*Plant Archives* Volume 20 No. 2, 2020 pp. 3905-3912 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

# THE EFFECT OF NANO NPK SPRAYING OF FYLLOTON EXTRACT AND SALICYLIC ACID ON THE ACTIVE COMPOUNDS OF *TAGETES PATULA*

**Zohoor Fuaad Abelaljabar* and Fanar Hashum Al_Hashemi**

College of Agriculture, University of Mosul, Iraq.

## Abstract

The study was conducted in the Lath house of the College of Agriculture/ University of Mosul for a period from 15 March to 15 October during the season 2019 on *Tagetes patula*. The experiment was carried out using three factors within R.C.B.D design, the first factor included nano Npk at four level 0, 30, 50, 65 mg/pot as well as the use of traditional fertilizer 314 mg/pot, the second factor was Fylloton extract (F) at (0,2) mL/L, the third factor was Salicylic acid (SA) at (0, 120) mL/L. The aim of this study is to demonstrate the effect of these factors on the percentage of active oil compounds from marigold flowers. The results indicated that nano3 (65 mg/pot) had been recorded the maximum percentage of active substances (camphor, Myrcene, Limonene) which reached (7.66, 5.78, 6.29) respectively. The foliar spray of F and SA separately or bilateral interaction was performed increasing the active compounds compared with untreated plants.

***Key words*** **:** *Tagetes patula*, nano NPK, Fylloton, Salicylic acid, active compounds, GLC.

## Introduction

Tagetes is one of the plants of the "Asteraceae" family, which is an annual herbaceous plant in summer, the plant reaches a height of 50cm. The original habitat is southern Europe and North Africa, despite the growth of wild marigolds in many countries of the world with different environments and conditions (Singh *et al.,* 2014). The importance of the plant in terms of coordination. It's used in decorating gardens and making bouquets. The flowers are beautiful appearance, looking suitable for harvesting as well as the coordination and aesthetic value of this plant, it's source for many of the vehicles used in the medical field that enter the pharmaceutical industries (Negi *et al.,* 2014).

The name (marigold) is believed to be derived from the word (Tages), it's one of the names of deities in ancient times. The marigold plant endures the conditions of acidic, clay and salty soils. The plant has an economic value in the production of active substances especially flavonoids and carotenoids in flowers that are used as colored and antioxidants in the food and pharmaceutical industries (Akshaya *et al.,* 2017). India is at the forefront of the countries producing marigold oil for use in many areas, including chemicals and agricultural materials, perfumes and cosmetics because the oil contain α-Terpinolene and Limonene (Rakesh, 2012). The genus of marigolds is one of the most important medicinal plant, which includes 56 species worldwide, 27 of them are annual and 29 perennials. It's characterized by abundant flowering during the summer and autumn as well as its strong aromatic smell when touching leaves and flowers. It's propagated with seeds that germinate during 3-5 days at a temperature of 24-27°C. (Maria *et al.,* 2006 and Sysoeva *et al.,* 2010). The plant contains a number of active components for the oil, including linalool, salicylaldehyde, tagetone, ocimene, 1,8 cineale, Myrcene, α-pinene, β-pinene, Limonene that used in treating stomach pain and anti-intestinal parasites, liver diseases (Maria *et al.,* 2006). The root of marigold release substances that have a killer effect for nematodes present in the soil, so, it's important to grow these plants in fruit farms and among ornamental plants as well as the aromatic sell repellent to insects. (Natarajan *et al.,* 2006, Amiraefat and Abd El-Nasser, 2018). It was found through

***Author for correspondence :*** E-mail: fwadzhur979@gmail.com

many studies that fertilization plays a major role in plant growth as nano-fertilizers that affect the growth rate by penetrating into plant tissue cells and increasing the rate of nutrient penetration required (AL-Juthery and Qusay, 2009). These Fertilizers can enter plant cells to transfer DNA and chemical substances into the cells. (Ghafariyan *et al.,* 2013). The positive effect of nano fertilizers to increase the nutritional quality of crops, reducing stresses in plants, lack of additional quantities, costs of fertilization and absorption from the root comparing with traditional fertilizers (Al-Juthery and Saadoun, 2018). Medicinal plants respond to spraying with yeast and salicylic acid by increasing vegetative, flowering growth and oil yields by increasing plant content of hormones such as auxins, cytokinins and gibberellins.

The aim of this study to find out the effect of spraying with yeast and salicylic acid on the content of active substances of oil.

## Materials and Methods

### Vegetal material

The research has been done in the lath house of horticulture department and landscape design for the period from 15 March to 15 October during the season 2019.

The planting of seeds began in germination trays containing peatmoss, as an agricultural medium in the green house on 21 March and in the four real leaves, the seedlings were transferred on 30 April to 20cm diameter plastic pots that contain garden soil and peatmoss with a ratio of 3:1. The medium weight in every pot was 7kg.

All experiment plants were pinched on 14 May and a preventive system was used to prevent fungal diseases such as pesticide (pentalon) at a concentration of 2ml/ Liter and also super acid with a concentration of 1ml/ liter to treat the red spider.

### The experiment was carried out with three factors

**First:** Fertilizer NPK nano at four levels: 0,30,50,65 mg/pot. In addition to using traditional fertilizer with 314 mg/pot. The fertilizer was added with water irrigation (up to 9 liters of water) and 200 ml/pot once a month. The first addition was on 20/5/2019 and then with consecutive periodic appointments, one month after the first addition.

**Second:** Yeast extract (Fylloton) symbolized by (F) the vegetative group was sprayed with (0, 2) mL/L until complete wetness on the second day of adding fertilizer every ten days starting from 21/5/2019.

**Third:** Salicylic Acid (SA) the vegetative group was sprayed with salicylic acid after preparing it in the form of aqueous mixture with (0, 120) mg/L, and the plants were sprayed separately until full wetness occurred every month on 22/5/ 2019, then by successive periodic appointments on month after the first spray.

The experiment was arranged according to randomized complete block design (RCBD) consisting of 20 treatments (4 plant each treatment) and three replicates. The average was taken for each appointment and compared according to Duncan test by probability level of 5%. Temperature was recorded during the research period (Table 1).

**Table 1:** Average of temperature during the research.

| The months | Temperature (C°) | |
|---|---|---|
| | Great | Small |
| March | 10.70 | 8.50 |
| April | 25.30 | 11.30 |
| May | 34.20 | 18.10 |
| June | 40.30 | 23.40 |
| July | 42.20 | 26.10 |
| August | 41.40 | 22.50 |
| September | 34.20 | 21.30 |
| October | 32.10 | 14.50 |

* Temperature was recorded from the weather service/Baghdad.

### Essential oils extraction

Dry flowers of *Tagetes patula* was gave during the harvest at July month, this date was exceeded significantly in number flowers, diameter and dry flowers weight (g). July harvest was chosen for the purpose of conducting the process of extracting the volatile oils. The shade dried flowers of marigold had been subjected to hydro distillation using a Clevenger - kind equipment for 3.5 hours mixing 10g of flowers in 250ml of distilled water.

The volatile oils collected have been dried over anhydrous sodium sulphate and persevered at 40º awhile the evaluation was carried out (Clevenger, 1928).

### Chromatographic separation of essential oils by GLC

Analysis of the essential oils was carried out on model (Shimadzor, 2010) Japanese origin, by use of flame Ionization detector (FID), A capillary separation type (DM_5Ms) with dimensions (30m * 0.25mm * 0.25 um). The temperature of injector and detector had been (280, 340) ºC respectively the gradual temperature of the separation column starts from (100-300) °C with an increase rate of 10 degrees / minute. Inert nitrogen gas was used as a transfer gas at a rate 100 kpa. The injection volume turned in 10ML.

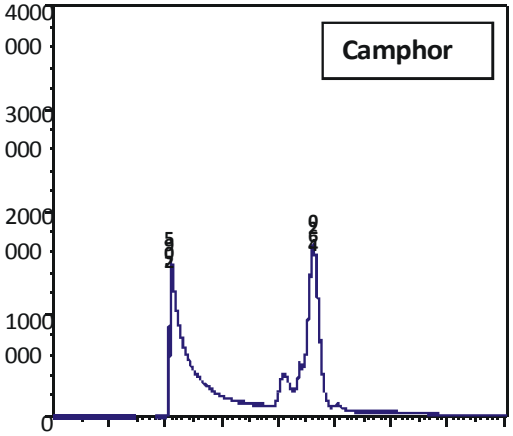
### Identification of compounds

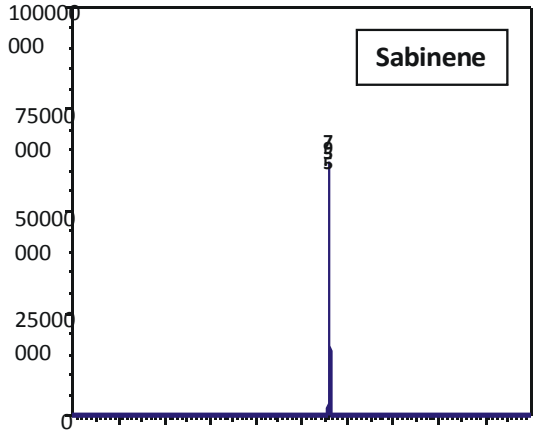
Volatile oils have been identified in dry flowers by gas liquid chromatography, comparison of their retention time ($R_t$) with those of the known solution (standard) which include: Camphor, Sabinene, Myrcene, Terpinene, Limonene, Linalool, α-pinene, Tagetone and Nerol were also identified depending on peak area values (Fig. 1).

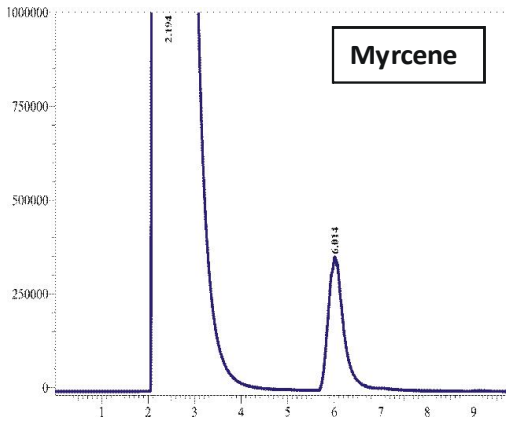
## Results and Discussion

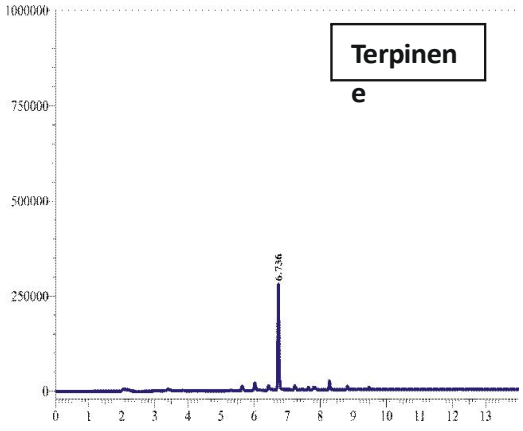
Essential oils had been extracted from dry flower (10 g) of *Tagetes patula* varied in percentage of active constituents (Table 2). Several studies looking at the chemical composition of many medical plants oils was indicated a vast difference, which may be due to many reasons. *i.e.* various climatically condition, seasonal variation, geographical locations, developmental stage of the plant (ontogeny) therefore harvesting time is one of the most important factors influencing oil (Cerven Zheljazkov, 2009).

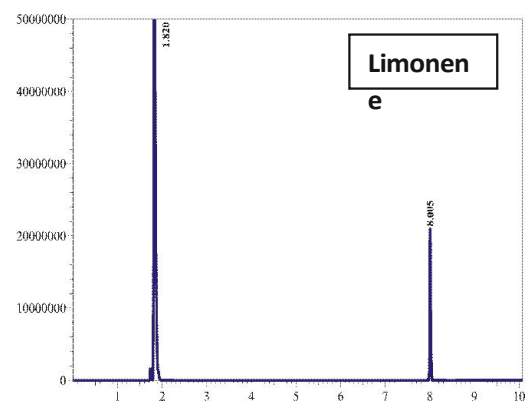
All standard compounds were emerged with all the parameters used in the research, which are nano fertilization and spraying with F and SA, whether when used alone or as a bilateral or triangular overlap of the three factors.

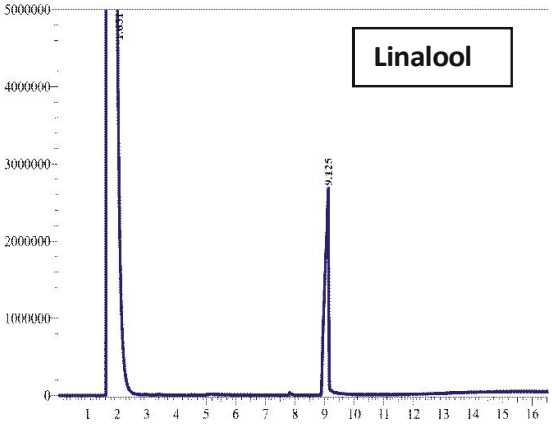


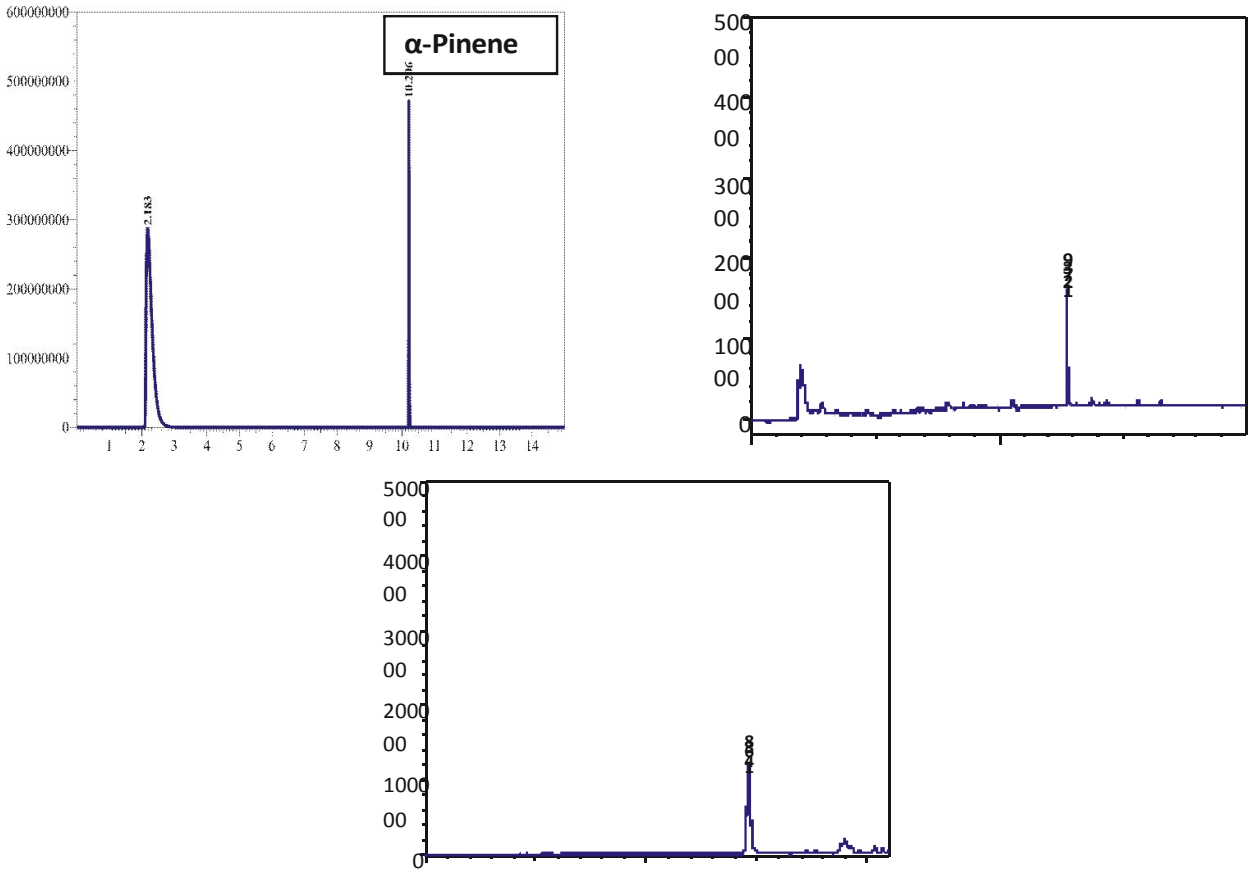



**Fig 1:** GLC chromatograms of standard essential oils.

Table 2 shown that nano fertilization at the third level (65 mg/pot) gave the highest concentration of active essential oils (Myrcenes 5.78% and limonene 6.29%) (Fig. 2). The used of third level resulted to increase the active compounds of oil compared to the second, first, traditional, untreated plants as a compounds (Camphor, Myrcene, Terpinene, Limonene, Linalool, α-Pinene, Tagetone and Nerol) which reached (7.66, 5.78, 6.66, 6.29, 5.77, 8.97, 4.86, 21.33)% respectively. This is due to the role of N,P and k to increasing vegetative and flowering growth, which leads to increasing primary metabolites and secondary products including the active of components of the oil, so the chemical energy resulting from the process of photosynthesis was exploited for other processes inside the plant, including the production of the main components of the a volatile oils that are produced from the primary pathway of Acetyl CoA through the mevalonic acid that consists of monoterpenes and the PEP (phosphoenol purvate) compound through the shikimic acid pathway that necessary to form oil compounds (Herrmann and Weaver, 1999, Taiz and Zeiger, 2002).

The use of nano fertilizer promote growth more effectively compared to conventional fertilizers (Fig. 3) and it is able to release nutrients in response to an environmental factor and in reaction to fluctuations in the environment such as temperature and moisture etc. (Al-Juthery *et al.,* 2019).

The spraying with F and SA separately showed an increase in the concentration of active substances in oil. The results also showed in table 3 that the bilateral interaction between both factors was performed increasing the concentration of active substances too, compared with untreated plants (Fig. 4, 5, 6, 7). In addition, the enhancing effect of yeast extract (F) on the percentage of active substances of marigold plants may be due to it's a rich source of phytohormone, sugar, vitamins, enzymes, amino acids and minerals as well as it has a catalytic role in cell division, protein. The catalytic effect of yeast may be attributed to its stimulating effect of enzymes, production of phytohormone, improvement of nutrient absorption and the release of $Co_2$ which imports the photosynthesis (Saffa *et al.,* 2011). These results are in agreement with Mahmoud *et al.,* (2008) for *Melissa officinalis* L. and Safaa *et al.,* (2017) for *Ocimum basilicum* whom reported that foliar spray of yeast resulted in the highest percentage of active substances using the range of (50-6) ml/L. while the role of SA is attributed to the nitrogen metabolism and to the

**Table 2 :** Effect of NPK (nano and traditional), foliar spray of Fylloton extract (F), Salicylic acid (SA) and their interaction on oil chemical composition of *T. patula.*

| Fertilizer | Active compounds | Camphor | | Sabinene | | Myrcene | | Terpinene | | Limonene | | Linalool | | α-Pinene | | Tagetone | | Nerol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Standard Rt(min.) | 4.620 | | 5.597 | | 6.014 | | 0.736 | | 8.005 | | 9.125 | | 10.306 | | 12.739 | | 14.638 |
| | Treatment | Conc% | RRT | Conc% | RRT | Conc% | RRT | Conc% | RRT | Conc% | RRT | Conc% | RRT | Conc% | RRT | Conc% | RRT | RRT |
| Control | Zero | 6.96 | 4.45 | 5.23 | 5.48 | 0.56 | 5.48 | 5.23 | 6.72 | 1.08 | 7.93 | 5.09 | 9.13 | 8.35 | 10.30 | 3.79 | 12.50 | 14.72 |
| | F2 | 7.25 | 4.67 | 5.48 | 5.82 | 0.59 | 6.02 | 4.84 | 7.04 | 1.15 | 8.29 | 5.23 | 9.52 | 8.55 | 10.71 | 3.87 | 13.29 | 15.11 |
| | SA | 7.89 | 4.67 | 6.97 | 5.82 | 0.81 | 6.02 | 6.97 | 7.05 | 1.35 | 8.30 | 5.89 | 9.52 | 9.24 | 10.72 | 4.60 | 13.30 | 15.12 |
| | F2 × SA | 7.36 | 4.47 | 5.69 | 5.56 | 0.64 | 5.83 | 5.69 | 6.75 | 1.20 | 7.96 | 5.38 | 9.17 | 8.62 | 10.34 | 3.99 | 12.54 | 14.77 |
| Traditional | Traditional | 8.06 | 4.50 | 7.45 | 5.60 | 0.83 | 5.84 | 7.45 | 6.79 | 1.38 | 8.009 | 6.04 | 9.21 | 9.38 | 10.38 | 4.74 | 12.59 | 14.82 |
| | F2 × Traditional | 8.24 | 4.51 | 7.80 | 5.62 | 0.87 | 5.86 | 7.80 | 6.82 | 1.43 | 8.05 | 6.28 | 9.26 | 9.45 | 10.43 | 4.83 | 12.65 | 14.87 |
| | SA × Traditional | 9.52 | 4.58 | 9.68 | 5.70 | 1.26 | 5.93 | 9.68 | 6.91 | 1.96 | 8.15 | 7.49 | 9.36 | 10.47 | 10.55 | 5.79 | 12.78 | 24.56 | 14.98 |
| | SA×F2×Traditional | | | | | | | | | | | | | | | | | | |
| Nano1 | Nano1 | 7.48 | 4.58 | 6.23 | 5.70 | 0.68 | 5.93 | 6.23 | 6.91 | 1.24 | 8.15 | 5.41 | 9.36 | 8.79 | 10.55 | 4.20 | 12.78 | 20.55 | 14.98 |
| | Nano1 × F2 | 8.33 | 4.55 | 8.22 | 5.66 | 0.92 | 5.90 | 8.22 | 6.86 | 1.48 | 8.09 | 6.39 | 9.30 | 9.52 | 10.48 | 4.96 | 12.70 | 22.58 | 14.94 |
| | Nano1 × SA | 4.48 | 4.67 | 8.35 | 5.82 | 0.97 | 6.02 | 8.35 | 7.04 | 1.53 | 8.29 | 6.47 | 9.52 | 9.66 | 10.71 | 5.24 | 12.96 | 22.63 | 15.13 |
| | Nano1×F2×SA | 9.75 | 4.65 | 9.82 | 5.79 | 1.31 | 6.01 | 9.82 | 6.54 | 2.33 | 8.25 | 7.55 | 9.31 | 10.66 | 10.66 | 5.88 | 13.27 | 24.88 | 15.18 |
| Nano2 | Nano2 | 7.56 | 4.59 | 6.48 | 5.72 | 0.72 | 5.94 | 6.48 | 6.94 | 1.26 | 8.18 | 5.59 | 9.40 | 8.86 | 10.58 | 4.35 | 12.82 | 20.79 | 15.02 |
| | Nano2 × F2 | 8.62 | 4.70 | 8.56 | 5.84 | 1.03 | 6.05 | 8.56 | 7.07 | 1.60 | 8.32 | 6.66 | 9.55 | 9.79 | 10.74 | 5.33 | 13.33 | 22.78 | 14.05 |
| | Nano2 × SA | 8.97 | 4.70 | 8.78 | 5.85 | 1.10 | 6.05 | 8.78 | 7.08 | 1.68 | 8.33 | 6.89 | 9.57 | 9.88 | 10.76 | 5.47 | 13.08 | 23.46 | 15.17 |
| | Nano2×F2×SA | 9.87 | 4.59 | 10.00 | 5.72 | 1.40 | 5.94 | 10.13 | 6.94 | 4.41 | 8.18 | 7.60 | 9.40 | 10.74 | 10.58 | 5.96 | 13.19 | 25.00 | 15.02 |
| Nano3 | Nano3 | 7.66 | 4.68 | 1.58 | 5.82 | 5.78 | 6.03 | 6.66 | 7.05 | 6.29 | 8.30 | 5.77 | 9.53 | 8.97 | 10.72 | 4.86 | 11.86 | 21.33 | 13.31 |
| | Nano3 × F2 | 9.33 | 4.68 | 2.57 | 5.82 | 1.15 | 6.03 | 9.00 | 7.05 | 1.74 | 8.30 | 7.12 | 9.53 | 10.25 | 10.72 | 5.58 | 11.86 | 23.78 | 13.31 |
| | Nano3 × SA | 9.41 | 4.65 | 2.66 | 5.80 | 1.20 | 6.00 | 9.35 | 7.02 | 1.88 | 8.27 | 1.36 | 9.50 | 10.33 | 10.69 | 5.63 | 12.93 | 24.05 | 17.98 |
| | Nano3×F2×SA | 9.95 | 4.46 | 3.12 | 5.54 | 1.44 | 8.81 | 10.13 | 6.71 | 2.46 | 7.91 | 1.68 | 9.11 | 10.89 | 10.27 | 6.02 | 2.90 | 25.32 | 14.74 |

increasing effectiveness of the enzyme Nitrate reductase, So, the nitrogen metabolism is one of the factors affecting the accumulation of proline acid (Umebese *et al.,* 2009).

Also, nitrogen is important in formation of energy compounds and enzymatic companions that have a role in stimulating Acetyl CoA from pyruvic acid to reach the production of isoprenoids and then terpenes of all kind as well as stimulating the cinnamic pathway that encourages phenyl propanoids (xiao-sun *et al.,* 2004 and Minmin *et al.,* 2007). This result was supported by Hafsa *et al.,* (2015) who stated that spraying with salicylic acid increased the concentration of α-pinene and Eugenol for *Dianthus caryophyllus* L.

Similar interactive effect of SA on active compounds has been reported in the thyme plant by (Abdollah *et al.,* 2013) why mentioned that foliar application of SA was gave increased of active compounds (α-pinene, Camphor, Sabinene, Myrcenes and Terpinene).

(Table 2) and (Fig. 8) shown that triangular between nano fertilizer at the second level(50 mg/pot) with F (2 ml/L) and SA (120 mg/L) was gave the highest concentration of active substances (Sabinene, Terpinene, Linalol) which reached (10, 10.13, 7.60)% respectively.

Also, the treatment with nano fertilizer at the third level (65 mg/pot) with F (2ml/L) and SA (120 mg/L) gave the highest values of active substances (Camphor, α-pinene, Tagetone, All and Nerol) which reached (9.95, 10.89, 6.02, 25.32) % respectively. This is due to the combined effect of nano fertilization, Fylloton and SA in to

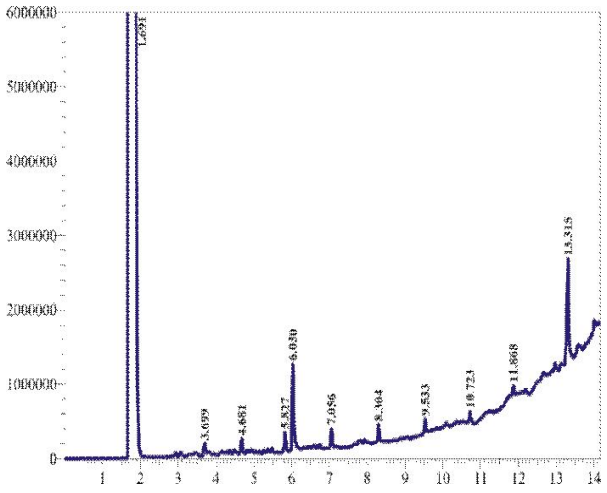


**Fig 2:** GLC chromatograms of essential oil content for treatment nano3.

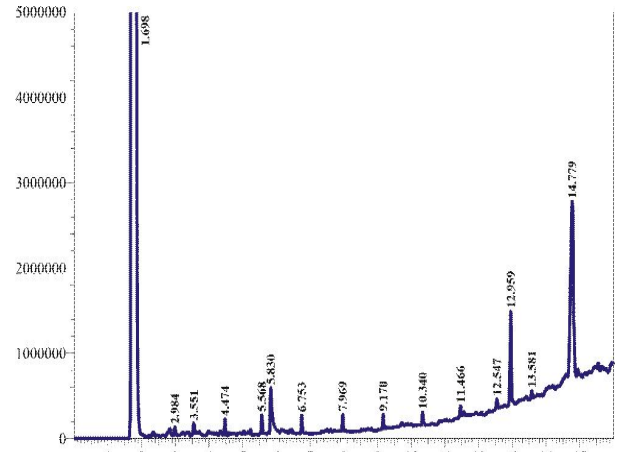


**Fig 3:** GLC chromatograms of essential oil content for treatment traditional fertilizer.

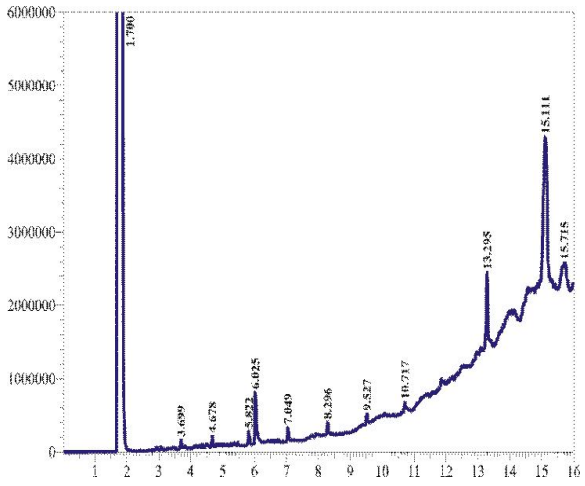


**Fig 4:** GLC chromatograms of essential oil content for treatment F2.

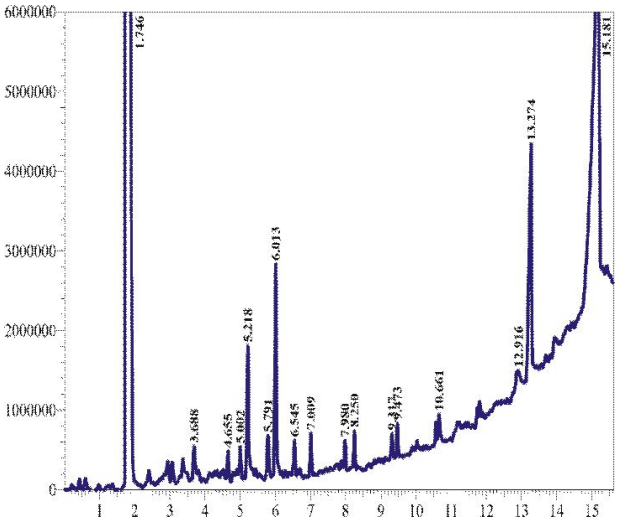


**Fig 5:** GLC chromatograms of essential oil content for treatment SA2.

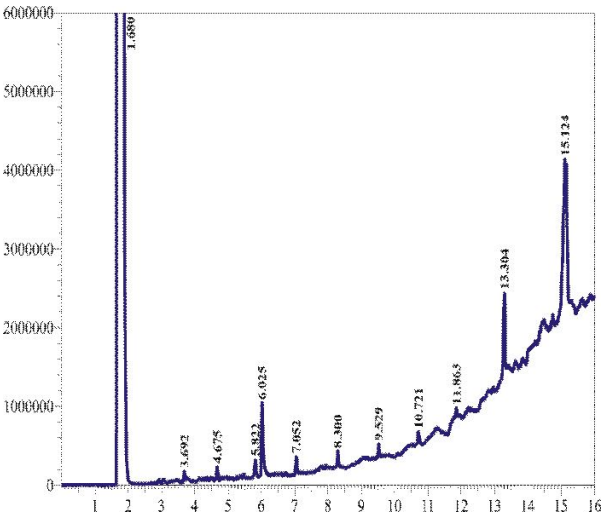


**Fig 6:** GLC chromatograms of essential oil content for treatment F2 + SA2.

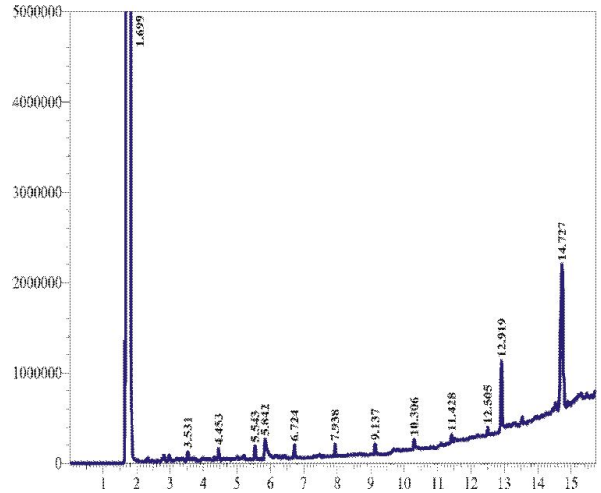


**Fig 7:** GLC chromatograms of essential oil content for treatment control.

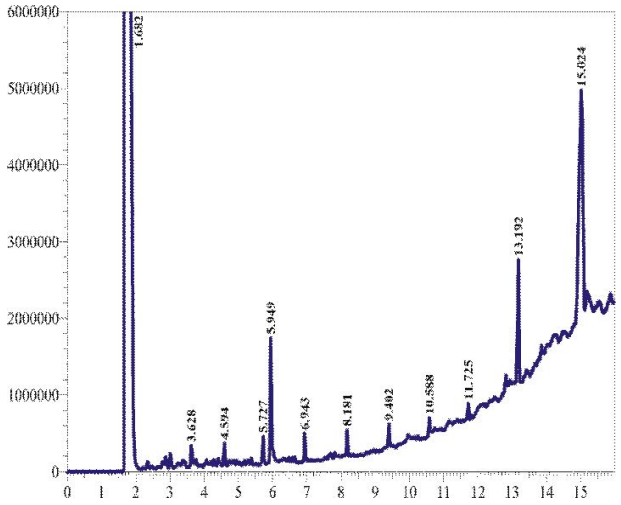



**Fig 8:** GLC chromatograms of essential oil content for treatment nano 2 + F2 + SA2.

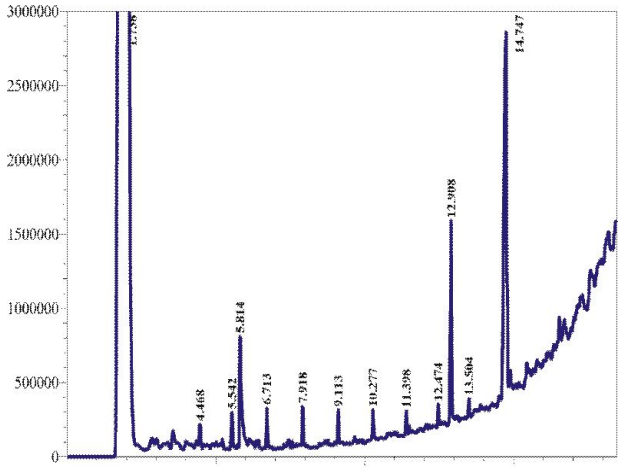



**Fig 9:** GLC chromatograms of essential oil content for treatment nano3 + F2 + SA2.

increase the concentration of active compounds and as stated in the interpretation of one of these factors individually.

## Conclusion

From the study, it was concluded that NPK nano fertilizer, Fylloton extract (F) and Salicylic acid (SA) have positively affect the chemical composition of volatile oils from the flowers of marigold compared to the control. All standard of essential oils was appeared in most treatments, so the interaction between fertilization (nano2 or nano3 + F + SA) had been increased the concentration of (Camphor, Sabinene, Terpinene, Linalool, α-pinene, Tagetone and Nerol).

## Acknowledgment

Author Zohoor and Dr. Fanar was very grateful to the University of Mosul, Collage of Agriculture and Forestry for their providing facilities, which helped to improve the quality of this work.

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