Bulletin of Environment, Pharmacology and Life Sciences

Bull. Env. Pharmacol. Life Sci., Vol 2 (5) April 2013: 79-85
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Online ISSN 2277-1808

Original Article

Effects of Potassium and Phosphorus Fertilizers on Arsenic Accumulation and Plant Growth of two Basil Cultivars

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ABSTRACT

In this study, the effects of phosphorus and potassium fertilizer were examined on As toxicity and plant growth in two basil variety. The experimental design was a factorial with two basil varieties including local of Zabol and Keshkeni Luvelou as the first factor, and the addition of phosphorous and potassium fertilizers to soil at three levels: 50, 150 and 250 mg K kg^{-1} soil as second factor. Arsenic sulphate was added to all the treatments at a uniform rate equivalent to 15 mg As kg^{-1} soil. The experiment was conducted in 2011 at the Zabol University greenhouse in Zabol, south Iran. Before reproductive phase beginning, plant height, number of lateral branches, number of leavesper plant and fresh weight and leaf area per pot were recorded. In addition, As concentration in plant tissues were measured. Morphological traits were affected by varieties at 1% level of probability. All growth parameters (except fresh weight) significantly changed between two varieties. In both varieties, arsenic concentration in aerial parts reduced with increasing phosphorus addition. At different potassium fertilizer, the greatest As concentration was observed in the least potassium addition.

Keywords:*Medicinal Plants, HeavyMetals, Essential Oil, Chemical Fertilizers.*

Received 12.03.2013 Accepted 13.04.2013

INTRODUCTION

Arsenic is a heavy metal that is considered toxic when present in very low concentrations, on the order of parts per billion (ppb). Arsenic has been used in embalming fluids, herbicides, insecticides, defoliants, and is typically found in the vicinity of metal smelters. Arsenic is released into the environment from anthropogenic sources such as mining activities and the application of arsenic-based pesticides and wood preservatives, in both inorganic and organic forms. Arsenite [As(III)] and arsenate [As(V)] are the inorganic phytoavailable forms of arsenic in soil and water. Plants vary in their sensitivity and resistance to arsenic [1]. In developing countries heavy metal pollution is very serious due to mining, smelting and tannery industries [2]. Heavy metal pollution does not only affect the production and quality of crops, but also influences the quality of the atmosphere and water bodies, and threatens the health and life of animals and human beings [3]. The use of herbal medicines has been on the rise in recent years due to their low prices and the lack of awareness of people about their adverse side effects. There is a common concept among people that herbal medicines have no side effects and that “being natural in origin, herbs are safe”. The assimilation of heavy metals in plants is obvious because of widespread heavy metals in the soil due to geo-climatic conditions [4]. Medicinal plants are the raw material for many herbal formulations and popular supplements. Heavy metals have a great tendency to accumulate in human organs over prolonged periods of time. Quality and safety standards for herbal medicine that clearly stipulate the maximum allowable value of heavy metals in herbal medicines have been enacted and put into effect by many countries [5]. However, since most of these standards are determined by referring to the quality standards for foods, they are not based on the research on herbal medicines [6].The uptake and accumulation of heavy metals may have impacts on medicinal plants that are different from their impacts on farm crops, Therefore, it is necessary to improve quality standards for herbal medicines by examining and revising the maximum allowable values of heavy metals in medicinal plants, using research based on medicinal plants [7]. Interaction of arsenic and phosphorous in soil and its uptake by plants is complex. As and P compounds similarities in the soil causes the soil to be competitive in their absorption. Phosphate and arsenate exhibit similar physiological behaviors and compete directly for absorption sites on soil particles [8]. As can be substituted for P in plants, but it is unable to carry out the role of P in energy transfer, the plant reacts as if there is a P deficiency. Thus, as plant Arsenic increases, the plant reacts by increasing P uptake [9]. Potassium can cause stabilization of arsenic in the soil and prevent its accumulation in the plant [10]. Komar [11] reported a positive correlation between potassium and arsenic accumulation. Carbonell, *et al* [12] during assessment of arsenic adsorption in tomato plants found that with increased uptake of this element, the occurrence of injuries due to high accumulation of arsenic in the plant's reduced the ability to absorb the element. This decrease was due to roots damage which reduced its uptake and transfer from roots to shoots. In another report arsenic was found to reduce potassium absorption in tomato plants [13].

Sweet basil (*Ocimum basilicum* L.) is an important essential oil plant distributed all over world. The plant is cultivated in various agroclimatic zones, has extensive use as a flavoring agent, in perfumery and in the pharmaceutical industry. Essential oil derived from the plant exhibits various therapeutic properties. Plant grows luxuriantly in soils contaminated with heavy metals such as cadmium (Cd), lead (Pb), copper (Cu) and arsenic (As). Plant roots take up As from soil and translocate it to aerial parts. High As concentrations in edible parts of plants pose serious health risks [14]. The objectives of this paper are: a) to clarify the accumulation of As in sweet basil and the influence of P and K in soil; b) to elucidate the responses of sweet basil growth and yield ;and c) to provide suggestions for the establishment of environmental safety standards for As in sweet basil.

MATERIAL AND METHODS

Site description

The experiment was conducted in 2011 using the greenhouse and field facilities of the University of Zabol, in Southeast Iran (61°29 N, 31°2 E, 450 m above sea level), which has a warm and arid climate with a mean annual temperature of 23 °C and average annual precipitation of 63 mm. The sandy loam soil [19% clay (<2 μm), 21% silt (2-20 μm), 41% fine sand (20-200 μm) and 19% coarse sand (200-2000 μm)], with a pH of 7.1, organic mater 1.45%, N-No3 6 ppm, P (Olsen) 12 ppm, and K 185 ppm (0-30 cm depth)] used in this study was collected from experimental agriculture farm of Zabol University. The soil was collected from the first 10cm of the soil surface, air-dried and afterwards sieved at 2mm.

Experimental Design

The experimental design was a factorial with two basil variety including local of Zabol and Keshkeni Luvelou as first factor, and addition of phosphorous and potassium fertilizers to soil at three levels: 50, 150 and 250 mg K kg^{-1} soil as second factor. Arsenic sulphate was added to all the treatments at a uniform rate equivalent to 15 mg As kg^{-1} soil. Seeds were sown in 20 cm height and 15 cm diameters pots and after that pots were placed randomly in the greenhouse, and when seedling were grown to 3 cm height plants in the pots were thinned up to five plant in each pot. During the plant growth pots were irrigated once every three days. Before reproductive phase begining plant height, number of lateral branches, number of leaves and lateral branches per plant and total fresh and dry weight of plant shoots, the leaf area per pot were recorded. In addition As concentration in plant tissues were measured.

Determination of As concentration

For As analysis, dried plant tissues were ground using a ball mill and then digested with HNO3/HClO4 (3:1 [v/v]) [13]. The As concentration in digested solutions was determined by a flame atomic absorption spectrophotometer (Pu9100x-Philips).

Statistical analyses

Obtained data were analyzed using SAS 9.2 software and means comparison was performed based on Duncan's multiple range tests at 5% of probability level. For drawing graphs and tables EXCEL software was used.

RESULTS AND DISCUSSION

Plants Height

Analysis of variance showed that the effect of variety, As addition and their interaction on plants height was significant at 1% probability level (Table 1). Means comparison showed the superiority of local variety than breeding variety (Table 2). In general, for different least of P and K fertilizers with As, the highest plant height was observed at 50 mg/kg soil K, and the lowest was at 250mg/kg soil P with As. Means comparison of interactive effect showed that among P and K fertilizer levels, with increase from 50 to 150 and 250 mg/kg soil plant height in both varieties reduced. Minimum difference in height between two varieties was observed in control treatment (no fertilizer), which although no fertilizer used, but had better growth than most treatments of fertilizers combined with arsenic (Fig. 1).

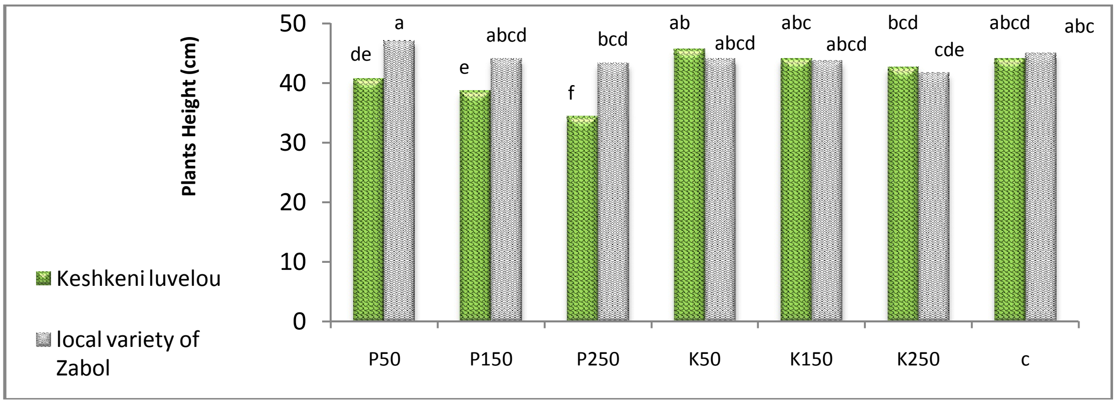

Fig 1. Interaction Varity*Fertilizers on the plant height (cm).

Leaves Number Per Plant

The results showed that effect of P and K fertilizers along with As was significant on leaves number at 5% of probability level, but their interaction was not statistically significant (Table 1). Means comparison of variety effect showed significant difference of local variety with bred one (Table 2). The highest and the least number of leaves per plant was observed at 50 mg P kg^{-1} soil and 250 mg P and K kg^{-1} soil, respectively (Table 3).

Lateral Branches Per Plant

As shown in Table 2 effect of variety and interaction between variety and P and K fertilizers along with As was significant at 1% of probability level, but effect of P an K fertilizers along with As on lateral branches was not significant. Among fertilizers level, the highest and the least lateral branches was for consumption of 150 mg P kg-1 soil with As and 250 mg P Kg-1 soil along with As, respectively (Table 3). Means comparison of interactive effects showed that P fertilizer levels had different effect on two varieties, so that the highest effect on lateral branches in bred variety was achieved from average fertilizer level, but in local variety, number of lateral branches was equal at 50 and 150 mg kg-1 soil. In K fertilizered potfor bred variety, maximum effect was observed in 50 mg K kg-1 soil, but in local variety150 mg K kg-1 had the greatest effect on lateral branches (Fig. 2).

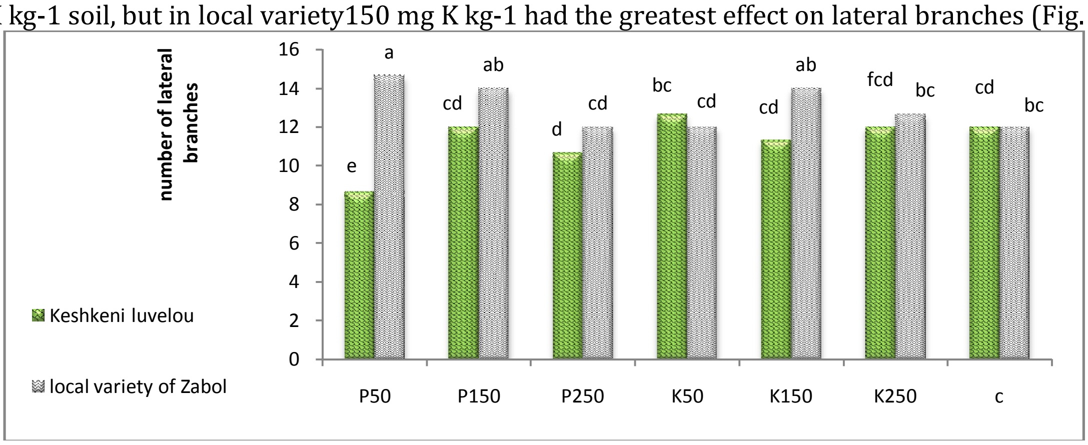

Fig 2. Interaction Varity*Fertilizers on the number of lateralbranches.

Fresh Weight of Aerial Parts

Analysis of variance showed significant difference of variety and fertilizers levels at 1% of probability level on aerial parts fresh weight, while there interaction was also significant (Table 2). Bred variety in terms of shoot fresh weight showed significant difference with local variety (Table 3). Means comparison showed highest weight are achieved from 50mg/kg P and K with As and control treatment (Table 6-4). Interaction effects means comparison showed that with increasing P and K levels, fresh weight was reduced in both cultivars. Better growth was observed fro bred variety in control treatment than P and K fertilizers with As, but in local variety growth at 50 mg/kg p and K was higher than control treatment (Fig 3).

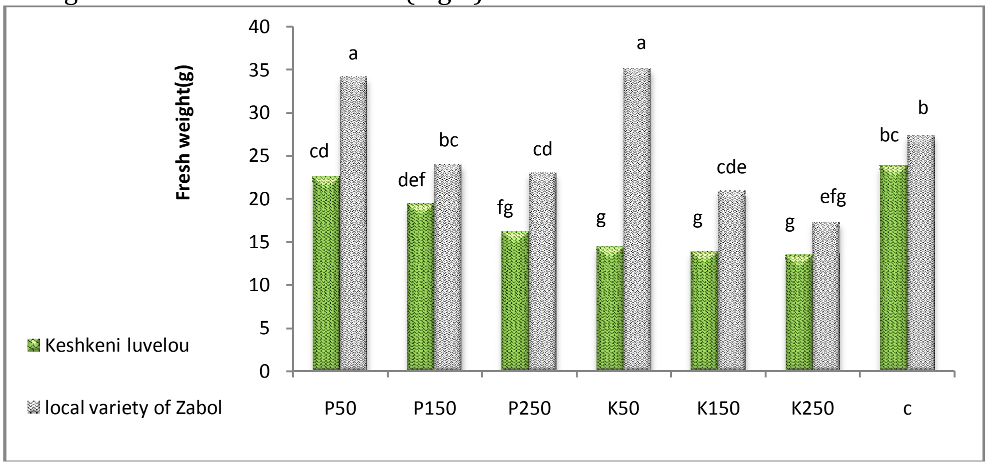

Fig 3.Interaction Varity*Fertilizers on the Fresh weight(g).

Leaf Area

The effect of variety, fertilizers level and their interaction on leaves area was significant at 1% of probability level (Table 2). Zabol local variety showed significant difference with bred variety. Highest leaves area observed at 250 mg/kg soil P with As that could be due to lower leaves density and their widening (Table 3 & 4). In their interaction, the highest leaves area of both cultivars achieved from 250 mg/kg soil P combined with As (Fig 4).

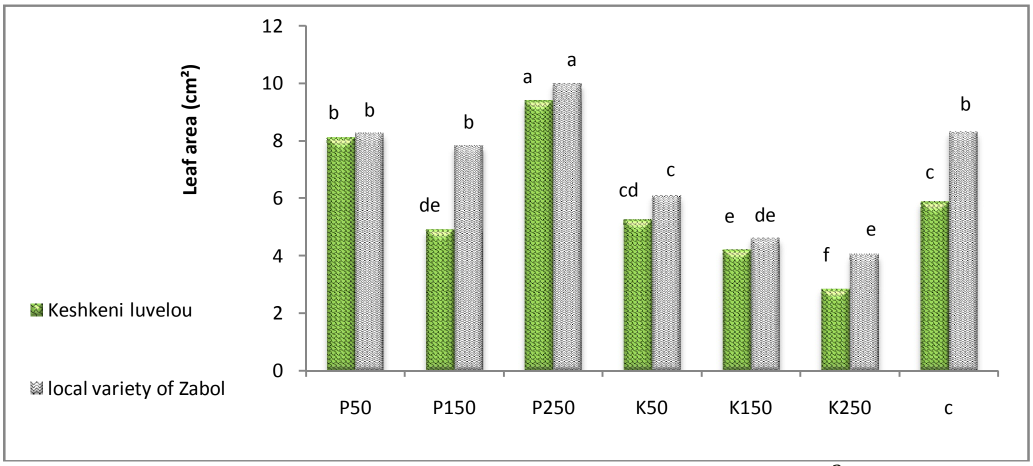

Fig 4. Interaction Varity*Fertilizers on the Leaf area (cm^2).]

Arsenic Concentration

Results showed that effect of variety and P and K fertilizers along with As was significant on As concentration in shoots; while their interaction was not significant (Table 2). Means comparison of main effect of variety showed that As concentration in vegetative parts of Keshkeni Luvelou is significantly different from local variety, which could be another reason of better growth of local variety besides its compatibility to the region (Table 3). The highest As concentration observed at 50 mg P kg-1 soil and the least concentration was observed at 250 mg P kg-1 soil (Table 4 and Fig. 5).

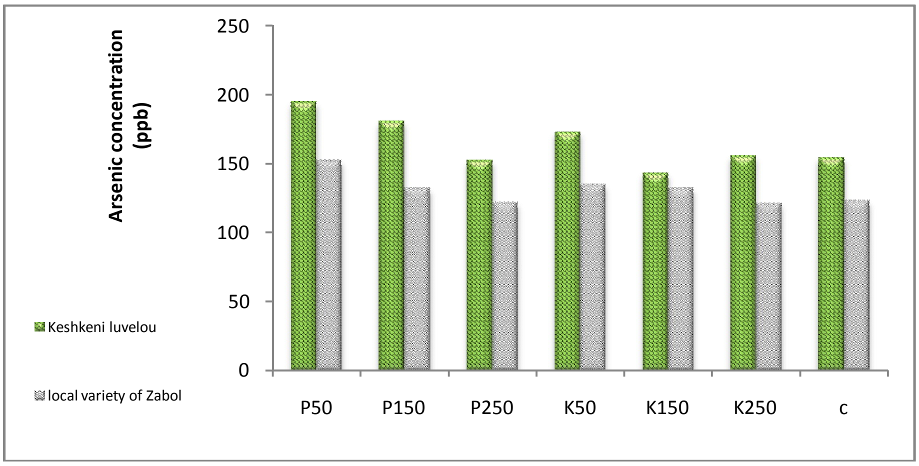

Fig 5.Interaction Varity*Fertilizers on theArsenic concentration (ppb).

Table 2. Analysis of variance of quantitative and qualitative characteristics on basil affected by P and K

SOV	DF	Plant height	Leaves number per plant	Lateral branches per plant	Fresh weight	leaf area	Arsenic Concentration
				Means Square			
Replication	2	73/23	31/45	2/95	2/32	4/49	192/77
Variety	1	** 74/66	**1337/35	**30/85	**1/33	**15/55	**11857/4
Fertilizer	6	** 27/43	*329/15	ns1/96	**5/43	**26/98	**1152/6
Varity×Fertilizer	6	** 26/16	92/57 ns	**7/30	**2/43	**1/62	213/7 ns
Error	26	4/34	120/99	1/00	0/06	0.16	202/9
C.V. (%)		4/87	18/81	8/21	8/16	6/25	9/62

fertilizers with As.

ns, * and ** not significant, significant at 5 and 1%, respectively.

Table3. Basil varieties mean comparison of quantitative and qualitative characteristics.

Variety	Plant height)cm(	Leaves number per plant	Lateral branches per plant	Fresh weight (g)	leaf area (cm²)	Arsenic Concentration (ppb)
KAshkani LOLO	41/42 b	52/81 b	11/33 b	2/91 a	5/79 b	164/8 a
Local of Zabol	44/09 a	64/09 a	13/04 a	3/27 a	7/01 a	131/2 b

Means with similar letter are not significant at the 5% probability level

Table 4. Means comparison of quantitative and qualitative characteristics affected by K fertilizer with As.

Treatments (mg/kg)	Plant height)cm(	Leaves number per plant	Lateral branches per plant	Fresh weight (g)	leaf area (cm2)	Arsenic Concentration (ppb)
P50	43/83 b	63/00 abc	29/32 a	8/35 a	8/17 b	173/5 a
P150	41/33 b	60/66 abc	22/71 c	8/85 a	6/36 d	156/4 b
P250	38/83 c	48/66 c	20/30 d	6/21 b	9/63 a	137/1 c
K50	44/83 a	67/66 a	25/43 b	9/45 a	5/67 e	153/1 bc
K150	43/83 ab	54/16 abc	18/38 de	6/18 b	4/40 f	137/9 bc
K250	42/16 ab	50/33 bc	16/50 e	4/38 c	3/50 g	138/6 bc
control	44/00 a	64/66 ab	26/61 b	8/15 a	7/08 c	138/75 bc

Means with similar letter are not significant at the 5% probability level.

In general, landrace of Zabol due to its compatibility to the region had better growth than the European cultivar, but at the control both varieties that As content of 15b mg/kg soil solely added to the soil, reduced local variety growth compared to other treatments, which may be due to lack of heavy metals contamination in this region. Studies on As showed that this element induce stress to plant and thus prevent plant growth. It can be said that P and K interaction in other treatments prevent negative effect of As on local cultivars [14], while in control treatment of European variety a type of growth stimulation was observed. This stimulating effect of As low levels on growth was similar to those achieved by Chen and Liu [15] and Jian, *et al* [16]on rice in which low As levels resulted in growth stimulation but contradicts with results obtained from wheat, maize, cucumber and cabbage [10] and sunflower [17] in which this stimulating effect did not happen. In a pot study that was conducted on pigweed, As levels of more than 15 mg/kg soil showed negative effect on seeds germination and leaves number [18]. Miteva et al. [19] reported the negative effect of As on tomato through limiting the growth through limiting roots and shoots growth. Their results showed that increased As concentrations reduce plants height and the highest reduction was in 100 mg/kg soil, but no significant difference was not found between 25 and 50 mg/kg soil. High As levels had a negative effect on Jatropha curcas (Euphorbiaceae) growth, but this reduction was lower in treatments containing As, N, P and K [20]. Fresh and dry weight reduction of shoots and roots with increased fertilizers levels can be due to lower growth of these plants at higher P and K levels that are consistent with those achieved from Artemisia [21]. Although quantitative traits of local Zabol variety was significantly different from European cultivar of Keshkeni Luvelou, but in compare with studies conducted in different regions of Iran on basil, presented results of three cultivars of basil, all results in terms of morphologic characters are very different, which represents the greater effectiveness of this variety from As toxicity [22]. Plants adaptation to heavy metal replacement is one of mechanisms associated with toxicity alleviate in plants. Toxicity and resistance to heavy metals by plants occur due to interaction of nutrients like Ca, P and K with heavy metals. One of the main roles of these nutrients in plants is to neutralize the toxic effect of heavy metals [23].

CONCLUSION

It was observed that P and K fertilizers can reduce the concentration of arsenic in edible parts of plants and reduce its toxicity. The interaction between P and As showed that increased applied P reduced As levels that reflects the competition between their absorption. At low P and K in soil, As uptake increased. Zabol local variety in most growth traits was better than the European one, which is due to its compatibility to studied region. Local variety absorbs less As from the soil, but had significant negative effect on growth than other treatments. Unlike the local variety, in control treatment of bred variety, As showed some simulative effect on vegetative growth.

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How to Cite this Article
Rahimi M., Poor M. R. A., Reza F., Rahman M. (2013).Effects of Potassium and Phosphorus Fertilizers on Arsenic Accumulation and Plant Growth of two Basil Cultivars. *Bull. Env. Pharmacol. Life Sci.*, Vol 2 (5): 79-85