

## The effects of Nano-fertilizers on Growth Indicators, Yield and Percentage of Essential Oil in Mentha Piperita Medicinal Plant

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### Abstract

According to the conducted research, the combination of chemical fertilizers with organic and biological sources of nano-fertilizers has had favorable results in increasing the production efficiency of agricultural products, which can be a way towards organic agriculture and ultimately sustainable agriculture. In this research, the effects of nano-fertilizer on growth indicators, yield and percentage of essential oil in Mentha piperita medicinal plant were investigated in greenhouse conditions and the results of this experiment showed: All nano fertilizers alone had a positive effect on all investigated traits, but among them, the treatment of 4 liters per hectare had the most effect among other fertilizers. Also, there is a significant difference between the effect of the third level of iron and zinc nano fertilizer on the main trait of essential oil yield. Did not see, As a result, if the purpose of growing plants of this family is to produce essential oil, the most suitable fertilizer is the third level of iron and zinc nano fertilizer and the second level of iron, zinc and copper nano fertilizer. The results of this experiment showed that all nano fertilizers alone had a positive effect on all investigated traits But among them, the treatment of 4 liters per hectare had the greatest effect among other fertilizers Also, no significant difference was observed between the effect of the third level of iron and zinc nano fertilizer on the main attribute of essential oil yield, if the purpose of growing plants of this family is essential oil yield, the most suitable third level fertilizer is iron and zinc nano fertilizer and the second level is iron, zinc and copper nano fertilizer.

**Keywords:** Nano Fertilizer; Performance; Growth; Essential Oil; Mentha Piperita

## Introduction

Today, the consumption of medicinal plants and herbal medicines is increasing day by day in the world. The methods of improving the quality and quantity of medicinal plants are among the things that have always been of interest. Considering the limited resources and increasing human population, it is necessary to pay attention to the methods of increasing the production efficiency of medicinal plants [1]. The use of chemical fertilizers as the fastest way to compensate for the lack of soil nutrients is common in the world. The correct and appropriate consumption of different types of fertilizers (chemical, animal, vegetable compost or green manure, etc.) is the most important and fundamental way to maintain and improve soil fertility conditions and increase the yield of agricultural products [2]. In traditional farming methods, using a limited number of chemical fertilizers such as nitrogen and phosphorus fertilizers to reach the maximum possible production is not fully effective. The high costs of using chemical fertilizers and the pollution of soil and water, as well as the decrease in the quality of agricultural products due to the use of these fertilizers, have caused environmental problems and also the lack of suitable food combinations in the products [3]. However, chemical fertilizers cannot be removed from agricultural ecosystems at once. In this regard, the use of degradable and natural resources with organic origin along with chemical fertilizers can be considered as a suitable solution to replace the use of chemical fertilizers, which in turn causes fertility and preservation of soil structure, biological activity, water conservation and Finally, the physical and chemical structure of the soil is modified [4].

In order to achieve the goals of sustainable agriculture, redesigning agricultural systems is of particular importance. In sustainable agriculture, simply paying attention to soil tests in order to recommend fertilizer is not considered an infrastructure issue [5]. This research investigates the effects of nano fertilizers on growth indicators, yield and percentage of essential oil in *Mentha piperita* medicinal plant [6]. In this research, the effects of nano-fertilizers on growth indicators, yield and percentage of essential oil in *Mentha piperita* medicinal plant were investigated in greenhouse conditions, and the results of this experiment showed that all nano-fertilizers alone had a positive effect on all investi-

gated traits [7] but among them, the treatment of 4 liters per hectare had the greatest effect among other fertilizers. Also, no significant difference was observed between the effect of the third level of iron and zinc nano fertilizer on the main trait of essential oil yield (Lin, 2008).

## Materials and Methods

This research was carried out in the form of pot cultivation in greenhouse conditions in Iran country, Arak city, Islamic Azad University, Arak branch, with coordinates, latitude 34 degrees 5 minutes and longitude 49 degrees 42 minutes and altitude 1757 meters above sea level. It was implemented in the crop year 2013. In this experiment, the effect of humic acid and gibberellic acid on the growth characteristics and production of essential oil in *Mentha piperita* medicinal plant was investigated in order to reduce the consumption of chemical fertilizers.

In this design, the statistical method of factorial testing was used in the majority of completely random blocks. In order to measure the desired factors (plant height, no leaf, no stem, leaf fresh weight, leaf dry weight, root fresh weight, root dry weight, biological yield, essential oil percentage, essential oil yield), 1 plant is selected from each replication and the average of the characteristics of 3 plants measured in 3 repetitions is the characteristics of the desired factor in each plot.

By using a precise metal ruler and placing it on the soil surface next to the plant, the height of the plant was measured for 1 sample in each repetition. Then, the average trait of 3 plants in 3 repetitions of measured plant height was calculated for the treatments. To measure the number of leaves, the number of leaves of 1 plant was counted in each replication. Yellow and dried leaves were not included. Then the average trait of 3 plants measured in 3 repetitions and the average number of leaves per plant is calculated. In order to measure this trait, the number of branches of 1 plant was counted in each replication. Then the average trait of 3 plants measured in 3 repetitions and the average number of branches per plant were calculated. To measure the fresh weight of leaves, the fresh weight of the leaves of 1 plant was weighed in each repetition. Then, the average trait of 3 plants measured in 3 repetitions was calculated, and the

average fresh weight of leaves per plant was calculated. To measure the fresh weight of leaves, the fresh weight of the leaves of 1 plant was weighed in each repetition. Then, the average trait of 3 plants measured in 3 repetitions was averaged and re-weighed in a dry oven. The roots of 1 plant from each pot are removed from the soil after the treatment, and after washing each root, the weight and average is taken. The number obtained is the average wet weight of the root.

3 wet roots from 3 repetitions are placed in the oven and after drying, the average is taken. The obtained number is the average dry weight of the root. The aerial part of one plant from one treatment of 3 repetitions, which is a total of 3 plants, is from the soil surface. We cut it and weigh it to get the performance of a pot. To measure the amount of essential oil of leaves and branches as essential oil producing organs, 50 grams of dried leaves and branches were powdered from each pot of each treatment in each repetition and essential oil was extracted. The amount of essential oil of the obtained samples was multiplied by the specific weight of the essential oil and thus this attribute was measured. This index was calculated from the product of the amount of dry matter of the branch in the percentage of essential oil of the branch and the amount of dry matter of the leaf in the percentage of leaf essential oil, for each experimental unit.

*Mentha piperita* L. is one of the most important, popular and widely used medicinal plants. From two thousand years ago until now, different types of mint have been used as spices and medicine, but the human use of peppermint goes back to 250 years ago. The leaves, vegetative body and essential oil of this plant have been mentioned as medicine in most of the reputable centers. The annual consumption of mint essential oil in the world reaches seven thousand tons. In this case, the United States of America has dedicated one of the largest agricultural areas to mint cultivation, and in 1992, about 3,200 tons of essential oil were extracted from 43,000 hectares of cultivated land (Amidbigi 2004 and Aflatuni 2005).

Cytological studies have shown that peppermint *Mentha piperita* L. ( $2n = 72$ ) is a type of triple hybrid that was created by combining the species (*M. aquatica* L. ( $2n =$

96) and ( $2n = 48$ ) *M. spicata* L. (2005) The *Mentha spicata* species is also a plant derived from the combination of the *Callicarpa longifolia* species. Among these plants, the presence of menthol has been proven in the essential oil of the *Phalaris aquatica* species it is called *Mentha piperita* (Omidbeigi, 2014).

*Mentha* is an herbaceous and perennial plant. The long leaves are 3 to 9 cm long and 1 to 3 cm wide, oval, broad, serrated on the sides and dark green in color. The veins of the leaves are blue and have many branches in the leaf blade. There are holes containing essential oil on both sides of the leaf. The flowers of mint are light purple and in the form of complex clusters on the cycles where each complex cycle contains 6 to 7 flowers (Omidbeigi, 2014). Their inflorescences are adapted in a way that facilitates pollination by insects, although the flowers of the mentioned family are originally bisexual, high degrees of male sterility are seen in many species, which is due to the existence of sterile stamens. Capsule fruit, small with tiny endosperm and dark red in color. The seed of this plant has no vegetative power (Omidbigi 1384 and Naqdibadi 1381). *Mentha* has a quadrangular stem. It is observed in purple color due to the presence of anthocyanins. The length of the stem is different and depending on the climatic conditions, it is between 30 and 100 cm. The upper part of the stem has more branches than the lower part. Stolons and rhizomes (underground organs that are alive in the cold of winter and have light activity) have numerous nodes that are the place of growth of thin roots and as a result of the formation of small plants around the mother base. The underground organs of *mentha* are white, thin and 5 to 20 cm long. The stolons come out of the rhizome in the form of numerous long and white branches. stolons are organs that are used for plant reproduction. From the growth of rhizomes, aerial organs (stems and leaves) are formed (picture 1-1). The root of *mentha* is not very deep and is scattered on the surface of the soil (Omidbeigi, 2014).

*Mentha* remains in one place for 2 to 3 years. Therefore, the frequency of cultivation is important for this plant. *Mentha* should be cultivated alternately with plants that have a short growing cycle and are harvested shortly after planting. For this reason, cereals and legumes are suitable for this work. After harvesting these plants, it is better

to leave the land fallow for a while. After harvesting mentha, plants should be planted that do not reduce the quality of the soil (the soil after harvesting mentha is of good quality). Cultivation of mentha should be done in lands that can be irrigated. Because mentha cultivation is not possible in low water areas. Four years after harvesting mint, it can be planted again in the same field (Omidbeighi 2014).

During the growth and production of effective substances, mentha needs a large amount of substances and nutrients. Research shows that the right amount of nitrogen significantly increases the mentha essential oil. For the production of mentha on a large scale, the presence of elements such as phosphorus and potash in sufficient quantity is also necessary. In summer (for autumn cultivation) or in autumn (for spring cultivation), we add 20 to 30 tons of fully decomposed manure along with 50 to 90 kg/ha of phosphorus oxide and 60 to 90 kg/ha of nitrogen fertilizer to the soil (Omidbeighi, 2014). If mentha is cultivated alternately with grains, deep plowing (25 to 30 cm deep) is done in the autumn season after harvesting the grains and adding the animal fertilizers needed by the plant. After plowing, the required chemical fertilizers are sprinkled with the soil and they are sent deep into the soil with a disk. In spring, you should avoid the use of tools that reduce soil moisture (Omidbeighi 2014).

Mentha does not produce seeds like any other two-veined plant, and its reproduction is done vegetatively by rooting (stolon), taking stem cuttings, or separating the stem from the mother plant. Cultivation and propagation of mentha through underground organs can be done in autumn or spring. Because the soil has enough moisture in the fall season and almost no irrigation is needed. The beginning of this season (October) is considered a good time for mentha cultivation. After passing through the cold period, the plant enjoys rapid growth and development from the beginning of spring. The most suitable time for spring planting is mid-spring (late May - early June). Some researchers do not consider spring cultivation suitable and believe that in this method, not only the growth of the plant is delayed and the crop can be harvested only once in the first year, but also the essence of plants is reduced in spring cultivation. But some of them have shown that the time of planting has no effect on the performance of the vegetative body

and the active substance of mentha, and when autumn planting is not possible due to unfavorable weather conditions, spring planting can be used. In propagation through rooting, the distance between the rows will be 50 to 60 cm. The optimal depth for root planting is 10 to 12 cm. The distance between two plants along the row will be 20 to 30 cm (Omidbigi 2017).

Adequate nutrients and sufficient irrigation and weed control during plant growth are necessary. To develop the surface of the leaves, it is recommended to use soluble nutrients in the form of spraying on the surface of the plants. The amount of irrigation depends on weather conditions and soil type. In each stage, the amount of irrigation should not be less than 40 to 60 mm. Weeds during growth may cause disturbances in the growth of mentha, therefore, weed control by both chemical and mechanical methods should always be considered. Early spring is the right time for mechanical harvesting of weeds and turning the soil between the rows in order to aerate the roots (Omidbeighi 2014).

Effective plant substances are classified into four main groups: alkaloids, glycosides, volatile oils (essences) and other effective substances (Omidbeighi, 2016). Apart from essential oils, aromatic plants also contain other compounds such as hormones, vitamins, antibiotics and disinfectants (Jaimand, 2015). Since essential oil extraction takes place in a range of inferior and often dicotyledonous plants, it indicates that the accumulation of terpene compounds was one of the characteristics of early plants. Among tropical and temperate dicotyledonous plants, there is the ability to accumulate terpenes in a wide range of the mentha family (Naghdebadi, 2012). Essential oil is made and stored in the vegetative body at the beginning of mentha growth. As the plant grows, the rate of essential oil synthesis increases. The leaves have 2 to 2.7% essential oil and the flowers have 4 to 6% essential oil, the stems usually have no essential oil. On average, the amount of essential oil in the aerial parts of the plant is reported to be 1 to 1.5%. The composition of essential oil reaches more than 20 types, the most important of which is menthol (40 to 60%). The highest amount of menthol is in the essential oil extracted from young leaves. The essential oil of flowers has a small amount of menthol and its most important composition is mentofuran (10 to

12%). Menthone (in the amount of 15 to 25%), piperitone (in the amount of 0.1 to 1.5%), pulgone (mostly present in young leaves), pinene, sabinene, cineole and methyl acetate can be mentioned among other ingredients of mentha essential oil. According to the reports, the amount of menthol is the main criterion for determining the quality of Mentha piperita essential oil (Platonic), the necessary condition for having an essential oil of good quality is to have at least 45% menthol, 15-18% menthone and a lot of isomenthol, and undesirable compounds such as menthofuran and polygone are less than 2 and 4%. The growing cycle of mentha (from the beginning of growth to the flowering stage) lasts 80 to 100 days. At first, the plants grow slowly, while after 2 to 3 weeks their growth accelerates. The underground organs of the plant are superficial and the resulting branches are scattered around. Flowers appear in early summer (July). After the first harvest, if the climatic conditions are suitable, the plants will flower again.

Mentha piperit and more specifically its essential oil is widely used in medicine. In short, it has properties such as: analgesic, antimicrobial, anti-nausea, antiviral, anti-inflammatory, antioxidant, anti-itching, anti-fever, anti-septic, Antispasmodic, antitussive, anti-ulcer, anti-flatulent, reducing or increasing sexual desire, allergenic, calcium

blocker, heart tonic, choleric, pain reliever, diuretic, detoxifier, laxative, expectorant, insecticide, stomach tonic, stimulant He pointed out that it is soothing, vasodilating and anti-helminthic. Also, the aroma of mentha is used to make unpalatable medicines taste better. Menthol of Mentha piperita essence has antibacterial properties and is used in the preparation of solutions for washing the mouth and throat. Tea prepared with mentha drops is useful in relieving cold symptoms and as an antitussive, and has weak diuretic and anti-viral properties. Consuming mentha food products is effective in eliminating fecal tract inflammation, and pills containing mentha with its essential oil have anti-cough properties.

It seems that allergic side effects caused by the consumption of Mentha piperita are very rare, or if they occur, they are very mild (Salehi Surmoghi, 2016).

## Results

According to the process carried out in the test and From the variance analysis of plant height in Mentha piperita medicinal plant, it was found that the use of nano fertilizers (iron, copper and zinc) had a significant effect on plant height at the probability level of 1% (Table 1-4).

**Table 1:** Variance analysis of Mentha piperita traits under the influence of different levels of nano fertilizers (iron, zinc, copper)

cover crown	Essential oil performance	percentage of essential oil	dry body weight	fresh body weight	Dry weight	Fresh weight	Number of stems per plant	Bush height	s.o.v
239.85**	15.124**	0.7682**	15.6048**	157.92**	1.3208**	65.235**	0.1605**	0.1605**	repetition
3780.1**	120.64**	3.2357**	29.2977**	853.39**	11.215**	179.16**	2175.3**	2175.3**	effect of ironA
9919.1**	42.381**	1.2298**	5.6104**	418.16**	8.0957**	105.93**	1115.9**	1115.9**	effect of CopperB
2545.3**	57.537**	1.6342**	5.4533**	246.58**	7.9851**	100.08**	631.46**	631.46**	effect of ZincC
423.62**	4.7925*	0.1574*	3.2611**	29.999*	0.8756*	15.826**	74.104**	74.104**	reaction(B.A)
22.528**	2.9816**	0.0773**	1.6638**	25.735*	0.1399**	1.5306**	26.901**	26.901**	reaction(C.A)
276.70**	1.4051**	0.0414**	0.0859**	3.3371**	0.3707**	2.0864**	13.234**	13.234**	reaction(B.C)
124.66**	4.6294**	0.1259**	0.4864**	15.385**	0.3121**	7.5308**	25.290**	25.290**	reaction(C.B.A)
100.083	1.7046	0.07263	0.4148	9.4017	0.2604	1.9654	1.0722	1.1348	experimental error
9.49	12.41	12.66	7.27	9.76	10.17	7.28	12.12	3.33	Coefficient of variation

Examining the table comparing the average height of *Mentha piperita* medicinal plants under the influence of different levels of nano iron fertilizers showed that the second level of nano iron fertilizer (4 liters per hectare) was

39.89 cm higher than the first level of nano iron fertilizers (2 liters per hectare. ) with 33.81 cm and control and a significant difference was observed between them (Table 1) (Table 2).

**Table 2:** Average comparison of different characteristics of *Mentha piperita* under the influence of simple effects of iron, copper and zinc

cover crown(cm <sup>2</sup> )	Essential oil performance	percentage of essential oil (%)	Dry weight of shoot (g)	Fresh weight of shoot (g)	Leaf dry weight (g)	leaf fresh weight (g)	Number of stems	Plant height (cm)	treatment
91.689b	8.074b	1.73b	7.74b	25.36c	4.27b	16.33c	6.85c	22.22c	without iron
112.84a	11.66a	2.35a	9.23a	32.39b	5.31a	20.19b	8.41b	33.81b	2 liters per hectare of iron
111.46a	11.80a	2.30a	9.60a	36.47a	5.46a	21.22a	10.4a	39.89a	4 liters per hectare of iron
86.226b	9.107a	1.91c	8.36b	27.13c	4.42c	17.00b	7.37b	24/78c	without copper
105.02ab	11.51a	2.33a	8.95a	32.22b	5.49a	20.74a	9.07a	34.00b	2 liters per hectare of copper
124.56a	10.92b	2.14b	9.26a	34.87a	5.14b	20.00a	9.19a	37.15a	4 liters per hectare of copper
94.363b	8.867b	1.86b	8.34b	28.02b	4.39b	17.07c	7.59b	26.44c	without zinc
108.79a	11.03a	2.20a	9.03a	32.40a	5.22a	19.93b	8.81a	34.07b	2 liters per hectare zinc
112.83a	11.65a	2.33a	9.19a	33.81a	5.42a	20.74a	9.22a	35.41a	4 liters per hectare zinc

According to the results of variance analysis of essential oil yield in *Mentha piperita* medicinal plant, it was found that the use of nano fertilizers (iron, copper and zinc) had a significant effect on the fresh weight of aerial parts at the probability level of 1% (Table 1-4).

Examining the comparison table of the average yield of *Mentha piperita* medicinal plant essential oil under the influence of different levels of nano iron fertilizers showed that the second level of nano iron fertilizer (4 liters

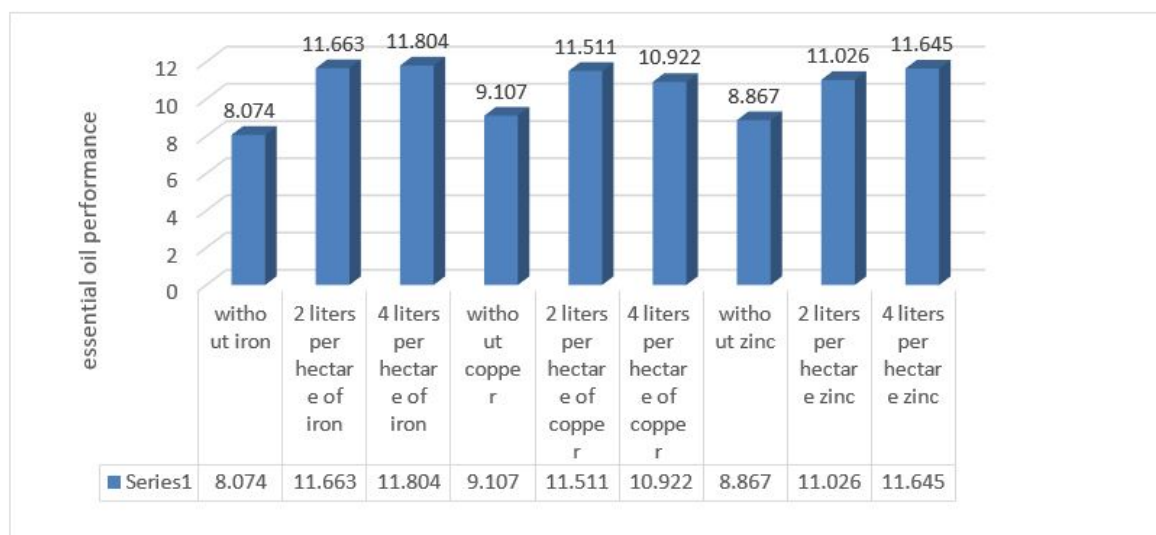
per hectare) used 11.80 grams more essential oil than the first level of nano iron fertilizer (2 liters per hectare) with 11.66 grams and controls and no significant difference was observed between them. (Table 1) (Table 2)

Examining the comparison table of the average yield of *Mentha piperita* medicinal plant essential oil under the influence of different levels of nano copper fertilizers showed that the first level of nano copper fertilizer (2 liters per hectare) with 2.33 grams of essential oil yields more

than the second level of nano copper fertilizers (4 liters per hectare).) with 2.14 grams and controls and a significant difference was observed between them. (Table 1) (Table 2)

Examining the comparison table of the average yield of *Mentha piperita* medicinal plant essential oil under

the influence of different levels of nano zinc fertilizers showed that the second level of nano zinc fertilizer (4 liters per hectare) with 2.33 grams more essential oil yield than the first level of nano zinc fertilizer (2 liters per hectare) with 2.20 grams and control and no significant difference was observed between them (Table 1) (Table 2).



**Figure 1:** The effect of separate effect of nano fertilizer (iron, copper and zinc) on the average yield of essential oil in *Mentha piperita* plant

Examining the comparison table of the average yield of *Mentha piperita* medicinal plant essential oil under the influence of different levels of nano fertilizers (iron, copper, zinc) (showed that the second level of nano iron fertiliz-

er (4 liters per hectare) was used with 36.47 grams and the second level of nano zinc fertilizer (4 liter per hectare) with 2.33 grams of essential oil yield was more than other nano fertilizers and controls. (Table 1) (Table 2).

**Table 3:** Average comparison of different characteristics of *Mentha piperita* under the influence of the three effects of iron, copper and zinc

cover crown(cm <sup>2</sup> )	Essential oil performance	Percentage of essential oil (%)	Dry weight of shoot (g)	Fresh weight of shoot (g)	Leaf dry weight (g)	leaf fresh weight (g)	Number of stems	Plant height (cm)	treatment
81.94b	7.556d	1.69f	7.74e	23.70e	3.81e	15.11d	6.67c	19.89g	B1C1
93.14b	9.256c	1.92ef	8.54cd	27.83d	4.51d	17.11bc	7.44bc	25.67f	B1C2
99.97ab	10.51bc	2.11cde	8.79bcd	29.86cd	4.92cd	18.78bc	8.00b	28.78d	B1C3
101.4ab	9.500c	1.98de	8.44d	28.21d	4.68d	18.22bc	8.00b	27.22e	B2C1
116.8a	12.38a	2.44ab	9.13abc	33.67ab	5.77ab	21.67a	9.44a	37.11b	B2C2
120.4a	12.64a	2.58a	9.28ab	34.78ab	6.02a	22.33a	9.78a	37.67b	B2C3
99.79ab	9.54c	1.90ef	8.84ad	32.13bc	4.70d	17.89bc	8.11b	32.22c	B3C1
116.4a	11.43ab	2.22cde	9.42ab	35.69a	5.39bc	21.00a	9.56a	39.44a	B3C2
118.2a	11.79ab	2.31abc	9.50a	36.80a	5.32bc	21.11a	9.89a	39.78a	B3C3

Iron, copper and zinc (the results showed that the use of the third level of iron and the second level of copper and the third level of zinc (A3B2C3) had the greatest effect on the performance of essential oil, which was consistent with the results of (Zareie. 2011).

It should be noted that the level (A3B2C) is on the same level as the level (A2B2C3). And this triple combined level is suggested to increase the performance of the essential oil in the *Mentha piperita* medicinal plant.

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