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Boron (H_3BO_3) Toxicity in Bean (*Phaseolus vulgaris* L.) Germination

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Author's contribution

This whole work was carried out by the author AC.

Original Research Article

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ABSTRACT

Aim: The beneficial and harmful effects of the chemical compound depend on the organism physiology but high levels of boron have harmful effects for most of the plants. Chemical compound limits life cycle activities. In the study, the toxic effects of Boron (H_3BO_3) on the germination of common bean (*Phaseolus vulgaris* L.) seeds were investigated.

Study Design: The experimental design comprised complete randomized blocks (CRD) with 3 replicates. The results were evaluated by analysis of variance using the Statistical Analysis System software and treatments means were considered significantly different at $p < 0.05$. Mean separation was evaluated by Least Significant Difference. Correlation coefficients between all possible combinations were determined and the results indicated that all the studied parameters had significantly positive or negative relationship.

Place and Duration of Study: The present experiment was conducted at Gaziantep University Vocational School of Higher Education in Nurdagi, Gaziantep/Turkey between July and August 2012.

Methodology: Gina and Sarikiz common bean cultivars were placed in the temperature adjustable plant growth cabinet at 25°C with 1-2-3-4-5% Boron Concentration and distilled water-as control-(2,5 μ S/cm). In this study Germination percentage (GP), germination index (GI), seed length (SL), vigor Index (VI), difference from control (DFC) and fitotoxicity parameters were investigated.

Results: Germination Percentage, Germination Index, Seed Length, Vigor Index, Difference from Control and Fitotoxicity parameters varied between 0.00-90.00% , 0.00-5.09, 0.00-5.64cm, 0.00-505.43, 0.00-100% and 0.00-100% respectively. Significant

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differences were found between cultivars for Germination Percentage, Germination Index, Seed Length, Vigor Index. On the other, the only significant difference in interaction (Variety X Boron Concentration) was detected in vigor index.

Conclusion: Increasing in boron concentration could decrease germination of common bean. Fitotoxicity has negative and significant relationship with Germination Percentage, Germination Index, Seed Length, Vigor Index however other all possible correlation combinations was significant and positive.

Keywords: Boron, H₃BO₃, toxicity, bean, Phaseolus vulgaris, germination

1. INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is the most widely distributed and consumed legume species of the genus *Phaseolus*, a genus comprised of some 70 species [1]. Among the domesticated species, *P. vulgaris* accounts for more than 90% of the cultivated crop and it is the most widely consumed grain legume in the world [2].

Phaseolus is cultivated on all continents except Antarctica, under very diverse cultivation conditions. Among Asian Countries China, Iran, Japan and Turkey are the major producers of the common bean [3, 4, 5].

Boron certainly required normal growth and development of plants [6] and plants must have boron for a life cycle [7]. There are some functions of boron in plants, for example it has task in structural and metabolic processes [8]. In addition, it important for plant in extracellular matrix [9].

This element is essential microelement for plant but it direct or indirect affects a lot mechanism such as leaf photosynthesis, nitrogen metabolism, cell membrane function, cell membrane biosynthesis, cell elongation and division, plant development etc[10, 11, 12]. On the other boron has a special importance for successful pollen germination and pollen tube growth [13]. It is a structural component in growing plant tissues [14]. It reported that Boron deficiency caused root growth inhibition [15]. It is necessary for the plants, but high concentrations of this micro-element have phytotoxic effect [16]. If it reaches toxic level, it inhibits primarily root growth through limiting cell elongation rather than cell division [17].

In this study, effects of toxic levels of Boron (H₃BO₃) were investigated on bean (*Phaseolus vulgaris* L.) germination.

In the research the Gina and the Sarikiz cultivars are widely use in Turkey. In the study, Gina and Sarikiz, spread all over the country, cultivars were used. And this cultivars are early dwarf (Gina) and early (Sarikiz).

Objectives of the research are the toxic effects of Boron (H₃BO₃) on the germination of common bean (*Phaseolus vulgaris* L.) seeds.

2. MATERIALS AND METHODS

2.1 Seed Material

In the research, nationally registered bean cultivars used (Gina and Sarikiz). The study was conducted at Gaziantep University Vocational School of Higher Education in Nurdagi, in climate controlled cabinet during July and August 2012.

2.2 Boron Concentrations and Seed Treatments

Common bean seeds were surface sterilized with 5% sodium hypochlorite (NaOCl) and washed thoroughly with distilled water [18]. And then, seeds were germinated in 120 mm diameter and 20 mm height, sterilized petri dishes. Petri dishes were washed with tap water followed by a rinsing with distilled water and then sterilized at 160°C for 2 hours in the hot air sterilizer [19].

Boron solutions (H_3BO_3) were prepared as 0(control)-1-2-3-4-5% with using distilled water (2,5 $\mu S/cm$). The petri dishes were arranged in a completely randomized design (CRD) with three replications.

20 bean seeds were put in each petri dish on double layer Whatman paper. 15 ml of the solution was applied to each petri dish. On the other hand, germination cabinet sprayed against fungi with 5g/L Captan (N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide) and it adjusted 25°C temperature and 50% relative humidity. Petri dishes were placed in germination cabinet for 9 days [20]. Petri dishes were observed daily and 5 ml distilled water (2,5 $\mu S/cm$) was added to the each petri dishes.

2.3 Germination Percentage (GP)

Germination percentage was recorded the 9th day. Germination percentage was calculated with the following formula:

$$GP \% = \frac{\text{Number of germinated seeds}}{\text{Number of total seeds}} \times 100$$

2.4 Germination Index (GI)

GI was calculated as described by the Association of Official Seed Analysts [21],

$$GI = \sum \frac{G_t}{T_t}$$

In this formula G_t is the number of seeds germinated on day t and T_t is the number of days.

2.5 Seedling length (SL)

Seedling lengths of the seeds in petri dishes were measured in centimeters with caliper.

2.6 Vigor Index (VI)

Vigor index (VI) was calculated according to [22] as follows:

$$VI = [\text{Seedling length (cm)} \times GP (\%)]$$

2.7 Difference from Control (DFC)

DFC was calculated by using the formula of [23]:

$$DFC \% = \frac{\text{Germination of control (\%)} - \text{Germination of treatment (\%)}}{\text{Germination of control (\%)}} \times 100$$

2.8 Phytotoxicity

The parameter was calculated from a formula as follows [24]:

$$\text{Phytotoxicity \%} = \frac{\text{Radicle length of control} - \text{Radicle length of test}}{\text{Radicle length of control}} \times 100$$

2.9 Statistical Analysis

The experimental design was comprised with complete randomized blocks (CRD) with three replicates. The results were evaluated by analysis of variance using the Statistical Analysis System software v.9.0 [25], and treatments means were considered significantly different at $p < 0.05$. Mean separation was evaluated by Least Significant Difference (LSD) [26].

3. RESULTS AND DISCUSSION

Significant differences were found between cultivars in terms of all measurement parameters except DFC and Fitotoxicity. And boron solutions were statistically different from each other for all studied features. Variety X Boron concentration interaction was statistically significant detected in vigor index parameter (Table 1).

Table 1. Summary of the analysis of variance for all the analyzed parameters

| Source of Variation | D F | Mean Square | | | | | |
|--|-----|-------------|----------|----------|------------|-------------|--------------|
| | | GP | GI | SL | VI | DFC | Fitotoxicity |
| Variety | 1 | 506.25* | 3.809** | 3.349** | 35250.1** | 0.828 | 188.47 |
| Boron Concentrations | 5 | 8332.92** | 24.483** | 23.282** | 172683.8** | 11939.049** | 8698.67** |
| Variety X Boron Concentration Interactions | 5 | 152.92 | 0.520 | 0.290 | 8008.5** | 209.999 | 74.02 |
| Error | 24 | 113.89 | 0.383 | 0.158 | 1287.7 | 313.669 | 60.40 |
| CV (%) | | 22.27 | 24.99 | 18.77 | 22.89 | 42.02 | 13.11 |

* $P < 0.05$; ** $P < 0.001$

3.1 Germination Percentage (GP)

Germination rate decreased with increasing concentration of boron and cv. Gina and cv. Sarikiz germination percentage was zero in 5% and 4% H_3BO_3 concentrations, respectively. Especially Gina cv. was resistant to boron toxicity. Because the cultivar germinated until 4% boron concentration and mean GP value was 51.67% (Table 2). GP decreased when the boron concentration increased (Table 3 and Fig. 1). Similar results reported by [27].

Similarly studying the effect of seed priming on germination, showed that concentrations exceeding 0.04 M boric acid significantly reduced the germination rate [28]. [29] reported that high doses of micronutrients due to their toxic effects, caused an increase of abnormal and dead seedlings.

Table 2. According to cultivars Germination Percentage, Germination Index, Seed Length, Vigor Index, Difference from Control and Fitotoxicity means

| Cultivars | GP (%) | GI | SL (cm) | VI | DFC (%) | Fitotoxicity (%) |
|-------------------|-------------|-------------|-------------|--------------|--------------|------------------|
| Gina | 51.67 A | 2.80 A | 2.42 A | 188.07 A | 42.30 | 56.98 |
| Sarikiz | 44.17 B | 2.15 B | 1.81 B | 125.49 B | 42.00 | 61.56 |
| LSD values | 7.34 | 0.43 | 0.27 | 24.69 | 12.18 | 5.35 |

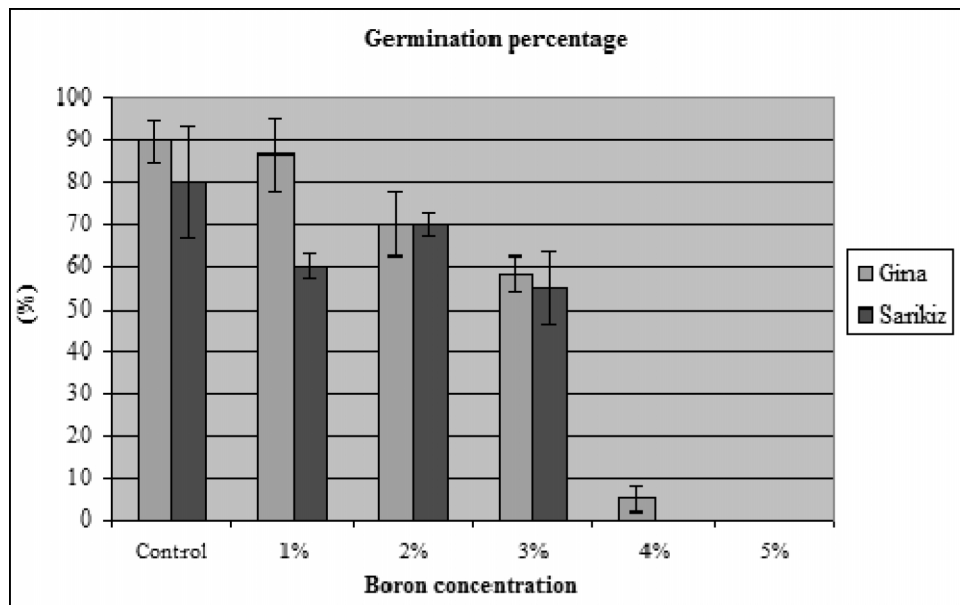


Fig. 1. Relationship between germination percentage and boron concentrations in two bean cultivars

Table 3. According to boron concentrations Germination Percentage, Germination Index, Seed Length, Vigor Index, Difference from Control and Fitotoxicity means

| Boron Conc.(%) | GP | GI | SL | VI | DFC | Fitotoxicity |
|----------------|---------|--------|--------|----------|----------|--------------|
| 0 (Control) | 85.00A | 4.77 A | 5.21 A | 438.14 A | 0.00 C | 0.00 E |
| 1 | 73.33AB | 4.10 A | 3.51 B | 265.60 B | 12.40 BC | 31.84 D |
| 2 | 70.00B | 3.26 B | 2.05 C | 143.58 C | 14.96 BC | 60.00 C |
| 3 | 56.67C | 2.66 B | 1.59 C | 90.80 D | 28.49 B | 69.64 B |
| 4 | 2.50D | 0.08 C | 0.34 D | 2.54 E | 97.06 A | 94.15 A |
| 5 | 0.00D | 0.00 C | 0.00 D | 0.00 E | 100.00 A | 100.00 A |
| Means | 47.92 | 2.48 | 2.12 | 156.78 | 42.15 | 59.27 |
| LSD values | 12.72 | 0.74 | 0.47 | 42.76 | 21.10 | 9.26 |

3.2 Germination Index (GI)

GI values similarly changed to GP. When the boron concentration increased, GI values decreased (Table 3 and Fig. 2). Germination was completely destroyed in 3% and 4% boron levels for Sarikiz and Gina cultivars, respectively (Table 2.). Highest value of mean GI was detected in control (4.77). Germination index parameter changed parallel with GP.

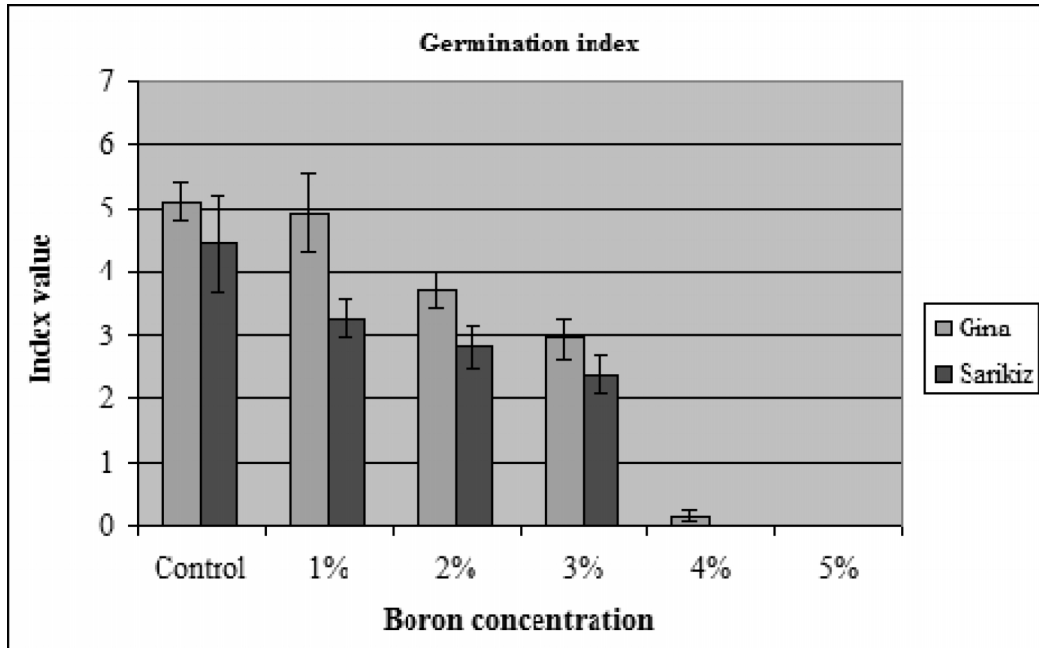


Fig. 2. Relationship between germination index and boron concentrations in two bean cultivars

3.3 Seedling length (SL)

According to cultivar means the highest SL value was observed at control treatment (5.21 cm) and the lowest value was observed at %5 boron concentration treatment (0.00 cm). In terms of cultivars, Gina seedling were more length (2.42 cm) than Sarikiz seedling. (1.81 cm) (Table 2 and Fig. 3). It was also found that root and shoot length of seedlings varied significantly between cultivars [27]. SL rate decreased with increasing concentration of boron[27]. B concentration increased the secondary root emergence inhibited [30].

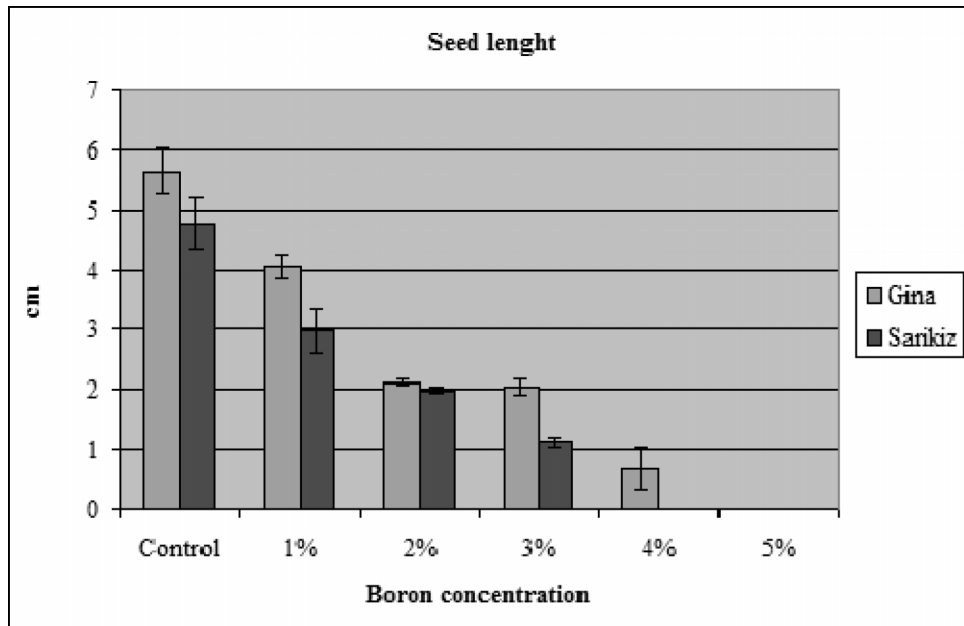


Fig. 3. Relationship between seed length and boron concentrations in two bean cultivars

3.4 Vigor Index (VI)

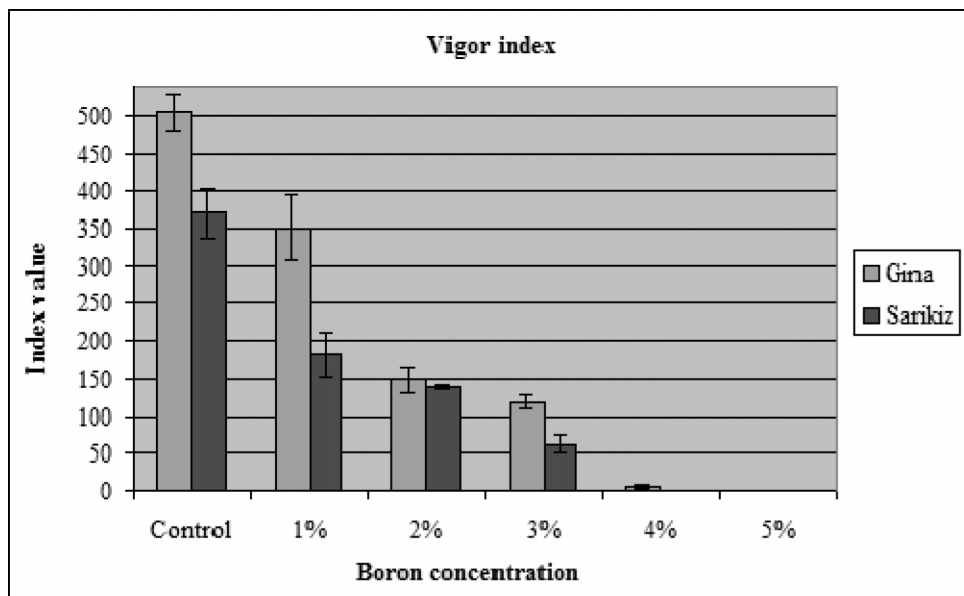


Fig. 4. Relationship between vigor index and boron concentrations in two bean cultivars

The highest VI, was observed in the control (438.14) and lowest was 5% boron (0.00). These values parallel GP and SL because VI is a value calculated from the parameters (SL and GP). In terms of genotypes, statistically significant difference was found between the cultivars (Table 1 and Fig. 4). Gina cv. vigor mean value was 188.07 and Sarikiz cv. was 125.49. Gina and Sarikiz cultivars are different statistically for Boron response. A lot of chemical compounds in high concentration significantly decreased the vigor index [31,32,33].

3.5 Difference from Control (DFC %)

The DFC increases when the boron concentration increased which meant increased boron concentration caused a decrease in germination (Table 3 and Fig. 5). If germination ratio is zero, DFC value is 100%. DFC value was increased in high level some chemical compounds [31].

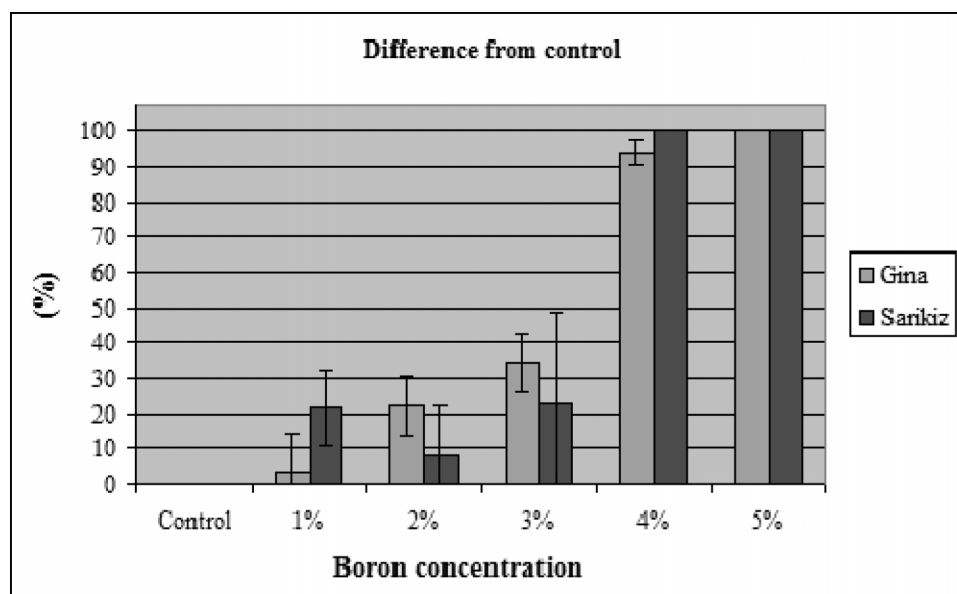


Fig. 5. Relationship between difference from control and boron concentrations in two bean cultivars

3.6 Phytotoxicity

According to means, 5% boron concentration caused maximum phytotoxicity level (100%). On the other Gina cv. more resistant to boron than Sarikiz. Gina cultivar has shown resistance to the level of 5% boron but Sarikiz cv. was lost all germination ability in 4% boron (Fig. 6). Phytotoxicity increased when chemical compounds increased [24]. Studies have indicated that excessive B can disrupt membranous structures [34, 35]. Boron 15 and 20 ppm seemed to be associated with B toxicity thus appeared to be stressful and accelerated adverse growth [36].

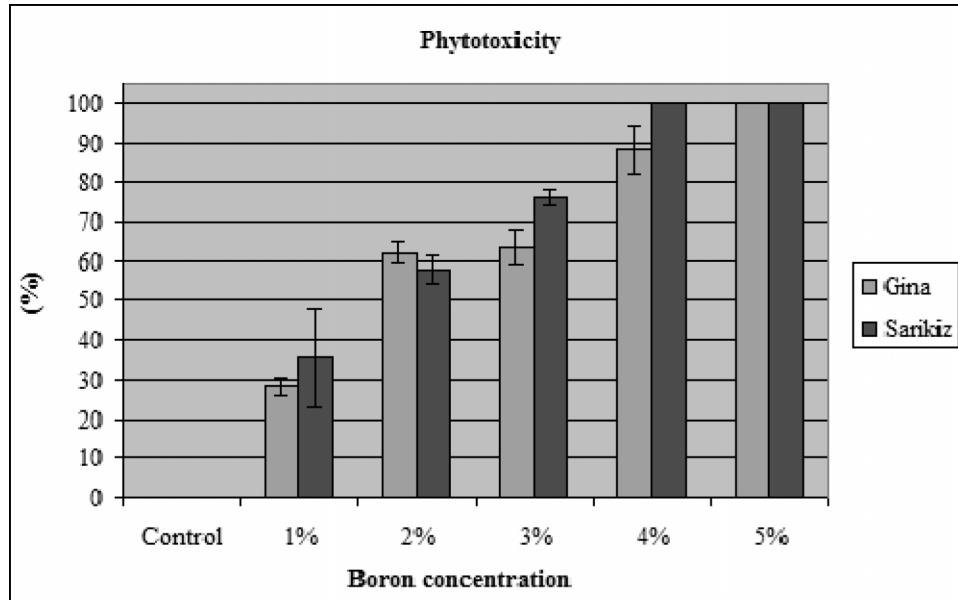


Fig. 6. Relationship between phytotoxicity and boron concentrations in two bean cultivars

3.7 Correlation Coefficients

Correlation coefficients between all possible combinations were determined and they were positive or negative statistically significant. GP was found to have positive correlation GI (0.9898), SL (0.8880), VI (0.8493) and GP was found to have negative correlation DFC (-0.9992) and Fitotoxicity (-0.8913) parameters (Table 4). GI showed positive correlation with SL (0.9392), VI (0.9086) and negative correlation with DFC (-0.9843) and Fitotoxicity (-0.9421). SL exhibited positive correlation with VI (0.9949) and it exhibited negative correlation with DFC (-0.8708), Fitotoxicity (-0.9999). The results indicate that VI has a highly significant negative correlation with DFC (-0.8292) and Fitotoxicity (-0.9943). On the other hand DFC-Fitotoxicity correlation coefficient was positive and significant (0.8742). DFC-Fitotoxicity and other germination parameters has adverse relationship due to their rates shows seeds poisoning rate. If the level of germination increased, decreased DFC and Fitotoxicity.

Table 4. Correlation matrix for analyzed variables (Germination percentage (GP), germination index (GI), seed length (SL), vigor Index (VI), difference from control (DFC) and fitotoxicity)

| | GP | GI | SL | VI | DFC | Fitotoxicity |
|--------------|-----------|-----------|-----------|-----------|---------|--------------|
| GP | 1.0000 | | | | | |
| GI | 0.9898** | 1.0000 | | | | |
| SL | 0.8880* | 0.9392** | 1.0000 | | | |
| VI | 0.8493* | 0.9086* | 0.9949** | 1.0000 | | |
| DFC | -0.9992** | -0.9843** | -0.8708* | -0.8292* | 1.0000 | |
| Fitotoxicity | -0.8913* | -0.9421** | -0.9999** | -0.9945** | 0.8742* | 1.0000 |

4. CONCLUSION

Germination percentage, germination index, seed length, vigor index statistical significantly different in terms of cultivar and variety x boron concentration interaction was significant in vigor index. The overall results indicated that increasing in boron concentration could decrease germination of common bean. This decrease continued up to the level of 4% and 5% in Sarikiz and Gina cv, respectively. No germination was at this point. If more boron concentration is increased, no germination is observed. According to correlation analysis DFC and Fitotoxicity has negative and significant relationship with Germination Percentage, Germination Index, Seed Length, Vigor Index but other all possible correlation combinations was significant and positive.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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