



## Original Article

# The Effect of Foliar Application of Humic Acid and Nanofertilizer (Pharmks<sup>®</sup>) on Yield and Yield Components of Black Cumin (*Nigella sativa* L.)

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## Abstract

In a sustainable agriculture system, application of the fertilizers which are nature friendly and suitable for plants is essential. This becomes more important when dealing with medicinal plants. Therefore, a factorial experiment in a randomized complete block design with three replications was set up in the 2012-2013 on *Nigella sativa* L. Treatments were nanofertilizer (Pharmks<sup>®</sup>) (at 2 levels 0, 1 ml/l) and humic acid (at 4 levels zero, 1, 3, 6 ml/l) which were applied at three times of plant growth. Results showed that different levels of humic acid imposed a significant effect on number of capsule per plant, number of seeds per capsule, seed weight, seed yield, biological yield and harvest index. But there were no significant effects on number of seeds per plant and weight of 1000 seed. The highest yield to levels 3, 6 ml/l humic acid treatments respectively. Nanofertilizer (Pharmks<sup>®</sup>) application significantly increased the yield and yield components of *N. sativa*. Combined treatment at various levels had significant effect on seed weight, seed yield, biological yield and harvest index respectively but had no significant effect on other traits. The lowest yield was detected at 1 ml/l humic acid and control treatments respectively. Generally, it seems that application of nanofertilizer (Pharmks<sup>®</sup>) and humic acid due to having nutritional ingredients and different physiological effects improves *N. sativa* performance and reduces environmental pollution and it could be used as a natural material to increase and stabilize field crop production.

**Key words:** Humic acid, *Nigella sativa*, Yield and yield components, Nano fertilizer (Pharmks<sup>®</sup>)

## Introduction

The ever-increasing tendency to the use of medicinal plants in the world has grown concerns about their cultivation and production processes. As medicinal plants are more compatible with the nature, special interest and attention has recently been given to herb therapy, and use of medicinal plants, being limited by the rise of pharmaceutical drugs, has become again common and widespread due to a number of reasons [1]. *Nigella sativa* L. is one of the herbs that has a variety of uses and has been being used in Iran's traditional medicine since old times. Today this

plant is considered as one of the most important kinds of medicine. Therefore, it is of great importance to conduct some researches on the herbs around the country due to different ecological requirements. *N. sativa* belonging to buttercup family, with the scientific name of Ranunculaceae, is an annual, dicotyledonous, herbaceous plant. This plant is widespread with in North Africa, southern Europe, the Mediterranean regions to India and Southeast and West Asia, and Australia, but researchers believe its origin as being in the Middle East and West Asia [2,3]. In several studies, *N. sativa* has been reported to have anti-oxidative, anti-inflammatory, strengthening of immune

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system, and anti-histamine and oil extract properties. Furthermore, several effects such as lowering blood sugar, lipids, and hypertension, excretion of bile and uric acid, protection of liver, kidney and cardiovascular tissues as well as anti-seizure, anti-cancer, anti-microbial and anti-parasitic effects related to this plant have been reported. Thus, considering the importance of *N. sativa* in pharmaceuticals, food and cosmetics industries, it is strongly needed to conduct comprehensive studies on the cultivation and development of the plant [4,5]. Organic agriculture is an integrated farming system, based on ecological principles, in which product quality is more important than its quantity. Organic agriculture system can be considered as an alternative to conventional systems and lead to the development of sustainable agriculture and environmental health [6]. Tendency to production of medicinal plants and demand for natural products, especially in organic farming circumstances, is increasing in the world. Organic cultivation of medicinal plants has negative effects on their quality and yield [7]. Environmental factors have an obvious impact on the growth, yield and quality of herbal in gradients. One of the environmental factors affecting the quantity and quality of medicinal plants is feeding [8]. With proper nutrition of plants, we can increase efficiency of inputs, while preserving environment, improving water quality, reducing erosion and conserving biodiversity. Furthermore, avoiding the unnecessary use and over-consuming of nutrients, we'd be able to reduce production costs to a minimum, and this could be taken into account as a way to sustainable agriculture. Fertilizer management is a key factor in the successful cultivation of medicinal plants [9]. Application of chemical fertilizers in agriculture causes environmental problems such as soil physical degradation and soil nutrients imbalance. Thus, application of various organic fertilizers is increasing [10]. The results from studies have shown that the organic fertilizer improves the physical, chemical and biological soil properties and increases crop yield. Humic acid, with a molecular weight of 30000-300000 Dalton, and folic acid, with a molecular weight of less than 30,000 Dalton, can form stable and insoluble complexes and soluble complexes with micronutrients [11]. The organic compound is composed of humic acid, folic acid, and is humin. These compounds have been derived from a variety

of sources such as peat, compost, worm compost and municipal solid waste. Humic acid is good to enhance the biological activity of the soil, and minerals are absorbed better and faster this way. It enhances the activity of soil microorganisms, increases plant resistance against diseases and strengthens the root system [12]. Nanotechnology is one of the new technologies that are at the early stage of its development. The main difference between nanotechnology with other technologies is related to the materials' scale and structures that are specified for this technology. Nanotechnology products contain a mixture of particles with dimensions between 1 nm to 100 nm that could change their physical and chemical properties of their raw materials [13]. All knowledge areas are affected by nanotechnology and this is typical of agricultural science. One of the most important applications of nanotechnology in various fields and orientations in agricultural and soil and water is to use nanofertilizers for plant nutrition [14]. Nanofertilizers provide the field plants with the nutrients in three ways: 1) nutrients are coated by nanoporous materials; 2) are covered by a thin layer of polymer; 3) are released in the form of a nanoscale particle or emulsion [15]. The use of nanofertilizers increases the efficiency of nutrients use, reduces soil toxicity, minimizes the negative effects from over-consuming of fertilizers and decreases the frequency of fertilizer applications. Using nanofertilizer, the time and speed of nutrients dispersion is coordinated by food requirements of the plant and thus, the plant will be able to absorb the maximum amount of nutrients and therefore, while reducing the leaching of nutrients, the crop yield increases [16]. There are few reports about the impact of nanofertilizers on increasing the quantity and quality of plant growth. Pandey *et al* [17] reported the increased growth rate of *Cicer arietinum* L. where ZnO nanoparticles are applied. Shaalan [18] reported the increased yield and yield components of *N. sativa* in circumstances where biological fertilizers are applied. Cacilia *et al* [19] applied levels of (0, 100, 200 and 300) milligrams per liter of humic acid on *Thymus vulgaris* L. Results showed that humic acid in the plant decreased height and oil yield, but significantly increased its fresh and dry weight and antioxidant activity. Khazaei *et al* [20] investigated the effect of concentrations of 0, 100, 200 and 300 milligrams per liter of humic acid on shoot growth, yield and essential oil in the medicinal plant of *Hyssopus officinalis* L. This compound significantly increased

the essential oil, shoot, leaf number and plant biomass.

Doing studies over the effect of nanopharmax and humic acid fertilizer son the plant, no research findings were obtained. So, in order to use less chemical fertilizers to prevent environmental pollution and encourage farmers to use more organic fertilizer, the present study was carried out to evaluate the effect of foliar application of humic acid and nanopharmax fertilizer on the yield and yield components of *N. sativa* species.

## Material and Methods

This test was conducted in 2012-2013. Before testing, a farm was selected to determine the chemical properties of soil and then, soil sampling was conducted. Physicochemical properties of the soil of the test site have been shown in Table 1. The test treatments would include humic acid and nanopharmax fertilizer with the levels of 0, 1, 3 and 6 ml per liter and 0 and 1 ml per liter, respectively. Humic acid and nanopharmax fertilizer characteristics are shown in Tables 2 and 3. Land preparation was done in October. The land area was 80 square meters in which three blocks were designed. Eight plots were prepared with in each block and each plot was planted with 4 lines. Furrow sowing operations were carried out on October 29. The plants were thinned in 6-4 leaf stage. First irrigation after planting, and subsequent ones were done every 7 days until the end of the growing season. No herbicides were applied in this plan and weeding was done by hand. Fertilizer treatments foliar was applied at the 8-10 leaf stage and continued once every two weeks, three times until after flowering. The sprayings, including all the three times, were done in sunset to prevent the leaves from sunburn. Foliar application (*or* spraying) continued until the plants were well treated with the solution. When the harvest time arrived, the plants yellowed and capsules dried, having removed the side effects of such characteristics as number of capsules per plant, number of seeds per capsule, seed number per plant, seed weight, 1000-seed weights and seed yield, the biological yield was measured. The factorial experiment was conducted in a completely randomized block design with three replications.

for the variance analysis of test data and drawing graphs, Excel and *Minitab-16* software was used. All the averages were compared at the 5% level, according to LSD test.

## Results and Discussion

### Number of Capsules per Plant

The results obtained from variance analysis indicated that the effect of nanopharmax fertilizer treatments on the number of capsules per plant was significant at 1% level. Also, humic acid was significant at 5% level. The impact of the interaction of these two on the number of capsules per plant was not significant (Table 4). The results from average comparison (Table 5) showed that there were significant differences between nanopharmax fertilizer treatments and the control. Levels of 3 and 6 ml/l of humic acid showed a significant difference compared to the control, but the difference between the two levels was not significant. Also, there was no significant difference between level of 1 ml/l and the control. It sounds like that the decrease in the number of capsules in the 1ml/l humic acid treatment was because of the vegetative and generative growth of plant in this treatment. Aranson *et al* [21] reported the increased number of strawberry fruit due to humic acid application. Akbarnejad *et al* [22] reported an increase in the number of capsules per *N. sativa* plant due to the combined use of organic fertilizers, municipal compost and sewage sludge. They also claimed that the combined use of these treatments due to the more nutrients in the soil has increased the number of capsules. Keshavarz *et al* [23] stated that the nanoiron chelate fertilizer increased the number of spikes in wheat. Due to the complete fertilizer uptake and release of nutrients with the optimum speed throughout the growing season, nanofertilizers will increase the crop [23].

### Number of Seeds per Capsule

Nanopharmax and humic acid fertilizer treatments are significant at 1% level in the number of seeds per capsule. The interaction of the two over the number of seeds per capsule was not significant (Table 4).

**Table 1** Physicochemical properties of soil experiment

Soil texture	K (mg/kg)	P (mg/kg)	Total N (%)	Electrical conductivity (ds/m)	Soil pH
Clay loam	334	19.7	0.3	8.35	7.9

**Table 2** properties of humic acid used in this experiment

Commercial name	Cu (ppm)	MgO (ppm)	Organic material (%)	Humic acid & folic acid (%)	Amino acid (%)	K <sub>2</sub> O (%)	P <sub>2</sub> O <sub>3</sub> (%)	Ca (%)	Fe (ppm)	N (%)	B (ppm)	Zn (ppm)	vitamin (%)
Super humic	23	12	92	37	6	12	0.05	3	2500	2	10	410	0.7

**Table 3** Properties of nanopharmax fertilizer used in this experiment

compound concentration (%w/w)	CaO	F	SiO <sub>2</sub>	MgO	SO <sub>3</sub>	ZnO	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>	Na <sub>2</sub> O	MoO <sub>3</sub>	K <sub>2</sub> O	CuO	NiO	CoO	SrO	Total
	33.74	27.50	9.63	0.83	0.802	0.333	0.288	0.190	0.160	0.075	0.069	0.051	0.046	0.039	0.036	100

The results from average comparison (Table 5) suggest that there was a significant difference between nanopharmax fertilizer and the control sample. Concentration of 6 ml/l humic acid had the highest number of seeds per capsule showed a significant difference with the control sample. But this difference was not significant for the concentration of 3 ml/l. Furthermore, there were no significant differences between the concentrations of 1 and 3 ml/l. At low concentrations of humic acid, the number of seeds per plant decreased. This happened as a result of lack of proper plant growth in the treatment and then decreases in the number of capsules. The highest seed number was related to the treatments of nanofertilizer and humic acid with concentration level of 6 due to the availability of nutrients, especially nitrogen, at flowering and seed filling times [24]. Nazaran *et al* [24] reported an increase in the number of seeds per wheat spike under the impact of nanoiron chelated organic fertilizer on wheat. It can be concluded that increase in the number of seeds per capsule as affected by fertilizer treatments is resulted from improving plant growth conditions and increasing the nutrients. Ghorbani *et al* [25] reported that application of humic acid increased the number of seeds at corn row.

#### Number of Seed per Plant

The results obtained from variance analysis (Table 4) showed that the effect of nanopharmax fertilizer on the seed number per plant is significant at 1% level. The effects of humic acid and interaction of

humic acid and nanofertilizer on the seed number per plant were not significant. Results of average comparison (Table 5) revealed that there was a significant difference between nanofertilizer treatment and control sample. There were no significant differences between different humic acid concentrations and the control. Baghaei *et al* [26] reported that nanoiron chelated fertilizer increases the grain number per rice panicle. Nanopharmax fertilizer promotes the vegetative growth and increase the number of sub-branches per plant. As a result, the number of capsules increases, and thereby makes an increase in the number of seeds per plant [26]. Humic acid treatments had no significant effect on the seed number per plant. This is because the plant doesn't grow properly in this fertilizer treatment and consequently, both number of capsules and number of seeds decrease.

#### Seed Weight

The effect of nanopharmax fertilizer and humic acid treatments and their interaction the seed weight was significant at 1% level (Table 4). The results from average comparison (Table 5) indicated that there was a significant difference between nanofertilizer treatment and control sample. Also, there was a significant difference between the various humic acid concentrations and control sample. 3 and 6 ml/l concentrations of humic acid had higher weights than control sample and 1 ml/l concentration. By the way, there was no significant difference between 3 and 6 ml/l concentrations. Combined treatments indicated a significant difference compared to the control sample, but there was no significant difference between them

(Fig. 2). Sanchez *et al* [27] reported that humic compounds caused an increase in the grape berry weight. They believed that humic substances improve nutrient absorption through stimulating the ions and physiological metabolism in the plant and this has led to an increase in berry weight. Combined use of compost fertilizer, municipal solid waste and sewage sludge increased the seed weight in *N. sativa*.

They stated that the availability of nutrients for the plant increases production of assimilates for seeds, thereby increasing their weight [28].

#### 1000-Seed Weight

The effect of nanopharmax fertilizer treatments on the 1000-seed weight was significant at 1% level. The effect of humic acid and their interaction on the 1000-seed weight was not significant. Results of average comparison of this characteristic indicated that all treatments were placed in one group (Table

5). Shaban *et al* [29] reported that 1000-seed weight of wheat was not affected by application of humic acid. In the experiment by Prasad [30], foliar zinc oxide nanoparticles increased 1000-seed weight in peanut. The high efficiency of uptake and surface area of effective nanoparticles can better account for these particles. 1000-seed weight is one of the factors that is more influenced by genetic control and has high heritability and is less controlled by environmental factors [30].

#### Seed Yield

The effect of nanopharmax fertilizer and humic acid treatments on the seed yield was significant at 1% level (Table 4), but the interaction effect of nanopharmax fertilizer and humic acid on the seed yield was not significant. The results from average comparison (Table 5) indicated that there was a significant difference between nanofertilizer treatment and control sample.

**Table 4** Analysis of variance (mean squares) components of *Nigella sativa* L.

Source of variation	df	Mean squares							
		capsule per plant	Seeds per capsule	Seeds per plant	Seeds weight per plant	1000 seed weight	Seed yield	Biological yield	Harvest index
Block	2	19.04**	4.54 <sup>ns</sup>	6239**	0.0009 <sup>ns</sup>	0.01 <sup>ns</sup>	323.3 <sup>ns</sup>	601**	0.05**
Nano fertilizer	1	20.16**	468.1**	2046**	1.88**	0.09**	18537**	126440**	1.54**
Humic acid	3	4.11*	106.7**	1142 <sup>ns</sup>	0.41**	0.01 <sup>ns</sup>	3570.04**	12372**	9.09**
Nano fertilizer*humic acid	3	0.72 <sup>ns</sup>	16.72 <sup>ns</sup>	1335 <sup>ns</sup>	0.27**	0.01 <sup>ns</sup>	359.5 <sup>ns</sup>	3320**	8.92**
Error	14	0.756	9.11	4115	0.0111	0.0037	259.5	167	1.17

\*Is significant at the 5% level. \*\*Is significant at the 1% level. <sup>ns</sup>not significant.

**Table 5** Comparison of average yield and yield components under different levels of nanopharmax fertilizer and humic acid of *Nigella sativa* L.

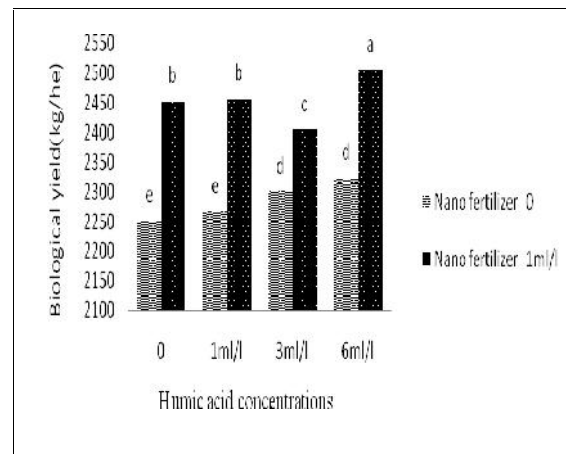
Treatments	Capsule per plant	Seeds per Capsule	Seeds Per plant	Seeds weight per plant (g)	1000 seed weight (g)	Seed yield (Kg/he)	Biological yield (Kg/he)	Harvest index (%)
Nano fertilizer								
control	8.4 b	68.4 b	592.8 b	1.6 b	3.1 b	841 b	2285.16 b	36.68 b
1 ml/l	9.8 a	75.3 a	757.4 a	2 a	3.2 a	908.41 a	2454.58 a	37.18 a
Humic acid								
control	8.3 b	68.8 c	629.5 a	1.55 b	3.1 a	846.3 b	2351.5 b	37.49 b
1ml/l	9 ab	71 c	646.7 a	1.65 b	3.1 a	854.7 b	2361.66 b	360.01 b
3ml/l	9.8 b	73.3 b	705.3 a	2 a	3/1 a	876.3 b	2352.5 b	36.72 b
6ml/l	11.2 a	75.3 a	708.8 a	2 a	3/1 a	900.8 a	2413.83 a	37.49 a

Similar letters in each column indicate no significant difference at  $P < 0.05$

6 ml/l concentration of humic acid had higher seed yield than control sample and 1 and 3 ml/l concentrations. Also, there was no significant difference between 1 and 3 ml/l concentrations and the control. According to Zhang *et al* [31], there was an increase in the growth of spinach when affected by nanoTiO<sub>2</sub> fertilizer. The results from the experiment conducted by El-Ghamry *et al* [32] indicated that humic acid can increase the seed yield in faba bean. The increased seed yield affected by humic acid and nanopharmax fertilizer can be attributed to the better vegetative growth, canopy development and consequently, more appropriate use of solar radiation and high photosynthesis [32].

#### Biological Yield

The effect of nanopharmax fertilizer and humic acid treatments and their interaction on the biological yield was significant at 1% level (Table 4). The results from average comparison (Table 5) indicated that there was a significant difference between nanofertilizer treatment and control sample. Also, there was a significant difference between the various humic acid concentrations and control sample. 3 and 6 ml/l concentrations of humic acid had higher biological yield than control sample and 1 ml/l concentration. By the way, there was no significant difference between 3 and 6 ml/l concentrations. The highest biological yield in different concentrations of humic acid was observed in combination with nanofertilizer surface in respect of simple effects (Figure 1). Results of the experiment done by Razazi *et al* [33] showed that nanoiron fertilizer caused an increased yield (of stigma) in the saffron. Ayas and Gulser [34] claimed that humic acid may increase the biological yield of spinach. They said that humic acid can make an increase in growth, height, and consequently biological yield of the plant through the increased nitrogen content. The results of the studies conducted by the researchers showed that increases in yield combined with applying organic fertilizers is resulted from increase in the available nitrogen, phosphorus and potassium contents in the plant, and increase in photosynthesis and growth of the plant [35]. Increased biological yield affected by humic acid and nanoPharmax fertilizer can be attributed to stimulated vegetative growth, increased straw yield and increased seed yield.



**Fig. 1** Interaction between nanopharmax fertilizer and humic acid on biological yield

#### Harvest Index (HI)

The effect of nanopharmax fertilizer and humic acid treatments and their interaction on HI was significant at 1% level (Table 4). The results from average comparison (Table 5) indicated that there was a significant difference between nanofertilizer treatment and control sample. 6 ml/l concentrations of humic acid had higher biological yield than control sample and 1 ml/l concentration. By the way, there was no significant difference between 1 and 3 ml/l concentrations and the control sample. Results of the experiment conducted by Mousa-Zadeh *et al* [36] showed that harvest index in *N. sativa*, as affected by organic fertilizers, increased. It appears that increased HI in the treatments of nanofertilizer and 3 ml/l concentration of humic acid has been due to the increased vegetative growth, thereby more assimilates being allocated to seed and a high HI was achieved. The reason for low HI in control treatment and 3 and 6 ml/l concentrations of humic acid is a result of low seed yield.

#### Conclusion

The results showed that the use of nanopharmax fertilizer and humic acid can have positive effects on yield components and yield of *N. sativa*. These effects could be a result of their physiological effects. Application of nanopharmax fertilizer and humic acid concentrations of 6 and 3 ml/l showed higher yield than other treatments. Intensifying the vegetative growth, these treatments increased the yield in *N. sativa* species. The combined treatments significantly increased the seed weight, dry substance, seed yield, biological yield and harvest

index. Therefore on the basis of the results of current study, it sounds like that nanopharmax fertilizer and humic acid can reduce the use of chemical fertilizers and environmental pollution. They also play an important role in achieving the goals of sustainable agriculture.

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## Original Article

# The Effect of Foliar Application of Humic Acid and Nanofertilizer (Pharmks<sup>®</sup>) on Yield and Yield Components of Black Cumin (*Nigella sativa* L.)

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## Abstract

In a sustainable agriculture system, application of the fertilizers which are nature friendly and suitable for plants is essential. This becomes more important when dealing with medicinal plants. Therefore, a factorial experiment in a randomized complete block design with three replications was set up in the 2012-2013 on *Nigella sativa* L. Treatments were nanofertilizer (Pharmks<sup>®</sup>) (at 2 levels 0, 1 ml/l) and humic acid (at 4 levels zero, 1, 3, 6 ml/l) which were applied at three time of plant growth. Results showed that different levels of humic acid imposed a significant effect on number of capsule per plant, number of seeds per capsule, seed weight, seed yield, biological yield and harvest index. But there were no significant effects on number of seeds per plant and weight of 1000 seed. The highest yield to levels 3, 6 ml/l humic acid treatments respectively. Nanofertilizer (Pharmks<sup>®</sup>) application significantly increased the yield and yield components of *N. sativa*. Combined treatment at various levels had significant effect on seed weight, seed yield, biological yield and harvest index respectively but had no significant effect on other traits. The lowest yield was detected at 1 ml/l humic acid and control treatments respectively. Generally, it seems that application of nanofertilizer (Pharmks<sup>®</sup>) and humic acid due to having nutritional ingredients and different physiological effects improves *N. sativa* performance and reduces environmental pollution and it could be used as a natural material to increase and stabilize field crop production.

**Key words:** Humic acid, *Nigella sativa*, Yield and yield components, Nano fertilizer (Pharmks<sup>®</sup>)

## Introduction

The ever-increasing tendency to the use of medicinal plants in the world has grown concerns about their cultivation and production processes. As medicinal plants are more compatible with the nature, special interest and attention has recently been given to herb therapy, and use of medicinal plants, being limited by the rise of pharmaceutical drugs, has become again common and widespread due to a number of reasons [1]. *Nigella sativa* L. is one of the herbs that has a variety of uses and has been being used in Iran's traditional medicine since old times. Today this

plant is considered as one of the most important kinds of medicine. Therefore, it is of great importance to conduct some researches on the herbs around the country due to different ecological requirements. *N. sativa* belonging to buttercup family, with the scientific name of Ranunculaceae, is an annual, dicotyledonous, herbaceous plant. This plant is widespread with in North Africa, southern Europe, the Mediterranean regions to India and Southeast and West Asia, and Australia, but researchers believe its origin as being in the Middle East and West Asia [2,3]. In several studies, *N. sativa* has been reported to have anti-oxidative, anti-inflammatory, strengthening of immune

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system, and anti-histamine and oil extract properties. Furthermore, several effects such as lowering blood sugar, lipids, and hypertension, excretion of bile and uric acid, protection of liver, kidney and cardiovascular tissues as well as anti-seizure, anti-cancer, anti-microbial and anti-parasitic effects related to this plant have been reported. Thus, considering the importance of *N. sativa* in pharmaceuticals, food and cosmetics industries, it is strongly needed to conduct comprehensive studies on the cultivation and development of the plant [4,5]. Organic agriculture is an integrated farming system, based on ecological principles, in which product quality is more important than its quantity. Organic agriculture system can be considered as an alternative to conventional systems and lead to the development of sustainable agriculture and environmental health [6]. Tendency to production of medicinal plants and demand for natural products, especially in organic farming circumstances, is increasing in the world. Organic cultivation of medicinal plants has negative effects on their quality and yield [7]. Environmental factors have an obvious impact on the growth, yield and quality of herbal in gradients. One of the environmental factors affecting the quantity and quality of medicinal plants is feeding [8]. With proper nutrition of plants, we can increase efficiency of inputs, while preserving environment, improving water quality, reducing erosion and conserving biodiversity. Furthermore, avoiding the unnecessary use and over-consuming of nutrients, we'd be able to reduce production costs to a minimum, and this could be taken into account as a way to sustainable agriculture. Fertilizer management is a key factor in the successful cultivation of medicinal plants [9]. Application of chemical fertilizers in agriculture causes environmental problems such as soil physical degradation and soil nutrients imbalance. Thus, application of various organic fertilizers is increasing [10]. The results from studies have shown that the organic fertilizer improves the physical, chemical and biological soil properties and increases crop yield. Humic acid, with a molecular weight of 30000-3000000 Dalton, and folic acid, with a molecular weight of less than 30,000 Dalton, can form stable and insoluble complexes and soluble complexes with micronutrients [11]. The organic compound is composed of humic acid, folic acid, and is humin. These compounds have been derived from a variety

of sources such as peat, compost, worm compost and municipal solid waste. Humic acid is good to enhance the biological activity of the soil, and minerals are absorbed better and faster this way. It enhances the activity of soil microorganisms, increases plant resistance against diseases and strengthens the root system [12]. Nanotechnology is one of the new technologies that are at the early stage of its development. The main difference between nanotechnology with other technologies is related to the materials' scale and structures that are specified for this technology. Nanotechnology products contain a mixture of particles with dimensions between 1 nm to 100 nm that could change their physical and chemical properties of their raw materials [13]. All knowledge areas are affected by nanotechnology and this is typical of agricultural science. One of the most important applications of nanotechnology in various fields and orientations in agricultural and soil and water is to use nanofertilizers for plant nutrition [14]. Nanofertilizers provide the field plants with the nutrients in three ways: 1) nutrients are coated by nanoporous materials; 2) are covered by a thin layer of polymer; 3) are released in the form of a nanoscale particle or emulsion [15]. The use of nanofertilizers increases the efficiency of nutrients use, reduces soil toxicity, minimizes the negative effects from over-consuming of fertilizers and decreases the frequency of fertilizer applications. Using nanofertilizer, the time and speed of nutrients dispersion is coordinated by food requirements of the plant and thus, the plant will be able to absorb the maximum amount of nutrients and therefore, while reducing the leaching of nutrients, the crop yield increases [16]. There are few reports about the impact of nanofertilizers on increasing the quantity and quality of plant growth. Pandey *et al* [17] reported the increased growth rate of *Cicer arietinum* L. where ZnO nanoparticles are applied. Shaalan [18] reported the increased yield and yield components of *N. sativa* in circumstances where biological fertilizers are applied. Cacilia *et al* [19] applied levels of (0, 100, 200 and 300) milligrams per liter of humic acid on *Thymus vulgaris* L. Results showed that humic acid in the plant decreased height and oil yield, but significantly increased its fresh and dry weight and antioxidant activity. Khazaei *et al* [20] investigated the effect of concentrations of 0, 100, 200 and 300 milligrams per liter of humic acid on shoot growth, yield and essential oil in the medicinal plant of *Hyssopus officinalis* L. This compound significantly increased

the essential oil, shoot, leaf number and plant biomass.

Doing studies over the effect of nanopharmax and humic acid fertilizer son the plant, no research findings were obtained. So, in order to use less chemical fertilizers to prevent environmental pollution and encourage farmers to use more organic fertilizer, the present study was carried out to evaluate the effect of foliar application of humic acid and nanopharmax fertilizer on the yield and yield components of *N. sativa* species.

## Material and Methods

This test was conducted in 2012-2013. Before testing, a farm was selected to determine the chemical properties of soil and then, soil sampling was conducted. Physicochemical properties of the soil of the test site have been shown in Table 1. The test treatments would include humic acid and nanopharmax fertilizer with the levels of 0, 1, 3 and 6 ml per liter and 0 and 1 ml per liter, respectively. Humic acid and nanopharmax fertilizer characteristics are shown in Tables 2 and 3. Land preparation was done in October. The land area was 80 square meters in which three blocks were designed. Eight plots were prepared with in each block and each plot was planted with 4 lines. Furrow sowing operations were carried out on October 29. The plants were thinned in 6-4 leaf stage. First irrigation after planting, and subsequent ones were done every 7 days until the end of the growing season. No herbicides were applied in this plan and weeding was done by hand. Fertilizer treatments foliar was applied at the 8-10 leaf stage and continued once every two weeks, three times until after flowering. The sprayings, including all the three times, were done in sunset to prevent the leaves from sunburn. Foliar application (*or* spraying) continued until the plants were well treated with the solution. When the harvest time arrived, the plants yellowed and capsules dried, having removed the side effects of such characteristics as number of capsules per plant, number of seeds per capsule, seed number per plant, seed weight, 1000-seed weights and seed yield, the biological yield was measured. The factorial experiment was conducted in a completely randomized block design with three replications.

for the variance analysis of test data and drawing graphs, Excel and *Minitab-16* software was used. All the averages were compared at the 5% level, according to LSD test.

## Results and Discussion

### Number of Capsules per Plant

The results obtained from variance analysis indicated that the effect of nanopharmax fertilizer treatments on the number of capsules per plant was significant at 1% level. Also, humic acid was significant at 5% level. The impact of the interaction of these two on the number of capsules per plant was not significant (Table 4). The results from average comparison (Table 5) showed that there were significant differences between nanopharmax fertilizer treatments and the control. Levels of 3 and 6 ml/l of humic acid showed a significant difference compared to the control, but the difference between the two levels was not significant. Also, there was no significant difference between level of 1 ml/l and the control. It sounds like that the decrease in the number of capsules in the 1ml/l humic acid treatment was because of the vegetative and generative growth of plant in this treatment. Aranson *et al* [21] reported the increased number of strawberry fruit due to humic acid application. Akbarnejad *et al* [22] reported an increase in the number of capsules per *N. sativa* plant due to the combined use of organic fertilizers, municipal compost and sewage sludge. They also claimed that the combined use of these treatments due to the more nutrients in the soil has increased the number of capsules. Keshavarz *et al* [23] stated that the nanoiron chelate fertilizer increased the number of spikes in wheat. Due to the complete fertilizer uptake and release of nutrients with the optimum speed throughout the growing season, nanofertilizers will increase the crop [23].

### Number of Seeds per Capsule

Nanopharmax and humic acid fertilizer treatments are significant at 1% level in the number of seeds per capsule. The interaction of the two over the number of seeds per capsule was not significant (Table 4).

**Table 1** Physicochemical properties of soil experiment

Soil texture	K (mg/kg)	P (mg/kg)	Total N (%)	Electrical conductivity (ds/m)	Soil pH
Clay loam	334	19.7	0.3	8.35	7.9

**Table 2** properties of humic acid used in this experiment

Commercial name	Cu (ppm)	MgO (ppm)	Organic material (%)	Humic acid & folic acid (%)	Amino acid (%)	K <sub>2</sub> O (%)	P <sub>2</sub> O <sub>3</sub> (%)	Ca (%)	Fe (ppm)	N (%)	B (ppm)	Zn (ppm)	vitamin (%)
Super humic	23	12	92	37	6	12	0.05	3	2500	2	10	410	0.7

**Table 3** Properties of nanopharmax fertilizer used in this experiment

compound concentration (%w/w)	CaO	F	SiO <sub>2</sub>	MgO	SO <sub>3</sub>	ZnO	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>	Na <sub>2</sub> O	MoO <sub>3</sub>	K <sub>2</sub> O	CuO	NiO	CoO	SrO	Total
	33.74	27.50	9.63	0.83	0.802	0.333	0.288	0.190	0.160	0.075	0.069	0.051	0.046	0.039	0.036	100

The results from average comparison (Table 5) suggest that there was a significant difference between nanopharmax fertilizer and the control sample. Concentration of 6 ml/l humic acid had the highest number of seeds per capsule showed a significant difference with the control sample. But this difference was not significant for the concentration of 3 ml/l. Furthermore, there were no significant differences between the concentrations of 1 and 3 ml/l. At low concentrations of humic acid, the number of seeds per plant decreased. This happened as a result of lack of proper plant growth in the treatment and then decreases in the number of capsules. The highest seed number was related to the treatments of nanofertilizer and humic acid with concentration level of 6 due to the availability of nutrients, especially nitrogen, at flowering and seed filling times [24]. Nazaran *et al* [24] reported an increase in the number of seeds per wheat spike under the impact of nanoiron chelated organic fertilizer on wheat. It can be concluded that increase in the number of seeds per capsule as affected by fertilizer treatments is resulted from improving plant growth conditions and increasing the nutrients. Ghorbani *et al* [25] reported that application of humic acid increased the number of seeds at corn row.

#### Number of Seed per Plant

The results obtained from variance analysis (Table 4) showed that the effect of nanopharmax fertilizer on the seed number per plant is significant at 1% level. The effects of humic acid and interaction of

humic acid and nanofertilizer on the seed number per plant were not significant. Results of average comparison (Table 5) revealed that there was a significant difference between nanofertilizer treatment and control sample. There were no significant differences between different humic acid concentrations and the control. Baghaei *et al* [26] reported that nanoiron chelated fertilizer increases the grain number per rice panicle. Nanopharmax fertilizer promotes the vegetative growth and increase the number of sub-branches per plant. As a result, the number of capsules increases, and thereby makes an increase in the number of seeds per plant [26]. Humic acid treatments had no significant effect on the seed number per plant. This is because the plant doesn't grow properly in this fertilizer treatment and consequently, both number of capsules and number of seeds decrease.

#### Seed Weight

The effect of nanopharmax fertilizer and humic acid treatments and their interaction the seed weight was significant at 1% level (Table 4). The results from average comparison (Table 5) indicated that there was a significant difference between nanofertilizer treatment and control sample. Also, there was a significant difference between the various humic acid concentrations and control sample. 3 and 6 ml/l concentrations of humic acid had higher weights than control sample and 1 ml/l concentration. By the way, there was no significant difference between 3 and 6 ml/l concentrations. Combined treatments indicated a significant difference compared to the control sample, but there was no significant difference between them

(Fig. 2). Sanchez *et al* [27] reported that humic compounds caused an increase in the grape berry weight. They believed that humic substances improve nutrient absorption through stimulating the ions and physiological metabolism in the plant and this has led to an increase in berry weight. Combined use of compost fertilizer, municipal solid waste and sewage sludge increased the seed weight in *N. sativa*.

They stated that the availability of nutrients for the plant increases production of assimilates for seeds, thereby increasing their weight [28].

#### 1000-Seed Weight

The effect of nanopharmax fertilizer treatments on the 1000-seed weight was significant at 1% level. The effect of humic acid and their interaction on the 1000-seed weight was not significant. Results of average comparison of this characteristic indicated that all treatments were placed in one group (Table

5). Shaban *et al* [29] reported that 1000-seed weight of wheat was not affected by application of humic acid. In the experiment by Prasad [30], foliar zinc oxide nanoparticles increased 1000-seed weight in peanut. The high efficiency of uptake and surface area of effective nanoparticles can better account for these particles. 1000-seed weight is one of the factors that is more influenced by genetic control and has high heritability and is less controlled by environmental factors [30].

#### Seed Yield

The effect of nanopharmax fertilizer and humic acid treatments on the seed yield was significant at 1% level (Table 4), but the interaction effect of nanopharmax fertilizer and humic acid on the seed yield was not significant. The results from average comparison (Table 5) indicated that there was a significant difference between nanofertilizer treatment and control sample.

**Table 4** Analysis of variance (mean squares) components of *Nigella sativa* L.

Source of variation	df	Mean squares							
		capsule per plant	Seeds per capsule	Seeds per plant	Seeds weight per plant	1000 seed weight	Seed yield	Biological yield	Harvest index
Block	2	19.04**	4.54 <sup>ns</sup>	6239**	0.0009 <sup>ns</sup>	0.01 <sup>ns</sup>	323.3 <sup>ns</sup>	601**	0.05**
Nano fertilizer	1	20.16**	468.1**	2046**	1.88**	0.09**	18537**	126440**	1.54**
Humic acid	3	4.11*	106.7**	1142 <sup>ns</sup>	0.41**	0.01 <sup>ns</sup>	3570.04**	12372**	9.09**
Nano fertilizer*humic acid	3	0.72 <sup>ns</sup>	16.72 <sup>ns</sup>	1335 <sup>ns</sup>	0.27**	0.01 <sup>ns</sup>	359.5 <sup>ns</sup>	3320**	8.92**
Error	14	0.756	9.11	4115	0.0111	0.0037	259.5	167	1.17

\*Is significant at the 5% level. \*\*Is significant at the 1% level. <sup>ns</sup>not significant.

**Table 5** Comparison of average yield and yield components under different levels of nanopharmax fertilizer and humic acid of *Nigella sativa* L.

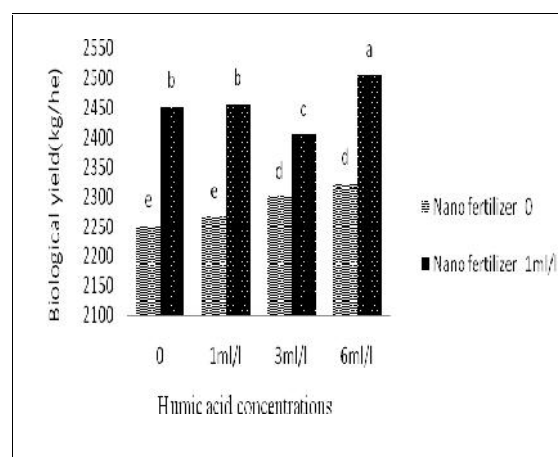
Treatments	Capsule per plant	Seeds per Capsule	Seeds Per plant	Seeds weight per plant (g)	1000 seed weight (g)	Seed yield (Kg/he)	Biological yield (Kg/he)	Harvest index (%)
Nano fertilizer								
control	8.4 b	68.4 b	592.8 b	1.6 b	3.1 b	841 b	2285.16 b	36.68 b
1 ml/l	9.8 a	75.3 a	757.4 a	2 a	3.2 a	908.41 a	2454.58 a	37.18 a
Humic acid								
control	8.3 b	68.8 c	629.5 a	1.55 b	3.1 a	846.3 b	2351.5 b	37.49 b
1ml/l	9 ab	71 c	646.7 a	1.65 b	3.1 a	854.7 b	2361.66 b	360.01 b
3ml/l	9.8 b	73.3 b	705.3 a	2 a	3/1 a	876.3 b	2352.5 b	36.72 b
6ml/l	11.2 a	75.3 a	708.8 a	2 a	3/1 a	900.8 a	2413.83 a	37.49 a

Similar letters in each column indicate no significant difference at  $P < 0.05$

6 ml/l concentration of humic acid had higher seed yield than control sample and 1 and 3 ml/l concentrations. Also, there was no significant difference between 1 and 3 ml/l concentrations and the control. According to Zhang *et al* [31], there was an increase in the growth of spinach when affected by nanoTiO<sub>2</sub> fertilizer. The results from the experiment conducted by El-Ghamry *et al* [32] indicated that humic acid can increase the seed yield in faba bean. The increased seed yield affected by humic acid and nanopharmax fertilizer can be attributed to the better vegetative growth, canopy development and consequently, more appropriate use of solar radiation and high photosynthesis [32].

### Biological Yield

The effect of nanopharmax fertilizer and humic acid treatments and their interaction on the biological yield was significant at 1% level (Table 4). The results from average comparison (Table 5) indicated that there was a significant difference between nanofertilizer treatment and control sample. Also, there was a significant difference between the various humic acid concentrations and control sample. 3 and 6 ml/l concentrations of humic acid had higher biological yield than control sample and 1 ml/l concentration. By the way, there was no significant difference between 3 and 6 ml/l concentrations. The highest biological yield in different concentrations of humic acid was observed in combination with nanofertilizer surface in respect of simple effects (Figure 1). Results of the experiment done by Razazi *et al* [33] showed that nanoiron fertilizer caused an increased yield (of stigma) in the saffron. Ayas and Gulser [34] claimed that humic acid may increase the biological yield of spinach. They said that humic acid can make an increase in growth, height, and consequently biological yield of the plant through the increased nitrogen content. The results of the studies conducted by the researchers showed that increases in yield combined with applying organic fertilizers is resulted from increase in the available nitrogen, phosphorus and potassium contents in the plant, and increase in photosynthesis and growth of the plant [35]. Increased biological yield affected by humic acid and nanoPharmax fertilizer can be attributed to stimulated vegetative growth, increased straw yield and increased seed yield.



**Fig. 1** Interaction between nanopharmax fertilizer and humic acid on biological yield

### Harvest Index (HI)

The effect of nanopharmax fertilizer and humic acid treatments and their interaction on HI was significant at 1% level (Table 4). The results from average comparison (Table 5) indicated that there was a significant difference between nanofertilizer treatment and control sample. 6 ml/l concentrations of humic acid had higher biological yield than control sample and 1 ml/l concentration. By the way, there was no significant difference between 1 and 3 ml/l concentrations and the control sample. Results of the experiment conducted by Mousa-Zadeh *et al* [36] showed that harvest index in *N. sativa*, as affected by organic fertilizers, increased. It appears that increased HI in the treatments of nanofertilizer and 3 ml/l concentration of humic acid has been due to the increased vegetative growth, thereby more assimilates being allocated to seed and a high HI was achieved. The reason for low HI in control treatment and 3 and 6 ml/l concentrations of humic acid is a result of low seed yield.

### Conclusion

The results showed that the use of nanopharmax fertilizer and humic acid can have positive effects on yield components and yield of *N. sativa*. These effects could be a result of their physiological effects. Application of nanopharmax fertilizer and humic acid concentrations of 6 and 3 ml/l showed higher yield than other treatments. Intensifying the vegetative growth, these treatments increased the yield in *N. sativa* species. The combined treatments significantly increased the seed weight, dry substance, seed yield, biological yield and harvest

index. Therefore on the basis of the results of current study, it sounds like that nanopharmax fertilizer and humic acid can reduce the use of chemical fertilizers and environmental pollution. They also play an important role in achieving the goals of sustainable agriculture.

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