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EFFECTS OF FOLIC ACID ON SEED GERMINATION PROPERTIES AND SEEDLING GROWTH OF WHEAT

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Abstract:

In order to determine the folic acid role in seed germination stage and some seedling growth of wheat, two compactly randomized design (CRD) experiments with 4 replications was conducted. In the first experiment, different levels of Sulfamethoxazole (5, 10, 20 and 30 micro molar) as inhibitor of folic acid production on the seed germination and seedling growth of wheat were used. Analysis of variance indicated significant differences among different levels of Sulfamethoxazole for germination rate, seedling growth, seed reserve utilization, the efficiency of mobilized seed reserve into plant tissue and seed reserve depletion percentage. In the second experiment, different combinations of folic acid and Sulfamethoxazole with control were used. Results of this experiment indicated significant differences among treatments and verified finding of the first experiment. Fitting linear regression of different concentrations of Sulfamethoxazole solution as the independent variable and the measured traits as the dependent variable indicated significant relations with relatively high amount of coefficient of destination except germination percentages. The positive effects of folic acid on growth and development of seeds indicates its important role in biochemical and physiological process in plant cells. Generally, we conclude that using Sulfamethoxazole as biosynthesis inhibitor of folic acid, can suppress most living process in seed germinating period such as the germination rate, seed reserve utilization, the efficiency of mobilized seed reserve into plant tissue and seed reserve depletion percentage.

Keywords: biosynthesis inhibitor; Sulfamethoxazole; *Triticum aestivum* L.

1. Introduction

Seeds and specially cereal grains are a vital component of the human's diet in allover of world. Seed germination could lead to the vegetative plant growth initiation. Seed germination is the first critical stage in the any plant life cycle and determines optimal plant density, crop uniformity and management options [7]. The seed germination phenomenon incorporates those events that commence with the absorbent of water by the quiescent dry seed and terminate with the elongation of the embryonic axis ë18ç. The food reserve of seed necessary for suitable germination is stored in the endosperm. When the seed uptakes water, enzymes are activated and digest the starch in the endosperm to soluble sugar. The cotyledon absorbs the sugar and passes it to the rapidly growing both radicle and shoot [15]. In other word, the sign that germination is complete is the penetration of the structures surrounding the embryo by the radicle which is called commonly germination.

During wheat germination, the aleurone synthesizes hydrolytic enzymes, including α-amylase, protease, phosphates, nuclease and lipase into the starchy endosperm. All enzymes synthesis is induced by the gibberellic acid, which emanates from the embryo. For example, α-amylase is synthesized at the rough endoplasmic reticulum (RER) and it is secreted through a vesicular route terminating in exocytosis at the plasma membrane. The α-amylase catalyzes the hydrolysis of endospermic starch and resulted carbohydrates with the other hydrolysis products, precede the heterotrophic growth of the wheat seedling. The growth rate of germinated wheat seed is high and cells are divided very rapidly. Therefore, it is necessary to have a balance between embryo demands to fundamental components and their production via hydrolytic enzymes activities.

Environmental stresses such as drought and salinity severely effete plant growth and development and limit plant production and the performance of plants. These stresses with decreasing available water supply which could be uptake from environment cause to sever decrease in hydrolytic enzymes activities in seed germination stage [4]. The seed germination and early-seedling growth are important stages in the plant growth. These stages are the most sensitive stages environmental changes which have been used in environmental studies [26]. Thus, production of fundamental components which are needed to embryo growth is limited due to water supply shortage. Also, metabolism factors such as vitamins and coenzymes cause to non-balance between demand and supply of fundamental components and decrease of seedling growth [21]. Folic acid is one of the important metabolism factors can be influence wheat seedling growth.

Folic acid is forms of the water-soluble vitamin B9 and is not biologically active, but its importance is due to tetrahydrofolate and other derivatives after its conversion to dihydrofolic acid [6, 2]. Increasing interest of some plant researchers in folic acid derivatives is due to its multiple functions [24]. These compounds are involved in photosynthesis [8], biochemical conversions of nitrogen, carbon, and sulfur, synthesis and catabolism of protein amino acids [16] and nucleic acids [15]. According to the Lehninger et al. [14], folic acid is active in plants only in its reduced form, as tetrahydrofolic acid (tetrahydrofolate) and tetrahydrofolic coenzymes. Stakhova et al. [24] reported that folic acid plays an important role in the regulation of the metabolism of plant cells. They described that increased content of folic acid in the leaves stimulates the synthesis of the dependent amino acids and so increases the yield and quality of the seeds of pea (Pisum sativum L.) and barley (Hordeum vulgare L.)

Sulfamethoxazole is synthetic folate antagonists that are effective antimicrobials as individual agents. In other word, it inhibits normal bacterial utilization of p-aminobenzoic acid for the synthesis of folic acid, an important metabolite in DNA synthesis [11]. The aim of this investigation is to research the effect of different levels of Sulfamethoxazole as inhibitor of folic acid production on the seed germination and seedling growth of wheat.

2. Material and Methods

The experimental plant used in the present investigation was bread wheat (*Triticum aestivum* L.), cultivar Omid being widely cultivated all over Iran. Twenty wheat seed samples were chosen based on standard germination higher than 85%. Seeds were then soaked for 2 hours in tap water, surface sterilized with 2.5% (v/v) commercial bleach solution (sodium hypochlorite) for 3 minutes and then were rinsed with distilled water for three times. All seed samples had been treated with the same fungicides. The seeds were then germinated in sterilized Petri dishes, 90 mm in diameter, on Whatmann filter-paper moistened with 10 mL of either double-distilled water (control) or different increasing concentrations of Sulfamethoxazole solution (5, 10, 20 and 30 micro molar). According to the obtained results, 20 micro molar of Sulfamethoxazole concentration was selected as the optimum level of folic acid inhibitor. In the second experiment, eight treatments consist on; T1, Sulfamethoxazole (20 micro molar) as inhibitor; T2, Folic acid with 100 micro molar concentration; T3, Folic acid with 200 micro molar concentration; T4, Folic acid with 400 micro molar concentration; T5, 100 micro molar folic acid plus inhibitor; T6, 200 micro molar folic acid plus inhibitor; T7, 400 micro molar folic acid plus inhibitor and T8, control (without any folic acid or Sulfamethoxazole) were performed.

For statistical analysis, a completely randomized block design with 4 replications was used to compare the different concentrations of Sulfamethox and control treatment. Petri dishes were subsequently moved to a germinator and kept in the dark, at 21±1 ºC, 58±2 percent humidity. After incubation petri dishes were checked three times per day for up to 8 days. The petri dishes were controlled in two day intervals for solutions content. The seeds considered as germinated when their radicle length was approximately 2 mm or more. Germination percentages were obtained on the 8th days using radicle extrusion (ISTA, 1996). The germination rate (GR) was calculated following to Wardle et al. [25]. Dry weight of seedlings and remnant cotyledons as the seedling growth (SG) was obtained after oven drying at 75 ºC for 48 hours on the 8th day.

Seedling growth components including seedling growth, seed reserve utilization (initial seed dry weight - dry weight of the cotyledons remnant), the efficiency of mobilized seed reserve into plant tissue (seedling dry weight / the seed reserves utilization) and seed reserve depletion percentage (utilized seed reserve / initial seed dry weight) were calculated by Soltani et al. [22]. In the first experiment, a linear regression analysis was performed to obtain the relationships between mean values of the measured traits and different increasing concentrations of Sulfamethoxazole solutions.

In the second experiment, dry weight of roots and shoots, and three seedling growth components including seedling growth, seed reserve utilization, the efficiency of mobilized seed reserve into plant tissue were measured. For these traits analysis of variance and mean comparison using Duncan Multiple Range Test (DMRT) at a probability level of 0.05 were done. Residual analysis was performed to testing assumptions of normal distribution and homogeneity of error in regression analysis. All of the dataset were tested for skewness, kurtosis, Kolmogorov-Smirnov test and Q-Q plot for normality. The data were statistically analyzed by SPSS 14.0 computer program.

3. Results and Discussion

The results of normality tests (Kolmogorov-Smirnov) and residual analysis for some of the measured traits indicated data normality and providing assumptions of normal distribution and homogeneity of error in both experiments (data are not shown). For abnormal data which did not fit a normal distribution, the data transformation process (arcsine and logarithmic) were used. Figure 1A and 1B show germination and seeding growth of wheat in different concentrations of Sulfamethoxazole three and seven days respectively. It is clear that with increasing Sulfamethoxazole concentrations, growth properties of wheat decreased.

Analysis of variance showed that different concentrations of Sulfamethoxazole have not significant effect on seed germination percentages and results of mean comparison verified this finding (Figure 2A) while germination rate was affected due to increasing of Sulfamethoxazole concentrations and decreased significantly (Figure 2B). The germination rate was significant between control and concentration 30 micro molar of Sulfamethoxazole. It has been reported that some seed treatments such as priming can improve germination rate but can not improve seed germination percentages [20, 3]. It seems that such treatments induce a range of biochemical changes in the seed that required initiating the germination process [1] and so accelerate germination rate. Therefore, it could be conclude that folic acid has some roles in breaking of dormancy, metabolism of inhibitors, imbibitions and enzymes activation processes.

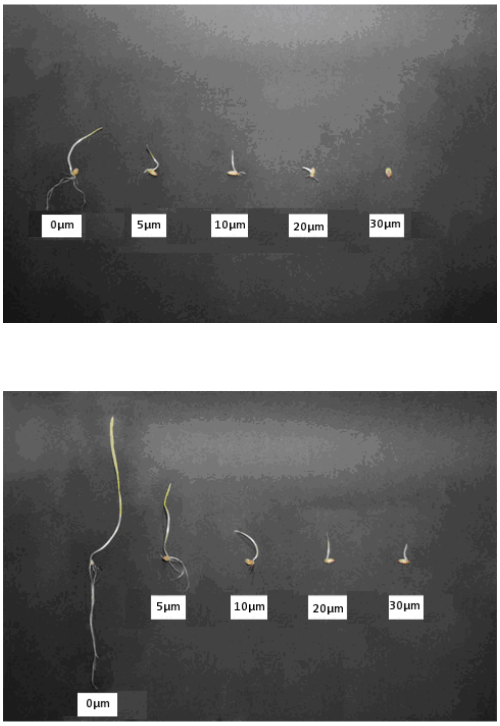


Figure 1. Comparison of germination and seeding growth of wheat in different concentrations of Sulfamethoxazole for up (A) after three days and down (B) after seven days.

Table 1. Analysis of variance for liner regression and its parameters

Traits	GP	GR	SG	SRU	EMSR	SRDP
			Analysis of Variance			
Regression	0.865	2.037**	0.0086*	0.0000348*	6.836*	144.77*
Error	4.202	0.028	0.0004	0.0000049	0.726	20.53
			Regression parameters			
Constant	94.162**	15.348**	0.1699**	0.02718**	6.310**	55.46**
Slop	-0.039ns	-0.059**	-0.0039*	-0.00024*	-0.109*	-0.50*
SE† Constant	1.437	0.1171	0.01424	0.001557	0.597	3.18
SE Slop	0.085	0.0069	0.00084	0.000092	0.035	0.19
R-Square	6.4	96.0	87.5	70.2	75.8	70.2

† SE, Standard Error; **, * and ns, respectively significant at the 0.01and 0.5 probability level and non-significant

GP, germination percentages; GR, germination rate; SG, seedling growth; SRU, seed reserve utilization; EMSR, efficiency of mobilized seed reserve into plant tissue; SRDP, seed reserve depletion percentage.

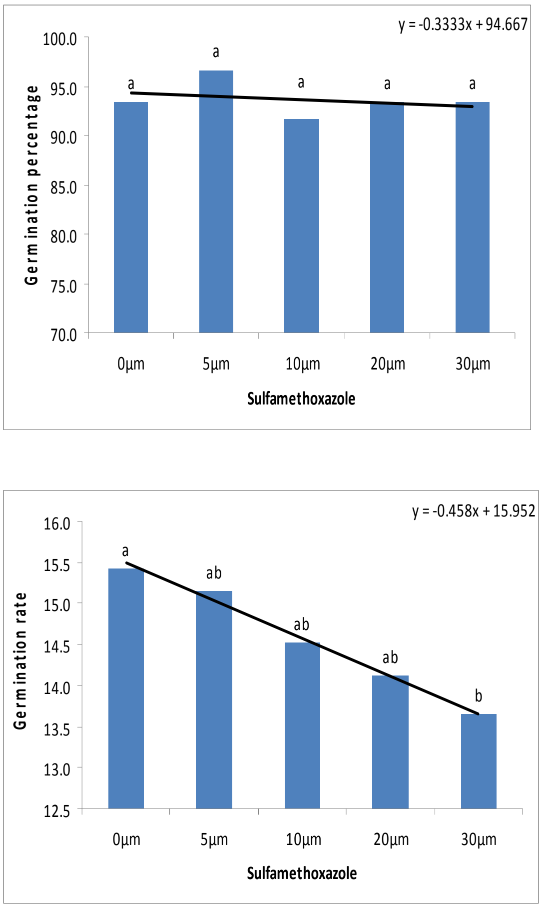


Figure 2. Comparison of mean values basis on the Duncan multiple range test at a probability level of 0.05 and fitted liner regression line with equation for up (A) germination percentages and down (B) germination rate. Means followed by the same letter(s) are not significantly different at 0.05 level of probability.

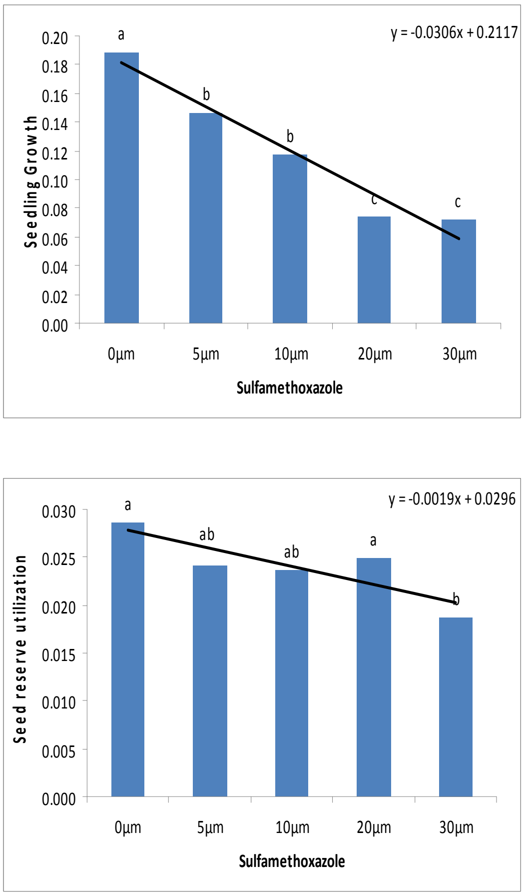


Figure 3. Comparison of mean values basis on the Duncan multiple range test at a probability level of 0.05 and fitted liner regression line with equation for up (A) seedling growth and down (B) seed reserve utilization. Means followed by the same letter(s) are not significantly different at 0.05 level of probability.

Analysis of variance (ANOVA) for linear regression of different concentrations of Sulfamethoxazole solution as the independent variable and the germination percentage as the dependent variable indicated a non significant relation with low amount (6.4) of coefficient of destination (Table 1).

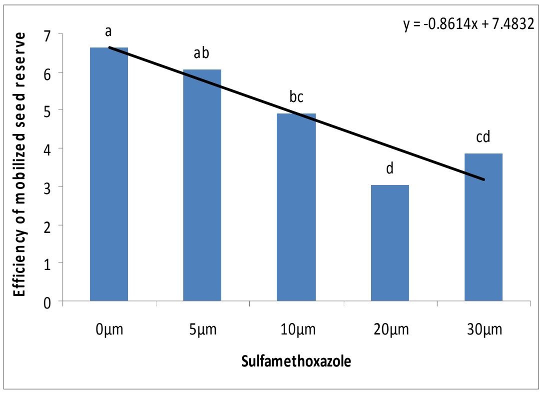
It seems that there is a minimum amount of folic acid in seed endosperm and accelerates the initial steps of seed germination. ANOVA for linear regression of Sulfamethoxazole and the germination rate indicated a significant relation at 0.01 probability level (Table 1).

Also testing of linear regression parameters (constant and line slop) via T-test showed that both of these parameters were significant at 0.01 probability level. The amount of coefficient of destination (R-square) was high for the above linear regression model and indicated the high valid of fitted model. According to this model, with increasing of one unit in concentration of Sulfamethoxazole, 0.059 percent of germination rate was reduced. Stakhova et al. [24] reported that there were maximum amount of folic acid in pea and barley seeds after 6-8 days of germination which the highest metabolic activity associated with synthetic processes. Dry weight of wheat seedlings remnant cotyledons (the seedling growth) was decreased due to increasing of biosynthesis inhibitor of folic acid (Figure 3A). Also, results of the ANOVA for linear regression of different concentrations of Sulfamethoxazole and the seedling growth quantity showed a significant relation at 0.05 probability level (Table 1). The T-test results indicated that constant was significant at 0.01 probability level. Significant of constant parameter revealed that despite of using Sulfamethoxazole as biosynthesis inhibitor of folic acid, amount of this vitamin has not been zero and there is a little amount in seed. Due to proper amount of R-square in this relation, the used fitted model had high validity for explanation of observed variation (Table 1). Therefore, with increasing of one micro-mole in Sulfamethoxazole, 0.0039 g of seedling growth was decreased. Stakhova et al. [24] in studying the of effect of folic acid on pea and barley reported that barley responded to folic acid higher that pea in seed content of free amino acids.

The main attraction processes in seeds of pea affect amino acids and proteins while in seeds of barley, attraction are related with carbohydrate accumulation [12, 13]. Therefore, it seems that inter-specific differences are responsible of these phenomenons and using biosynthesis inhibitor of folic acid could not completely prevent folic acid-dependent activities. The seed reserve utilization was decreased with increasing of concentrations of Sulfamethoxazole (Figure 3B) but these differences were significant only between control and concentration 30 micro molar of Sulfamethoxazole. ANOVA for linear regression of different concentrations of Sulfamethoxazole and the seed reserve utilization showed a significant relation at 0.05 probability level (Table 1).

Although this significant relation was in the borderline but it seems that this phenomena was due to low observations and degrees of freedom. The high amount of R-square for this regression model is verifying above conclusion. Also testing of linear regression constant via T-test showed that this parameter was significant at 0.01 probability level. However, according to this model it could be decaled that with increasing of one unit of Sulfamethoxazole concentration, 0.00024 g of the seed reserve utilization was reduced. Relatively, similar results have been reported by some authors such as Soltani et al. [23] chickpea in Mohammadi et al. [17] in soybean. Also, Stakhova et al. [24] declared that high concentration of folic acid in seed germination is necessary sufficient for intensive biosynthesis of amino acids, proteins, nucleotides, and nucleic acids.

The efficiency of mobilized seed reserve into plant tissue was decreased in concentration 10 micro molar and more of Sulfamethoxazole in comparison to control treatment (Figure 4A). The fitted regression model was significant at 0.01 probability level. The T-test results verified ANOVA results and indicated that both parameters (constant and slop) were significant at 0.01 probability level (Table 1). The amount of coefficient of destination was proper (75.8 percent) for the above linear model and so with increasing of one micro-molar of Sulfamethoxazole concentration, 0.109 percent of the efficiency of mobilized seed reserve into plant tissue was reduced. Soltani et al. (2006) found that the increase of osmotic pressure cause to reduction of seed reserve mobilization, but can not affect significantly on the conversion efficiency of mobilized reserves in seedling growth of wheat in drought and salt stresses conditions.



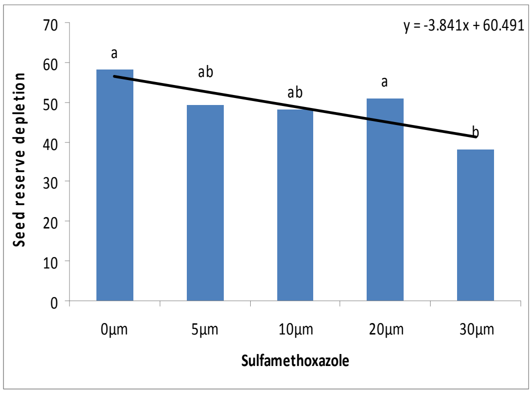


Figure 4. Comparison of mean values basis on the Duncan multiple range test at a probability level of 0.05 and fitted liner regression line with equation for up (A) efficiency of mobilized seed reserve into plant tissue and down (B) seed reserve depletion percentage. Means followed by the same letter(s) are not significantly different at 0.05 level of probability.

The seed reserve depletion percentage was decreased with increasing of concentrations of Sulfamethoxazole (Figure 4B) but these differences were significant only between control and concentration 30 micro molar of Sulfamethoxazole.

Analysis of variance for regression of different Sulfamethoxazole levels and seed reserve depletion percentage indicated a significant relation at 0.05 probability level (Table 1). Similar to seed reserve utilization this significant relation was in the borderline but it seems that this phenomenon was due to low observations and degrees of freedom. Therefore, with regarding the high amount of R-square (70.2%) and low observations and degrees of freedom, it could be find that with increasing of one micro-molar of Sulfamethoxazole concentration, 0.5 percent of the seed reserve depletion percentage was decreased. Our results can demonstrate that the important effects of folic acid in early seed development and formation of seedling. Stakhova et al. [24] reported that the high plant productivity could be caused by an increase of exogenous folic acid application on planted seeds.

Table 2. Analysis of variance and comparison of mean values basis on the Duncan multiple range test

SOV	df	DWR	DWSH	SG	SRU	EMSR
Treatment	7	0.00889*	0.01062*	0.0002806**	0.0000962**	0.0715700**
Error	24	0.00013	0.00007	0.0000027	0.0000005	0.0012921
		0.21	0.12	0.07	0.11	0.15
Mean comparison						
T1		0.0035 C	0.0128 C	0.01194 D	0.00080 C	0.06725 D
T2		0.1028 A	0.1210 A	0.03004 A	0.01119 A	0.37242 A
T3		0.1015 A	0.1205 A	0.02979 A	0.01110 A	0.37310 A
T4		0.0948 A	0.1198 A	0.02926 A	0.01073 A	0.36698 A
T5		0.0055 C	0.0308 B	0.01328 CD	0.00181 BC	0.14000 BC
T6		0.0053 C	0.0288 B	0.01608 B	0.00170 BC	0.10787 CD
T7		0.0323 B	0.0223 BC	0.01555 BC	0.00273 B	0.17789 B
T8		0.0930 A	0.1168 A	0.02968 A	0.01049 A	0.35361 A

**, significant at the 0.01 probability level. Means followed by the same letter(s) are not significantly different at 0.05 level of probability. DWR, dry weight root; DWSH, dry weight shoot; SG, seedling growth; SRU, seed reserve utilization; EMSR, efficiency of mobilized seed reserve into plant tissue. T1, Sulfamethoxazole (20 micro molar) as inhibitor; T2, Folic acid with 100 micro molar concentration; T3, Folic acid with 200 micro molar concentration; T4, Folic acid with 400 micro molar concentration; T5, 100 micro molar folic acid plus inhibitor; T6, 200 micro molar folic acid plus inhibitor; T7, 400 micro molar folic acid plus inhibitor and T8, control (without any folic acid or Sulfamethoxazole)

The folic acid as an important vitamin is an ancillary initiator of glutamic acid synthesis and when its activity induces, synthesis off other amino acids, because glutamate is an allosteric activity regulator for multiple-enzyme systems [19]. Also, the synthesis of methionine may increase with increasing of folic acid level [9]. Finally, folic acid in the form of folic coenzymes involves in synthesis of glycin and high amont of free glycin initiate synthesis in the plants of porphyrins and their derivatives, chlorophylls [16]. The positive effects of folic acid on growth and development of plant species indicates its important role in biochemical and physiological process in plant cells. Burguieres et al. [6] reported that folic acid application stimulate the phenolic related antioxidant response in seed germination of pea which is modulated via the praline related pentose phosphate pathway. Thus, positives effects of folic acid on germination presses via improving biochemical reactions have been demonstrated.

The folic acid improves agronomic performance and the other morphological traits. Stakhova et al. [24] reported that application of folic acid in form exogenous treatment increases the productivity through improving the yield, weight, and quality of the seed in both of pea and barley. Burguieres et al. [6] indicated that germination percentage and some seedling properties such as shoot weight and both shoot and root height were increased with folic acid treated plants. Also the same authors reported that the improving of seed vigor by folic acid cause to improving both agronomic and biochemical properties in pea.

In the second experiment, all of the studied traits were significant according to ANOVA (Table 2). Mean comparison of dry weight of roots and shoots using Duncan Multiple Range Test indicated that treatments T2, T3 and T4 (applying exogenous folic acid in media) have not significant differences with control (Table 2). It seems that wheat has good potential to biosynthesis of its folic acid and did not need to exogenous folic acid. On the other hand, dry weights of shoots were increased in treatments which had both exogenous folic acid and inhibitor in comparison to T1. This situation was seen for dry weights of roots between T1 and T7.

Our results show that with adding different levels of folic acid, seedling growth is increasing. The seedling growth was significantly increased to 26% in T7 (400 micro molar folic acid plus inhibitor) in comparison to 7.6% in T1 (20 micro molar Sulfamethoxazole). This 360 percent increase of seedling growth (between T1 and T7) cause to only 12% increase in seed reserve utilization (Table 2). The Sulfamethoxazole has been instead of Para Amino Benzoic Acid in biosynthesis of folic acid and prevents from its transformation to Tetra Hydro Folate form. It has expected that the Sulfamethoxazole can prevent from folic acid biosynthesis and on the other hand it has expected that exogenous folic acid can compensate folic acid shortage in seed germination. But in all treatments which contain both exogenous folic acid and Sulfamethoxazole inhibitor, maximum seedling growth was seen only 26%. It seems that exogenous folic acid can compensate folic acid shortage in seed germination to some extent but its high concentration acts as a toxin. Relatively similar results can be found for seed reserve utilization and the efficiency of mobilized seed reserve into plant tissue (Table 2).

4. Conclusions

It seems that an inhibitor of folic acid biosynthesis like Sulfamethoxazole can effectively prevent from its production and so suppress the growth. This suppression is relatively severe and using exogenous folic acid can not compensate all of inhibitor effects or folic acid high concentration shows another inhibitor factor to the growth. Generally, we can conclude that using Sulfamethoxazole as biosynthesis inhibitor of folic acid, can suppress most living process in seed germinating period such as the germination rate, seed reserve utilization, the efficiency of mobilized seed reserve into plant tissue and seed reserve depletion percentage. Also, if there was economical explanation, Sulfamethoxazole could be regarded as the herbicide in future for weed control in agricultural fields. However this conclusion maybe needs to further investigation from both biochemical and agrochemical aspects as well as environmental risks.

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