terpenes and phenolics, flavonoids, flovcnols 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 (SOEO), 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 can cert and two human lung cancer, cell line significantly inhibition [22]. (3), The different mutal ratio of compounds contained the may excert 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 tumer cells [26]. EO, has been development of alternative strategies to reduce bacteriosides, virocides, pesticides as well as elicitation to upraise biomass production and quality [27]. Qunsequantly, 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 pharaceutical 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 biotic stresses [17, 20]. Elicitation, also quali- quantitative 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].

Elicitators, application can be used to increase SMs production in the plants and to enhance its qualitative values for fresh produces 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 phytoalex in [58-60, 35]. Successful practical application of initiating sMs, phytoalxins PAs formation and accumulation by elicitors confirmed the validity and practability 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 inhanced 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 axide 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], virucideal [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. Therefor biofertlizers 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] cabable to improving plant growth yield production and quality [97] and reducing problems associated with the use of chemical fertilizers [98, 99].

Many reports have showen 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 meniral'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- nutirents, rish source of B- carotene, Protein, Vitamin C calcium and potassium and acts as good source of ntural 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 heavey 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 manly 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 deseases 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 systims 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, counarines, total phenols, total flavoncids [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) inhancing 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 an 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/m^2 (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/m^2, and moringa dry leaves extract (ML), 5 g/m^2 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 dryed for determination dry biomass yield, Kg/m^2.

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±0.5C in the absence of light.

Total penolic content (TPC) assay:

TPC was assayed by folin – ciocaleau 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% Na_2CO_3 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% $NaNO_3$ 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 ruutin 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}) $AlCl_3$ 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 spectrophotometricaly 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./m^2 (EoYg./m^2):

Eqyg/m^2 were determined by multiplying dry leaves yield, g./m^2 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- alkones, 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 firsty 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

Biomas, Essential Oil Yield Production

3.1. Biomass, Dry Leaves Yield, g/m^2 (DLY, g/^{2})

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/m^2 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] ixhibited positive impacts on yield productivity as biofertilizers.

Table 1. *S. officinalis Dry leaves yield, g/m² essential oil% (EO%), essential yield, g/m² (EOY, gm²) 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. /m²			Essential oil%			Essential oil yield, g. /m²		
	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).

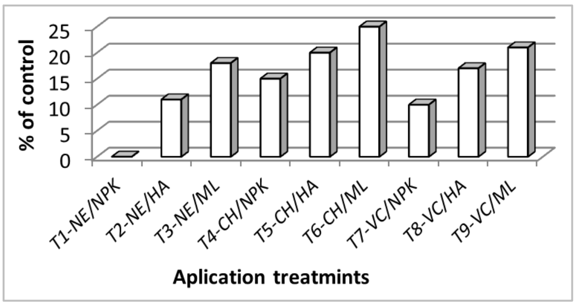

Figure 1. *Dry leaves yield, g/m² for sage elicited by BH, VC under NPK, HA, ML.*

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.

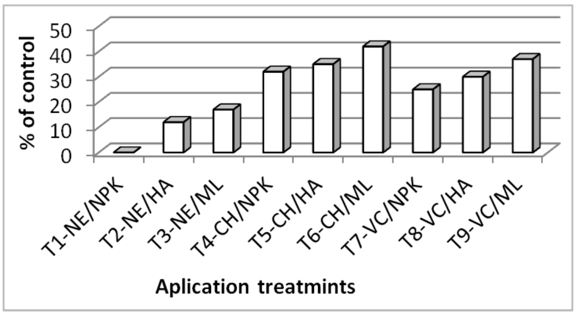

Figure 2. *Essential oil percentage for sage elicited by BH, VC Under NPK, HA, ML.*

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

Multi repeating CH or VC elicitation along ML, HA, NPK envoked significant appraise EOY, g/m² as percent over control (T_1 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, O% 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].

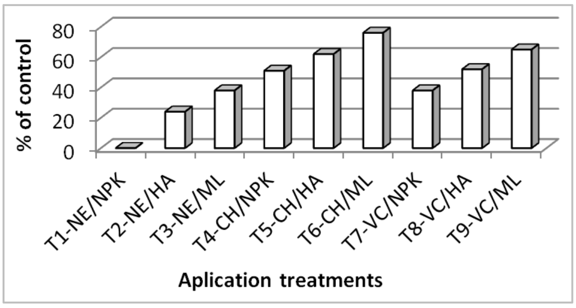

Figure 3. *Essential oil yield, g/m² for sage elicited by BH, VC Under NPK, HA, ML.*

4. Bioactive Secondary Production and Quality

Multi- repeating CH or VC elicitation entegrated with biofertilizers ML, HA and traditional NPK agrochemical fertilizer envoked 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.

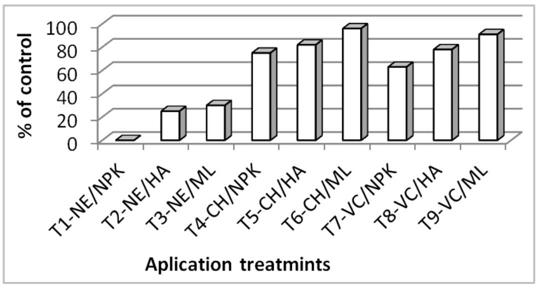

Figure 4. *Total phenolic, mgGA/g dry leaves for sage elicited by BH, VC under NPK, HA, ML.*

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.

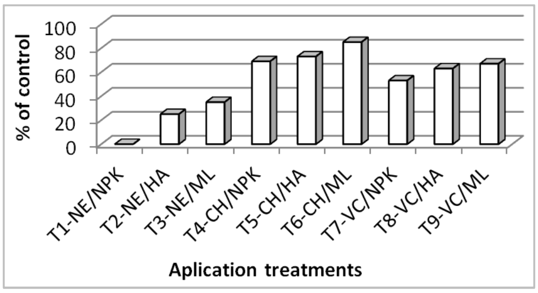

Figure 5. *Total flavonoids, mg/g.dry leves leaves for sage elicited by BH, VC under NPK, HA, ML.*

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.

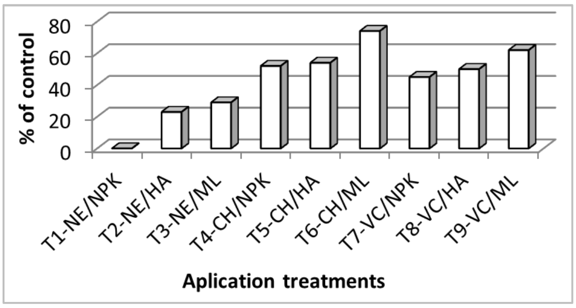

Figure 6. *Total flavonoids, mg RU/g.dry leves leaves for sage elicited by BH, VC under NPK, HA, ML.*

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 diseasis 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 antioxidan**t** 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	156.0 (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, O% T3 (NE/ML), T2 (NE/ HA), T1 (NE/NPK). Respectively;

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].

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 extractfor pooled mean of two seasons 2019 and 2020.*

Application Treatment	Caroph yllene	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.

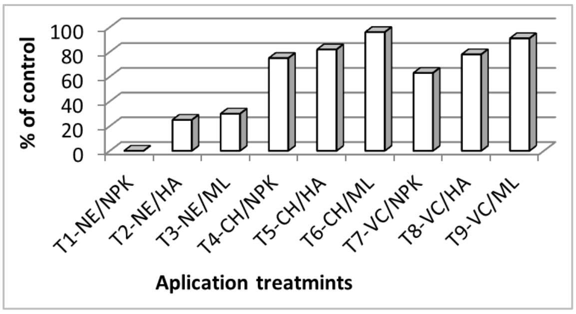

Figure 7. *Antioxidant activity for sage elicited by BH, VC Under NPK, HA, ML.*

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 microbioside and insecticide [65] which spur plant defense system inducing the immune system lead to promot yield and inhancing health benefits [67] which confermed 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 thenth, this strategy supported strong evidence its potentiality to achieve entigrated and sustainable development *S. officinalis* under biofertilizer that excel agrochemical fertilizer agriculture system.

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