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# Activity of the Recommended and Optimized Rates of Pyridate on Chickpea - *Mesorhizobium mediterraneum* Symbiosis

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**Abstract**

Crop-rhizobium symbiosis can be influenced by leaching of herbicides which is unavoidable after their application. Due to an adjuvant which might help to develop the low-use-rate of herbicide, an experiment was carried out to compare the impact of the recommended rate (1200 g active ingredient $ha^{-1}$) and the optimized rate (282.15 g active ingredient $ha^{-1}$) of pyridate on the biological properties of eight chickpea cultivars inoculated with *Mesorhizobium mediterraneum*, grown in pots. Based on the required rate of herbicide to give 95% control of common lambsquarters (*Chenopodium album* L.) value, the efficacy of pyridate improved up to 3.87-fold by adding methylated rapeseed oil to spray solution. The 'Desi' cultivar had significantly higher nodulation than 'Kabuli' cultivar. In general, toxicity of the recommended rate was higher than the optimized rate. With the exception of root dry weight, all of the measured parameters were significantly affected by the recommended rate of pyridate in varying degrees. The symbiotic properties of chickpea cultivars were affected more than 10% at the recommended dose. The reduced nodulation ranged from 29% to 73% among cultivars exposed to pyridate at the recommended dose. The 'Desi' cultivar was more sensitive than the 'Kabuli' to the recommended rate of pyridate. We may conclude that effective low-use-rate of pyridate via applying of activator adjuvants should be noted.

***Keywords:*** adjuvant, herbicide, *Mesorhizobium mediterraneum*, nodulation, toxicity

## Introduction

Chickpea, *Cicer arietinum* L., is the third most important leguminous crop in the world (Mohammadi *et al.*, 2005). There are two major types of chickpeas, 'Kabuli' and 'Desi'. The former have larger and cream-colored seeds with thin seed coats, while the latter have smaller, dark- or brown-colored seeds with thick seed coats (Gaur *et al.*, 2012). Both types form a highly specific symbiosis with its rhizobial partner, namely *Mesorhizobium mediterraneum* or *Mesorhizobium ciceri* (Prevost and Antoun, 2008), that fixes atmospheric nitrogen in exchange for crop carbohydrates (Fernaddez-Aparicio *et al.*, 2009). The amount of fixed nitrogen by *M. mediterraneum* is equivalent to an application of about 150 N $ha^{-1}$ in a chickpea crop (Datta *et al.*, 2011); in other words, about 82% plant-N derives from atmosphere (Unkovich and Pate, 2000). In Iran, therefore, a propensity is being increased to involve legumes as a source of nitrogen in short-term rotation systems with wheat (Mohammadi *et al.*, 2005); above all, the legumes can be a cycle-breaker against graminaceous crop diseases (Malik, 2010).

An open canopy architecture, dwarf stature and slow development are intrinsic plant characteristics of chickpea, decreasing its ability to compete with weeds (Radicetti *et al.*, 2012). As a result, the tendency of the farmer is to use herbicides to solve this problem because they have helped them to increase yields while reducing labor (Macias *et al.*, 2001). Because less than 5% of these magic solutions are estimated to reach the target, with the remainder being deposited on the soil (Eerd *et al.*, 2003), the potential environmental and toxicological risks of widespread application of them are highly considered in decreasing the rhizobial growth (Liebman *et al.*, 2004). Later, the phytotoxic effects of quizalafop-p-ethyl and clodinafop (Ahemad and Khan, 2010), chlorsulfuron (Anderson *et al.*, 2004), linuron, methabenzthiazuron, and terbutryn (Khan *et al.*, 2006), simazine and prometryn (Kumar *et al.*, 1981), 2,4-D, fluchloralin, and isoproturon (Aamil *et al.*, 2004), fluchloralin and pendimethalin (Pahwa and Prakash, 1992), and isoxaflutole (Datta *et al.*, 2006; 2007; 2008; 2009; 2011) on chickpea and its symbionts have been reported. Besides, there are reports that symbiotic nitrogen fixation is affected with herbicide application on other pulse crops (Niina, 2008).

In Iran, herbicide pyridate O-(6-chloro-3-phenyl-4-pyridazinyl)S-octyl carbonothioateat 1200 g active ingredient $ha^{-1}$ is labeled only for use post-emergence in chickpea to control broadleaf weeds such as common lambsquarters, *Chenopodium album* L. This is a cosmopolitan summer annual weed (Solymosi and

Lehoczki, 1989) and a serious constraint to increased production in chickpea in northeast Iran (Sarparast and Sheykh, 2010). In other countries, it is labeled in peanuts, corn, cereals, mint, and rice (Monaco *et al*., 2002). The herbicidal activity of pyridate (group 6/$C_3$) is associated with inhibition of photosynthesis by binding to the quinine B-binding site on the $D_1$ protein of the photosystem II complex in chloroplast thylakoid membranes. This reaction obstructs electron transfer from quinine A to quinine B and layoffs carbon dioxide fixation and production of ATP and $NADPH_2$, which are necessary for plant growth (Stein *et al*., 1984).

From an Integrated Weed Management strategy perspective, it is important to develop the effective low-use-rate of herbicides (Kudsk, 2008). Hence, it seems that the use of adjuvants is really a suitable manner (Rashed-Mohassel *et al*., 2009; Aliverdi *et al*., 2009) so that certain herbicides are sold with a particular adjuvant together in a combi-pack. For instance, in the case of herbicide pinoxaden, the use of methylated rapeseed oil, a methylated rapeseed oil, is essential in order to obtain safe and successful use of this herbicide (Burgis and Harvey, 2012). This adjuvant is also able to improve the efficacy of other herbicides. Rashed-Mohassel *et al*. (2011) found that the performance of sethoxydim against wild oat (*Avena fatua*) was increased 1.49-fold in the presence of methylated rapeseed oil. Parsa *et al*. (2013) found that the performance of haloxyfop-P-methyl and imazethapyr against velvetleaf (*Abutilon theophrastii*) and johnsongrass (*Sorghum halepense*) were increased 3.49-fold and 2.04-fold, respectively.

So far, not enough data are available on the phytotoxic effects of herbicide pyridate on chickpea *M. mediterraneum* symbiosis. However, based on the available information, the influence of agrochemicals on a pulse crop and its symbionts is depended upon the interactions among the *Rhizobium* species, crop genotypes and the type and rate of agrochemicals (Ahemad and Khan, 2010). Hence, this research was conducted in two experiments with two objectives of (*i*) characterizing the rate-response curves of common lambsquarters to pyridate alone and with methylated rapeseed oil to determine the optimized rate; (*ii*) assessing the effect of the recommended and optimized rates of pyridate on the growth and nodulation of eight chickpea cultivars inoculated with *M. mediterraneum*.

## Materials and methods

### *Experiment 1: Optimizing rate of herbicide*

The seeds of common lambsquarters were collected from plants in the field near the Research Greenhouse at Ferdowsi University of Mashhad, Iran. Before using the seeds in the experiment, to increase the seed coat permeability of common lambsquarters, they were acid-scarified in concentrated sulfuric acid (98%) for 1 min; and then rinsed with distilled water (Andersen, 1968). Then, the treated seeds were sown in potting trays (3 cm inner diameter and 5 cm in depth) filled with moistened peat. The potting trays were placed in the greenhouse and watered with tap water to prevent moisture stress. The temperature in the greenhouse varied from 18±4 °C during the day and 12±3 °C at night. When the seedlings were in first leaf stage, they were transplanted to plastic pots (20 cm upside diameter and 30 cm in depth) filled with a mixture of sand, clay loam soil, and peat (1:1:1 by volume). Afterwards, the pots were placed in the greenhouse under the same conditions mentioned above. Pots were irrigated when needed. At two leaf stage, the seedlings were thinned to four per pot. At the three leaf stage, the treatments were exerted using an overhead trolley sprayer (Matabi 121030 Super Agro 20 L sprayer; Agratech Services-Crop Spraying Equipment, Rossendale, UK) equipped with an 8002 flat fan nozzle calibrated to deliver 200 L $ha^{-1}$ at 2 bar spray pressure.

The treatments were consisted of herbicide pyridate at 0, 75, 150, 300, 600, and 1200 g active ingredient $ha^{-1}$ (600 g pyridate $L^{-1}$) alone and with methylated rapeseed oil (47% methylated rapeseed oil) at 0.5% (v/v), according to the label, against common lambsquarters. The applied rates were 0%, 6%, 12%, 25%, 50%, and 100% of the labeled rate for Iran. Four weeks after spraying, the biomass of experimental units was harvested fresh and dry weight was determined after the fresh samples were oven-dried at 75 °C for 48 h. The response of fresh and dry weight to herbicide rate was assumed by a log-logistic model that was already described by Streibig *et al*. (1993) and Tind *et al*. (2009). On the basis of the nonlinear regression parameters, using logistic dose-response model of $Y = C + \{D - C / 1 + \exp [b (\log X - \log ED)]\}$; where Y is the response (e.g., dry weight), C is the lower limit, D is the upper limit corresponding to the response of the untreated control, b is the slope of the line, the $ED_{50}$ and $ED_{95}$ values were calculated, denoting the required rate of pyridate to give 50% and 95% control, respectively. The required rate of pyridate along with methylated rapeseed oil in which 95% control was obtained, was chosen as optimized rate in subsequent experiment.

### *Experiment 2: Comparing effect of rates*

The chickpea cultivars seeds were obtained from the Seed Bank of Research Department of Plant Science, Ferdowsi University of Mashhad, Iran. Before using the seeds in the experiment, each of ten chickpea cultivars seeds were disinfected with sodium hypo chloride (5%) for a period of 5 min, and then dried with paper towels after washing with distilled water. Before planting, the seeds were dipped for a period of 1 hour into a suspension *M. mediterraneum* (obtained from the Mehr-e-Asia Biotechnology Co., Tehran, Iran) at a rate of 12 g inoculants per 1 kg seeds according to label. Ten seeds inoculated of each chickpea cultivar were grown in plastic pot (35 cm inner diameter and 50 cm in depth) at 1 cm depth that was filled with an autoclaved mixture of loam soil and sand (1:10 by volume). The seedlings were thinned to three per pot at the one-leaf stage. When chickpea cultivars were in third leaf stage, they were sprayed as already described above. The experiment was set up as a completely randomized design with 8×3 factorial combinations: the eight chickpea cultivars obtained from Mashhad Chickpea Collection (six 'Kabuli' types (K-MCC-252, K-MCC-283, K-MCC-358, K-MCC-360, K-MCC-395, and K-MCC-950) and two 'Desi' types (D-MCC-49 and D-MCC-362)) and three herbicide rates (control, the recommended rate (1200 g active ingredient $ha^{-1}$) and the optimized rate (282.15 g active ingredient $ha^{-1}$). There were three replications. The treatments were applied at two leaf stage using the method mentioned above.

The biomass of experimental units was harvested at thirty days after herbicide treatment. At harvest time, growth parameters (shoot and root dry weights); nodulation (nodule number, nodule dry weight and nodule largest diameter); amount of N (shoot N, root N) and amount of phosphorus (shoot phosphorus, root phosphorus) were measured. Plant shoots were harvested at ground level and roots were then hand washed. The numbers of nodules per plant were counted after careful hand-washing of the roots. Dry weights of nodules, shoots and roots were determined as already described above. Phosphorus and nitrogen content in roots and shoots were determined at thirty days after harvest using the method of Jackson (1967) and micro-Kjeldahl (Iswaran and Marwah, 1980), respectively. This trial was repeated. Since similar results were nearly detected in both studies, data were averaged across the two trials and analyzed in accordance with the first experiment. At all measurements, the data were changed to individual plant and subjected to analyze of variance using the SAS software.

**Results and Discussion**

*Experiment 1*

Dose-response curves of common lambsquarters treated with pyridate alone or in the presence of the methylated rapeseed oil are showed in Fig. 1. The $ED_{50}$ and $ED_{95}$ values based on the fresh weight of common lambsquarters for pyridate were 226.29 and 970.94 g active ingredient of pyridate $ha^{-1}$, respectively. Whereas, these values based on the dry weight were 192.85 and 1092.02 g active ingredient of pyridate $ha^{-1}$, respectively. As judged by the $ED_{50}$ and $ED_{95}$ values given in Tab. 1, the addition of the methylated rapeseed oil decreased both values significantly, indicating an increase in the efficacy of pyridate to control common lambsquarters. As according to dry weight, the $ED_{95}$ value for pyridate was decreased 3.87-fold by adding this adjuvant. Rashed-Mohassel *et al.* (2011) and Parsa *et al.* (2013) asserted that the methylated rapeseed oil was able to enhance the activity of some herbicides. Generally, it is widely believed that the benefit of an oil adjuvant can be related to their ability (*i*) to increase the drying period of the spray droplets during their fly time before impacting the leaves, (*ii*) to prevent bouncing off after impacting the leaves, (*iii*) to decrease contact angle of the droplet on the leaf surface and improve the spreading of them on the leaf surface, (*iv*) to delay crystallization of the active ingredient on the leaf surface, (*v*) to reduce the volatization and photo-decomposition period of the active ingredient, (*vi*) to act as a penetrant agent on the leaf epicuticular wax, and mainly (*vii*) to enhance the penetrability of the active ingredient into the plants (Rashed-Mohassel *et al.*, 2010; 2011; Izadi-Darbandi *et al.*, 2013).

The rate of 282.15 g active ingredient of pyridate $ha^{-1}$ was chosen as the optimized rate to compare with the recommended rate (1200 g active ingredient of pyridate $ha^{-1}$) in the further experiment.

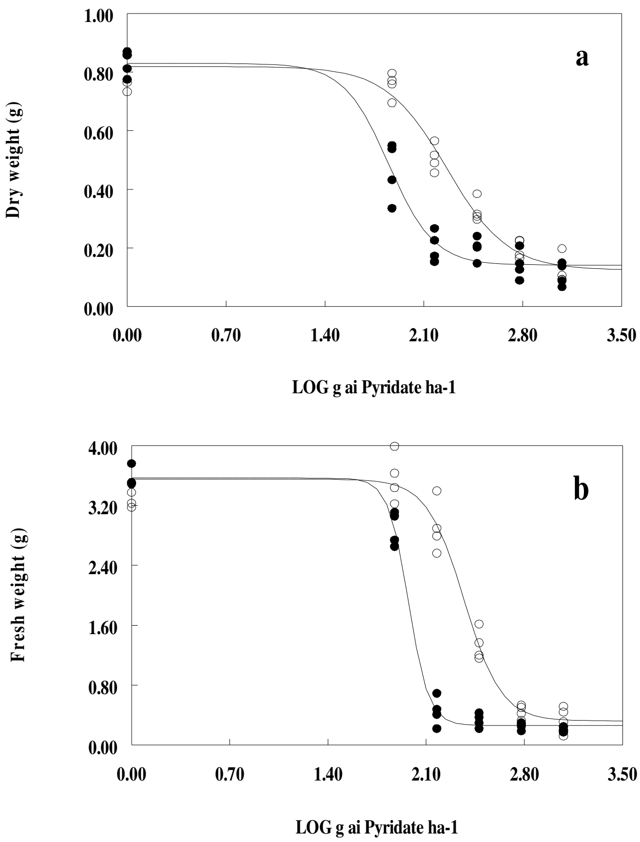


Fig. 1. Dose-response curves for herbicide pyridate alone (○) and in the presence of methylated rapeseed oil (●) against common lambsquarters. The lines were fitted on data belong to shoot dry weight (a) and shoot fresh weight (b).

*Experiment 2*

Rhizobial colonization of chickpea roots was observed in all experimental units. Generally, the analysis of variance revealed (Tab. 2) that with the exception of root dry weight; the shoot dry weight, number of nodules, nodules dry weight, shoot N, root N, shoot P, and root P per plant were affected significantly by the treatment factor in varying degree. All of the measured parameters were significantly affected by the cultivar factor. Moreover, with the exception of root P per plant, the interaction between cultivar and treatment were significant in all of the measured parameters.

Based on shoot dry weight, the 'Desi' cultivars (D-MCC-49 and D-MCC-362) grew heavier than the 'Kabuli' cultivars (Tab. 2). The recommended and optimized rates of pyridate had no effect on the shoot dry weight of K-MCC-252 cultivar. In other 'Kabuli' cultivars (K-MCC-283, K-MCC-358, K-MCC-360, K-MCC-395, and K-MCC-950), a significant reduction in the shoot dry weight was observed with application of the recommended rate of pyridate. The decrease was about 37%, 41%, 44%, 35%, and 49%, respectively. In the 'Desi' cultivars, this parameter was significantly reduced by both the recommended rate and the optimized rates of pyridate. The decrease was about 53% and 55% for the recommended rate in D-MCC-49 cultivar and 35% and

Tab. 1. Estimated regression parameters for the dose-response curves (in Fig. 1) of common lambsquarters (*Chenopodium album* L.) treated with herbicide pyridate alone and in the presence of methylated rapeseed oil at 0.5% concentration of spray volume

| Treatment | *b* | *C* | *D* | $ED_{50}$ | $ED_{95}$ |
|---|---|---|---|---|---|
| | | (g plant$^{-1}$) | (g plant$^{-1}$) | (g active ingredient ha$^{-1}$) | |
| | | | Shoot fresh weigh | | |
| Pyridate | 7.54 (1.55) | 3.49 (0.11) | 0.32 (0.12) | 226.29 (11.70) | 970.94 (10.22) |
| Pyridate + methylated rapeseed oil | 13.79 (1.21) | 3.56 (0.06) | 0.25 (0.04) | 96.14 (6.29) | 207.34 (7.81) |
| | | | Shoot dry weight | | |
| Pyridate | 4.64 (0.75) | 0.16 (0.02) | 0.81 (0.02) | 192.85 (10.50) | 192.85 (10.50) |
| Pyridate + methylated rapeseed oil | 6.28 (1.85) | 0.12 (0.02) | 0.83 (0.03) | 82.39 (9.51) | 82.39 (9.51) |

* The concentration that was chosen as the optimized rate. The standard errors are in parentheses (p=0.05). The parameter of b is the slope of the line, C is the lower limit, D is the upper limit corresponding to the response of the untreated control, and $ED_{50}$ or $ED_{95}$ is the required rate of herbicide to give 50% or 95% control.

Tab. 2. Effect of the recommended and optimized rates of pyridate on biological properties of the chickpea cultivars inoculated with *M. mediterraneum*

| Cultivar | Treatment | Dry weight | | Nodulation | | Nitrogen content | | Phosphorus content | |
|---|---|---|---|---|---|---|---|---|---|
| | | Shoot (g/plant) | Root (g/plant) | Number (no./plant) | Dry weight (mlg/plant) | Shoot (mg/g) | Root (mg/g) | Shoot (mg/g) | Root (mg/g) |
| K-MCC-252 | Control | 0.98$^{ef}$ | 0.30 e-g | 37$^{cd}$ | 89$^{c}$ | 26$^{f-i}$ | 17 g$^{-i}$ | 0.20$^{gh}$ | 0.12$^{gh}$ |
| | The recommended rate | 0.92$^{fg}$ | 0.35$^{b}$ | 26$^{ef}$ | 52$^{e}$ | 19$^{j-l}$ | 9$^{k-m}$ | 0.11$^{l}$ | 0.07$^{ij}$ |
| | The optimized rate | 1.02$^{df}$ | 0.28 g$^{h}$ | 40$^{b}$-$^{d}$ | 93$^{bc}$ | 23$^{h-j}$ | 14$^{i-k}$ | 0.20$^{gh}$ | 0.14 f$^{h}$ |
| K-MCC-283 | Control | 1.21$^{bc}$ | 0.31$^{c-g}$ | 49$^{ab}$ | 89$^{c}$ | 34$^{b-d}$ | 27$^{cd}$ | 0.23$^{d-g}$ | 0.16$^{c-g}$ |
| | The recommended rate | 0.76$^{gi}$ | 0.29$^{gh}$ | 22$^{fg}$ | 45$^{ef}$ | 17$^{lm}$ | 7$^{m}$ | 0.15 i$^{j}$ | 0.10$^{hi}$ |
| | The optimized rate | 1.22$^{bc}$ | 0.36$^{b}$ | 41$^{b-d}$ | 90$^{bc}$ | 30$^{d-f}$ | 22$^{d-g}$ | 0.26 a$^{-c}$ | 0.19$^{a-e}$ |
| K-MCC-358 | Control | 1.28$^{ac}$ | 0.36$^{b}$ | 33$^{de}$ | 83$^{cd}$ | 22 i$^{-k}$ | 13$^{i-k}$ | 0.21$^{fg}$ | 0.15 d$^{-g}$ |
| | The recommended rate | 0.75$^{hi}$ | 0.33$^{b-d}$ | 16$^{g}$ | 34$^{f}$ | 18$^{k-m}$ | 8$^{k-m}$ | 0.12$^{kl}$ | 0.05$^{j}$ |
| | The optimized rate | 1.19$^{bc}$ | 0.33$^{b-d}$ | 49$^{ab}$ | 92$^{bc}$ | 19$^{j-l}$ | 10$^{j-m}$ | 0.18$^{h}$ | 0.12$^{gh}$ |
| K-MCC-360 | Control | 1.18$^{bd}$ | 0.24$^{i}$ | 40$^{b-d}$ | 88$^{cd}$ | 28$^{e-g}$ | 20$^{d-g}$ | 0.25$^{c-e}$ | 0.19$^{a-e}$ |
| | The recommended rate | 0.66$^{i}$ | 0.34$^{b-d}$ | 15$^{g}$ | 56$^{e}$ | 18$^{k-m}$ | 8$^{k-m}$ | 0.12$^{kl}$ | 0.10$^{hi}$ |
| | The optimized rate | 1.02$^{df}$ | 0.30 e-g | 47$^{a-c}$ | 80$^{cd}$ | 27$^{f-h}$ | 18$^{f-i}$ | 0.27$^{a-c}$ | 0.20$^{a-c}$ |
| K-MCC-395 | Control | 1.16$^{bd}$ | 0. 31$^{c-g}$ | 42$^{b-d}$ | 82$^{cd}$ | 33$^{b-d}$ | 25$^{c-e}$ | 0.22$^{e-g}$ | 0.15$^{d-g}$ |
| | The recommended rate | 0.75$^{hi}$ | 0.30$^{e-g}$ | 17$^{fg}$ | 53$^{e}$ | 15$^{lm}$ | 5$^{m}$ | 0.11$^{l}$ | 0.04 j |
| | The optimized rate | 1.13$^{ce}$ | 0.28$^{gh}$ | 39$^{cd}$ | 82$^{cd}$ | 32$^{c-e}$ | 23$^{d-f}$ | 0.21$^{fg}$ | 0.14 f-h |
| K-MCC-950 | Control | 1.31$^{f}$ | 0.29$^{gh}$ | 43$^{b-d}$ | 88$^{cd}$ | 23$^{h-j}$ | 15$^{h-j}$ | 0.26$^{a-c}$ | 0.19 a-e |
| | The recommended rate | 0.67$^{i}$ | 0.28$^{gh}$ | 17$^{fg}$ | 45$^{ef}$ | 18$^{k-m}$ | 8$^{k-m}$ | 0.11$^{l}$ | 0.12 gh |
| | The optimized rate | 1.05$^{df}$ | 0.29$^{gh}$ | 42$^{b-d}$ | 81$^{cd}$ | 25$^{g-i}$ | 16$^{hi}$ | 0.24$^{c-f}$ | 0.17 b-f |
| D-MCC-49 | Control | 1.42$^{a}$ | 0.35$^{b}$ | 57$^{a}$ | 104$^{ab}$ | 35$^{bc}$ | 29$^{b}$ | 0.29$^{a}$ | 0.21 ab |
| | The recommended rate | 0.66$^{i}$ | 0.34$^{b-d}$ | 21$^{fg}$ | 38$^{f}$ | 16$^{lm}$ | 6$^{m}$ | 0.14$^{jk}$ | 0.12 gh |
| | The optimized rate | 0.92$^{fg}$ | 0.26$^{hi}$ | 54$^{a}$ | 103$^{ab}$ | 37$^{b}$ | 29$^{b}$ | 0.26$^{a-c}$ | 0.19 a-e |
| D-MCC-362 | Control | 1.40$^{a}$ | 0.39$^{ab}$ | 56$^{a}$ | 106$^{a}$ | 43$^{a}$ | 35$^{a}$ | 0.26$^{a-c}$ | 0.19 a-e |
| | The recommended rate | 0.63$^{i}$ | 0.33$^{bf}$ | 15$^{g}$ | 44$^{ef}$ | 14$^{m}$ | 4 m | 0.14$^{jk}$ | 0.10 hi |
| | The optimized rate | 0.87 f$^{-h}$ | 0.42$^{a}$ | 48$^{ab}$ | 112$^{a}$ | 42$^{a}$ | 35$^{a}$ | 0.29$^{a}$ | 0.22 a |
| LSD Treatment (*df* = 2) | | 0.06$^{**}$ | 0.01$^{ns}$ | 3.32$^{***}$ | 4.84$^{***}$ | 1.46$^{**}$ | 1.96$^{**}$ | 0.01$^{**}$ | 0.01$^{*}$ |
| LSD Cultivar (*df* = 7) | | 0.09$^{*}$ | 0.02$^{***}$ | 5.42$^{*}$ | 7.90$^{**}$ | 2.38$^{***}$ | 3.20$^{**}$ | 0.02$^{**}$ | 0.02$^{**}$ |
| LSD Treatment × Cultivar (*df* = 14) | | 0.17$^{**}$ | 0.03$^{**}$ | 9.40$^{**}$ | 13.69$^{**}$ | 4.13$^{**}$ | 5.55$^{*}$ | 0.03$^{**}$ | 0.04$^{ns}$ |

K- and D- is abbreviation of 'Kabuli' and 'Desi' cultivars, respectively. Mashhad Chickpea Collection is shorted to MCC. df is degree of freedom. The recommended rate was 1200 g active ingredient of pyridate ha$^{-1}$ and the optimized rate was 282.15 g active ingredient of pyridate ha$^{-1}$ + methylated rapeseed oil at 0.5% concentration of spray volume. Means in a column followed by same letter are not significantly different at the 0.05 probability level determined by Least Significant Difference Test (LSD). $^{*}$: Statistical significance (p≤0.05); $^{**}$: Highly statistical significant (p≤0.01); $^{***}$: Very highly statistical significant (p≤0.001); $^{ns}$: No statistical significant.

38% for the optimized rate in D-MCC-362 cultivar, respectively. The highest root dry weight and the lowest root dry weight were observed in D-MCC-362 and K-MCC-360 cultivars, respectively (Tab. 2). Although the application of the recommended rate on K-MCC-252 and K-MCC-360 cultivars led to stimulation in root dry weight, both treatments had no significant effect on the root dry weight in other cultivars. Generally, the results revealed that the shoot:root ratio from plants treated with herbicide was slightly lower relative to control (data not shown). It is established that many pesticides induce a reduction in biological $N_2$ fixation via their noxious effect on rhizobial bacteria (Liebman *et al*., 2004); as a result, any factor which unfits growing conditions results in a reduced shoot:root ratio (Harris, 1992); that is, root grows more in weight than shoot. Similar results have also been reported (Niina, 2008).

Between two types of chickpea, the 'Desi' cultivars (D-MCC-49 and D-MCC-362) had higher nodulation (nodule number and dry weight) than the 'Kabuli' cultivars (Tab. 2). In all chickpea cultivars, the number of nodules per plant was significantly decreased by applying the recommended rate of pyridate. This parameter was found to be reduced by about 29%, 51%, 51%, 62%, 59%, 60%, 63%, and 73% in K-MCC-252, K-MCC-283, K-MCC-358, K-MCC-360, K-MCC-395, K-MCC-950, D-MCC-49, and D-MCC-362 cultivars exposed to pyridate at the recommended dose, respectively. There was a similar trend for the dry weight of nodules per plant (Tab. 2). The application of pyridate at the recommended dose reduced significantly by about 41%, 49%, 59%, 36%, 34%, 48%, 63%, and 58% as compared with the control, respectively. The number and the dry weight of nodules per plant were significantly unaffected with the optimized rate in all cultivars. The decrease in both nodule number and dry weight in this experiment indicates that symbiosis breaks down at the early stages of symbiosis establishment. The result obtained in this study indicates that shoot N was more than root N in all chickpea cultivars (Tab. 2). The shoot N of all chickpea cultivars was generally affected by the recommended dose of pyridate, but in K-MCC-358 cultivar; it was not statistically significant. The optimized rate of pyridate had no effect on the shoot N content of all cultivars. A similar trend for the root N, shoot P, and root P was also observed. As can be observed (Tab. 2), when pyridate was used at the recommended dose, these parameters were decreased as compared with both control and the optimized dose of pyridate.

Although the effect of pyridate on chickpea - *M. mediterraneum* symbiosis was not found in the literature, the reduced nodulation by some herbicides, especially the photosystem II- inhibiting herbicides, in chickpea have already reported as listed above. The literature review indicated that legume-symbionts symbiosis may directly and indirectly affected by herbicides via (1) altering the balance of leacetic acid in host plant, leading to lower root nodulation (Kremer and Means, 2009); (2) reducing root biomass, leading to limit the number of available sites for rhizobia to attach to root hairs (Anderson *et al*., 2004); (3) altering carbohydrate preparation to the root nodules; (4) altering the activity of a critical enzyme in the root nodules, called nitrogenase (Niewiadomska and Sawicka, 2002); (5) inhibiting or inactivating the biochemical signaling by either symbionts or plants required to initiate nodule development (Ahemad and Khan, 2011); and/or (6) reducing the capacity of cell division, leading to inhibit nodule development (Datta *et al*., 2009).

## Conclusion

In this study, we demonstrated the deleterious effects of pyridate on chickpea - *M. mediterraneum* symbiosis. Generally, the 'Desi' cultivars were more sensitive than the 'Kabuli' cultivars to pyridate application. In our study, several symbiotic properties of chickpea cultivars were affected more than 10% at the recommended dose. Because chickpea - *M. mediterraneum* symbiosis was not affected by the optimized dose of pyridate, thus, we may conclude that: effective low-use-rate should be noted; the application of activator adjuvants can be a suitable manner to decrease unfavorable effects of herbicides; because a higher leaching of herbicide is imaginable in light texture soils, the intensive application of herbicide should be avoided as much as possible; leaching of herbicide should be minimized by some adjuvants.

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