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Influence of phosphorus and soil amendments on black seed (*Nigella sativa* L.) oil yield and nutrient uptake

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ABSTRACT

In order to investigate the effects of sulfur (S) oxidation and vermicompost (V) application on black seed (*Nigella sativa* L.) oil yield and its nitrogen and phosphorus uptake, a two-year field experiment was conducted at Faculty of Agriculture, Ferdowsi University of Mashhad, Iran in a calcareous soil during 2013 and 2014. A randomized complete block design arranged in factorial with four replications was used to analyze 12 treatments. The calcareous soil amendments (control, V + *Thiobacillus thiooxidans* (T), S + T and V + S + T) and three levels of P (0, 30 and 60 kg ha^{-1}) were considered as the first and the second experimental factors, respectively. All calcareous soil amendments (V+ T, S+ T and V + S + T) caused a significant reduction in soil pH, and a significant increase in electrical conductivity (EC). In addition, calcareous soil amendments showed a considerable effect on soil available P content. The highest grain, oil and essential oil yields were obtained from V + S + T treatment. The soil amendments significantly increased N and P concentration in plants and improved P harvest index. Although a positive correlation was observed between P and N concentration, there was a negative correlation between grain yield and P use efficiency. It seems that any approach that would allocate more P to seeds, during seed development on the mother plants, would increase final yield in black seed.

1. Introduction

Black seed (*Nigella sativa* L.), an annual flowering plant from the Ranunculaceae family, is often cultivated in semi-arid areas (D'Antuono et al., 2002), especially, in Iran (Ghamarnia et al., 2010). The black seed distribution is extended from southern and eastern-rim of the Mediterranean basin countries such as Egypt and Turkey to India (Atta, 2003; Gharby et al., 2015; Tuncturk et al., 2011). The black seed has been used as a medicinal plant because of its specific anti-microbial, anti-tumor and anti-inflammatory properties (Ali and Blunden, 2003; Salem, 2005). In addition, powerful antioxidant compounds in the seeds have been documented (Erkan et al., 2008). Black seed oil is considered as a new source of edible oil (Piras et al., 2013).

Phosphorus (P) is one of the most influential factors to improve the performance and quality of black seed (Mohamed et al., 2000; Rana et al., 2012; Shirmohammadi et al., 2014). According to the results of Tuncturk et al. (2011), in P deficient soils, the recommended dose of P for obtaining maximum grain yield is 30–40 kg ha^{-1}. However, soils with high amount of calcium carbonate often exhibit low P availability, which is one of the most important problems in crop nutrition, especially, in arid and semi-arid regions of Iran (Sameni and Kasraian, 2004a,b). Hence, the P uptake by black seed might be affected by excessive amounts of calcium carbonate in soil.

Calcareous soil is defined as "having the presence of significant quantities of free excess lime (calcium or magnesium carbonate) with pH greater than neutral, typically 7.5–8.5" (Hopkins and Ellsworth, 2005). In some cases, the low P uptake in calcareous soils does not mean a low level of P in the soil, in other words, due to formation of poorly soluble P complexes with calcium, available P can become unavailable as calcium phosphate (Hopkins and Ellsworth, 2005; Ozturk et al., 2005). In highly calcareous soils of Iran, calcium carbonate equivalent reaches up to 650 g kg^{-1} (Adhami et al.,

Abbreviation: PAE, phosphorus acquisition efficiency; PUTE, phosphorus utilization efficiency; PUE, phosphorus use efficiency; PHI, phosphorus harvest index.

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2006). Low P solubility under such conditions may decrease P use efficiency (PUE) (Korkmaz et al., 2009).

In general, PUE represents the amount of dry matter produced per unit of absorbed P, in other words, PUE describes plants' capability to P absorption, transportation and internal utilization (Manske et al., 2001; Shenoy and Kalagudi, 2005). In this regard, P acquisition efficiency (PAE) (uptake of P from soil) and P utilization efficiency (PUTE) (productivity per unit P taken up) are considered as PUE components (Bayuelo-Jiménez and Ochoa-Cadavid, 2014). Therefore, PUE in alkaline or calcareous soils highly depends on the plants ability to uptake P from the soil and consequently on its acquisition efficiency.

Using some approaches such as application of sulfur (S) (Handreck, 1986), S-oxidizing bacteria particularly *Thiobacillus thiooxidans* (T) (Pith Otero et al., 1995; Salimpour et al., 2010; Vidyalakshmi et al., 2009) and supplying P from organic sources (Hosseinpur et al., 2011) such as vermicompost (V) (Doan et al., 2013; Mohammady Aria et al., 2010) can improve P availability and organic matter content in soil, and promote sustainable production of black seed in arid and semi-arid regions of Iran. Sulfur oxidation by *T. thiooxidans*, an obligate chemolithotroph, is the most important step of S cycle, which can improve P availability by plants grown in calcareous soils (Vidyalakshmi et al., 2009). In brief, sulfuric acid derived from S oxidation, reacts with calcium carbonate and produces calcium sulfate. As a result of this reaction, phosphorus solubility in soil increases (Sameni and Kasraian, 2004a; Ullah et al., 2013). Moreover, due to positive correlation between S application and nitrogen (N) uptake (Fismes et al., 2000; Salvagiotti et al., 2009), Olsen-P and N mineralization (Parfitt et al., 2005) and also P and N uptake (Duan et al., 2004), S biological oxidation may promote P solubility and N uptake in calcareous soil.

The current experiment was aimed to evaluate the effects of S, V, and T on soil chemical characteristics and black seed yield and yield components. In addition, P and N uptake, PUE and P harvest index (PHI) were examined in response to experimental treatments.

2. Material and methods

2.1. Site description

The experiment was conducted at the experiment station of Faculty of Agriculture, Ferdowsi University of Mashhad (latitude: 36°15′N; longitude: 59°28′E; elevation: 985 m) during 2013 and 2014 growing season. Experimental site was located in a semi-arid region, Khorasan Province, Northeast of Iran. Total precipitation in 2013 and 2014 (From March to July) was 112.30 and 168.80 mm, respectively. During this period, average temperature was 20.53 °C for 2013 and 20.41 °C for 2014. The physical and chemical soil characteristics of the experimental site are presented in Table 1.

Table 1
Main physical and chemical characteristics of the studied soil.

Soil analysis	2013	2014
Physical		
Clay (%)	48.46	49.11
Silt (%)	31.95	34.21
Sand (%)	19.59	16.68
Chemical		
Organic carbon (%)	0.33	0.39
Total N (%)	0.08	0.08
Olsen-P (mg kg^{-1})	10.59	6.71
Available K (mg kg^{-1})	173.36	184.68
pH	8.39	8.48
EC (dS m^{-1})	0.75	0.73
$CaCo_3$ (%)	11.17	14.91

Soil texture class (0–30 cm): clay.

2.2. Experimental design

A randomized complete block design arranged in factorial with four replications was used. The calcareous soil amendments (control, V (10 t ha^{-1}) + T, micronized S (20 t ha^{-1}) + T and V + S + T) and three levels of P (0, 30 and 60 kg ha^{-1}) were the first and the second experimental factors, respectively.

2.3. Agronomic practices

The land lied fallow before both years of the experiment. Seed bed was prepared using plough and disk. Plots were designed with 3 m long and 2 m width, 0.5 m apart each other. Between blocks, 1 m alley was kept to eliminate the influence of lateral water movement. V (pH 7.59, organic carbon (OC) 34.76%, total N 2.31%, and total P 1.78%) and S 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}, respectively (from urea fertilizer). Furthermore, 30 kg N ha^{-1} from urea was applied as top dress at the six true-leaf stage.

Seed sowing was performed (19th March in 2013 and 15th March in 2014) on both sides of the furrows. Seedlings were thinned at the four-true leaf stage to 200 plants m^{-2}. The first irrigation was done after seed sowing with weekly irrigation until physiological maturity stage. Weeds were removed by hand during growing seasons.

2.4. Data collection

At flowering stage, soil samples were collected from each plot separately (0–30 cm depth) and analyzed for pH (pH meter, METROHM model 632), EC (EC meter, JENWAY model 4310) and P content (Olsen method, using spectrophotometer, Jenway Model, 4510). Plant height, leaf area index and plant dry weight were determined using eight plants randomly from each pot. In addition, P and N concentration in plant were determined (Murphy and Riley method using Spectrophotometer, Jenway Model, 4510 and Kjeldahl method using Kjeltec-PECO-Psu 55, respectively).

At maturity stage, eight plants from each plot were selected randomly and plant height and follicle number per plant were recorded. Final biological and grain yields were measured by harvesting 2 m^2 of the central part of each plot. In addition, oil and essential oil percentages were determined.

PAE, PUTE, PUE and PHI were calculated using following equations:

$$PAE(\%) = \left(\frac{Pt}{Pa}\right) \times 100 \quad (1)$$

$$PUTE(kg\ grain\ kg^{-1}Pt) = \frac{GY}{Pt} \quad (2)$$

$$PUE(kg\ grain\ kg^{-1}Pa) = \frac{GY}{Pa} \quad (3)$$

$$PHI\ (\%) = \frac{Pg}{Pt} \times 100 \quad (4)$$

Pt = kg of P in the total plant per m^2 at maturity stage; Pa = kg of soil available P per m^2; GY = kg of grain produced per m^2; Pg = kg of grain P per m^2.

Soil available P content (at maturity stage) was determined based on soil bulk density of each plot at the depth of 30 cm. Soil pH, EC and available P content of the soil were also determined.

2.5. Statistical analyses

All data were subjected to analysis of variance (ANOVA) using SAS 9.3 software (SAS, 2011). When *F* test indicated statistical significance at $P < 0.01$ or $P < 0.05$, the least significant difference (LSD) was used to separate the means.

3. Results and discussion

3.1. Soil characteristics as influenced by P levels and soil amendments at flowering stage of black seed

The effect of calcareous soil amendments (V+T, S+T and V+S+T) and P fertilizer rates on soil characteristics and black seed traits are shown in Table 2. Although the effect of P fertilizer was not significant on soil pH and EC, calcareous soil amendments showed a significant effect on traits as indicated in Table 2. All calcareous soil amendments (V+T, S+T and V+S+T) caused a significant reduction in soil pH, while significantly increased EC (Table 2). For instance, S+T and V+S+T treatments decreased soil pH by 11.22 and 7.79%, respectively. Similarly, soil EC increased by 0.55 and 0.37 dS m^{-1} due to S+T and V+S+T treatments, respectively (Table 2). These changes might be due to S biological oxidation, which leads to sulfuric acid generation (Vidyalakshmi et al., 2009). Sulfuric acid, the chemical which cause low pH, more P solubility and increased soil EC, reacts with calcium carbonate to form calcium sulfate (Heydarnezhad et al., 2012; Sameni and Kasraian, 2004a). Soil pH reduction and increased P solubility on account of S application have been previously reported by others (Modaihsh et al., 1989; Mohammady Aria et al., 2010; Soaud et al., 2012).

Due to S oxidation and calcium sulfate formation, before using any S-based product in calcareous soils, initial soil EC and damage threshold value in plant should be taken into account. In general, a damage threshold value in black seed was determined at 2 dS m^{-1} (Ghamarnia et al., 2012). Therefore, it appears that an increase in soil EC up to 1.2 dS m^{-1} (Table 2) will not affect the black seed growth and yield.

According to Table 2, soil available P content significantly increased with increasing P application. In addition, all calcareous soil amendments showed a considerable effect on soil available P content. For instance, under no P fertilizer application, V+S+T treatments increased soil available P more than four times, compared with control treatment (Table 2). In this regard, Soaud et al. (2012) reported that application of S decreases soil pH and improves soil available P content, 32 days after applying. On the other hand, the effect of V+S+T on increasing the available P content was more pronounced compared with V+T and S+T treatments (Table 2).

In addition to positive effect of S oxidation on soil available P content (Modaihsh et al., 1989; Ullah et al., 2013), V can also be effective in improving soluble P (Mahmoud and Ibrahim, 2012). In this regard, application of V as an organic source of P (Valdez-Pérez et al., 2011), supplies carbon for S-oxidizing bacteria (Mohammady Aria et al., 2010) and produces carbon dioxide and some organic acids such as citric acid during decomposition process (Biswas and Narayanasamy, 2006). Therefore, it can be effective for increasing soil available P content. Effective role of V in reducing soil pH and increasing available P has also been reported by Parthasarathi et al. (2008).

3.2. Black seed traits as affected by P levels and soil amendments at flowering stage

According to the results, V+T, S+T and V+S+T treatments increased plant height, leaf area index and dry matter production compared with control treatment (Table 2). Irrespective of P fertilizer application, the highest plants with the highest leaf area index and dry matter were obtained when V+S+T treatment was applied. Increase in plant dry weight due to application of cattle manure amended with S and T have been reported by Yadegari and Barzegar (2011) in lemon balm (*Melissa officinalis* L.).

As mentioned before, in calcareous soil, application of V helps to create a healthy root system by providing nutrients for plants, supplying energy for bacteria and producing carbon dioxide (Mohammady Aria et al., 2010) and organic acids which are able to reduce soil pH (Biswas and Narayanasamy, 2006).

P and N concentration in plant (g kg^{-1}) and P and N content per plant (g m^{-2}) increased due to V+T, S+T and V+S+T treatments (Table 2). For instance, application of S+T and V+S+T increased P concentration in plants by 20.35 and 48.49%, respectively, compared with control treatment (Table 2). Such results, not only indicate high potential of the soil (in terms of calcium carbonate) in P fixation, but also demonstrate that S-oxidizing bacteria play an important role in pH regulation and improvement of soil available P content (Soaud et al., 2012). On the other hand, the highest N content per plant (11.47 g m^{-2}) was observed by applying V+S+T in combination with 60 kg ha^{-1} P (Table 2).

The preference of V+S+T treatment compared with V+T or S+T in terms of N and P content per plant (Table 2) indicate positive effect of integrated application of organic fertilizers and S.

There was a positive correlation between soil available P content with P concentration in plants (Fig. 1A) and soil available P content with N concentration in plants (Fig. 1B). Similar correlations were found between P and N concentration in plants (Fig. 2A), P content per plant and N concentration in plants (Fig. 2B), P concentration in plant and N content per plant (Fig. 2C) and P and N content per plant (Fig. 2D).

Calcareous soil quality is very closely linked to its chemical properties (Najafi-Ghiri et al., 2013 Zhao et al., 2009). On the other hand, growth and yield in black seed is highly dependent on the availability and balanced uptake of P and N (Rana et al., 2012). Hence, the positive correlation between P and N concentration suggests that calcareous soil amendments application and pH reduction could improve soil P availability, promote N uptake and improve growth in black seed. These results are in agreement with those of Duan et al. (2004) who found a positive correlation between P and N uptake in spring wheat. Similar results have been reported by Parfitt et al. (2005). Koocheki and Seyyedi (2015) have found a positive and significant correlation between PAE and NAE as well as between PHI and NHI in saffron (*Crocus sativus* L.). Furthermore, the effectiveness of S to improve N uptake in wheat has been reported by Salvagiotti et al. (2009).

3.3. Effects of P levels and soil amendments on soil characteristics at black seed maturity stage

Similar to flowering stage, calcareous soil amendments showed a significant effects on soil pH, EC and soil available P content (Table 3). The S+T treatment revealed the strongest effects on pH reduction and EC enhancement. In addition, the highest soil available P content was observed in V+S+T treatment (Table 3).

The pH reduction, at the end of the growing season, on account of calcareous soil amendments might be due to S application along with V, which promote S biological oxidation and improve P uptake during growing season. However, high buffering capacity of the calcium carbonate in soil may leads to smaller pH swings over time (Sibbet, 1995). Hence, the calcareous soil amendments should be applied regularly in order to maintain the fixed pH, especially in highly calcareous soils (Sameni and Kasraian, 2004b).

Table 2
Effect of phosphorus (P) rate and calcareous soil amendments (A) on some studied traits of soil and black seed at flowering stage.

Treatments	Soil			Black seed						
	pH	EC ($dS\,m^{-1}$)	Olsen-P ($mg\,kg^{-1}$)	Plant height (cm)	Leaf area index	Plant dry weight ($g\,m^{-2}$)	P concentration in plant ($g\,kg^{-1}$)	P content per plant ($g\,m^{-2}$)	N concentration in plant ($g\,kg^{-1}$)	N content per plant ($g\,m^{-2}$)
P ($kg\,ha^{-1}$)										
0	7.98	1.00	15.68	44.57	1.00	749.41	2.84	2.26	8.30	6.46
30	7.99	0.97	19.52	52.63	1.12	871.15	3.00	2.70	9.42	8.31
60	7.99	1.02	21.40	54.22	1.20	939.02	3.05	2.96	9.67	9.12
LSD (*P* = 0.05)	0.070	0.057	0.700	1.965	0.056	56.373	0.114	0.220	0.344	0.568
A										
C	8.47	0.74	9.98	41.33	0.69	530.56	2.31	1.24	8.16	4.44
V + T	8.14	0.83	21.54	54.04	1.28	979.35	3.34	3.29	9.19	9.08
S + T	7.52	1.29	18.32	47.04	1.03	773.06	2.78	2.16	9.32	7.21
V + S + T	7.81	1.11	25.63	59.46	1.42	1129.81	3.43	3.87	9.86	11.12
LSD (*P* = 0.05)	0.081	0.066	0.808	2.269	0.065	65.094	0.131	0.254	0.397	0.656
P × A										
C + 0			5.37	33.63	0.49	361.52				2.52
V + T + 0			18.97	45.00	1.14	820.53				6.81
S + T + 0			15.57	41.00	0.91	683.81				5.79
V + S + T + 0			22.82	58.63	1.45	1131.77				10.72
C + 30			11.30	44.50	0.75	574.76				4.80
V + T + 30			21.64	57.25	1.32	1028.71				9.83
S + T + 30			18.46	47.00	1.06	764.65				7.46
V + S + T + 30			26.69	61.75	1.38	1116.49				11.16
C + 60			13.27	45.88	0.84	655.41				6.02
V + T + 60			24.00	59.88	1.40	1088.81				10.59
S + T + 60			20.93	53.13	1.11	870.70				8.38
V + S + T + 60			27.39	58.00	1.44	1141.15				11.47
LSD (*P* = 0.05)			1.399	3.930	0.112	112.700				1.137
Y	ns	ns	**	ns	ns	ns	ns	ns	ns	ns
P	ns	ns	**	**	**	**	**	**	**	**
A	**	**	**	**	**	**	**	**	**	**
P × A	ns	ns	**	**	**	**	ns	ns	ns	**
Y × P	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
Y × A	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
Y × P × A	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.

3.4. Effects of P levels and soil amendments on yield and associated traits of black seed at maturity stage

Plant height was affected by calcareous soil amendments and P application (Table 3). Increase in plant height was attributed to an increase in levels of P fertilizer. Moreover, V+T, S+T and V + S + T treatments increased plant height (Table 3). Irrespective of P fertilizer application, no significant difference was observed between V + T and V + S + T in terms of plant height (Table 3).

Generally, the maximum growth and yield of crops is influenced by the balance of nutrient uptake (Koocheki and Seyyedi 2015; Kuzmina, 1997). The importance of N in black seed growth (Shah, 2007) and positive correlation between S and N uptake and also P and N uptake have been reported previously (Duan et al., 2004; Salvagiotti et al., 2009). Therefore, increase in plant height might be due to S biological oxidation and increase in P solubility which make plants able to uptake more N from the soil.

Irrespective of P fertilizer rate, S + T treatment increased follicle number per plant (Table 3). Increase in plant height and seed number in psyllium (*Plantago ovata* L.), grown on calcareous soil amended by S, have been reported by Mosavi-Nik (2012). Furthermore, V application along with T with or without S increased follicle

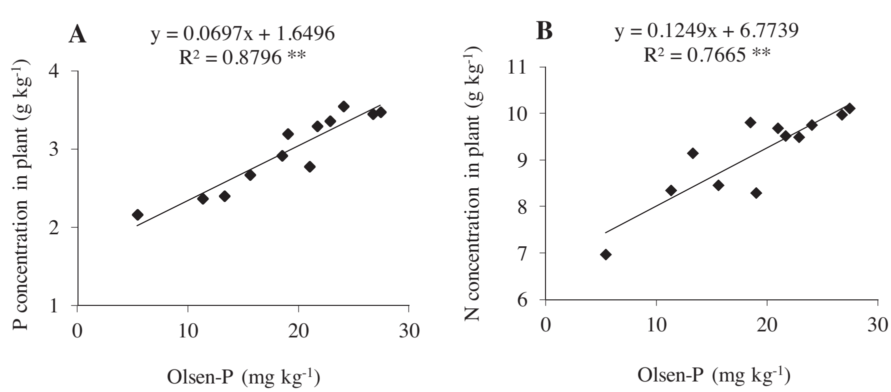

Fig. 1. Relationships between Olsen-P and P concentration in plant (A) and Olsen-P and N concentration in plant (B). Values are means of eight observations. The asterisks ** indicate statistical differences at $P \leq 0.01$.

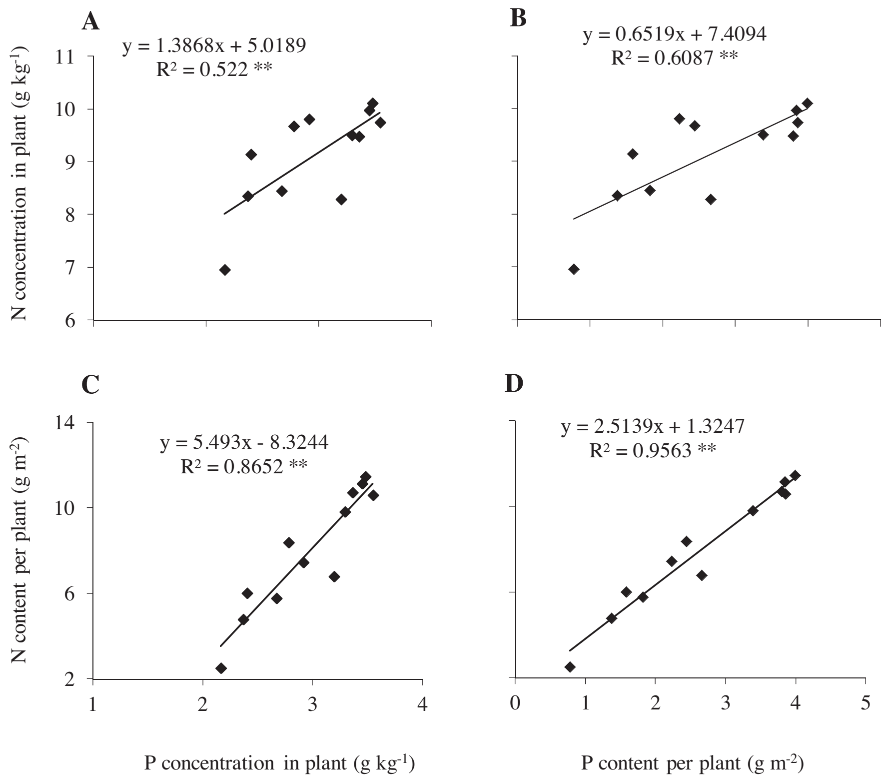

Fig. 2. Relationship between P and N concentrations in plant (A), P content per plant and N concentration in plant (B), P concentration in plant and N content per plant (C), and P content per plant and N content per plant (D). Values are means of eight observations. The asterisks ** indicate statistical differences at $P \leq 0.01$.

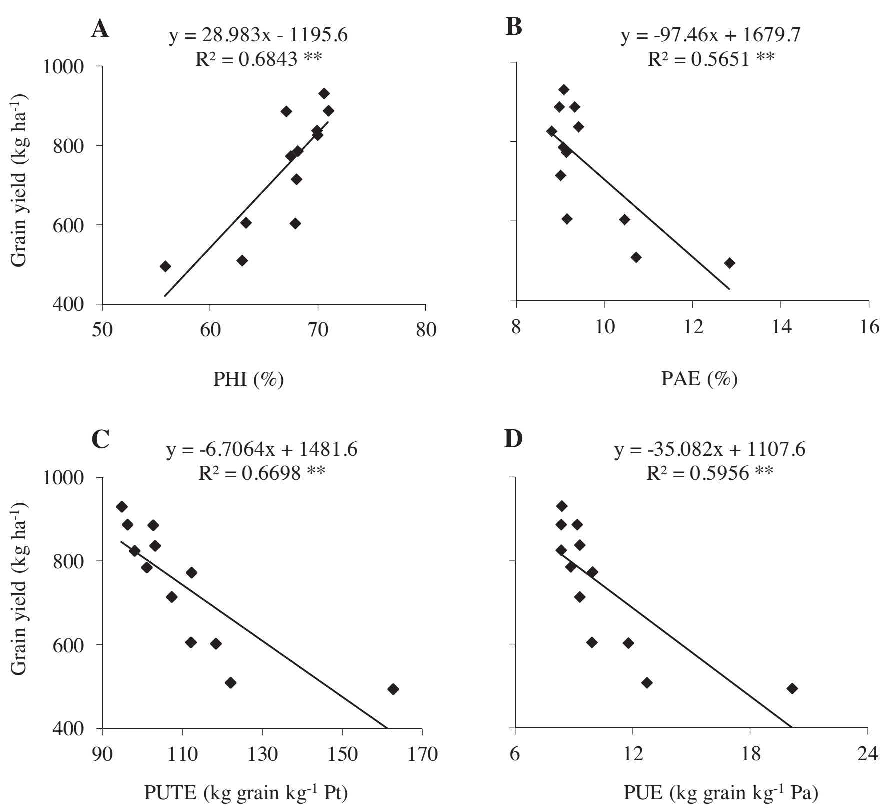

Fig. 3. Relationship between P harvest index (PHI) and grain yield (A), P acquisition efficiency (PAE) and grain yield (B), P utilization efficiency (PUTE) and grain yield (C), and P use efficiency (PUE) and grain yield (D). Pt: kg of P in the total plant per m^2; Pa: kg of soil available P per m^2. Values are means of eight observations. The asterisks ** indicate statistical differences at $P \leq 0.01$.

Table 3
Effects of phosphorus (P) rate and calcareous soil amendments (A) on some studied characteristics of soil and black seed at maturity stage.

	Soil			Black seed									
Treatments	pH	EC ($dS\,m^{-1}$)	Olsen-P ($mg\,kg^{-1}$)	Plant height (cm)	Number of follicle per Plant	Biological yield ($kg\,ha^{-1}$)	Grain yield ($kg\,ha^{-1}$)	Oil yield ($l\,ha^{-1}$)	Essential oil yield ($l\,ha^{-1}$)	PHI (%)	PAE (%)	PUTE (kg grain kg^{-1} Pt)	PUE (kg grain kg^{-1} Pa)
P ($kg\,ha^{-1}$)													
0	8.02	1.02	15.30	54.88	47.09	1944.65	691.37	230.20	1.80	63.34	10.10	122.24	12.27
30	8.01	0.96	18.53	59.31	52.84	2019.22	738.54	254.13	2.22	67.88	9.51	106.99	9.91
60	7.96	1.01	20.58	63.78	56.34	2147.41	787.86	271.27	2.48	69.05	9.34	102.87	9.32
LSD (P = 0.05)	0.069	0.056	0.830	1.723	2.508	120.980	56.088	19.778	0.196	2.655	0.893	5.813	0.904
A													
C	8.46	0.77	9.12	49.04	36.46	1456.47	537.26	171.82	1.18	62.13	11.32	134.12	14.86
V + T	8.14	0.86	20.86	64.33	60.92	2227.36	813.42	280.11	2.48	69.04	9.10	104.26	9.17
S + T	7.60	1.22	17.84	59.21	48.13	1963.01	703.23	240.15	2.08	66.40	9.06	106.63	9.33
V + S + T	7.80	1.13	24.71	64.71	62.88	2501.53	903.11	315.38	2.93	69.46	9.11	97.78	8.62
LSD (P = 0.05)	0.080	0.065	0.958	1.989	2.896	139.700	64.765	22.838	0.227	3.066	1.032	6.712	1.044
P × A													
C + 0				41.75	25.88							162.41	20.12
V + T + 0				60.25	57.38							112.10	9.92
S + T + 0				54.50	44.88							111.93	9.89
V + S + T + 0				63.00	60.25							102.50	9.16
C + 30				48.88	38.00							121.77	12.71
V + T + 30				64.88	63.00							102.89	9.28
S + T + 30				58.88	45.88							107.14	9.29
V + S + T + 30				64.63	64.50							96.15	8.35
C + 60				65.50	45.50							118.20	11.76
V + T + 60				67.88	62.38							97.80	8.32
S + T + 60				64.25	53.63							100.81	8.82
V + S + T + 60				66.50	63.88							94.70	8.37
LSD (P = 0.05)				3.446	5.015							11.630	1.809
Y	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
P	ns	ns	**	**	**	**	**	**	**	**	ns	**	**
A	**	**	**	**	**	**	**	**	**	**	**	**	**
P × A	ns	ns	ns	**	**	ns	ns	ns	ns	ns	ns	**	**
Y × P	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	ns
Y × P × A	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. Pt: kg of P in the total plant per m^2; Pa: kg of soil available P per m^2; C: control; V: vermicompost; S: sulfur; T: *Tiobacilus* bacteria; Y: year; PAE: phosphorus acquisition efficiency; PUTE: phosphorus utilization efficiency; PUE: phosphorus use efficiency; PHI: phosphorus harvest index.

number per plant (Table 3). Similar results have been documented by Yadegari and Barzegar (2011), who found that integrated application of S, T and cattle manure increase stem branches and flower number in lemon balm (*Melissa officinalis* L.).

Biological yield, grain, oil and essential oil yields of black seed were affected by calcareous soil amendments and P fertilizer application (Table 3). Although application of 30 kg P ha^{-1} had no significant effects on biological and grain yields, application of 60 kg P ha^{-1} significantly increased above mentioned traits. In this regard, Rogério et al. (2013) have reported that increase in P_2O_5 increases grain and oil yields in crambe (*Crambe abssynica* Hoechst). Moreover, all calcareous soil amendments improved biological, grain, oil and essential oil yields (Table 3). For instance, V + S + T treatment increased grain yield twice of the control treatment (Table 3).

Although P fertilizer and calcareous soil amendments showed a significant effects on P concentration and PHI at flowering stage, the results showed a significant reduction in PAE, PUTE and PUE due to application of all soil amendments (Table 3). Moreover, increase in P fertilizer rate decreased PAE, PUTE and PUE. However reduction in PAE was not statistically significant (Table 3). For instance, under no P fertilizer condition, application of V + S + T decreased PUTE by 36.89%, compared with control treatment (application of any soil amendments). Among treatments, the highest PUE (20.12 kg grain kg^{-1} Pa) was recorded in control treatment (Table 3).

Interestingly, for every unit increase in PHI, grain yield increased (Fig. 3A). Nonetheless, there was a negative correlation between PAE, PUTE and PUE with grain yield (Fig. 3B–D).

The relationships between PAE, PUE and grain yield can be studied from different aspects. For example, under P deficient conditions, cultivars with greater ability to absorb nutrients such as P show more PAE, and produce higher grain yield (Shenoy and Kalagudi, 2005; Veneklaas et al., 2012). On the other hand, it has been reported that an increase in P fertilizer rate may lead to a significant reduction in the PAE (Rahim et al., 2010). As for positive correlation between soil P availability and P uptake and negative correlation between PAE and black seed yield, it appears that under P deficient conditions, black seed is able to use P more efficiently. With regard to critical role of P in improving quantitative and qualitative traits of black seed (Mohamed et al., 2000; Tuncturk et al., 2011), it can be concluded that in calcareous soil, increase in grain yield is more correlated with PHI.

4. Conclusions

P concentration in plant is low in calcareous soil. Under such conditions, biological S oxidation, promoted by organic material such as V, plays an important role in P solubility and uptake. Increased P uptake stimulates N uptake by plants. Therefore, with regard to positive correlation between grain yield and PHI, it seems that any approach that would allocate more P to seeds, during seed development on the mother plants, would increase black seed final yield.

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References

Adhami, E., Maftoun, M., Ronaghi, A., Karimian, N., Yasrebi, J., Assad, M.T., 2006. Inorganic phosphorus fractionation of highly calcareous soils of Iran. Commun. Soil Sci. Plant Anal. 37, 1877–1888.

Ali, B.H., Blunden, G., 2003. Pharmacological and toxicological properties of *Nigella sativa*. Phytother. Res. 17, 299–305.

Atta, M.B., 2003. Some characteristics of nigella (*Nigella sativa* L.) seed cultivated in Egypt and its lipid profile. Food Chem. 83, 63–68.

Bayuelo-Jiménez, J.S., Ochoa-Cadavid, I., 2014. Phosphorus acquisition and internal utilization efficiency among maize landraces from the central Mexican highlands. Field Crops Res. 156, 123–134.

Biswas, D.R., Narayanasamy, G., 2006. Rock phosphate enriched compost: an approach to improve low-grade Indian rock phosphate. Bioresour. Technol. 97, 2243–2251.

D'Antuono, L.F., Moretti, A., Lovato, A.F.S., 2002. Seed yield, yield component, oil content and essential oil content and composition of *Nigalla sativa* L. and *Nigella damascena* L. Ind. Crops Prod. 15, 59–69.

Doan, T.T., Ngo, P.T., Rumpel, C., Nguyen, B.V., Jouquet, P., 2013. Interactions between compost, vermicompost and earthworms influence plant growth and yield: a one-year greenhouse experiment. Sci. Hortic. 160, 148–154.

Duan, Z., Xiao, H., Dong, Z., Li, X., Wang, G., 2004. Combined effect of nitrogen–phosphorus–potassium fertilizers and water on spring wheat yield in an arid desert region. Commun. Soil Sci. Plant Anal. 35, 161–175.

Erkan, N., Ayranci, G., Ayranci, E., 2008. Antioxidant activities of rosemary (*Rosmarinus officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. Food Chem. 110, 76–82.

Fismes, J., Vong, P.C., Guckert, A., Frossard, E., 2000. Influence of sulfur on apparent N-use efficiency, yield and quality of oilseed rape (*Brassica napus* L.) grown on a calcareous soil. Eur. J. Agron. 12, 127–141.

Ghamarnia, H., Jalili, Z., Daichin, S., 2012. The effects of saline irrigation water on different components of black cumin (*Nigella Sativa* L.). Int. J. AgriSci. 2, 915–922.

Ghamarnia, H., Khosravy, H., Sepehri, S., 2010. Yield and water use efficiency of (*Nigella sativa* L.) under different irrigation treatments in a semi-arid region in the West of Iran. J. Med. Plants 4, 1612–1616.

Gharby, S., Harhar, H., Guillaume, D., Roudani, A., Boulbaroud, S., Ibrahimi, M., Ahmad, M., Sultana, S., Ben Hadda, T., Chafchaouni-Moussaoui, I., Charrouf, Z., 2015. Chemical investigation of *Nigella sativa* L. seed oil produced in Morocco. J. Saud. Soc. Agric. Sci. 14, 172–177.

Handreck, K.A., 1986. Gypsum and superphosphate as sources of sulfur for plants in containers. Sci. Hortic. 30, 19–35.

Heydarnezhad, F., Shahinrokhsar, P., Shokri Vahed, H., Besharati, H., 2012. Influence of elemental sulfur and sulfur oxidizing bacteria on some nutrient deficiency in calcareous soils. Int. J. Agric. Crop Sci. 4, 735–739.

Hopkins, B., Ellsworth, J., 2005. Phosphorus availability with alkaline/calcareous soil. Salt Lake City, UT In: Western Nutrient Management Conference, 6, pp. 88–93.

Hosseinpur, A.R., Biabanaki, F.S., Alikhani, H.A., 2011. Impact of poultry manure application on phosphorus desorption in some calcareous soils. Commun. Soil Sci. Plant Anal. 42, 208–219.

Koocheki, A., Seyyedi, S.M., 2015. Relationship between nitrogen and phosphorus use efficiency in saffron (*Crocus sativus* L.) as affected by mother corm size and fertilization. Ind. Crops Prod. 71, 128–137.

Korkmaz, K., Ibrikci, H., Karnez, E., Buyuk, G., Ryan, J., Ulger, A.C., Oguz, H., 2009. Phosphorus use efficiency of wheat genotypes grown in calcareous soils. J. Plant Nutr. 32, 2094–2106.

Kuzmina, N.A., 1997. Nitrogen: phosphorus and potassium concentrations and their balance of durum wheat plants during different growth stages. Plant Nutr. Sustain. Food Prod. Environ. 78, 97–98.

Mahmoud, E.K., Ibrahim, M.M., 2012. Effect of vermicompost and its mixtures with water treatment residuals on soil chemical properties and barley growth. J. Soil Sci. Plant Nutr. 12, 431–440.

Manske, G.G.B., Ortiz-Monasterio, J.I., van Ginkel, M., González, R.M., Fischer, R.A., Rajaram, S., Vlek, P.L.G., 2001. Importance of P uptake efficiency versus P utilization for wheat yield in acid and calcareous soils in Mexico. Eur. J. Agron. 14, 261–274.

Modaihsh, A.S., Al-Mustafa, W.A., Metwally, A.I., 1989. Effect of elemental sulphur on chemical changes and nutrient availability in calcareous soils. Plant Soil 116, 95–101.

Mohamed, S.A., Medani, R.A., Khafaga, E.R., 2000. Effect of nitrogen and phosphorus applications with or without micronutrients on black cumin (*Nigella sativa* L.) plants. Ann. Agric. Sci. 3, 1323–1338.

Mohammady Aria, M., Lakzian, A., Haghnia, G.H., Berenji, A.R., Besharati, H., Fotovat, A., 2010. Effect of Thiobacillus, sulfur, and vermicompost on the water-soluble phosphorus of hard rock phosphate. Bioresour. Technol. 101, 551–554.

Mosavi-Nik, M., 2012. Effect of drought stress and sulphur fertilizer on quantity and quality yield of psyllium (*Plantago ovata* L.) in Baluchestan. J. Agroecol. 4, 170–182.

Najafi-Ghiri, M., Ghasemi-Fasaei, R., Farrokhnejad, E., 2013. Factors affecting micronutrient availability in calcareous soils of Southern Iran. Arid Land Res. Manage. 27, 203–215.

Ozturk, L., Eker, S., Torun, B., Cakmak, I., 2005. Variation in phosphorus efficiency among 73 bread and durum wheat genotypes grown in a phosphorus-deficient calcareous soil. Plant Soil 269, 69–80.

Parfitt, R.L., Yeates, G.W., Ross, D.J., Mackay, A.D., Budding, P.J., 2005. Relationships between soil biota, nitrogen and phosphorus availability, and pasture growth under organic and conventional management. Appl. Soil Ecol. 28 (1), 1–13.

Parthasarathi, K., Balamurugan, M., Ranganathan, L.S., 2008. Influence of vermicompost on the physico-chemical and biological properties in different types of soil along with yield and quality of the pulse crop–blackgram. Iran. J. Environ. Health Sci. Eng. 5, 51–58.

Piras, A., Rosa, A., Marongiu, B., Porcedda, S., Falconieri, D., Dessi, M.A., Ozcelik, B., Koca, U., 2013. Chemical composition and in vitro bioactivity of the volatile and fixed oils of *Nigella sativa* L. extracted by supercritical carbon dioxide. Ind. Crops Prod. 46, 317–323.

Pith Otero, A., Curutchet, G., Donati, E., Tedesco, P., 1995. Action of *Thiobacillus thiooxidans* on sulphur in the presence of a surfactant agent and its application in the indirect dissolution of phosphorus. Process Biochem. 30, 747–750.

Rahim, A., Ranjha, A.M., Waraich, E.A., 2010. Effect of phosphorus application and irrigation scheduling on wheat yield and phosphorus use efficiency. Soil Environ. 29, 15–22.

Rana, S., Singh, P.P., Naruka, I.S., Rathore, S.S., 2012. Effect of nitrogen and phosphorus on growth: yield and quality of black cumin (*Nigella sativa* L.). Int. J. Seed Spices 2, 5–8.

Rogério, F., da Silva, T.R.B., dos Santos, J.I., Poletine, J.P., 2013. Phosphorus fertilization influences grain yield and oil content in crambe. Ind. Crops Prod. 41, 266–268.

Salem, M.L., 2005. Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. Int. Immunopharmacol. 5, 1749–1770.

Salimpour, S., Khavazi, K., Nadian, H., Besharati, H., Miransari, M., 2010. Enhancing phosphorous availability to canola (*Brassica napus* L.) using P solubilizing and sulfur oxidizing bacteria. Aust. J. Crop Sci. 4, 330–334.

Salvagiotti, F., Castellarín, J.M., Miralles, D.J., Pedrol, H.M., 2009. Sulfur fertilization improves nitrogen use efficiency in wheat by increasing nitrogen uptake. Field Crops Res. 113, 170–177.

Sameni, A.M., Kasraian, A., 2004a. Effect of agricultural sulfur on characteristics of different calcareous soils from dry regions of Iran. II. Reclaiming effects on structure and hydraulic conductivity of the soils under saline-sodic conditions. Commun. Soil Sci. Plant Anal. 35, 1235–1246.

Sameni, A.M., Kasraian, A., 2004b. Effect of agricultural sulfur on characteristics of different calcareous soils from dry regions of Iran. I. Disintegration rate of agricultural sulfur and its effects on chemical properties of the soils. Commun. Soil Sci. Plant Anal. 35, 1219–1234.

SAS, 2011. SAS for Windows Version 9.3. SAS Institute Inc., Cary, NC, USA.

Shah, S.H., 2007. Influence of nitrogen and phytohormone spray on seed, inorganic protein and oil yields and oil properties of *Nigella sativa* L. Asian J. plant Sci. 6, 364–368.

Shenoy, V.V., Kalagudi, G.M., 2005. Enhancing plant phosphorus use efficiency for sustainable cropping. Biotechnol. Adv. 23, 501–513.

Shirmohammadi, E., Khaje, M., Talaei, G.H., Shahgholi, H., 2014. Effect of biological phosphate and chemical phosphorus fertilizer on yield and yield components of Black cumin (*Nigella sativa* L.). Agric. Sci. Dev. 3, 279–283.

Sibbet, S.S., 1995. Managing high pH calcareous, saline, and sodic soils of the Western Pecan growing region. HortTechnology 5, 222–225.

Soaud, A.A., Al Darwish, F.H., Saleh, M.E., El-Tarabily, K.A., Sofian-Azirun, M., Rahman, M.M., 2012. Effects of elemental sulfur phosphorus micronutrients and *Paracoccus versutus* on nutrient availability of calcareous soils. Aust. J. Crop Sci. 5, 554–561.

Tuncturk, M., Tuncturk, R., Yildirim, B., 2011. The effects of varying phosphorus doses on yield and some yield components of black cumin (*Nigella Sativa* L.). Adv. Environ. Biol. 5, 371–374.

Ullah, I., Jilani, G., Irfan-ul-Haq, M., Khan, A., 2013. Enhancing bio-available phosphorous in soil through sulfur oxidation by *Thiobacilli*. Br. Microbiol. Res. J. 3, 378–392.

Valdez-Pérez, M.A., Fernández-Luqueño, F., Franco-Hernandez, O., Flores Cotera, L.B., Dendooven, L., 2011. Cultivation of beans (*Phaseolus vulgaris* L.) in limed or unlimed wastewater sludge, vermicompost or inorganic amended soil. Sci. Hortic. 128, 380–387.

Veneklaas, E.J., Lambers, H., Bragg, J., Finnegan, P.M., Lovelock, C.E., Plaxton, W.C., Price, C.A., Scheible, W.-R., Shane, M.W., White, P.J., Raven, J.A., 2012. Opportunities for improving phosphorus-use efficiency in crop plants. New Phytol. 195, 306–320.

Vidyalakshmi, R., Paranthaman, R., Bhakyaraj, R., 2009. Sulphur oxidizing bacteria and pulse nutrition—a review. World J. Agric. Sci. 5, 270–278.

Yadegari, M., Barzegar, R., 2011. The effect of sulphur and *Thiobacillus* on nutrient absorption: growth and essential oil in lemon balm (*Melissa officinalis* L.). J. Med. Plants 1, 35–40.

Zhao, Y., Wang, P., Li, J., Chen, Y., Ying, X., Liu, S., 2009. The effects of two organic manures on soil properties and crop yields on a temperate calcareous soil under a wheat–maize cropping system. Eur. J. Agron. 31, 36–42.