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Improving of seed quality of black seed (*Nigella sativa* L.) by increasing seed phosphorus content in a calcareous soil

Seyyed Mohammad Seyyedi[a], Parviz Rezvani Moghaddam[a], Mohammad Khajeh-Hossieni[a], and Hamid Shahandeh[b]

[a]Department of Agronomy, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran; [b]Department of Soil & Crop Sciences, Texas A&M University, College Station, TX, USA

ABSTRACT
Phosphorus (P) sufficiency during seed formation and development can affect the quality of seeds production. For increasing P content of black seed (*Nigella sativa*) in a calcareous soil, series of experiments were conducted in completely randomized factorial design with 4 replications at the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran, in 2012 and 2013. The combinations of vermicompost (V), sulfur (S), and *Thiobacillus* bacteria (T) were mixed with a calcareous soil fertilized with 0, 30 and 60 kg P ha^{-1} in pots and incubated for 63 days. At the end of incubation period, black seeds were sown in the pots and plants were grown to maturity. Results showed that S+T and V treatments were significantly increased soil available P, emergence, plant P content, seed yield and vigor of the seeds production. There were positive relationship between soil and plant P concentration with the quality of seeds production.

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KEYWORDS
Mean emergence time; nutrient priming; sulfur; radicle emergence test; seed vigor

Introduction

Black seed (*Nigella sativa* Linn.) as an annual herbaceous plant from the Ranunculaceae family is grown in Mediterranean countries and western part of Asia, particularly in arid and semi-arid regions of Iran (D'Antuono et al., 2002; Ghamarnia et al., 2010). It is well known for its pharmacological values as a natural remedy for some illnesses including asthma, hypertension, diabetes, dizziness, rheumatism, cough, headache, fever (Ali and Blunden, 2003; Mehta et al., 2009), and antimicrobial and antioxidant activities (Burits and Bucar, 2000; Erkan et al., 2008; Mehta et al., 2009). The seeds are also used as condiment in cousins in East Asia and Mediterranean regions.

The seed quality can be influenced by the availability of nutrient presented in the soil during the growth and the development of mother plant to produce seeds for the next generation (Sawan et al., 2011; Krueger et al., 2013). Phosphorous (P) is one of the most limiting nutrients stored in seeds that is needed for a successful plant establishment and subsequently higher yield (Modi, 2002; Sawan et al., 2011; White and Veneklaas, 2012).

Black seed is generally grown in alkaline and calcareous soils, where P availability is low due to the formation of soluble calcium phosphate minerals (Korkmaz et al., 2009; Soaud et al., 2011; Tuncturk et al., 2011). Therefore, black seed quality and production can be severely affected by P deficiencies in the soils (Mohamed et al., 2000; Sameni and Kasraian, 2004; Tuncturk et al., 2011) in which it can be improved by numbers of fertilizer P management strategies. For example, applying organically complex P in the form of organic fertilizer has been frequently practiced for increasing P in calcareous soils

CONTACT Parviz Rezvani Moghaddam rezvani@um.ac.ir Department of Agronomy, Faculty of Agriculture, Ferdowsi University of Mashhad, P.O. Box 91779-8974, Mashhad, Iran.

(Mohammady Aria et al., 2010; Hosseinpur et al., 2011; Shah et al., 2013). In addition, an application of sulfur (S) with oxidizing bacteria has also shown to decrease soil alkalinity and consequently increase P availability (Salimpour et al., 2010; Heydarnezhad et al., 2012).

In nutrient deficient soils, seed nutrient priming with solutions containing limited nutrient is another technique that more recently has been used to enhance seed vigor and emergence (Al Mudaris and Jutzi, 1999; Ajouri et al., 2004; Imran et al., 2013). For example, priming of barley (*Hordeum vulgare* Linn.) (Ajouri et al., 2004), okra (*Abelmoschus esculentus* L.) Moench) (Shah et al., 2011) and mung bean (*Vigna radiata* Linn.) (Shah et al., 2012) seeds with P nutrient significantly improved seed germination and emergence.

The objective of this research was to investigate the possibility of increasing seed P content of black seed grown in a calcareous soil by increasing P availability in the soil through addition of different soil amendments and seed P priming. The Increasing P content of the seed could enhance the growth and the quality of produced seeds.

Material and methods

The study was conducted at series of laboratory and greenhouse pot experiments at Faculty of Agriculture, Ferdowsi University of Mashhad, Iran, between October 2012 and May 2013. A clay calcareous soil was collected from depth of 0—30 cm (Table 1) from the Farm of the Faculty (36°15′ N; 59°28′E). The collected soil was sieved (2 mm) and prepared for incubation with different soil treatments in pots with 2 kg volumes and seeded with black seeds obtained from the local market.

Experiment one

The Effects of elemental S, *Thiobacillus* bacteria (T), and cow vermicompost (V) on enhancing P availability of a calcareous soil was studied in this experiment. The calcareous soil was fertilized with diammonium phosphate at rates of 0, 30 and 60 kg P ha^{-1} and amended with V, S at 10, and 20 t ha^{-1}, respectively. The different combinations of V, S, V+S, V+T, S+T, V+S+T and P rates were prepared. The amended pots were completely randomized factorial design with four replications. Pots soil were periodically mixed and kept at alternative temperature of 25/ 17°C (day/ night) and 2/3 of water field capacity (FC) for 63 days.

In the incubation period, soil samples were collected from pots at intervals of 0, 21, 42, and 63 days and analyzed for available P (Olson-P, Spectrophotometer, Jenway Model 4510, Bibby Scientific Limited, Stone, UK), pH (744 pH meter, Metrohm Ltd., Herisau, Switzerland), and electrical conductivity (EC) (model Jenway 4310).

Experiment two

Pots were fertilized with urea at rate of 60 kg nitrogen (N) ha^{-1} at the end of incubation period (63 days). Applied N was corrected for N added from diammonium phosphate fertilizer. Seeds of black seed were planted in pots and pots brought to FC. Plants were thinned to four plants in each pot two weeks after sowing. Pots were irrigated periodically to FC by weighing. Seeds of black seed plants were harvested at maturity, 85 days after sowing. 1000-seed weight, seed weight per plant and seed P content of the harvested seeds were determined. Plant and soil were also analyzed for plant P content (spectrophotometer, Jenway Model, 4510), and soil available P, pH and EC.

Germination and vigor tests were performed (on Whatman filter paper in 9-cm Petri dishes) using standard germination test (with 2 mm radicle growth) and radicle emergence (RE) test (ISTA, 2012).

Table 1. Some physical and chemical properties of soil and cow vermicompost used in the experiment.

Sample	Clay (%)	Silt (%)	Sand (%)	Total P (%)	Olsen-P (mg kg^{-1})	pH (1:2)	EC (dS/m)	$CaCO_3$ (%)
Soil	48.46	31.95	19.59	0.056	10.59	8.39	1.75	11.17
Cow vermicompost	—	—	—	1.13	138.13	8.24	8.15	—

For determine the final germination percentage (FGP), counts of germinated seed were made daily for 14 days in a germinator at 25°C, using four replications of 25 seeds. Mean germination time (MGT) was calculated using the following formula (Khajeh-Hosseini et al., 2009):

$$MGT = \Sigma n.t/\Sigma n$$

Where in: n = number of seeds germinated at each day, t = number of days from beginning of germination.

Seed tests for final mergence percentage (FEP) and mean emergence time (MET) were also performed by sowing 15 seeds in fertilized pots and counting seeds emerged daily for the next 25 days under greenhouse conditions. MET was calculated using the same formula of MGT, but using counts of emergence (Demir et al., 2008; Khajeh-Hosseini et al., 2009).

Experiment three

The effect of seed priming on black seed emergence was tested in this experiment. Seeds in which showed emergence below 60% in the previous experiment were selected. Seeds were primed by soaking in distilled water for 48 hours (water-primed) and in 500 mM monopotassium phosphate monobasic (KH_2PO_4) solution for 12 hours (P-primed). Treated seeds were rinsed with distilled water and allowed to air-dry in the laboratory to around 13% moisture content, monitored by weighing. Seeds were planted in pots and were arranged in completely randomized design with 4 replications of 15 seeds. Seeds in pots were evaluated for FEP and MET for 21 days with similar procedure in the experiment two.

Statistical analyses of the data were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Statistically significant mean values were separated by the Least Significant Difference (LSD) test at 0.05 level of probability.

Results

Experiment one

At all levels of P application rate, changes in soil pH for all treatments were similar to soil pH, decreased initially and then stayed relatively constant throughout the incubation period except in control when soil pH remained constant (Figure 1). The highest drop in pH occurred in S treated soil with or without V, T or V + T treatment. The pH of S treated soil reduced 0.9 after 21 days of incubation, as compared to control (Figure 1). Addition of V by itself did not change soil pH significantly but when applied with T, soil pH lowered by 0.6. Higher oxidation activity of bacteria in V treatments could be related to readily available source of carbon in organic fertilizer. However, the effect of T in oxidation of S and S related compounds were short lived and almost ended after 21 days after incubation. High buffer capacity of calcareous soil used probably limited the oxidation reaction.

Soil P increased significantly with addition of soil amendments of V, S, T, or their combinations though soil available P of control remained constant during the incubation period (Figure 1). The largest increase in available P was observed at 21 days of incubation with V treatment. For example, addition of V, V+S, V+T, and V+S+T almost quadrupled the amount of Olson-P in soil as compared to control (Figure 1). Addition of S and S+T to the soil also increased soil available P but not as much as V treatment. Application of S almost tripled the EC of the soil (by 1895 $\mu S\ cm^{-1}$) (Table 2).

Experiment two

The results of soil amendments at different P rates for increasing black seed production and seed quality are presented in Table 3. In general, regardless of P application rate, quality of black seed produced in V treated soil was significantly higher than other soil P treatments. For example, at different P rates, the highest significant effects on increasing the seed weight per mother plant was observed by V+S+T

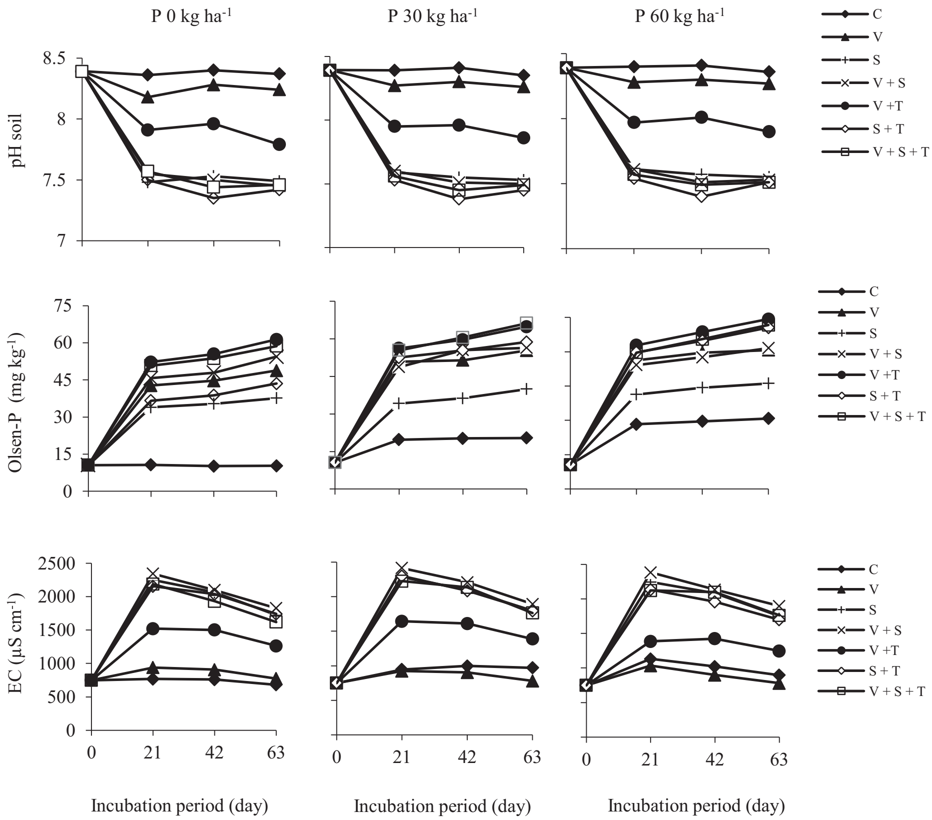

Figure 1. Effects of soil P amendment at three levels of P application on soil pH and EC and Olsen-P during incubation period.

Table 2. Effects of soil P amendment at three levels of P application on soil pH and EC and available P at harvest day of black seed (148 days after onset of incubation time).

Soil P treatments			
Rate (kg ha^{-1})	Amendments	pH	EC (μS cm^{-1})
0	Control (C)	8.17	711
	Vermicompost (V)	8.04	837
	Sulfur (S)	7.62	1592
	V + S	7.61	1574
	V + *Thiobacilus* bacteria (T)	7.89	1081
	S + T	7.53	1595
	V+ S+ T	7.63	1451
30	C	8.17	710
	V	8.04	834
	S	7.62	1547
	V + S	7.61	1578
	V + T	7.86	1128
	S + T	7.56	1564
	V+ S+ T	7.61	1431
60	C	8.17	714
	V	8.06	883
	S	7.61	1601
	V + S	7.57	1568
	V + T	7.64	1212
	S + T	7.52	1504
	V+ S+ T	7.57	1393
	LSD (5%)	0.12	92.65

Table 3. The effects of soil P treatments on some quality of characteristics of black seed.

Soil P treatments Rate (kg ha^{-1})	Amendments	1000-seed weight (g)	Seed weight per plant (mg)	P concentration (g kg^{-1})	P uptake (mg)	Final germination (%)	Normal seedling (%)	*RE test (%)	MGT (day)	Final emergence (%)	MET (day)
0	Control (C)	2.28	42.62	2.83	0.51	90.0	55.0	42.5	6.10	17.8	13.50
	Vermicompost (V)	2.60	64.07	3.35	1.14	92.5	70.0	57.5	5.17	35.6	10.94
	Sulfur (S)	2.33	58.74	3.14	0.87	90.0	62.5	45.0	5.55	31.1	10.90
	V + S	2.56	78.60	3.57	1.55	90.0	87.5	75.0	4.40	71.1	9.99
	V + *Thiobacilus* bacteria (T)	2.61	79.96	3.50	1.56	90.0	90.0	70.0	4.48	68.9	6.96
	S + T	2.40	59.20	3.17	1.01	92.5	80.0	62.5	5.14	31.1	10.63
	V+ S+ T	2.58	94.65	3.63	1.78	90.0	87.5	82.5	4.40	82.2	10.26
30	C	2.35	61.09	2.97	0.74	92.5	80.0	67.5	5.31	35.6	10.69
	V	2.60	86.67	3.36	1.38	90.0	87.5	82.5	4.50	60.0	9.48
	S	2.45	69.33	3.43	1.15	90.0	87.5	85.0	4.55	53.3	9.71
	V + S	2.62	97.81	3.72	1.84	97.5	87.5	97.5	4.44	68.9	9.23
	V + T	2.64	99.60	3.57	1.81	90.0	87.5	77.5	4.64	75.6	9.02
	S + T	2.53	71.68	3.37	1.25	95.0	87.5	82.5	4.66	62.2	9.68
	V+ S+ T	2.69	105.04	3.70	1.93	90.0	90.0	90.0	4.44	80.0	8.89
60	C	2.45	62.79	3.00	0.79	92.5	72.5	67.5	5.07	55.6	11.09
	V	2.67	94.36	3.39	1.64	90.0	87.5	75.0	4.59	68.9	9.39
	S	2.49	76.26	3.37	1.24	92.5	87.5	80.0	4.62	55.6	9.18
	V + S	2.65	96.39	3.73	1.84	92.5	92.5	92.5	4.36	75.6	9.28
	V + T	2.62	92.65	3.57	1.55	92.5	92.5	87.5	4.43	75.6	9.23
	S + T	2.55	84.89	3.40	1.47	90.0	87.5	80.0	4.59	60.0	8.80
	V+ S+ T	2.66	101.85	3.76	1.79	90.0	90.0	90.0	4.33	77.8	9.17
	LSD (5%)	0.04	4.77	0.11	0.14	9.59	7.96	13.40	0.30	6.20	0.68

*: Germination after 5 days of setting the test.

treatment (approximately 70%), as compared to control, in which accompanied by significant increasing for 1000 seed weight and P concentration and content in the mother plant (Table 3). On the other hand, addition of S or T individually or in combination with V treatment, improved the seed production of black seed differently. There was no significant difference in seed quality between addition of S or T, but there was a significant difference among V treatments when S and T was added (Table 3).

Similar to seed production, the seed quality of characteristic of black seed (normal seedlings, MGT, FEP and MET) were significantly affected in response to P supply by V only or in combined to S oxidation, expect for FGP (Table 3).

There was a positive and significant relationship between soil available P and P concentration in black seed plant ($R^2 = 0.73^{**}$) (Figure 2). This relationship that represented data calculated from all

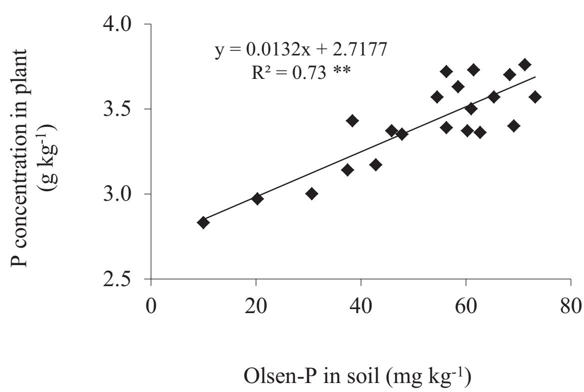

Figure 2. Relationships between available P in soil and P concentration in black seed plant.

soil P treatments could also be extended to seed quality characteristics measured (FGP, FEP, MGT and MET). For instance, when P concentration in black seed plant was related to seed characteristics, except for FGP, there was a significant correlation among other characteristics (Figure 3). The relationship between plant P concentration and MGT and MET were negative while for 1000- seed weight, seed weight per plant, percentage of normal seedlings and FEP were positive (Figure 3).

Seed vigor was also assessed by RE test in this experiment for further evaluation of the effects of P on mother plant on the quality of producing seeds (Figure 4). The relationship between plant P concentration and germination percentages after 4 to 12 days of the germination test are shown in Figure 4.

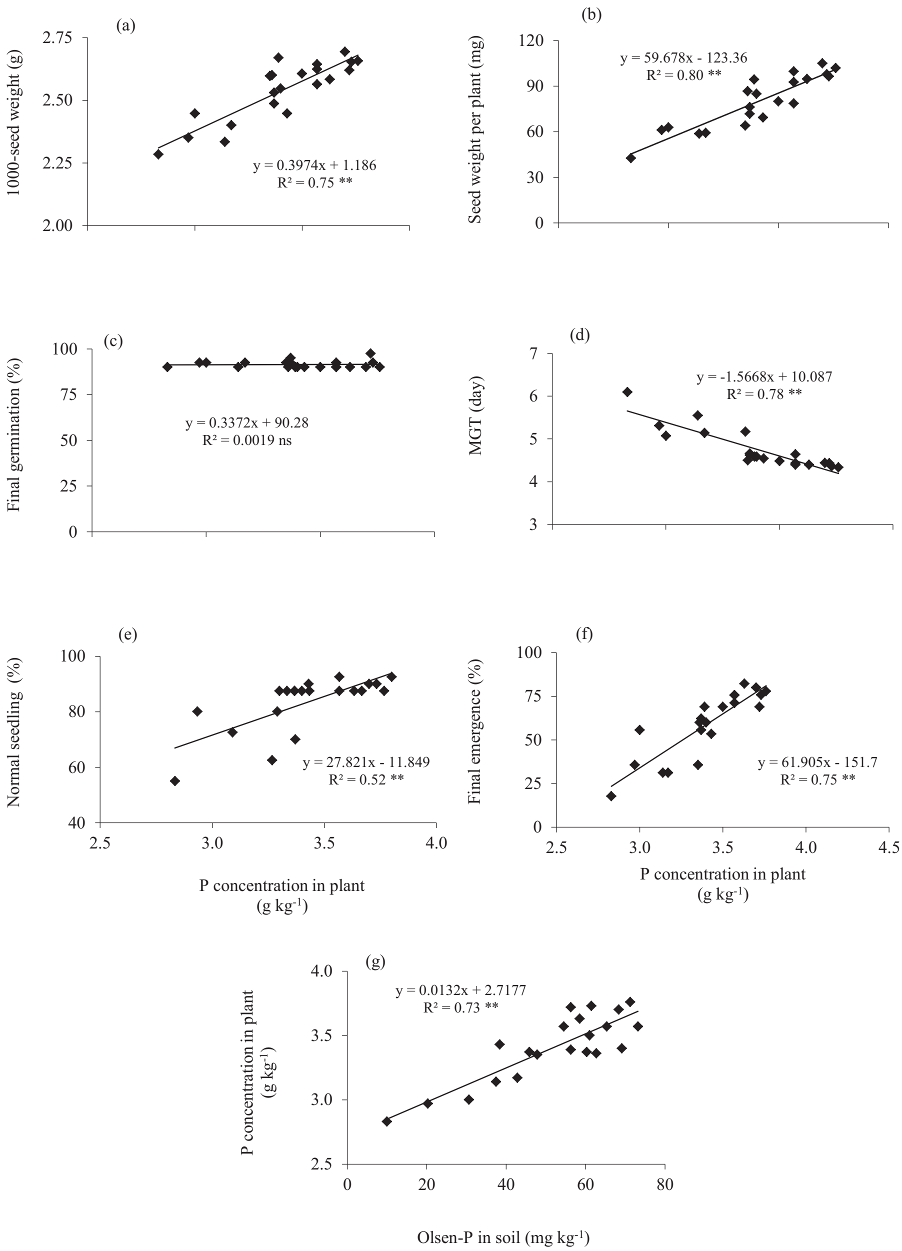

Figure 3. Relationships between P concentration per mother plant and A) 1000- seed weight, B) seed weight per mother plant, C) germination (%), D) mean germination time (MGT), E) normal seedlings (%), F) final emergence (%) and G) mean emergence time (MET).

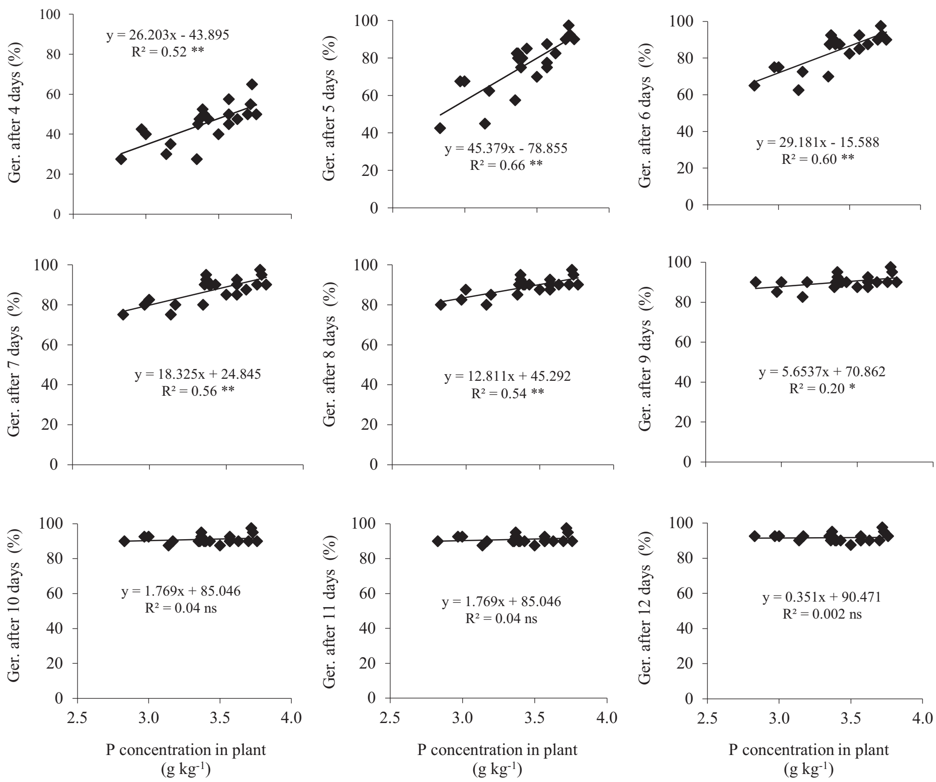

Figure 4. The evaluation of seed vigor (radicle emergence test) affecting by P concentration in mother plant.

There was a positive and significant relationship between plant P concentration and germination from four to nine days after germination test but in later days it was no significant. The highest correlation was observed after five days ($R^2 = 0.66^{**}$).

FGP in 5 days after seeding was used as seed vigor test and related to MET, FEP, and 1000 seed weight (Figure 5). There was a positive and significant relationship between seed emergence qualities and seed vigor influenced by P status of black seed. It can be also indicating the successful evaluation of black seed vigor based on RE test.

Experiment three

The effect of black seed priming on seed emergence is shown in Table 4. The seed treatments which were selected for priming all had emergence below 60% in the previous experiment. Seed P- priming

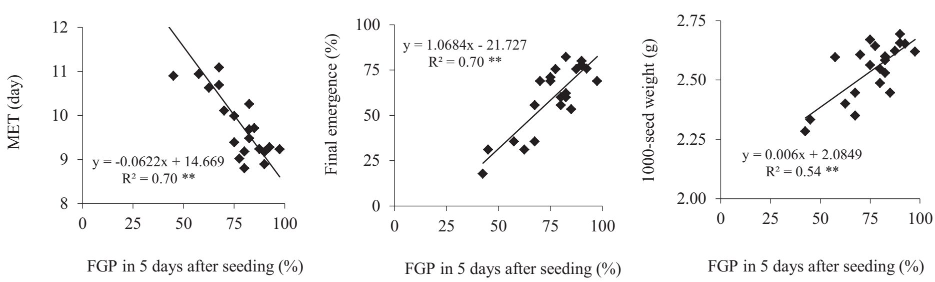

Figure 5. The relationship between seed vigor (final germination percentage (FGP) in 5 days after seeding) and A) mean emergence time (MET), B) final emergence (%) and C) 1000-seed weight.

Table 4. The effects of soil P and seed priming treatments on final emergence percentage and mean emergence time (MET).

Soil P treatments	Seed priming treatment	Final emergence (%)	MET (day)
Control (C)	Unprimed	17.8	13.50
	Water-primed	20.0	13.56
	P-primed	42.2	12.60
Vermicompost	Unprimed	35.6	10.94
	Water-primed	33.3	10.84
	P-primed	48.9	10.81
Sulfur (S)	Unprimed	31.1	10.82
	Water-primed	35.6	10.57
	P-primed	51.1	9.71
S + *Thiobacilus* bacteria	Unprimed	31.1	10.55
	Water-primed	33.3	10.60
	P-primed	62.2	10.43
C + 30 kg P ha^{-1}	Unprimed	35.6	10.69
	Water-primed	37.8	10.29
	P-primed	55.6	10.29
S + 30 kg P ha^{-1}	Unprimed	53.3	9.71
	Water-primed	55.6	9.96
	P-primed	64.5	9.49
C + 60 kg P ha^{-1}	Unprimed	55.6	11.09
	Water-primed	55.6	11.04
	P-primed	68.9	9.78
S + 60 kg P ha^{-1}	Unprimed	55.6	9.18
	Water-primed	57.8	9.42
	P-primed	71.1	9.05
LSD (5%)	—	5.46	0.78

resulted in higher and faster emergence (shorter MET). The largest difference among P primed and non-primed soil P treatment was observed in control treatment, where P-priming almost tripled the FEP of the seeds in the control. P-priming affected the black seed emergence in S and T treated soil with or without soil P fertilization (Table 4).

However, among all treatments (experiment two), P 30 kg ha^{-1} + V+ S+ T showed highest soil emergence of 80% and shortest MET of 8.89 days. Therefore, under conditions of soil P deficiency, it may be concluded that increasing the soil available P can be considered as an important strategy to produce the high seed quality of black seed, as compared to P priming treatment of produced seeds.

Discussion

Experiment one

The increase in P content of V treated soil can be related to initial rich P concentration of V and in S treated soil to acidification and release of bonded or partially fixed P in calcareous soil (Vidyalakshmi et al., 2009; Mohammady Aria et al., 2010). For example, with similar V application rates in V+S+T, V+T, and V treatments, the pH of the soil after 21 days of incubation were 7.42, 7.79, and 8.18 respectively. Similar results on effects of S and compost in lowering pH and increasing P availability in calcareous soil as have been reported by Lopez-Aguirre et al. (1999) and Heydarnezhad et al. (2012).

In no P application conditions, Olsen-P was higher in S treatment (38 mg kg^{-1}) compared to 30 and or 60 kg P ha^{-1} (20 and 31 mg kg^{-1}, respectively) 63 days after oxidation. This is an indication that P applied in previous years had been inverted by calcium carbonate ($CaCO_3$) in soil and it became available with lowering soil pH.

Application of S or V to soils was also increased soil salinity as determined by EC in this experiment. Similar results had been found by Heydarnezhad et al. (2012) when 10 t S ha^{-1} was applied to a calcareous soil. However, the increase in soil EC probably may not affect the potential black seed germination in the experiment. Salinity threshold value for black seed germination and growth was reported to be 2 dS m^{-1} (Ghamarnia et al., 2012).

Experiment two

Results of experiment two supported the previous incubation findings that V+S+T treatment had been the highest effect on P availability. Similar results have been reported that application of S to soil increased P availability, yield, and seed quality of soybean (Glycine max Linn.) (Mostafavian et al., 2008).

P plays a certain role in chlorophyll biosynthesis, cell division and development of meristematic tissues and also is required for the production of key molecules such as adenosine triphosphate (ATP) (Schachtman et al., 1998). Seed P reserving, the only P source available to germination and faster growth of initial root, are mobilized and translocated to seedling tissues (White and Veneklaas, 2012). Pacheco et al. (2012) stated the seeds enriched with P can be considered as a strategy for improving growth and grain yield of common bean (*Phaseolus vulgaris* Linn.). Similar results on effect of P in improving the seed quality characteristics had been reported by Modi (2002), Bishnoi et al. (2007) and Sawan et al. (2011). Therefore, considering the high significant correlation between Olsen-P and P concentration in mother plant can be concluded that increasing soil-P availability is a main approach to improve the seed quality characteristics of black seed in a P-deficient calcareous soil.

Experiment three

In P deficiency condition during seed formation and development, the quality of produced seed can be improved by seed priming strategies, such as "nutrient priming." Ajouri et al. (2004) reported that P priming can improve the germination and early seedling growth of barley (*H. vulgare*) as a result of increasing seed P concentration. Shah et al. (2012) stated that the significant effects of seed priming with P solutions on increasing P concentration, dry weight and height of mung bean (*V. radiata*) seedlings can be due to optimum availability of P to seedling immediately after emergence. These findings support our results.

Conclusions

The P deficiency during seed formation and development can affect the quality of produced seeds. To supply sufficient P in mother plant of black seed grown in calcareous different strategies were used. Soil fertilization with P was not adequate and addition of amendments like V, S and T were required. The best selection among soil P treatments was addition of V in combination of S. The produced seeds from different soil P treatments were further evaluated by the seed quality characteristics. The state of available P in the soil and seeds of mother plant should be considered as a quality indicator for successful seed production. High correlation between FGP in 5 days after seeding with MET and FEP, RE test may be an appropriate indicator to evaluate the *Nigella sativa* seed vigor as approved for maize by ISTA.

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