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Effects of phosphorus and seed priming on seed vigor, fatty acids composition and heterotrophic seedling growth of black seed (*Nigella sativa* L.) grown in a calcareous soil

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ABSTRACT

In order to investigate the effects of increasing soluble phosphorus (P) on seed vigor, fatty acids composition and heterotrophic seedling growth of black seed (*Nigella sativa* L.) in a calcareous soil, a two-year field experiment was conducted at Faculty of Agriculture, Ferdowsi University of Mashhad, Iran, in 2013 and 2014. The fertilizer resources (vermicimpost (V) + *Tiobacilus thiooxidans* (T), Sulfur (S) + T, V + S + T and control) and three levels of P (0, 30 and 60 kg ha^{-1}) were the first and second experimental factors, respectively. In the second experiment, selection treatments (which showed emergence below 60% in the previous experiment) and seed priming (no-priming, water-priming and three levels of P-priming of 100, 300 and 500 mM KH_2Po_4) were the first and second experimental treatments, respectively. The resources of soil amendment (V + T, S + T and V + S + T) significantly decreased the P concentration in seed coat and mean germination time. However, 1000 seed weight, seed vigor, P concentration in embryo and intact seed were significantly increased. The soluble P had significant decreasing effects on linolenic acid. There was the highest significant relationship between linolenic acid and seed vigor ($R^2 = 0.74^{**}$). In addition, seed reserve depletion percentage, seed reserve utilization efficiency and emergence percentage significantly increased by P-priming treatments. There was a positive correlation between weight of mobilized seed P and emergence percentage of black seeds ($R^2 = 0.90^{**}$).

1. Introduction

Black seed (*Nigella sativa* L.) offers a wide range of chemical compounds including fixed oil, proteins, essential oil, mucilage, alkaloids, crude fiber, saponin, minerals and vitamins (Al-Kayssi et al., 2011; Ramadan, 2007). Recently, black seed has identified as a valuable source of edible oil (Piras et al., 2013) with 24.8–29.2% saturated and 69.7–73.5% unsaturated fatty acids (Atta, 2003).

Seed vigor is one of the most important factors affecting seedling establishment and final production (Krueger et al., 2013; Sawan et al., 2011). There are increasing evidence that mother plant nutrition not only affect seed production, but also can affect the seed vigor (Bishnoi et al., 2007; Sawan et al., 2011).

The seed reserves (which is rich in starch, storage proteins and oils) affect normal emergence through effect on heterotrophic seedling growth (HSG) (Elamrani et al., 1992). The HSG (mg per seedling) can be described as the product of the following two components: (1) "the weight of mobilized seed reserve (WMSR), and (2) the conversion efficiency of mobilized seed reserve to seedling tissue, i.e., the production of seedling dry matter per unit of usage of seed reserve. The first component can be further divided into (1) initial seed weight, and (2) the fraction of seed reserve which is mobilized, i.e., seed depletion ratio" (Soltani et al., 2006). Hence, the amount of nutrient that can be taken up by mother plant, determine seed chemical composition and therefore physiological aspects of produced seeds (Modi, 2002). The main effect of nutrition management on seed quality is through its influence on seed reserves potential. In this context, it has been reported that environmental conditions and nutritional status can affect oil content and fatty acid composition in plants such as canola (Gao et al., 2014) and sweet pepper (Xu et al., 2002).

Abbreviations: GP, germination percentage; MGT, mean germination time; EP, emergence percentage; MET, mean emergence time; HSG, heterotrophic seedling growth; WMSR, weight of mobilized seed reserve; WUSR, weight of un-utilized seed reserve; SRDP, seed reserve depletion percentage; SRUE, seed reserve utilization efficiency; WISP, weight of initial seed P; WMSP, weight of mobilized seed phosphorus; WUSP, weight of un-utilized seed phosphorus; SPDP, seed phosphorus depletion percentage; SPUE, seed phosphorus utilization efficiency.

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Seed phosphorus (P) content plays a key role in improving seed physiological aspects (Modi, 2002; Sawan et al., 2011) and seedlings metabolism (Modi, 2002; White and Veneklaas, 2012). This pivotal role has been confirmed in soybean (Bishnoi et al., 2007) and common bean (Pacheco et al., 2012). In general, phytate is the major storage form of P in seeds (Lickfett et al., 1999). Phytic acid content in seeds is affected by genetic and edaphic factors and highly correlates with plant-available P in soil. In other words, P availability is one of the most crucial environmental factors (Koocheki et al., 2014), affecting seed P content (Fujita et al., 2013). On the other hand, considering the vital role of lipids and proteins in seed germination and seedling establishment (Nonogaki, 2008; Soltani et al., 2002), improving nutritional status of mother plants in terms of P uptake, especially in arid and semi-arid regions, where calcareous soils are prevalent (Adhami et al., 2006), would improve the seed vigor and seedling heterotrophic growth.

In cases where mother plants are unable to uptake enough nutrient, nutrient-priming, especially P-priming, is a practical method to promote physiological aspects of produced seeds (Shah et al., 2011, 2012). In fact, if there is P deficiency during mother plant growth, seed soaking in P solution significantly affect physiological aspects of the seeds (Al Mudaris and Jutzi, 1999). It has been reported that under P deficiency conditions, which typically occurs in calcareous soils, priming enhances seed P content and increases germination percentage and seedling dry weight in barley (Ajouri et al., 2004).

With regard to all mentioned above, the current study was aimed to investigate seed physiological indexes, oil, essential oil and protein percentage as well as fatty acid composition of black seed and understand how these parameters correlate with seed vigor in calcareous soils. In addition, the effect of P-priming on HSG was assessed.

2. Materials and methods

2.1. Impact of phosphorus and soil amendments on seed quality characteristics

A two-year field experiment with four replications and 12 treatments was conducted at Faculty of Agriculture, Ferdowsi University of Mashhad, Iran, in 2013 and 2014 (Table 1). The fertilizer resources (vermicimpost (V) + *Tiobacilus thiooxidans* (T), Sulfur (S) + T, V + S + T and control) and three levels of P (0, 30 and 60 kg ha^{-1}) were the first and second experimental factors, respectively. Some chemical properties of used V in the study are given in Table 1.

The land lied fallow before both years of the experiment. Seed bed was prepared by plough and disk. Then plots were designed with 3 m long and 2 m width, 0.5 m apart each other. V (10 t ha^{-1}) and S (20 t ha^{-1}) along with T were applied before seed sowing. Diammonium phosphate was incorporated into the soil at the same time. Since diammonium phosphate contains 18% net N, corrections were made using 54, 27 and 0 kg N ha^{-1} (from urea fertilizer), respectively. Furthermore, 30 kg N ha^{-1} from urea was applied as top dress at the six true-leaf stage.

Seed sowing was performed on both sides of the furrows. The first irrigation was done after seed sowing with weekly irrigation until physiological maturity. Weeds were removed by hand during growing seasons.

Harvested seeds were evaluated for germination percentage (GP), mean germination time (MGT), emergence percentage (EP), mean emergence time (MET), seed vigor, 1000-seed weight, seed coat to embryo ratio and P concentration in seed coat, embryo and intact seed. In order to remove seed coat, the intact seeds were soaked in distilled water up to 1 h. The intact seeds were germinated in a germinator at 25 °C for 14 days to determine GP (ISTA, 2012) and MGT (Khajeh-Hosseini et al., 2009). Seed vigor was calculated according to RE test described by ISTA (2012). In order to determine EP and MET (Demir et al., 2008; Khajeh-Hosseini et al., 2009), the seeds were sown in plastic pots filled with 1.4 kg soil with good texture and organic matter content. The pots were placed in a greenhouse at 25/17 °C day/night temperature for 21 days when real leaves appeared.

For oil extraction, the seeds were ground to powder and then extracted with *n*-hexane (1:4 wt/vol) by agitation in a dark place at ambient temperature for 48 h. The solvent was evaporated in vacuo at 35 °C to dryness (Farhoosh et al., 2009).

The fatty acid composition of black seed oil was determined by gas chromatography (YOUNG LIN – Acme 6000 GC, The Republic of Korea) with fame-ionization detector (FID) and capillary column (60 m × 0.32 mm i.d.; film thickness was 0.25 μm). The carrier gas was hydrogen at 1 mL/min column flow and 1:20 split ratio. Injector and detector temperatures were 300 and 320 °C, respectively. Oven temperature was at 250 °C. Oil composition (percent of fatty acid in total seed oil) was calculated from the compound peak areas (Nzikou et al., 2009).

2.2. Impact of seed priming on HSGT and HSGP

After determining EP in the first experiment, priming was performed on the seeds with emergence below 60%. Selected treatments (emergence below 60%) and seed priming no priming, water-priming, P-priming (100, 300 and 500 mM KH_2Po_4) were considered as first and second factors, respectively.

Water-priming and P-priming were done for 48 h and 12 h in distilled water and KH_2Po_4 solutions, respectively. After priming, the seeds were rinsed with distilled water and dried at room temperature (Shah et al., 2012). P concentration in intact seeds was determined again.

In order to calculate HSG based on total seed reserve (HSGT) (Eqs. (1)–(3)), standard germination test was done on primed seeds and then seedlings dry eight was recorded (Soltani et al., 2006).

$$\text{Weight of mobilized seed reserve (WMSR) (mg seed}^{-1}) = \text{Initial seed dry weight} - \text{un-utilized seed dry weight} \quad (1)$$

$$\text{Seed reserve depletion percentage (SRDP)} = \left(\frac{\text{WMSR}}{\text{Initial seed dry weight}}\right) \times 100 \quad (2)$$

$$\text{Seed reserve utilization effeciency (SRUE) (\%)} = \left(\frac{\text{Seedling dry weight}}{\text{WMSR}}\right) \times 100 \quad (3)$$

In order to determine initial seed dry weight, 25 seeds from each treatment were weighed with four replicates (seed fresh weight) and then samples were dried at 103 °C for 24 h and weighed again to calculate seed moisture content (Eq. (4)). Initial seed dry weight was calculated according to Eq. (5).

$$\text{Seed moisture content} = \text{seed fresh weight} - \text{seed dry weight} \quad (4)$$

$$\text{Initial seed dry weight} = \text{seed fresh weight} - \text{seed moisture content} \quad (5)$$

Table 1
Some physical and chemical properties of field soil and vermicompost.

Sample	Clay (%)	Silt (%)	Sand (%)	Organic carbon (%)	Total N (%)	Olsen-P ($mg\ kg^{-1}$)	Total P (%)	Available K ($mg\ kg^{-1}$)	pH	EC ($dS\ m^{-1}$)	$CaCo_3$ (%)
Soil (2013)	48.46	31.95	19.59	0.33	0.08	10.59	0.06	173.36	8.39	0.75	11.17
Soil (2014)	49.11	34.21	16.68	0.39	0.08	6.71	0.05	184.68	8.48	0.73	14.91
Vermicompost	–	–	–	34.76	2.31	–	1.78	–	7.59	14.10	–

In addition to initial seed P content, seedling P content, un-utilized seed P content and HSG based on seed P (HSGP) were calculated (Eqs. (6)–(8)).

$$\text{Weight of mobilized seed P (WMSP) (mg seed}^{-1}) = \text{weight of initial seed P (WISP)} - \text{weight of un-utilized seed P (WUSP)} \quad (6)$$

$$\text{Seed P depletion percentage (SPDP)} = \left(\frac{\text{WMSP}}{\text{WISP}}\right) \times 100 \quad (7)$$

$$\text{Seed P utilized efficiency (SPUE) (\%)} = \left(\frac{\text{Weight of P in seedling tissue}}{\text{WMSP}}\right) \times 100 \quad (8)$$

EP and MET after priming were determined similar to the first experiment.

2.3. Statistical analyses

The data were subjected to an analysis of variance using SAS 9.3 software. To determine the difference amongst means, least significant difference (LSD) was used at the level of 0.05.

3. Results and discussion

3.1. Impact of phosphorus and soil amendments on seed quality characteristics

As can be seen from Table 2, there was a positive correlation between P concentration in intact seed ($g\ kg^{-1}$) and GP from third to tenth days, however after tenth day there was no significant correlation. Among these days, the highest correlation was observed on the fourth days ($R^2 = 0.88^{**}$). Therefore, according to RE test, GP on the fourth days was determined as seed vigor assessment.

According to Table 3, seed quality indexes were affected by P application and soil amendments, except for GP. P application (30 and 60 $kg\ ha^{-1}$) significantly increased seed weight (Table 3). In addition, soil amendments (including V + T, S + T and V + S + T) increased 1000-seed weight compared with control treatment. Among soil amendments, V+S+T increased seed weight more than other soil amendments (Table 3).

In general, small seeds are unable to emergence and establish a strong seedling due to low nutrient reserve (Hojjat, 2011). On the other hand, biological sulfur oxidation (Soaud et al., 2012; Vidyalakshmi et al., 2009) and application of organic fertilizer such as vermicompost (Hejazi Mehrizi et al., 2015; Mohammady Aria et al., 2010) are considered as an effective approaches in order to increase P solubility in calcareous soils. Effective role of V in reducing soil pH and increasing available P have also been reported by others (Azarmi et al., 2008; Parthasarathi et al., 2008). Hence, under conditions of P limitation, increase in P availability during plant growth period can improve seed yield and quality (Sawan et al., 2011). In this regard, Tuncturk et al. (2011) have reported that application of 40 $kg\ ha^{-1}$ P could increase seed weight of black seed grown in calcareous soil.

The results indicated that although 1000 seed weight increased due to P fertilizer and soil amendments application, seed coat to embryo ratio significantly decreased (Table 3). Furthermore, above mentioned treatments decreased seed coat to intact seed ratio and increased embryo to intact seed ratio (Table 3). On the other hand, with increasing P availability on account of diammonium phosphate (30 and 60 $kg\ ha^{-1}$) and other soil amendments (V + T, S + T and V + S + T) application, P concentration in seed coat and also P percentage of seed coat to intact seed decreased, while P concentration in embryo and intact seed increased (Table 3). For instance, by applying V + S + T treatment, P concentration in seed coat decreased by 19.8%, compared with control treatment, whereas P concentration in embryo increased by 50.5% (Table 3). The highest P concentration in embryo and intact seed was observed when 60 $kg\ ha^{-1}$ diammonium phosphate was applied along with V + S + T (Table 3).

P is one of the main elements in biological membranes and organic compounds such as phospholipids, ADP and ATP. Therefore, it plays an important role in energy storage and transfer as well as oxidation reactions in plants (Schachtman et al., 1998; White and Veneklaas, 2012). During germination process, phytate converts into mineral P by phytase enzyme (Hegeman et al., 2001). Hence, P content in seeds is a crucial factor in germination and seedlings metabolism (Modi, 2002; White and Veneklaas, 2012). It has been reported that there is a direct relationship between seed size and P concentration in intact seed (Liao and Yan, 1999). Therefore, larger seeds with higher P concentration germinate faster, use resources more efficient and produce the stronger seedlings (White and Veneklaas, 2012).

Although P fertilizer and soil amendments application had no significant effects on GP, MGT and MET significantly decreased (Table 3). Moreover, EP and seed vigor increased due to P fertilizer and soil amendments application (Table 3). Generally, among studied treatments, the highest MGT and the lowest seed vigor was related to control treatment. Application of V + T and S + T increased seed vigor by 25.5 and 16.4%, respectively, in comparison with control treatment (Table 3).

Besides environmental conditions and genetic factors, seed vigor highly depends on nutrient reserves in the seeds (Peltonen-Sainio et al., 2006; White and Veneklaas, 2012). As mentioned earlier, due to pivotal role of P in energy transfer reactions (Schachtman et al., 1998), increment in seed vigor of black seed might be due to increase in P solubility and availability during growing period of mother plants.

Since most of P concentration in intact seed (~95%) is reserved in embryo (Table 3), so it appears that seed vigor of black seed depends on P allocation in the embryos. A positive linear correlation between 1000 seed weight and P concentration in embryo and also a negative linear correlation between 1000 seed weight and P concentration in seed coat (Fig. 1) confirm this suggestion.

Phospholipids play an important role in membrane stability and metabolism (Dowhan, 1997; Gniazdowska et al., 1999). Therefore, increase in seed coat to intact seed ratio and P concentration in seed coat to P concentration in intact seed ratio might be an adaptability mechanism in response to low P conditions.

Table 2
Evaluation of seed vigor (RE test) of black seed affecting by seed P concentration in mother plant.

	Germinaqtion (%)											
	Third days	Fourth days	Fifth days	Sixth days	Seventh days	Eighth days	Ninth days	Tenth days	Eleventh days	Twelfth days	Thirteenth days	Fourteenth days
P concentration in intact seed (g kg $^{-1}$)	0.426 *	0.880 **	0.862 **	0.873 **	0.8423 **	0.6713 **	0.499 *	0.413 *	0.314 ns	0.203 ns	0.203 ns	0.203 ns

Values (R^2) are means of eight observations. The asterisks *, **, or ns indicate statistical differences at $P \leq 0.05$, $P \leq 0.01$, or non-significant, respectively.

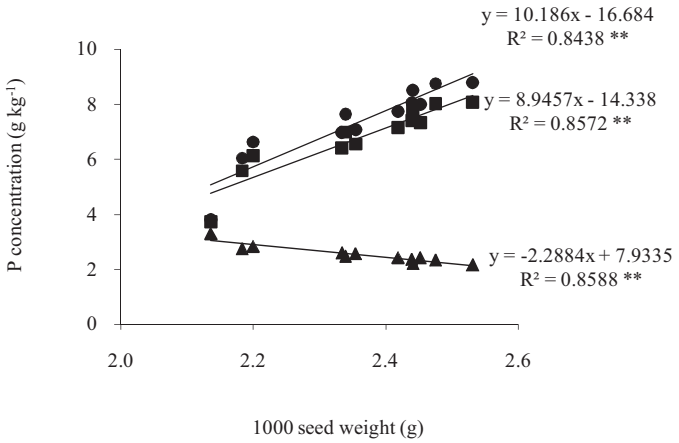

Fig. 1. Relationships between 1000 seed weight and P concentration in coat (triangle), embryo (circle) and intact seed (square). Values are means of eight observations. The asterisks ** indicate statistical differences at $P \leq 0.01$.

A negative correlation was found between 1000 seed weight and seed coat to embryo ratio (Fig. 2A), while there was a positive correlation between percentage of seed coat to intact seed and seed coat to embryo ratio (Fig. 2B). In addition, although there was a positive correlation between 1000 seed weight and seed vigor (Fig. 2C), by increasing the percentage of seed coat to intact seed, seed vigor was decreased (Fig. 2D).

By definition, "germination of a seed commences with the uptake of water and is completed with the appearance of the embryo, in most species radicle first, through the surrounding structure(s). (Nonogaki et al., 2010). Although water absorption take places in dead seeds, however it is not a physical phenomenon, but seed coat and chemical structure of seed can be affect the water absorption (Bradford, 1990; King and Ashton, 1985; Mehanna and Martin, 1985). Hence, the negative effect of seed coat on seed vigor might be due to increasing P concentration in seed coat hence reducing water absorption rates.

There was positive correlations between crude protein, oil and essential oil percentage and seed vigor (Fig. 3A–C). Positive correlations between protein or oil percentage and seed vigor confirms that these reserves compounds can be affect seed metabolism and germination directly (Pahlavani et al., 2009). However, due to wide spectrum of organic compounds found in black seed essential oil (Jrah Harzallah et al., 2011), the relationships between essential oil and seed vigor is not clearly known. Generally, antioxidant properties of essential oil in black seed (Erkan et al., 2008; Sen et al., 2011) can improve the seed vigor.

According to the results, black seed contains 33.66% oil, and there is a stronger correlation between oil percentage and seed vigor (R^2 = 0.87 **), compared to crude protein (R^2 = 0.70 **) or

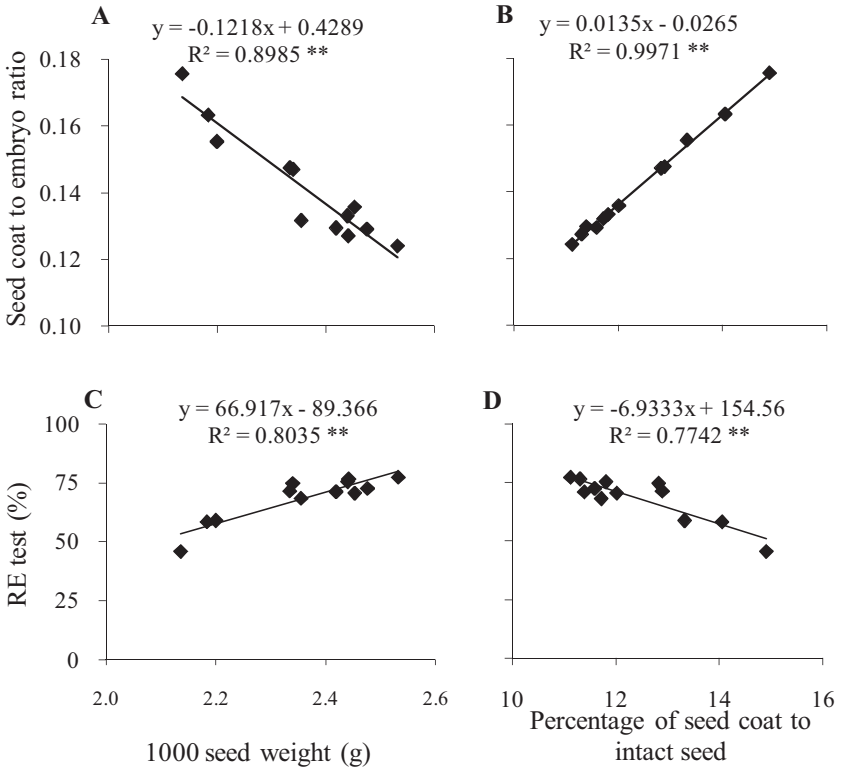

Fig. 2. Relationships between seed quality characteristics of black seed in the experiment. Values are means of eight observations. The asterisks ** indicate statistical differences at $P \leq 0.01$.

Table 3
Effects of phosphorus (P) rate and soil amendments (A) on some seed quality characteristics of black seed in the experiment.

Treatments	Germination (%)	MGT (day)	RE test (%)	Emergence (%)	MET (day)	1000 seed weight (g)	Seed coat to embryo ratio	Percentage of seed coat to intact seed	Percentage of embryo to intact seed	P concentration (g kg^{-1})			P percentage of seed coat to intact seed	P percentage of embryo to intact seed
										Seed coat	Embryo	Intact seed		
P (kg ha^{-1})														
0	98.04	4.59	61.08	70.50	11.67	2.28	0.15	12.84	87.16	2.78	6.32	5.89	6.97	93.03
30	98.58	4.35	70.33	74.96	11.15	2.36	0.14	12.57	87.43	2.48	7.63	7.00	4.70	95.30
60	98.58	4.29	74.04	78.63	11.06	2.44	0.13	11.84	88.16	2.35	8.08	7.40	3.95	96.05
LSD (P = 0.05)	1.211	0.060	2.966	4.133	0.187	0.094	0.005	0.338	0.338	0.232	0.458	0.399	0.561	0.561
A														
C	97.72	4.82	58.56	59.00	12.31	2.22	0.16	13.95	86.05	2.88	5.61	5.24	8.56	91.44
V + T	99.06	4.24	73.50	81.22	10.93	2.41	0.13	11.61	88.39	2.38	7.89	7.25	3.94	96.06
S + T	98.22	4.41	68.17	75.89	11.15	2.33	0.15	12.73	87.28	2.58	7.43	6.82	4.92	95.08
V + S + T	98.61	4.17	73.72	82.67	10.78	2.48	0.13	12.73	88.63	2.31	8.44	7.74	3.42	96.58
LSD (P = 0.05)	1.398	0.069	3.425	4.773	0.215	0.108	0.005	0.391	0.391	0.268	0.529	0.460	0.647	0.647
P × A														
0 + C	97.67	5.32	45.83	49.33	13.04	2.14	0.18	14.91	85.09	3.30	3.81	3.73	13.14	86.86
0 + V + T	98.33	4.31	68.33	79.67	11.15	2.35	0.13	11.72	88.28	2.56	7.08	6.55	4.67	95.33
0 + S + T	99.00	4.53	59.00	71.67	11.49	2.20	0.16	13.33	86.67	2.84	6.64	6.13	6.21	93.79
0 + V + S + T	97.17	4.19	71.17	81.33	11.00	2.42	0.13	11.39	88.61	2.43	7.76	7.15	3.87	96.13
30 + C	98.33	4.74	58.33	57.50	12.05	2.18	0.16	14.06	85.94	2.75	6.05	5.58	7.04	92.96
30 + V + T	99.33	4.18	75.50	80.67	10.79	2.44	0.13	11.81	88.19	2.36	8.07	7.40	3.88	96.12
30 + S + T	97.33	4.34	74.83	77.33	11.04	2.34	0.15	12.83	87.17	2.47	7.65	6.98	4.52	95.48
30 + V + S + T	99.33	4.15	72.67	84.33	10.73	2.48	0.13	11.59	88.41	2.33	8.76	8.02	3.37	96.63
60 + C	97.17	4.41	71.50	70.17	11.85	2.33	0.15	12.90	87.10	2.60	6.98	6.42	5.48	94.52
60 + V + T	99.50	4.22	76.67	83.33	10.84	2.44	0.13	11.31	88.69	2.21	8.51	7.80	3.27	96.73
60 + S + T	98.33	4.34	70.67	78.67	10.93	2.45	0.14	12.02	87.98	2.42	8.01	7.33	4.01	95.99
60 + V + S + T	99.33	4.17	77.33	82.33	10.61	2.53	0.12	11.12	88.88	2.16	8.81	8.07	3.02	96.98
LSD (P = 0.05)		0.119	5.932				0.009	0.677	0.677		0.916		1.121	
Y	ns	*	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns
P	ns	**	**	**	**	**	**	**	**	**	**	**	**	**
A	ns	**	**	**	**	**	**	**	**	**	**	**	**	**
P × A	ns	**	**	*	*	ns	*	*	*	ns	*	ns	**	**
Y × P	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y × A	ns	**	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y × P × A	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

The asterisks *, **, or ns indicate statistical differences at $P \leq 0.05$, $P \leq 0.01$, or non-significant, respectively. C: Control; V: Vermicompost; S: Sulfur; T: *Tiobacilus* bacteria; Y: Year.

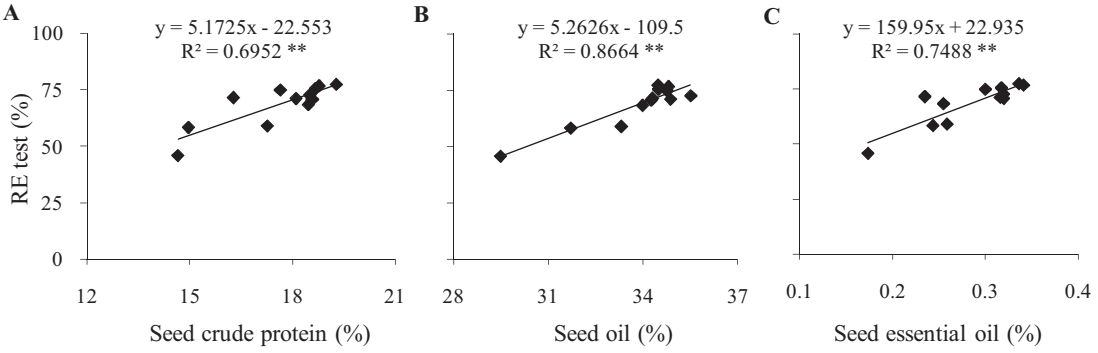

Fig. 3. Relationships between seed crude protein and seed vigor (A), seed oil and seed vigor (B), and seed essential oil and seed vigor (C). Values are means of eight observations. The asterisks ** indicate statistical differences at $P \leq 0.01$.

essential oil ($R^2 = 0.75^{**}$). Therefore, it appears that seed vigor in black seed depends on oil content or oil structure rather than protein or essential oil.

Besides the specific role of P in seed quality, S plays an important role in improving nitrogen uptake (Salvagiotti et al., 2009) and oil biosynthesis (Ahmad et al., 2000). Therefore, in calcareous soils, S application can affect seed vigor directly (by oil and protein synthesis) or indirectly (by increasing P availability in soil).

As can be seen from Table 4, the unsaturated fatty acids amounted to more than 80% of total fatty acid content. *Cis*-inoleic (54.3%) and oleic acids (24.6%) were the major unsaturated, while palmitic acid (10.4%) was the main saturated fatty acid (Table 4).

All fatty acids were affected by soil amendments, Except for palmitic and oleic acids. In addition, the effect of P application on fatty acids was not significant, except for palmitoleic, *cis*-linolenic, linolenic, eicosenoic and arachidic acids (Table 4).

Unsaturated fatty acids showed different responses to experimental treatments. For instance, cis-linoleic acid increased with increasing P availability and soil amendments application, whereas linolenic and eicosenoic acids decreased (Table 4). Under no P application, linolenic acid percentage decreased two times in control treatment, compared with V + S + T (Table 4).

The relationships between seed vigor and unsaturated fatty acid are shown in Fig. 4A–F. The correlation between cis-linoleic acid with seed vigor was positively significant, while the correlation

Table 4
The effects of phosphorus (P) rate and soil amendments (A) on fatty acids composition in black seed oil.

Treatments	Unsaturated acids (%)						Saturated acids (%)				
	Palmitoleic	Oleic	Linoleic		Linolenic	Eicosenoic	Myristic	Palmitic	Stearic	Arachidic	Behenic
			Cis	*Trans*							
P (kg ha^{-1})											
0	0.37	24.45	53.99	0.43	0.46	0.64	0.06	11.10	3.55	0.43	4.35
30	0.28	24.44	54.27	0.43	0.39	0.60	0.02	11.01	3.57	0.38	4.42
60	0.32	24.36	54.47	0.48	0.35	0.53	0.04	11.43	3.54	0.36	4.31
LSD ($P = 0.05$)	0.047	0.440	0.328	0.094	0.018	0.048	0.038	0.471	0.080	0.051	0.147
A											
C	0.41	24.34	53.43	0.59	0.62	0.72	0.00	10.97	3.65	0.49	4.53
V + T	0.26	24.45	54.63	0.39	0.32	0.52	0.09	11.19	3.46	0.36	4.33
S + T	0.41	24.55	53.58	0.44	0.40	0.59	0.05	11.54	3.70	0.43	4.30
V + S + T	0.22	24.31	55.34	0.37	0.25	0.51	0.02	11.03	3.41	0.28	4.28
LSD (P = 0.05)	0.055	0.508	0.379	0.108	0.020	0.055	0.044	0.544	0.093	0.059	0.170
P × A											
0 + C	0.51	24.12	53.18	0.63	0.74	0.83	0.00	10.56	3.58	0.58	4.59
0 + V + T	0.29	24.66	53.71	0.37	0.33	0.56	0.19	11.64	3.53	0.38	4.33
0 + S + T	0.45	24.50	54.09	0.26	0.47	0.57	0.05	11.48	3.60	0.45	4.09
0 + V + S + T	0.24	24.50	54.98	0.45	0.30	0.59	0.00	10.71	3.50	0.33	4.41
30 + C	0.33	24.42	53.55	0.56	0.59	0.69	0.00	11.29	3.63	0.41	4.53
30 + V + T	0.26	24.54	55.07	0.35	0.33	0.56	0.04	10.62	3.50	0.39	4.34
30 + S + T	0.34	24.69	53.33	0.53	0.39	0.69	0.03	11.27	3.82	0.46	4.46
30 + V + S + T	0.21	24.09	55.93	0.31	0.23	0.46	0.00	10.88	3.31	0.26	4.33
60 + C	0.39	24.49	53.58	0.58	0.53	0.64	0.00	11.06	3.73	0.48	4.48
60 + V + T	0.23	24.15	55.11	0.44	0.30	0.46	0.03	11.30	3.34	0.32	4.33
60 + S + T	0.45	24.46	53.32	0.54	0.34	0.53	0.07	11.87	3.69	0.39	4.34
60 + V + S + T	0.21	24.32	55.09	0.35	0.22	0.49	0.05	11.50	3.41	0.26	4.10
LSD ($P = 0.05$)			0.657	0.188	0.035	0.096	0.076		0.160	0.102	
Average	0.32	24.41	54.24	0.45	0.40	0.59	0.04	11.18	3.55	0.39	4.36
Y	**	ns	ns	ns	**	**	**	ns	ns	**	**
P	**	ns	*	ns	**	**	ns	ns	ns	*	ns
A	**	ns	**	**	**	**	**	ns	**	**	*
P × A	ns	ns	**	*	**	**	**	ns	**	*	ns
Y × P	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y × A	*	ns	ns	ns	**	ns	**	ns	*	**	ns
Y × P × A	*	ns	ns	ns	**	ns	**	ns	ns	*	**

The asterisks *, **, or ns indicate statistical differences at $P \leq 0.05$, $P \leq 0.01$, or non-significant, respectively. C: Control; V: Vermicompost; S: Sulfur; T: *Tiobacilus* bacteria; Y: Year.

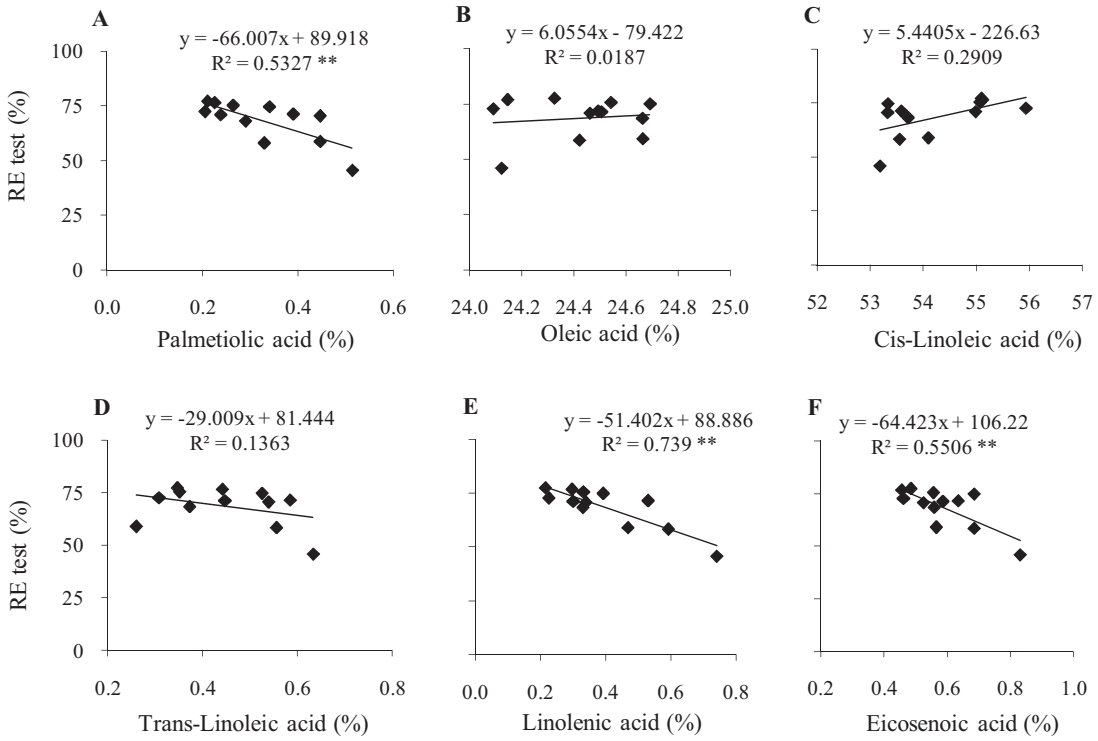

Fig. 4. Relationships between seed vigor and some unsaturated fatty acids of black seed. Values are means of eight observations. The asterisks ** indicate statistical differences at $P \leq 0.01$.

between palmitolic, linolenic and eicosenoic acids with seed vigor was negative. According to Fig. 5A–E, among saturated fatty acids, arachidic acid showed a negative and significant correlation with seed vigor.

Although, linolenic, eicosenoic and arachidic acids comprise small amount (0.40, 0.59 and 0.39%, respectively) of overall black seed fatty acids, the highest correlation was observed between these fatty acid and seed vigor (0.74, 0.55 and 0.51%, respectively).

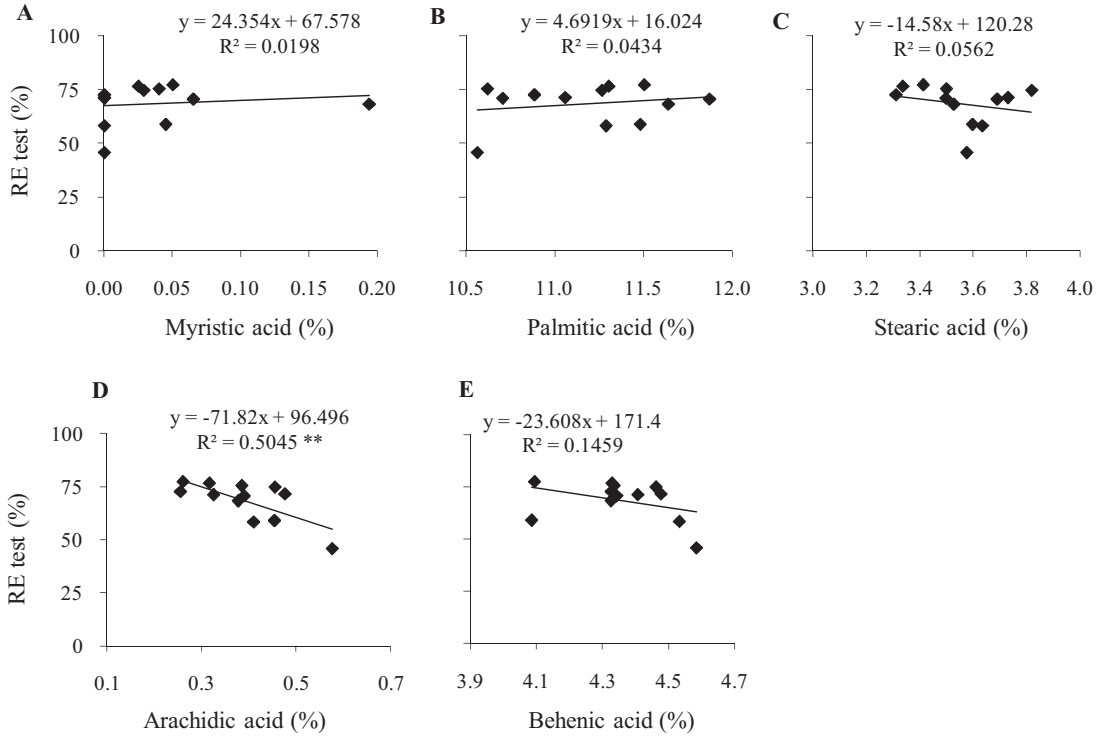

Fig. 5. Relationships between seed vigor and saturated fatty acids of black seed. Values are means of eight observations. The asterisks ** indicate statistical differences at $P \leq 0.01$.

Table 5
Effect of seed priming solutions on heterotrophic seedling growth (based on total seed reserve) of black seed.

Treatments	Seedling dry weight (mg)	Weight of un-utilized seed reserve (mg seed $^{-1}$)	Weight of mobilized seed reserve (mg seed $^{-1}$)	Seed reserve depletion (%)	Seed reserve utilization efficiency (%)
Selection Treatments (S)					
First treatment	0.44	0.79	1.46	64.89	29.14
Second treatment	0.49	0.80	1.50	65.20	31.92
LSD (P = 0.05)	0.038	0.018	0.077	1.270	3.002
Priming solution (P)					
No priming	0.21	0.80	1.37	62.82	15.80
Water-priming	0.25	0.84	1.36	61.82	18.35
100 mM KH_2PO_4	0.58	0.77	1.50	65.86	39.31
300 mM KH_2PO_4	0.67	0.77	1.58	67.34	42.15
500 mM KH_2PO_4	0.59	0.77	1.60	67.37	37.02
LSD (P = 0.05)	0.060	0.029	0.121	2.009	4.746
Year (Y)	ns	ns	*	*	ns
S	**	ns	ns	ns	ns
P	**	**	**	**	**
S × P	ns	ns	ns	ns	ns
Y × S	ns	ns	ns	ns	ns
Y × P	ns	ns	ns	ns	ns
Y × S × P	ns	ns	ns	ns	ns
CV (%)	18.46	5.12	11.53	4.36	21.93

The asterisks *, **, or ns indicate statistical differences at $P \leq 0.05$, $P \leq 0.01$, or non-significant, respectively. Selection treatments showed emergence below 60% in the previous experiment.

Hence, it seems that linolenic acid is more effective on seed quality rather than other fatty acids.

The recent findings describe the special role of lipids in germination and seedling growth (Abbade and Takaki, 2012; Nonogaki, 2008; Soltani et al., 2002). However, the response of seed vigor to fatty acid composition is not clearly known. In general, negative correlation between linolenic acid and seed vigor might be related to the role of this fatty acid in oil unsustainability properties. In general, high linolenic acid content increases oxidation rate and decreases oil sustainability (Yun and Surh, 2012).

3.2. Impact of seed priming on HSGT and HSGP

Although, the effect of fertilizers on HSGT (HSG based on total seed reserve) related indexes was not significant, the priming effect was significant on all these indexes (Table 5). According to the results, water-priming had no significant effect on mentioned indexes (Table 5).

It has been reported that water-priming improves crop seed quality under conditions of low moisture or water stress (Demir and Vandeventer, 1999; Li et al., 2011; Sağlam et al., 2010; Sharma et al., 2014). Therefore, it seems that water-priming is not an effective approach for black seed production under P-deficient conditions.

According to Table 5, P-priming up to 300 mM significantly increased seeding dry weight and WMSR and decreased WUSR. Similar to WMSR, P-priming increased SRDP and SRUE (Table 5). The highest effect was related to concentration of 300 mM in the priming solution, so that SRDP and SRUE slightly decreased with increasing P-priming concentration up to 500 mM (Table 5).

The technique of 'P seed priming' consists of soaking seeds in P solutions instead of pure water which improves seed vigor and crop development during early seedling growth (Ajouri et al., 2004). This could be due to the quick availability of P to the seedling under any P deficiency in the soil (Ajouri et al., 2004; Jamil et al., 2014). The positive effects of P-priming in improving germination related traits and seedling emergence of barley (Ajouri et al., 2004), okra (Shah et al., 2011) and mungbean (Shah et al., 2012 Umair et al., 2011) has been previously reported. Korkmaz (2006) also observed an increase in the germination percentage and rate in lettuce as a result of seed priming in KH_2PO_4 solution.

Generally, WUSR is an important criterion in assessing seed vigor, as WUSR reduction leads to allocate more assimilates to seedling, through effect on WMSR. Hence, low WUSR represents this fact that more seed reserves are affected by hydrolysis enzymes (Elamrani et al., 1992; Soltani et al., 2006). In other words, seed dry weight is not an exact criterion to assess seed vigor rather than WMSR. In addition, increase in SRUE represents more assimilates allocation to seedlings and causes high seed vigor. In turn, SRUE is affected by energy availability to protein synthesis and structural compounds in seedling tissue. Therefore, under P deficit conditions, P-priming might be a good method to increase seedling dry matter per unit of seed reserve.

HSGP (HSG based on seed P content) related indexes are indicated in Table 6. From the results, P-priming increased P concentration in intact seed seedling, SPDP, SPUE and EP. Conversely, MET significantly decreased due to P-priming. For instance, in first selected treatment, P-priming in rate of 300 mM increased WMSP more than twice, compared with control treatment (Table 6).

As mentioned earlier, P plays an important role in energy supplying and cell biosynthesis reactions, especially during germination and seedling establishment (White and Veneklaas, 2012). Hence, study on HSGP can be clearly described the effect of P-priming on seedling emergence and growth, rather than HSGT. In this context, significant correlation between WMSP and EP (Fig. 6A), SPUE and EP (Fig. 6B), WMSP and MET (Fig. 6C), and SPUE and MET (Fig. 6D) can be a reason for this result. Nonetheless, according to the results, the higher correlation between WMSP and MET (R^2 = 0.89**) than that of SPUE and MET (R^2 = 0.57**), implies that WMSP is more effective on seed quality than SPUE.

In general, SPUE was less than SRUE by 34.7%, which indicates that P is allocated to seedlings with lower efficiency, compared with total seed reserve. Specific role of P in ATP structure (Schachtman et al., 1998) is one of the main reasons. In other words, respiration is major reason for reducing seedling dry weight for every unit of WMSP (Soltani et al., 2006). Lower SPUE compared with SRUE indicates that P is more effective in cell respiration process rather than total seed reserve.

Table 6
Effect of seed priming treatments on heterotrophic seedling growth (based on seed P), emergence and mean emergence time (MET) of black seed.

Treatments [a]	In laboratories						In greenhouse	
	P concentration in intact seed ($g\,kg^{-1}$)	P concentration in seedling ($g\,kg^{-1}$)	Weight of un-utilized seed P ($mg\ seed^{-1}$)	Weight of mobilized seed P ($mg\ seed^{-1}$)	Seed P depletion (%)	Seed P utilization efficiency (%)	Emergence (%)	MET (day)
Selection Treatments (S)								
First treatment (FT)	5.59	5.10	0.31	0.010	78.37	21.62	64.00	11.79
Second treatment (ST)	6.74	5.44	0.32	0.013	82.99	19.63	68.73	11.45
LSD ($P = 0.05$)	0.203	0.270	0.014	0.0006	1.333	2.192	5.914	0.211
Priming solution (P)								
No priming	4.66	4.62	0.31	0.008	74.04	13.21	53.42	12.55
Water-priming	4.63	4.44	0.30	0.008	73.49	15.28	60.33	12.02
100 mM KH_2PO_4	6.82	5.30	0.31	0.013	84.27	23.70	71.92	11.39
300 mM KH_2PO_4	7.25	6.18	0.32	0.015	85.53	28.26	73.42	11.10
500 mM KH_2PO_4	7.45	5.82	0.32	0.015	86.09	22.66	72.75	11.05
LSD ($P = 0.05$)	0.321	0.426	0.023	0.0009	2.108	3.466	9.351	0.333
S × P								
FT + no priming	3.73	4.20	0.29	0.006	70.19	13.89	49.33	13.04
FT + water-priming	3.56	3.80	0.30	0.005	67.53	18.29	59.00	12.24
FT + 100 mM KH_2PO_4	6.44	5.83	0.31	0.012	83.91	25.18	69.50	11.46
FT + 300 mM KH_2PO_4	7.02	6.09	0.32	0.014	84.81	28.99	71.17	11.14
FT + 500 mM KH_2PO_4	7.19	5.60	0.32	0.014	85.43	21.74	71.00	11.06
ST + no priming	5.58	5.03	0.34	0.010	77.90	12.53	57.50	12.05
ST + water-priming	5.71	5.09	0.30	0.010	79.46	12.27	61.67	11.80
ST + 100 mM KH_2PO_4	7.20	4.77	0.32	0.014	84.62	22.22	74.33	11.31
ST + 300 mM KH_2PO_4	7.48	6.26	0.32	0.015	86.24	27.53	75.67	11.07
ST + 500 mM KH_2PO_4	7.72	6.04	0.32	0.016	86.75	23.58	74.50	11.04
LSD ($P = 0.05$)	0.456	0.603		0.0015	2.981			0.472
Year (Y)	ns	ns	ns	ns	ns	ns	ns	ns
S	**	*	ns	**	**	ns	ns	**
P	**	**	ns	**	**	**	**	**
S × P	**	**	ns	**	**	ns	ns	*
Y × S	ns	ns	ns	ns	ns	ns	ns	ns
Y × P	ns	ns	ns	ns	ns	ns	ns	ns
Y × S × P	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	7.36	11.41	10.24	11.42	3.69	23.71	19.69	4.35

The asterisks *, **, or ns indicate statistical differences at $P \leq 0.05$, $P \leq 0.01$, or non-significant, respectively. Selection treatments showed emergence below 60% in the previous experiment.

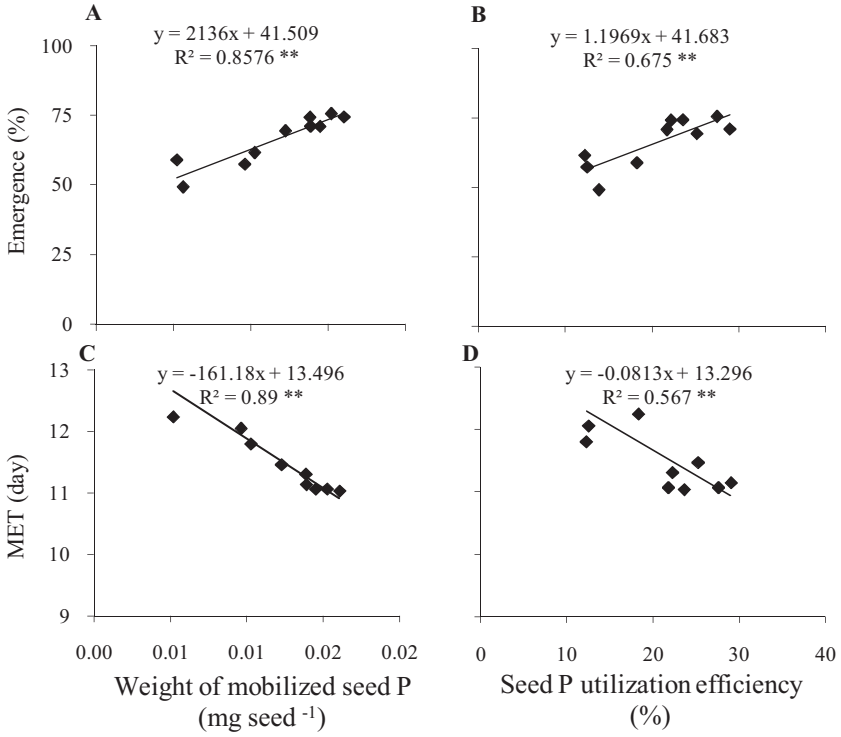

Fig. 6. Relationships between weight of mobilized seed P (WMSP) and emergence (A), seed P utilization efficiency (SPUE) and emergence (B), WMSP and MET, and SPUE and MET. Values are means of eight observations. The asterisks ** indicate statistical differences at $P \leq 0.01$.

4. Conclusion

In arid and semi arid regions, where calcareous soils are abundant and P deficiency is prevalent, biological S oxidation along with P supplying from vermicompost, is one of the most important factors in improving seed physiological aspects. On the other hand, under P deficit conditions, increase in seed coat P content compared with total P is known as a useful mechanism to enhance seed tolerance to unfavorable conditions. It seems, under P deficit conditions, black seed mother plants prefer to allocate more P to the seed coat possibly to produce seeds with improved longevity, while under normal conditions, P is allocated to the embryo to improve seed vigor. In addition, improving nutritional status affects oil percentage and decreases some undesirable fatty acids such as linolenic acid. Since the highest negative correlation was found between linolenic acid and seed vigor ($R^2 = 0.74^{**}$), linolenic acid percentage can be proposed as an index to describe seed vigor. Furthermore, we found that P-priming at 300 mM concentration increases seedling EP and decreases MET through increasing WMSP, WMSR, SPUE and SRUE.

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