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## **RESEARCH PAPER**

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# The impact of seed priming and integrated plant nutrition on yield and yield components of black mustard

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### Abstract

A field experiment was conducted to evaluate the effects of seed priming ( $P_1$ : unprimed,  $P_2$ : hydro-priming and P<sub>3</sub>: priming with 1% KNO<sub>3</sub> for 8 hours at  $20\pm1^{\circ}$ C) and chemical fertilizer of control (F<sub>1</sub>), 250Kg/ha urea + 200Kg/ha triple superphosphate (F<sub>2</sub>) and bio-fertilizer levels of nitrogen fixing (Azoto Barvar 1) and phosphate solubilizing bacterial (Barvar 2), 125Kg/ha urea + 100Kg/ha triple superphosphate (50%) + bio-fertilizer, respectively) on black mustard (Brassica nigra L.) performance in 2016. A factorial experiment was arranged with randomized complete block design with three replications. Results showed that primed seeds significantly resulted in higher number of plants per unit area, compared to unprimed seeds. Seed priming and fertilizers improved number of grains per plant, biological and grain yields per unit area of black mustard. Also, the highest biological and grain yields, pods per plant and number of grains per plant were achieved in integrated plant nutrition containing the reduced chemical fertilizer (50%) by application of biological nitrogen and phosphorus fertilizers (100%). Additionally, integrated plant nutrition resulted in decreasing 50% chemical fertilizer application confirming its important role towards environmentally friendly cropping systems such as sustainable agriculture and organic farming systems. In this study, number of seeds per plant had more effect on grain yield, compared to 1000-grain weight. The highest positive correlation with grain yield was recorded for biological yield, followed by grains per plant, suggesting that these traits have the major roles in determining final yield of this medicinal plant.

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#### Introduction

Medicinal plants were considered as rich sources of secondary metabolites that can be used in pharmaceutical industry. Mustard is an annual plant which belongs to the Brassicaceae family (Abul-Fadl *et al.*, 2011). The glucosinolates (thioglucosides) in mustard seeds are pseudo-thioglucosides containing nitrogen and sulphur (Fahey *et al.*, 2001). Mustard seed is widely used as a condiment; however, its advantageous chemical composition and relatively low price offer wide possibilities for utilization as additives in human food and in animal feeds (Abul-Fadl *et al.*, 2011).

Different techniques could be used to enhance crop yield. In crop production, stand establishment determines plant density, uniformity and management options (Cheng and Bradford, 1999). Seed priming is a useful technique which can be used to improve seedling establishment field performance of crops (Ghassemi-Golezani et al., 2011). Seed priming process is a pre-sowing treatment that exposes seeds to a certain solution for a certain period that allows partial hydration, but radicle emergence does not occur (Rosental et al., 2014). Factors such as water potential, priming duration, temperature, seed vigor, plant species and seed primed storage condition affect the seed response to priming. Thus, the optimization of the priming technique is important to achieve the best results (Parera and Cantliffe, 1994; Maiti and Pramanik, 2013).

The positive effects of seed priming have also been reported in many crops, such as maize (*Zea mays* L.) (Abraha and Yohannes, 2013), mung bean (Ghassemi-Golezani *et al.*, 2014), chickpea (*Cicer arietinum* L.) (Ghassemi-Golezani and Hosseinzadeh-Mahootchi, 2013), milk thistle (*Silybumm arianum* L.) (Ghassemi-Golezani *et al.*, 2016).

Integrated Plant Nutrient Management (IPNM) is also one of the most important methods that increase

plant production (Fretz, 1976). The field level management practices considered under the heading of IPNM would include the use of farmyard manures, natural and mineral fertilizers, soil amendments, agro forestry, green manures, biological fertilizers, cover crops, intercropping and crop rotations. They have made improvements in the biological, physical and chemical proper ting of their soils that farmers can expect to get the full benefits from the supply of additional plant nutrients, in the form of organic and inorganic fertilizer, to their crops. Chemical fertilizers have various negative environmental effects such as soil, water and air pollution, which increase environmental production cost (Moradi et al., 2011). Bio-fertilizer as essential components of organic farming, play a vital role in maintaining long term fertility and sustainability of soil (Mishra et al., 2013). Bio-fertilizers have the ability to access a major part of nutrients for growing plant along with growth promoting factors (Cordovilla et al., 1999).

Rhizobium and phosphate solubilizing bacteria significantly increased yield of faba bean (*Vicia faba* L.) and a synergetic effect was observed when the two types of microorganisms were combined (Rugheim and Abdelgani, 2012). Crop yield and growth increased 20 to 30% by replacing chemical nitrogen and phosphorus with bio-fertilizers (Vessey, 2003). Thus, the objective of this research is to investigate the effects of seed priming and integrated plant nutrition on grain yield and yield components of black mustard in the field.

#### Materials and methods

#### Field conditions

The experiment was conducted in 2016 at the Research Farm of the Faculty of Agriculture, University of Tabriz, Iran (Latitude 38°05'N, Longitude 46°17'E, Altitude 1360m above sea level with the mean annual rainfall of 285mm). Some physical and chemical properties of soil in experimental area were shown in Table 1.

Table 1. Some physical and chemical properties of soil in experimental area.

Texture	EC	pН	CaCO3	OC	Ν	Р	K
Sandy Loam	0.77 dS/m	7.75	14.8 %	0.9 %	0.1 %	13 mg/Kg	3.82 mg/Kg

#### Experimental design and treatments

A factorial set of treatments were arranged based on RCBD with three replications. Factors were three levels of seed priming ( $P_1$ ,  $P_2$ ,  $P_3$ : control and primed with distilled water and 1% KNO3 solution, respectively) and four fertilizer levels [ $F_1$ ,  $F_2$ ,  $F_3$ ,  $F_4$ : control, 250Kg/ha urea + 200Kg/ha triple super phosphate (100%), nitrogen fixation (Azoto Barvar 1) + phosphate solubilizing (Barvar 2) bacteria, and 125Kg/ha urea + 100Kg/ha triple superphosphate (50%) + two biological fertilizers, respectively]. Rates of chemical fertilizers were determined on the basis of soil analysis (Table 1).

Seeds were divided into three sub-samples, one of which was kept as control (non-primed,  $P_1$ ) and two other samples were soaked in distilled water ( $P_2$ ) and 1% KNO3 ( $P_3$ ) solution in an incubator adjusted on  $20\pm1^{\circ}$ C in dark conditions for 8 hours and then seeds were dried at 20-23°C for 3 hours. Each unprimed and primed seed lots were divided into two parts, one of which was kept as control (non-inoculated). The other part was inoculated according to instruction on their package. Chemical fertilizers were applied as strip method.

All the seeds were treated with Benomyl at a rate of 2g/kg and then were sown by hand in about 1-2cm depth of a sandy loam soil with a density of 80 seeds m<sup>2</sup> on 26th April 2016. Each plot consisted of 8 rows of 2.5m length; spaced 25cm apart. All plots were immediately irrigated after sowing and subsequent irrigations were carried out after 70mm evaporation from class A pan. Weed control was frequently carried out by hand during crop growth duration.

#### Measurements

At maturity, plants in 1m<sup>2</sup> of the middle part of each plot were harvested and number of pods per plant, grains per pod, grains per plant, 1000-grain weight and grain yield per unit area were determined. Above ground biomass was oven-dried at 75°C for48 hours and then, biological yield per unit area was recorded.

#### Statistical analysis

Data analysis and mean comparisons were conducted using MSTAT-C software and Duncan multiple range tests at  $P \le 0.05$ . Excel software was used to draw figures.

#### **Results and discussion**

The highest number of plants per unit area was achieved from the primed seeds with water. However, the differences between  $P_2$  and  $P_3$  plants were not statistically significant (Fig. 1). Reduction of germination imbibition periods, faster and higher emergence as a result of seed priming led to better stand establishment. The efficiency of seed priming for improving seedling emergence was also reported in Lallemantia (*Lallem antiaiberica* Fish et May) (Ayyari *et al.*, 2016).



**Fig. 1.** Mean plants/m<sup>2</sup> of black mustard affected by seed priming.

Different letters indicating significant difference at  $p \le 0.05$ .

Between factors, only fertilizer significantly influenced the number of pods per plant and 1000grain weight (Table 2). Biological and grain yields and the number of grains per plant were significantly affected by seed priming and fertilizer.

The number of pods per plant was increased as fertilizer was applied. The highest pods per plant were obtained from chemical fertilizer (100%) and 50% chemical fertilizer + bio-fertilizers. Minimum number of pods per plant was observed in control. Although, seed inoculation with bio-fertilizers (Barvar 1 and Barvar 2) resulted in enhancing pods per plant compared to control, but it was no significant. Increasing number of pods per plant as a result of application of fertilizer could be attributed to the enhancing nutrients uptake, reducing competition, important photosynthesis and reduce the percentage of flower shedding in this condition. Soybean seed inoculation was also increased pods per plant (Zhang *et al.*, 2002).

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С		MS						
Source of	Df	Pods per	1000-grain	Grains	Grains per	Biological	Grain	Harvest
Variation		plant	weight	per pod	plant	yield	yield	Index
Replication	2	164.25	1.43	3.62	14129.70	10877.20	1191.26	0.05
Seed priming (A)	2	177.75	0.073	4.48	126637.60**	53265.94**	6125.02**	4.98
Fertilizer (B)	3	780.25**	1.22**	3.60	339058.10**	115499.37**	11002.65**	2.33
A*B	6	52.64	0.19	0.616	10279.90	9878.23*	998.04*	0.77
Error	22	58.37	0.15	3.19	15059.27	4027.93	400.25	3.32
CV (%)	-	12.27	11.10	13.26	15.07	11.50	12.28	6.18

Table 2. Analysis of variance of field traits of black mustard affected by seed priming and fertilizer.

\* and \*\*: significant at p  $\leq$  0.05 and p  $\leq$  0.01 respectively

No significant difference in grain weight of plants from primed and unprimed seeds suggest that 1000grainweight of plants from primed seeds was not changed by higher density of plants per unit area (Fig. 1) and more grains per plant (Table 3). 1000-grain weight was reduced by application of fertilizers. The highest reduction was caused by chemical fertilizer rather than by bio-fertilizers. Maximum 1000-grain weight was achieved in control  $(F_1)$ . Reducing seed weight of plants by application of fertilizer could be attributed to more number of pods per plant, grains per plant (Table 3) and thus reduce the share assimilate for each grain. Reducing seed weight by increasing application of N fertilizer was reported by Rathke *et al.* 2009.

Table 3. Means of yield components an	l grain yield of black mustard aff	fected by seed priming and fertilizer.
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Treatment	Pods per	1000-grain weight	Grains per pod	Grains per plant	Harvest Index
	plant	(gr)			(%)
Seed priming					
P1	58.25a	3.50a	12.58a	748.49b	31.71a
P2	64.75a	3.35a	13.05a	<b>892.80</b> a	<b>32.5</b> 1a
P3	61.00a	3.46a	13.45a	825.41ab	<b>31.64</b> a
Fertilizer					
F1	51.55c	3.86a	<b>12.74</b> a	651.20b	31.55a
F2	66.99a	3.17bc	14.00a	<b>936.10</b> a	<b>31.86</b> a
F3	57.66bc	3.69ab	13.05a	752.81b	31.67a
F4	64.89ab	3.10c	13.96a	903.11a	31.79a

Different letters indicate significant difference at  $p \le 0.05$ .

P1, P2, P3: Control, Seed priming with water and KNO3 respectively

 $F_1$ ,  $F_2$ ,  $F_3$ ,  $F_4$ : Control (0%), Chemical fertilizers (100%), Biological fertilizers (100%) and Chemical fertilizers (50%) + Biological fertilizers (100%) respectively

The highest grains per plant were produced by plants from primed seeds. However, the differences between  $P_2$  and  $P_3$  plants were not statistically significant. Seed priming techniques increased grains per plant by 19.28% ( $P_2$ ) and 10.28% ( $P_3$ ), compared with plants from unprimed seeds. Increasing grains per plant from primed plants could be attributed to faster emergence, efficient use of resources as soil nutrient and production of vigorous plants with high efficiency. Seeds primed improved number of grains per plant in fennel (*Foeniculum vulgare* Mill.) (Shabbir *et al.*, 2013). Maximum number of grains per plant was produced by plants from 100% chemical fertilizer ( $F_2$ ). However, the differences between  $F_2$  and  $F_4$  plants were not statistically significant. Application of 100% chemical fertilizer ( $F_2$ ), bio-fertilizer ( $F_3$ ) and 50% chemical fertilizer + bio-fertilizer ( $F_4$ ) increased the number grains per plant up to 43.75, 15.60 and 38.68% in comparison with plants from control ( $F_1$ ), respectively. Increment in number of grains per plant as a result of application of chemical and bio fertilizer can be attributed to the production of plant growth promoting hormones, higher and better uptake of water and mineral by roots, enhancing photosynthetic efficiency and produce more pods per plant (Table 3). Enhancing number of grains per plant in inoculated seeds of soybean was also reported by Kazemi *et al.* (2005).

Biological and grain yields per unit area of plants from unprimed and primed seeds were increased under all fertilizer levels. The highest biological and grain yields were achieved by the fertilized and nonfertilized plants from hydro-primed seeds. Plants from unprimed seeds  $(P_1)$  with no fertilization  $(F_1)$ had the lowest plant biomass and grain yield. Biological and grain yields of P1, P2 and P3 plants were enhanced by application of all fertilizers, particularly by chemical fertilizers. However, the difference between 100% chemical fertilizer  $(F_2)$  and 50% chemical fertilizer + bio-fertilizer was not statistically significant. No significant differences between chemical and bio-fertilizers in plant biomass and grain yield of plants from hydro-primed seeds were observed (Fig. 2).

Improving grain yield of black mustard due to seed priming (Fig. 2B) could be related with higher number of plants per unit area (Fig. 1), the number of grains per plant (Table. 3) and biomass (Fig. 2B) of the resultant plants. The superiority of plants from hydro-primed seeds in grain yield per unit area resulted from higher seedling establishment and production of more grains per unit area, compared to those from unprimed seeds (Ghassemi-Golezani *et al.*, 2012; Ayyari *et al.*, 2016). Shabbir *et al.* (2013) also found that different priming techniques improve number of umbels per plant, grains per umbel, 1000grain weight, biological and grain yields in fennel.

Enhancing grain yield of black mustard due to application of all fertilizers (Fig. 2B) could be related with more pods per plant, grains per plant (Table 3) and biomass (Fig. 2B) of the resultant plants. Biofertilizers and nitrogen and phosphate fertilizers are activator of some enzymatic systems and can influence plants growth and production. In the field trials, single and dual N-fixing *B. subtilis* and *P. solubilizing*, *B. megaterium* inoculations significantly increased pods number, grain yield and total biomass of chickpea compared with the control treatment, equal to or higher than N, P, and NP treatments (Elkoca *et al.*, 2008). In this study, grains number per plant had more effect on grain yield, compared with 1000-grain weight.



**Fig. 2.** Mean biological yield (A) and grain yield (B) per unit area for plants from primed and unprimed seeds of black mustard under different fertilizer application. Different letters indicate significant difference at  $p \le 0.05$ . P<sub>1</sub>, P<sub>2</sub>, P<sub>3</sub>: Control, Seed priming with water and KNO<sub>3</sub> respectively

F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub>: Control (0%), Chemical fertilizers (100%), Biological fertilizers (100%) and Chemical fertilizers (50%) + Biological fertilizers (100%) respectively

Correlation of pods per plant and grains per pod with grains per plant and correlation of pods per plant, grains per plant, biological yield and harvest index with each other and with grain yield were positive and significant. The highest positive correlation with grain yield was recorded for biological yield, followed by grains per plant (Table 4). This suggests that grains per plant and biological yield had the major roles in determining final grain yield of black mustard.

	Pods per	1000-grain	Grains	Grains	Biological	Grain	Harvest
	plant	weight	per pod	per plant	yield	yield	Index
Pods per plant	1						
1000-grain weight	-0.63**	1					
Grains per pod	0.28	-0.32	1				
Grains per plant	0.86**	-0.83**	0.70**	1			
<b>Biological</b> yield	0.66**	0.37	0.35	0.77**	1		
Grain yield	0.63**	-0.41	0.41	0.79**	0.95**	1	
Harvest Index	$0.42^{*}$	-0.18	0.14	0.44*	0.79**	0.71**	1

Table 4. Correlation coefficients of different field traits.

\*and\*\*: Statistically significant at  $p \le 0.05$  and  $p \le 0.01$  respectively

#### Conclusion

Seed priming, particularly hydro-priming and fertilizer levels improved grain yield of black mustard via higher number of plants per unit area, grains per plant and biological yield per unit area. Application of 50 % chemical fertilizer + bio-fertilizers can reduce the use of chemical fertilizer. In this study, seed number per plant had more effect on grain yield, compared with 1000-grain weight. Thus, replacing part of the chemical fertilizer by bio-fertilizer could improve black mustard yield. Finally, it was concluded that hydro-priming and bio-fertilizer can reduce the environmental risk and increase field performance of medicinal plants such as black mustard.

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