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# Effect of some growth stimulators on productivity, chemical and biological properties of *Coleus amboinicus* Lour. Cultivated under desert conditions

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**Abstract**
During the two successive seasons, 2020 and 2021, this investigation was carried out on *Coleus amboinicus* to study the effect of spraying some plant stimulators on growth, yield, active constituents, and biological activities. The experiment included six foliar spray treatments (control, moringa extract, blue green algae extract, compost tea, active dry yeast extract, and Azolla extract). The applications were arranged in a randomized complete block design with three replicates. The measured data involved growth and yield parameters (plant height, fresh weight of herb per plant, fresh yield of herb per hectare, dry weight of herb per plant, and dry yield of herb per hectare), chemical parameters (essential oil percent, oil content per plant, oil yield per hectare, oil composition, total phenolic content, total flavonoid content), and biological activities (antioxidant and anticancer). The results represented that spraying *C. amboinicus* with all stimulators significantly increased growth, yield, essential oil percent, oil content per plant, and oil yield per hectare over control. The significant promising treatment was sprayed with blue green algae extract, which recorded the best traits. Moreover, the results showed that using blue green algae extract revealed the highest total phenolic content in the plant while utilizing compost tea represented the highest total flavonoid content. Consequently, using compost tea enhanced *in vitro* antioxidant and anticancer activities of *C. amboinicus*.

**Keywords:** *Coleus amboinicus*, biostimulators, yield, active constituents, biological activities

## Introduction
*Coleus amboinicus* (synonym: *Plectranthus amboinicus*) is a member of the Lamiaceae family. It grows up to 1 m in height. The stem is fleshy, about 30-90 cm, either with long rigid hairs or densely covered with soft, short, erect hairs. Old branches are smooth. Leaves are 5-7 cm by 4-6 cm, fleshy, simple, broad, egg/oval-shaped with a tapering tip. The margins are coarsely crenate to dentate-crenate except in the base. They are thickly studded with hairs (pubescent), with the lower surface possessing the most numerous glandular hairs, giving a frosted appearance. The petiole is 2-4.5 cm. The aroma of the leaves can be described as a spicy combination of the aromas of oregano, thyme, and turpentine. The leaf's taste is similar to the oreganos but with a sharp mint-like flavor. The plant is considered native to Africa, the Arabian Peninsula, and India. However, it is widely cultivated and naturalized elsewhere in the tropics, used as a spice for seasoning meat dishes and in food products and ornamental plants. Common names in English include French thyme, Indian mint, Mexican mint, Cuban oregano, and Spanish thyme. *Coleus aromaticus* is considered an antispasmodic, stimulant, and stomachic and treats headaches, fever, epilepsy, and dyspepsia. It is used to treat indigestion, diarrhea, nervous tension, insect bites, toothache, earache, rheumatism, whooping cough, and bronchitis. The plant also finds significant importance in modern medicine [1-7].
Plant growth stimulators support crops during the different growth phases. Stimulators consist of essential nutrients, hormones, amino acids, proteins, vitamins, and other natural elements that boost the plants during a particular growth phase. They also make crops less susceptible to diseases and diverse abiotic stresses. They work quickly and effectively. Each simulator has different dosage and usage instructions.

The most crucial growth stimulants used as a spray-on plant are compost tea, blue-green algae extract, active dry yeast extract, moringa leaf extract, and Azolla extract. Many researchers have pointed out its importance in increasing the productivity of medicinal and aromatic plants and their contents of active constituents [8] on Dutch fennel; [9] on dill; [10] on lemongrass, and [11] on curly parsley.

*C. amboinicus* is a drought-tolerant plant and does not have to water regularly. So, we target to introduce the cultivation of this plant in our new reclamation lands, where it bears the lack of irrigation water as a new medicinal plant and spice, which contributes to the development of exports and the pharmaceutical industry. The research aims to study spraying with some biostimulants on growth, yield, phenolic constituents, and biological activities *(in vitro* antioxidant and anticancer effects).

## Materials and Methods

The experiment was carried out at Ahmed Orabi Agricultural Cooperative Association, Cairo-Ismailia Desert Road, during the two successive seasons 2020 and 2021. The chemical analysis of irrigation water of the farm was as follows (EC=316 ppm, $CO_3^{--}$ =0.00, $HCO_3^-$ =1.46 meq/l, $Cl^-$ =1.19 meq/l, $SO_4^{--}$ =2.68 meq/l, $Ca^{++}$=3.10 meq/l, $Mg^{++}$= 1.32 meq/l, $Na^+$= 0.82 meq/l, $K^+$=0.09 meq/l). The physical analysis of experimental farm soil was as follows (sand=53.00%, silt= 41.50%, clay=5.50%, soil texture=sandy). The chemical analysis of experimental farm soil was as following (pH=8.25, EC=921.60 ppm, $CO_3^{--}$ =0.00, $HCO_3^-$ =0.50 meq/l, $Cl^-$ =8.50 meq/l, $SO_4^{--}$=5.28 meq/l, $Ca^{++}$=4.50 meq/l, $Mg^{++}$= 2.50 meq/l, $Na^+$= 7.00 meq/l, $K^+$=0.28 meq/l).

The seedlings of *Coleus amboinicus* were obtained from the Faculty of Agriculture, Al-Azhar University. The soil was prepared before planting by adding organic fertilizer at 24 $m^3$ compost/hectare. The distance between rows was 75 cm, and between plants within a row was 50 cm (26667 plants/hectare). The seedlings were planted on the 25 of March. The irrigation system of the experiment was drip irrigation with a rate of 4 l/h. Half of the recommended chemical fertilization was added to all experimental treatments, and the same amount was repeated after each cut [12].

The research included six spraying material treatments:- control, *Moringa oleifera* leaf extract, blue green algae extract, compost tea, active dry yeast extract, and Azolla extract. It was planned in a randomized complete block design with three replicates. Moringa leaves extract was applied at a concentration of 6 g/l, spraying with blue green algae extract of *Spirulina platensis* was used at a 5 ml/l, compost tea was ready by soaking compost in water (1: 4 v/v) for 24 hours as plants were treated with water extract of the compost, spraying with active dry yeast extract was utilized at a concentration of 10 g/l, Azolla extract at a concentration of 9 g/l [13, 14, 10, 15]. Spraying with growth stimulants was carried out a month after transplanting, and the spraying was repeated after each harvest. The analyses of some chemical properties for different growth stimulators are shown in Tables (1-5).

All the obtained data were subjected to analysis of variance method according to [16]. The differences among means were compared using the LSD test at the probability of 5% level. The herb was cut two times in the season. The first one was on 28 June and the second was on 1 October. The plants were harvested by cutting the herb at 10 cm height, leaving some branches for regrowth. The parameters under evaluation were as the following mention.

### Growth and yield data

Plant height (cm), fresh weight of herb/plant (g), fresh yield of herb/hectare (ton), dry weight of herb/plant (g), and dry yield of herb/hectare (ton).

### Chemical analyses data

#### Essential oil parameters

Essential oil percentage in the fresh leaves [17], essential oil content/plant (ml), essential oil yield/hectare (l), and chemical composition of essential oil by Gas Chromatography/Mass Spectrometry system (GC/ MS).

**Table 1:** The analysis of moringa leaf extract (mg $g^{-1}$ DW)

| Total amino acids | P | Ca | $K_2O$ | Fe | Mn | Cu |
|---|---|---|---|---|---|---|
| 198.30 | 4.13 | 5.11 | 24.60 | 2.00 | 4.00 | 0.44 |

**Table 2:** The analysis of aqueous extract of *Spirulina platensis*

| N (%) | P (%) | $K_2O$ (%) | Fe (mg/l) | Mn (mg/l) | Zn (mg/l) | Cu (mg/l) |
|---|---|---|---|---|---|---|
| 1.60 | 0.01 | 0.90 | 269.00 | 89.00 | 81.00 | 54.00 |

**Table 3:** The analysis of aqueous extract of compost tea

| N (%) | P (%) | $K_2O$ (%) | Fe (mg/l) | Mn (mg/l) | Zn (mg/l) | Cu (%) |
|---|---|---|---|---|---|---|
| 1.15 | - | 0.78 | 12.5 | 3.00 | - | - |

**Table 4:** The analysis of active dry yeast

| Protein (%) | Carbohydrates (%) | Nucleic acids (%) | Minerals (%) | Lipids (%) |
|---|---|---|---|---|
| 47 | 33 | 8 | 8 | 4 |

**Table 5:** The analysis of Azolla extract

| N (%) | P (%) | $K_2O$ (%) | Mn (ppm) | Cu (ppm) | Zn (ppm) | B (ppm) |
|---|---|---|---|---|---|---|
| 0.07 | 0.02 | 1.50 | 59 | 0.87 | 5.19 | 1.70 |

### Determination of total phenolic content

The total phenol content was determined using the Folin–Ciocalteu method as described by [18], with minor modifications to be carried out in microplates. Briefly, 10 µL of each of the examined extracts (5 mg/mL) and the standard was mixed with 100 µL of Folin-Ciocalteu reagent (Diluted 1: 10) in a 96-well microplate. Then, 80 µL of 1 M $Na_2CO_3$ was added and incubated at room temperature for 20 min in the dark. At the end of incubation time the resulting blue complex color was measured using microplate reader FluoStar Omega at 630 nm. Meanwhile, gallic acid (1 mg/mL) was used as the standard and serially diluted in concentration of 25, 50, 100, 200, 400, 500, 800 and 1000 µg/mL for the construction of the calibration curve. The total phenolic content of the tested samples was expressed in terms of µg gallic acid equivalents/mg dry extract (µg GAE/mg DE). Data were represented as means ± SD.

### Determination of total flavonoid content

The total flavonoid content of the tested extracts was analyzed using $AlCl_3$ assay that was previously reported by [19] with some modifications to be carried out in 96-well microplate. Quercetin stock solution of 1 mg/mL in MeOH

was prepared, and 12 serial dilutions were prepared in concentration range (0.625 – 640 µg/mL) to be used as a standard for generate the calibration curve. 15 µL of sample (5 mg/mL)/standard was placed in a 96-well microplate. Then, 175 µL of methanol was added followed by 30 µL of 1.25% $AlCl_3$. Finally, 30 µL of 0.125 M $C_2H_3NaO_2$ was added and incubated for 5 min followed by the determination of absorbance using FluoStar Omega Microplate Reader at 420 nm. Data were given as means ± SD and the results were presented as µg quercetin equivalents/mg dry extract (µg QE/mg DE).

**Preparation of plant extracts for HPLC and bioassays**

Three treatments including; control and the bio-stimulator treatment with highest flavonoid content as well as the treatment have the highest phenolic content were only investigated. The alcoholic extracts of their plant aerial parts were prepared by macerating 50 g plant powder from each treatment in methanol (three times) after drying in air and grinding steps. The methanol extract in each treatment was concentrated until dryness using rotary evaporator to obtain a semisolid residue.

**HPLC analysis**

Standard stock solutions of four flavonoids (quercetin, kaempferol, hesperidin, and diosmin) and six phenolic acids (gallic acid, vanillic acid, cinnamic acid, *p*-coumaric acid, ferulic acid, and chlorogenic acid) in addition to quinic acid were prepared in methanol at a concentration of 1 mg/mL and stored in the refrigerator at 5 °C until use. All stock solutions were further diluted 10 times with methanol before HPLC injection. The extract of each analyzed treatment (100 mg) was dissolved in methanol (10 mL) and passed through a 0.45 µm pore size filter. 1 mL of the filtrate was diluted to 10 mL (1 mg/mL) and 20 µl of this sample was injected. HPLC analysis was performed with Thermo HPLC (Ultimate 3000) with a diode array detector DAD-3000, and associated DELL-compatible computer supported with Cromelion7 interpretation program. The phenolic compounds were detected in the range of 230–400 nm with a flow rate of 1 mL/min. The column was operated at a temperature of 25 °C. Separations were carried out in dual pumping system by varying the proportion of acetonitrile acidified by 0.05% trifluoroacetic acid (solvent A) and distilled water (solvent B). The gradient elution program was as follows: 82% to 80% B (v/v) in 5 min, to 60% B at 12 min and finally to 82% B at 20 min. The injection volume for all samples was 20 µL [20]. The phenolic compounds were analyzed by matching the retention time and their spectral characteristics against those of standards.

**Biological parameters**

***In vitro* anticancer activity**

*In vitro* anticancer activity of methanol plant extracts against human colon carcinoma (HCT-116) and human Lung carcinoma (A-549) cell lines were determined by MTT colorimetric assay. The two cancer cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/mL gentamycin. The cells were maintained at 37 ºC in a humidified atmosphere with 5% $CO_2$ and were sub-cultured two to three times a week. The tumor cell lines were suspended in medium at concentration $5 \times 10^4$ cells/well in Corning® 96-well tissue culture plates and incubated for 24 hr. The tested extracts were then added into 96-well plates (triplicates) to achieve ten concentrations form each extract. Six vehicle controls with media or 0.5% DMSO were run for each 96 well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT test [21]. Briefly, the media was removed from the 96-well microplate and replaced with 100 µL of fresh culture Roswell Park Memorial Institute (RPMI) 1640 medium without phenol red then 10 µL of the 12 mM MTT stock solution [5 mg of MTT in 1 mL of PBS (Phosphate Buffered Saline)] to each well including the untreated controls. The 96 well plates were then incubated at 37 °C and 5% $CO_2$ for 4 h. An 85 µL aliquot of the media was removed from the wells, and 50 µL of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37 °C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)] × 100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. Vinblastine sulfate was used as a positive control. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. $IC_{50}$ value of the tested extracts was estimated from graphic plots of the dose response curve for each concentration using Graphpad Prism software (San Diego, CA. USA) [22]. Results are expressed as the mean value of triplicate data points ± SD.

***In vitro* antioxidant Activity**

The antioxidant activity of the examined extracts was determined at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University using three different *in vitro* assays including DPPH (2, 2'-diphenyl-1-picryl-hydrazyl hydrate), FRAP (ferric reducing ability Power), and ABTS (2,2´-azino-bis(3-ethylbenzothiazoline-6-sulphonate). The $IC_{50}$ value of the tested extracts was deduced and expressed as $IC_{50}$ ± SD (triplicate).

**DPPH Free Radical Scavenging Assay**

Freshly prepared (0.004% w/v) methanol solution of DPPH was prepared and stored at 10 ºC in the dark. A methanol solution of the tested extracts was prepared. A 40 µL aliquot of the methanol solution was added to 3 mL of DPPH solution. Absorbance measurements were recorded immediately with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). The absorbance of the DPPH radical without antioxidant (control) and the reference compound (ascorbic acid) were also measured. All the determinations were performed in three replicates ($n$ = 3) and averaged [23]. The percentage inhibition (PI) of the DPPH radical was calculated according to the equation:

$$PI = [(A_C – A_T)/A_C \times 100]$$

Where

$A_C$: Absorbance of the control at t = 0 min, $A_T$: absorbance of the sample + DPPH at t = 16 min [24].

$IC_{50}$ of each tested extract (the concentration required to 50% DPPH radical scavenging activity) was estimated from graphic plots of the dose response curve using Graphpad Prism software (San Diego, CA. USA).

**FRAP Assay**

The ferric reducing ability Power of the examined extracts was evaluated using the method of [25]. This method is based on the reduction of ferricyanide relative to different concentrations of extract sample. Samples in 1mL of methanol were mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%, w/v). After 20 min of incubation at 50 °C, the reaction mixture was acidified with 2.5 mL of trichloroacetic acid (10%, w/v). The reaction mixture was centrifuged at 1000 *xg* for 10 min. The supernatant solution (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of freshly prepared ferric chloride (0.1%, w/v). The absorbance of the resulting solution was measured at 700 nm versus a blank using a spectrophotometer (Milton Roy, Spectronic 1201). BHT was used as reference standard. The reducing capability percentage (%) was calculated as follows

$$\text{Reducing capability (\%)} = 100 - [(A_O - A_S)/A_O \times 100]$$

Where

$A_O$: absorbance of the control solution; $A_S$: sample absorbance.

**ABTS Radical Scavenging Assay**

The determination of antioxidant activity by ABTS radical scavenging method was performed according to the procedure described by [26]. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution (1.8 mM) (Sigma, PN: A3219) with 0.63 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Then, the solution was diluted with ethanol until absorbance reached to 0.700 (± 0.030) at 734 nm. Measurements were performed at ambient temperature. The extracts were diluted at a ratio of 1: 10 with 80% methanol. Later, 190 µL of radical solution was mixed with 10 µL of diluted extracts in microplates. The absorbance at 734 nm was measured for every 1 min until 13 min following initial mixing. Appropriate solvent blanks were run in each assay. 2.5 ppm of Butylated hydroxyanisole (BHA) and 80% methanol were used as the standard antioxidant and the negative control, respectively. Experiments were performed three times with three replicates for each sample. The percent free radical scavenging activity was calculated according to the following formula;

$$\text{\% Free Radical Scavenging Activity} = [(A_N - A_S) \times 100]/A_N$$

Where

$A_N$: final absorbance values of the negative control; $A_S$: final absorbance values of the sample.

**Results and Discussion**

**Effect on growth and yield attributes**

Data presented in Tables (6 and 7) declared the influence of different stimulators on the development and yield of *C. amboinicus*. In general, the use of growth stimulants led to significant increases in plant height, fresh and dry herb weight per plant, fresh and dry herb yield per hectare. In the first cut, the highest significant increments were from blue green algae extract followed by compost tea and active dry yeast extract treatments. At the same time, the differences among them were non-significant. The values of blue green algae extract treatment were 275.83 g, 7.36 ton, 91.90 g, 2.45 ton, the data of compost tea treatment were 261.83 g, 6.98 ton, 87.3 g, 2.33 ton, the values for active dry yeast extract treatment were 244.83 g, 6.53 ton, 81.60 g, 2.18 ton for the fresh weight of herb/plant, fresh yield of herb/hectare, dry weight of herb/plant, and dry yield of herb/hectare, respectively. The differences between readings of moringa extract and Azolla were not significant. In the second cut, the same trend was observed. The significant highest increases in growth and yield characters were detected by spraying blue green algae extract followed by compost tea and then active dry yeast extract. The findings of blue green algae extract were 338.33 g, 9.02 ton, 112.78 g, 3.01 ton, the results of compost tea treatment were 251.00 g, 6.69 ton, 83.67 g, 2.23 ton, the measurements of active dry yeast extract were 247.17 g, 6.59 ton, 82.39 g, 2.20 ton for the fresh weight of herb/plant, fresh yield of herb/hectare, dry weight of herb/plant, and dry yield of herb/hectare, correspondingly. The differences between compost tea and active dry yeast were not significant. Also, the differences between moringa and Azolla values were not significant in most cases. The efficiency of growth stimulants on the productivity of plants depends on the concentration used and the quality of entered raw materials in their manufacture. Based on the methodology of this research, we observed that using blue green algae extract in both seasons was significantly superior to other stimulants, which might be due to several reasons. The positive result of spray with *Spirulina platensis* extract on yield could be attributed to its high contents of macro and microelements and amino acids. The presence of natural carotene, xanthophyll phytopigments, and hormones such as auxins and cytokinins were considered essential for plants and allowed them to withstand adverse stresses. These results agreed with [8, 10, 11, 27-30].

**Table 6:** Effect of biostimulators treatments on plant height (cm), fresh weight of herb/plant (g), and fresh yield of herb/hectare (ton) of *Coleus amboinicus* (mean values of the two seasons)

| Treatments | Plant height | | Fresh weight of herb/plant | | Fresh yield of herb/hectare | |
|---|---|---|---|---|---|---|
| | 1st cut | 2nd cut | 1st cut | 2nd cut | 1st cut | 2nd cut |
| Control | 51.33 | 43.50 | 123.00 | 160.83 | 3.28 | 4.29 |
| Moringa extract | 64.17 | 55.00 | 169.00 | 215.00 | 4.51 | 5.73 |
| Blue green algae extract | 84.17 | 76.33 | 275.83 | 338.33 | 7.36 | 9.02 |
| Compost tea | 73.67 | 66.17 | 261.83 | 251.00 | 6.98 | 6.69 |
| Active dry yeast extract | 70.17 | 62.67 | 244.83 | 247.17 | 6.53 | 6.59 |
| Azolla extract | 54.00 | 50.17 | 176.00 | 223.17 | 4.69 | 5.95 |
| LSD 0.05 | 5.51 | 4.10 | 46.31 | 43.95 | 1.24 | 1.17 |

**Table 7:** Effect of biostimulators treatments on dry weight of herb/plant (g) and dry yield of herb/hectare (ton) of *Coleus amboinicus* (mean values of the two seasons)

| Treatments | Dry weight of herb/plant | | Dry yield of herb/hectare | |
|---|---|---|---|---|
| | 1st cut | 2nd cut | 1st cut | 2nd cut |
| Control | 41.00 | 53.61 | 1.09 | 1.43 |
| Moringa extract | 56.30 | 71.67 | 1.50 | 1.91 |
| Blue green algae extract | 91.90 | 112.78 | 2.45 | 3.01 |
| Compost tea | 87.30 | 83.67 | 2.33 | 2.23 |
| Active dry yeast extract | 81.60 | 82.39 | 2.18 | 2.20 |
| Azolla extract | 58.70 | 74.39 | 1.57 | 1.98 |
| LSD 0.05 | 15.41 | 14.65 | 0.41 | 0.39 |

## Chemical analyses

### Volatile oil parameters

In the first cut, it was evident from Table (8) that the significant highest volatile oil percentage resulted from spraying with active dry yeast extract followed by blue green algae extract and moringa leaves extract as they gave 0.31, 0.24, and 0.18%, in that order. In the second cut, the top volatile oil percentage obtained from the blue green algae treatment followed by Azolla and active dry yeast as their values were 1.62, 0.75, and 0.71%, respectively. Concerning the volatile oil content and volatile oil yield (Table 8), these parameters took a similar trend to that of the oil percentage of both cuts. These values in the first cut were 0.76, 0.66 and 0.30 ml/plant; 20.27, 17.60 and 8.00 l/hectare. In the second cut, it produced 5.48, 1.76, and 1.67 ml/plant; 146.14, 46.93, and 44.53 l/hectare. The beneficial effect of Spirulina extract on increasing the volatile oil yield of plants coincided with the work of [31] on *Origanum vulgare*.

Table (9) showed the volatile oil investigation using GC/MS. The main coleus oil constituents were p-Mentha-1,4(8)-diene, cymene, terpinene, l-fenchone, carvacrol, copaene, caryophyllene, humulene, germacrene D, cadina-1(10),4-diene, α-cadinol, 6-epi-shyobunol, and Neoisolongifolene, 8,9-dehydro. The foliar spray of stimulators increased some volatile oil components over control, such as l-fenchone, carvacrol, germacrene D-4-ol, cubenol, tau.-cadinol, α-cadinol, and 6-epi-shyobunol as the following:- using active dry yeast extract resulted in the highest germacrene D-4-ol (4.04%). Azola extract gave a maximum 6-epi-shyobunol (10.61%) than control and other stimulants. Also, using blue green algae extract recorded the highest oil content of l-fenchone (6.91%), carvacrol (5.78%, which is an essential flavor and spicy component), cubenol (0.56%), tau.-cadinol (1.62%), α-cadinol (7.93%) than control and other stimulators. These results agreed with [9] on dill plants observed that spraying blue green algae extract could improve the quality of seed yield by increasing the oil content of d-limonene concentration (as limonene is responsible for the seed pleasant fresh odor). Therefore, increase its grade in the food industry.

**Table 8:** Effect of biostimulators treatments on essential oil percentage, essential oil content/plant (ml), and essential oil yield/hectare (l) of *Coleus amboinicus* (mean values of the two seasons).

| Treatments | Essential oil percentage | | Essential oil content/plant | | Essential oil yield/hectare | |
|---|---|---|---|---|---|---|
| | 1st cut | 2nd cut | 1st cut | 2nd cut | 1st cut | 2nd cut |
| Control | 0.15 | 0.46 | 0.19 | 0.74 | 5.07 | 19.73 |
| Moringa extract | 0.18 | 0.63 | 0.30 | 1.36 | 8.00 | 36.27 |
| Blue green algae extract | 0.24 | 1.62 | 0.66 | 5.48 | 17.60 | 146.14 |
| Compost tea | 0.07 | 0.31 | 0.18 | 0.78 | 4.80 | 20.80 |
| Active dry yeast extract | 0.31 | 0.71 | 0.76 | 1.76 | 20.27 | 46.93 |
| Azolla extract | 0.09 | 0.75 | 0.16 | 1.67 | 4.27 | 44.53 |
| LSD 0.05 | 0.02 | 0.03 | 0.10 | 0.36 | 2.57 | 9.42 |

**Table 9:** Chemical constituents (%) of essential oils

| No | Compounds | Control | Moringa extract | Blue green algae extract | Compost tea | Active dry yeast extract | Azolla extract |
|---|---|---|---|---|---|---|---|
| 1 | 4(10)-Thujene | - | 0.35 | - | - | - | - |
| 2 | 3-Carene | 1.12 | 1.47 | 1.36 | 0.67 | 1.00 | 0.90 |
| 3 | p-Mentha-1,4(8)-diene | 5.88 | 8.04 | 5.22 | 2.60 | 3.82 | 4.62 |
| 4 | Cymene | 18.16 | 20.40 | 12.60 | 9.17 | 13.12 | 13.27 |
| 5 | Terpinene | 5.10 | 9.31 | 10.36 | 1.82 | 2.82 | 3.00 |
| 6 | L-Fenchone | 3.81 | 6.83 | 6.91 | 6.33 | 5.01 | 4.53 |
| 7 | α-Linalool | - | - | 0.28 | - | - | - |
| 8 | Terpinen-4-ol | - | 0.41 | 0.51 | - | - | - |
| 9 | Carvacrol | 5.15 | 4.19 | 5.78 | 4.77 | 4.43 | 3.52 |
| 10 | Copaene | 4.75 | 3.77 | 3.97 | 4.56 | 4.07 | 4.62 |
| 11 | Caryophyllene | 3.51 | 3.04 | 3.47 | 4.16 | 3.76 | 3.79 |
| 12 | Humulene | 10.92 | 10.02 | 11.12 | 13.56 | 12.26 | 12.64 |
| 13 | Germacrene D | 10.56 | 8.48 | 9.25 | 13.46 | 11.89 | 12.94 |
| 14 | Elemene | 0.75 | 0.64 | 1.03 | 1.62 | 1.10 | 1.33 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 15 | α-Muurolene | 0.50 | 0.63 | 0.76 | 0.86 | 0.62 | 0.33 |
| 16 | 8-Isopropenyl-1,5-dimethyl-1,5-cyclodecadiene | - | - | - | 0.39 | - | 0.35 |
| 17 | Cadinene | - | - | 0.31 | - | - | - |
| 18 | Cadina-1(10),4-diene | 5.35 | 5.41 | 6.51 | 7.13 | 6.03 | 5.93 |
| 19 | Cadina-1,3,5-triene | 0.45 | - | - | 0.56 | 0.35 | - |
| 20 | Germacrene D-4-ol | 2.34 | 1.75 | 1.37 | 3.66 | 4.04 | 4.07 |
| 21 | Cubenol | 0.46 | 0.39 | 0.56 | 0.51 | 0.37 | |
| 22 | tau.-Cadinol | 0.90 | 1.06 | 1.62 | 1.41 | 1.05 | 0.53 |
| 23 | α-Cadinol | 5.12 | 5.33 | 7.93 | 7.85 | 5.23 | 3.54 |
| 24 | 6-epi-shyobunol | 6.33 | 3.42 | 3.04 | 7.20 | 10.47 | 10.61 |
| 25 | Longipinocarvone | - | 0.29 | - | - | - | - |
| 26 | α-Neoclovene | 0.66 | - | 0.41 | 0.45 | 0.51 | 0.47 |
| 27 | Neoisolongifolene, 8,9-dehydro | 5.74 | - | 0.39 | - | 6.36 | 6.22 |
| 28 | Cycloisolongifolene, 8,9-dehydro- | 0.52 | 4.13 | 5.21 | 6.87 | 0.51 | 0.47 |
| 29 | Caryophyllene oxide | 1.50 | 0.63 | - | 0.38 | 0.76 | 1.55 |
| 30 | (-)-Spathulenol | 0.42 | - | - | - | 0.42 | 0.77 |
| Total identified compounds | | 100 | 99.99 | 99.97 | 99.99 | 100 | 100 |

**Estimation of phenolic compounds**

Phenolic compounds are considered the most important and major non-volatile compounds in *C. amboinicus* [32]. Therefore, herein we started by investigating the total phenolic and total flavonoid contents of the plant extract in all bio-stimulator treatments and compared them with the control one. As can be seen from Table 10, the total phenolics and flavonoids of *C. amboinicus* aerial parts in the control treatment were 39.64 ± 2.26 µg GAE/mg DE and 20.03 ± 0.62 µg QE/mg DE, respectively. A previous study showed the existence of total phenolic content (49.9 mg gallic acid equivalent (GAE)/g extract) and total flavonoids (26.6 mg rutin equivalent (RE)/g extract) [33]. However, other previous research has documented that the total phenolic content was higher in the stem extract (9.6 mg/g) compared to the leaf extracts (8.4 mg/g) and the root extracts (5.4 mg/g) [34]. Our results indicated that spraying the plant with all bio-stimulator treatments except moringa extract enhanced the contents of both total phenolics and total flavonoids, with concentrations ranging from 39.80 to 45.64 µg GAE/mg DE and from 20.17 to 23.17 µg QE/mg DE, respectively (Table 10). Moreover, among them, it can be noticed that using blue green algae extract revealed the highest total phenolic content in *C. amboinicus* (45.64 ± 4.52 µg GAE/mg DE). However, utilizing compost tea represented the highest total flavonoid content (23.17 ± 2.26 µg QE/mg DE).

Thereafter, according to the above findings *C. amboinicus* phenolic compounds upon using the three treatments; control treatment and the one with highest total phenolic content (blue green algae extract) as well as the one with highest total flavonoid content (compost tea) were identified. Identification of phenolic compounds in each treatment *via* HPLC was done by comparison of their retention's time and UV absorption spectrum with those of the standards. The results obtained demonstrated just the annotation of quercetin, kaempferol, gallic acid, *p*-coumaric acid, chlorogenic acid, and quinic acid in *C. amboinicus* aerial parts of the control treatment. However utilizing blue green algae extract, two flavonoids (quercetin, kaempferol), six phenolic acids (gallic acid, vanillic acid, cinnamic acid, *p*-coumaric acid, ferulic acid, and chlorogenic acid), and quinic acid were identified in the plant aerial parts. Meanwhile, In case of using compost tea, eight out of ten standard phenolic compounds used for chemical characterization of plant aerial parts extract were found, besides quinic acid. They are quercetin, kaempferol, hesperidin, diosmin, gallic acid, *p*-coumaric acid, ferulic acid, and chlorogenic acid. The literature reported the presence of *p*-coumaric acid, gallic acid, and quercetin in *C. amboinicus*, along with other phenolic constituents (phenolic acids and flavonoids) [32- 34].

**Table 10:** Total phenolics and total flavonoids of *Coleus amboinicus* extracts

| Extracts | Total phenolic content (µg GAE/mg DE) | Total flavonoid content (µg QE/mg DE) |
|---|---|---|
| Control | 39.64 ± 2.26 | 20.03 ± 0.62 |
| Moringa extract | 25.43 ± 2.05 | 18.62 ± 0.57 |
| Blue green algae extract | 45.64 ± 4.52 | 22.50 ± 0.38 |
| Compost tea | 40.30 ± 1.83 | 23.17 ± 2.26 |
| Active dry yeast extract | 39.80 ± 1.53 | 20.17 ± 0.47 |
| Azolla extract | 39.87 ± 1.63 | 20.96 ± 0.87 |

**Biological Parameters**

***In vitro* antioxidant and anticancer activities**

Based on their rich content of flavonoids and phenolics, the three extracts, i.e. control (1W), blue green algae extract (3W), and compost tea (4W) were selected and tested for their biological activities (anticancer and antioxidant).

The antioxidant activities of the plant extracts were tested by using different assays, i.e. DPPH, FRAP and ABTS (Table 11). The treatment of plants with compost tea (4W) was observed to exhibit the maximum antioxidant activities (DPPH, FRAP and ABTS) with $IC_{50}$ values of 37.86, 42.02 and 69.14, respectively, when compared with the control plant and the positive control. Meanwhile, the plants treated with blue green algae extract (3W) showed antioxidant activities (DPPH, FRAP and ABTS) with $IC_{50}$ values of 68.97, 115.45and 89.63, respectively. Supporting our results, the aqueous extract of the plant was found to have significant superoxide- scavenging, nitric oxide-scavenging and ferrous ion-chelating capacity, [35], in addition to high DPPH antioxidant activity which might be attributed to the rich content of polyphenolic compounds in the plant extract [36]. Other studied by [37] have demonstrated a lower antioxidant activity in the ethanolic extract of *P. amboinicus*, which might be related to lower contents of flavonoids and phenolics. Moreover, the essential oil *P. amboinicus* have been reported to exert significant antioxidant activities, which might be due to the presence of some volatile compounds, reported herein, such as carvocrol and thymol [38].

Regarding the anticancer activities, we observed that all the extracts showed cytotoxic activity against the tested cell lines (A-549 and HCT-116) (Table 12). Among the potent extracts, the treatment of plants with compost tea (4W) decreased the cell viability and showed maximum cytotoxic activities against both A-549 and HCT-116 with $IC_{50}$ values of 37.95 and 53.71, respectively. Meanwhile, the application of blue green algae extract (3W) has also decreased the cell viability against A-549 and HCT-116 with $IC_{50}$ values of 61.80 and 100.46 respectively. Similar to our results, the antitumor activity of *P. amboinicus* extracts has been previously reported [39], whereas it was found that the growth of Sarcoma-180 tumor in mice treated with hexane extract of the plant was significantly inhibited. This was comparable to metrotexat, a cancer-treating drug which may cause harmful side effects but reduces 100% of tumoral growth. Thus, the use of hexane extract of the plant might be safer than such synthetic drugs. Moreover, the ethanolic extract of the plant showed remarkable anticancer activity against human lung cancer cell line (A549) [40]. However, other studies by [34] have demonstrated high $IC_{50}$ against hepatocellular and breast carcer cell lines compared to doxorubicin, which suggested that they could be inactive as cytotoxic drugs.

**Table 11:** Antioxidant activities of *Coleus amboinicus* extracts

| Extracts/ Ascorbic acid | Antioxidant activity Mean ± SD | | |
|---|---|---|---|
| | $IC_{50}$ (µg/mL) | | |
| | DPPH | FRAP | ABTS |
| Control | 75.94 ± 5.18 | 136.08 ± 6.28 | 137.72 ± 5.46 |
| Blue green algae extract | 68.97 ± 4.23 | 115.45 ± 5.73 | 89.63 ± 4.91 |
| Compost tea | 37.86 ± 2.92 | 42.02 ± 3.84 | 69.14 ± 3.12 |
| Ascorbic acid (positive control) | 10.62 ± 0.84 | 18.72 ± 1.26 | 15.14 ± 1.08 |

**Table 12:** Evaluation of the $IC_{50}$ of *Coleus amboinicus* extracts against A-549 and HCT116 cancer cell lines.

| Extracts/Vinblastine sulfate | $IC_{50}$ (µg/mL) | |
|---|---|---|
| | A-549 | HCT-116 |
| Control | 94.23 ± 6.71 | 119.62 ± 7.96 |
| Blue green algae extract | 61.80 ± 4.98 | 100.46 ± 6.48 |
| Compost tea | 37.95 ± 2.67 | 53.71 ± 3.85 |
| Vinblastine sulfate (positive control) | 24.65 ± 1.87 | 3.51 ± 0.43 |

* The activity was shown as $IC_{50}$ value, which was the concentration of the tested extract (µg/mL) that decreased the number of viable cells by 50%. Results are expressed as $IC_{50}$ ± SD (n = 3).

## Conclusion

Overall, foliar spray of *C. amboinicus* with blue green algae extract (*Spirulina platensis* extract) produced the highest fresh and dry yield, essential oil yield with the highest percentage of carvacrol. In addition, using blue green algae extract and compost tea showed the highest total phenolic and flavonoid contents of the plant methanol extract, respectively. Accordingly, the treatment of *C. amboinicus* with compost tea improved *in vitro* antioxidant (DPPH, FRAP, and ABTS) and anticancer activities against lung (A549) and colon (HCT116) cancer cell lines.

## References


1. Warrier PK, Nambiar VP, Ramankutty C. Indian Medicinal Plants, 1st ed, Orient Longman Limited, Madras. 1995;4:315-317.
2. Small E. Culinary herbs. National Research Council of Canada NRC Research Press, 1997, 488.
3. Dragland Steinar, *et al*. Several culinary and medicinal herbs are important sources of dietary antioxidants. J Nutr. 2003;133(5):1286–1290.
4. Faleiro L, Graca M, Gomes S, Costa L, Venancio F, Teixeira A, Figueiredo A, *et al*. Antibacterial and Antioxidant Activities of Essential Oils Isolated from *Thymbra capitata* L. (Cav.) and *Origanum vulgare* L. J. Agric. Food Chem. 2006;53(21):8162–8168.
5. Boning CR. Florida's Best Herbs and Spices: Native and Exotic Plants Grown for Scent and Flavor, Pineapple Press Inc, 2010, 75.
6. Centre for Agriculture and Biosciences International. *Plectranthus amboinicus* (Indian borage), Datasheet, Invasive Species Compendium, 2017.
7. Royal Botanic Gardens, Kew. Plants of the World Online. *Coleus amboinicus* Lour. Retrieved 2021-08-30. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names: 20765-1
8. Abd El-Aleem W, Hendawy SF, Hamed ES, Toaima WIM. Effect of planting dates, organic fertilization and foliar spray of algae extract on productivity of Dutch fennel plants under Sinai conditions. Journal of Medicinal Plants Studies. 2017;5(3):327-334.
9. Toaima WIM, Osman YAH, Hamed ES. Comparative study for the effect of spraying with blue green algae extract on some Russian dill varieties under El-Maghara region - North Sinai conditions. Journal of Medicinal Plants Studies. 2017;5(2):112-119.
10. El-Mahrouk EM, Abido AI, Radwan FI, Hamed ES, El-Nagar EE. Vegetative growth and essential oil productivity of lemongrass (*Cymbopogon citratus*) as affected by NPK and some growth stimulators. International Journal of Botany Studies. 2018;3(6):48-55.
11. Abd El-Aleem W, Hamed ES, Toaima WIM. Effect of blue green algae extract on three different curly parsley varieties under Sinai conditions. Bulgarian Journal of Agricultural Science. 2021;27(5):887–895.
12. Hamed ES. Studies on planting of some medicinal plants in the desert. M.Sc. Thesis, Fac. Agric. Kafr El-Sheikh, Tanta University., Egypt, 2004.
13. Kassem AHM, Abd El-Aal AM. Minimizing the effect of soil salinity on fennel plant using cyanobacteria and compost. J. Product. & Dev. 2016;21(2):153-178.
14. Toaima WIM. Effect of organic fertilization and active dry yeast on productivity of three-lobed sage (*Salvia fruticosa* Mill.) plants under Siwa Oasis conditions. Egyptian J. Desert Res. 2014;64(1):153-166.
15. El-Sayed AA, Sakr WRA, Hamouda AM, Saad El-Deen FSA. Response of *Pelargonium graveolens* L. Herit plant to NPK chemical Fertilization and spraying

with natural leaf extracts of *Moringa oleifera* and *Aloe vera*. J. Product. & Dev. 2018;23(3):411- 428.
16. Snedecor GW, Cochran WG. Statistical methods. The Iowa State Univ., Press, Ames, IA, 1982.
17. British Pharmacopoeia. Determination of volatile oil in drugs. The Pharmaceutical Press, London, UK, 1963.
18. Hassan AR. Chemical profile and cytotoxic activity of a polyphenolic-rich fraction from *Euphorbia dendroides* aerial parts. South African Journal of Botany. 2022;147:332–339.
19. El-Kousy SM, Emam SS, Hassan AR, Sanad IM. Metabolites profiling of *Limonium tubiflorum* (Delile) Kuntze var *tubiflorum via* UPLC-qTOF-MS technique in relation to its cytotoxic activity. Jordan Journal of Biological Sciences 2021;14(4):663–669.
20. Selim S *et al*. Innovating the synergistic assets of β-amino butyric acid (BABA) and selenium nanoparticles (SeNPs) in improving the growth, nitrogen metabolism, biological activities, and nutritive value of *Medicago interexta* sprouts. Plants. 2022;11(3), 306.
21. Hassan AR, Ashour A, Amen Y, Nagata M, El-Toumy SA, Shimizu K. A new cycloartane triterpene and other phytoconstituents from the aerial parts of *Euphorbia dendroides*. Natural Product Research 2022;36(3):828-836.
22. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods. 1983;65(1-2):55–63.
23. El-Tantawy HM, Hassan AR, Taha HE. Antioxidant potential and LC/MS metabolic profile of anticancer fractions from *Echium angustifolium* Mill. aerial parts. Journal of Applied Pharmaceutical Science 2021;11(12): 200–208.
24. Yen GC, Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. Journal of agricultural and food chemistry. 1994;42(3):629–632.
25. Selim S, *et al*. Pits of Date Palm: Bioactive Composition, Antibacterial Activity and Antimutagenicity Potentials. Agronomy. 2021;12(1):54.
26. Zrig A, *et al*. Melatonin priming as a promising approach to improve biomass accumulation and the nutritional values of *Chenopodium quinoa* sprouts: A genotype-based study. Scientia Horticulturae. 2022;301:111088.
27. Amer HM, Marrez DA, Salama AB, Wahba HE, Khalid KA. Growth and chemical constituents of cardoon plant in response to foliar application of various algal extracts. Biocatalysis and Agricultural Biotechnology. 2019;21:101336.
28. El-Gohary AE, Amer HM, Salama AB, Wahba HE, Khalid KA. Growth and chemical profile of clary sage (*Salvia sclarea* L.) in response to algae and banana peel extracts. Bulletin of the National Research Centre. 2020;44(1):1-9.
29. Perez-Madruga Y, Lopez-Padron I, Reyes-Guerrero Y. Algae as a natural alternative for the production of different crops. Cultivos Tropicales 2020;41(2). NA-NA.
30. Sabry RM, EL-Gohary A, Elsayed AA. Growth and essential oil quality of lemon verbena aerial parts (*Aloysia citriodora*) in response to foliar application of royal jelly and algae extracts. Journal of Essential Oil Bearing Plants. 2021;24(6):1279-1290.
31. Moghith WMA. Effect of organic and biofertilization on the growth, production and the chemical constituents of *Origanum vulgare* L. plants, MSc. Thesis, Fac. Agric., Tanta Univ., Egypt, 2016.
32. Arumugam G, Swamy MK, Sinniah UR. *Plectranthus amboinicus* (Lour.) Spreng: botanical, phytochemical, pharmacological and nutritional significance. Molecules. 2016;21(4):369.
33. Bhatt P, Joseph GS, Negi PS, Varadaraj MC. Chemical composition and nutraceutical potential of Indian borage (*Plectranthus amboinicus*) stem extract. Journal of Chemistry, 2013, 1-7.
34. El-hawary SS, El-sofany RH, Abdel-Monem AR, Ashour RS, Sleem AA. Polyphenolics content and biological activity of *Plectranthus amboinicus* (Lour.) spreng growing in Egypt (Lamiaceae). Pharmacognosy Journal. 2012;4(32):45-54.
35. Kumaran A, Karunakaran J. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. Food Chem. 2006;97:109-114.
36. Bhatt P, Negi PS. Antioxidant and antibacterial activities in the leaf extracts of Indian borage (*Plectranthus amboinicus*). Food Nutr. Sci. 2012;3:146–152.
37. Khanum H, Ramalakshmi K, Srinivas P, Borse BB. Synergistic antioxidant action of oregano, ajowan and borage extracts. Food Nutr. Sci. 2011;2:387–392.
38. Manjamalai A, Grace DVB. Volatile constituents and antioxidant property of essential oil from *Plectranthus amboinicus* (Lour). Int. J Pharm. Biol. Sci. 2012;3:445–458.
39. Gurgel AP, da Silva JG, Grangiero AR, Oliveira DC, Lima MP, Silva AC, *et al*. *In vivo* study of the anti-inflammatory and antitumor activities of leaves from *Plectranthus amboinicus* (Lour.) Spreng (Lamiaceae). J. Ethnopharmacol. 2009;125:361–363.
40. Ramalakshmi P, Subramanian N, Saravanan R, Mohanakrishnan H, Muthu M. Anticancer effect of *Coleus amboinicus* (Karpooravalli) on human lung cancer cell line (A549). Int. J Dev. Res. 2014;4:2442-2449.