

## Effects of Seed Placement in Soil on Virulence of *Orobanche crenata* on Faba Bean

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### ABSTRACT

*Orobanche crenata* Forsk, a major constraint to faba bean (*Vicia faba* L.) production, albeit recently introduced into Sudan, is widely distributed and has become a pest of national importance. The present investigation, undertaken at the College of Agricultural Studies, Khartoum North, was set to investigate the effects of seed placement in soil on the parasite virulence and influence on selected faba bean growth attributes. Seeds buried at a depth of 5 cm resulted in the heaviest infestation and highest emergence of the parasite. Seed placement at 10 and 15 cm soil depths did not impair attachment of the parasite, but curtailed emergence. *O. crenata* from seeds placed in the surface soil displayed late emergence and produced no seeds, whereas those from seeds buried at 5 and 10 cm depths produced considerable number of capsules (16-29 capsules/plant). *O. crenata* from seeds placed at 15 cm soil depth remained subterranean and produced no seeds. *O. crenata* infestation from seeds placed in the surface soil had no adverse effects on faba bean growth attributes. However, those from seeds buried at 5, 10 and 15 cm reduced all measured growth attributes and infestation resulting from seeds placed at 5 cm depth was, invariably, the most damaging. The results suggest that in newly infested areas soil inversion or adoption of no-till curtail replenishment of the parasite seed bank and reduce crop damage.

**Keywords:** Orobanchaceae, *Vicia faba*- soil depth

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### INTRODUCTION

Faba bean (*Vicia faba* L), a Fabaceae, has its centre of origin in the Mediterranean region and West and Central Asia (Hatwin and Webb, 1982). The seeds, used both as food and feed, are important source of good quality protein (Crépona *et al.*, 2010). Furthermore, the crop plays an important role in improving soil fertility through influx of biologically fixed nitrogen (Elgilany *et al.*, 2007). Weeds constitute a major constraint to faba bean production in the Mediterranean region, and in eastern and southern Europe (Parker and Riches, 1993). Among weeds the holoparasite, *Orobanche crenata* Forsk (crenate broomrape), an Orobanchaceae, is one of the serious constraints of faba bean in North Africa, Nile Valley and sub-Saharan Africa countries where more than 30% of faba bean is produced (Maalouf, 2011). In Sudan *O. crenata* was first reported in 2001 in a localized area, in the Northern States. However, within 5 years the infested area, in the northern States, increased by over a 1000-fold and the weed has become a national problem (Babiker *et al.*, 2007).

The parasite, lacking chlorophyll and well developed roots, is totally dependent on its host for organic carbon, minerals and water. The parasite is devastating and complete collapse of faba is not uncommon under heavy infestations specially when coupled with water stress (Parker and Riches, 1993). Heavy infestations by the parasite may force farmers to plant less profitable crops and/or abandon their land. In addition to yield losses and reduction in cultivated areas the parasite reduces grain and hay quality as well as marketability of the produce.

Copious seed production, prolonged seed viability and the need for special germination requirements make *O. crenata* a difficult weed to control (Mauromicale *et al.*, 2001). In common with other root parasitic weeds no single measure provides effective control and an approach based on integrated crop management strategy is imperative (Babiker *et al.*, 2007). Current measures of control focus on depletion of the parasite seed reserves and on curtailment of seed movement. Deep ploughing and /or soil inversion were reported to influence *Phelipanche ramosa* infestation in tomato (*Solanum lycopersicum* L.) and tobacco (*Nicotina tabacum* L) (Linke, 1999). Furthermore, seed position in the soil profile was reported to influence conditioning and parasitism in the closely related root parasitic weeds *Striga asiatica* and *S. hermonthica* (Bebawi *et al.*, 1984 and Babiker, 1987)

The present investigation was therefore set to study the effects of seed placement in soil, on *O. crenata* virulence and influence on selected faba bean growth attributes.

## MATERIALS AND METHOD

*O. crenata* seeds, collected from under faba bean in the River Nile State were provided by Dr. Abdellaha N. K. from the Agriculture Research Corporation, Sudan.

The experiment was undertaken during 2007/2008 and 2008/2009 winter seasons at the College of Agriculture Studies, Sudan University of Science and Technology. Plastic pots (13 cm i. d.), with perforations at the bottom, were filled with a mixture of soil collected from the College farm and river sand (1:1 v/v). The soil-sand mix was artificially inoculated with *O. crenata* seeds (8 mg/pot). The seeds were placed in the surface soil or, 5, 10 and 15 cm below. A control treatment without the parasite seeds was included for comparison. Seeds of faba bean cv. Hediba 93 (5 seeds/pot) were sown on the 10<sup>th</sup> of November in both seasons and immediately irrigated. Faba bean seedlings were thinned to three plants per pot two weeks after crop emergence. Subsequent irrigations were made every two days. Treatments were arranged in a Randomized Complete Block Design (RCBD) with four replicates. At harvest emerged *O. crenata* shoots were counted and cut at the soil surface. Faba bean shoot height was measured and the plants were cut at the soil surface. Faba bean roots were carefully washed with tap water and attached *O. crenata* shoots and tubercles were counted and separated for determination of dry weight. Faba bean shoots, roots, and *O. crenata* shoots and tubercles were air-dried and weighed. The data, collected, were analyzed by analysis of variance. Means were separated for significance by the Duncan's Multiple Range Test (DMRT) at 5% level using Mstac computer programme.

Due to seasonal differences data on *O. crenata* dry weight, plant height, number of leaves, number of nodes, root dry weight and shoot dry weight were analyzed separately, while data on *O. crenata* attachment, emergence, *O. crenata* capsules and number of branches on faba bean were subjected to combined analysis.

## RESULTS AND DISCUSSION

### Effects on *O. crenata*

*O. crenata* infestation, as revealed by total attachments, was influenced by seed placement (Fig 1). Seed placed in the surface soil resulted in the lowest infestation (2 *Orobanchae* shoots /pot) while those placed at 5 cm depth effected the highest infestation (14 *Orobanchae* shoots /pot). Seed placement at 10 cm depth resulted in *Orobanchae* attachment comparable to those placed at 15 cm soil depth. However, *O. crenata* emergence was strongly influenced by seed burial depth (Fig 1). Numbers of emerged *Orobanchae* shoots was 1, 10, 2 and 0 for seeds placed in the surface soil and 5, 10 and 15 cm below, respectively. When *Orobanchae* seeds were placed at 10 and 15 cm soil depths, most of the *Orobanchae* seedlings remained subterranean (Fig 1).

*Orobanchae* shoots arising from seed placed in the surface soil displayed very late emergence and produced negligible number of capsules (Fig 2). However, those resulting from seeds placed at 5 and 10 cm depth showed considerable number of capsules 29 and 16 capsules/ plant, respectively. *Orobanchae* shoots arising from seeds placed at 15 cm soil depth remained subterranean and produced no capsules.

*O. crenata* shoot dry weight was lowest (1.5 g /pot) for those arising from seeds placed in the surface soil. However, seed placement at 5 cm soil depth resulted in the highest dry weight (Fig 3 a and b). *Orobanchae* shoots resulting from seeds placed at 10 cm soil depth, though lower in number, and those from seeds placed at 15 cm, albeit remained subterranean, displayed dry weight comparable to those arising from seeds placed at 5 cm depth.

#### **Effects on faba bean**

*O. crenata*, invariably reduced faba bean height, number of nodes, number of leaves, and number of branches (Tables 1 and 2). However, the magnitude of the reduction was dependent on seed placement depth. *O. crenata* infestations, resulting from seeds placed in the surface soil and those placed at 10 and 15 cm soil depths, reduced faba bean height by 3. - 9%, 16 -17% and 9-16%, respectively. However, the observed reductions were not significant. Infestation resulting from seeds placed at 5 cm soil depth, significantly reduced faba bean height and the observed reduction was 34 and 36% in the first and second season, respectively (Tables 1 and 2).

*O. crenata* infestation from seeds placed in the surface soil and at 10 and 15 cm soil depth reduced the number of nodes in faba bean by 2 - 9%, 15- 25% and 15 -17.%, respectively (Tables 1 and 2). Infestation resulting from seeds placed at 5 cm depth inflicted maximum and significant reductions (32 and 38%).

Infestations resulting from seeds placed in the surface soil or those placed at 10 and 15 cm soil depths reduced number of leaves, albeit not significantly (Tables 1 and 2). However, infestation from seeds placed at 5 cm depth resulted in a significant reduction amounting to over 30% in both seasons.

*O. crenata* infestations from seeds placed in the surface soil and those placed at 15 cm depths caused non-significant reduction in number of branches in faba bean (Table 1). Infestations resulting from seeds placed at 5 and 10 cm soil depths, however, reduced branching in faba bean significantly.

*O. crenata* infestation arising from seeds placed in the surface soil did not reduce faba bean shoot dry weight (Fig 4 a and b). Seeds placed at 5, 10 and 15 cm depths reduced shoot dry weight significantly and the observed reduction declined with seed placement depth. Infestation resulting from seeds placed at 5, 10 and 15 cm depths inflicted 49 and 60%, 43 and 44% and 32 and 36% reductions, respectively.

Faba bean root dry weight showed similar trends to shoot dry weight in response to *O. crenata* seed placement. *O. crenata* infestation resulting from seeds placed in the surface soil had no effect on root dry weight in the first season. However, in the second season considerable, albeit non significant, reduction was observed (Fig 5 a and b). Infestations resulting from seeds placed at 5, 10 and 15 cm soil depths caused significant reductions (37-61%), irrespective of season. *Orobanchae*

infestations arising from seeds placed at 5 cm depth resulted in maximal reductions (51 and 61 %) (Fig 5 a and b).

#### **Discussion:-**

The result revealed that *O. crenata* seeds distribution within the soil profile had profound effects on parasitism, seed bank replenishment and host damage (Figs 1-5 and Tables 1 and 2). *O. crenata* seeds placed in the surface soil resulted in the least infestation, the least number of emerged shoots and underground attachments and did not affect significantly any of the measured faba bean growth attributes (Figs 4 and 5 and Tables 1 and 2). The low infestation and consequently the low emergence of the parasite and least inflicted damage to faba bean may be attributed to interruption of the conditioning process associated with rapid drying of the surface soil may interrupt conditioning of the parasite seeds. Babiker *et al.* (1987) reported a similar finding with *S. hermonthica*. However, it is also plausible that light may curtail germination of seeds placed in the surface soil. Parker and Riches, (1993) reported a negative effect of light on germination of *O. crenata*. It is also (Grenz *et al.*, 2005) reported the low germination of seeds placed in the surface to less frequent contact with the host roots or exposure to none sufficient amounts of root exudates to trigger germination.

*Orobanch*e seeds placed at 5 cm depth resulted in the heaviest infestation and was the most damaging to faba bean (Table 1 and Fig 4-5). These results are in line with those previously reported for *S. hermonthica* where most of the damage was associated with seed placed at 5-10 cm soil depth (Eltayeb, 1998). Babiker *et al.*, (1987) showed that *Striga* seeds buried at 5 cm soil depth displayed relatively consistent and considerable germination (56 - 79%), while those buried at or below 15 cm depth were less responsive to germination stimulants.

Seed placement at 10 cm soil depth resulted in moderate reduction in *O. crenata* attachment, but considerably curtailed emergence as only 20 % of the attached parasite emerged above the soil surface (Fig 1). Placement of seeds at 15 cm soil depth resulted in considerable attachment of *O. crenata* to the host roots; however, emergence of the parasite was completely suppressed in both seasons.

Failure and /or reduction of *O. crenata* emergence following seed placement at 10 and 15 cm soil depth are in line with previous reports, on the a closely related root parasitic weed, *S. asiatica*. *S. asiatica* emergence in maize, was reduced by soil placement. Maximum emergence of the parasite occurred only when the seeds were placed in the top 10 cm of soil and no emergence or reduced emergence were observed when the parasite seeds were placed at or below 11 cm depth (Bebawi *et al.*, 1984). A similar finding was reported for *P. ramosa* on tomatoes and tobacco as few shoots were reported to emerge when the parasite seeds were buried in soil at 10 cm or more (Ahmed and Donlad, 1994). Linke (1999) reported that emergence of root parasitic weeds from deeper soil depths require substantial food reserves that are hardly provided by the comparatively thin roots at such depths. Of interest in this respect is the similarity in dry weight between *O. crenata* shoots resulting from seeds placed at 5 and 15 cm, despite the exclusively subterranean nature of growth of the latter (Fig. 1). This finding, albeit does not refute the argument made by Linke (1999), suggests possible involvement of other factors in curtailment of emergence from deeper soil depths and probably mechanical impedance may account, at least in part, for the observed low emergence.

Despite the limited emergence of *O. crenata* shoots when the seeds were placed at 10 and 15 cm below the soil surface the inflicted losses on faba bean growth attributes were considerable (Tables 1 and 2). This observation corroborates the notion that *Orobanch*e spp. by virtue of connection to host phloem create an active sink that diverts translocation of assimilates to the infested roots. Such observation may explain the susceptibility of the parasites to phloem mobile herbicides, applied to the host foliage (Goldwasser and Kleifeld, 2004). Furthermore, the damage to the host caused by infestation resulting from deeply buried seeds despite lack of emergence of the parasite is in line

with the notion that root parasitic weeds, in general, inflict their maximum damage on the host while they are still below the ground.

The results demonstrated clearly that damage to the host is influenced by position of seeds in the soil profile and that infestation resulting from seeds placed at 5 cm depth was, invariably, the most suppressive (Tables 1 and 2). Deep burial of the parasite seeds and placement in the surface soil reduced emergence of the parasite and the damage it inflicted on the host. Furthermore, the results suggest that, in newly infested fields, soil inversion or adoption of no-till planting may reduce replenishment of the parasite seed reserves in soil and the host.

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**Table 1. Influence of *O. crenata* seed placement on selected faba bean growth attributes (Season 2007/2008)**

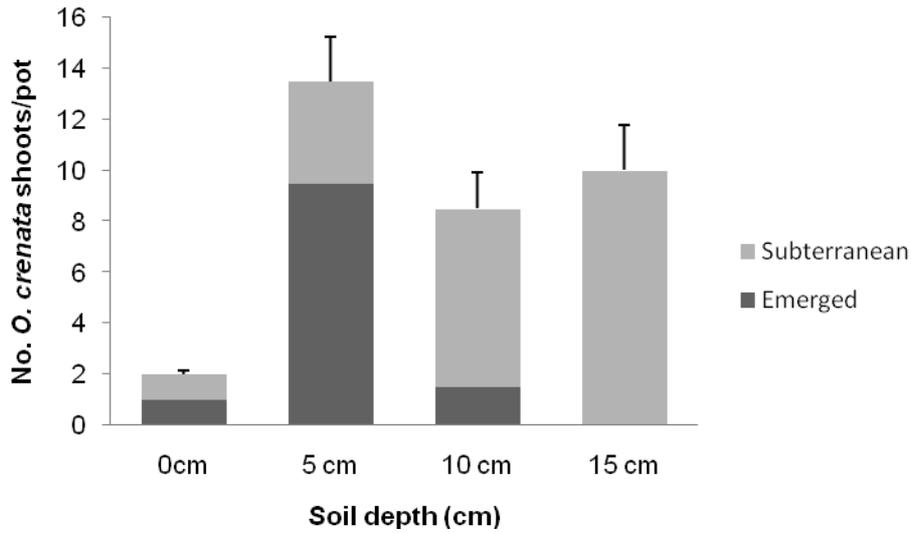
Seed placement (depth cm)	Plant height (cm)	Number of nodes/plant	Number of leaves/plant	Number of branches/plant
Uninfected	62.2 a	34.9 a	37.1 a	1.9 a
Surface soil	60.2 a	34.2 a	36.8 a	1.8 ab
5	41.3 b	21.6 b	23.7 b	1.4 bc
10	51.4 ab	26.4 ab	28.5 ab	1.3 c
15	52.0 ab	29.8 ab	32.3 ab	1.7 abc
SE ( $\pm$ )	3.8	3.42	3.39	0.11
CV%	14.4%	23.3%	21.4	18.4

Means within a column followed by the same letter(s) are not significantly different according to the Duncan's Multiple Range Test at 5%.

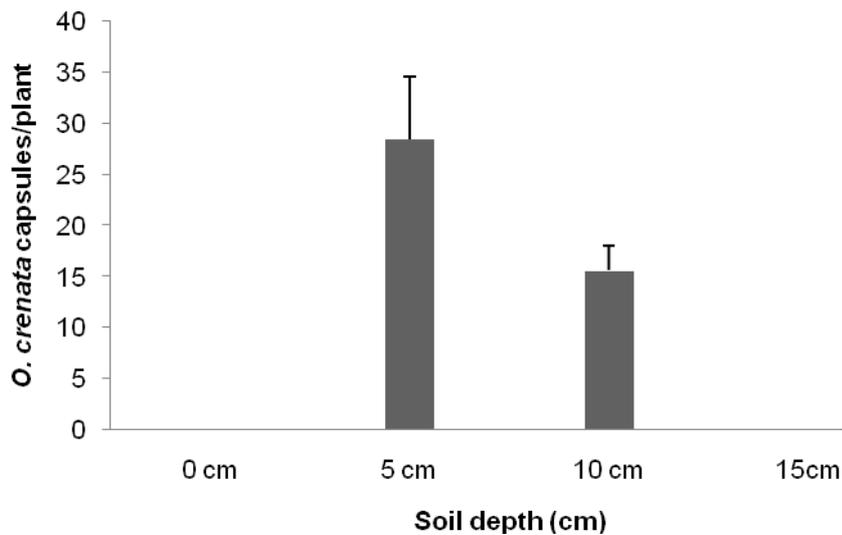
**Table2. Influence of *O. crenata* seed placement on selected faba bean growth attributes (Season 2008/2009)**

Seed placement (depth cm)	Plant height (cm)	Number of nodes/plant	Number of leaves/plant
Uninfected	38.5 a	24.8 a	27.4 a
Surface soil	35.1 ab	22.6 a	24.9 a
5	24.8 b	16.9 b	19.1 b
10	32.5 ab	21.6 ab	23.8 a
15	35.0 ab	20.5 ab	22.9 ab
SE ( $\pm$ )	3.8	1.5	1.4
CV%	22.8%	14.2%	12.0%

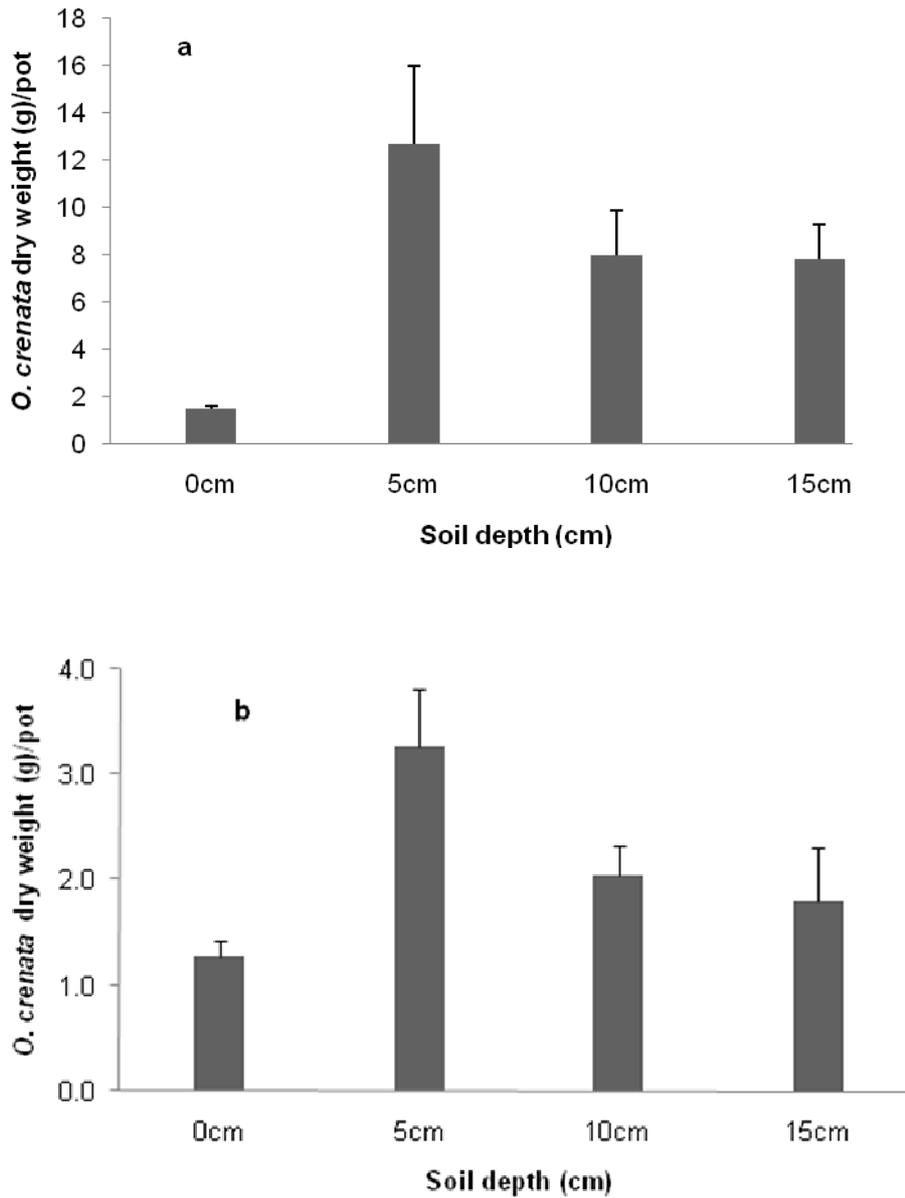
Means within a column followed by the same letter(s) are not significantly different according to the Duncan's Multiple Range Test at 5%.



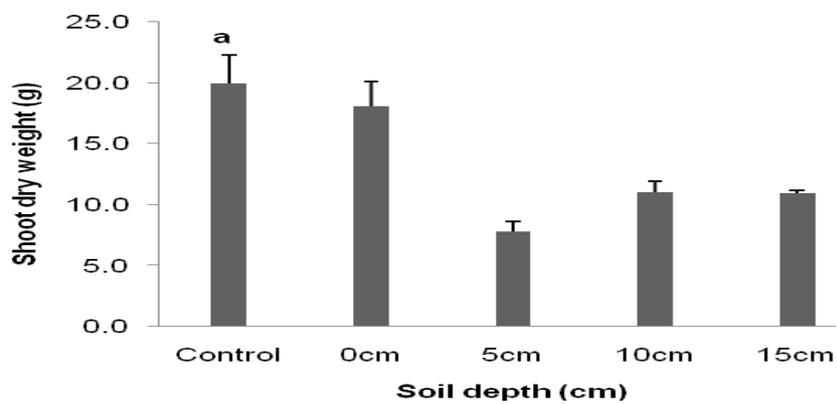
**Fig. 1** Influence of seed placement in soil on attachment and emergence of *O. crenata* on faba bean. [Combined analysis season 2007/2008 and season 2008/2009). Vertical bars represent Standard error].

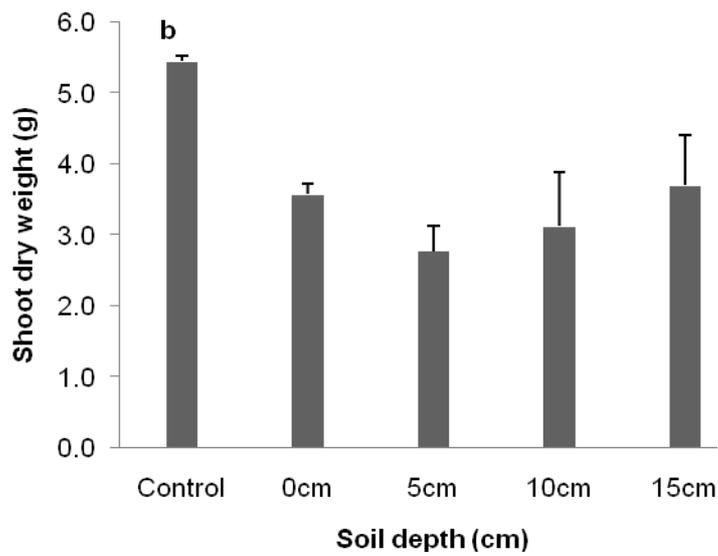


**Fig. 2** Effects of seed placement in soil on capsules production by *O. crenata* [Combined analysis season 2007/2008 and season 2008/2009). Vertical bars represent Standard error]

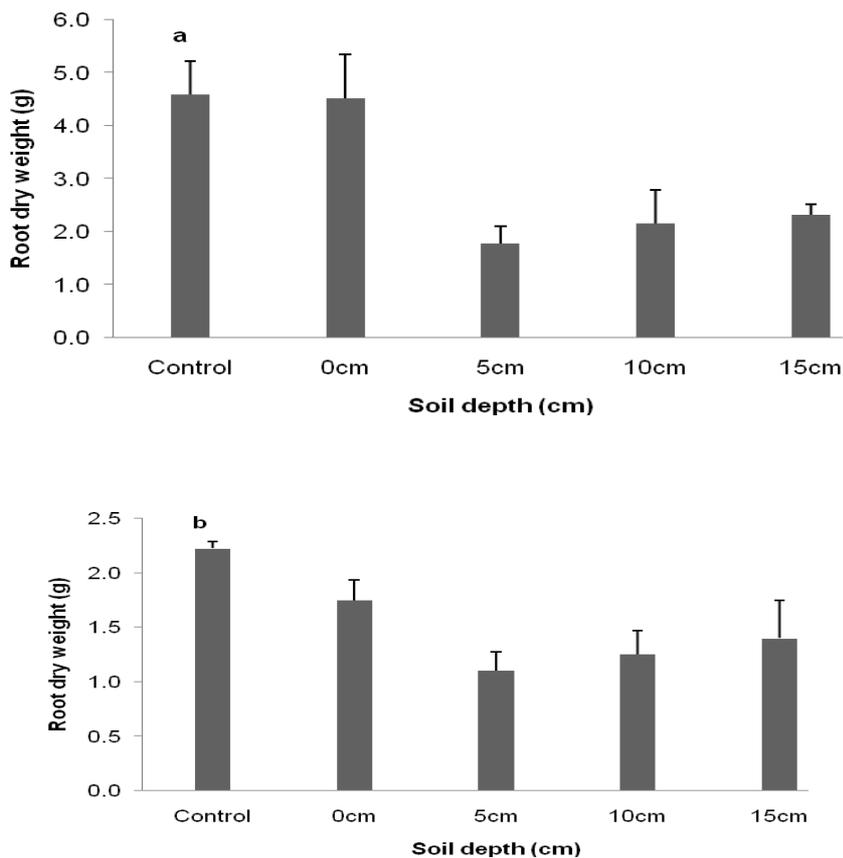


**Fig.3** Effects of seed placement in soil on dry weight of *O. crenata* shoots and tubercles. (a season 2007/2008 and bseason 2008/2009). Vertical bars represent Standard error.





**Fig. 4** Effects of *O. crenata* seed placement in soil on faba bean shoot dry weight. (a season 2007/2008 and b season 2008/2009). Vertical bars represent Standard error.



**Fig. 5** Effects of *O. crenata* seed placement in soil on faba bean root dry weight. (a season 2007/2008 and b season 2008/2009). Vertical bars represent Standard error

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