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RESEARCH ARTICLE

# Evaluation of PRE and POST Herbicides on Growth Features, Nodulation, and Nitrogen Fixation of Three Cultivars of Chickpea (*Cicer aritinium* L.)

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## Abstract

It is exceptionally difficult to have productive farming without the use of herbicides. It has been found that they create difficulties in the growth and biological nitrogen fixation of some legume crops after their application. Adverse impacts may result from a direct effect of the herbicide on rhizobial growth and/or an indirect effect on plant growth. In order to study the effect of some PRE and POST herbicides on nodulation, nitrogen fixation, and growth characteristics of three chickpea cultivars, a greenhouse trial was conducted in a completely randomized design with factorial arrangement and three replications. Pyridate, imazethapyr, and trifluralin were applied with the recommended rates of 1100, 100, and 720 g ai ha$^{-1}$, respectively, and also non-treated control on three chickpea cultivars (ILS482, Hashem, and Kaka). The results showed that trifluralin and pyridate herbicides had the highest and the least negative impact on growth characteristics of three examined cultivars. Pyridate showed a positive effect on vegetative characteristics but nodulation and total nitrogen contents in the shoots of chickpea cultivars decreased significantly. Results also revealed that root and shoot growth, nodulation, and nitrogen fixation diminished significantly when imazethapyr and trifluralin were applied to Hashem and ILC482 cultivars. It seems that pyridate is an appropriate herbicide for broadleaf weed control in chickpea fields.

**Key words :** Herbicides, nitrogen fixation, chickpea, nodulation

## Introduction

Chickpea is a cool-season crop belonging to the family fabaceae (Boydston et al. 2018; Jefferies et al. 2016). Chickpea production on the Iranian prairies is challenging because of multiple devastating stressors, including adverse environmental conditions, disease pressure, and weed incidence. It plays an essential role in improving soil fertility by fixing the atmospheric nitrogen. Chickpea can supply 80 to 120 kg N ha$^{-1}$ to the soil (Mafakheri et al. 2012; Shakouri et al. 2012). After harvest, chickpea leaves a substantial amount of residual nitrogen for subsequent crops, as well as a certain amount of organic matter to maintain and improve soil health and fertility (Kantar et al. 2007).

Chickpeas compete poorly with weeds because of their slow growth and limited canopy development, resulting in yield losses of 48 to 97% when weeds are not controlled (Al-Thahabi et al. 1994; Boydston et al. 2018; Mohammadi et al. 2005; Paolini et al. 2006). Chickpea is commonly grown as a rotation crop in west and northwest Iran to enhance soil nitrogen and to break graminaceous crop disease cycles. It develops slowly and has an open canopy architecture and low stature, reducing its ability to compete with weeds (Brigido et al. 2007; Mohammadi et. 2005; Zargar et al. 2017). One of the principal benefits of cultivation of a legume crop is the symbiotic N fixation. Hence, it is important in investigating herbicides efficacy, either directly on rhizobium species, or indirectly by affecting the rhizobium–plant symbiosis (Datta et al. 2009; Duy et al. 2018).

Residual levels of herbicides can significantly inhibit

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**Table 1.** Herbicide treatment descriptions.

| Active ingredients | Trade name | Rate (g ai ha$^{-1}$) | Concentration /Formulation | Chemical name | Mechanism of action |
|---|---|---|---|---|---|
| Pyridate | Lentagran® | 1100 | 450 g/kg WP | O-(6-chloro-3-phenyl-4-pyridazinyl) S-octyl carbonothioate | Photosystem II Inhibitors |
| Imazethapyr | Pursuit® | 100 | 100g/L SL | 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarbo xylic acid | ALS Inhibitors |
| Trifluralin | Trefelan™ | 720 | 480 g/L EC | 2,6-dinitro-N,N-dipropyl-4 (trifluoromethyl)benzenamine | Microtubule Inhibitors |

Abbreviations: WP, wettable powder; SL, soluble liquids; EC, emulsion concentrate.

nodulation and N fixation by legumes and may have negative effects on the N balance of legume–cereal rotations. Mallik and Tesfai (1985) found that nodulation and N fixation in soybean (*Glycine max* L.) were affected adversely by application of trifluralin, 2,4-DB, alachlor, glyphosate, and metribuzin. Singh and Wright (1999) in a research examined the effect of some pre-emergence herbicides (terbutryn/terbuthylazine, trietazine/simazine, and prometryn) and a POST herbicide (bentazone) on nodulation, symbiotic N fixation, growth and yield of pea (*Pisum sativum* L.), they revealed that all pre-emergence herbicides diminished nodulation, total nitrogenize activity, root and shoot dry weight, N content, and seed yield. Although various herbicides are used for weed control in chickpea in other countries including trifluralin, ethalfluralin, and simazine in the United States and Australia (Lees 2004) and imazethapyr in Turkey (Kantar et al. 2007), only linuron and pyridate have been registered for weed control in chickpea in Iran (Shahbazi et al. 2019; Bayat et al. 2019). Therefore, introducing the proper herbicide for a grain crop such as chickpea in a developing country such as Iran is essential because it is an important section of agricultural systems in Iran where the area under sowing of chickpea have been 400,000 hectares.

Lack of proper herbicide for controlling the broadleaf weeds in chickpea fields is one of the most important challenges of chickpea production in Iran. Therefore, the main aim of the present study was to evaluate the phytotoxic effects of lentagran, Pursuit, and treflan herbicides on growth characteristics, nodulation, and N content on three varieties of chickpea (ILS482, Hashem, and Kaka) under greenhouse condition.

# Material and Methods

A composite soil sample was collected at 0-30 cm depth (latitude 36° 14' N, longitude 59° 40' E) at an altitude of 985 m above sea level. A total of 4 mm sieved soil samples were used for a pot experiment and 2 mm sieved soil samples were used for soil physical and chemical analysis. Soil texture was determined by the hydrometer method (Klute and Dirksen 1986), soil pH, and soil electrical conductivity (EC) measured in saturated paste extract (using METROM-model 632 and JENWAY-model 4310, respectively), soil organic matter (Walkley and Black 1934), total nitrogen (Page et al. 1982), available phosphorus (Olsen et al. 1954), available potassium (Richards 1954), iron, zinc, magnesium, and copper concentrations were assessed by DTPA-TEA extraction (Lindsay and Norvell 1978) and measured by atomic absorption (Shimadzu, AA 670).

A completely randomized experiment with factorial arrangement was conducted in greenhouse conditions with three replications. The first factor was the recommended dose of three different herbicides: pyridate 'postemergence' 1100 g ai ha$^{-1}$, imazethapyr 'pre-emergence' 100 g ai ha$^{-1,}$ and trifluralin 'pre-emergence' 720 g ai ha$^{-1}$ and without herbicide. Herbicide treatment descriptions are summarized in Table 1. The second factor was the chickpea cultivars (ILS482, Hashem, and Kaka). Plastic plant pots (Dimensions: 20 cm diameter top, 16 cm diameter base, and 18 cm depth) with drainage holes were filled with 4 kg sandy loam, non-sterile soil with low salinity (EC= 3.2 dS/m).

Trifluralin herbicide was mixed with soil samples 5 days before planting. Chickpea seeds were surface sterilized with 3% sodium hypochlorite for 5 min and washed several time with sterilized water. Three seeds were sown in each plastic pot. Lentagran and Pursuit herbicides were sprayed at the three-leaf stage and 3 days after planting, respectively, using a Tee Jet Spray Nozzle # 8001. Totally, 36 pots were maintained at 12-h day/night photoperiod at 26°/16°C. The soil moisture in pots was adjusted at 70% of field capacity by distilled water.

Plants were harvested after 3 months. Plant height, leaves, pod, nodules numbers, root and shoot dry weight, wet nodule weight, and nitrogen content in shoot and root were measured. Before analysis, all collected data were examined for normality and constant variance. MSTAT-C program was used for data analysis and Duncan's test applied for comparing the means with a P-value of less than 0.05.

# Results and Discussion

The results of the soil analysis showed that soil texture was sandy loam and the amount of clay, silt, and sand were 18, 25, and 57%, respectively. Soil pH and electrical conductivity of soil saturation extract was 7.4 and 3 dS/m, respectively (Table 2). With regarding to soil analysis results,

**Table 2.** Soil physical and chemical properties of the experimental soil.

| Soil properties | Unit | |
|---|---|---|
| Texture | – | Sandy loam |
| pHs | – | 7.43 |
| ECs | dS $m^{-1}$ | 3.12 |
| OM | % | 1.10 |
| Total N | mg $kg^{-1}$ | 302.10 |
| Available P | mg $kg^{-1}$ | 10.19 |
| Available K | mg $kg^{-1}$ | 186.04 |
| Fe | mg $kg^{-1}$ | 9.03 |
| Zn | mg $kg^{-1}$ | 0.62 |
| Mn | mg $kg^{-1}$ | 14.54 |
| Cu | mg $kg^{-1}$ | 0.75 |

chemical fertilizer was not applied to the soil sample before planting.

POST application of lentgran at the recommended rate did not have any symptoms of visual phototoxicity on three chickpea cultivars in sequential observations of plants. It had no significant adverse effect on plant vitality. The most visual phototoxicity on all three chickpeas was observed when they were treated by imazethapyr. Imazethapyr herbicide application showed the most phototoxicity on three chickpea cultivars at the recommended rate which caused yellowing of plants, delay in growth, flowering and pod formation, and reduction in plant height. Most chickpea varieties are susceptible to the negative effects of imidazolinone herbicides, to which imazethapyr belongs (Cardina et al. 1986; Jefferies et al. 2016). Taran et al. (2010) examined the response of various chickpea genotypes to imidazolinone herbicides and they observed severe chlorosis on some of the accessions. In another study by Taran et al. (2013), they noted that applying imazethapyr can cause severe damage to chickpea plants negatively affecting seed yield and protracting the duration for maturation.

Trifluralin herbicides suspended all growth stages in all three chickpea cultivars but the adverse effect of trifluralin herbicides was the greatest on the Kaka cultivar. Physiological changes of 24 chickpea (*Cicer arietinum* L.) genotypes evaluated by Kaur et al. (2017), indicated that herbicide application intensely affects the leaves by altering the morphology.

All morphological characteristics (except for nodule wet weight) and shoot and root nitrogen content of three examined chickpea cultivars were statistically significant at $P \leq 0.05$ (Table 3). Plant height, leaf number, pod number, shoot and root dry weight, nodule number, and nitrogen content in the shoot and root of three chickpea cultivars was affected differently by herbicide application. Maximum and minimum root and shoot nitrogen contents were measured in Hashem and Kaka cultivars, respectively. Genetic diversity among the cultivars is the main factor which controls growth parameters, moreover individual response of each cultivar to applied herbicide or other environmental factors can be different.

Shoot dry weight increased 5% when chickpea plants were treated by pyridate but diminished 54 and 8%, respectively, when treated with imazethapyr and trifluralin herbicides. The effect of pyridate on the shoot dry weight of the chickpea varieties is similar to the noted effect by Khan et al. (2006) when terbutyrn was applied at both recommended rate and double rate significantly improved dry shoot weight by 31 and 28%, respectively. Pyridate had no significant effect on shoot dry weight of three chickpea cultivars but the application of imazethapyr and trifluralin herbicides caused a reduction on this parameter (Fig. 1). Miller (2003) observed that imazamox applied as a POST herbicide caused 21 and 28% injury on the green pea crop at the respective rates of 0.036 and 0.045 kg $ha^{-1}$. In the same experiment, trifluralin along with two other pre-emergence herbicides clomazone and sulfentrazone caused 15-19% crop injury in one of the test years.

**Table 3.** Mean comparisons of morphological characteristics, shoot and root nitrogen contents of three chickpea cultivars at the end of the experiment.

| Measured parameters | Chickpea cultivars | | | | | |
|---|---|---|---|---|---|---|
| | ILC482 | | Hashem | | Kaka | |
| Plant height (cm) | 24.88 | c | 51.42 | a | 38.42 | b |
| Leaves number (per pot) | 237.58 | a | 156.42 | b | 229.75 | a |
| Pod number (per pot) | 10.42 | a | 0.92 | c | 7.50 | b |
| Pod wet weight (per pot) | 2.14 | a | 0.31 | b | 1.67 | a |
| Shoot dry weight (per pot) | 3.14 | b | 3.46 | a | 2.56 | c |
| Root dry weight (per pot) | 6.23 | b | 7.07 | a | 6.07 | b |
| Nodule number (per pot) | 175.92 | a | 170.17 | a | 112.42 | b |
| Nodule wet weight (per pot) | 3.61 | a | 3.50 | a | 3.33 | a |
| Root nitrogen % | 0.48 | b | 0.55 | a | 0.43 | c |
| Shoot nitrogen % | 1.237 | a | 1.20 | a | 1.07 | b |

Numbers with the same letters indicate no significant difference $P < 0.05$.

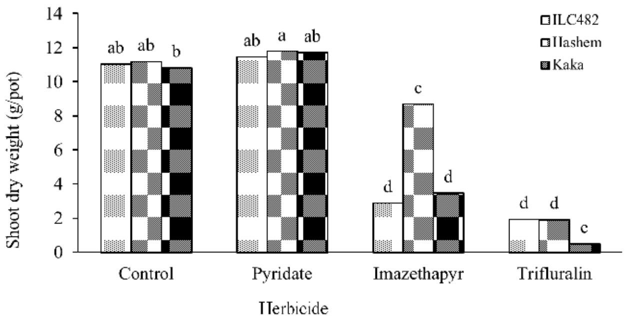


**Fig. 1.** Shoot dry weight responses of the three chickpea cultivars (ILC482, Hashem, and Kaka) to pyridate, imazethapyr, and trifluralin herbicides.
Same letters above the columns indicate no significant difference $P \leq 0.05$.

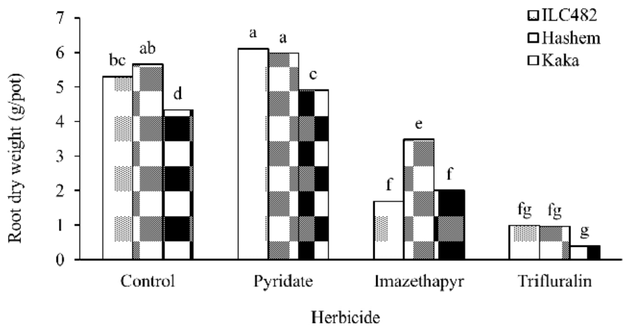


**Fig. 2.** Response of root dry weight of chickpea cultivars (ILC482, Hashem, and Kaka) to pyridate, imazethapyr, and trifluralin herbicides.
Same letters above the columns indicate no significant difference $P \leq 0.05$.

A substantial reduction in shoot dry weight of three cultivars was observed when they were treated by trifluralin. Imazethapyr also induced a reduction in shoot dry weight of three cultivars but Hashen was less affected compared to ILC482 and Kaka. In a study by Jefferies et al. (2016), application of imazethapyr plus imazamox significantly diminished height and node development in conventional chickpea cultivars. Herbicide timing arrested vertical growth until 21 days after application, eventually culminating in overall height reduction in the conventional varieties. There are many scientific reports about the direct effect of trifluralin on cell division. Hence, Fernandes et al. (2007) revealed that trifluralin can stimulate cellular damage. They also reported that trifluralin had a direct effect on the microtubules and it leads to the production of many polyploidy cells.

Similar to shoot dry weight, trifluralin herbicides had the greatest adverse impact on the root dry weight of chickpea cultivars which dropped to 87% compared to the control. On the other hand, pyridate enhanced root dry weight of all chickpea cultivars 11% higher than the control. The three cultivars had about a 55% reduction in root dry weight compared to the control when imazethapyr was applied. Boydston et al. (2018) reported favorable chickpea tolerance to pyridate when it caused no significant injury nor yield

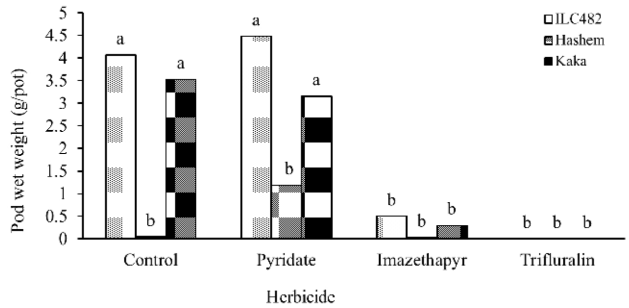


**Fig. 3.** Response of pod wet weight of chickpea cultivars (ILC482, Hashem, and Kaka) to pyridate, imazethapyr, and trifluralin herbicides.
Same letters above the columns indicate no significant difference $P \leq 0.05$.

loss, whilst the other two POST herbicides acifluorfen and fomesafen injured chickpea plants until final evaluation. The findings of the research in tandem with the results observed by Khan et al. (2006) where terbutyrn applied in the recommended rate significantly increased dry root weight by 67 and 33%, respectively. The effect of herbicides on the root dry weight of three cultivars was more or less similar to the shoot dry weight (Fig. 2).

Singh and Wright (2002) investigated the effect of terbutryn/terbuthylazine, bentazone, and prometryn on nodulation and growth of two varieties of peas at recommended and double recommended doses. Results showed that terbutryn/terbuthylazine and bentazone at recommended dose reduced the root dry weight by 35 and 25%, whereas at double the recommended dose the reduction was 43 and 44%, respectively. Pyridate had a positive efficacy on the pod wet weight of chickpea cultivars. The pod wet weight of chickpea cultivars dropped 82 and 100% when imazethapyr and trifluralin herbicides were applied at the recommended dose, respectively (Fig. 3).

By using trifluralin herbicide, no pod was observed in three chickpea cultivars. Hashem was the most sensitive among three cultivars, even in the control treatment (non-treated), Hashem had a lower pod wet weight than the pyridate treatment. ILC482 had the highest pod wet weight (4.5g / pot) when it was treated by pyridate.

Chickpea cultivars were nodulated in all treatments. The highest number of the root nodules was attained for the control (300/pot) and then for pyridate (220/pot). In imazethapyr and trifluralin the root nodule numbers were reduced to 60 and 90% of the untreated control, respectively. The highest number of root nodules (370 nodules per pot) was attained for the control treatment on the roots of Hasehm, but the lowest root nodule number (10/pot) was observed in Kaka cultivar when it was treated by trifluralin (Fig. 4). Lentagram not affected the root nodules number of ILC482 and Kaka, but imazethapyr and trifluralin reduced the root nodule numbers in three cultivars ($P \leq 0.05$). The highest root nodule number was obtained for Hashem in the non-treated control, but the lowest one was observed in Kaka when it was treated by trifluralin (Fig. 4).

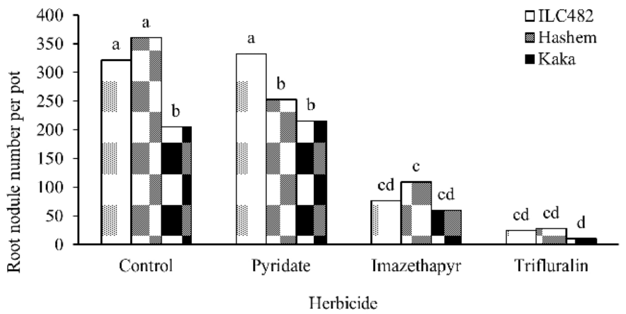


Fig. 4. Response of root nodule number to pyridate, imazethapyr, and trifluralin herbicides in chickpea cultivars (ILC482, Hashem, and Kaka).
Same letters above the columns indicate no significant difference $P \leq 0.05$.

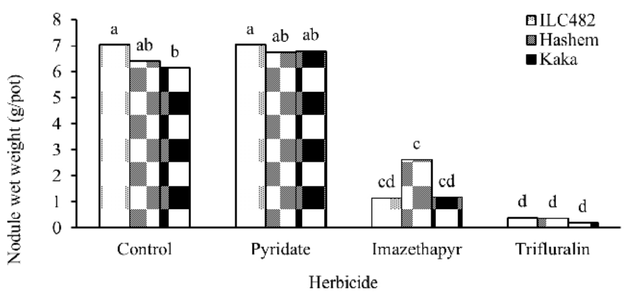


Fig. 5. Response of nodule wet weight of chickpea cultivars (ILC482, Hashem, and Kaka) to pyridate, imazethapyr, and trifluralin herbicides.
Same letters above the columns indicate no significant difference $P \leq 0.05$.

Pyridate improved the wet weight of root nodules for three cultivars. Unlike pyridate, imazethapyr and trifluralin reduced the nodule wet weight 74 and 91%, respectively, in the chickpea cultivars. Drew and Wilson (2005) reported the negative effects of imazethapyr and ethalfluralin in injury on the chickpea plants (Fig. 5).

The non-treated control and pyridate had the maximum nodule wet weight among the other treatments. The least nodule wet weight was observed for trifluralin, so that the reduction in the nodule wet weight chickpea cultivars were 94, 93, and 97%, respectively. The reduction of the nodule wet weight for ILC482, Hashem, and Kaka were 83, 59, and 80%, respectively, when they were treated by imazethapyr compared to the control. Trifluralin and pyridate had the most and the least effect on nodule wet weight of three examined cultivars. Imazethapyr had a great adverse efficacy on nodule wet weight in all chickpea cultivars, nevertheless the amount of the reduction was not as low as trifluralin. Imazethapyr showed less negative effect on Hashem compare to ILC482 and Kaka. Pyridate was a suitable herbicide for all three cultivars because it had no any negative effect on nodule wet weight of three chickpea cultivars (Fig. 5).

All herbicides had an adverse efficacy on nitrogen content of shoots for three chickpea cultivars. The application of lentagram, imazethapyr, and trifluralin affected a drop in the

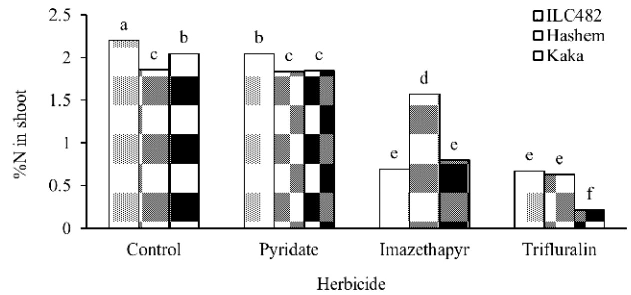


Fig. 6. Response of nitrogen content of chickpea cultivars (ILC482, Hashem, and Kaka) to pyridate, imazethapyr, and trifluralin herbicides.
Same letters above the columns indicate no significant difference $P \leq 0.05$.

shoot nitrogen by 6, 50, and 78%, respectively (Fig. 6). In Shahbazi et al. (2019), both pyridate and imazethapyr had some considerable negative effects on the chickpea plants. However, of the six herbicides they tested, pyridate had the least negative effects (10%) and contrastingly the most severe injury was caused by imazethapyr (40%). The results of our experiment confirm in a similar pattern that, of the two POST herbicides pyridate has the least adverse effects on the chickpea crop. With reference to the root nodule number and the nodule wet weight, the results were not unexpected.

There is a positive correlation between active nodules and biological nitrogen fixation. Nitrogen content of ILC482 and Kaka dropped significantly while the nitrogen content Hashem did not change when pyridate was used. Datta et al. (2009) revealed that the isoxaflutole herbicide reduced total N content of the sensitive chickpea cultivar by 21%. At the recommended rate of isoxaflutole, the herbicide reduced the N fixation capacity of the sensitive chickpea cultivar by 51% compared with the tolerant chickpea cultivar (33%). The lowest amount of total nitrogen content was detected in Kaka cultivar when it was treated by trifluralin herbicide (Fig. 6).

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## Conflicts of interest

No potential conflict of interest was reported by the authors.

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