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Effect of Humic and Salicylic acid on Productivity of basil (*Ocimum basilicum* L.) plants

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Abstract: The aim of this investigation was to investigate the impacts of humic acid (H) and Salicylic acid foliar application (SA) on growth (plant height, number of branches/plant, number of leaves, fresh and dry weight of /herb and volatile oil percentage and it content of Basil (*Ocimum basilicum* L.) seedlings were investigated during period from August 2024 to January 2025 in Misurata city, Libya.Four levels of humic acid including 0, 2, 4 and 6 ml/L in main plots. Four levels of Salicylic acid (SA) were assigned in sub-plots at (0, 100, 200 and 300 ppm) as well as their interactions. The results demonstrated that the use of humic and salicylic acids significantly improved the growth parameters, and oil extraction. Regarding this concern, the highest results were recorded through the addition of a high level of humic acid (6 ml/L) along with foliar spray of Salicylic acid (300 ppm) in comparison to the control during period growing. The volatile oil's GC-MS analysis revealed that the usage of biostimulant applications also had an impact on the main ingredients. When plants were sprayed with 300 ppm of salicylic acid and a higher standard humic acid (6 ml/L), the proportions of key components were generally greater than those of untreated plants.

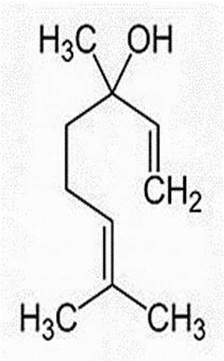
Key words: Humic acid, Salicylic acid and Basil (*Ocimum basilicum* L.).

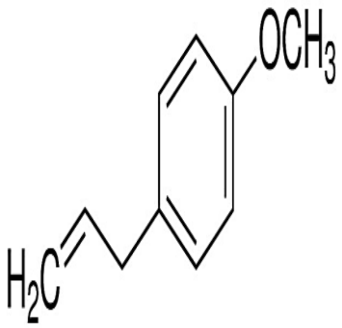
1. Introduction

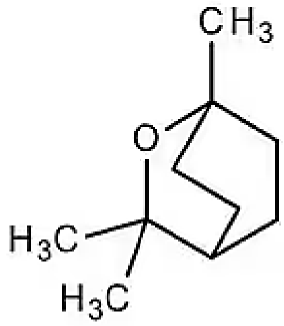
Throughout history, indigenous tribes have utilized medicinal plants to heal both human and animal ailments. Based on their ethnopharmacological uses and applications, isolated chemicals from medicinal plants have been used to create most contemporary medications in more recent years (**Batanouny *et al.*, 1999; Cragg and Newman, 2013; Mukherjee and Heinrich 2008 and Jardak *et al.*, 2017).** Natural products have been playing an increasingly important role in drug research, not only when the bioactive chemicals are used as direct therapeutic agents but also when they are used as a basic model for novel biologically active molecules or as a raw material for drug synthesis (**Mendonça, 2006 and Swain, 1972)**. To put this resource on par with traditional pharmaceutical goods, however, a lot of scientific and applied research is needed to validate and use plants as phytopharmaceuticals (**Batanouny *et al.*, 1999)**.

In addition, only roughly 10% of the estimated 250,000 plant species in the world have been thoroughly investigated for possible medical use (**Cragg and Newman, 2002**). Additionally, by 2050, it is likely that some 60,000 species would go extinct, making the hunt for novel chemicals with medicinal potential imperative (**Mukherjee and Heinrich, 2008**).

The Lamiaceae family includes the annual spice plant basil (*Ocimum basilicum* L.), whose fragrant leaves are used fresh or dried to flavor food, drink, and confections. Basil also contains important essential oil. It is grown all over the world and has commercial importance. It has long been used in traditional medicine to cure a wide range of conditions, including kidney problems, warts, worms, headaches, coughing, diarrhea, and constipation. It can be applied externally to treat acne and as an ointment for bug bites (**Baydar 2013 and Omidbeigi 2000)**. The essential oil, which is mostly utilized in the food and fragrance industries, also has antibacterial, antifungal, antiparasitic and antiviral qualities and is associated with a number of bioactive substances. Camphor, 1,8-cineole and Linalool are a few of its known physiologically active components (**Gürgan & Adilo, 2021 and Faiza *et al.*, 2022)**.







Linalool estragole 1, 8-cineole

The main compounds of basil volatile oil

Humic acid is a serious component of brown humus that affect the physical quality and chemical content of soil. Also, Humic substances typically consist of heterogeneous mixtures of converted bio compounds that exhibit a supramolecular structure that can be isolated into smaller molecular components by sequential chemical fractionation (**Cordell and Colvard, 2005)**. One of the complicated compounds that emerges from the dilating process of plant and animal matter is humic acid. Humin, fulvic acid, and humic acid make up the majority of these compounds. These compounds are crucial for plant nutrition and soil fertility. Humic acid positively affect plant growth because it helps in the permeability of cell membranes, stimulate enzymatic reactions, improve mitosis and stimulate intracellular vitamins (**Pettit, 2008)**.

Spraying Fennel plants *Foeniculum vulgare* Mill with humic acid (0, 1, 2.5, 3.5) ml L-1 led spraying at a concentration of 3.5 ml L-1 to significant superiority in the height of the plant, the branches number and the dry weight of the plant (137.11 cm, 8.83 branches plant 1, 168.87 g), respectively, showed that the addition of a humic acid with a 7 ml^{-1} concentration recorded the highest significant values in all vegetative growth qualities of rosemary plants (**Al-Ajili, 2014)**. When compared to the control plant (one that did not receive humic acid), humic acid treatments enhanced the vegetative growth features of caraway plants, including plant height, branch number per plant, and plant dry weight. Caraway plants treated with humic acid exhibit increased vegetative development features, including plant height, branch count, and herb dry weight/plant (**Calixto, 2005**). The highest humic acid concentration (10 ml/l) was the most beneficial in increasing branch number/plant, plant height, herb dry weight and number of umbels/plants in treated anise plants treated with humic acid at 2.5, 5.0, and 10.0 ml/l as a foliar spray (**Aly *et al*., 2022**).

A phenolic plant hormone (salicylic acid) is regulates numerous physiological processes relevant to plant development and growth (**Qaiser *et al.*, 2010)**. Salicylic acid is involved in several processes, including thermogenesis, environmental stress tolerance, and DNA damage/repair, the production of fruits, seed germination, and more, according to many researches **(Dempsey and Klessig, 2017)**. Since SA effectively promotes the growth and manufacture of bioactive substances, it is a common elicitor molecule in aromatic and medicinal plants **(Gorni *et al.,* 2020).** As stated by **(Idrees *et al.,* 2010 and Khan *et al.,* 2015),** these include transpiration, photosynthesis, stomata control, protein and chlorophyll synthesis, the activity of enzymes, and the absorption of nutrients. Due to its classification as "a substance generally recognised as safe" by the Food and Drug Administration (FDA), salicylic acid might be used in commercial medicinal species cultivation targeted at the phytomedicine market **(Divya *et al.,* 2014)**. The findings demonstrated that applying salicylic acid topically alters plant growth and metabolism over the long run **(Erna and Adisa, 2017)**.

Examining the effects of various biostimulants and their interactions on the essential oil and plant growth parameters of basil (*Ocimum basilicum* L.) plants was the aim of this study. It also sought to determine the best treatment for improving these traits and confirm the right rate of salicylic acid and humic acid to provide the highest productivity in order to generalize these materials to the productivity of other aromatic and medicinal plants.

2. Materials and Methods

2.1. Explanation of the study site

This study aims to Effect of Humic Acid and salicylic acid on the Productivity of Basil (*Ocimum basilicum* L.) plants be conducted during the period from August 2024 to January 2025 in Misurata city, Libya.

2.2. Design of Experiment

A split plot design with 3 replicates was worked in this investigation, where humic acid was used as organic fertilizer and the main plots (A) included 4 treatments, while the salicylic acid treatments (B) were allocated to the sub-plots and were also four treatments, thus the interaction coefficients were 16. Basil shoots were transferred to the experimental site on August 15, in 15 cm diameter pots containing 3 rows, with a distance of 60 cm between each row, each row containing 6 plants. Humic acid levels of 0, 2, 4 and 6 ml/L added three times in soil during the growth period. The salicylic acid treatments were as follows: control (no plants sprayed) and (100,200 and 300 ppm). Sweet basil plants were foliar sprayed with the salicylic acid, either three times as follows; for the 1^{st}, 2^{nd} and 3^{rd} sprays, the dates are September 15, September 30, and October 15, respectively. Foliar spraying was applied to the plants till runoff. Every other cultural custom was observed as usual.

2.3. Sampling and data collection

On January 1^{st}, three plants were chosen at random from each plot to measure the following parameters: plant height (cm), number of leaves per plant, and number of branches per plant. Additionally, the weight of the fresh and dry herbs in grams per plant, their volatile oil content, and their volatile oil yield (ml/plant) were noted. The volatile oil yield was then calculated by multiplying the volatile oil content by the herb yield (g) per plant.

2.4. Volatile oil isolation from herb

Using a Clevenger apparatus, 100 g of each replicate of every treatment were weighed and hydrodistilled (HD) for three hours (**Clevenger, 1928**). A relative percentage (volume/weight) was used to determine the essential oil content. Additionally, using dry weight, the total essential oils were computed as milliliters per 100 plants. For chemical identification, anhydrous sodium sulfate was used to dry the essential oils that were isolated from basil plants for each treatment.

2.5. Statistical analysis

All of the data collected for this study were organized, documented, and statistically evaluated utilizing the L.S.D. test at 5% to determine the differences between all treatments (**MSTAT- C 1986).**

3. Results

3.1. Growth and yield parameters

The presented data in Tables (1and 2) reveal that utilizing humic acids (HU) at any level significantly increased plant height, branch number and fresh and dry weights of herb (g/plant) of basil (*Ocimum basilicum* L.) plants is clear that plants treated with humic acids at the high level (6 ml/liter) registered the high-rise growth parameter values which increased plant height by 46.99 % augmented number of branches and leaves by 48.06 % & 33.49, elevated herb fresh weight by 20.11 % and increased herb dry weight by 32.23 % over the control, during the study, respectively. Additionally, the ability of organic fertilization to increase the growth aspects found in this study was revealed by (**Omer *et al.,* 2020** on caraway, **Rania *et al.,* 2022** on rue plant and **Rania *et al.,* 2020** on coriander plant).

The information provided in Tables (1,2) on the treatments with showed that, foliar application with salicylic acid resulted in positive in plant growth traits. The utilized of spraing with salicylic acid t resulted in higher values of plant height (cm), branch number and fresh and dry weights (g/plant) of Basil (*Ocimum basilicum* L.) plants. In general, the foliar application with the salicylic acid (300 ppm) proved to be more effective in enhancing vegetative growth and yield parameters, than those noticed by control and other ones. The increases percentage of plant height, branches and leaves numbers and herb fresh and dry weight as ranged 17.19, 33.78, 22.56, 16.26 and 23.16 % over un-treated ones, respectively.

The increases in growth parameters about by the use of stimulant substances in this study were further demonstrated by **Abdul-Hafeez and Soliman (2020), Umesha *et al.,* (2021) and Arpitha *et al.,* (2024** on black cumin (*Nigella sativa* L.), **Jalalzadeh (2024)** on cumin and **Erna and Adisa (2017)** on basil (*Ocimum basilicum* L.) plants,

As for the interaction impact, it was a significant influence on the vegetative growth parameters as shown in the following tables (1, 2). It was found that using a mixture of the two substances together (humic acid and salicylic acid) led to further improvement in growth and productivity values growth traits (plant height, branches as well as leaves number and herb fresh and dry weights) of basil (*Ocimum basilicum* L.) plants ,when compared to each substance alone, compared to other treatments. The best effects were when adding humic acid at a high rate (6 ml/liter) with foliar spraying with salicylic acid at a concentration of 300 ppm.

3.2. Volatile oil Yield

Humic acids (H) applied to Basil (*Ocimum basilicum* L.) plants had a favorable effect on volatile oil % and volatile oil content of the herb in mg/L. Clearly, were significantly raised due to the use of humic acid (H) at all levels, concerning volatile oil % and herb content (ml), in relative to untreated plants. Clearly, plants grown in humic acids at the high level registered the maximum values the which increased volatile oil % and volatile oil content by 18.90 and 70.11 over the check treatment, as clearly mentioned in Table 3.

The role of organic manure in increasing oil yield parameters detected in this study was, also insured by **Rates (2001) and Ronga (2019)** on basil plant, **Rotblatt (2000) and Rouphael and Colla (2018)** on caraway (*Carum carvi* L.), **Aly (2022)** on anise and **Salim and Chin (2008)** on (*Mentha piperita* L.).

In relation to Salicylic acid (SA) treatments, the given data in Table 3 showed that oil yield parameters (volatile oil percentage and plant content (ml) of Basil (*Ocimum basilicum* L.) plants were positively responded to adding Salicylic acid, Apparently, treating the plants with foliar application of Salicylic acid gave a significant augment in volatile oil % and plant content (ml) by 11.52 and 38.76 over the check treatment compared to untreated plants. Numerically,

The primitive impact of biostimulants treatments on oil yield aspects revealed in this research was, also mentioned on coriander (*Coriandrum sativum* L.) **Ali *et al.* (2023)** and Sharma ***et al.,* (2023)** on coriander plant and **Darzi *et al.* (2012)** and **Hegazi *et al.* (2015)** on *Anethum graveolens.*

Table (1). Effect of Humic acid (H) and Salicylic acid (SA), as well as, their interactions on Plant height, branches number and Leaves number/plant at two cuts of basil plants

Salicylic acid (B)	Humic acid (A)														
	plant height (cm)					branches number/plants					Leaves number/plant				
	H1	H2	H3	H4	Mean	H1	H2	H3	H4	Mean	H1	H2	H3	H4	Mean
SA1	38.00	48.00	54.33	55.33	48.92	4.900	6.067	7.150	7.733	6.463	44.00	51.33	55.00	58.00	52.08
SA2	39.33	50.00	56.67	58.67	51.17	5.450	6.700	7.367	8.283	6.950	49.00	53.67	59.33	65.33	56.83
SA3	43.00	53.00	60.00	62.67	54.67	6.667	7.750	8.417	9.417	8.063	51.67	57.67	60.00	68.33	59.42
SA4	45.00	55.00	63.00	66.33	57.33	7.250	8.000	8.833	10.500	8.646	53.33	64.00	65.33	72.67	63.83
Mean	41.33	51.50	58.50	60.75		6.067	7.129	7.942	8.983		49.50	56.67	59.92	66.08	
L.S.D 0.05	A:2.3 B:2.6 AB:5.2					A:5.59 B:4.35 AB:8.70					A:5.59 B:4.35 AB:8.70				

Humic acid H1 = 0, H2 = 2 and H3 = 4 and H4= 6 ml/ L., SA = Salicylic acid (SA1 = 0, SA2=100, SA3=200 and SA4=300 ppm)

Table (2). Effect of Humic acid (H) and Salicylic acid (SA), as well as, their interactions on Herb fresh and dry weight g/ plant at two cuts of basil plants

Salicylic acid (B)	Humic acid (A)									
	Herb fresh weight g/ plant					Herb dry weight g/ plant				
	H1	H2	H3	H4	Mean	H1	H2	H3	H4	Mean
SA1	65.67	71.00	74.67	77.67	72.25	15.00	16.00	17.50	19.01	16.88
SA2	69.67	73.00	80.00	83.67	76.58	15.63	17.00	20.00	21.67	18.58
SA3	71.67	75.67	83.00	86.33	79.17	16.42	17.83	20.50	22.00	19.19
SA4	75.00	82.33	87.67	91.00	84.00	18.00	20.00	21.83	23.33	20.79
Mean	70.50	75.50	81.33	84.67		16.26	17.71	19.96	21.50	
L.S.D 0.05	A:3.96 B:4.23 AB:8.46					A:1.78 B:1.20 AB:2.39				

Humic acid H1 = 0, H2 = 2 and H3 = 4 and H4= 6 ml/ L., SA = Salicylic acid (SA1 = 0, SA2=100, SA3=200 and SA4=300 ppm)

Table (3). Effect of Humic acid (H) and Salicylic acid (SA), as well as their interactions on Essential oil % and Essential oil (ml)/ plant plant at two cuts of basil plants

Salicylic acid (B)	Humic acid (A)									
	Essential oil %					Essential oil (ml)/ plant				
	H1	H2	H3	H4	Mean	H1	H2	H3	H4	Mean
SA1	0.550	0.580	0.617	0.650	0.599	0.087	0.060	0.118	0.126	0.098
SA2	0.580	0.607	0.633	0.677	0.624	0.061	0.103	0.126	0.140	0.108
SA3	0.590	0.623	0.657	0.707	0.644	0.097	0.111	0.135	0.156	0.125
SA4	0.609	0.646	0.683	0.733	0.668	0.105	0.120	0.148	0.170	0.136
Mean	0.582	0.614	0.647	0.692		0.087	0.098	0.132	0.148	
L.S.D 0.05	A:0.039 B:0.023 AB:0.046					A:0.024 B:0.021 AB:0.041				

Humic acid H1 = 0, H2 = 2 and H3 = 4 and H4= 6 ml/ L., SA = Salicylic acid (SA1 = 0, SA2=100, SA3=200 and SA4=300 ppm)

3.3. Volatile oils components

In Table (4) and Figures (1, 2 and 3) the gas chromatographic analysis (GC/MS) results of basil (*Ocimum basilicum* L.) oil obtained from the study proved that it consists of (9) compounds. Linalool, estragole, 1.8-cineole, and eugenol have the largest percentages of volatile oil compounds when compared to the other compounds, according to the values of the oil's chemical components. The highest average for the Linalool compound (44.38) was recorded in (H4) Humic acid (6 ml/L) + (SA4) Salyslic acide (300 ppm), followed by treatment Humic acid at (6 ml/l) + (SA3) Salyslic acide (200 ppm) which recorded (43.12), followed by treatment Humic acid at (4 ml/L) + (SA4) Salyslic acide (300 ppm) which reached (42.98), while the highest average was for compound estragole (18.15) in treatment Humic acid at (6ml/L) + (SA4) Salyslic acide (300 ppm), followed by treatment humic acid at (6ml/L) + (SA3) Salyslic acide (200 ppm, which recorded (17.87), which had the highest percentages for the compound. also the highest average was for compound 1.8-cineole (13.11) and Eugenol (8.24) in the same treatment. This shows that the treatments had a noticeable impact on the amounts of some of the key components of basil (Ocimum basilicum L.) oil. Certain molecules are also clearly affected by the treatments.

Table (4). Effect of Humic acid and Salicylic acid combinations on essential oil (E.O) components of Basil (Ocimum basilicum L.) plants

Treatments / E.O components	H1+SA1	H3+SA3	H3+SA4	H4+SA3	H4 +SA4
1.8-cineole	12.22	12.38	12.55	12.88	13.11
Linalool	42.22	42.82	42.98	43.12	44.38
α-terpinol	1.02	1.05	1.08	1.12	1.20
Bornyl acetate	1.12	1.25	1.55	1.88	2.14
Estragole	16.25	16.82	17.25	17.87	18.15
Trans-a' ergamotene	5.12	5.33	4.68	4.23	4.18
Germacrene-D	2.42	2.57	2.25	1.77	1.53
Alfa-copaene	5.42	5.33	4.13	3.19	2.24
Eugenol	6.88	7.15	7.75	8.24	8.16

Humic acid H1 = 0, H2 = 2 and H3 = 4 and H4= 6 ml/ L., SA = Salicylic acid (SA1 = 0, SA2=100, SA3=200 and SA4=300 ppm)

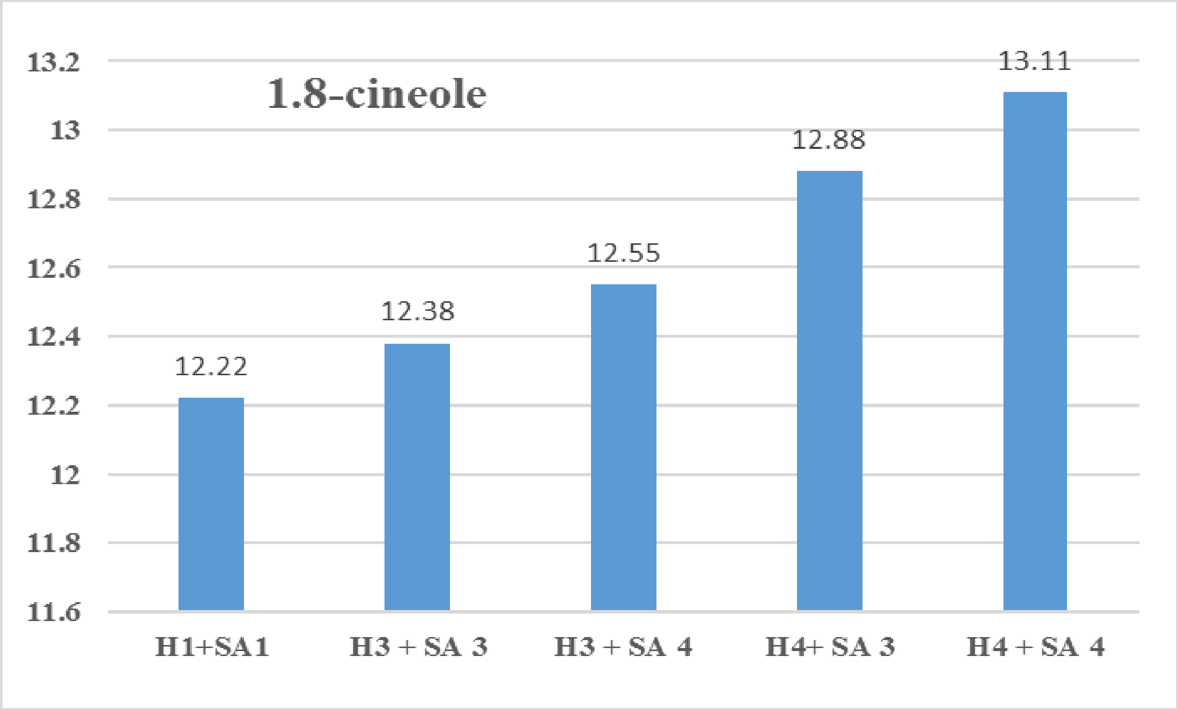


Fig. (1). Effect of Humic acid and Salicylic acid combinations on 1.8-cineole % of Basil *(Ocimum basilicum* L.) oil

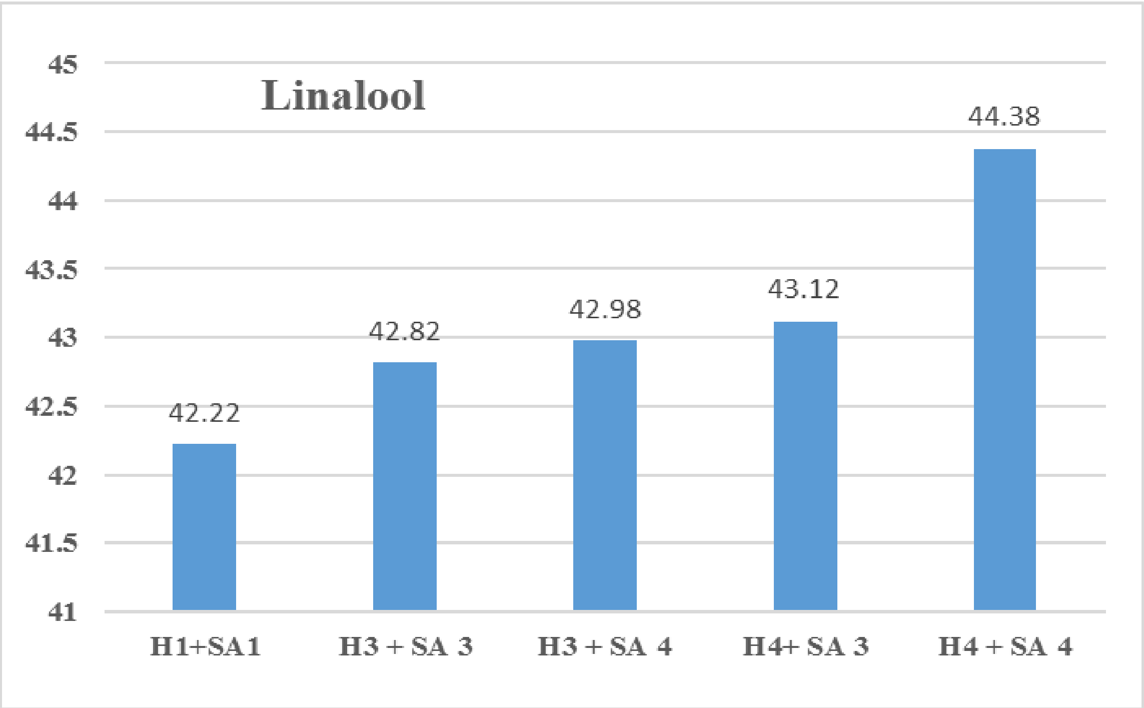


Fig. (2). Effect of Humic acid and Salicylic acid combinations on Linalool % of Basil (*Ocimum basilicum* L.) oil.

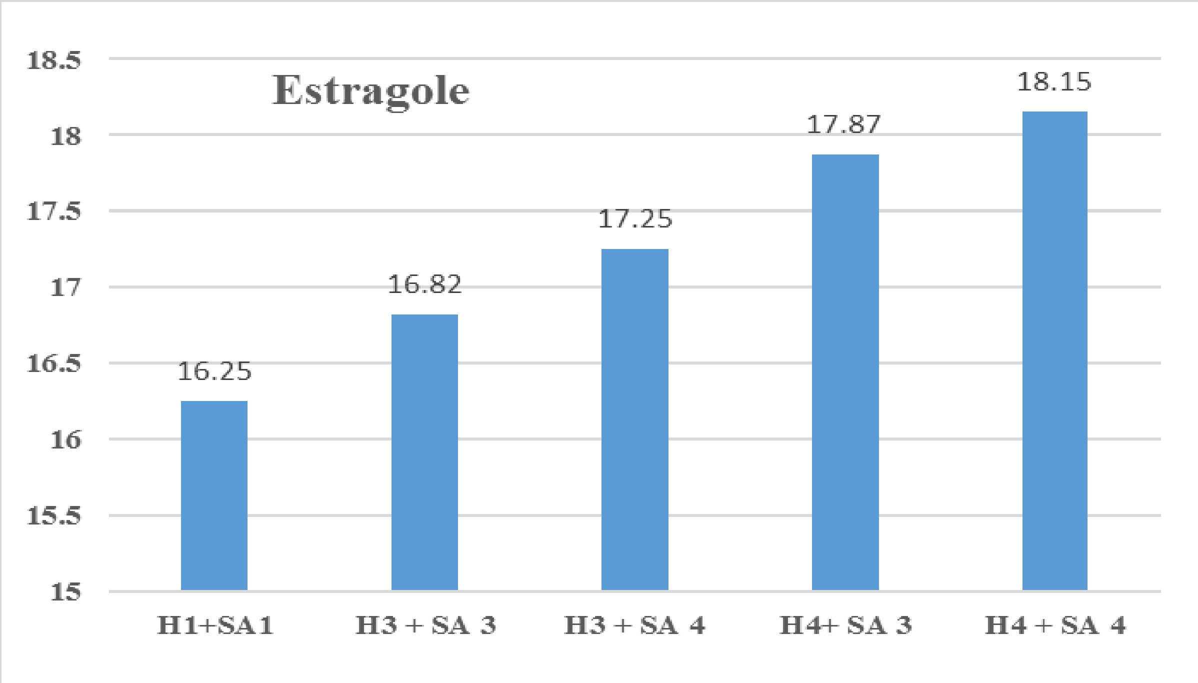


Fig. (3). Effect of Humic acid and Salicylic acid combinations on estragole % of Basil (*Ocimum basilicum* L.*)* oil

4. Discussion

The superior plant growth by adding humic acid is attributed to its vital role in providing the plant with the macro- and micro-nutrients necessary to enhance the plant's metabolism and development. It can directly improve plant growth by accelerating photosynthesis and increasing water and nutrient absorption and plant productivity **Panda (2006),** Furthermore, organic compounds are expected to raise chlorophyll levels in green plants, assisting in chlorosis resistance and photosynthesis, **Nardi *et al*. (2002)**, humic acid can offer defense against certain harmful compounds that limit growth when added to the soil. According to **Ezzat *et al.* (2009)**, humic acid lowers the rate of transpiration, which keeps the water content in plant tissues higher. This may benefit the metabolism, physiological functions, photosynthetic rate, and many other critical processes that have a direct impact on plant growth. The superiority of humic acid may indicate that attributed to the role in nutrient translocation, protein synthesis and foliar growth as indicated by **Chen *et al.* (2004).** Humic acid stimulates plant development by absorption of minor and major nutrients, enzymes activation, membrane permeability changes, synthesis of protein and biomass production stimulation (**Ulukan, 2008).** Humic compounds are great suppliers of nutrients; they also enhance the soil chemical and physical qualities and reduce erosion Soil pH, which leads to nutrient solubilization and enhances its availability while minimizing loss nutrients via leaching and encourages the growth of Microorganism abundance and activity **Bdrnardi *et al.* (2008)**.

It has been determined that salicylic acid is a plant hormone. It regulates the metabolism of plants **Romani *et al.* (1989) and Miao *et al.* (2015).** According to **Mahdy (1994),** the increase in plant essential oils may be caused by salicylic acid -stimulated vegetative growth, the number of leaf oil glands, the amount of carbohydrates, as well as the positive effect of salicylic acid on metabolism and the activities of the enzymes involved in the biosynthesis of mono- or sesqueterpenes. Promoting flowering, delaying senescence, and increasing the rate of cell metabolism, it may be necessary for and/or play a significant function as a coenzyme. Additionally, it has a strong defence against a variety of plant pathogens, including parasitic plants, nematodes, fungus, and bacteria **Rafiee *et al.* (2016).** Salicylic acid influences ethylene production, stomata movement, and retains the effect of ABA on leaf abscission, as demonstrated by **Romani *et al.* (1989).** Salicylic acid attaches itself to a protein-like receptor on the plasma membrane of the cell. A series of translocable intracellular signals are stimulated by this salicylic acid-receptor interactions, causing the plant to mount various defence mechanisms **Halder *et al.* (2019)** and **Janda *et al.*, (2020)**. This effect is explained by the SA's accessibility inside the plant, which enhances signals and facilitates complex regulatory activities by interacting with many physiological and biochemical events. **Haquea *et al.* (2022)**.

5. Summary and Conclusion

One of the most important results reached from this investigation is that the different levels of humic acid used led to an improvement in the productivity of the basil (*Ocimum basilicum* L.) plant. Growth, herb yield and oil yield of the Basil plants significantly improved with different concentrations of Salicylic acid, compared to control. Humic acid and the salicylic acid interaction had a significant effect on growth, yield and oil index. The best treatment was humic acid at high level (6ml/l) combined with salicylic acid (300 ppm).

From the results obtained in this study, it can be recommended to use humic and salicylic acids to improve the basil plants. It is also suggested to use these two substances safely to improve the productivity of other important medicinal and aromatic plants. It can also be recommended to search for other substances that have the same positive effect on plant productivity.

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