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Leaf mineral nutrients composition and primary bud necrosis disorder in fruiting and de-fruited 'Askari' grapevine

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ABSTRACT

Primary bud necrosis (PBN) of grapevine, which is a physiological disorder, seriously decreases the fruit yield of vineyards. This research was carried out to determine the effects of mineral deprivation on increasing the incidence of PBN in 'Askari' table grapes in Sisakht region of Southwest of Iran. For this purpose, the changes of mineral elements in leaves and PBN percentage were estimated on both fruiting and de-fruited 'Askari' vines. The experiment followed a factorial experiment in a randomized completely block design consisting of 10 × 2 (10 sampling dates × 2 treatments (fruited and de-fruited)) and four replicates was conducted. Eighty leaf samples were collected from vine clusters located in four blocks of commercial vineyard every 10 days starting 40 days after bud break (DAB) until 130 DAB. PBN was first studied by a hand lens and then by a microscope after dissecting the buds. The results showed that the initial symptoms of PBN appeared at 60 DAB. The PBN disorder progresses as bud development proceeds, with the higher percentage of PBN being observed in de-fruited vines than fruiting ones. In both fruiting and de-fruited vines at 60 DAB, the amount of iron content was close to the critical deficiency level whiles the concentration of elements such as Zn, B and Mn, was below the critical range. Furthermore, with increasing the concentration of Zn, B and Mn in leaves, PBN percentage significantly decreased.

Keywords: Nutrition, primary bud necrosis, Sisakht, *Vitis vinifera* L., Vineyards.

وضعیت عناصر غذایی برگ و بافت‌مردگی جوانهٔ اولیهٔ در بوته‌های میوه‌دار و بی‌میوه انگور عسکری

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چکیده

بافت‌مردگی جوانهٔ (Bud necrosis) یک ناهنجاری فیزیولوژیک در انگور است که منجر به کاهش عملکرد می‌شود. این پژوهش به منظور تعیین اثر کمبود عناصر غذایی معدنی بر افزایش بروز مرگ جوانه اولیه انگور عسکری در منطقه سی‌سخت در جنوب‌غربی ایران بود. بدین منظور تغییرات عناصر معدنی در برگ و درصد بروز مرگ جوانه در بوته‌های میوه‌دار و بدون میوه تعیین گردید. آزمایش به‌صورت فاکتوریل در قالب طرح بلوک‌های کامل تصادفی شامل ده تیمار زمان نمونه‌برداری و دو تیمار میوه‌دار و بدون میوه در چهار تکرار اجرا شد. تعداد ۸۰ نمونه برگ مقابل خوشه، هر ۱۰ روز یکبار از ٤۰ روز تا ۱۳۰ روز بعد از شکفتن جوانه جمع آوری شد. ابتدا ارزیابی مرگ جوانه اولیه به وسیله لنز دستی و سپس با یک میکروسکوپ دیجیتالی بعد از برش جوانه، صورت گرفت. نتایج نشان داد که نخستین نشانهٔ ناهنجاری در٦۰ روز پس از شکفتن جوانه‌ها مشاهده شد. پیشرفت ناهنجاری مرگ جوانه در مراحل رشد و نمو جوانه، در بوته‌های بی میوه نسبت به میوه‌دار در سطح بالاتری بود. در هردو تیمار بوته میوه‌دار و بی‌میوه، حدود ٦۰ روز بعد از شکفتن جوانه، میزان سطح آهن به حد بحرانی نزدیک بود درحالیکه غلظت سایر عناصر همچون روی، بور و منگنز در زیر حد بحرانی قرار داشتند. بنابراین با افزایش غلظت عناصر روی، بور و منگنز در برگ، درصد ناهنجاری مرگ جوانه به‌طور معنی‌داری کاهش یافت.

واژه‌های کلیدی: تاکستان، تغذیه، مرگ جوانه اولیه، سی‌سخت، .*Vitis vinifera* L

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Introduction

Grape (*Vitis vinifera* L.) is considered as one of the most important fruit crops of Iran which is produced in different climates from temperate to tropical. Iran has been ranked as the 9th largest producer of grape with 2.2 million tons of fruit in 2012 (FAO, 2012). Unfortunately, the production value per area is relatively low due to poor cultural practices such as improper application of macro and micro nutrients, inadequate information for site and cultivar selection and complicated soil conditions (Bains, *et al*. 1981). In many parts of Iran, vineyards soils are calcareous which decreases the availability of micronutrients and exposes the vines to severe nutritional disorders. Primary bud necrosis (PBN) is a serious problem in some vineyards of Iran, and 'Askari' as important table grapes seems susceptible to PBN (Kavoosi, *et al*. 2012). Grapevines have compound buds containing primary (the most productive), secondary and tertiary buds; under normal conditions just primary bud grows. Whenever primary bud is damaged, secondary or in some cases tertiary buds break and grow. As a result of damages to such buds the bud break will decreases and the number of fruitful shoot and consequently the yield per vine are significantly reduced.

Mineral nutrition directly or indirectly affects the quantity and quality of bunches in table grapes. Lack of certain nutrient elements in plants also causes nutritional disorders that are similar to necrosis. For instance, deficiency of boron usually kills cells in meristematic regions (Collier & Tibbetts, 1983). Researchers have investigated the relationship between mineral nutrients and flower bud formation in different fruit species (Hartmann, *et al*, 1966; Priestely, 1977; Golomp & Goldschidt, 1981). Stressful conditions during flower bud formation may cause malformation and reduce bunch numbers in the following spring. This includes nutritional deficiency or excess and water stress (Jackson and Lombard, 1993; Srinivasan & Mullins, 1981). In addition to mineral nutrient deficiencies, overly vigorous vines might be expected to have high PBN (Dry & Coombe, 1994; Lavee *et al*., 1981; Wolf & Warren, 1995) because of actively growing shoot tips that are strong photosynthetic sinks. Carbohydrate resources and mineral nutrients preferentially go to the growing tips rather than to the buds and fruit bunches (Candolfi- Vasconcelos & Koblet, 1990).

Naito *et al*. (1987) observed equal amounts of the mineral nutrients in strong and weak shoots of 'Kyoho' as well as shoots of BN-resistant cultivars such as 'Delaware' and Muscat Bailey A' 25, 40 and 57 days after bud break. They reported that the levels of boron in the lateral buds of 'Kyoho' grape had no correlations with BN. The relationship between micronutrient status in fruiting and de-fruited vines with bud necrosis, which is a serious problem in some vineyards in Iran and all over the world, is unclear. Therefore, the objective of this study was to evaluate the changes in leaf micronutrient contents of fruiting and de-fruited vines in relation to PBN incidence in table grape 'Askari'.

Materials and methods

The experiment was conducted on Askari' table grape in Sisakht region (30^o, 51', 57'' N; 51^o, 27', 24'' E, 2200 m above sea level) Southwest of Iran in 2007-2008. The average annual precipitations are 676.7mm. The temperature ranges from -7.8 °C to 35.3°C. The average minimum and maximum temperature in growing season was 13 °C and 29.5°C, respectively. The vines were 18 years old grown on their own roots, trained as a head system, and spaced 2.5 × 3 m. They were pruned as spur pruning leaving 60 buds on each vine. The experiment followed as factorial in randomized completely block design consisting of two factors (10 date of sampling × 2 treatments of fruited and de-fruited) with four replications. The selected vines (40 vines) were divided in two groups; the first group was left intact while in the second group all flower clusters were removed on appearance on the shoots. All cultural practices were uniformly used across blocks following standard instructions.

Composite soil samples were taken, air-dried, ground and passed through a 2-mm sieve to obtain the needed soil fractions for determining physical and chemical soil properties. Texture was determined by Bouyoucos hydrometer method (Day, 1965) after dispersing soil with sodium hexametaphosphate. Electrical conductivity (EC) was measured on a 1:2.5 ratio extract with an EC meter and expressed as ds/m^{-1}. The pH was measured potentiometrically in water and in 1M CaCl^2 at the ratio 1/2.5 soil water and soil - CaCl^2. Organic carbon was determined by wet oxidation method of Walkley and Black (Nelson**,** 1982) and converted to organic matter (OM) by multiplying by a factor of 1.724. Kjeldahl method (Bremner, 1982) was employed to determine total nitrogen. Concentration of soil nutrients (Phosphorus and Potassium) were used to calculate the soil nutrients from concentration basis to an amount or nutrient stock basis (soil P and soil K stocks) based on the procedure that Enideg (2008) described. The method used for the determination of the elements (Fe, Zn, B, Mn, Mo and Cu) in soil solution was the atomic absorption spectrophotometer (Varian Model Spectra 400 Plus) (table 1).

Eighty leaf samples composed of twenty leaf samples from fruiting and de-fruited vines in four blocks were taken from the opposite of the cluster.

Also all samples were taken opposite to the clusters or node 3 to 12 at 10 days intervals starting 40 days after bud break (DAB) until 130 DAB and transported in perforated paper bags to the laboratory of Department of Horticulture, Shiraz University. The sample surfaces were dried with clean blotting papers, placed in paper bags, and dried in a forced-air oven at 70°C for 72 hours. The dried samples were ground in a stainless steel Wiley mill. The oven-dried-ground samples were wet digested in a mixture of nitric-perchloric acids (HNO_3:$HClO_4$ (4:1) and K, Ca, Mg, Fe, Mn, Zn and Cu concentrations in the digest were quantified by Atomic Absorption Spectrophotometry (Varian Model Spectra-400 Plus) (Ulger *et al*. 2004).

Table 1. Physical and chemical soil properties of Vineyard

Depth (cm)	EC (ds/m)	T.N.V (%)	pH	Saturation percentage (S.P.%)	Texture	Organic carbon (O.C.%)
0-30	1.5	28.75	7.2	78.6	CL-L	3.5
30-60	1.5	37.5	7.4	48.3	CL-L	0.78

Continued table 1. Physical and chemical soil properties of Vineyard

Depth (cm)	N (%)	P (mg/kg)	K (mg/kg)	B (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
0-30	0.33	80	2320	0.17	10.4	0.73	0.69
30-60	0.08	37.2	1070	0.22	12.3	1.01	0.95

Bud samples were taken at 10 days intervals starting 40 DAB until 130 DAB. A 10 x hand lens was applied to check compound bud situation and for taking photos, a digital microscope (Dinolite-AM413T) was used. A damaged bud showing green tissue for the primary, secondary, and tertiary bud were considered normal.

Conversely, a bud with damage shows a Dark/brown discoloration (Figure1). SAS package program (SAS Inc. Raleigh, NC. USA) was used to analyze the data. Means were separated using Duncan's Multiple Range Test at $p \leq 0.01$.

Results and Discussion

Micronutrient contents and PBN incidence

Analyses of variance showed that effect of fruiting and de-fruited treatments, Organ type and date of sampling on microelements changes in ‘Askari’ table grape were significant ($p \leq 0.01$) (Table 2). The results in Figure 2 show that at 40 and 50 DAB there was no symptom of PBN. The first symptoms appeared at 60 DAB and eventually with more bud development, the PBN disorder developed more. The highest PBN was observed at 130 DAB.

PBN percentage was significantly higher in ‘de-fruited’ vines than in ‘fruiting’ ones (Figure 3). Interaction effect of sampling date with fruiting status of vines showed that except for 40 and 50 DAB, other sampling dates have higher rate of the PBN in ‘de-fruited’ vines compared to ‘fruiting’ ones (Figure 4).

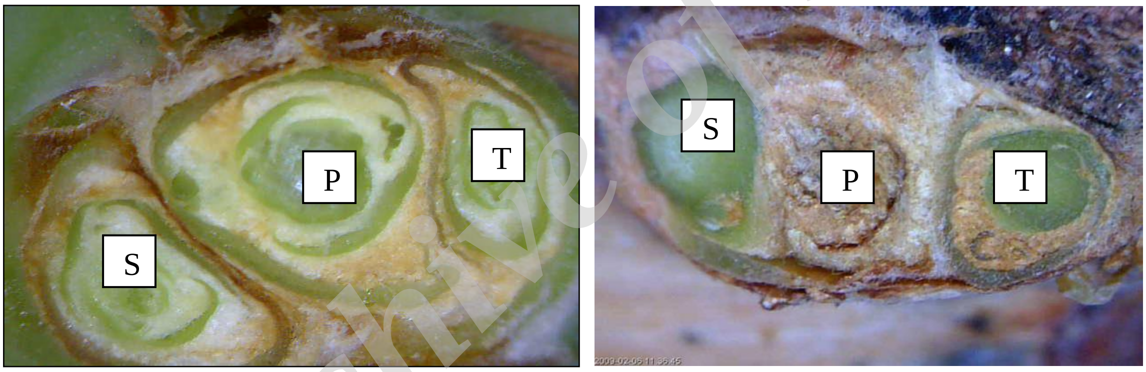


Figure 1. Cross section of a compound bud showing all buds alive (Left), and damaged-primary bud (Right) in ‘Askari’ grapevine. Primary (P), Secondary (S) and Tertiary (T).

Table 2. Variance analysis (ANOVA) of the effects of fruiting and de-fruited treatments, organ type and date of sampling on microelement levels in ‘Ansari’ table grape

Source of Variation	df	Mean Square					
		Fe	Zn	B	Mn	Mo	Cu
Replication	2	322.19**	251.41**	319.40**	14.83**	0.0457^{ns}	230.85**
Treatment	1	3957.05**	196.06**	3428.71**	0.2516**	0.0583^{ns}	2.97*
Organ	2	26833.79**	336.80**	4930.45**	223.05**	0.4424**	293.01**
Date	9	3792.36**	184.87**	7362.06**	6.04**	0.0895**	128.81**
Treatment × Organ	2	2933.01**	52.66**	4430.10**	0.9620**	0.0521**	0.3042**
Treatment × Date	9	2059.67**	67.27**	1882.32**	2.08**	0.0786**	23.57**
Date × Organ	18	4038.24**	89.75**	4729.87**	4.50**	0.0751**	16.36**
Treatment × Organ × Date	18	2782.26**	63.95**	4136.55**	2.56**	0.0780**	20.13**
Error	118	1.4680	0.1123**	5.2094**	0.0093**	0.0168**	0.5724**
C.V (%)		1.87	1.77	4.63	4.09	5.22	5.75

ns, *, **: Non significantly differenc and significantly differenc at 5 and 1% probability levels, respectively.

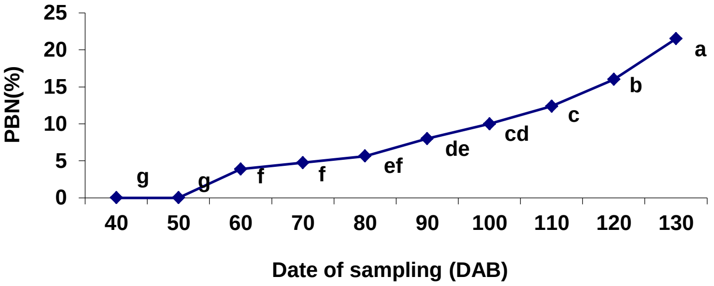


Figure 2. PBN percentage of ‘Askari’ cultivar at different dates of sampling.

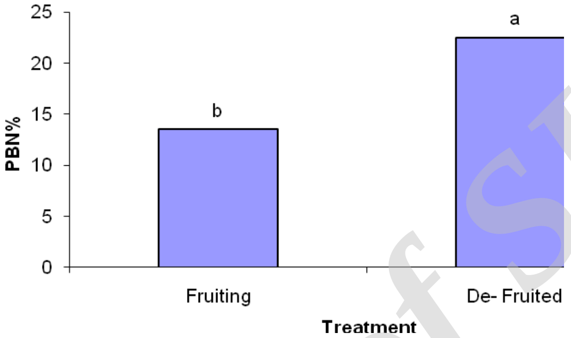


Figure 3. PBN percentage in the fruiting and de-fruited of ‘Askari’ cultivar.

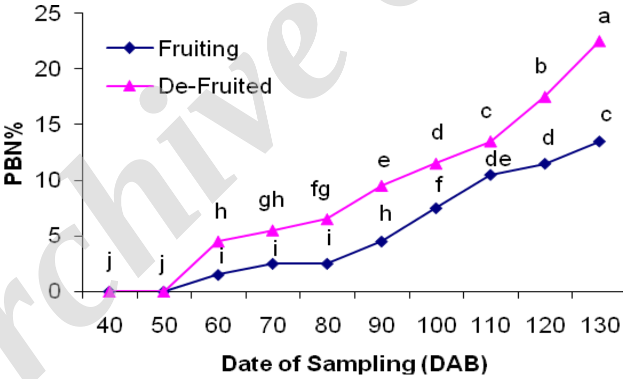


Figure 4. Interaction of fruiting and de-fruited treatment with date of sampling on the PBN percentage of ‘Askari’ cultivar.

From the onset of PBN, occurring at 60 DAB, changes in microelements represent the high and low levels of some elements compared to those in normal ranges. In both fruiting and de-fruited vines at 60 DAB, the amount of Fe was near the critical ranges, but other elements such as Zn, B and Mn were in less than normal level at this time (Table 3).

The overall changes in micronutrients in the fruiting and de-fruited vines, compared to critical range, showed that the iron was greater than the critical range but Zn, B, Mn and Mo were below the normal range during the growing season (Table 4).

Significant correlations between the low levels of Zn, B and Mn with PBN incidence were observed. However, a negative correlation between the decreasing in Zn, B and Mn and the increase in then percentage was observed (Figure 6).

There was a correlation between the level of microelements (Zn, B and Mn) and the percentage of PBN incidence. With decrease in the leaf contents of these elements, the percentage of PBN increased (Figure 7).

Table 3. Changes in levels of leaf microelements at 60 DAB in fruiting and de-fruited vines against the critical range in 'Askari' grapevine

Treatment	Micro elements					
	Fe	Zn	B	Mn	Mo	Cu
Critical range	40	25	30	20	0.45	11
Fruited	44.5	16.32	19.42	15.59	0.18	11.8
De-Fruited	42.58	14.88	18.45	14.47	0.17	12.4

Table 4. Microelements changes in fruiting and de-fruited vines in comparison to leaf analysis critical range in 'Askari' grapevine

Treatment	Micro elements					
	Fe	Zn	B	Mn	Mo	Cu
Critical range	40	25	30	20	0.45	11
Fruited	53.08	19.97	27.65	19.32	0.24	13.02
De-Fruited	50.45	17.88	25.92	17.39	0.27	13.27

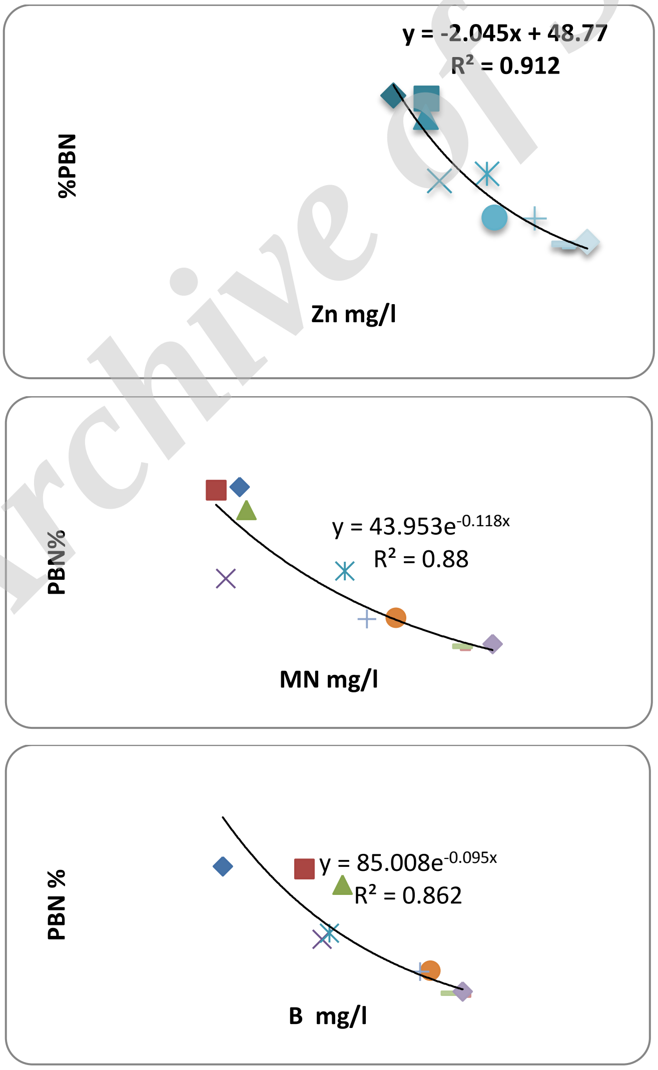
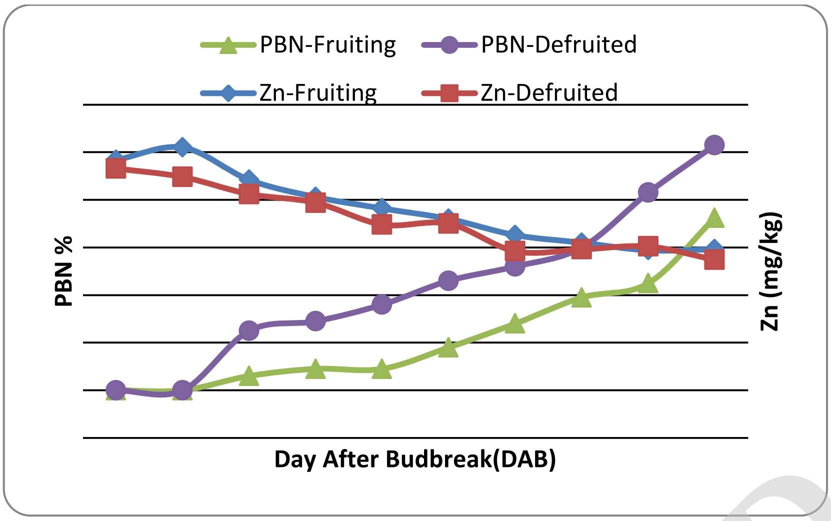
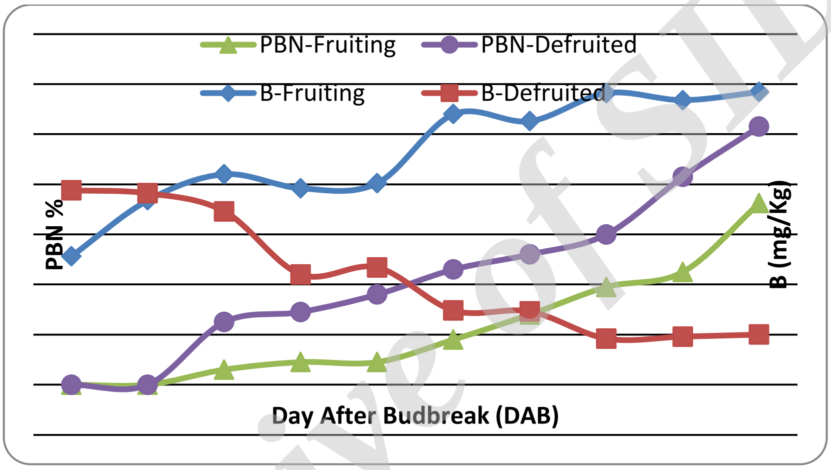


Figure 6. Linar regression between the Zn, Mn, B and %PBN incidence in 'Askari' grapevine





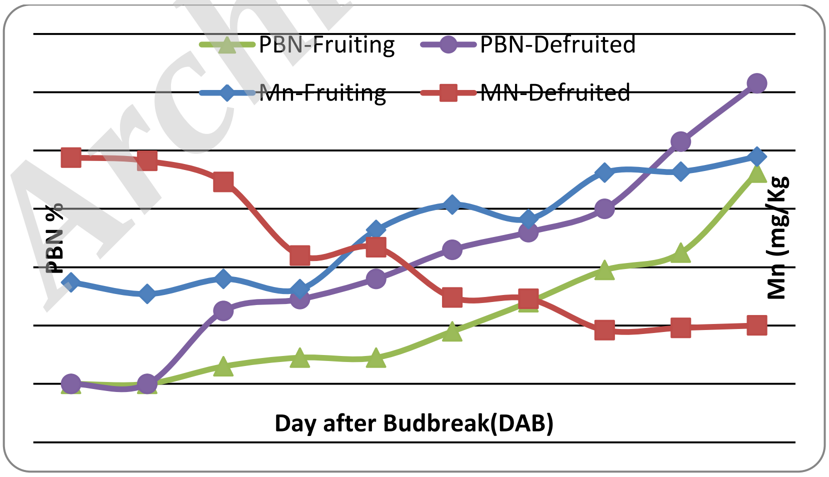


Figure 7. Changes of microelements in fruiting and de-fruited vine in different date of samplings related to %PBN.

Figure7 shows that the PBN rate has increased with the sampling date in both fruiting and de-fruited vines, with the rate of PBN being higher in fruiting vines than de-fruited ones. Also, the concentration of Zn reduced with the advancing sampling date in 'fruiting' and 'de-fruited' vines; however, the amount of Mn and B reduced just in 'de-fruited' vines.

The incidence of PBN in vineyards has been studied in some countries, indicating that this physiological disorder can significantly decrease fruit yield (Kavoosi, *et al.* 2013). Grapevines are adapted to a wide range of soil types and have fewer mineral deficiency problems and a lower plan food demand than many other horticultural crops (Perez Harvey, 2008). In other words, a normal fertile soil with essential nutrients for vine growth is needed. Deficiencies of micro elements drastically affect grape yield and quality, and excessive amounts of some nutrients can also be harmful (Perez Harvey, 1993). There are conflicting reports in the literature regarding the effect of mineral nutrients on BN. From these reports, it is not possible to conclude whether the nutrient factor is the cause, the effect, or independent of the BN problem. It was, therefore, hypothesized that mineral nutrients may be locally deficient in the lateral buds and that such deficiencies might lead to BN. Studies conducted in India have indicated that excessive fertilizer application and irrigation result in flower bud killing in ‘Anab-e-Shahi’ grapes (Bains *et al*., 1981; Bindra & Chohan, 1975). Zinc and iron deficiencies are the most common micro elements nutritional disorders that have been observed in many vineyards of Iran. Another common deficiency includes manganese, boron, copper, molybdenum. Most vineyards of Sisakht region, where the soil temperature is very low in spring, i.e., when the grapes need to make the most of the mentioned elements, absorption of nutrients from the soil by the roots is reduced. There is also considerable amount of lime (≥ 60%) in the soil with high pH (7.9). Under these conditions, vines cannot uptake iron and other micronutrients including zinc and boron. Bud mortality, as a proven disorder in the vineyard, induces low fertility, poor bud break in next spring and eventually reduces the productivity of the ‘Askari’grape.

Several external factors such as temperature, light, availability of nutrients and water may have an impact on the fertility of the grapevine buds (Shikhamany, 1999; Dry, 2000; Mullins *et al*., 2000). In addition to nutrient deficiencies, PBN disorder is more common in vigorous vine, because the tip of the shoots is too strong for photosynthetic assimilates. Therefore, actively growing shoot tips compete for carbohydrates and minerals with bunches. Mineral deficiency in Riesling and Chardonnay grape cultivars showed that PBN disorder is not due to deficiencies in essential elements (Vasudevan, 1997). Contrary to some reports, the role of mineral elements in the fertility and mortality of ‘Askari’ grape buds in the vineyards surveyed is shown. Insufficient amounts of some micronutrients such as Zn, B and Mn, which were below the normal range in the developing buds in our findings, were related to PBN disorder. The possible involvement of these elements in root growth and hormone synthesis can be effective in bud development and the above mentioned disorder. The possible involvement of these micro elements in root growth and therefore in cytokinins biosynthesis could explain their influence in floral stimulus (Jako, 1976).

Conclusion

In general, our findings show the role of zinc, boron and manganese deficiency in PBN incidence in ‘Askari’ table grape. Also with increasing the concentrations of Zn, B and Mn in leaves, PBN percentage significantly decreased. The amount of PBN in de-fruited vines was more than that of fruiting vines.

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