



## Effect of salicylic acid, nano-iron chelate and pseudomonas on quality and quantity of rapeseed yield

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### Abstract

A factorial experiment has been conducted in randomized complete block design with 3 replications to analyze effect of salicylic acid, nano-iron chelate and pseudomonas bacterium on quality and quantity of rapeseed yield in Dare-Shahr County (Ilam Province) in 2013. Experiment's factors included 3 levels of salicylic acid (0, 0.5, 1mM), as the first factor, and foliar spraying of constant concentration of nano-iron chelate (3kg of ferrous sulfate per hectare) and non-application and pseudomonas fluorescence (non-insemination and insemination). The highest content of rapeseed oil produced by using 1mM of salicylic acid was 45.1% which showed a 24% increase in comparison to non-spray treatment. Concerning pseudomonas bacterium effect, highest content of oil produced by inseminating was 44.8%. General findings of the experiment revealed positive effect of salicylic acid on rapeseed yield and yield components and dry matter (DM), so that 1mM was the best concentration of salicylic acid. Also findings suggested positive effect of nano-iron chelate either when it used alone or in a combination with salicylic acid and pseudomonas fluorescence bacterium.

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## Introduction

Rapeseed (*Brassica napus* L.) is one of the most important oilseeds. Rapeseed, after soya and palm oil, is the third-largest source of vegetable oil in the world. Rapeseed nutrition is an important factor affecting seed yield, oil content and seed quality. Rapeseed oil is the only vegetable oil contains sulfurized fatty acid. Also it is high in unsaturated fatty acids (Mohammadi *et al.* 2011; O'Hara *et al.* 2009).

Salicylic acid is an endogenous growth regulator which belongs to natural phenolic compound group and plays an important role in regulating physiological processes of plants, namely; induction of flowering, plants growth, ethylene synthesis, informing on opening and closing of stoma and respiration. Salicylic acid also increases the tolerance to salt of wheat seedlings and Dicotyledonous like pea. Salicylic acid begins cell division and enlargement process in which other regulators like auxin takes part as well. There are reports on effect of salicylic acid on increasing crop yield such as soya, cowpea and pea (Majd, 2006). DolatAbadi *et al.* (2011), also stated that salicylic acid could plays a key role in increasing tolerance to environmental stresses.

Essential plant nutrient elements are divided into two groups of macronutrients and micro nutrients. First group includes: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulphur (S). Micronutrients are essential for plant growth, they include: boron (B), copper (Cu), iron (Fe) manganese (Mn), molybdenum (Mo) and zinc (Zn) (Hergert *et al.* 1996). Large parts of Iran's arid and semiarid regions contain calcareous soil and as the result are lacking in micronutrients, zinc and iron in particular (Malakouti, 2000). Iron is an essential element in oxidation-reduction reactions in which approximately 85% of cell iron is in connection with chloroplast. If iron is not sufficient, number of photosynthetic pigments and amount of chlorophyll will be decreased (Morales *et al.* 1995). Providing such nutrient elements in soil will lead to nutrient

balance in plant and increase of production and crop quality at the end (KhalidBarin&EslamZadeh, 2001). In farm and soils lacking in iron, it is not effective to apply iron but an increase in seed yield will be achieved through foliar spraying of iron (Marschner, 1995).

Further, the application of modern scientific methods is necessary to respond to demands of ever growing population in the world. To this end, agricultural management systems have to be reviewed and new systems which assign high priority to long-term sustainability accompanied by maintaining short-term production must be designed (Mirhashemi *et al.* 2009). Ecological agriculture is one of those new scientific methods in which quality of products is given more importance than quantity of them. Ecologic agricultural systems as well as low-input farming could be taken into account as suitable alternatives for current agricultural systems due to their effects on developing sustainable agriculture and preserving the health of the environment (Khoram Del *et al.* 2008).

The application of bio-fertilizer is significant to keep the biological balance of soil so as to maximize positive biological relationships of system. Bio fertilizers are solid, liquid or semi-solid materials which contain one or more useful microorganism or their metabolites used to provide nutrient elements required for plant, to preserve the health of plant or to improve physical and chemical properties of soil (TohidiMoghadam *et al.* 2007).

Therefore this research aimed at investigating effect of salicylic acid, nano-Iron chelate and pseudomonas on quality and quantity of rapeseed yield.

## Materials And methods

### Area study

This experiment carried out in a farm situated 10Km away from Dare-Shahr County, Ilam Province during 2012-13. Determining the properties of soil, samples were taken from 0-30cm depths of soil before

performing the experiment and its properties analyzed. Sowing date was October 22, 2010. And

Hyola-401 used in this experiment.

*Soil analysis*

**Table 1.** Physical and chemical properties of experimental filed soil.

Salinity (Ds/m)	pH	Percent of organic Carbon	Total percent of Nitrogen	Available Potassium (ppm)	Available Phosphor (ppm)	Text ure	Percent saturati on	Percent clay	Percent silt	Percent sand
1.3	7.1	1.01	20	212	4.6	Loam	44.5	17	35	48

*Treatments*

A factorial experiment has been conducted in randomized complete block design with 3 replications. Experiment’s factors included 3 levels of salicylic acid (0, 0.5, 1mM), as the first factor, and foliar spraying of constant concentration of nano-iron chelate (3kg of ferrous sulfate per hectare) and non-application and pseudomonas fluorescence (non-insemination and insemination). Salicylic acid and nano-iron chalet have been sprayed at flowering stage through dissolving of specific concentration in water. Each plot consisted of 6 rows, each of which was 40cm apart from next one and intra-row distance between seeds was 6cm. Size of each plot in experiment was 4(length) x3(width). Two side rows made the margin in each plot and 4 middle rows used as sample of the experiment. There was 3m between blocks (replication). And seeds have been planted ata depth of 2-3cm.

And finally,number of pods per plant, seeds per pod and 100-seed weight, seed yield, biological yield and seed oil has been estimated. First and last rows were eliminated from sampling and only rows in the middle of each plot were sampled.

Soxtecapparatus was applied to determinefat (oil) content in 1gr of seed. First the seeds were ground by electric mill, then 1gr of the homogenized seeds were inserted into cartouche through paper filter and its opening closed by cotton. And it was placed into a glass half-full of Hexan and then into the apparatus. Hexan was distilled after 1h and 45mins, seeds fatty oil dissolved in Hexan and deposited in glass. Next the glasses were taken out of the apparatus and put

into oven for 24hs. After this phase they were put into desiccator and their weights, after being constant, measured. Finally oil content was calculated by the following equation:

$$\text{(Equation 1): } fat = \frac{m_3 - m_2}{m_1} \times 100$$

M<sub>3</sub>: secondary weight of glass

M<sub>2</sub>: primary weight of glass

M<sub>1</sub>: weight of sample

Fat: oil content

*Statistical analysis*

Variance analysis conducted on the data according to split-plot design in randomized complete block and comparison of means has been made through Duncan’s multiple range test. SAS and EXCEL software have been used to this end.

**Results and discussion**

Results showed that salicylic acid had a significant effect on number of pods per plants, number of seeds per pod, 1000-seed weight, biological yield, seed yield and oil content of seed. Also nano-iron chelate had significant effect on 1000-seed weight, seed yield, biological yield and oil content. Pseudomonas bacterium had a significant effect on all the characteristics under study except number of pods in plant (table 2).

The highest number of pods per plant produced by using 1mM of salicylic acid was 125.8 which showed a 24% increase in comparison to non-spray treatment (table 3). And highest number of pods per plant produced by using nano-iron chelate was 134.5 which

showed a 16% increase in comparison to treatment of non-application (table 3).

The highest number of seeds per pod produced by using 1mM of salicylic acid was 19.3 which showed a 37% increase in comparison to non-spray treatment (table 3). And highest number of seeds per pod produced by using nano-iron chelate was 17.01 which showed a 14% increase in comparison to treatment of non-application (table 3). And the highest number of seeds per pod produced by inseminating pseudomonas bacterium was 18.6 which showed a 19% increase in comparison to non-insemination treatment (table 3).

The highest 1000-seed weight produced by using 1mM of salicylic acid was 4.29gr which showed a 32% increase in comparison to non-spray treatment (table 3). And highest 1000-seed weight produced by using nano-iron chelate was 3.83gr which showed a 25% increase in comparison to treatment of non-application (table 3). And the highest 1000-seed weight produced by inseminating bacterium was 3.83gr which showed a 26% increase in comparison to non-insemination treatment (table 3).

The highest seed yield produced by using 1mM of salicylic acid was 1973kg per hectare which showed a 31% increase in comparison to non-spray treatment (table 3). And highest seed yield produced by using nano-iron chelate was 1832kg per hectare which showed a 23% increase in comparison to treatment of non-application (table 3). And the highest seed yield produced by inseminating bacterium was 1817kg per hectare which showed a 26% increase in comparison to non-insemination treatment (table 3).

The highest biological yield produced by using 1mM of salicylic acid was 7518kg per hectare which showed a 36% increase in comparison to non-spray treatment (table 3). And highest biological yield produced by using nano-iron chelate was 6590kg per hectare which showed a 24% increase in comparison to treatment of non-application (table 3). And the highest biological

yield produced by inseminating bacterium was 6590kg per hectare which showed a 15% increase in comparison to non-insemination treatment (table 3).

The highest oil content produced by using 1mM of salicylic acid was 45.1% which showed a 24% increase in comparison to non-spray treatment (table 3). And highest oil content produced by using nano-iron chelate was 44.7% which showed a 17% increase in comparison to treatment of non-application (table 3). And the highest oil content produced by inseminating bacterium was 44.8% which showed a 16% increase in comparison to non-insemination treatment (table 3).

The number of pods per plant was decreased as the result of a decline in the number of pods per main and side shoots. The number of pods per plant is more sensitive to draught stress than other yield components. Bahl and Jean (1997) put that “seeds are the most important part of a plant to increase biological yield”. Sing and Yusha (2004), stated that “wheat seeds treated with salicylic acid solution (1-3mM) produces plants with higher moisture content and higher number of grains per spike in normal and stress conditions on par with untreated plants”. Also “wheat seeds treated with salicylic acid solution (5-10mM) produces plants with higher grains per spike and higher 1000-seed weight and number of pigments increased by increasing the concentration of salicylic acid” (Hayat *et al.* 2004). There are also reports on effect of salicylic acid on increasing crop yield in cowpea and pea. Most of environmental stresses will disturb the hormone balance in plants. Changes in hormone levels are the reason behind most of physiological and morphological effects caused by stresses. Ethylene is a natural plant hormone whose amount will be increased at the time of environmental stresses (Shakirova *et al.* 2003).

According to research, salicylic acid is likely to improve plant tolerance to environmental stresses by affecting ethylene biosynthesis (Ahmad *et al.* 1995). It seems that salicylic acid dealing with stress by increasing tolerance to stress through stimulating

enzymes activities which leads to yield components increase and consequently seed yield increase. Ahmad et.al, (1005), suggested that “pretreatment of seed with plant growth hormones such as salicylic acid not only increase tillering and emergence index but also affect the final yield in normal and stress conditions.

Salispour (2006), said that applying iron micronutrient led to an increase in number of grains per spike of wheat and pod seeds in rapeseed plant. Baghaeet.al, (2011), put that foliar spray of iron had significant effect on number of seeds in ear of rice. Low 100-seed weight in low-volume of foliar treatment is caused by seeds competition to achieve more nutrient and reduction of carbohydrate reserve which lead to a decrease in number of reproductive cells and low 100-seed weight consequently (Sing, 2000). Salispour (2006), indicated that applying iron in various areas increased wheat grain yield up to 317kg on par with control treatment. This researcher quoted other researchers findings that showed applying sequestrene in wheat farms increased wheat grain yield up to 20%. Faranjnia and KhorshidiBenam (2007), reported that applying micronutrients led to improve wheat tolerance to salinity. Soil application and foliar application are increased in average and high level of salinity respectively. Shariatmadari (2010), described that applying iron caused a significant increase in wheat grain yield. This increase was probably caused by the iron role in activating electron transport chains of both photosystem I and photosystem II. Shariatmadari (2010), stated that foliar spray of iron sulfate with salinity below 9 Ds/m had little effect on increasing sunflower seed yield but the highest seed yield was observed at the highest level of salinity (9 Dm/m). It revealed that higher level of iron sulfate salinity would have stronger effect on seed yield. Baghaeet.al, (2011), asserted that foliar spray of iron had a significant effect on dry rice and the DM increased during various period of spraying i.e. onset of tillering, ending of stem elongation and ending of heading and on par with non-spray treatment. The researchers explained that nano-iron chelate was

likely to increase DM through affecting yield components, especially number of spikes per square meter and number of seeds per spike, and spike length characteristic. Marschner (1995), expressed that in chloroplast lacking iron, absorption rate of photosynthesis carbon dioxide would be decrease due to reduction of photochemical capacity. Decrease of chlorophyll and damage of photosynthesis electron transport chain contributed to slowing growth and reducing sugar in plant (Bright *et al.* 1995).

Researchers reported in a study that wheat and rapeseed insemination with azotobacter in greenhouse condition would substitute up to 50% of nitrogen required by plant. Furthermore, during the recent years, wide application of azotobacter (a bio-fertilizer) has showed considerable effect on wheat yield, corn yield and other crops (Zabihi *et al.* 2009). According to Ahmad et. al, (2010): increasing sunflower seed yield under the effect of stabilized nitrogen and phosphorus fertilizer have been probably caused by increasing metabolic activities of bio-fertilizers (which increase the net photosynthesis rate) and producing growth promoter hormones by bacteria which have been led to higher yield at the end. It was also reported that growth promoter bacteria could increase plant height and yield through producing phytohormones, increasing accessibility to soil nutrients, facilitating nutrient absorption by reducing the absorption of nutrient of toxic heavy elements and improving tolerance to diseases. On the other hand, enzymatic activity of growth promoter bacteria plays an important role in microbial, physical and chemical properties of soil which leads to the increase in plant growth (Shokat *et al.* 2006). Also Falahiet.al, (2009), quoted from other researchers that “among bio-fertilizers studied in research, treating pseudomonas had a significant effect on rapeseed yield. Sharifi and Haghnia (2007), also reported that the highest seed yield in treatment of bio-fertilizer application showed a 15.5% increase on par with control treatment. Researchers’ work revealed that seed insemination with plant growth promoter bacteria caused a progressive increase in

catalyzing of enzyme activity and chlorophyll and carotene pigments before and after flowering in photosynthesis process as well as energy production and finally, plant growth improvement by using organic fertilizer on par with other treatments, and control treatment in particular (Marcus *et al.* 2005).

Zahir *et al.* (2000), assumed that production of Indole-3-acetic acid by different types of Azotobacter and production of auxin, gibberellic acid and isogibberellic acid by azospirillum lipoferum

bacterium led to the increase in rapeseed growth. Mirzakhani *et al.* (2008), found that safflower seed insemination with Free-living azotobacter and a Symbiotic fungus, producing mycorrhiza contributed to high seed and oil yield. Findings of ShoghiCalkhoran *et al.* (2010), revealed that; although sunflower oil yield, by bacterial insemination (azotobacter and azospirillum), was higher than non-insemination treatments, there was no significant difference in crop yield of the said treatments statistically.

**Table 2.** Analysis of variance of some recorded traits under the effect of research treatments.

Source variation	DF	No. of pods per plant	No. of seeds per pod	100-seed weight	Biological yield	Seed yield	Seed oil
Replication	2	2.77 ns	0.84 ns	0.042 ns	83354 ns	6597389 ns	2.170 ns
Salicylic acid	2	156.15*	46.73**	3.202**	442840**	18413481**	14.937**
Nano-iron chelate	1	133.40 ns	8.83 ns	0.412**	104324**	7295221**	28.903**
Pseudomonas Bacterium	1	59.29 ns	45.18**	0.424**	54161**	2433600**	38.574**
Salicylic acid & Nano-iron chelate	2	26.73 ns	1.44 ns	0.001 ns	342 ns	146645 ns	0.089 ns
Salicylic acid & Pseudomonas Bacterium	2	11.71 ns	1.49 ns	0.047 ns	15 ns	36756 ns	4.224 ns
Nano-iron chelate & Pseudomonas Bacterium	1	80.70 ns	1.11 ns	0.021 ns	120 ns	377037 ns	0.738 ns
Salicylic acid & Nano-iron chelate & Pseudomonas Bacterium	2	24.33 ns	1.60 ns	0.020 ns	106 ns	56108 ns	0.915 ns
Total error	22	32.63	2.62	0.026	1380	6597389	1.485
CV (%)	-	4.3	9.2	4.2	2.1	5	3.7

\*\* And\* and ns, respectively significant at the one percent and five percent level, and no significant difference

**Table 3.** Effect of salicylic acid, nano-iron chelate and Pseudomonas Bacterium on rapeseed yield , yield components and oil content.

		No. of pods per plant	No. of seeds per pod	100-seed weight (gr)	Seed yield (kg/ha)	Biological yield (kg/ha)	Seed oil (%)
Salicylic acid (mM)	0	128.7 c	15.41 c	3.29 c	1589 c	5046 c	42.9 b
	0.5	133.3 b	17.83 b	3.60 b	1773 b	6427 b	43.5 ab
	1	135.8 a	19.32 a	4.30 a	1974 a	7518 a	45.1 a
Nano-iron chelate	Non-Application	130.7 b	17.02 a	3.62 b	1724 b	5880 b	43.0 b
	Application	134.5 a	18.01 a	3.83 a	1832 a	6780 a	44.8 a
Pseudomonas Bacterium	Non-insemination	131.3 b	16.40 b	3.62 b	1740 b	6071 b	42.08 b
	Insemination	133.9 a	18.64 a	3.84 a	1818 a	6591 a	44.9 a

Means with same letter in each column are significantly different at probability level of 5 percent

## Conclusion

The general findings of the research showed that salicylic acid has positive effect on rapeseed yield, yield components and DM, so that 1mM recognized as the best concentration of salicylic acid. Furthermore the findings revealed that nano-iron chelate has also positive effect either when applied lonely or in combination with salicylic acid and pseudomonas fluorescens bacterium.

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