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Evaluating Biotic Elicitation with Phenylalanine and/or Yeast for Rosemary (*Rosmarinus officinals* L.) Sustainable Improvement under Traditional and Organic Agriculture

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Abstract

Rosemary (*Rosmrinus officinals*) is one of the most important medicinal plants was cultivated for two subsequent seasons field experiment trial (March 2018, 2019) designed as factorial split-plot design with three replicates. The main factor 4 biotic elicitors: control (E_1), phenylalanine (E_2), yeast (E_3) and (E4), E2 + F3 Whereas, the sub-main factor, four fertilizers: (F1) NPK, (F2) 1/2NPK + PGPB and humic acid + PGPB (F_3), and moringa dry leaves extract. + PGPB (F_4). Statistical analysis for collected data revealed significant promotion for growth traits leading to significant increment biomass yield, secondary metabolites production and quality. Total phenolics, total flavonoids and essential oil its terpenes contents in which solitary, E2, E3 acted positive significant impact while E4exhiboted significant positive impact over E1 whereas, F1-4 achieved significant increment in which, biofertilser f4 > f3 > f2 over NPK biofertilser, while paired E1-4 with F1-4 performed E4E4 exceeded E3F3 exceeded E3F2 that exceeded E1F1. Therefore, multi-repeating elicitation with E2, 3, 4 coupled with Biofertilizers F2, 4 could be considered as eco-friendly innovative reliable practical application for sustainable improvement and sustainable use that exceeded significantly over traditional agriculture NPK alone or 1/2 NPK-PGPB for *R. officinals*.

Keywords

Rosemary, Aromatic Plant, Medicinal Plant, Elicitation, Biotic Elicitor

1. Introduction

Rosemary (*Rosmarin officinalis* L), RM, is a perenial aromatic and medicinal plant belong to the family Lamiaceae and originated from the Mediterranean region. However, it could found all over the world. It may be used as a spice in cooking, as a natural preservative in the food industry, and as ornamental and medicinal plant [1] [2]. RM, is one of the most important aromatic and medicinal plant in the world. It is grown under a wide range of climates, endogenous to Europe. Asia and Afica, mainaly in areas surrounding the Mediterranean Sea [3].

In folk medicine, *R. officinalis* has been used to treat headaches, poor circulation, epilepsy, [4]. Rosemary essential oil (RMEO) was reported to possess strong antioxidant and antimicrobial possess strong antioxidant and antimicrobial properties as wells as wound healing activity [5] [6]. Moreover, topical application of tea tree and RMEO has been documented with satisfactory safety and efficacy [4]-[9]. There is a lot of literature on the usefulness of bioactive supstances of RM plants, in order to the medicinal, pharmaceutical and food industries [10] Therefore many studies were interested in secondary metabolites (SMs) RM plant for their great beneficial effects for human health [11]. RM, has been used as health care supplements to treat arthritis, diabetes, memory loss and hair restoratio [12]. RM has great potential due to the different biological activities of its secondary metabolites (SMs), especially EO and polyphenols which have antidiabctic spasmolytic, carminative, hepatoprotective, antiviral and carcinogenic activities [11]. Furthermore, the aromatic RM herb is added to different types of food to improve the flavor and its organoleptic properties, stringent and food preservative and its antioxidant properties are still uses to extend the shelf-life of prepared foods [13] [14].

R. officinalis, can promote several pharmacological effects demonstrated by this plant [2], ability to attenuate asthma, atherosclerosis, cataract, renal, colic, hepatoxcity, peptic ulcer, inflammatory diseases, ischemic heart diseases [15] [16] control of hypercholesterolemia and oxidative stress and relief of physical and mental flatig [17], myocardial blood pressure reduction with rosmarinic acid [16], antiulcer action [18]. Lipid peroxidase reduction in heart and brain, [19]. antiangiocarnosal [20], prevention of problems related to the atherclerosis [21] anti-cancer and antiproliferative effects [22] [23] [24], antiviral [25] and antimicrobial actions [26] hepatoprotective [27], neuroprotective [28] and radioprotiective anti-mutagenic capacities [29], glycaemia reduction [30] muscle relaxant and treatment for cutaneous allergy [31], ability to treat depressive behavior [32], reducting the reactive oxygen (ROS) in Hela cells without cell toxicity [33].

R. officinalis, Contains a number of phytochemicals including rosmaric acids console, carnosic and oleanolic acid, unsolic acid. These compounds and some of their derivative have been demonstrated to have multiple physiological activities such as antioxidant [34] [35] anticancer [36] [37]. Anti-diabetic [38] [39] and could be a therapeutic agent in neurodegenerative disease treatment [40] [41] [42].

The antioxidant and antimicrobial activities of RM extract are mainly due to carnosic and carnosol [43] [44]. Carnosic acid is the major phenolic diterpene compound in RM leaves that stabilizes unsaturated fatty acids and thus related their deterioration [44] [46] [47], RMEO contain components with insecticidal properties that can be used as pesticides for stored product pests [48], RM extracts could scavenge free radical effects against boldenone induced damage in heart [49]. Also, RM infusion protect against hypodermic-ischemia [50]. RMEO, had bacteriostatic effect at a low concentration which is important for application in the food industry and did not have a germicidal effect on bacterial cells [51].

Elicitation application, can be used to increase SMs production and quality [52] [53] [54] [55] through enhance the transcription of biosynthetic genes involved in SMs biosynthetic pathway [56] [57] [58] that has potential importance particularly on human health benefits [59] [60] and play a major role in adaptation of plants to the changes environmental overcoming biotic and abiotic stresses, [61] [62]. Modified growth and development, can have benifical effects on morphological, physiological, biochemical characteristics than increased biomass yield production and quality [63] [64], Effortful studies has been undertaken for motivtation. innate ability of elicitation in producing extent of bioactive SMs and/or biomass production [65] Alos elicitation has been applied to stimulate the medicinal plants production through organic and agrochemical management contributing [66], Elicitation have related yet distinct role in regulation of plant and pathogen attack, that cause hug loss in yield production under agrochemical traditional agriculture [67], Biotic and abiotic stress/eliators, induces, the production of oxygen derived radicals such as H_2O_2 (hydrogen peroxide), superoxide molecules, hydroxyl (OH) and/or oxygen radicals (O) that are the first line of defense for stressed plant [68] which are often implicated to induce systemic resistance (ISR) by regulating the expression genes involved for production and accumulation of SMs, phytoalexins (PAs) which non specific toxins characterized brood spectrum bio-anti-pesticides and bio-anti-micro biocides making them improving against microbial diseases and pests infestation [64] [69] [70] [71].

In the traditional cultivation methods, the excessive use of pesticides and chemical fertilizers leads to an imbalance of nutrient contents in the soil, an increase in vegetative quality and yield. In the face of increasingly serious invironmental and food problems, organic agriculture is considered to be an effective solution. Since the beginning of green revolution the agriculture has changed by excessive use of fertilizers pesticides, microbiocides [72] [73] in order to increase productivity this agrochemical has been indiscriminately used not only in grain and horticulture plants [66]. Organic agriculture (OA) has been growth in recent years, reaching a 300% increased of production unite between 2010-2018 [74], this trend, both in production and consumption. Agrochemicals and pesticides impact the environment, preventing sustainable development [75] [76] [77]. Several studies indicated an association between the increase use pesticides and health-related problems such as incidence of fatal malformation, child hood and Juvenile cancer, impacts on hearing loss and numerous occupational contamination events of renal registered in the health system [78] There is a contradiction when talking about the safe use of pesticides, microbiocides for the application of the product [79].

Over recent decades organic farming practices have more widely respected globally, leading to significant increase in certified organic farm [80] [81]. According to the recent survey by [82], a total of 69.8 million hector were organically managed and 93 countries had organic regulation at the end. Organic agriculture practices include a focus on soil health, ecological process and biodiversity without relying on use synthetic chemicals impute [83]. Grown organically medicinal plant by using different organic fertilizers, produced best results in many investigations [84]. It has been reported that organic fertilizers enhanced dry weight, yield, total phenolic, total flavonoids and vitamin C. besides, microorganism can be act as elicitors and increase in the biological and pharmaceutical activities as well as overcoming biotic and abiotic stresses [85]. Such as phenolic flavonoids and terpenes content, microbial activity, chlorophyll content, nutrient uptake, plant growth and development [86] [87] [88] [89]. Biofertilizers has physiological role towards sustainable agriculture in reducing physiological role towards sustainable agriculture in reducing problems associated with the use of agrochemicals [90].

Plant growth promoting bacterium (PGPB) can influence directly or indirectly the general morphology of plants, as they have the ability to recognize tissues and different mechanisms of action [91] [92]. They can act on iron sequestration and phosphorus solubilization, atmospheric nitrogen, hormone production [93], Systemic resistance to pathogens, tolerance to biotic and abiotic stress [94] [95]. Bio fertilizers, suppressed plant diseases, inducing systemic resistance (ISR) against pathogens [71] [95], Diminish environmental concerns associated with the use of synthetic fertilizers to be environmentally co-friendly, the application of bio fertilizers [96] [97], Cabable of improving plant growth, yield production and quality [98], Reducing problems associated with use of chemical fertilizers [90] [99], The application of bio stimulant such as microorganisms rhizobacterium, humic acid, moringa, neem leaves in vivo cultivation of medicinal plants open the opertunity for the development of organic fertilizers for agroecolgoical system, aiming at good quality raw material without pesticides, and/or microbiocides, with, increased concentration of SMs biologically and pharmacologically [100]-[106].

To the best of our knowledge, no findings have been reported on malti-repeating elicitation technology with biotic elicitors for promoting sustainable agriculture medicinal and oromatic plants, under tradiational and organic system. Therefore, the aim of this study was to evaluate potential synergistic elicitation impacts with phenylalanine and yeast to sustainable improvement rosmarene (*Rosonarins officnals* L.) biomas production and quality under traditional and organic agriculture systems.

2. Material and Methods

2.1. Biotic-Elicitor Application

R. officinals 2-month old plants were foliarily, with phenylalanine (essential amino acid), 125 ppm (E_2), yeast, 1.5 g/L (F_3), and (E4) in targeted in tegrated E2 + E3, with Tween 80, o.1%, plants were sprayed only with tween 80 solution, as control l (F1).

2.2. Fertigation Management

Four fertilizer (F1-4), chemical NPK, 20 g/m^2 (F_1), traditional chemical fertilizer, as control (F_1) and 10 g/m^2 NPK, tinoclulated seeds (F2) with(PGPB) mixture of nitro.fixing bacteria (Azotobacter SP+ Azospirillum Sp) and biophosphorus bacteria (*Bacillus* Sp + *Pseudomonasp*). (F3), humic acid, 20 g/m^2 + inoculated seeds with-(PGPB), (F4) moriga dry leave extract, 20 g/m^2, + inoculated Seeds with PGPB. Such F1-4, 30 m^3/L from solution of 5% from each Fe, Zn, Mn, Mg, Cu, were added. Fertigation was under taken monthly from sowing up tell one month before harvesting.

2.3. Execute Field Experiment

Inoculated and non inoculated seeds with PGPB were cultivated two subsequent seasons field experiment trials, 2018 and of 2019, were designed as factorial split-plot based on randomized complete block design with 3 replications. Four elicitors (E_{1-4}) as main plot and 2-bio fertilizers (F_3, F_4). RM seeds were sown 20 March at both seasons, in plots 3 × 2.5 m^2 size in rows 50, 60 cm enter and entra-spacing. Irrigation and fertigation management through surface drip irrigation system. Resultant plants aged 60, 90, 120, 150, 180 days were foliarly sprayed with (E_{1-4}) and harvested at September 2018, 2019.

2.4. Biometeric growth traits

1) Five randomized selected, plants were recorded for plant height (PH, Cm), number of branches/plant (NBP), fresh herb/plant (FHP, g.) dry herb/plant (DHP, g.), fresh leaves per plant (FLP, g.) and dry leaves per plant (DLP, g.).

2) Biomass yield traits: fresh herb yield, Kg/m^2 (FHY, Kg/m^2), dry herb yield, Kg/m^2 (DHY, Kg/m^2), fresh leaves yield, Kg/m^2 (FlY, Kg/m^2) and dry leaves yield, kg/m^2 (DLY, Kg/m^2) were also recorded.

2.5. Quli-Quantitative Bioactive Secondary Metabolites (BSMs) Evaluation

Phenolic Compounds

Extraction procedure:

Dry leaves samples of RM powder (15 g.) were placed in the filter cartridge (paper No. 89) in a classical soxhlet 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 RM extracts were stored in glass vials with Teflon sealed, at 20 ± 0.5 C in the absence of light.

Total penolic content (TPC) assay:

TPC was assayed by folin-ciocaleau clorimetric method [107], methanolic extracts (0.1 ml) was mixed with 2.5 ml. distilled water followed by the addition of 1 ml (2N) folin-Cicalteau ragnet. Then 0.5 ml 20% Na_2CO_3 was added 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 curve with 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 by [90] the methanol leaves extract standard (0.25 ml) were mixed with 1 - 475 ml distilled water. Ten 0.075 ml 5% $NaNO_3$ solutions were added. After 5 min, the absorption was measured at 510 nm using spectrophotometer the absorbance was expressed as mg. of catechin equivalents per gram dry leaves weight.

Main phenolic compound:

The rosmarynic acid and carnosic acid content of rosmary dry leaves extract were determined by HPLC conditions of device mobile phase. (A, methanol + B, 10 mM 850 ml. acetic acid) and (150 ml acetonitrile mixture) elution condition linear gradiant, flow 1 - 1 mm^{-1}, coloumin type Zorbax, 5 Um. 15 cm. X4 - 6 mm, detector: waters 2487 daul absorbance UV 285 nm, injection volume 25 UL.

Essential oil % (EO%):

EO was determined according to [108] 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 (EOY g./m^2):

EOY, g/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 with FID and mass spectrophotometer detectors using a HP-5MS 5% capillary column (30.00 m X0.25, 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 split ratio was 40:1. The injector temperature was set at 300˚C. The acquisition range was 50 - 550 m/Z in electron impact (EI) mode using an ionization voltage of 70ev. 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 (RI), determined with 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 [109]. The relative amounts of individual components were calculated based on CG peak area (FID response).

Statistical analysis:

Statistical analysis for the interaction between two subsequent season (2019 and 2021) were found to be not significant. Therefore, The pooled mean values of 2 years for all the traits were subjected to statistical analysis of variance was done for all traits whereas, the calculated least significant differences, LSD at 1% level were used for comparison between mean treatments.

3. Results and Discussion

3.1. Growth Traits

Multi-repeating elicitation with E_{1-4} under F_{1-4} caused significant positive impact on growth traits $E_3 > E_2 > E_1$ while E_4 actuated significant synergistic impact at $F_4 > F_3 > F_2 > F_1$, therefore, the interaction, $F_4E_4 > F_3E_3 > F_2E_2 > F_1E_1$ as represented in **Table 1**, exceedingly multi-repeating elicitation under bio fertilizer ($F_{3,4}$) over traditional ($F_{2,1}$) Chemical fertilizers, However PGPB, application reduced NPK (F1) these data were in accordance with that has been reported by [4] [97] [110] [111] [112] [113] [114].

3.2. Biomass Yield Traits

$E_{2,3}$ invoked significant increment in DLY, g/m^2 and EOY, g/m^2 whereas E_4 achieved synergistic positive impact under F_{1-4} aside F_{1-4} resulted in significant improvement aside $F_4 > F_3 > F_2 > F_1$ hence, $F_4E_4 > F_3E_3 > F_2E_2 > F_1E_1$ significantly as represented **Table 2** and illustrated **Figure 1**. These obtained data were in agreement with that has been investigated. [58] [110] [111] [115].

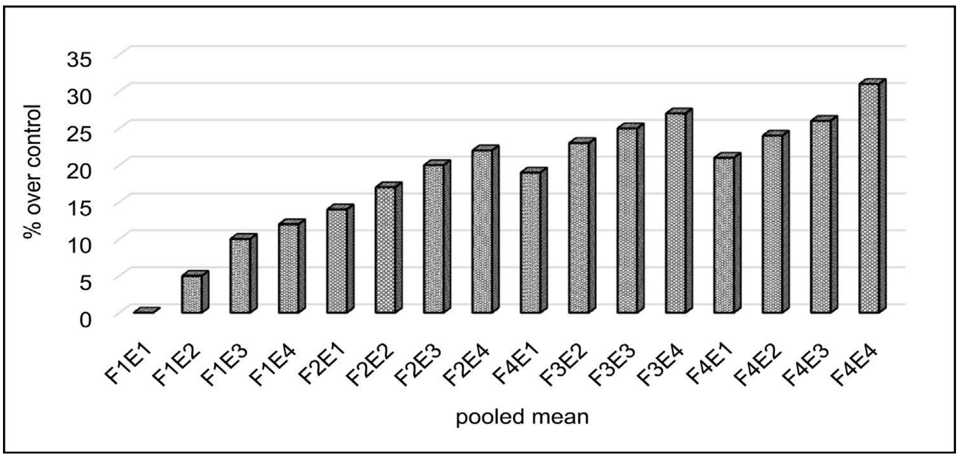

Figure 1. Pooled mean DLY, g/m^2 as % over control under E1-4 interacted with F1-4 for *R. officinals*.

Table 1. Mean growth traits at two subsequent seasons for *R. officinals* in response to multi-repeating elicitation with 4 biotic elicitors under 2-traditional fertilizer (F1, 2) and 2-organic, biofertilizers (F3, 4).

Application Treatment	Plant hight, cm			Number branches			FLYP, g.		
	2018	2019	Pooled mean	2018	2019	Pooled mean	2018	2019	Pooled mean
F_1E_1	70.7	98.7	84.70 (0)	95.8	102.5	99.15 (0)	350.5	432.3	391.4 (0)
2	75.6	105.6	90.60 (+7)	100.6	107.6	104.10 (+5)	368.3	435.9	411.1 (+5)
3	77.5	107.9	92.70 (+9)	104.4	111.7	108.05 (+9)	385.5	475.5	430.5 (+10)
4	78.9	109.8	94.35 (+11)	109.2	116.8	113.00 (+14)	392.6	484.2	434.4 (+12)
F_2E_1	79.8	111.5	95.65 (+13)	111.1	118.9	115.00 (+16)	399.6	492.8	446.2 (+14)
2	81.3	113.5	97.40 (+15)	114.9	123.0	118.95 (+20)	410.1	505.8	457.9 (+17)
3	82.7	115.5	99.10 (+17)	120.7	129.2	124.95 (+26)	420.6	518.7	469.7 (+20)
4	86.8	118.4	101.60 (+20)	125.4	134.2	129.80 (+31)	427.6	527.5	477.6 (+22)
F_3E_1	86.3	120.4	103.35 (+22)	116.8	125.1	120.90 (+22)	417.9	514.4	466.2 (+19)
2	88.3	123.4	105.85 (+25)	121.6	130.2	125.90 (+27)	431.1	531.7	481.4 (+23)
3	87.8	125.3	107.55 (+27)	126.4	135.3	130.85 (+32)	438.1	540.4	489.3 (+25)
4	92.6	129.3	110.95 (+31)	129.3	138.4	133.85 (+35)	445.1	549.2	497.2 (+27)
F_4E_1	89.2	124.3	106.75 (+26)	122.6	131.2	126.90 (+28)	424.1	523.1	473.8 (+21)
2	91.2	127.3	109.25 (+29)	127.4	136.3	131.85 (+33)	434.6	536.1	485.4 (+24)
3	93.3	130.2	111.75 (+32)	130.2	139.4	134.80 (+36)	441.6	544.7	493.2 (+26)
4	95.4	133.2	114.30 (+35)	132.2	141.5	136.85 (+38)	459.2	566.3	5128 (+31)
LSD1%	**0.23**	**0.28**	**0.26**	**0.25**	**0.31**	**0.27**	1.9	2.6	2.4

E1-4: O control, phenylalanine yeast and integrated with phenylalanine yeast, respectively; F1-4 NPK chemical, traditional fertilizer, 1/2NPK + PGPB, humic acid + PGPB, and moringa dry leaves extract + PGPB, respectively.

Table 2. Dry leave yield, g/m^2 (DLY, g/m^2) and essential oil yield, g/m^2 (EOY, g/m^2) for. *R. officinalis* at 2-subsequent cultivated seasns under traditional and organic fertilizer and multi-repeating illicitation with biotic elicitors.

Application Treatment	DLY, g./m^2			EO DlY, g./m^2		
	2018	2019	Pooled mean	2018	2019	Pooled mean
F_1E_1	337.9	335.2	346.5 (0)	273.8	301.8	387.8 (100)
2	354.8	372.9	363.8 (+5)	445.6	522.2	483.9 (125)
3	371.7	390.7	381.2 (+10)	550.6	563.2	556.9 (144)
4	378.4	397.8	388.1 (+12)	582.1	644.8	613.4 (158)
F_2E_1	385.2	404.9	395.0 (+14)	429.3	490.1	459.7 (119)
2	395.3	415.5	405.4 (+17)	549.1	607.4	578.3 (149)
3	405.5	426.2	415.8 (+20)	641.4	707.2	674.3 (174)
4	412.2	433.3	422.7 (+22)	688.0	740.4	714.2 (184)
F_3E_1	402.1	433.3	412.4 (+19)	570.8	630.3	600.5 (155)
2	415.6	436.9	426.2 (+23)	673.9	742.9	708.4 (183)
3	422.4	440.0	433.1 (+25)	742.7	783.2	762.9 (197)
4	429.1	451.1	440.0 (+27)	797.9	816.3	807.1 (208)
F_4E_1	408.8	429.7	419.3 (+21)	621.7	683.7	652.7 (168)
2	418.9	440.4	429.6 (+24)	691.4	743.6	717.5 (185)
3	425.7	447.5	436.5 (+26)	813.6	828.8	821.2 (212)
4	442.6	465.3	453.9 (+31)	890.4	897.5	894.0 (231)
LSD1%	11.1	12.7	9.4	9.1	11.3	10.6

E1-4: O control, phenylalanine yeastand coubled phenylalanine and yeast, respectively; F1-4 NPK chemical, traditional fertilizer, 1/2NPK + PGPB, humic acid + PGPB, and moringa dry leaves extract + PGPB, respectively.

There is no best and disease incidence in the 2 seasons field experiments, in accordance to multi-repeating elicitation with E2-4 and F2-4, whom could induce systemic resistance (ISR) that lead to protect and biological control for elicitated and bio fertilized plants [69] [100] [102] [111] [113] [116] [117].

3.3. Biomas Yield Quality Traits

Secondary metabolites (SMs), total phenol content (TPC), total flavonoid content (TFC). **Table 3** and major phenolic acid, romasrynic acid (RCA) and carnosic (CTC). **Table 4** declared that E3 > 2 achieved significant increase for (TPC), TFC, RCA, CTC while E4 resulted synergistic increament in these traits under F_1-F_4 aside $F_4E_4 > E_3F_3 > E_2F_2 > E_1F_1$ significantly, as represented (**Table 3**, **Table 4**).

SMs, EO components were listed **Table 5** that declared, ciniol (20.33%, linolool (16.57%), α-penine (15.50), compere (5.80%), limonine (3.22%), P-cymene (2.42%), tepineol (2.82%), caryophllene (1.70%) and terpiniol (1.51%). E2,3 performed significant increase ciniol, liniol, α-penine as well as total components for EO while E4 resulted synergetic increment under F_{1-4}. Aside $E_4F_4 > E_3F_3 > E_2F_2 > E_1F_1$ significantly. As represented **Figure 2** It has been extensively declared that biotic elicitor enhanced SMs production and quality [71] [100] [102]. Also, bio fertilizer enhanced SMs production and quality [110] [111] [118] Since plant pathogens cause huge yield losses. Plant defense often depends in toxic SMs that inhibit pathogen and overcoming biotic and abiotic stresses [69] [70] [110] [119] [120].

Table 3. Total phenolics compound/ and total flavonoids (TFC) compounds for *R. Offcinalis* cultivated 2-subsequent seasns under traditional and organic system in respone to multi repeating biotic elicittion.

Application Treatment	TPC			TFC		
	2018	2019	Pooled mean	2018	2019	Pooled mean
F_1E_1	30.47	35.75	33.11 (100)	2.66	3.72	3.19 (100)
2	41.13	48.26	44.69 (135)	3.45	4.83	4.14 (130)
3	47.53	55.77	51.65 (156)	3.59	5.02	4.30 (135)
4	53.32	62.56	57.94 (175)	3.77	5.28	4.52 (142)
F_2E_1	31.99	37.53	34.76 (105)	3.05	4.27	3.66 (115)
2	45.40	53.26	49.33 (149)	3.64	5.09	4.37 (137)
3	51.18	60.06	55.62 (168)	3.85	5.39	4.62 (145)
4	55.45	65.06	60.25 (182)	4.01	5.61	4.81 (151)
F_3E_1	33.82	39.68	36.75 (111)	3.21	4.50	3.85 (121)
2	49.36	57.91	53.63 (162)	3.83	5.35	4.59 (144)
3	54.23	63.63	58.93 (178)	3.99	5.58	4.78 (150)
4	58.80	68.99	63.90 (193)	4.44	6.21	532 (167)
F_4E_1	35.95	42.18	39.06 (118)	3.32	4.65	3.98 (125)
2	52.71	61.84	57.28 (173)	4.20	5.87	5.04 (158)
3	56.36	66.13	61.25 (185)	4.33	6.06	5.19 (163)
4	60.02	70.42	65.22 (197)	5.81	4.66	6.51 (175)
LSD1%	0.21	0.27	0.25	0.06	0.8	0.07

E1-4: O control, phenylalanine yeast and coubled phenylalanine and yeast, respectively; F1-4 NPK chemical, traditional fertilizer, 1/2NPK + PGPB, humic acid + PGPB, and moringa dry leaves extract + PGPB respectivel.

Table 4. Pooled mean (2018, 2019 seasons) for major phenolic acids, rosmarynic acid (RAC) and carnosic acid (CSC) under E1-4 interacted with F1-4 for R-officinals.

Application Treatment	RAC			CTC		
	2018	2019	Pooled mean	2018	2019	Pooled mean
F_1E_1	16.51	13.62	15.06 (100)	112.15	95.74	103.94 (100)
2	20.63	17.02	18.82 (125)	159.25	135.95	147.60 (142)
3	21.95	18.11	20.03 (133)	168.22	143.61	155.91 (150)
4	23.11	19.06	21.08 (140)	188.41	160.84	174.62 (168)
F_2E_1	17.83	14.70	16.26 (108)	128.97	110.10	119.53 (115)
2	21.79	17.97	19.88 (132)	177.19	151.26	164.22 (158)
3	23.93	19.74	21.83 (145)	185.04	157.97	171.50 (165)
4	25.92	21.38	23.65 (157)	192.89	164.67	178.78 (172)
F_3E_1	18.49	15.26	16.87 (112)	143.55	122.54	133.04 (128)
2	23.11	19.06	21.08 (140)	185.04	157.97	171.50 (165)
3	24.93	20.56	22.74 (151)	190.65	162.75	176.70 (170)
4	27.24	22.47	24.84 (165)	202.99	173.65	188.32 (181)
F_4E_1	18.99	15.66	17.32 (115)	148.03	126.37	137.20 (132)
2	25.09	20.70	22.89 (152)	194.01	165.63	179.82 (173)
3	27.41	22.61	25.01 (166)	207.47	177.11	192.29 (185)
4	29.38	24.24	26.8 (178)	216.44	184.77	200.40 (193)
LSD1%	0.17	0.12	0.15	0.85	0.72	0.81

E1-4: O control, phenylalanine), yeast and coubled phenylalanine and yeast, respectively; F1-4 NPK chemical, traditional fertilizer, 1/2NPK + PGPB, humic acid + PGPB, and moringa dry leaves extract + PGPB, respectively.

Table 5. Essential oil contents for pooled mean (2018, 2019 seasons) for R-officinals under F1-4 interacted with E1-4.

Application Treatment	EO components										
	α-Penine	Comphere	Limonine	Cineol	Terpinene	p-cymene	Linlool	Terpineol	Caryophllene	α-Terpineol	Total
F_1E_1	15.50	5.80	3.22	20.33	1.12	2.42	16.57	1.51	1.70	2.82	70.99
2	16.12	6.03	3.35	21.14	1.16	2.52	17.23	1.57	1.77	2.93	73.82
3	16.59	6.21	3.45	21.75	1.20	2.59	18.05	1.62	1.82	3.02	76.30
4	16.90	6.32	3.51	22.16	1.31	2.64	18.06	1.65	1.85	3.07	77.47
F_2E_1	15.97	5.97	3.32	20.94	1.15	2.49	17.07	1.56	1.75	2.90	76.76
2	16.43	6.15	3.41	21.55	1.19	2.57	17.56	1.60	1.76	2.99	75.21
3	16.74	6.26	3.48	21.96	1.21	2.61	17.90	1.63	1.82	3.05	76.66
4	17.52	6.55	3.64	22.97	1.27	2.74	18.72	1.71	1.85	3.19	80.16
F_3E_1	16.28	6.09	3.38	21.35	1.18	2.54	17.40	1.59	1.79	2.96	74.56
2	17.05	6.38	3.54	22.36	1.23	2.66	18.23	1.66	1.87	3.10	78.08
3	17.36	6.50	3.61	22.77	1.26	2.71	18.56	1.69	1.90	3.16	79.52
4	17.83	6.67	3.70	23.38	1.29	2.78	19.06	1.74	1.96	3.24	81.65
F_4E1	16.59	6.21	3.45	21.75	1.20	2.59	17.73	1.62	1.82	3.02	75.98
2	17.98	6.73	3.74	23.58	1.30	2.81	19.22	1.75	1.97	3.27	82.35
3	18.29	6.84	3.80	23.99	1.32	2.86	19.55	1.78	2.01	3.33	83.77
4	18.91	7.08	3.93	24.80	1.37	2.95	20.22	1.84	2.07	3.44	86.61
LSD 1%											0.42

E1-4: O control, phenylalanine yeast and coubled phenylalanine and yeast, respectively; F1-4 NPK chemical, traditional fertilizer, 1/2NPK + PGPB, humic acid + PGPB, and moringa dry leaves extract + PGPB, respectively.

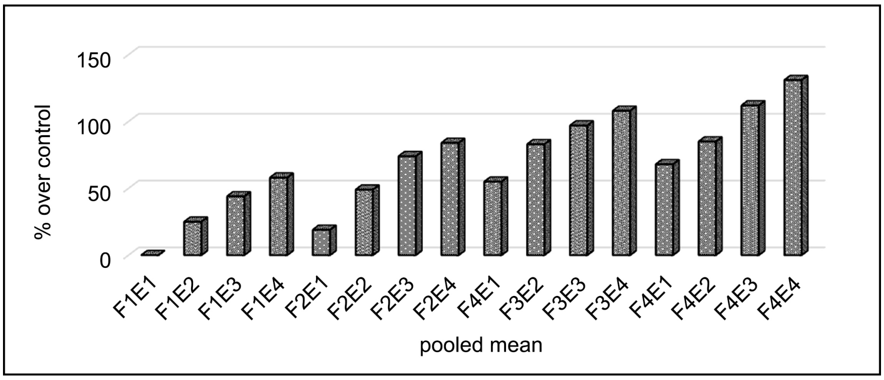

Figure 2. Pooled mean EOY, g/m^2 as % over control under E1-4 interacted with F1-4 for *R. officinals.*

4. Conclusion

According to our study it declared multi-repeating elicitation with yeast (E_3), phenylolanine (E_2) and coupled (E_{2+3}) under organic system, bio fertilizers (humic acid + PGPB, F_3; Moringa dry leaves extract + PGPB, F_4) ensured sustainable development and sustainable reliable practical application, without NPK system (F1), While F2 reduced NPK, application.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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