

Biochemical and cytogenetic effects of Imazethapyr on *Cicer arietinum* L.

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ABSTRACT

Weeds are a serious obstacle to the production of chickpea. Imazethapyr (IM) herbicide is used to control weeds in most of the pulses, including chickpea. Mitotic abnormalities, chromosomal behavior, and protein content in chickpea (*Cicer arietinum* L.), due to IM treatment, were studied. The chickpea seeds (variety JG-11) were germinated in sterilized Petri dishes, 9 cm in diameter, on Whatman filter paper moistened with 10 ml of either Hoagland nutrient solution (control) or five concentrations of IM (0.5, 1, 2.5, 5, and 10 ppm). From the cytologic point of view, observations demonstrated that the mitotic frequency in root meristematic cells diminished, and that abnormality frequency increased parallel to the increase in concentrations of IM. The herbicide was highly mito-inhibitory and induced chromosomal irregularities, such as stickiness, lagging, scattering, and chromosome bridges. The endosperm and root-shoot axis' protein content decreased with increasing of the herbicide concentration in all the treatments. It can be argued that IM produces undesirable side effects during mitosis in chickpea's somatic cells and biochemical parameters.

1. INTRODUCTION

Agricultural field is exposed to various pests, especially weeds, which are of the greatest restraining factors to the efficient crop production, resulting in a severe reduction in all crop yields. Today's weed control tactics are crop rotations, manual weeding, mechanical practices, and herbicide application [1]. The usage of chemicals to control weeds is not new to mechanized agricultural farming. However, herbicide residues impose a serious negative impact on the environment, non-target organisms, especially man and animal. Furthermore, genetic changes induced by herbicides include structural changes (chromosomal aberrations) in chromosomes and chromatids [2].

Imazethapyr (IM) is a broad-spectrum imidazolinone herbicide, which inhibits acetolactate synthase (ALS) enzyme, applied as a built-in pre-plant, pre-emergence, and early pre-emergence for the control of annual grass, broadleaf weeds, and perennial sedges

in chickpeas and other leguminous fields [3,4]. The herbicide leaves a great effect on the normal behavior of chromosomes during mitosis and induces different types of abnormalities. Their frequency depends on the concentration and the duration of treatment, i.e., increasing concentration and duration of treatment leads to a parallel increase in the frequencies of chromosome abnormalities [1].

Many legumes have been reported by a number of authors to extend the effects of IM, such as kharif black gram [5], green gram [6], lentil [7], soybean [8,9], and oilseed rap [10].

Chickpea (*Cicer arietinum* L., Leguminosae) is the second omnipresent grown legume after soybean worldwide [11,12]. However, Nedumaran and co-workers have reported 11 million hectares with 8 million tons of production as the third most important food legume ever grown [13]. Besides, as an important source of food for man and animal, chickpea also plays an important role in the conservation of soil fertility, mainly in the dry, rain-fed areas, through symbiotic nitrogen fixation [14,15]. The plant is, however, a weak competitor to weeds, due to its slow growth rate and narrow leaf area development at primary stages of the crop growth and establishing. The current investigation

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was carried out to study the effects of IM on the early growth parameters of chickpea seedlings, especially chromosomal aberrations and biochemical alterations.

2. MATERIAL AND METHODS

The seed samples of certified varieties of chickpea (*C. arietinum* L.) variety JG-11 ($2n = 2x = 16$ chromosomes) [16] were attained at the University of Agricultural Sciences, GKVK, Bangalore, India. The herbicide, IM (Pursuit 10% SL), was obtained at BASF, India.

One hundred healthy-looking chickpea grains were washed carefully and surface-sterilized through sodium hypochlorite solution containing 2% chlorine for 5 minutes, followed by a thorough wash in sterile distilled water for 10–12 times to remove the extra chloride [17,18]. Ten seeds were then left in each Petri plate of 9 cm, sterilized with sulfuric acid, containing two layers of Whatman No.1 filter paper and 5 ml of Hoagland or a treatment solution of IM at five different concentrations, i.e., 0.5, 1, 2.5, 5, and 10 ppm of active ingredient prepared in Hoagland nutrient solution, under clean lab condition at $26\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ for 4 days at dark [18,19].

Dose selection was done based on field prescribed concentrations, which could affect 10%–95% of seedlings with logarithmic intervals. From the 5th day onwards, the germinated seedlings were exposed to a 12 hours light intensity and grown-up for more than 15 days. Out of the 400 seeds, four replicates were kept in each dose. The plants were watered with distilled water, when required.

Cytological studies were done on the root tips of both the treated and control germinated seedlings, by collecting and pretreating with 8-hydroxyquinoline for 4 hours; then, they were fixed in Carnoy's solution II (alcohol: chloroform: acetic acid in 6:3:1 ratio) for 24 hours. The fixed root tips were preserved in a 70% ethanol in the refrigerator for further studies [20]. Fiskesjo [21] method was employed for calculating the mitotic index and the frequency of abnormalities, by examining 500 cells per slide and calculating approximately 2,000 cells [18]. For each concentration, three replicates were made. Lowry et al. [22] method, with some modifications described by Hoseiny-Rad et al. [18], was used to determine the total protein content of seedlings. Statistical analyses by variance analysis were done, using the SPSS package with Tukey's honestly significant difference test at a 5% level.

3. RESULTS AND DISCUSSION

The effects of IM on the mitosis stages and chromosomal abnormalities in root tip cells of chickpea are summarized in Table 1. In the current study, with an increase in concentrations of IM herbicide in chickpea, the mitotic index decreased, compared to the control. The mitotic index decreased from 11.4% to 7.15% at a dosage of 0.5–10 ppm, while the maximum mean value was observed in the control (11.5%). Although mitotic cells were observed in the treated root tips, they were relatively lower than the control root tips, indicating a disturbance of IM in the normal sequence of cell division, due to the reduction of mitotic activity

Table 1: Imazethapyr induced mitosis stages and chromosomal abnormalities in root tip cells of chickpea.

IM concentration (ppm)	Total number of cells	Mitotic frequency (%)	Prophase (%)	Metaphase (%)	Ana-Telophase (%)	Total abnormalities (%)	Laggard chromosome (%)	Scattered chromosome (%)	Chromosomal bridge (%)	Sticky chromatin (%)
Control	6120	11.5 ± 0.85 ^a	50.76 ± 2.35 ^f	23.62 ± 1.65 ^b	25.31 ± 1.87	2.10 ± 0.15	0.50 ± 0.03	0.10 ± 0.01	0.00 ± 0.00	0.60 ± 0.03
0.5	6090	11.4 ± 0.56 ^b	53.46 ± 3.45 ^e	22.73 ± 1.23 ^a	23.81 ± 2.11	2.30 ± 0.13	0.00 ± 0.00	0.30 ± 0.02	0.70 ± 0.03	1.30 ± 0.09
1	6100	10.61 ± 0.45 ^c	52.07 ± 4.21 ^e	22.91 ± 1.45 ^c	25.02 ± 1.96	2.50 ± 0.14	0.31 ± 0.02	0.50 ± 0.02	0.00 ± 0.00	1.70 ± 0.12
2.5	5800	9.44 ± 0.74 ^d	52.71 ± 5.31 ^d	25.18 ± 1.89 ^a	22.11 ± 1.36	6.80 ± 0.21	1.20 ± 0.09	1.40 ± 0.08	2.10 ± 0.14	2.10 ± 0.21
5	5850	8.03 ± 0.35 ^e	60.49 ± 4.36 ^b	20.33 ± 1.21 ^c	19.18 ± 1.57	11.50 ± 0.51	1.80 ± 0.11	2.50 ± 0.019	2.90 ± 0.13	4.30 ± 0.31
10	5700	7.15 ± 0.24 ^f	62.26 ± 6.14 ^a	18.81 ± 1.45 ^f	18.93 ± 1.23	15.20 ± 0.31	2.30 ± 0.017	5.30 ± 0.021	3.40 ± 0.20	5.20 ± 0.36

Mean ± standard deviation (SD) followed by the same superscript are not statistically significant between the concentrations, when subjected to SPSS package, according to Tukey's mean range test at 5% level.

in the treated plants [18]. Other herbicides such as Imazethapyr on *Vicia faba* [1], fusilade on lentil [23], IM on wheat [18], IM on *Allium cepa* [24], and Butachlor on *Triticum aestivum* L. [20] have shown such effects.

Such mitodepressive effect may be due to an interference in the normal process of mitosis as a decrease in the number of dividing cells, which prevents or reduces the number of cells to enter the prophase stage [25]. It was suggested that ALS-inhibiting herbicides would block the synthesis of valine, leucine, and isoleucine *in vivo*, and the branched-chain amino acids deficiencies could cause a fall in protein synthesis, which, in turn, could slow down the rate of cell division, a process that eventually leads to the cell death [24].

In the somatic cells, the mitosis inhibition and pro-metaphase blockage were seen in treated plants (Table 1). The prophase was 62.26% in 10 ppm treatment, compared to 50.76% in the control one. The frequency of metaphase, anaphase, and telophase was decreased among all the treatments. Blocking the mitosis in meristematic regions is of known mitotic poisoning mechanisms in herbicides. The IM inhibited mitosis and stopped the division process at the pro-metaphase in wheat [18]. Our results are in line with the effects of fusilade in *A. cepa* affected by IM [24], which showed an increase in prophase as well as the number of root meristematic cells. The accumulation of dividing cells at prophase with IM might be an indication of the blockage of the process at the end of the prophase [24]. In the treated plants, mitotic stages are present, but sometimes one or more stages will be absent or aberrant, due to disruption of cell division [23].

The frequency of occurrence of chromosomal abnormalities increased with an increase in the concentration of IM, i.e., in treatments, it was between 2.3% and 16.2%, at the dosage of 0.5–10 ppm compared to 1.2% in control plants (Table 1 and Fig. 1). The sticky chromosomes, lagging chromosomes, scattered chromosome, and chromatin bridge were of the most common type of observed anomalies (Fig. 1a–h). Lagging chromosome was not seen at the dosage of 0.5 ppm in chickpea, while it showed a minimum frequency (0.31%) at the dosage of 1 ppm and a maximum frequency (2.3%) at the dosage of 10 ppm. The frequency of occurrence of scattered chromosomes increased from 0.10% in control to 5.35% in 10 ppm herbicide concentration. At 10 ppm herbicide concentration, 5.2% of sticky chromosomes was observed. Chromosome stickiness is caused by an improper folding of the chromosome fiber into single chromatid and chromosome. As a result, there is an intermixing of fibers, while chromosomes are attached to each other by sub-chromatid bridges [18,26]. The results on chromosome stickiness and clumping are in line with other studies on chemicals such as Imazethapyr in *V. faba* [1], maleic hydrazide in *Trigonella foenum-graecum* [25], atrazine in *A. cepa* [27], IM on wheat [18], and IM on *A. cepa* [24]. The depolymerization of the nucleic acid caused by mutagenic treatments or partial dissociation, as well as the modification of the nucleoprotein organization, has been justified for such sticking, which render their separation and free movement incomplete; so, they stay connected by the bridges [18,25].

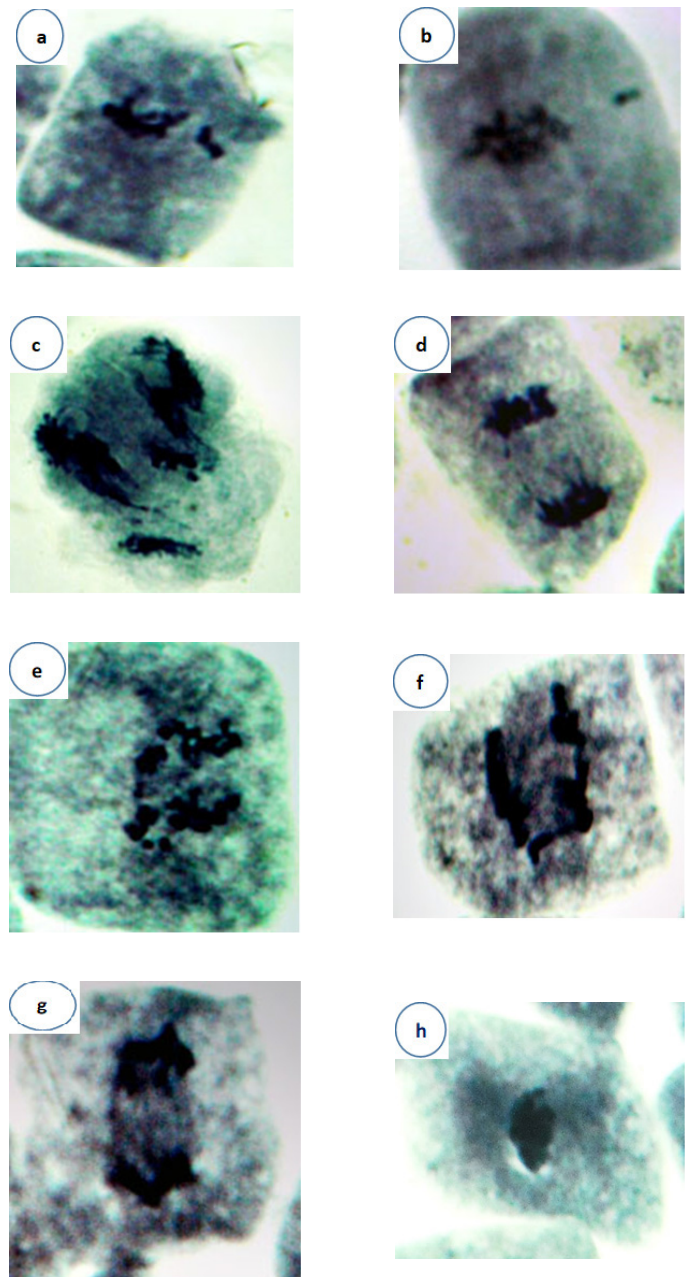


Figure 1: Chromosomal abnormalities in root tip cells of chickpea induced by different concentrations of Imazethapyr. (a) Scattered chromosome (10ppm), (b) Scattered chromosome (0.5 ppm), (c) Scattered chromosome (5 ppm), (d) Laggard chromosome (1 ppm), (e) Laggard chromosome (5 ppm), (f) Chromosome bridge (2.5 ppm), (g) Chromosome bridge (10 ppm), and (h) Sticky chromosome (0.5 ppm).

IM was found to be effective for bridging, particularly at high doses, while control and 1 ppm treatment showed no chromosome bridging. Some chemicals such as Imazethapyr on *V. faba* [1], atrazine on *A. cepa* [27], and IM on wheat [18] have induced chromosome bridges. Furthermore, different effects of IM on *A. cepa* at variable times of exposure to herbicide have been reported [24]. The presence of chromosomal bridges may be attributable to the linkage or the formation of dicentric chromosomes, caused by disruption and assembly [25].

Table 2: Effect of Imazethapyr on total protein content in chickpea.

Conc.(ppm)	Days	Shoot-root axis				Cotyledon			
		4 th	8 th	12 th	15 th	4 th	8 th	12 th	15 th
		Total protein (mg/g fresh weight)							
Control		26.28 ± 0.18 ^b	28.15 ± 0.31 ^b	29.22 ± 0.88 ^b	49.27 ± 1.20 ^a	81.25 ± 1.20 ^f	66.51 ± 0.87 ^f	42.2 ± 0.76 ^f	30.61 ± 0.88 ^f
0.5		25.51 ± 0.92 ^a	33.95 ± 1.20 ^a	34.40 ± 2.92 ^a	47.50 ± 0.65 ^b	83.36 ± 0.99 ^e	82.91 ± 0.31 ^e	74.52 ± 0.52 ^e	56.16 ± 0.54 ^e
1.0		26.31 ± 0.28 ^a	27.61 ± 0.98 ^e	29.61 ± 0.33 ^b	42.10 ± 1.10 ^e	85.25 ± 1.10 ^d	83.53 ± 0.77 ^d	76.34 ± 0.25 ^d	58.83 ± 1.40 ^d
2.5		24.43 ± 0.52 ^c	26.32 ± 1.30 ^d	27.92 ± 0.45 ^b	30.60 ± 0.75 ^d	93.88 ± 2.12 ^c	91.12 ± 0.36 ^c	85.61 ± 0.95 ^c	81.28 ± 0.95 ^c
5.0		22.25 ± 0.61 ^d	25.42 ± 0.63 ^e	25.50 ± 1.03 ^c	26.20 ± 1.20 ^e	96.71 ± 1.35 ^b	95.44 ± 0.54 ^b	89.88 ± 1.21 ^b	88.58 ± 1.12 ^b
10.0		20.34 ± 0.22 ^c	21.11 ± 0.55 ^f	22.72 ± 0.81 ^d	23.60 ± 0.45 ^f	101.05 ± 3.21 ^a	100.14 ± 2.98 ^a	99.11 ± 1.01 ^a	96.41 ± 1.11 ^a

Mean ± SD followed by the same superscript are not statistically significant between the concentrations, when subjected to SPSS package, according to Tukey's mean range test at 5% level.

Lagging chromosome in affected plants has been reported including *V. faba* treated with Imazethapyr [1], and atrazine [24]. Wheat and *A. cepa* treated with IM revealed that the early movement of chromosomes and laggards may be due to a failure in the normal organization of the spindle apparatus [18,25].

The frequency of occurrence of scattered chromosomes increased from 0.10% in control to 5.35% in 10 ppm herbicide concentration. Copper mine wastes induced some abnormalities, such as chromosome scattering in the root tip cells of *A. cepa* L. [28]. Also, such effects have been reported in wheat [18]. The mitotic activity decline is of common effects of most herbicides studied.

There was a significant reduction in a total protein of the root-shoot axis, when seeds were exposed to a dosage of 0.5–10 ppm of IM. On 4th and 15th days, the percentage of the total protein decreased from 23% to 3% and 52% to 4%, respectively, when compared to the control. In cotyledons and on those days and at the same dosage (0.5–10 ppm of IM), the percentage of the total protein increased from 3% to 24% and 83% to 215%, respectively, when compared to the control (Table 2). Protein metabolism is involved in the breaking of seed dormancy because seed storage proteins play a fundamental role in reducing nitrogen, carbon, and amino acids in growing tissues [29]. The slow degradation of the protein in the treated cotyledons compared to the control can be because of an inhibition of the proteolytic activity of such enzymes [30]; while the significant reduction in the protein content of the treated shoot-root axis compared to the control indicates that this may be due to the inhibition of dipeptidase activity [31]. In the treated seedlings, a decrease in the rate of protein synthesis along with an increase in the rate of protein degradation was detected, which may be responsible for the observed rapid protein loss. These results are consistent with those of Gaston, Zabalza et al. [32] who demonstrated that IM could interfere with protein metabolism during the germination and the early growth. Zabalza et al. [12] observed that the soluble protein content of the plants treated with IM did not decrease, and that the synthesis of fresh proteins was inhibited in the treated plants, which showed that there would be protein synthesis but from amino acids recovered mainly by protein turnover. The reduction in plant protein content may be due to a higher rate of protein denaturation and the degradation of the existing protein to amino acid, or a reduced afresh protein synthesis, which is also corroborated by the findings of Souhai et al. [33].

4. CONCLUSION

In general, it can be concluded that IM has damaging effects on the root tip cells of chickpea. In addition, certain irreversible cytogenetic effects increased in plants treated by herbicides. In order to run a better weed control, the accomplishment of further tests on selecting the best possible herbicides, having environmentally friendly properties, as well as their best method of application, is suggested as a necessary experimental further step to be taken to avoid any more adverse impacts on the crop health.

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