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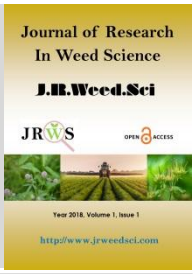

Studying magnetic field and Bentazon on Kautsky curve of bean (*Phaseolusvulgaris* L.)

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Abstract

Bentazon herbicide is a post-emergent herbicide and a photosynthesis inhibitor in broadleaf weed. However, different parameters of Bean Kautsky curve changed significantly. Seeds were treated magnetically at three levels normal, 100 mT and 150 mT (3 hours) and weed control (weeding by hand), weed free (untreated), control by 25 percent herbicide, or 0.6 $l.ha^{-1}$, 50 percent herbicide or 1.2 $l.ha^{-1}$, and 100 percent herbicide or 2.5 $l.ha^{-1}$. Due to the major paramagnetic components of bean seeds, affected the parameter minimum quantum yield of PS Π (Fv/Fm) significantly ($p<0/01$) as in the treatment of magnetic field 150 mT and 2.5 $l.ha^{-1}$ herbicide decreased significantly (0.61) although Bentazon is a selective herbicide of bean. However, besides posing damage to photooxidation and center PS Π, 150 mT magnetic fields lengthened the time to reach maximum fluorescence (493.3) and maximized the stress to the parameter Fo and Fo/Fm. In the case that no herbicide was applied, it amounted to the maximum fluorescence at the least time (183.3). Also, in the absence of herbicides (both weeding and weed free), basic quantum efficiency were always normal (0.14 to 0.21); however, by using the herbicides, it exceeded its normal range. Therefore, the selective of the herbicide did not mean the immunity of the plant against the herbicide completely. In general, 100 mT magnetic fields were known to be an effective treatment for plant's exposure to Bentazon herbicides and stronger field had the opposite effect.

Keywords: Fluorescence, Fv/Fm, Herbicide, Kautsky curve

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1. Introduction

Using of magnetic fields (seeds, water, herbicide, etc) is a good strategy for increasing the efficiency of water, herbicide, Germination, Vegetative and reproductive growth of the crop (Kordas, 2002). Elements existing in bean include calcium, potassium, sodium, iron, and phosphorus. With the exception of iron (ferromagnetic), other elements of seed beans are paramagnetic (Ibrikci et al. 2003). That's why this plant shows a stronger response to the magnetic field. Permanent magnetic field also causes such significant changes in Fluorescence range and temperature in bean leaves that FIR and leaf temperature changes (ΔT) enhance with an increase in magnetic field strength. The increase in the temperature changes is known to be resulted from the relationship between magnetic field and increased ions velocity (Jovanic and Sarvan, 2004). Some researchers also believe that magnetic field, through increasing the osmotic pressure, leads to enhanced water absorption and thus, to improve germination of beans (Cakmak et al. 2010).

Bentazon herbicide is the only post-emergence herbicide that has been used for weed control in beans for many years in Iran. It belongs to Benzothiadiazole group of herbicides and inhibits PSII of broadleaf weeds (Anonymous, 2004) because it acts as a barrier to electron transfer at Plastoquinone and thereby interferes with their photosynthesis. However, because of the application of rapid metabolism in crops, the herbicide is turned into non-toxic compound (Mousavi et al. 1995). The remarkable point is whether crop will be damaged after spraying selective herbicide or it will be totally immune, and whether the magnetic field can have an effect on it, also how bean responds to the herbicides in this condition, change the efficacy of the herbicides when magnetic field used as well, in other words, how the details of photosynthesis and Kautsky curve vary in the case that the magnetic field is applied. Among ways to quickly and accurately measure the performance of herbicides, it can be referred to the investigation of Kautsky curve and parameters such as maximum quantum efficiency of PSII (Fv/Fm) (0.7 to 0.8 in healthy plant), basic fluorescence (Fo), time to reach maximum fluorescence (Tfm), basic quantum efficiency (Fo / Fm) (with the normal range of 0.14- 0.21) and relative changes in the fluorescence at the j-step (Fvj), logarithm of the area between Kautsky curve and Fm (log area) (Devine and Shukla, 2000). The purpose of this study is to assess some of the Kautsky curve parameters for bean using the magnetic field at some doses of Bentazon herbicide.

2. Materials and Methods

The experiment based on RCBD with three replications for each treatment was done in a field at the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran, in 2016. Seeds were treated magnetically at three levels normal, 100 mT and 150 mT (exposed to magnetic fields for 3 hours) and the second treatments included weed control (weeding (by hand), weed free (untreated), control by 25 percent herbicide, or 0.6 $l.ha^{-1}$, 50 percent herbicide or 1.2 $l.ha^{-1}$, and 100 percent herbicide or 2.5 $l.ha^{-1}$ (with the recommended dose of 2-3 liters

per ha). Hence seeds were placed in a transparent plastic bag and bags were put directly between the poles of magnet by 120 min. The magnetic field generating device consisted of two magnets (10*15 cm), each one 180 mT (Figure 1) where two opposite poles of two magnets attract each other. Intensity of magnetic field changed with adjusting the distance between the poles. It was measured by using a micrometer scew Tslameter Leybold-Heraeus Model 51652 Gauss/Tesla Meter (Feizi et al. 2008).

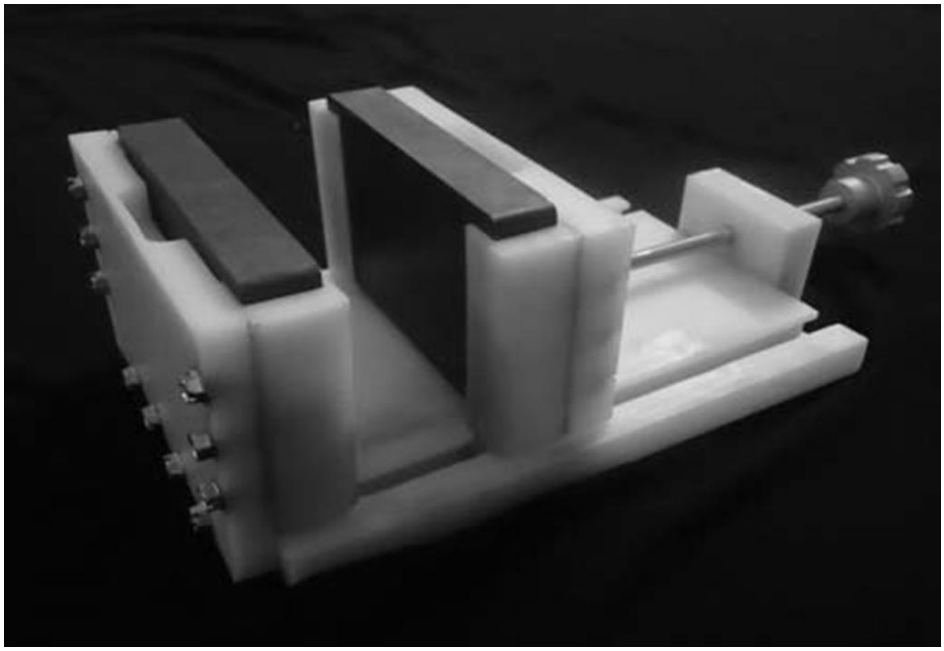

Figure 1- Device for inducing of different intensities of magnetic field.

The plants were sprayed at the 2-4 leaf stage with 2000 L ha^{-1} of spray solution using a flat fan of 8001 nozzle at a pressure of 300 k Pa. Chlorophyll fluorescence was measured by a chlorophyll fluorometer (Handy PEA, Hansatech Instruments, King's Lynn, Nor folk, UK) which emits a light of 650 nm wave length with an intensity of 3000 µmol photons $m^{-2}s^{-1}$ for 10 seconds on darkadapted leaves (30 minutes dark adapted by covering the leaves with a clip). The parameters analyzed in this experiment were Fv/Fm, Fo, Tfm, Fo/Fm and Fvj, where Fm is the maximum fluorescence, F0 is ground state fluorescence time to reach maximum fluorescence (Tfm), basic quantum efficiency (Fo / Fm) and relative changes in the fluorescence at j-step (Fvj) Data were analyzed by SAS ver 9.2 software and the means were compared by LSD tests at the probability level of 5 percent.

3. Results and Discussion

The application of magnetic field to bean seeds at the time of sowing, along with different doses of Bentazon herbicide, affected significantly ($p<0/01$) through the Kautsky curve of beans. Different parameters such as maximum quantum efficiency of PSII (Fv / Fm), log

Area, relative changes in the fluorescence at j-step (Fvj), the ground state (Fo), time to reach maximum fluorescence (Tfm), basic quantum efficiency (Fo / Fm) and Fm (Table 1).

Bentazon is a selective herbicide for bean, so the Fv / Fm parameter was in normal (0.7-0.8 range of natural plant) at different treatments of seed and control management (Table 1). But it decreased in the maximum herbicide (the recommended dose) - intensity of the magnetic field (150 mT) treatment significantly even bean damaged (0.6). It seems maximum of both treatments have had antagonistic effect on the Fv / Fm parameter of bean. Therefore it could be say that the magnetic field applied on the bean seeds and its permanence effect (due to the paramagnetic components of bean seeds), especially Magnetoteropism or special effect of magnetic field on germination of bean seeds (Cakmak et al. 2010; Maffei 2014), increased germination and root length of bean seeds treated with magnetic field were observed in different concentrations of calcium chloride (Sakhnini, 2007), are acceptable but it was reported that bean seed produced more vigorous seedlings just in 100 mT magnetic fields (Rajendra, 2005).

In another study, the authors stated that the use of magnetic fields may affect the flow of calcium (paramagnetic element). On the other hand, previous studies have proved the positive and direct effects of magnetic field on photosynthesis (De Souza et al. 2010), the content of chlorophyll pigments (Novitskii et al. 2014) and thus increased quantum yield of PS Π. The results also inducted that the magnetic field could have a positive effect on the Fv / Fm up to certain intensity, means 100 mT. It cannot be that concluded the maximum quantum efficiency increases in accordance with raised dose of Bentazon herbicide. The reduction of Fv / Fm below the normal value (0.75) represented a photo-oxidation and damage to PSII centers (Genty et al. 1989). It is known that, this reduction was caused by increased variable fluorescence (Fv) or increased Fo and decreased Fm. Given these observations, it could be concluded that 100 mT magnetic fields influenced maximum quantum efficiency of bean photosystem. But at reduced doses of herbicides, generally between different treatments of normal seed and 100 and 150 mT magnetic field (with the exception of 150 mT magnetic treatment of seeds - the recommended dose of herbicide), the values Fv / Fm were not significantly different (Table 1). Adverse effects of stronger magnetic fields were also proved on other plant species. In a study, for example, weight and number of strawberries were assessed in magnetic fields of 96, 192 and 384 mT under greenhouse conditions. Fruit yield per plant treated with 96 mT magnetic field (246.07 grams) significantly increased compared with control (208.05 g). But in 384 mT magnetic field, fruit yield per plant (202.5 g) decreased in comparison with other intensities (Esitken and Turan, 2004).

Besides the treatments of herbicide doses and magnetic field, had a significant effect ($p=0.01$) on the logarithm area of bean significantly and the biggest of log area showed at the without herbicide-seed treatments (Table 1). It could be said that by using herbicide the logarithm area Kautsky Curve of bean decreased significantly ($p= 0.01$).

According to the results, in the absence of herbicide, parameter Fo was always higher in normal seeds of beans than in seeds treated with magnetic field (Fig 1). However, in the lowest doses (502.7) and the recommended dose (574.7) - the maximum intensity of the magnetic field, Fo amounted to the highest level (Figure 2). With amplifying the dose, Fo was gradually increased in all treatments of bean seeds just as what happened when the plant was under stress, because, as we know, an increase in this parameter will result in lower maximum quantum efficiency (Govindjee, 1995). The results also confirmed it (Fig 2). It may be resulted from the stress on the plant due to the drop of osmotic potential because of herbicide use and possibly higher intensity of magnetic field (negative effect of a strong magnetic field that was out of tolerance of beans). Based on these results, it can be said that although the crop was resistant to its specific herbicide, parameter Fo decreased due to using the recommended dose of herbicide.

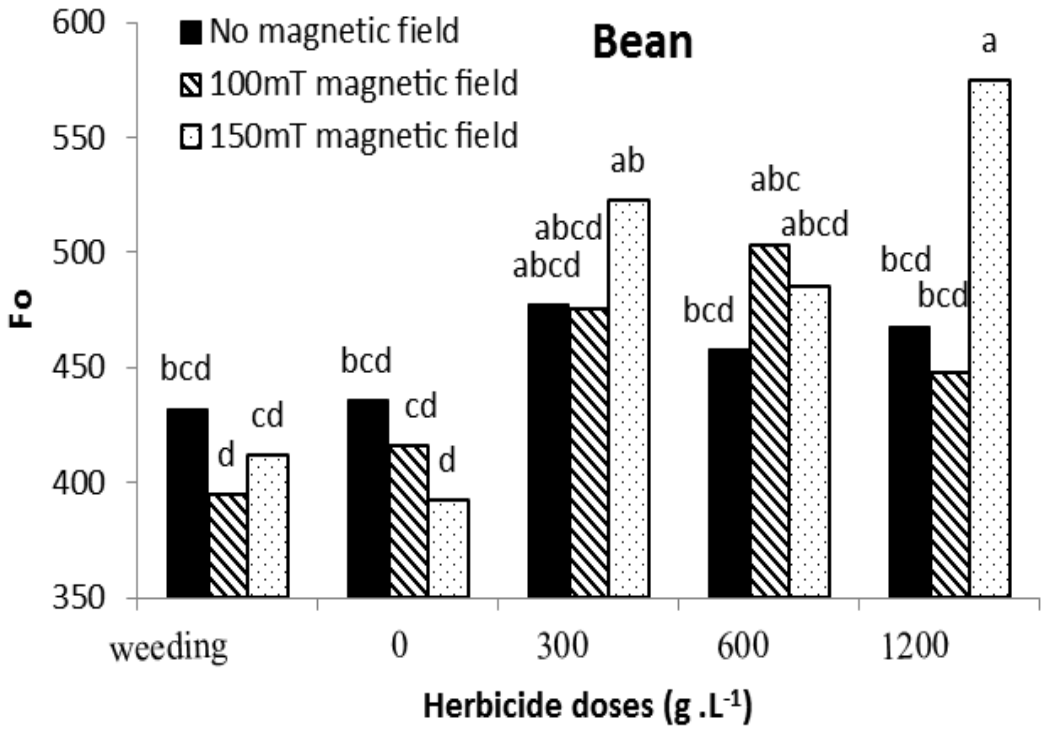

Figure 2- Effects of magnetic field and herbicide doses of Bentazon on Fo parameter of Kautsky curve at Bean field.

Another parameter associated with measuring the efficacy of herbicides is fluorescence parameters at j-step or (Fvj). If a plant is sensitive to herbicide, the parameter decreases. Despite bean's resistance to Bentazon herbicide, the bean's response in terms of the parameter Fvj was slightly different in this study, due to the use of magnetic field. The absence of herbicide + 100 mT magnetic field led to a significant reduction (0.65) Fvj compared to other treatments, but using the recommended dose of herbicide and stronger

fields resulted in a significant increase (0.88) up to 27 percent in this parameter (Table 1). However, Fvj was one of the quantitative parameters of Kautsky curve that was associated with efficacy and appeared to show no desired response to treatments in this experiment. Other studies have also reported that this parameter, despite its inhibiting effect on Metribuzin herbicides at PSII, showed less reaction compared with the parameters Fv/Fm and Area (Elahifard et al. 2013). In another study, the parameter was less responsive compared to other parameters such as Area to the dose of the herbicide used. For example, in an experiment (Avarseji et al. 2012), Despite the fact, that herbicide used was not PS II inhibitor, Kautsky curve was extremely impressed and the parameter (Fvj) reacted less than parameter Area to the increased dose of Clodinafop herbicide.

As this fact that the true Fo level is only observed when the first stable electron acceptor of photosystem II called Qa is fully oxidised. This requires thorough dark adaption. Fo occurs at time base O. it is the almost instantaneous (nanoseconds range) rise to an origin level of fluorescence upon illumination using the fluorimeter. However it is possible to estimate the Fo level to a high degree of accuracy using a mathematical algorithm (Wim et al. 1994). Results of this parameter have been recorded (Table 1).

The results also showed that the treatments of herbicide doses and magnetic field, had a significant effect (p= 0.01) on the time to reach maximum fluorescence (Tfm) (Table 1) and the impact was the most lasting in the treatments by maximum magnetic field + recommended dose of herbicide (499.3), and the shortest in treatments by maximum magnetic field + weed control (183.3) (Table 1). As a result, the maximum magnetic field and the lack of use of herbicides allowed the plant to reach the maximum fluorescence at the shortest time, but in the case of using recommended dose of herbicides, it required the longest time (Table 1). So it can be stated that crop's specific herbicide does not insure its full immunity against the herbicide and that the plant is partly damaged due to the use of herbicides.

The study of the basic quantum efficiency (Fo / Fm) showed that in all treatments of bean seeds + weeding and no use of herbicides, value of Fo / Fm was in the normal range, but when the herbicides were used, this parameter exceeded the normal range (0.14 to 0.21) and indicated a significant increase (p=0.01) (Table 1) and raised in parallel to increase in the herbicide dose (Table 1).

Table 1- Effects of magnetic field and herbicide doses of Bentazon on some parameters of Kautsky curve at Bean field

Seed treatment	Herbicide Dose	Fv/Fm	Log Area	Fvj	Fo	tFm	Fo/Fm	Fm
Normal	Weeding	0.80±0.01	4.50 ±0.055	0.69 ±0.014	432.3±13.9	186.6±32.7	0.19±0.005	2204±40.7
	0	0.78±0.001	4.46 ±0.013	0.68 ±0.006	436±14.5	200±25.1	0.21±0.0004	2021±110
	300	0.73±0.02	4.29 ±0.16	0.78 ±0.037	477.3±20.3	213.3±26.6	0.26±0.02	1825.6±199.3
	600	0.72±0.04	4.24 ±0.14	0.83 ±0.065	479±39.4	310±49.3	0.27±0.035	1761.3±81
	1200	0.76±0.03	4.42 ±0.046	0.77 ±0.016	468±33.5	270±5.7	0.24±0.028	1974±143.9
Magnetic (100mT)	Weeding	0.81±0.02	4.54 ±0.063	0.66 ±0.027	395±17.8	196.6±14.4	0.18±0.013	2097.3±88.6
	0	0.82±0.01	4.57 ±0.070	0.65 ±0.019	416.3±16.2	203.3±25.9	0.18±0.009	2315±88.1
	300	0.74±0.03	4.45 ±0.088	0.75 ±0.024	475±7.7	260±29.9	0.25±0.032	1938.6±266.9
	600	0.80±0.04	3.75 ±0.033	0.71 ±0.003	502.6±1.4	283.6±2.3	0.25±0.0003	1999.3±0.34
	1200	0.76±0.01	4.56 ±0.051	0.68 ±0.010	447.6±9.2	281.6±6	0.23±0.008	1887.6±105.5
Magnetic (150mT)	Weeding	0.81±0.001	4.54 ±0.003	0.67 ±0.005	412±13.1	183.3±12	0.18±0.009	2203±60.1
	0	0.80±0.01	4.51 ±0.035	0.67 ±0.015	392.6±22.8	240±15.2	0.20±0.009	1958.3±118.7
	300	0.72±0.01	4.38 ±0.0001	0.77 ±0.013	523±11.8	280.6±12.1	0.26±0.007	1950±11.5
	600	0.72±0.03	4.36 ±0.008	0.77 ±0.011	485.3±22.6	256.6±33.7	0.27±0.028	1771.6±109.6
	1200	0.61±0.04	4.07 ±0.138	0.88 ±0.033	574.6±36.5	493.3±121.2	0.38±0.045	1509.3±121.6
Analysis of variance								
Seed (S)		**	Ns	**	Ns	Ns	**	Ns
Management (M)		**	**	**	**	**	**	**
S*M		*	**	**	*	Ns	**	Ns
C.V %		5.5	4.6	6.0	8.0	26	15.5	10.7
R^2 %		70	62	77	71	64	73	56

ns, * and** are non-significant and significant at 5 and 1 probability levels, respectively.

The parameter increased more at every dose in 100 mT magnetized seed in comparison to normal seed treatments. The increase was much higher in more intensive treatments (150 mT) and reached to its maximum (0.38) in the highest dose of herbicides (the recommended dose) (Table 1). Reduction of the basic quantum efficiency below this range is regarded as plant stress (Rohâcek, 2002). But the basic quantum efficiency can be increased due to various reasons such as osmotic stress (for any reason including Plastoquinone (Qa) and photosystem of PS I. Since it was proved that the magnetic field influenced the dehydration of seed at germination stage in different plants affected by the magnetic field, (Moon and Sook, 2000; Vincze et al. 2003; Paul et al. 2006; Herranz et al. 2013; Manzano et al. 2013), it was likely that this effect would continue at later stages of the plant growth and stimulate potassium ion entry and thus adjust the osmotic pressure and finally change the quantum efficiency in beans. On the other hand, it may be relevant to potassium (which is a paramagnetic element). As a result, stimulation of potassium was reciprocal affected by the magnetic field and affected the parameter.

Conclusions

It can be concluded that the paramagnetic nature of bean elements improves its response to the Bentazon herbicide at 100 mT magnetic field in response to the parameter Fv / Fm. Also, at the recommended dose of herbicide + 100 mT magnetic fields increases the maximum yield of PSII, but more stronger fields (150 mT) had an opposite effect and led to photo-oxidation and damage to the PS II center. The plant tolerated the maximum level of stress in recommended dose of herbicide + 150 mT magnetic fields in terms of the parameter Fo and much time was needed to achieve the maximum fluorescence. The parameter of basic quantum efficiency also coped with maximum osmotic stress, while parameters Fo and Tfm were not significantly different in recommended dose + 100 mT magnetic field, and control. However, in weeding treatment + 150 mT magnetic fields achieved the maximum fluorescence with the least possible time. In the case where herbicide was not used (weeding, weed free), basic quantum efficiency was always normal, but as soon as the herbicide was used, it exceeded the normal scope. Therefore, the specificity of herbicide is not related to the full immunity of the crop against the herbicide concerned.

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Conflict of interest

The authors confirm that there are no known conflicts of interest associated with this study.

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