PHYSIOLOGICAL VARIATIONS IN HERBICIDE TOLERANCE AMONG CHICKPEA (*Cicer arietinum* L.) GENOTYPES

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

INTEGRATED MASTER OF SCIENCE (HONS.)
in
BOTANY
(Minor Subject: Biochemistry)

By

Kawaljit Kaur
(L-2011-BS-27-IM)

Department of Botany
College of Basic Sciences and Humanities

LUDHIANA-141004

2016

CERTIFICATE-I

This is to certify that the thesis entitled, **"Physiological variations in herbicide tolerance among chickpea (*Cicer arietinum* L.) genotypes"** submitted for the degree of **Integrated Master of Science (Hons.)**, in the subject of **Botany** (Minor subject: **Biochemistry**) of Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Kawaljit Kaur (L-2011-BS-27-IM)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

_____

Major advisor
Dr. Jagmeet Kaur
Senior Botanist
Department of Botany
Punjab Agricultural University
Ludhiana- 141004

CERTIFICATE-II

This is to certify that the thesis entitled, **"Physiological variations in herbicide tolerance among chickpea (*Cicer arietinum* L.) genotypes"** submitted by **Kawaljit Kaur (L-2011-BS-27-IM)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Integrated Master of Science (Hons.)**, in the subject of **Botany** (Minor subject: **Biochemistry**) has been approved by the Student's Advisory Committee along with Head of the Department after an oral examination of the same.

_____
(Dr. Jagmeet Kaur)
Major Advisor

_____
Dr. Sunita Sheokand
External Examiner
Professor Plant Physiology
Department of Botany and Plant Physiology
CCS Haryana Agricultural University
Hisar- 125004

_____
(Dr. Seema Bedi)
Head of the Department

_____
(Dr. Neelam Grewal)
Dean Post Graduate Studies

ACKNOWLEDGEMENT

All the unfathomable analogies humble thanks giving are preferred to Almightily WAHEGURU, the most gracious and compassionate, whose blessing and exaltation flourished my thoughts and thrived my ambitions to eventually shape up the cherished fruit of my modest endeavors to this manuscript.

I deem it an utmost pleasure to be able to express the heartiest gratitude and deep sense of indebtness to my esteemed Major Advisor ***Dr. Jagmeet Kaur****, Senior Botanist, Pulses section, Plant Breeding and Genetics PAU, Ludhiana for her unfailing patience, skillful, affectionate and generous guidance. Acknowledgement perhaps is too narrow in scope to express my reverence for her indelible help to elucidate my problems, unreserved enthusiasm and providing me the necessary research facilities for this study.*

My sincere thanks is reserved for ***Dr. Seema Bedi****, Head, Department of Botany for providing me with the necessary facilities. I feel elated to acknowledge the cooperation extended by esteemed members of advisory committee,* ***Dr. Nirmaljit Kaur****, Senior Botanist, Department of Botany,* ***Dr. Sarvjeet Singh****, Senior Plant breeder, Department of Plant breeding and genetics,* ***Dr. Satvir Kaur****, Assistant Biochemist, Department of Biochemistry,* ***Dr. Seema Bedi,*** *Professor (Dean PG's Nominee) Department of Botany, for their invaluable insights and suggestion during the tenure of my work.*

I would like to thank all the special people who have taught me starting with my school teachers, my undergraduate teachers, my graduate teachers and examiners. It was my pleasure to work with my seniors Sukhpreet Kaur and Priya, they have been an invaluable support day in, day out during these years. Words are not enough to express my thanks to my abiding friends Heena Rajpal, Chahat Sama, Prabhjot Kaur,Vaishali Sharma, Gagandeep Deol, Gaganjit Kaur, Dimple Mandla, Manjeet Kaur, Harman Mehta and Gurjeet Kaur for their valuable support.

A Father is someone who wants to catch you before you fall, but instead picks you up, brushes you off, and lets you try again. Special thanks to my Dad for ingraining in me the ways of logic which have become an intricate part of who I am and who I will be. It's a long journey, and he has always stood beside me giving all the support and encouragement. I owe a lot to my mother, who helped me at every stage of my personal and academic life, and longed to see this achievement come true. Special thanks to my younger sister Anikdeep Kaur for her unflagging love and care throughout my life.

Help rendered to me by the field staff of Department of Plant Breeding and Genetics is worth appreciating. I am grateful to Raghuveer uncle for their timely help and cooperation.

All may not have been mentioned but none is forgotten.

(Kawaljit Kaur)

Title of the Thesis	**:**	Physiological variations in herbicide tolerance among Chickpea (*Cicer arietinum* L.) genotypes
Name of the student and Admission No.	**:**	Kawaljit Kaur (L-2011-BS-27-IM)
Major Subject	**:**	Botany
Minor Subject	**:**	Biochemistry
Name and Designation of Major Advisor	**:**	Dr. Jagmeet Kaur Senior Botanist
Degree to be Awarded	**:**	Integrated M.Sc. (Hons.)
Year of award of Degree	**:**	2016
Total Pages in Thesis	**:**	62 + Vita
Name of University	**:**	Punjab Agricultural University, Ludhiana – 141004, Punjab, India.

ABSTRACT

The present investigation was carried out in field area of the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana during *rabi* season 2015-16 to evaluate the variations in herbicide tolerance in chickpea genotypes. A field experiment was conducted in factorial randomized block design using twenty four genotypes and three treatments (unsprayed, imazethapyr and carfentrazone-ethyl). According to our studies, both herbicides caused change in phenological development of plants. The early flowering and maturity was found in herbicide tolerant genotypes. Significant variations were recorded in physiological parameters (leghaemoglobin content, leaf area index, photosynthetic efficiency, hill reaction activity, cellular respiration and malondialdehyde content) during reproductive phase displaying that herbicides affect physiological processes in the plant. There was an upregulation in malondialdehyde content in sensitive genotypes. A negative effect of herbicidal treatments on yield attributes (yield/plant, pods/plant and 100-seed weight) was observed. From physiological parameters and yield attributes, genotypes were assorted as tolerant and sensitive from each treatment. Six genotypes (three sensitive GL 12021, GLW 125, GL 27023 and three tolerant GLW 11, GL 10047, PDG 4) following carfentrazone-ethyl treatment and (three sensitive JG 1362, GL 28203, GL 12021 and three tolerant GL 11026, GLW 44, PBG 5) following imazethapyr treatment were further evaluated for biochemical evaluation. It was shown that during reproductive phase, total soluble sugars, total proteins, total free amino acids and proline content in leaves were elevated in tolerant genotypes of each treatment. Anti-oxidative defence system was studied from leaves and it was found to be hiked in tolerant genotypes. This work emphasizes that genpotypes GLW 11, GL 10047, PDG 4 and GL 11026, GLW 44, PBG 5 are apt for the post-emergence herbicide tolerance.

Keywords: Chickpea, Imazethapyr, Carfentrazone-ethyl, Genotypes, Growth, Physiological variations, biochemical estimations

_____ _____

Signature of Major Advisor **Signature of the student**

ਖੋਜ ਪ੍ਰਬੰਧ ਦਾ ਸਿਰਲੇਖ	:	ਛੋਲਿਆਂ ਦੀਆਂ ਕਿਸਮਾਂ ਵਿਚ ਨਦੀਨਨਾਸ਼ਕ ਸਹਿਣਸ਼ੀਲਤਾ ਲਈ ਫਿਜਾਲਜੀ ਵਿਭਿੰਨਤਾ
ਵਿਦਿਆਰਥੀ ਦਾ ਨਾਂ ਅਤੇ ਦਾਖ਼ਲਾ ਨੰਬਰ	:	ਕਵਲਜੀਤ ਕੌਰ (ਐੱਲ-2011-ਬੀ.ਐੱਸ.-27-ਆਈ.ਐੱਮ)
ਮੁੱਖ ਵਿਸ਼ਾ	:	ਪੌਦਾ ਵਿਗਿਆਨ
ਨਿਮਨ ਵਿਸ਼ਾ	:	ਜੀਵ-ਰਸਾਇਣ
ਮੁੱਖ ਸਲਾਹਕਾਰ ਦਾ ਨਾਮ ਅਤੇ ਅਹੁਦਾ	:	ਡਾ. ਜਗਮੀਤ ਕੌਰ ਸੀਨੀਅਰ ਬੋਟਨਿਸਟ
ਮਿਲਣ ਵਾਲੀ ਡਿਗਰੀ	:	ਇੰਟੀਗ੍ਰੇਟਿਡ ਮਾਸਟਰ ਆਫ਼ ਸਾਇੰਸ (ਆਨਰਜ਼)
ਡਿਗਰੀ ਮਿਲਣ ਦਾ ਸਾਲ	:	2016
ਖੋਜ ਪ੍ਰਬੰਧ ਦੇ ਕੁੱਲ ਪੰਨੇ	:	62 + ਵੀਟਾ
ਯੂਨੀਵਰਸਿਟੀ ਦਾ ਨਾਮ	:	ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ - 141004 ਪੰਜਾਬ, ਭਾਰਤ ।

ਸਾਰ

ਮੌਜੂਦਾ ਖੋਜ ਤਜ਼ਰਬਾ ਛੋਲਿਆਂ ਦੀਆਂ ਕਿਸਮਾਂ ਵਿੱਚ ਨਦੀਨਨਾਸ਼ਕ ਸਹਿਣਸ਼ੀਲਤਾ ਵਿਭਿੰਨਤਾ ਨੂੰ ਜਾਨਣ ਲਈ ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ ਦੇ ਪਲਾਂਟ ਬਰੀਡਿੰਗ ਅਤੇ ਜੈਨੇਟਿਕਸ ਵਿਭਾਗ ਦੇ ਤਜ਼ਰਬਾ ਖੇਤਰ ਉੱਪਰ ਹਾੜ੍ਹੀ 2015-16 ਦੌਰਾਨ ਕੀਤਾ ਗਿਆ । ਤਜ਼ਰਬਾ 24 ਕਿਸਮਾਂ ਅਤੇ ਤਿੰਨ ਉਪਚਾਰਾਂ ਨੂੰ ਵਰਤ ਕੇ ਫੇਕਟੋਰੀਅਲ ਰੈਂਡੋਮਾਇਜਡ ਬਲਾਕ ਡਿਜ਼ਾਇਨ ਵਿੱਚ ਕੀਤਾ ਗਿਆ। ਸਾਡੀ ਖੋਜ ਮੁਤਾਬਕ ਦੋਵੇਂ ਨਦੀਨਨਾਸ਼ਕ ਪੌਦਿਆਂ ਦੇ ਫੀਨੋਲੋਜੀ ਵਿਕਾਸ ਵਿਚ ਬਦਲਾਅ ਲਿਆਉਂਦੇ ਹਨ। ਨਦੀਨਨਾਸ਼ਕ ਸਹਿਣਸ਼ੀਲ ਕਿਸਮਾਂ ਵਿਚ ਨਿਸਾਰ ਅਤੇ ਪਕਾਈ ਅਗੇਤੀ ਪਾਈ ਗਈ। ਫ਼ਸਲ ਦੀ ਸੋਭ ਦੀ ਅਵਸਥਾ ਉੱਪਰ ਫਿਜਾਲਜੀ ਮਾਪਦੰਡ (ਲੈਗਹੀਮੋਗਲੋਬਿਨ ਮਾਤਰਾ, ਪੱਤਾ ਤਰਜਕੀ ਖੇਤਰ, ਪ੍ਰਕਾਸ਼ਸੰਸ਼ਲੇਸ਼ਣ, ਕੁਸ਼ਲਤਾ, ਹਿਲ ਰਿਏਕਸ਼ਨ ਐਕਟੀਵਿਟੀ, ਸੈਲੂਲਰ ਰੈਸਪੀਰੇਸ਼ਨ) ਵਿਚ ਅਰਥਪੂਰਨ ਵਿਭਿੰਨਤਾ ਦਾ ਪਾਇਆ ਜਾਣਾ ਸਾਬਿਤ ਕਰਦਾ ਹੈ ਕਿ ਨਦੀਨਨਾਸ਼ਕ ਪੌਦੇ ਦੇ ਫਿਜਾਲਜੀ ਕਿਰਿਆ ਨੂੰ ਪ੍ਰਭਾਵਿਤ ਕਰਦੇ ਹਨ । ਸੰਵੇਦਨਸ਼ੀਲ ਕਿਸਮ ਵਿਚ ਮੈਲੇਨੈਲਡੀਹਾਈਡ ਦਾ ਵਾਧਾ ਸੀ । ਨਦੀਨਨਾਸ਼ਕ ਉਪਚਾਰ ਦਾ ਝਾੜ ਦੇ ਮਾਪਦੰਡਾਂ ਉੱਪਰ ਉਲਟ ਅਸਰ ਪਿਆ। ਹਰ ਇਕ ਉਪਚਾਰ ਵਿਚੋਂ ਫਿਜਾਲਜੀ ਮਾਪਦੰਡ ਅਤੇ ਝਾੜ ਦੇ ਮਾਪਦੰਡਾਂ ਦੇ ਆਧਾਰ ਤੇ ਜੀਨੋਟਾਇਪ ਨੂੰ ਸਹਿਣਸ਼ੀਲ ਜਾਂ ਨਾਜੁਕ ਦਾ ਦਰਜਾ ਦਿੱਤਾ ਗਿਆ। ਕਾਰਫੈਂਟਰਾਜੋਨ ਈਥਾਇਲ ਉਪਚਾਰਿਤ ਛੇ ਜੀਨੋਟਾਇਪ (ਤਿੰਨ ਸ਼ਹਿਣਸ਼ੀਲ, ਤਿੰਨ ਨਾਜੁਕ), ਇਮੇਜ਼ਥਾਇਪਰ ਉਪਚਾਰਿਤ ਛੇ (ਤਿੰਨ ਸ਼ਹਿਣਸ਼ੀਲ ਅਤੇ ਤਿੰਨ ਨਾਜੁਕ) ਕਿਸਮ ਨੂੰ ਅਗਾਂਹ ਜੀਵ ਰਸਾਇਣਿਕ ਮੁਲਾਂਕਣ ਲਈ ਚੁਣਿਆ ਗਿਆ। ਪੱਤਿਆਂ ਵਿਚ ਕੁਲ ਸ਼ੂਗਰ, ਕੁਲ ਪ੍ਰੋਟੀਨ, ਕੁਲ ਫਰੀ ਅਮੀਨੋ ਏਸਿਡ ਅਤੇ ਪਰੋਲੀਨ ਦੀ ਮਾਤਰਾ ਦਾ ਪੱਧਰ ਵੱਧ ਪਾਇਆ ਗਿਆ । ਪੱਤਿਆਂ ਵਿਚ ਐਂਟੀਆਕਸੀਡੈਂਟ ਰੱਖਿਆ ਪ੍ਰਣਾਲੀ ਨੂੰ ਘੋਖਿਆ ਗਿਆ ਅਤੇ ਪਾਇਆ ਗਿਆ ਕਿ ਸਹਿਣਸ਼ੀਲ ਜੀਨੋਟਾਇਪ ਵਿਚ ਇਹ ਵੱਧ ਸੀ। ਇਹ ਦਰਸਾਉਂਦਾ ਹੈ ਕਿ ਜੀਨੋਟਾਇਪ GLW 11, GL 10047, PDG 4, GL 11026, GLW 44 ਅਤੇ PBG 5 ਉੱਗਣ ਤੋਂ ਬਾਅਦ ਨਦੀਨਨਾਸ਼ਕ ਦੇ ਸਹਿਣਸ਼ੀਲਤਾ ਲਈ ਚੁੱਕਵਾਂ ਸੀ।

ਮੁੱਖ ਸ਼ਬਦ: ਛੋਲੇ, ਕਾਰਫੈਂਟਰਾਜੋਨ ਈਥਾਇਲ, ਕਿਸਮ, ਇਮੇਜ਼ਥਾਇਪਰ, ਵਿਕਾਸ, ਫਿਜਾਲਜ਼ੀ ।

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

CONTENTS

CHAPTER	TITLE	PAGE NO.
I	INTRODUCTION	1-3
II	REVIEW OF LITERATURE	4-13
III	MATERIALS AND METHODS	14-22
IV	RESULTS AND DISCUSSION	23-47
V	SUMMARY	48-50
	REFERENCES	51-62
	VITA	

LIST OF TABLES

Table No.	Title	Page No.
3.1	List of chickpea genotypes	16
3.2	List of selected chickpea genotypes used in the study	18
4.1	Phenophasic development of chickpea genotypes in response to herbicides	24
4.2	Visual scoring of vigour in response of various herbicides after 7 Days after spray	26
4.3	Variation in shoot and root length (cm) under herbicidal treatments in chickpea genotypes	27
4.4	Variation in root and shoot dry weight (g/plant) after herbicide application	29
4.5	Number of leaves, senesced leaves and leaves dry weight (g/plant) under herbicide treatments	30
4.6	Variation in dry weight of nodules (g/plant) and leghaemoglobin content (mg/g) under herbicide application	32
4.7	Hill reaction activity (mg-1 chl h-1), cellular respiration (%) and malondialdehyde content (μ moles g^{-1} dw) in leaves of chickpea genotypes at flowering stage	35
4.8	Yield per plant (g), 100-seed weight and pods per plant of chickpea genotypes	38

LIST OF FIGURES

Figure No.	Title	Page No.
4.1	Photosynthetic rate (µmole CO_2 m^{-2} s^{-1}) and Leaf area index (cm^2) of chickpea genotypes under various treatments.	33
4.2	Total soluble sugars accumulated in leaves of chickpea genotypes after the application of carfentrazone-ethyl	39
4.3	Total soluble sugars accumulated in chickpea genotypes after the application of imazethapyr	39
4.4	Changes in total soluble protein of chickpea genotypes after carfentrazone-ethyl application	40
4.5	Changes in total soluble protein of chickpea genotypes after imazethapyr treatment	41
4.6	Proline content in chickpea genotypes after application of carentrazone-ethyl	41
4.7	Proline content in chickpea genotypes after imazethapyr treatment	42
4.8	Total free amino acids in chickpea genotypes following carentrazone-ethyl application	43
4.9	Total free amino acids in chickpea genotypes following imazethapyr application	43
4.10	Superoxide dismutase activity in leaves of chickpea genotypes in response to carfentrazone-ethyl treatment	44
4.11	Superoxide dismutase activity after application of imazethapyr	44
4.12	Catalase activity in leaves of chickpea genotypes after carfentrazone-ethyl treatment	45
4.13	Catalase activity in leaves of chickpea genotypes after imazethapyr treatment	45
4.14	Peroxidase activity in leaves of chickpea genotypes under carfentrazone-ethyl treatment	46
4.15	Peroxidase activity in leaves of chickpea genotypes after imazethapyr treatment	46

Chickpea crop

CHAPTER I

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a member of the Fabaceae family and contributes more than 20% to world pulse crop production (Dana *et al* 2000). Chickpea is an annual cool-season crop, the plant that ranges in height from one to three feet. It has an indeterminate and branched growth habit, erect or spreading, with hairy leaves, stems, and seed pods that secrete highly acidic exudates. It typically has a bluish green or dark green color, but some types are olive in appearance. Chickpea is a self-pollinated crop with flowers that are borne singly at the tip of axillary branches and that vary in color from white to purple to faded blue. Its cultivation is mainly concentrated in semi arid environments. Chickpea is the second most produced pulse worldwide after dry beans (Garg *et al* 2016). It is cultivated in more than 51 countries in the world on area over 13.5 Mha with an annual global yield of about 967.6 kg/ha that makes more than 20% of world pulse production (FAOSTAT 2015). India is the largest producer of chickpea in the world, accounting for 67.02% of world's population (Ghosh *et al* 2015). The other major chickpea growing countries include Turkey, Pakistan, Australia, Ethiopia, Iran, Mexico, Canada and USA. This is basically a *rabi* crop, sown in months of September-November and harvested in the months of February-April. Maturity period ranges from 95-140 days after sowing.

Chickpea has the highest nutritional composition and is free from anti-nutritive components compared to any other dry edible grain legumes (Mallikarjuna *et al* 2007). It is a rich source of protein and carbohydrates and its protein quality is better than other legumes such as pigeon pea, black gram and green gram (Papalamprou *et al* 2009). It is also rich in fibre and minerals (phosphorus, calcium, magnesium, iron and zinc) and carotenoids (Abbo *et al* 2005). Its lipid fraction is high in unsaturated fatty acids (Williams and Singh 1987).

The presence of weeds is one of the major constraints for low seed yield of chickpea. Due to its initial slow growth and wide row spacing which provides ample scope for weed infestation, it often suffers severe weed competition. Therefore, control of weeds during critical period of crop weed competition is very important to avoid severe yield losses. Weeds compete with crop mainly for space, solar radiation, nutrients, water and carbon dioxide. Through competition, weeds damage crop and cause reduction in yield of the crop. Control of weeds is a basic requirement and major component of management in most production systems (Young *et al* 1994). Broadleaf control options in pulse crops such as chickpeas (*Cicer arietinum* L.) are very limited. Weed management has been practiced through manual labor, which is time consuming; therefore, chemical weed control was stimulated. The choice of best herbicide, the proper time of application and proper dose is an important consideration for lucrative returns (Fayed *et al* 1998). The advent of herbicides has been hailed as one of the

most important advances in agriculture (Pike 1991). In general, compared with other pulses, chickpea develops relatively slowly when plants are young and it has an open canopy architecture and low stature (Knights 1991). There were significant yield losses reported in chickpea (up to 84%) due to weeds, more severely (up to 98%) in autumn-sown chickpea. Therefore, Weed control with herbicides would be advantageous for optimizing input efficiency in a particular crop, by reducing the population of weeds. Certain families of herbicides may inhibit the process of photosynthesis. Others may inhibit the synthesis of chlorophyll or amino acids vital to plant's growth, still other groups may cause leaks to plant cells resulting in plant kill.

Imazethapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid) is an imidazolinone compound used as a selective herbicide to control most annual grasses and certain broad-leaf weeds. This herbicide is applied as pre-emergence and early post-emergence for control of annual and perennial grass and broad-leaf weeds in chickpea and other legume fields. It inhibits the activity of Acetolactate synthase (ALS), which is involved in the synthesis of the branched-chain amino acids (leucine, isoleucine, and valine). ALS herbicides are readily absorbed by both roots and foliage and translocated in both xylem and phloem to the site of action at growing points (Peterson *et al* 2001). Chemical control through application of ALS-inhibiting herbicide remains the method of choice for control of *E. heterophylla* in soybeans.

Carfentrazone-ethyl (α,2-dichloro-5-[4-(difluoromethyl)- 4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoic acid, ethyl ester) is a phenyl triazolinone herbicide that inhibits protoporphyrinogen oxidase (protox inhibitor)-PPO in the chlorophyll biosynthesis pathway causing lipid peroxidation and membrane disruption and ultimately kills sensitive weeds (Dayan *et al* 1997). Carfentrazone-ethyl is a new active ingredient in the aryl triazoline class of chemistry and was discovered in Food Machinery Corporation (FMC) laboratories. Carfentrazone-ethyl is sometimes referred to as a *PPO inhibitor*.The herbicidal action on susceptible plants is rapid, depending on environmental conditions, with initial symptoms appearing as quickly as one day after treatment and plant mortality generally occurring within seven days of application. Carfentrazone-ethyl is registered by the U.S. Environmental Protection Agency as a post-emergent herbicide in wheat, barley, rye, oats, corn, soybean, rice, and for use in turf and ornamental sites. Carfentrazone-ethyl is a post-emergence, broadleaf herbicide for use in winter cereals. It is a rapid knockdown herbicide with activity observed 1 to 4 days after treatment. The product degrades quickly in the soil and does not provide any residual activity against weeds. Carfentrazone-ethyl belongs to the aryl triazolinone group and is included in group G for the herbicide mode of action. (Nechet *et al* 2008).

According to Willoughby *et al* (1996), use of herbicides must provide adequate weed

control without adversely affecting seedling emergence and survival. Tolerance of seedling to herbicides is dependent on the herbicide dose, cultivar and environmental conditions (VanGessel *et al* 2000). Once in the phloem and translocated to the site of action, the Imidazolinone inhibit ALS, causing death of meristematic cells resulting in plant death (Little and Shaner 1991). As photosynthesis did not decline in the short term, it is reasonable to assume that the treated plants maintained sufficient photosynthetic carbon assimilation rates to accumulate carbohydrates in both sources and in sinks (Zabalza *et al* 2004). The herbicide-induced stress may be of short-term or long-lasting. Herbicides may affect plant's physiology by inhibiting photosynthesis or associated biochemical processes (Miyazawa and Yahata 2006). Better growth attributes caused more accumulation and translocation of photosynthates by the crop, which resulted in more number and dry weight of nodules (Choudhary *et al* 2012).

Information regarding the Imazethapyr and Carfentrazone-ethyl herbicides' tolerance to chickpea is limited and physiology and biochemistry behind the tolerance of these herbicides in chickpea is not clear. So attempt is made to understand the physiological variations in herbicide tolerance in chickpea. Therefore, keeping in view of the above facts the present investigation was undertaken with the following objectives:

1. Screening of chickpea genotypes for herbicide tolerance under field conditions.
2. To investigate morpho-physiological and biochemical traits associated with herbicide tolerance

CHAPTER II

REVIEW OF LITERATURE

Chickpea (*Cicer arietinum* L.) is a highly valued commodity being an important source of proteins for human consumption in several developing countries (Zaccardelli *et al* 2013). Weeds are a constant problem in chickpea from years. Weeds not only compete with crops for water, nutrients, sunlight, and space but also harbor insect and disease pests; clog irrigation and drainage systems; undermine crop quality; and deposit weed seeds into crop harvests. If left uncontrolled, weeds can reduce crop yields significantly. Farmers can fight weeds with tillage, hand weeding, herbicides, or typically a combination of all techniques. Unfortunately, tillage leaves valuable topsoil exposed to wind and water erosion, a serious long-term consequence for the environment. For this reason, more and more farmers prefer reduced or no-till methods of farming. Similarly, many have argued that the excessive use of herbicides has led to groundwater contaminations, the death of several wildlife species and has also been attributed to various human and animal illnesses. Herbicides are chemical compounds used to kill or inhibit growth of undesirable plants (Quantick 1985). A successful herbicide should be safe to the crop with reliable performance and must not be influenced by soil or any environmental factors (Khair Eldin *et al* 1980). Compared with alternative means of weed control, such as mechanically weeding by hand or machine, herbicides are less expensive, often safer (especially in forestry), faster, and sometimes more selective.

However, if herbicides are not used properly, damage may be caused to crop plants, especially if high dose is used, or if spraying occurs during a time when the crop species is sensitive to the herbicide. Unintended but economically important damage to crop plants is sometimes a consequence of the inappropriate use of herbicides. The use of synthetic herbicides for weed control is a common practice in modern agricultural systems. However, herbicide effects can extend beyond the intended target species and influence non-target organisms. The effects of herbicides were known by the type and rates of its application, health and stage of plant growth, and other environmental variables. Kumar *et al* (2010) reported that metsulfuron methyl alone or in combination of other herbicides 2, 4-D and carfentrazone-ethyl gave effective control of broad leaf weeds and established the fact that morphological changes and disturbances in cell division due to impact of both the herbicides and their combinations.

Herbicide-tolerant (HT) crops offer farmers a vital tool in fighting weeds and are compatible with no-till methods, which help preserve topsoil. They give farmers the flexibility to apply herbicides only when needed, to control total input of herbicides and to use herbicides with preferred environmental characteristics. Herbicides affect the vegetative growth of plant in many ways. Herbicides also affects plant in terms of physiological and

biochemical processes. Generally herbicides are no toxic to plants if applied below label rate but when applied in higher doses, it becomes lethal to plants. However, some plants tolerate these herbicides on natural level. The growth stages and developmental stages are affected with the application of herbicides.

Imidazolinones (IMIs) have been widely used for weed control because of the limited soil persistence, favourable toxicological properties, and broad spectrum of weed control (Hanson *et al* 2007). Greater uptake of imidazolinone herbicides by plants under conditions of increased and excessive soil moisture, particularly, has been observed (Malefyt and Quakenbush l99l). Toker *et al* (2012) experimented with induced mutation to develop IMI resistant chickpea. The study was deemed successful when mutant *C. reticulatum* Ladiz had no IMI herbicide injury compared to the susceptible parents. Imazethapyr may cause less injury to lentil when applied below the label rate but lower doses are less effective in controlling weeds (Blackshaw and Esau 1991). Taran *et al* (2010) demonstrated that natural tolerance to imidazolinone herbicides exists in chickpea germplasm. The existence of tolerance to imidazolinone herbicides was recorded in maize, wheat, rice, oilseed rape, sunflower (Siyuan *et al* 2005) and field pea (Hanson and Thill 2001).

The mode of inheritance of tolerance to Acetolactate synthase (ALS) inhibiting herbicides has been reported to be relatively simple with a single, dominant nuclear-gene in some species (Van Eerd *et al* 2004) or a single, partially dominant gene in others (Kolkman *et al* 2004). Tiwari *et al* (2007) observed that imazethapyr at 75 g/ha controlled only broad leaved weeds in soybean. Application of imazethapyr at 0.10 kg/ha controlled the weeds most effectively than other herbicides in summer irrigated black gram (Mishra and Chandrabhanu 2006).

The growth attributes are also affected by herbicides. Most of the physiological processes *viz.,* cellular respiration, hill reaction, leghaemoglobin content in nodules, photosynthetic efficiency, lipid peroxidation, leaf area index have been influenced by herbicides. The literature relevant is presented under the following headings:

2.1 Impact of herbicide stress on growth attributes

2.1.1 Phenological Changes

Plants display a variety of morphological symptoms in relation to herbicide applications. The applied herbicides are known to accumulate in meristematic regions of a plant, consequently affecting growth within a few days and causing chlorosis and discoloration of young tissues within 1-3 weeks of application in susceptible species (Cobb 1992). Renner and Powell (1992) reported that imazethapyr applied POST caused 20% injury in pinto bean. Sanders *et al* (1998) reported that sequential applications of imazethapyr at 70 g/ha resulted in 30% injury in rice plants. However, other studies conducted in New Mexico and Alberta have shown that imazethapyr applied POST at 50 and 70 g ai ha^{-1} causes minimal

injury in pinto bean (Arnold *et al* 1993).

Masson and Webster (2001) reported that injury in rice plants was less than 16% when imazethapyr at 70 g/ha was applied to two- to three-leaf drill-seeded rice or water-seeded rice at pegging stage (green leaf tissue emerged from the seed and the root has extended downward into the soil). Lyon and Wilson (2005) evaluated chickpea crop for damage with pre-emergence application of imazethapyr. The reduction in plant height, chlorosis and delayed maturity was recorded when application rates were 0.053 kg a.i/ha. However, a lower rate @ 0.026 kg a.i/ha applied in combination with sulfentrazone, reduced injury symptoms to a commercially acceptable level.

The results of visual wheat injury at 15 days after herbicide applications (DAHA) showed that 2, 4-D plus carfentrazone-ethyl caused higher damage compared with other treatments. Almost similar pattern was observed at 30 DAHA in case of both weed damage and crop injury. It was found that increase in 2, 4-D plus carfentrazone-ethyl dose, the herbicide ability to damage weeds increased but this was at the expense of higher wheat injury (Baghestania *et al* 2007). Lyon *et al (*2007) reported that all herbicide treatments containing carfentrazone-ethyl caused localized leaf necrosis in proso millet. Gaur *et al* (2013) reported that the leaves which shows less burning were considered as tolerant (score 2) and with more burning or complete burning (score 5) were considered as sensitive respectively. Imazethapyr mainly kills the growing tips (apical meristem and young leaves) of the branches. Other abnormalities include elongation of branches (similar to tendrils) with very small or needle shaped leaves, delaying of flowering, deformation of flowers, poor pod setting and seed size. Secondary growth was also observed 20–25 days after herbicidal application leading to flowering and pod set. At 2 and 4 weeks after crop emergence (WAE), a significant damage was observed in dry bean with sulfentrazone alone or in combination with imazethapyr. At 2 WAE, sulfentrazone alone caused less injury than sulfentrazone + imazethapyr in black, cranberry, kidney, and white bean (Soltani *et al* 2014).

2.1.2 Growth Changes

An extreme higher concentration of 2,4-D or 2,4,5-T leads to the death of bean and sunflower plants or reduced growth in maize plants by occurrence of abnormalities in the nucleic acids and protein synthesis (Shaddad *et al* 1990). In soybean, with the imazethapyr application, fresh weight of shoots and roots increased, but dry weight decreased, indicating higher water concentrations in imazethapyr-treated plants (Scarponi *et al* 1996).

The absence of any adverse effect of imazethapyr on nodulation may also be attributed to the fact that imazethapyr has been reported to positively affect nodule initiation and not nodule development (Royuela *et al* 2000). Growth reduction of several plant species was reported after the application of triazine and triazinone herbicides (Dvorak and Remesova 2002). It has been reported that an increase in the concentration of IM significantly reduced

the growth of primary root meristems, fresh and dry weight, yield, and also the number of root nodules under field conditions (Gaston *et al* 2002).

The inhibition of cellular division by ALS inhibiting herbicides may cause a decrease in the root growth. An alteration of the intermediate metabolite pools or the ATP levels after the ALS inhibition could cause the inhibition of cellular division (Zabalza *et al* 2004) In earlier field studies conducted by several scientists, imazethapyr injured crops and injury increased with rate of application, sulfentrazone applied PRE at 420 g ha^{-1} did not have any effect on the shoot dry weight of black, brown, cranberry, kidney, otebo, pinto, white, and yellow eye beans but, at 840 g ha^{-1}, decreased shoot dry weight 30 to 40% (Hekmat *et al* 2007). Other PPO inhibitor herbicides such as saflufenacil applied PRE have been shown to reduce shoot dry weight 92 to 99% in adzuki, cranberry, lima, snap, and white bean (Soltani *et al* 2010). Nandan *et al* (2011) also reported that post-emergence application of imazethapyr at 25 g/ha at 15-20 DAS was safe to the blackgram. Post-emergence application of imazethapyr had no adverse effect on shoot as well as root dry weight/plant in Blackgram when recorded at flower-initiation stage.

Nirala *et al* (2012) and Ram *et al* (2013) determined that post-emergence application of imazethapyr can be used effectively in reducing the weed intensity and dry-matter production by weeds in soybean and blackgram. Ram *et al* (2013) concluded that higher concentrations of imazethapyr affects growth and yield of chickpea and lower concentrations were inefficient for effective weed control. Imazethapyr applied as post-emergence at 50 to 75 g/ha shows season-long control of many weeds without injuring soybean. Goud *et al* (2013) also determined that imazethapyr @ 75 g/ha had no adverse effect on nodule number and biomass relative to weedy check and hand-weeding in chickpea. Imazethapyr (IM) may be injurious to the non-target plant species i.e. main crop and its residue is known to persist in the soil affecting the succeeding crop. Post-emergence application of imazethapyr had no adverse effect on shoot as well as root dry weight/plant when recorded at flower-initiation stage (Aggarwal *et al* 2014).

2.1.3 Physiological attributes

Reactive oxygen species e.g. peroxides of polyunsaturated fatty acids generate malonialdehyde (MDA) on decomposition and in many cases MDA is the most abundant individual aldehydic lipid breakdown product (Esterbauer and Cheeseman 1990). Imidazolinone resistant soybean is evidence of rapid metabolic detoxification of IMI herbicide (Tecle *et al* 1993). The herbicide selectivity is based on the plants' ability and rate of metabolism. Hydrogen peroxide is a toxic compound produced as a result of scavenging of superoxide radical and its higher concentrations are injurious to plants, resulting in lipid peroxidation and membrane injury (Menconi *et al* 1995). Shim *et al* (2003) have shown that the chlorophyll concentration was less vulnerable to the herbicide treatment. The difference in

chlorophyll concentration at different doses showed a negative correlation of concentration with chlorophyll content. Degradation of Rubisco subunits may next decrease amounts of chlorophyll and the net photosynthetic rate (Mateos-Naranjo *et al* 2009). Khan *et al* (2004) suggested that herbicides when applied indiscriminately had variable effects on the legume production.

The variation in plant height among the various herbicidal treatments was not very conspicuous. There was an increase in leaf area index (LAI) with application of herbicides (Cheema and Akhtar (2005). They reported that herbicide application to wheat crop at 3-4 leaf stage have increased LAI by diverting competition among weeds and crop plants in favor of crop plants. Although IMI herbicides do not directly target photosynthesis, it has been suggested that treated plants may have a chlorophyll fluorescence response (Riethmuller-Haage *et al* 2006). MDA formation is considered as the general indicator of lipid peroxidation (Wang and Zhou 2006).

Post-emergence application of imazethapyr at 60 g/ha recorded significantly shortest plants, lowest LAI and dry matter production (Veeraputhiran and Chinnusamy 2008). Herbicides can prevent electron transfer in plant pigments that cause oxidative stress (Zhu *et al* 2009). Many herbicides are interfered with photosynthesis through blocking electron transport system and pigments in the chloroplast membranes are destroyed and plant face the loss of photosynthetic pigments (Velini *et al* 2010). At 30 DAS, the highest plant height was obtained in twice hand weeded treatment which was statistically at par with metribuzin @ *500* g ha and imazethapyr @150 g ha^{-1} treatment. And at 60 DAS, the highest plant height was obtained in hand weeded plot which was statistically at par with imazethapyr at 150 g ha^{-1} treatments. The treatment twice hand weeding at 15 DAS and 30 DAS registered higher LAI at all the crop growth stages and was considered to be the best treatment followed by imazethapyr at@ 150g a.i. ha^{-1} (Basu and Sengupta 2012). Plant height and leaf area index were reduced by treatment with Imazethapyr compared to the hand-hoeing treatment (Aboali and Saeedipour 2015).

2.1.4 Yield attributes

Bahn and Kukula (1987) reported that weeds cause considerable loss in yield of chickpea, although weeding by hand to prevent weed competition during the period before the development of a full canopy cover has invariably been most effective, but limitations of labour and high labour costs often prevent the adoption of this method. The proper time of application of herbicides is very important. An early application of a herbicide can damage the crop and on the other hand too delayed application will not be effective in controlling weeds as they may become tolerant at advanced age. Imazethapyr resulted in effective weed control and high yields in urdbean (Chin and Pandey 1991) and soybean (Angiras and Rana 1995). It was concluded that weed competition reduced seed yield of chickpea by 80%

(Tiwari *et al* 2001). Application of herbicides 30 days after sowing was most effective in decreasing dry matter of weeds and increasing grain yield, while effectiveness of herbicide decreased with a delay in their application (Prasad and Singh 1995). Wall (1996) reported that imazethapyr severely decreased lentil yields, also drawing attention to similar effects of imazamethabenz. Soybean injury by chlorimuron and imadazolinone herbicides typically results in decreased plant height and biomass, and can result in reduced yield (Newsom and Shaw 1995). Mohamed *et al* (1997) also reported that imazethapyr, terbutryn, prometryn and pendimethalin were well tolerated by lentil and controlled weeds successfully, enhancing yield. Windley *et al* (1999) also reported that post-emergence application of imazethapyr at 96 g/ha increased mungbean yield by 20.4% over unweeded control.

In Bubny biotype, which was resistant both to ALS inhibitors and atrazine, recorded the lowest hill reaction activity values among all biotypes examined by Hola *et al* (2004). Khan *et al* (2004) also reported that carfentrazone-ethyl was the best post-emergence herbicide for controlling weeds and recording higher yield and yield components compared to other herbicidal treatments and weedy check. Khan and Marwat (2006) investigated that crop density alone could not suppress the weeds below threshold level. Zand *et al* (2007) also attributed lower grain yield of the handweeded control than herbicide treated plots to possible damages of hand weeding on wheat. Tiwari *et al* (2007) conducted field trials during the rainy (*kharif*) seasons of 2001 and 2002 in Madhya Pradesh, India, to evaluate the efficacy of post-emergence herbicides against weeds in soybean (*Glycine max*) and reported in contrast that the post-emergence herbicides imazethapyr @75g ha^{-1} controlled only broadleaved weeds. Substantial control of weeds and significant increase in grain yield of chickpea with different herbicides has been reported (Yadav *et al* 2007). Qasem (2007) evaluated efficacy of different herbicides against wild-oat in wheat. The herbicides affected wheat plant height, dry weed biomass, biological yield and grain yield.). Baghestani *et al* (2007) reported that bromoxynil + MCPA (2-methyl-4-chlorophenoxyacetic acid) @ 150 g a.i. ha-1, 2,4-D plus MCPA, and 2,4-D + carfentrazone-ethyl @ 490 g a.i. ha-1 would be most effective to control weeds, increase grain yield and prevent herbicide resistance weeds. However, over dose of Carfentrazone-ethyl to control weeds would cause serious crop injury. Hossain *et al* (2008) reported that maximum grain yield can be obtained with Carfentrazone-ethyl 50 WG. Khan *et al* (2008) reported that with increasing crop plant population m^{-2} suppress weeds and increased yield. Veeraputhiran and Chinnusam (2008) also reported that the yield attributing characters of black gram *viz.,* number of pods per plant and number of grains/pod were higher under imazethapyr applied at 21 DAS. Ram and Singh (2011) also recorded that post-emergence application of imazethapyr 75 g/ha at 20–25 DAS resulted in significantly higher pods/plant than weedy check but were at par with 2 hand-weedings at 20 and 40 DAS in soybean. Nandan *et al* (2011) also recorded the highest weed-control efficiency in blackgram

with imazethapyr 25 g/ha at 15-20 DAS. The highest yield attributes, viz. branches/plant, pods/plant, seed weight/plant and 100-seed weight were recorded with the application of imazthapyr and quizalofop-ehtyl. Goud *et al* (2013) also observed that at higher concentration, imazethapyr and quizalofop-ethyl affect growth and yield of chickpea cv. KAK-2 and lower concentrations were inefficient for effective weed control.

Maximum grain yield (4.585 tons ha^{-1}) was recorded in carfentrazone-ethyl 50 WDG followed by Puma super 75 EW with grain yield of 4.413 tons ha^{-1}. The minimum yield 2.54 tons ha^{-1} was observed in *Partheinum hysterophorus* plots (Khan *et al* 2013). Aboali and Saeedipour (2015) too reported that in broad bean, the highest pod number per plant was observed in weed free treatment. All herbicide treatments had less pod numbers than free weed control. The least pod number per plant was found in weedy check with a 53.3% decrease compared to weed free treatment and the highest and the lowest 100 seed weight was recorded in hand weeding and weedy check by 96.18 and 85.08 g, respectively.

2.2 Biochemical changes

Herbicide inhibition causes a disruption in protein synthesis, which in turn leads to interference in DNA synthesis and cell growth. The proteolytic enzymes are synthesized in cotyledons during germination (Ashton 1976). The inhibition of acetohydroxyacid synthase by the imidazolinones could demonstrate the herbicidal effects of such compounds. If the imidazolinones inhibit the synthesis of valine, leucine, and isoleucine *in vivo*, there may be a rapid decrease in the pool size of such amino acids, which in turn may cause a decrease in protein synthesis (Shaner *et al* 1984). Egli *et al* (1985) reported that the inhibition of protein synthesis by herbicides is primarily due to disturbance of the absorption and retention of amino acids available for protein synthesis, interference with the incorporation of amino acids into protein, and/or the formation of enzymes responsible for protein synthesis and metabolism. The protein degradation to amino acids in the initial stages of seed germination helps in diverting amino acids towards the synthesis of new proteins/enzymes, cellular constituents or translocation to the growing axis. Persistence of imazethapyr depends on soil properties and may vary from 70 to 254 days (Vischetti 1995). Imazethapyr controlled *Cyperus rotundus* more effectively when applied to weeds 5 to 20 cm tall compared with weeds 30 cm tall (Richburge *et al* 1996). Shivay *et al (*1997) reported that there were non significant differences among the weedy check and herbicidal treatments before the application of herbicides. Scarponi *et al* (1997) found an increase in ammonia in broad bean treated with imazethapyr. Further, both imazethapyr and chlorimuron have been shown to decrease protein and branched-chain amino acid contents of legumes. It has been observed that damage in protein, deletion or addition of the some proteins in repetitive region might be the reason for these variations (Benmoussa *et al* 2000). Herbicides kill plants by disrupting essential physiological or biochemical process, usually through a specific interaction with a

single molecular target in the plant. (Devine and Preston 2000). When a plant is subjected to any biotic or abiotic stress factor, the first observed response is a decrease in its normal metabolic activities, along with a consequent reduction of growth. In this 'alarm phase' protein synthesis is an adversely affected anabolic process along with photosynthesis, transport of metabolites, uptake, and translocation of ions (Bonjoch and Tamayo 2001).

The weed resistance to these herbicides is associated with the changes in the protein structure and several ALS amino acid substitutions that confer herbicide resistance have been reported from various weed species (Tranel *et al* 2003). Application of the tank mixed herbicides reduced broad and narrow leaf weeds to a varying degree sometimes approaching to 100% control (Khan *et al* 2003). The direct effect of herbicides on protein or nucleic acid synthesis have not yet been discovered, probably because neither of these sites are primary site of action of any frequently applied herbicides (Khan *et al* 2006). A large number of metabolites like proline were accumulated in the plants, when they grow under various abiotic stresses (Ashraf and Foolad 2007).

It has been opined that, after the ALS inhibition by herbicides, protein synthesis can only continue through an increase in the protein turnover; and then the free amino acid pool shows a remarkable accumulation (Zabalza *et al* 2007). Furthermore, it was observed that IM had not decreased protein, and "de novo" synthesis of proteins had also inhibited in treated plants, showing that there would have been protein synthesis, but from the amino acids scavenged, mainly from the protein turnover. In addition, IM has been found to be a potent inhibitor of acetohydroxyacid synthase enzyme. This enzyme has an excellent herbicidal function and its phytotoxic effects can be reversed by exogenous application of valine, leucine, and isoleucine. This indicates that such imidazolinone herbicides are bound to the enzyme-pyruvate complex.

High doses of herbicide Imazethapyr can be harmful (Pasha 2013) and usage in large quantities for chickpea is dangerous (Hoseiny-Rad and Jagannath 2011). Moreover, they also reported that protein content of the leaves was decreased with increasing concentration of the herbicide. Imazethapyr also reduces photosynthesis and dry matter accumulation and showed low protein accumulation in leaves. Also, anthocyanins have reduced the protein. Herbicide inhibition causes a disruption in protein synthesis, which in turn leads to interference in DNA synthesis and cell growth. The inhibition of cellular division by ALS-inhibiting herbicides may cause a decrease in the root growth. An alteration of the intermediate metabolite pools or the ATP levels after the ALS inhibition could cause the inhibition of cellular division (Zabalza *et al* 2004).

2.3 Anti-oxidative Defence System:

Exposure of plants to unfavourable environmental conditions such as temperature extremes, heavy metals, drought, water availability, air pollutants, nutrient deficiency, or salt

stress can increase the production of ROS e.g., O_2, H_2O_2 and OH; which gets accumulated causing damage to cellular components, severely disrupting metabolic function. Prevention of oxidative damage to cells during stress is one of the mechanisms of stress tolerance (Kraus and Fletcher 1994), which is attributed to enhanced antioxidant enzyme activity. To protect themselves against these toxic oxygen intermediates, plant cells and its organelles like chloroplast, mitochondria and peroxisomes employ antioxidant defense systems. Increases in SOD activity has been observed in response to treatment with herbicides. The herbicide paraquat produces its cytotoxic effects via a free radical mechanism. Paraquat-resistant varieties of various species like tobacco, ryegrass and horseweed correlate with increased activities of SOD and other antioxidant enzymes than the corresponding sensitive genotypes (Matters and Scandalios 1986).

A great deal of research has established that the induction of the cellular antioxidant machinery is important for protection against various stresses (Khan and Singh 2008).The components of antioxidant defence system are enzymatic and non-enzymatic antioxidants. Stress induced ROS accumulation is counteracted by enzymatic antioxidant systems that include a variety of scavengers, such as superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD), glutathione S-transferase (GST), catalase (CAT) and non-enzymatic low molecular metabolites (Mittler *et al* 2004). CATs are tetrameric heme containing enzymes with the potential to directly dismutate H_2O_2 into H_2O and O_2 and are indispensable for ROS detoxification during stressed conditions (Garg and Manchanda 2009).

Kielak *et al* (2011) concluded that Roundup herbicide may be harmful to plants. Its phytotoxicity towards duckweed as a model plant caused an increase in CAT and APX activity, what may suggest that Roundup generated the oxidative stress within tissues of the treated plants. Reactive oxygen species are highly reactive and toxic to cells as they are capable of unrestricted oxidation of various cellular components. Therefore, the accumulation of ROS causes considerable damage to membrane lipids and other cellular components (Lukatkin 2003). It is widely believed that ROS are involved in the activation of cellular stress responses and defence systems (Desikin *et al* 2001). Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are the main enzymes that scavenge ROS in plants. They exert synergistic effects on the elimination of O^{2-} and H_2O_2 to maintain the balance of active oxygen metabolism in plants under environmental stress (Polle 2001).

Other than as an osmolyte, proline is considered as a potent antioxidant and potential inhibitor of Programmed cell growth. Therefore, it is regarded as non-enzymatic antioxidants that microbes, animals, and plants require to mitigate the adverse effects of ROS (Chen and Dickman 2005). Metalloenzyme SOD is the most effective intracellular enzymatic antioxidant which is ubiquitous in all aerobic organisms and in all subcellular compartments prone to ROS mediated oxidative stress. It is well established that various environmental stresses often

lead to the increased generation of ROS, where, SOD has been proposed to be an important in plant stress tolerance and provide the first line of defense against the toxic effects of elevated levels of ROS.

CHAPTER III

MATERIALS AND METHODS

The present investigation entitled "Physiological variations in herbicide tolerance among chickpea (*Cicer arietinum* L.) genotypes" includes the screening of twenty four genotypes of chickpea for herbicide tolerance under field conditions. The work was carried out in the field area of Pulses Section, Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Biochemical studies were performed on six (three sensitive and three tolerant) genotypes selected on the visual scoring and physiological observations. Seeds of chickpea were made available from Pulses Section, Plant Breeding and Genetics, PAU. The materials and methods employed in the present investigation have been described in this chapter.

Seeds of the following chickpea genotypes were used for field and laboratory studies for their growth behavior and yield attributes.

Table 3.1: List of Chickpea genotypes

Sr. No.	Genotypes	Sr. No	Genotypes
1	DKG 1030	13	GLW 187
2	JG 1362	14	GLW 188
3	GPF 2	15	GL 13016
4	GL 22044	16	GL 13022
5	GL 26016	17	PBG 1
6	GL 28202	18	PDG 4
7	GL 28203	19	PBG 5
8	GL 10047	20	FLIP-05-93C
9	GL 11026	21	GLW 11
10	GL 12003	22	DKG 876
11	GL 12021	23	GLW 125
12	GLW 44	24	GL 27023

Location- Ludhiana represents the Indo Gangetic plains and is situated at 36°-54'N latitude, 25°-48'E longitude and at a mean height of 247 meters above sea level.

Experiment: 1 Screening of chickpea genotypes for herbicide tolerance under field conditions

Field Layout

The present investigation was carried out on Chickpea (*Cicer arietinum* L.) sown on 10 November, 2015 in the experimental field of Pulses Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The crop was planted in 2 rows with row to row spacing of 40 cm using factorial randomized block design with three replications.

Observations:

3.1 Visual scoring

The genotypes were scored for stay green trait against herbicides on a 1-5 scale, where 1 is highly tolerant (no or very less burning or curling of leaves) and 5 is highly sensitive (complete burning of leaves leading to plant mortality or curling), 12-15 days after herbicide foliar application.

3.2 Phenophasic development

3.2.1 Days to flower initiation and 50% flowering

The data for days to flower initiation and 50% flowering were recorded as the number of days taken by the plants from the date of sowing to the period of 1st flower and 50% flowering respectively.

3.2.2 Days to pod initiation

The number of days taken by the plants from the date of sowing to the period of 1st pod was recorded.

3.2.3 Days to maturity

The number of days taken by the plants from the date of sowing to the period of maturity was recorded.

3.3 Growth attributes

3.3.1 Shoot length

The length of main stem was measured from the ground level to the tip of plant with meter rod manually and expressed in cms.

3.3.2 Root length

The root length was recorded with meter rod manually and expressed in cms.

3.3.3 Shoot dry weight

The plant samples were dried in a hot-air oven for 48 hours at 70±1ºC for till constant weight was obtained. The dry weight of shoots from three randomly selected plants from three replications was recorded using electronic balance. The mean values were taken and expressed in grams.

3.3.4 Root dry weight

Roots of oven-dried samples were weighed on electronic balance to record root dry

weight and expressed in grams.

3.3.5 Leaves dry weight

Samples were collected during developmental stage. Leaves were dried at 70±1ºC for a period of 48 hours till constant weight was obtained. The dry weight of leaves per plant were weighed from three randomly selected plants with replications and expressed in grams per plant.

3.3.6 Number of leaves

The numbers of leaves from three replications were counted manually from the plant samples of each treatment.

3.3.7 Number of senesced leaves

The numbers of senesced leaves due to herbicidal treatment were recorded from three replications.

3.4 Physiological traits

3.4.1 Leghaemoglobin content in nodules (Wilson and Reisenauer 1963)

The leghaemoglobin content was determined by using Drabkin's solution in nodules at flowering stage and expressed as mg/g FW nodules.

Extraction of nodule tissue

Fresh tissue of nodules (0.5 g) was crushed in a 10 ml round bottom centrifuge tube with 3ml drabkin's solution. The resulting mixture was centrifuged (15 min, 5000 rpm) so that large particles of nodule tissue settle down. The supernatant was transferred to 10 ml volumetric flask. The nodule tissue was extracted twice more and supernatant combined with the first one in the flask. The total volume was made to 10 ml with drabkin's solution, and centrifuged at 2000 rpm for 30 minutes. The absorbance of the clear supernatant was read on UV2600 spectrophotometer at 540 nm using drabkin's solution as a solvent blank.

3.4.2 Dry weight of nodules

Nodules from roots were collected at flowering stage and dried in the hot air oven at 80±1ºC for a period of 48 hours and weighed (g) by using electronic balance.

3.4.3 Leaf area index

Leaf area index (LAI) was recorded with the instrument 'Sun Scan Canopy Analyzer' during reproductive stage of crop.

3.4.4 Photosynthetic rate

Photosynthetic rate was recorded at flowering stage by using 'Portable photosynthesis system (LI-6400XT, LICOR). Rate of photosynthesis is expressed as µmol CO_2 m^{-2} s^{-1}.

3.4.5 Hill reaction (Cherry 1973)

Reagents

a) 0.067M Phosphate buffer (pH 7.5)

b) Potassium ferricyanide solution

c) 20% Trichloroacetic acid (TCA)

d) 0.035M Sucrose

Extraction: Leaf samples (0.1g) were taken and gently grounded in 5ml extraction medium (0.067M phosphate buffer, pH 7.5 containing 0.035M sucrose). During extraction and collection of leaves, the temperature was maintained at 0-4°C.

Estimation: Potassium ferricyanide solution was prepared by dissolving sodium chloride (1.02g) and potassium ferricyanide (13mg) in phosphate buffer (25ml). The reaction was started by mixing 0.5ml of supernatant from above extract with 2.5ml of ferricyanide solution. The tubes were kept in light (approx. 5000 lux) for 10 min and another similar set in dark. The reaction was stopped by adding 20% TCA (0.3ml). The absorbance was recorded at 420nm on spectrophotometer. Hill reaction was expressed as decrease in absorbance ΔOD mg^{-1} chl h^{-1}.

3.4.6 Cellular respiration (Steponkus and Lanphear 1967)

Fresh leaves (100mg) were collected and rinsed with distilled water and kept in test tube with 1ml of deionised water. Two sets were then heat treated as follows:

First set was kept at 25°C for 90 min. and second set was placed in water bath at 49°C for 90 min. Then 10 ml of TTC solution (0.8% TTC (Triphenyl tetrazolium chloride) in 0.05 M Sodium phosphate ($NaPO_4$) buffer, 7.4 pH was added per tube and vaccum infilterated for 10 minutes. The tissue was then incubated in TTC solution for 24 hours at 25°C in dark. After incubation, leaves were removed, rinsed with distilled water, placed individually in separate tubes using 4ml of 95% ethanol and submerged for 24 hours at 25°C in dark. The level of acquired temperature tolerance was determined by measuring % reduction to formazon using the formula given below:

$$\text{TTC \% reduction} = (OD_h/OD_c) \times 100$$

OD_h refers to mean optical density (530 nm) values for heat stressed set (49°C for 90 min)

ODc refers to mean optical density (530 nm) values for control set (25°C for 90 min)

3.4.7 Lipid peroxidation (Heath and Packer 1968)

Lipid peroxidation is measured in terms of malondialdehyde content

Reagents

Thiobarbituric acid (TBA) reagent: mixed 18% Trichloroacetic acid (TCA) with 0.45% TBA in 1:2 ratios.

Procedure

TBA reagent (10ml) was added to 100mg of dry leaf powder in test tube. The mixture was heated for 15 minutes over a water bath at 100°C and filtered hot through Whatman (no. 42) filter paper. Absorbance of the filterate was read at 532 nm and 600 nm. The non-specific absorbance at 600 nm was subtracted from that at 532 nm. The concentration of MDA was

calculated using an extinction coefficient of 155 mM $cm^{-1.}$

3.5 Yield and yield attributing traits

Yield and yield attributes were recorded at maturity from three randomly selected plants in each replication.

3.5.1 Number of pods per plant

The total numbers of pods were counted from three randomly selected plants at maturity and average was calculated to obtain number of pods per plant and expressed as number of pods per plant.

3.5.2 100-seed weight

The hundred seeds were taken at random from each treatment and then weighed in grams.

3.5.3 Yield/plant

Three plants were selected randomly from each replication of each treatment and yield per plant was recorded and expressed in grams.

Experiment: 2

Biochemical parameters related to herbicide tolerance

Table 3.2: List of selected chickpea genotypes used in the study

Sr. No.	Genotypes (Tolerant) for Carfentrazone-ethyl	Genotypes (Tolerant) for Imazethapyr	Sr. No.	Genotypes (Sensitive) for Carfentrazone-ethyl	Genotypes (Sensitive) for Imazethapyr
1.	GLW 11	GL 11026	1.	GL 12021	JG 1362
2.	GL 10047	GLW 44	2.	GLW 125	GL 28203
3.	PDG 4	PBG 5	3.	GL 27023	GL 12021

3.6 Biochemical Analysis (In leaves at reproductive stage)

3.6.1 Total soluble protein (Lowry *et al* 1951)

Reagents:

i. Reagent A: 2 % Na_2CO_3 in 0.1N NaOH.

ii. Reagent B: 0.5% $CuSO_4.5H_20$ in 1% sodium citrate

iii. Reagent C: (Reagent A + Reagent B in 50:1 ratios)

iv. Reagent D: Folin Ciocalteau phenol + Distilled water in 1:1 ratios)

v. 20% Trichloroacetic acid (TCA)

Extraction:

0.1g of dry leaf samples was extracted with 3ml of 0.1N NaOH. The samples were centrifuged in 10ml round bottom centrifuge tubes at 14000rpm for 10 minutes. Then the same amount of TCA is added to the supernatant of each tube. Precipitates formed were stored overnight and then again centrifuged at 14000rpm for 10 minutes. Precipitates which settled down at the bottom were extracted for estimation of proteins.

Estimation:

2ml of 0.1N NaOH was added to each tube. 0.1ml was taken from above extract and 0.9ml of distilled water was added. Then 5ml of Reagent C was added. After 10 minutes 0.5ml of Reagent D was added to test tubes and shaked well to mix properly. Absorbance was recorded on spectrophotometer at 520nm. The total soluble protein was expressed in mg/g dry weight.

3.6.2 Total soluble sugars (Dubois *et al* 1956)

Reagents:

i. 5% Phenol

ii. Chilled Concentrated H_2SO_4

iii. 80% Ethanol

Extraction: 100mg of dry leaf material was homogenized in 5ml of 80% ethanol and centrifuged at 3000 rpm for 15 minutes. The extraction procedure was repeated twice. The supernatants were pooled and the alcohol was evaporated with water bath at 100°C. The final volume was adjusted to 5ml with distilled water. This extract was used for estimation of total soluble sugars.

Estimation: To 0.2ml of extract, add 0.8ml of distilled water, then 1ml of 5% phenol was added. The solution was kept for 10 minutes followed by addition of 5ml of chilled Concentrated H_2SO_4 with constant shaking. After 10 minutes, the tubes were cooled to room temperature under running tap water. The absorbance was recorded at 490nm against 80% ethanol as blank. The concentration of total soluble sugar content was expressed as mg g^{-1} dry weight.

3.6.3 Proline content (Bates *et al* 1973)

Reagents:

i. 6M Orthophosphoric acid

ii. 3% Sulphosalicylic acid

iii. Acid Ninhydrin

iv. Glacial Acetic Acid

v. Toluene

Extraction: The tissue (0.2g) was extracted in 10ml of 3% Sulphosalicylic acid at room temperature and centrifuged at 3000 rpm for 10 minutes. The supernatant was collected for

estimation.

Estimation: To 2ml of supernatant, add 2.5ml of acid ninhydrin, (125mg of Ninhydrin mixed in 3ml of glacial acetic acid and 2ml of orthophosphoric acid, and then kept in oven at 70°C till a clear solution was formed), 2ml of 6M orthophosphoric acid and 2ml of glacial acetic acid. The mixture was boiled for 1 hour in hot water bath till pink colour appears. On cooling, 5ml of toluene was added and shaken on vortex. Again allow it to stand in separating funnel for another 10 minutes till the two layers get separated. Lower layer was discarded and upper pink layer was collected. The absorbance was recorded at 515nm by using toluene as blank. Proline is used as standard to make standard curve. Proline content is expressed in µmoles g^{-1}.

3.6.4 Peroxidase Activity (Shannon *et al* 1966)

Peroxidase catalyses the breakdown of H_2O at the expense of electron acceptors, such as ascorbate, quinones and cychrome c.

$$H_2O + AH_2 \xrightarrow{\text{peroxidase}} 2H_2O + A$$

Reagents:

i. 0.1M Potassium phosphate buffer (pH 7.5)

ii. 1% Polyvinyl pyrrolidine (PVP)

iii. 0.05M Guaicol in 0.1M of potassium phosphate buffer (pH 6.5)(freshly prepared)

iv. 0.8M H_2O_2 (fresh)

Extraction: Fresh leaf (100mg) of each genotype was extracted in 2ml of 0.1M potassium phosphate buffer (pH 7.5) containing 1% PVP using chilled pestle and mortar. There were three replications of each genotype. Homogenate was centrifuged in appendrof's tube at 10,000rpm at 4°C for 10 minutes and clear supernatant was used for enzyme assay.

Assay: In spectrophotometric cuvette, add 3ml of guaicol (0.05M) prepared in potassium phosphate buffer and 0.1ml of enzyme extract was added. The reaction was initiated by adding 0.1ml of 0.8M H_2O_2 and rate of change of absorbance was recorded at 470nm using spectrophotometer for 2 minutes at the interval of 30 seconds.

Peroxidase activity has been defined as change in absorbance $min^{-1}g^{-1}$FW.

3.6.5 Catalase (Chance and Maehley 1955)

Catalase is able to use one molecule of H_2O_2 as substrate or electron donor and another molecule of H_2O_2 as oxidant or electron acceptor.

$$2H_2O_2 \xrightarrow{\text{CAT}} 2H_2O + O$$

Reagents:

i. 50 mM sodium phosphate buffer (pH 7.5)

ii. H_2O_2 solution: 0.2ml of H_2O_2 was diluted to 50 ml with 50 mM sodium phosphate buffer (pH 7.5)

iii. 1% Polyvinyl pyrrolidine (PVP)

Extraction: The enzyme was extracted from fresh tissue (0.1 g) with 50 mM sodium phosphate buffer (pH 7.5) containing 1% polyvinyl pyrrolidine (PVP) and centrifuged at 10,000 rpm for 20 minutes at 4°C. Supernatant was used as enzyme extract

Assay: In spectrophotometric cuvette, 1.9 ml of 50 mM sodium phosphate buffer (pH 7.5) and 0.1 ml of enzyme extract was added. The reaction was initiated by adding 1 ml of utilization of H_2O_2 was recorded at intervals of 30 seconds for 2 minutes by measuring the decrease in absorbance at 240 nm. Catalase activity was expressed as µmoles of H_2O_2 decomposed/min/g of tissue.

3.6.6 Superoxide Dismutase (Marklund and Marklund 1974)

Superoxide dismutase catalyzes the disproportionation of superoxide anion to H_2O_2 and molecular oxygen.

$$O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Reagents:

i. 0.1M Potassium phosphate buffer (pH 7.5)

ii. 1% Polyvinyl pyrrolidine (PVP)

iii. 6 mM disodium EDTA

iv. 0.1M Tris-HCl (pH 8.2)

v. 6 mM Pyrogallol (Fresh solution was prepared for assay).

Extraction: The enzyme was extracted from 100mg of fresh leaf with 2ml of 0.1M potassium phosphate buffer (pH 7.5) containing 1% PVP. The homogenate was centrifuged at 10,000 g at 4°C for 10 minutes. Supernantant was used for enzyme assay.

Assay: To a spectrophotometric cuvette, 1.4 ml of 0.1M Tris-HCL buffer (pH 8.2), 0.5 ml of 6 mM EDTA, 1ml of 6 mM pyrogallol solution and 0.1 ml of enzyme extract was added. Absorbance was recorded at 420 nm after an interval of 30 seconds up to 2 minutes. The reaction mixture without pyragallol was taken as control. A unit of enzyme activity has been defined as the amount of enzyme causing 50% inhibition of auto-oxidation of pyrogallol observed in blank.

3.6.7 Estimation of free amino acids (Lee and Takahashi 1966)

Reagents: Prepared ninhydrin reagent by reagents A: B: C in the ratio of 5:12:2 (pH 5.5)

i. Reagent A: 1% ninhydrin in 0.5 M citrate buffer (pH 5.5)

ii. Reagent B: Pure glycerol

iii. Reagent C: 0.5 M citrate buffer

Extraction: 0.1g of dry leaf samples was extracted with 3ml of 0.1N NaOH. The samples were centrifuged in 10ml round bottom centrifuge tubes at 14000rpm for 10 minutes. The supernatant was further used for estimation.

Procedure: To 0.2 ml of the extract 3.8 ml of ninhydrin reagent was added. The reaction mixture was heated in water bath for 12 min and after cooling the test tubes under running water the absorbance was read at 570 nm against the blank. Using the standard curve of L-glycine (2-10 µg) run simultaneously, the concentrations of amino acid were determined.

Statistical analysis

The data recorded was statistically analyzed by CPCS1software. Comparison of the mean values of different treatments was made at 5% level of significance.

Chickpea appears to be most sensitive legume species to weeds which leads to lesser yields. For their control, herbicides applied sometimes causes detrimental effects on crops too. However, there are appreciable genotypic variations in herbicide tolerance, although these differences were considered insuffiecient to undertake breeding programs. It appears that if high doses of herbicides are given, plants get sensitive towards them.

So further work should be done for identification herbicide tolerant cultivars which can be further utilized as donor for breeding programs.

Legume species apart from being economy also withholds an important position in daily life whether in the form of food or livestock feed. Herbicides with the optimum doses increases the yields of crops while at higher doses, it results into yield reduction.

CHAPTER IV

RESULT AND DISCUSSION

The results of the investigation entitled "Physiological variations in herbicide tolerance among chickpea (*Cicer arietinum* L.) genotypes" are presented in this chapter. A set of 24 chickpea genotypes were raised in the field area of department of Plant Breeding & Genetics for studying various morphological, physiological and yield traits in relation to herbicide tolerance. Out of these three sensitive and three tolerant genotypes from each treatment were chosen for biochemical analysis. The results are presented in the form of tables and figures under these headings:-

4.1 Phenophasic development

4.2 Growth efficiency

4.3 Physiological traits

4.4 Yield and yield attributes

4.5 Physiological Evaluation

4.6 Biochemical estimations

4.1 Phenophasic Development

4.1.1 Days to flower initiation and 50% flowering, pod initiation and maturity

Herbicides affects phenological period by inducing delay in the life cycle. Phenological development has direct correlation with the herbicide application. Days pertaining to physiological stages i.e. number of days to flowering, 50% flowering, pod initiation and maturity of all 24 genotypes were recorded time to time during the season and presented in Table 4.1 in terms of days after sowing (DAS). The genotypic variation in phenophasic development was observed with foliar application of herbicides (imazethapyr @750ml ha^{-1} and carfentrazone-ethyl @375g ha^{-1}). The initiation of flowering ranged between 59-102 days after sowing in carfentrazone-ethyl treated plants, 61-108 in imazethapyr treated plants whereas flowering initiated between 57-95 DAS in unsprayed plants *i.e.,* control. The 50% flowering was observed in genotypes between 93-107 DAS under carfentrazone-ethyl treatment, 99-127 DAS under imazethapyr treatment whereas in control it was ranged from 85-108 DAS. The maximum delay to flowering initiation was shown by GLW 125 and GL 28203 under carfentrazone-ethyl and imazethapy treatments respectively. Flowering was rapid in control as compared to both herbicide treatments. Taran *et al* (2013) reported that flowering was delayed in chickpea after imazethapyr application. This may be affecting the meristematic region and causing the delay. Days to pod initiation ranged between 98-110 DAS in carfentrazone-ethyl treated plants, 106-123 DAS in imazethapyr treated plants whereas 92-108 DAS in Control. The crop got matured in 124-138 days after sowing under carfentrazone-ethyl treatment, the plants attained maturity ranged from 126-143 DAS under imazethapyr treatment, while it reached from 120-130 days in

Table 4.1: Phenophasic development of chickpea genotypes in response to herbicides

Genotypes	Days to flower initiation			50% flowering			Days to maturity			Days to pod inititation		
	Control	Imazethapyr	Carfentrazone-ethyl	Control	Imazethapyr	Carfentrazone-ethyl	Control	Imazethapyr	Carfentrazone-ethyl	Control	Imazethapyr	Carfentrazone-ethyl
DKG 1030	76	98	88	96	113	99	129	137	131	107	114	109
JG 1362	65	106	94	96	127	98	129	137	130	107	114	109
GPF 2	62	96	89	97	116	99	129	134	136	107	123	109
GL 22044	67	90	87	96	102	97	130	136	130	107	112	110
GL 26016	57	67	60	86	103	88	121	128	127	107	111	109
GL 28202	58	68	66	87	109	88	122	129	129	92	108	107
GL 28203	57	108	62	85	127	88	122	138	128	93	109	103
GL 10047	55	68	59	92	107	93	120	133	127	92	117	101
GL 11026	90	63	90	98	109	99	128	127	132	107	108	100
GL 12003	86	88	90	97	110	98	125	138	132	107	107	109
GL 12021	68	103	93	89	99	98	126	143	136	107	114	109
GLW 44	92	61	92	99	100	94	128	129	132	108	116	110
GLW 187	88	86	92	103	108	104	124	134	131	107	106	110
GLW 188	89	99	92	100	109	100	124	138	133	107	113	109
GL 13016	89	99	92	100	110	102	126	137	134	106	115	109
GL 13022	88	106	91	102	110	104	126	134	131	107	112	108
PBG 1	90	104	90	101	109	101	130	133	128	107	113	108
PDG 4	92	105	63	105	108	107	127	135	125	107	112	108
PBG 5	93	63	97	108	105	100	128	126	135	93	114	98
FLIP-05-93C	91	96	94	108	111	104	130	135	136	105	109	107
GLW 11	66	86	60	102	109	95	123	137	124	108	113	110
DKG 876	95	101	97	107	106	105	125	136	131	104	112	109
GLW 125	60	68	102	97	107	106	127	130	134	108	111	108
GL 27023	60	98	96	92	106	105	120	131	138	100	110	107
Mean	**76.4**	**88.6**	**84.8**	**97.6**	**109.2**	**98.8**	**125.8**	**134.0**	**131.3**	**104.2**	**112.2**	**107.3**
CD (5%)	G=0.66,T=0.23,G×T=1.14			G=0.55,T=0.19,G×T=0.95			G=0.71,T=0.25,G×T=1.22			G=0.61,T=0.22,G×T=1.06		

Moderately tolerant **Tolerant** **Sensitive**

Plate 1: Visual scoring of vigour

control. The maximum dealy in pod initiation was shown by GL 27023 and GL 12003 under carfentrazone-ethyl and imazethapyr effects. Delay in pod initiation may be due to slow growth rate of herbicide treated plants, which ultimately results in delayed maturity. According to Royuela *et al* (2000) the reason of this growth inhibition was carbohydrate starvation and blockage of acetolactate synthase catalysed reactions. Further, it seems that combined with the delay of flowering and maturity with the herbicide application may lead to lower yields.

4.1.2 Visual Scoring

Herbicide susceptibility was apparent from visual injuries on leaves and stunted growth for which the herbicide tolerance score was given to genotypes on the basis of stay green trait (Plate 1). After herbicidal spray, scoring was recorded at two stages (Table 4.2). At stage I, leaf burning and leaf yellowing was observed in carfentrazone-ethyl and imazethapyr treated plants respectively.

At stage II, recovery was shown by plants from each treatment. The herbicides restrict the enzymatic actions, resulting in the arrested cell division and leading to cell death. Mishra *et al* (2005) reported phytotoxic effects of imazethapyr in lentil, but with time, plants recovered. The crops are also known to recover from phytotoxic effects of herbicides according to Rao and Rao (2003).

4.2 Growth Efficiency

4.2.1 Root and Shoot length

The length measurements gave an indication of how growth was affected by the herbicide application. The root length varied from 7.0 to 11.1cm in carfentrazone-ethyl treated plants, 7.5cm to 13.5cm in imazethapyr treated plants whereas 9.0cm to 13.9cm in control. The maximum shoot length recorded was 24.8cm which declined to 12.3cm in carfentrazone-ethyl treated plants, whereas in imazethapyr treated plants, maximum was 22.7cm and decreased to 11.5cm. In the unsprayed conditions, it ranged from 16.8 to 35.7cm (Table 4.3). These results are in agreement with the findings of Marwat *et al* (2005) and Arif *et al* (2011) who indicated that the post-emergence herbicides had no significant effect on plant height in wheat. Plant heights of the carfentrazone-ethyl treated plants were 84 and 99% less than those of untreated controls for ivyleaf morningglory (Dayan *et al* 1997). Post-emergence application of imazethapyr at 60g/ha recorded significantly the shortest plants, lowest LAI and dry matter production (Veeraputhiran and Chinnusamy 2008). Highest plant height was recorded in control plots because of competition among weeds and wheat plants enforced to grow up the plant height higher than the actual height (Marwat *et al* 2005). Weeds present in present chickpea crop are presented in Plate 2. The decrease in chickpea plant height in weedy check plots clearly showed the weed competition effect on plant growth and development. This was due to the fact that weeds utilized the resources more efficiently than

Table 4.2 Visual scoring of chickpea genotypes in response to various post-emergence herbicides after 7 DAS

Genotype	Carfentrazone-ethyl	Imazethapyr	Carfentrazone-ethyl	Imazethapyr
DKG 1030	3.7	3.3	2.3	3.0
JG 1362	4.0	3.8	2.0	3.5
GPF 2	3.2	3.5	2.8	3.5
GL 22044	2.8	2.8	2.3	2.8
GL 26016	3.2	3.0	2.3	3.2
GL 28202	2.8	3.5	3.0	3.0
GL 28203	3.0	3.8	2.7	3.0
GL 10047	3.3	3.7	2.7	2.8
GL 11026	3.7	3.0	2.8	2.8
GL 12003	3.5	3.2	2.5	3.3
GL 12021	3.8	3.3	3.5	3.3
GLW 44	3.8	2.8	2.7	2.3
GLW 187	4.0	2.7	2.8	3.5
GLW 188	3.7	2.7	2.5	3.3
GL 13016	3.5	3.0	2.0	3.3
GL 13022	3.3	3.2	2.2	3.3
PBG 1	3.2	2.7	2.5	2.5
PDG 4	3.7	2.8	2.6	3.5
PBG 5	3.7	2.8	2.8	3.3
FLIP-05-93C	3.5	3.5	3.2	3.2
GLW 11	4.0	3.0	2.5	3.7
DKG 876	3.3	2.7	2.8	3.3
GLW 125	3.3	3.2	3.5	3.7
GL 27023	3.8	3.3	3.8	3.5
Mean	**3.5**	**3.1**	**2.7**	**3.2**

1-Very good, 2- Good, 3-Acceptable, 4- Poor, 5- Very poor

Table 4.3 Variation in shoot and root length (cm) under herbicidal treatments in chickpea genotypes

Genotypes	Root length (cm)			Shoot length (cm)		
	Control	Imazethapyr	Carfentrazone-ethyl	Control	Imazethapyr	Carfentrazone-ethyl
DKG 1030	10.5	9.3	8.5	22.5	16.8	18.2
JG 1362	12.5	11.6	9.8	23.7	14.5	19.3
GPF 2	12.5	10.6	9.8	22.0	17.8	16.8
GL 22044	13.8	11.5	9.5	20.3	18.2	16.0
GL 26016	9.3	9.1	7.3	35.7	20.0	18.8
GL 28202	12.0	11.3	9.1	26.5	14.5	22.8
GL 28203	10.9	7.5	9.6	30.8	14.3	15.2
GL 10047	12.3	8.6	10.5	31.8	19.0	24.5
GL 11026	11.6	13.5	9.6	24.3	22.7	17.3
GL 12003	11.5	11.3	8.8	27.5	19.0	21.2
GL 12021	11.5	8.6	7.3	27.5	11.5	13.2
GLW 44	13.9	13.3	7.8	24.8	22.5	20.0
GLW 187	10.3	9.3	8.5	22.6	15.5	21.0
GLW 188	11.3	10.1	8.1	23.3	17.8	21.7
GL 13016	10.8	9.1	8.6	26.5	15.5	21.7
GL 13022	10.3	9.3	9.0	19.8	17.3	17.7
PBG 1	11.8	10.0	8.8	25.8	14.5	23.0
PDG 4	9.8	11.0	10.3	16.8	17.7	24.8
PBG 5	9.0	12.0	9.3	25.0	21.0	17.7
FLIP-05-93C	10.3	10.3	8.8	19.7	16.8	16.8
GLW 11	11.3	11.0	11.1	27.8	14.7	25.7
DKG 876	10.6	10.3	9.5	20.7	16.5	21.2
GLW 125	11.8	8.5	7.1	23.0	15.0	14.8
GL 27023	9.6	9.1	7.0	23.5	17.1	12.3
Mean	**11.2**	**10.3**	**8.9**	**24.7**	**17.1**	**19.2**
CD (5%)	G=0.09,T=0.03,G×T=0.16			G=0.29,T=0.10,G×T=0.51		

Values are mean; critical difference (CD) at 5% level of significance; G- genotype; T-treatment

crop plants and suppressed the crop growth and thus resulted in decrease in their height (Ansar *et al* 2010). Khan *et al* (2003) reported that applying some herbicide applications did not affect plant height but other research (Larik *et al* 1999; Marwat *et al* 2003) observed that effects of various herbicides on plant height were significant.

4.2.2 Root and Shoot dry weight

Herbicide application resulted in the variation in dry weight of root and shoot as well. (Table 4.4). The root dry weight varied from 0.3g to 1.5g in carfentrazone-ethyl treated plants, 0.3g to 2.5g in imazethapyr treated plants while 0.9g to 2.8g in control. The shoot dry weight ranged from 0.7g to 2.0g in carfentrazone-ethyl treated plants, 0.8g to 2.4g in imazethapyr treated plants whereas 1.5g to 2.9g in Control. The maximum dry matter was was exhibited by GL 11026 with the imazethapyr application, showing its tolerance/recovery towards herbicide. Minimum dry matter was recorded with imazethapyr 160 g/ha and showing 92% reduction compared with control (Singh *et al* 2010). Among the herbicide treatments post- emergence application of oxyfluorfen (0.25 kg ha-1) at 20 DAS recorded lowest dry matter production at all the stages of crop due to phytotoxic effect on crop plant with application of oxyfluorfen (Haroun 2002) Glyphosate and fluchloralin at 2 and 0.5 µg, respectively, marginally increased the dry matter accumulation in roots but decreased the shoot biomass compared with that observed for control plants. In contrast, metribuzin and 2,4-D at 0.5 µg dramatically reduced the dry matter accumulation in roots (77% for metribuzin and 44% for 2,4-D) and shoots (53% for metribuzin and 21% for 2,4-D) and they were significantly lower than those obtained for the control treatment (Zaidi *et al* 2005). Dry matter accumulation was reduced by trifluralin, chlorimuron and clomazone (Marenco 1993).

4.2.3 Total number of leaves, senesced leaves and leaf dry weight

There was a non-significant difference between total number of leaves and senesced leaves after the herbicide application. The number of leaves in carfentrazone-ethyl treated plants ranged from 21-77, in imazethapyr treated plants, from 24-75 whereas in control, the amount was 47-96. The number of senesced leaves in carfentrazone-ethyl treated plants ranged between 2-9, 2-8 in imazethapyr and 2-6 in control. At the post flowering stage, the decrease in the photosynthetic rate as compared to the flowering and pre flowering stages can be attributed to the fact that the ability to photosynthesize increases temporarily and then often, before maturity begins to decrease, which may be because of senescent leaves (Sestak, 1991). Linuron and methabenzthiazuron were both phytotoxic to yacon when applied postemergence. Both chemicals caused severe leaf necrosis and leaf loss (Scheffer *et al* 2002). Herbicides reduced the leaf dry weight to great extent irrespective of genotypes. However, GL 12003 showed maximum dry weight with respect to both herbicides (Table 4.5). Leaves dry weight was ranged from 0.44g to 0.88g in carfentrazone-ethyl treated plants and 0.35g to 0.81g in Imazethapyr treated plants whereas 0.50g to 1.53g in control.

Plate 2: Weeds present in Chickpea crop followed by herbicidal applications

1. *Fumaria parviflora* (Common name- Fumitory)
2. *Oenothera drumundii* (Common name- Hook)
3. *Rumex obtusifolius* (Common name- bitter dock)
4. *Anagallis arvensis* (Common name- Scarlet pimpernal
5. *Cyperus rotundus* (Common name- nut sedge)
6. *Lepidium sativum* (Common name - halon)

Table 4.4 Variation in root and shoot dry weight (g/plant) after herbicide application.

Genotypes	Root dry weight			Shoot dry weight		
	Control	Imazethapyr	Carfentrazone-ethyl	Control	Imazethapyr	Carfentrazone-ethyl
DKG 1030	1.2	0.5	0.4	1.5	1.1	1.2
JG 1362	1.1	0.4	0.6	1.8	0.9	1.5
GPF 2	1.5	0.6	0.4	1.6	1.3	1.5
GL 22044	1.2	0.9	0.8	2.5	1.5	1.6
GL 26016	1.4	1.1	0.5	2.3	1.3	1.7
GL 28202	0.9	0.5	0.4	1.7	1.8	1.4
GL 28203	0.9	0.6	0.4	1.9	0.8	1.4
GL 10047	1.5	1.3	1.0	2.6	2.0	2.0
GL 11026	2.0	1.8	0.7	1.9	2.2	1.3
GL 12003	1.4	0.9	0.8	2.2	1.3	1.5
GL 12021	0.6	0.3	0.4	1.6	1.0	0.7
GLW 44	1.8	1.7	0.6	2.9	2.8	1.0
GLW 187	1.2	1.0	0.8	1.9	1.2	1.4
GLW 188	1.4	0.8	0.7	1.5	1.3	1.2
GL 13016	1.4	0.8	0.6	1.9	1.4	1.3
GL 13022	1.2	0.9	0.3	2.5	1.2	1.4
PBG 1	1.4	1.2	0.8	2.2	2.0	1.5
PDG 4	1.5	1.1	1.5	2.1	1.2	1.9
PBG 5	2.8	2.5	0.8	2.6	2.4	1.5
FLIP-05-93C	1.8	1.5	0.8	2.1	1.8	1.6
GLW 11	1.3	0.8	1.0	1.9	1.2	1.8
DKG 876	0.9	0.8	0.5	2.0	1.2	1.3
GLW 125	1.7	1.0	0.4	1.8	1.4	1.0
GL 27023	0.5	0.5	0.3	1.7	1.6	0.9
Mean	**1.4**	**1.0**	**0.6**	**2.0**	**1.5**	**1.4**
CD (5%)	G=0.09, T=0.03, G×T=0.16			G=0.09, T=0.03, G×T=0.16		

Table 4.5: Number of leaves, senesced leaves and leaf dry weight (g/plant) under herbicide treatments.

Genotypes	Number of leaves			Number of senesced leaves			Leaves dry weight		
	Control	Imazethapyr	Carfentrazone-ethyl	Control	Imazethapyr	Carfentrazone-ethyl	Control	Imazethapyr	Carfentrazone-ethyl
DKG 1030	67	44	49	3	4	4	0.86	0.60	0.70
JG 1362	67	50	55	4	6	7	0.80	0.35	0.71
GPF 2	52	44	51	3	5	5	0.84	0.52	0.62
GL 22044	73	67	68	3	6	4	0.68	0.54	0.60
GL 26016	54	40	45	5	3	5	0.88	0.42	0.55
GL 28202	47	30	38	3	6	6	0.78	0.62	0.76
GL 28203	57	42	50	5	5	4	0.97	0.42	0.75
GL 10047	59	24	45	5	8	7	1.01	0.57	0.73
GL 11026	72	65	49	3	3	3	0.87	0.67	0.72
GL 12003	62	42	54	6	7	8	0.50	0.81	0.88
GL 12021	64	52	47	5	5	3	0.65	0.39	0.44
GLW 44	57	47	46	3	4	4	0.79	0.45	0.74
GLW 187	65	75	54	2	2	2	0.88	0.62	0.72
GLW 188	67	55	64	6	4	5	0.91	0.58	0.70
GL 13016	73	47	59	6	4	4	0.75	0.47	0.63
GL 13022	96	56	77	3	4	5	1.53	0.56	0.60
PBG 1	76	70	66	4	3	3	1.15	0.75	0.64
PDG 4	77	58	72	4	4	4	1.00	0.62	0.79
PBG 5	76	64	63	5	5	9	1.27	0.64	0.71
FLIP-05-93C	76	61	51	5	4	8	1.44	0.61	0.62
GLW 11	49	24	21	6	5	4	0.88	0.68	0.70
DKG 876	78	53	52	3	3	4	0.73	0.64	0.70
GLW 125	61	52	50	5	5	5	0.78	0.44	0.50
GL 27023	47	39	38	5	8	6	0.60	0.48	0.54
Mean	**65.5**	**50.0**	**52.7**	**4.3**	**4.7**	**5.0**	**0.90**	**0.60**	**0.70**
CD (5%)	G=13.0, T=4.6, G×T=NS			G=2.23, T=NS, G×T=NS			G=0.02, T=0.008, G×T=0.041		

4.3 Physiological traits

4.3.1 Leghaemoglobulin content in nodules and nodules dry weight

Leghaemoglobin is a pink colored pigment present in the root nodules of leguminous plants, where it facilitates the diffusion of oxygen to symbiotic bacteriods in order to promote nitrogen fixation. Nodulation and leghaemoglobin were negatively affected by both herbicides. Leghaemoglobin content was significantly higher under controlled treatment. Rhizobium infects plant roots through root hairs and thus it was hypothesized that herbicides affecting root hair development might interfere with nodulation. The plants showed higher tolerance for carfentrazone-ethyl as compared to imazethapyr (Table 4.6). Maximum leghaemoglobin was found in GL 10047 (6.4mg/g) and minimum in GLW 125 (2.1mg/g) among carfentrazone-ethyl treated plants. On the other hand, in imazethapyr treated plants, the highest value was shown by PBG 5 (4.1mg/g) whereas GL 28203 (1.5mg/g) exhibited least value. Under controlled conditions, maximum value was observed for PBG 5 (9.3mg/g) while minimum was observed in GL 26016 (3.1 mg/g).

Anderson *et al* (2004) claimed that herbicides may negatively affect the legume-rhizobium relationship by: (i) directly affecting root and shoot biomass of the host plant thereby limiting the number of available sites for rhizobia to attach to, or by decreasing the carbohydrate supply to existing nodules, (ii) directly affecting rhizobial survival or growth that leads to a decreased potential for rhizobial infection on root hairs, (iii) inhibiting or inactivating the biochemical signaling that plants require to initiate nodule development; this inhibition could affect either rhizobia or plants, and (iv) inhibiting nodule development by reducing the capacity for cell division.

Legumes crops restore fertility to agricultural soils by capturing nitrogen from the atmosphere. The physiology of root nodules reflects a high degree of structural and metabolic integration between plant and microbial symbionts. It was found that both herbicide treatments had negative effect on symbiotic parameter like nodule dry weight. There was a little variation in nodule dry weight accumulation in chickpea genotypes in response to carfentrazone-ethyl and imazethapyr. Nodule dry weight showed slight increase from 0.08g in GL 12021 to 0.43g in PDG 4 for carfentrazone-ethyl treated plants. Whereas this variation in dry weight was observed to be 0.06g in JG 1362 to 0.26g in GL 10047 for imazethapyr treated plants and 0.11g in GLW 11 to 0.65g in PDG 4 in untreated conditions. Overall reduction in nodules dry weight/plant was recorded due to herbicide application.

The toxicity of various herbicides to nodule bacteria in vitro or legume plants varies widely, and often compounds with the greatest herbicidal activity are the most damaging to both rhizobium and legumes (Zaidi *et al* 2005) The reduction in nodules dry weight/plant was maximum (72.2%) in case of imazethapyr @ 40 g/ha at 45 DAS. Nodule specific weight was also higher under weed free treatment (6.84 and 8.89 mg/plant at 45 and 60 DAS,

Table 4.6 Variation in dry weight of nodules (g/plant) and leghaemoglobin content (mg/g) under herbicide application

Genotypes	Dry weight of nodules			Leghaemoglobin content		
	Control	Imazethapyr	Carfentrazone-ethyl	Control	Imazethapyr	Carfentrazone-ethyl
DKG 1030	0.46	0.09	0.21	3.5	2.9	2.4
JG 1362	0.33	0.06	0.21	4.2	2.1	3.6
GPF 2	0.33	0.08	0.13	4.0	2.3	2.7
GL 22044	0.30	0.10	0.22	3.5	2.7	3.1
GL 26016	0.28	0.13	0.11	3.1	2.7	2.9
GL 28202	0.27	0.10	0.19	3.1	2.5	2.4
GL 28203	0.27	0.07	0.14	3.3	1.5	2.9
GL 10047	0.28	0.26	0.28	7.2	2.8	6.4
GL 11026	0.33	0.19	0.25	4.3	3.8	3.3
GL 12003	0.30	0.24	0.26	3.6	3.1	3.0
GL 12021	0.22	0.06	0.08	3.5	2.2	2.9
GLW 44	0.41	0.19	0.38	4.8	4.1	3.1
GLW 187	0.33	0.15	0.17	3.4	3.0	3.2
GLW 188	0.17	0.14	0.15	3.7	3.0	3.2
GL 13016	0.27	0.13	0.16	5.1	2.6	2.9
GL 13022	0.36	0.12	0.19	5.0	2.6	3.0
PBG 1	0.35	0.09	0.26	4.3	3.4	3.6
PDG 4	0.65	0.13	0.43	5.2	3.5	4.1
PBG 5	0.25	0.14	0.20	9.3	3.9	3.5
FLIP-05-93C	0.28	0.15	0.23	5.1	2.8	3.9
GLW 11	0.11	0.20	0.24	3.5	2.7	3.6
DKG 876	0.32	0.16	0.18	4.2	2.7	3.0
GLW 125	0.29	0.11	0.08	3.1	2.4	2.1
GL 27023	0.31	0.10	0.14	3.3	2.5	2.9
Mean	**0.30**	**0.10**	**0.20**	**4.3**	**2.8**	**3.2**
CD (5%)	G=0.003 , T=0.009, G×T=0.01			G=0.10,T=0.03,G×T=0.18		

respectively) and the lowest was recorded in imazethapyr application. Application of herbicides in general reduced nodules specific weight (Kumar *et al* 2015). There was significant decrease in root dry weight accumulation in chickpea genotypes in reponse to isoxaflutole (Datta *et al* 2006).

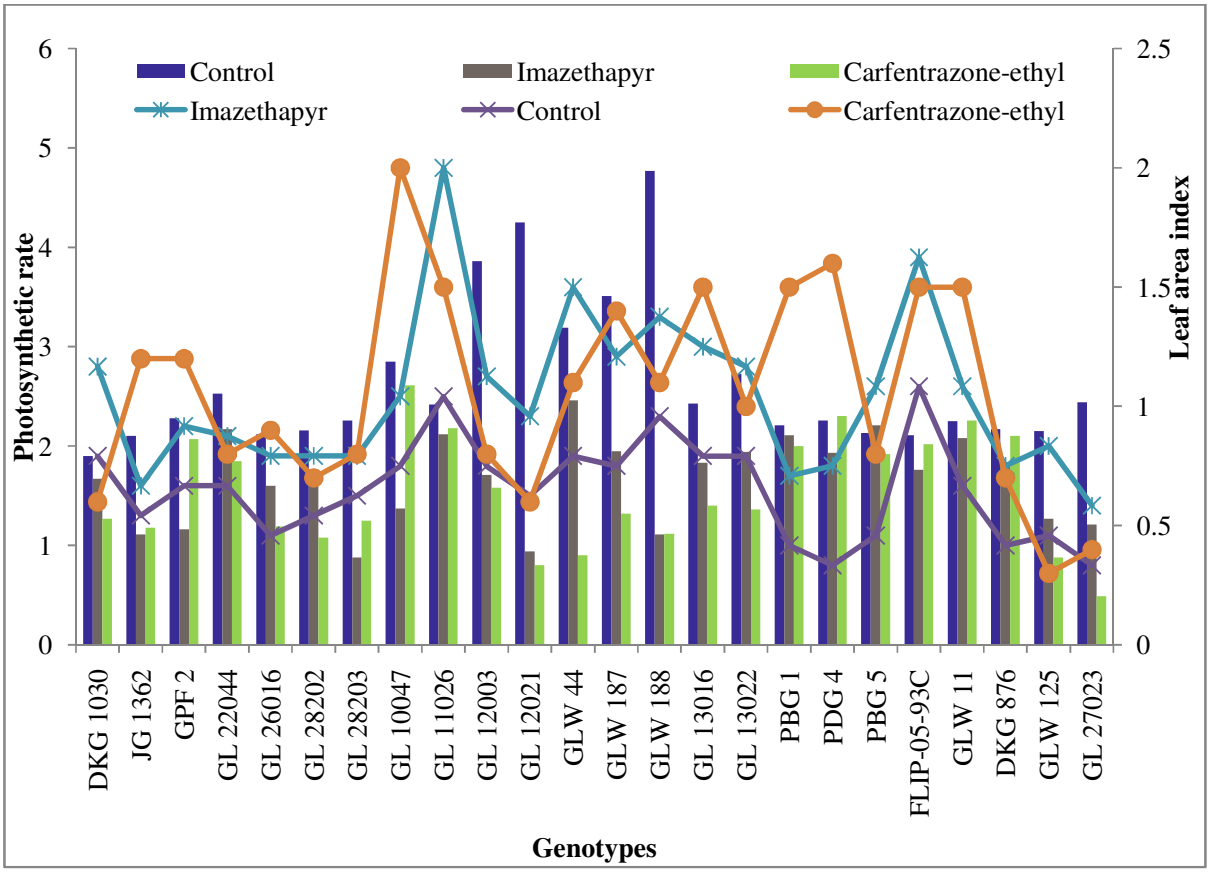

Fig. 1: Photosynthetic rate (µmole CO_2 m^{-2} s^{-1}) and Leaf area index ($cm^{2)}$ of chickpea genotypes under various treatments.

4.3.1 Photosynthetic rate

The photosynthetic rate is the fraction of light energy converted into chemical energy during photosynthesis in plants and algae. In this study, GL 10047 (2.61 µmole CO_2 $m^{-2}s^{-1}$) exhibited maximum photosynthetic rate, showing its tolerance but GL 27023 (0.49 µmole CO_2 $m^{-2}s^{-1}$) showed sensitivity towards carfentrazone-ethyl and GL 28023 had the least value while GLW 44 gives higher rate of photosynthesis among imazethapyr treated plants. In unsprayed plants, maximum photosynthetic rate was exhibited by GLW 188 (4.77 µmole CO_2 $m^{-2}s^{-1}$) and minimum was showed by DKG 1030 (1.90 µmole CO_2 $m^{-2}s^{-1}$). Overall, from herbicidal treatments, imazethapyr has an adverse effect on photosynthetic rate (Fig. 1). Bigot *et al* (2007) confirmed the strong inhibition of net photosynthesis and parallel decrease of stomata conductance and transpiration as well as the photosystem II activity decline in *Vitis vinifera* due to herbicide flumioxazin.

4.3.2 Leaf area index

Maximum leaf area index in Carfentrazone-ethyl treated plants was 2.0 and minimum was 0.3, whereas in Imazethapyr treated plants maximum was 2.3 and minimum was 0.3, whereas in Control treated plants maximum was 2.6 and minimum was 0.8 (Fig. 1). Pre emergence application of pendimethalin @ 0.9 kg ha-1 + post-emergence application of oxadiagryl @ 90 g ha-1 60 DAT and weed free treatment remain at par and recorded significantly higher plant height, leaf area index in onion (Rathod *et al* 2014). Plant height and leaf area index were reduced in the weedy treatment compared to the hand-hoeing treatment (Aboali and Saeerdipour 2015). A higher leaf area index (leaf area per unit ground area) implies a greater photon flux density (PFD) captured by canopy leading to higher chlorophyll content and photosynthetic production (Manzoor and Goutam 2014).

4.3.3 Hill reaction activity

One of the first steps of photosynthesis, known as the Hill reaction, is the splitting of water to donate two electrons to the reaction center; this reaction can be used to evaluate photosynthetic capability (Ye *et al* 2009). The Hill reaction represents the photochemical activity of either one (PS II) or both photosystems of the chloroplast and characteristically, involves the photochemical oxidation of water with evolution of oxygen in the presence of a suitable electron acceptor. Treatment of plants with carfentrazone-ethyl lead to the conclusion that minimum hill reaction activity (mg^{-1} chl h^{-1}), was found in GL 12021 (0.022) and maximum was detected in GL 10047 (0.089) (Table 4.7). While treatment with imazethapyr, GL 12021 (0.017) again showed least activity and PBG 5 (0.070) exhibited highest activity (Table 2). In untreated plants, GL 27023 and GL 12021 (0.096) displayed minimal effect and peaked in GL 13016 (0.140).

As the ability of chlorophyll a to split water in the reaction center of PSII680 decreased, the Hill reaction activity also decreased with increasing concentrations of a toxic substance (Ghasemi *et al* 2012).

4.3.4 Cellular respiration

Triphenyl tetrazolium chloride, TTC, or simply tetrazolium chloride (with the formula 2, 3, 5-triphenyl-2H-tetrazolium chloride) is aredox indicator commonly used in biochemical experiments especially to indicate cellular respiration. TTC is used to differentiate between metabolically active and inactive tissues. The white compound is enzymatically reduced to red TPF (1, 3, 5-triphenylformazan) in living tissues due to the activity of various dehydrogenases (enzymes important in oxidation of organic compounds and thus cellular metabolism), while it remains as white TTC in areas of necrosis since these enzymes have been either denatured or degraded. The extent of denaturation of dehydrogenase enzyme was comparatively higher for control than herbicidal treatment. The % reduction to formazon was found maximum in controlled conditions while its treatment

Table 4.7: Hill reaction activity (mg^{-1} chl h^{-1}), cellular respiration (%) and malondialdehyde content (µ moles g^{-1} dw) in leaves of chickpea genotypes at flowering stage

Genotypes	Hill reaction activity			Cellular Respiration			Malondialdehyde content		
	Control	Imazethapyr	Carfentrazone-ethyl	Control	Imazethapyr	Carfentrazone-ethyl	Control	Imazethapyr	Carfentrazone-ethyl
DKG 1030	0.108	0.035	0.057	28.6	25.5	24.5	8.96	8.60	16.88
JG 1362	0.125	0.023	0.048	32.3	19.8	19.9	9.52	21.19	12.26
GPF 2	0.128	0.04	0.074	41.1	30.8	23.0	7.92	14.17	16.87
GL 22044	0.131	0.031	0.056	28.6	23.4	21.9	7.18	10.93	12.52
GL 26016	0.134	0.027	0.030	33.3	27.6	17.5	7.02	11.40	13.87
GL 28202	0.115	0.041	0.061	83.0	31.2	15.1	8.44	8.61	24.22
GL 28203	0.124	0.029	0.060	31.2	20.1	19.7	4.94	28.01	10.98
GL 10047	0.121	0.068	0.089	32.8	32.6	30.9	6.84	11.96	10.08
GL 11026	0.104	0.065	0.046	40.2	34.1	23.0	6.11	11.26	11.47
GL 12003	0.103	0.042	0.08	52.7	27.4	26.0	10.17	13.48	16.31
GL 12021	0.096	0.017	0.022	44.2	20.3	17.5	8.87	17.62	21.01
GLW 44	0.126	0.058	0.049	37.4	33.4	24.7	8.23	10.48	12.28
GLW 187	0.103	0.035	0.068	29.4	26.4	24.1	10.18	13.57	16.77
GLW 188	0.085	0.034	0.062	36.6	28.1	26.8	7.08	10.63	16.44
GL 13016	0.140	0.023	0.036	30.3	29.9	20.5	9.90	12.83	15.62
GL 13022	0.101	0.052	0.039	29.1	25.7	21.5	7.59	13.91	14.45
PBG 1	0.123	0.038	0.066	34.7	29.5	23.4	14.10	14.95	11.21
PDG 4	0.111	0.042	0.081	31.2	28.5	31.8	9.69	11.27	10.78
PBG 5	0.115	0.070	0.061	60.9	35.4	18.1	8.76	9.13	10.19
FLIP-05-93C	0.102	0.031	0.054	62.3	31.6	23.9	7.63	10.64	12.53
GLW 11	0.107	0.023	0.082	63.1	27.1	29.1	8.12	12.87	10.53
DKG 876	0.109	0.029	0.073	25.3	22.7	17.3	7.55	15.47	11.21
GLW 125	0.114	0.047	0.032	46.5	22.5	15.8	6.62	10.60	28.27
GL 27023	0.096	0.059	0.034	63.8	23.6	13.8	7.76	11.44	31.27
Mean	**0.110**	**0.040**	**0.060**	**41.6**	**27.4**	**22.1**	**8.30**	**13.13**	**15.33**
CD (5%)	G=0.0004, T=0.001 G×T=0.022			G=0.60 , T=0.21, G×T=1.04			G=0.02,T=0.009,G×T=0.05		

with both the herbicides had shown a negative effect on cellular respiration. Highest respiration rate was shown by carfentrazone-ethyl treated plants in PDG 4 *i.e.,* 31.8% and least was shown in GL 27023 *i.e.,* 13.8% , On the other hand, in imazethapyr treated plants the maximum value observed was 35.4% for PBG 5 and minimum value noted was 19.8% for JG 1362. Whereas in control treatment, maximum and minimum values were 83% for GL 28202 and 25.3 % for DKG 876 respectively. Carfentrazone-ethyl and imazethapyr had a significant impact on all genotypes but had a minimal effect in control (Table 4.7). The reduced respiration rate after herbicide treatments may be explained by a lack of respiration substrates resulting from an impairment of photosynthetic assimilation.

Chodová and Zemánek (1971) reported that simazine herbicide gives a lower respiration rate as compared with the controls in the majority of cases, independent of the means of application of the herbicide.

4.3.5 Lipid peroxidation

The polyunsaturated fatty acids produced by membrane lipid peroxidation are degraded to form malondialdehyde (MDA) which has a deleterious effect on membrane lipids.Tolerance was a result of accumulation of antioxidants protecting membrane lipids against peroxidation. Malondialdehyde content was found to be highest under carfentrazone-ethyl treatment as compared to imazethapyr and control one (Table 4.7). However, under carfentrazone-ethyl treatment highest MDA content was observed in GL 27023 (31.27 µ moles g^{-1} dw) followed by GLW 125 (28.27 µ moles g^{-1} dw), GL 12021 (21.01 µmoles g^{-1} dw) while DKG 1030 (8.60 µmoles g^{-1} dw) showed maximum MDA among twenty four genotypes under imazethapyr treatment. In unsprayed plants, maximum was 14.10 µmoles g^{-1} dw in PBG 1 and minimum was 4.94 µ moles g^{-1} dw in GL 28203. Malondialdehyde (MDA) concentrations in chickpea genotypes remained relatively stable under control conditions.

Increased MDA levels may be due to the accumulation of Proto IX in the cytoplasm, which subsequently generates active oxygen species (AOS) upon light activation (Jung *et al* 2004). Changes in MDA concentration in a tissue is a good indicator of the structural integrity of the membranes of plants sensitive to protoporphyrinogen oxidase (PROTOX) - inhibiting herbicides (Loreto and Velikova 2001). It has been well known that MDA accumulation causes the oxidation of unsaturated fatty acids in plant membranes thereby impairing membrane permeability (Djebali *et al* 2005).

4.4 Yield and yield contributing attributes

Herbicide treatments affected yields significantly. The decrease in yield attributes and seed yield under these treatments may be because of the damage caused by herbicides. Due to better indendence of weeds in control, genotypes accumulate more dry matter, consequently greater translocation of photosynthtates to the reproductive parts and reflected in superiority of yield attributes and ultimately higher seed yield. A negative effect of herbicides on seed

yield was observed. Significant seed yield reduction was observed for both herbicides.

A negative effect of herbicides on seed yield was observed. Significant seed yield reduction was observed in sensitive genotypes irrespective of herbicide. The variation in yield can also be related to the recovery ability of the genotype against herbicide carfentrazone-ethyl. Irrespective of treatment, under carfentrazone-ethyl treatment, PDG 4 (14.0g) showed good response in terms of yield and least yield was found in GL 27023 (2.8g). PBG 5 exhibited maximum yield (5.5g/plant) and minimum yield was observed in GL 28203(1.4g/plant) under imazethapyr. For untreated plants maximum yield was 20.0g which was observed for genotype GL 28202, and minimum yield was 7.0g in GL 12021. Imazethapyr had a more pronounced effect on yield of chickpea plants as compared to carfentrazone-ethyl (Table 4.8).

The highest chickpea yield was recorded in weed free treatment and poor yield of chickpea was mainly due to toxic effect of imazethapyr on chickpea plants (Kumar *et al* 2015). Two hand weeding treatment registered the highest grain yield (1776 kg/ha), which was, however, on par with all imazethapyr treatments (Singh *et al* 2014). Similar findings were also reported by Taran *et al* (2013) in chickpea, in which they observed yield reduction and which was corresponded to the severity of visual injury symptoms. Wall (1996) reported that imazethapyr severely decreased lentil yields, also drawing attention to similar effects of imazamethabenz. In soybean, post-emergence herbicides caused yield loss upto 18% reported by Johnson (2002).

Maximum pods per plant under carfentrazone-ethyl treatment were 43 and minimum were 13, under imazethapyr treatment, maximum were 20 and minimum were 8, whereas in control, maximum were 57 and minimum were 22. Pods/plant is known to have significant positive correlation with grain yield in lentil (Singh *et al* 2009). Higher profitable pod yield of summer groundnut was also reported by Raj *et al* (2008) with keeping the crop in weed free condition. The results corroborate the findings of Vyas *et al* (2000) and Pandya *et al* (2005) and many others who reported enhanced soybean yield due to various weed control treatments. Pods per plant and 100-seed weight was higher in control as compared to other weed management treatments in soyabean (Peer *et al* 2013). Aboali and Saeerdipour (2015) reported that herbicide treatments had less pod numbers than free weed control the highest pod number per plant (18.75) was observed in weed free treatment.

Weed free treatment recorded the highest number of branches and pods per plant, 100-seed weight and also seed yield (0.98 t/ha) which were statistically at par with two hand weeding, pendimethalin fb pinoxaden and pendimethalin fb clodinafop (Kumar *et al* 2014). Among carfentrazone-ethyl treated plants, maximum 100-seed weight observed was 27.3 and

Table 4.8: Yield per plant (g), 100-seed weight (g) and pods/plant of chickpea genotypes

Genotypes	Yield per plant			100-seed weight			Pods per plant		
	Control	Imazethapyr	Carfentrazone-ethyl	Control	Imazethapyr	Carfentrazone-ethyl	Control	Imazethapyr	Carfentrazone-ethyl
DKG 1030	11.3	2.3	8.6	16.2	13.0	14.6	39	17	22
JG 1362	9.9	1.5	8.4	21.4	9.1	18.5	31	8	23
GPF 2	11.3	1.8	9.1	16.3	12.1	13.1	43	13	24
GL 22044	8.9	4.1	8.5	28.1	11.6	20.6	31	10	25
GL 26016	11.5	4.1	7.5	18.7	18.4	17.5	48	11	29
GL 28202	20.0	1.8	7.5	16.3	15.3	16.2	30	17	23
GL 28203	11.2	1.4	7.2	16.3	10.5	14.7	40	10	25
GL 10047	13.3	3.5	10.1	28.7	11.1	27.3	38	11	33
GL 11026	19.0	5.0	7.0	26.2	25.4	22.4	22	19	13
GL 12003	11.3	2.6	8.8	18.3	17.0	15.0	36	15	19
GL 12021	7.0	1.8	6.2	18.1	10.9	13.0	34	9	17
GLW 44	11.7	4.3	6.1	25.4	24.6	14.7	31	20	28
GLW 187	9.8	2.7	9.4	12.7	11.2	14.2	57	10	27
GLW 188	19.7	2.9	11.9	20.2	13.6	16.0	43	19	28
GL 13016	15.3	2.1	10.0	16.5	13.8	15.6	56	10	30
GL 13022	11.4	2.0	9.2	21.6	17.5	21.1	33	10	30
PBG 1	12.3	3.9	10.3	15.0	12.4	13.0	47	14	16
PDG 4	15.8	3.9	14.0	26.9	11.8	25.5	45	16	43
PBG 5	11.4	5.5	10.6	24.8	20.9	18.4	26	17	21
FLIP-05-93C	8.3	3.3	7.0	29.4	15.0	23.6	31	10	25
GLW 11	13.6	3.4	12.1	13.1	11.7	24.0	36	9	32
DKG 876	15.3	1.5	8.2	15.9	12.0	15.6	28	16	17
GLW 125	11.4	2.5	5.8	13.6	11.7	12.3	48	13	16
GL 27023	12.6	1.9	2.8	15.2	14.4	12.4	40	15	13
Mean	**12.6**	**2.9**	**8.6**	**19.8**	**14.4**	**17.5**	**38.0**	**13.3**	**24.1**
CD (5%)	G=0.25 , T=0.08, G×T=0.43			G=0.34, T=0.12, G×T=0.60			G=0.98,T=0.35,G×T=1.69		

minimum was 12.3, from imazethapyr treated plants, maximum was 25.4 and minimum was 9.1 and in control, maximum was 29.4 and minimum was 13.1. Hence, final yield was reduced due to low harvest indexes and reduced plant growth.

4.3 Biochemical traits (In leaves during reproductive phase)

4.3.1 Total soluble sugars

The accumulation of soluble sugars may indicate an adaptive response to both herbicides, as well as contributing to osmotic adjustment when plants were exposed to herbicidal stress. Royuela *et al* (2000) reported that Imazethapyr treatment leads to increase in leaf soluble carbohydrates. However, the decrease in soluble sugar concentrations

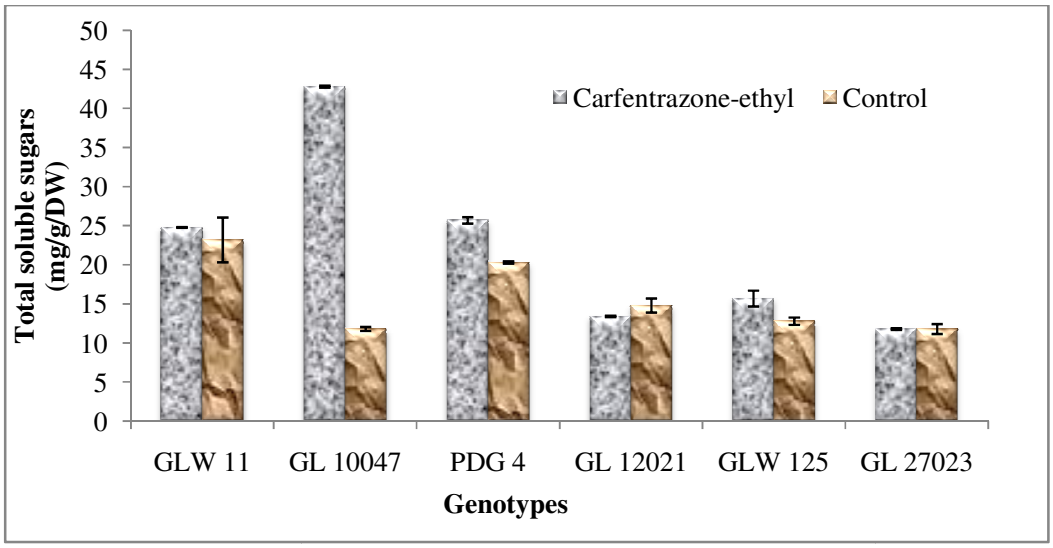

Fig. 4.2 Total soluble sugars in leaves of chickpea genotypes after the application of carfentrazone-ethyl. Vertical bars represent Standard Error (SE) from mean of replicates

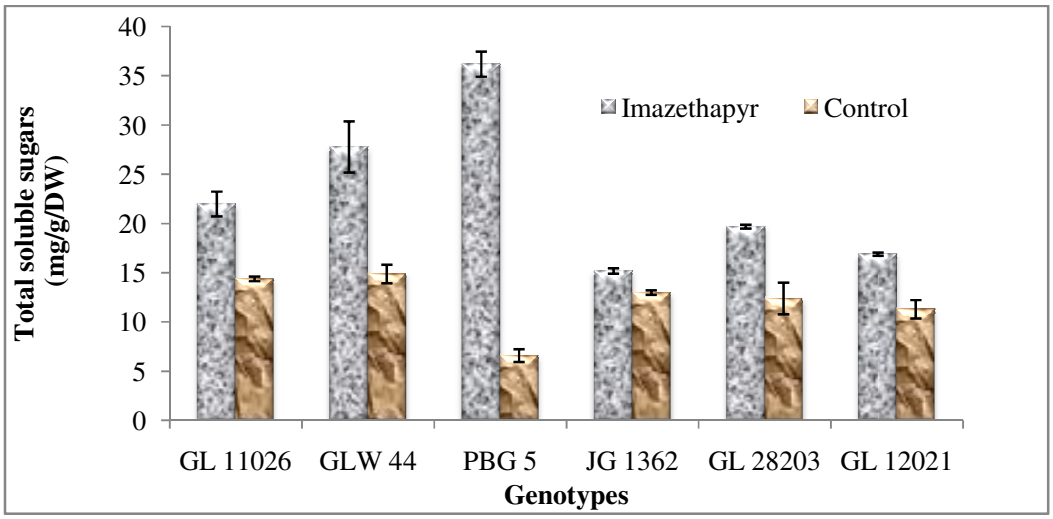

Fig. 4.3 Total soluble sugars in leaves of chickpea genotypes after the application of imazethapyr. Vertical bars represent Standard Error (SE) from mean of replicates

in leaves may also be associated with a reduction in photosynthetic activity. GL 10047 accumulated maximum sugars with carfentrazone-ethyl (Fig. 4.2) while PBG 5 showed highest total soluble sugars with imazethapyr treatment (Fig. 4.3). However, it can be assumed that the accumulation of carbohydrates was not being used for growth, maintenance, and translocation due to herbicidal applications. Amino acid accumulation has been observed in pea plants treated with lethal doses of glyphosate (Orcaray *et al* 2012). Similarly, an increase in free amino acid contents following ALS inhibition have been widely reported (Zabalza *et al* 2013).

4.3.2 Total soluble protein

The variation in protein content with herbicide application was very obvious (Fig 4.4 and Fig 4.5). GLW 11 (17.0 mg/g dw) showed higher accumulation of proteins with carfentrazone-ethyl treatment followed by GL 10047 and PDG 4 whereas GLW 44 (16 mg/g dw) exhibited more accumulation of soluble proteins with imazethapyr treatment. However, GLW 11 and GLW 44 showed tolerance to carfentrazone-ethyl and imazethapyt treatment respectively. Royuela *et al* (2000) also concluded that Imazethapyr caused a decrease in the soluble protein levels. Shabana *et al* (2001) also showed that protein content increased in alga treated with pendimethalin. Leaf soluble protein content in soyabean when treated with pendimethalin was significantly higher than weed-free check treatment. According to Zulet *et al* (2013), an increase in the amino acid content and decrease in the soluble protein content decrease are very well-known effects of herbicides inhibiting amino acid biosynthesis. The increased amino acid pool is thought to be derived from a rise in protein turnover,

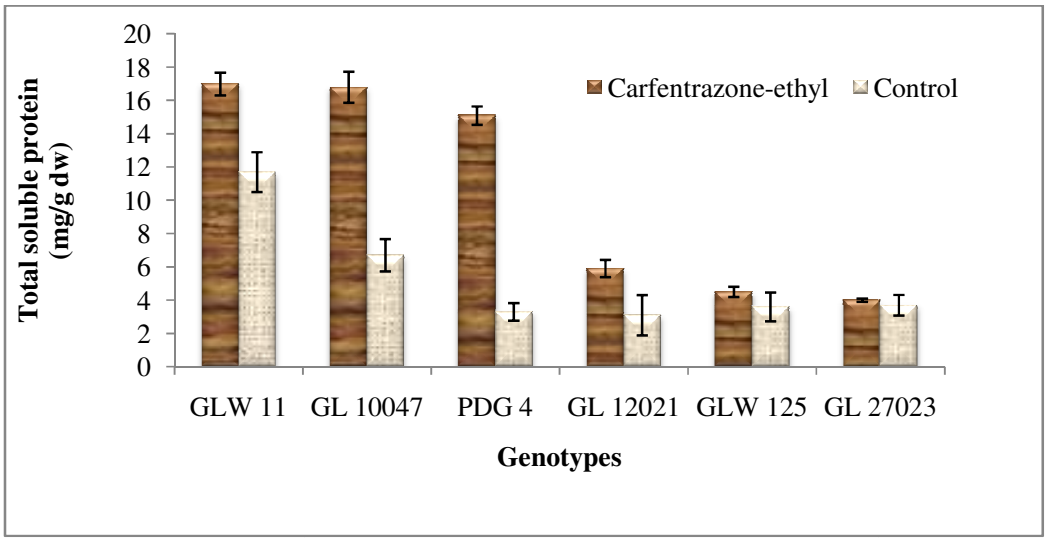

Fig. 4.4 Changes in total soluble protein of chickpea genotypes after carfentrazone-ethyl application. Vertical bars represent Standard Error (SE) from mean of replicates

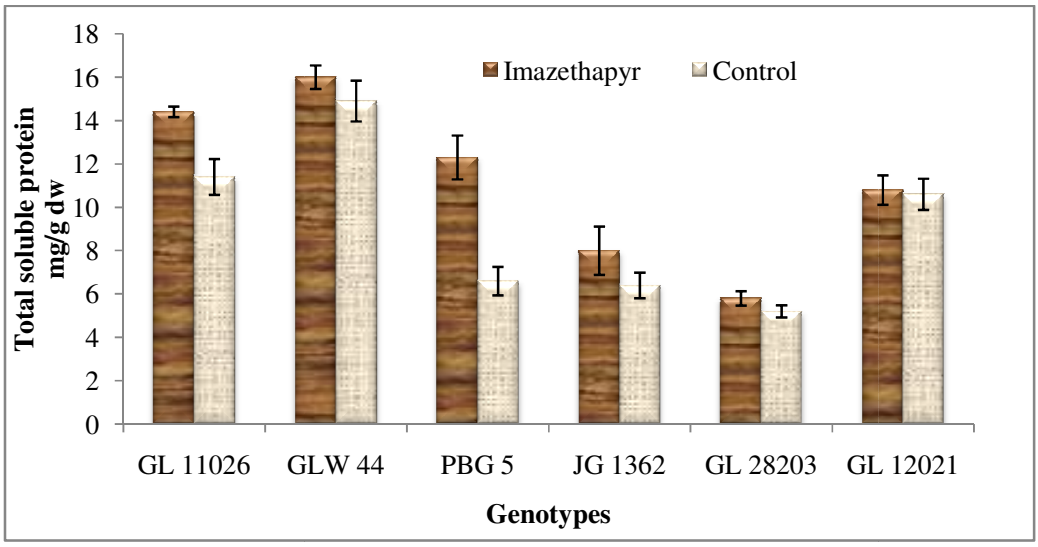

Fig. 4.5 Changes in total soluble protein of chickpea genotypes after imazethapyr treatment. Vertical bars represent Standard Error (SE) from mean of replicates

suggesting that proteases might be involved in protein degradation to provide plants with amino acids that cannot otherwise be synthesized due to herbicide inhibition. Further, they evaluated whether these increased free amino acid and decreased soluble protein contents were associated with major proteolytic activities.

4.3.3 Proline content

Proline plays a vital role in maintaining the osmotic balance in plants, so increases in free proline concentrations protected genotypes against the effects of both herbicides. Proline concentrations increased significantly when subjected to the herbicidal stress (Fig. 4.6 and 4.7).

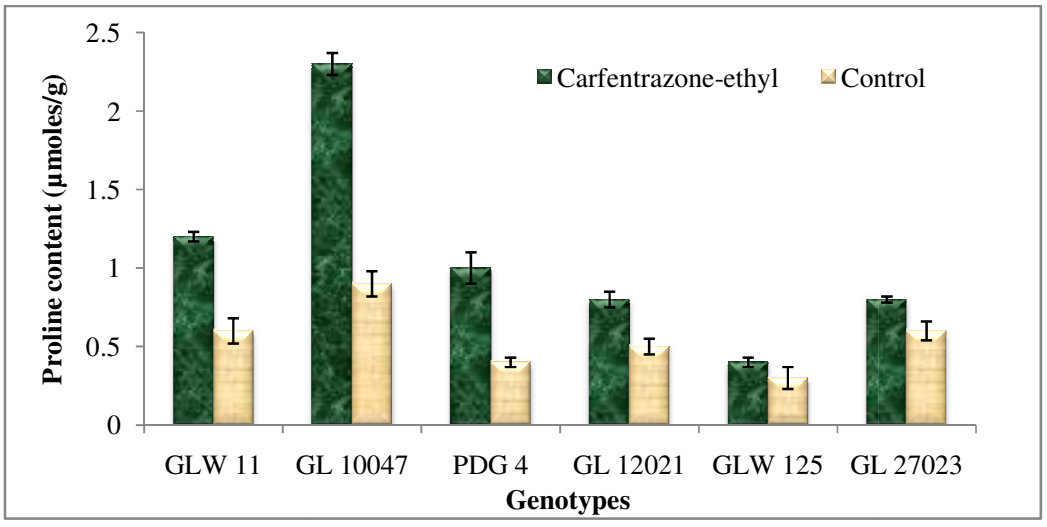

Fig. 4.6 Proline content in chickpea genotypes after application of carentrazone-ethyl. Vertical bars represent Standard Error (SE) from mean of replicates

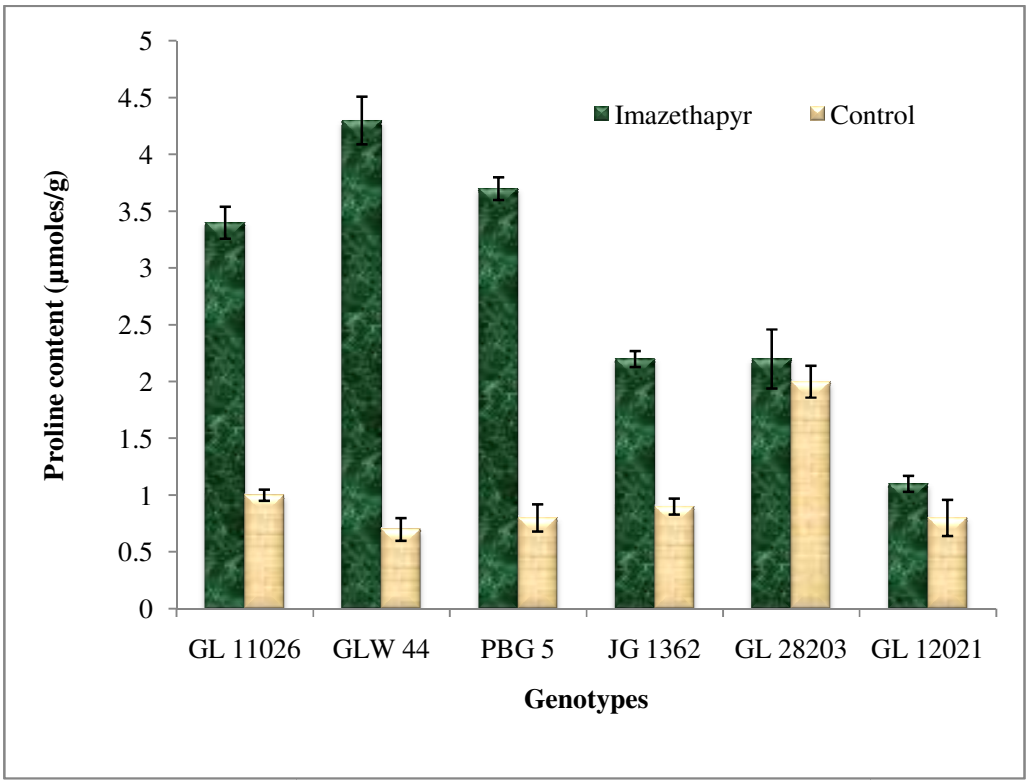

Fig. 4.7 Proline content in chickpea genotypes after imazethapyr treatment. Vertical bars represent Standard Error (SE) from mean of replicates

The maximum proline content was observed in GL 10047 (2.3) and minimum in GLW 125 (0.4) under carfentrazone-ethyl treatment, while with imazethapyr treatment, maximum proline content was observed in GLW 44 (4.3) and minimum in GL 12021 (0.8). Proline content was highest in the leaves of plants sprayed with the herbicides, which caused maximal electrolyte leakage. These results are similar to those of Zhang *et al* (2011) in which application of pesticide Omethoate significantly increased proline contents in wheat plants. Similarly, Du *et al* (2006) reported that proline content increased in rice plants treated with pesticide 1, 2, 4-trichlorobenzene. The present results suggest a positive link between herbicide toxicity and increased tissue proline and shows an adaptive role of proline in mitigating the damaging effects of herbicides.

4.5.4 Total free amino acids

GL 10047 (21.8) showed maximum amino acids (mg/g dw) with carfentrazone-ethyl treatment, showing tolerance and GLW 125 (7.5) found to be sensitive (Fig. 4.8), whereas under Imazethapyr treatment, maximum amino acids were observed in GL 11026 (20.6) and minimum in JG 1362 (6.2) presented in Fig. 4.9. It was observed that the genotypes showed high amino acids content and lesser protein content in plants. These results are in confirmation with results reported by Zulet *et al* (2013).

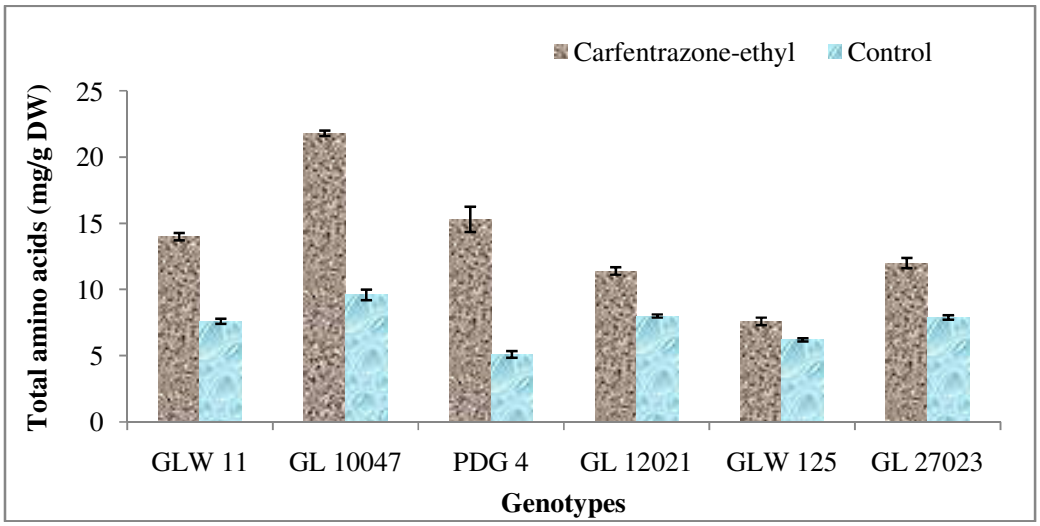

Fig. 4.8 Total free amino acids in chickpea genotypes following carentrazone-ethyl application. Vertical bars represent Standard Error (SE) from mean of replicates

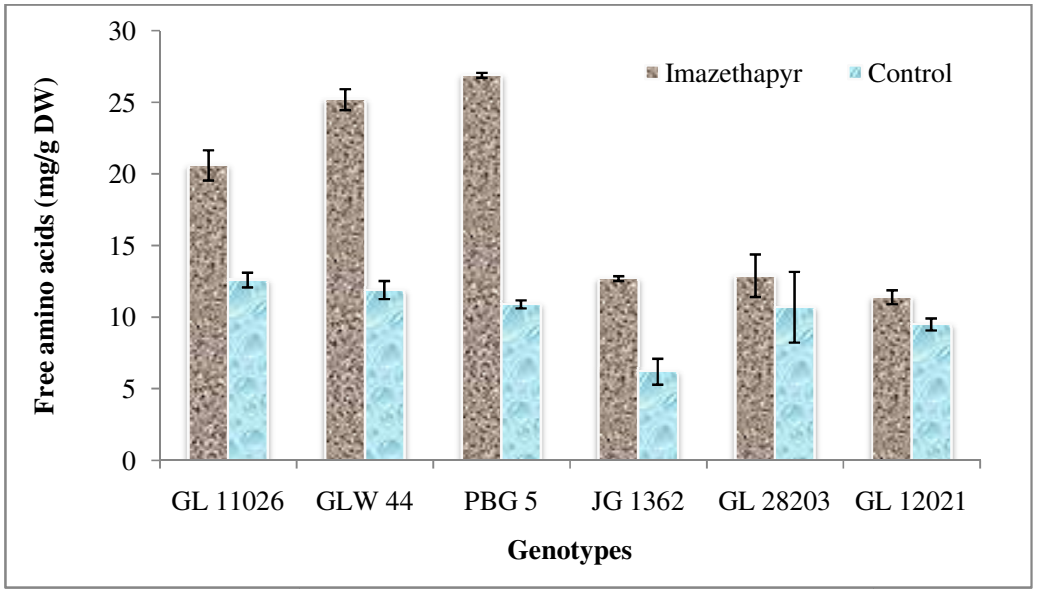

Fig. 4.9 Total free amino acids in chickpea genotypes following Imazethapyr application. Vertical bars represent Standard Error (SE) from mean of replicates

4.6 Anti-oxidant enzymes

4.6.1 Superoxide dismutase activity

Generally, herbicide treatment induced activity of antioxidative enzymes. Among Carfentrazone-ethyl treated plants, higher activity of SOD (Fig. 4.10) was found in tolerant genotype GLW 11 (269.0 unit enzyme/g FW) and decline in sensitive genotype GLW 125 (244.0 unit enzyme/g FW). In case of Imazethapyr treated plants, higher activity was showed by tolerant genotype PBG 5 (271.1 unit enzyme/g FW) and decrease in sensitive genotype GL 28203 (246.8 unit enzyme/g FW) presented in Fig. 4.11.

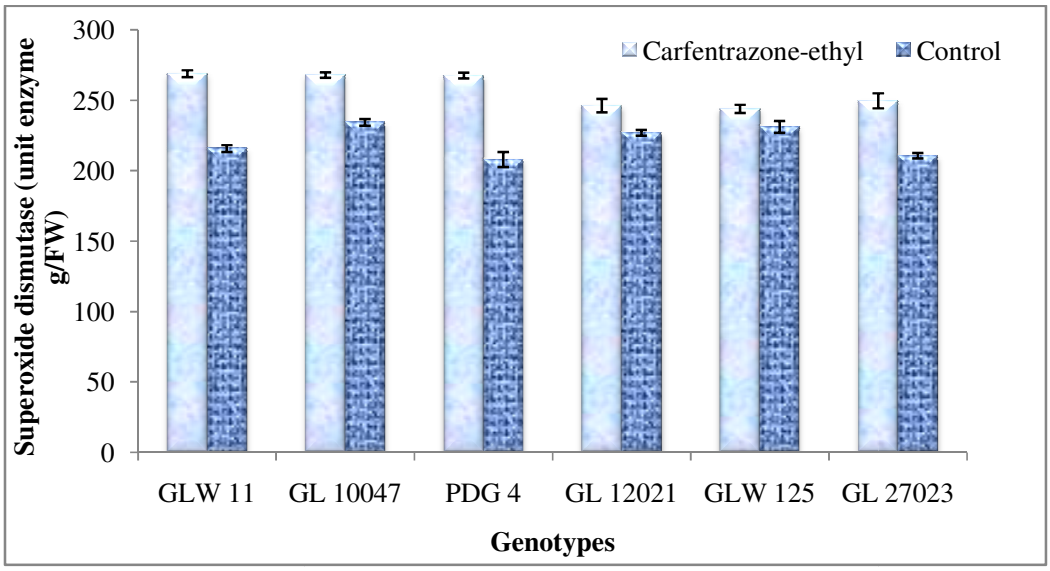

Fig. 4.10 **Superoxide dismutase activity in leaves of chickpea genotypes in response to carfentrazone-ethyl treatment. Vertical bars represent Standard Error (SE) from mean of replicates**

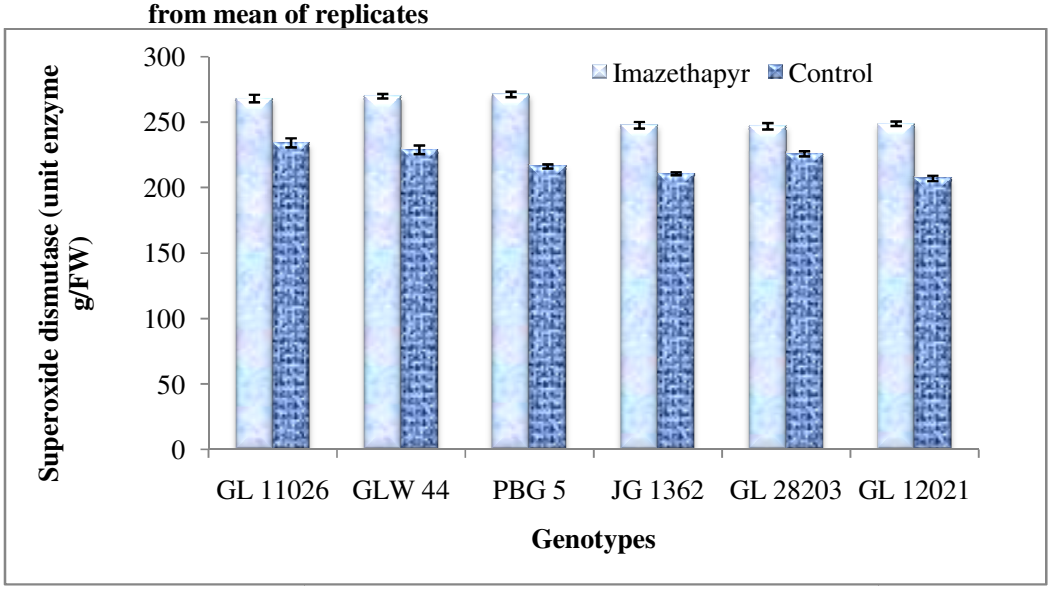

Fig. 4.11 **Superoxide dismutase activity after application of imazethapyr. Vertical bars represent Standard Error (SE) from mean of replicates**

Tang *et al* (2006) reported that foliar application of methamidophos herbicide increased the activities of superoxide dismutase (SOD) and catalase (CAT) in Chinese cabbage as compared to unsprayed control plants. Moreover, the activities of SOD, POD and CAT increased significantly in wheat and rice plants by applications of 1, 2, 4-trichlorobenzene and in bitter gourd to cope up with the biotic stresses by application of dimethoate (Mishra *et al* 2009).

4.6.2 Catalase activity

Catalase is an intracellular enzyme and it decomposes hydrogen peroxide to water and oxygen (García *et al* 2007). CAT scavenges H_2O_2 by breaking it down directly to form

water and oxygen, and an increase in its activity is related with increase in stress tolerance. Higher activity of catalase was exhibited in tolerant genotype GLW 11 (1204.9 ΔA/min/g FW) and lower in sensitive genotype GL 27023 (380.3 /min/g FW) under Carfentrazone-ethyl treatment (Fig. 4.12), whereas under Imazethapyr treated plants (Fig. 4.13), higher activity was found in tolerant genotype GL 11026 (1299.1 ΔA/min/g FW) and lesser in sensitive genotype JG 1362 (413.6 ΔA/min/g FW). Lü *et al* (2004) reported that quinclorac, an herbicide that stimulates the induction of 1-aminocyclopropane-1-carboxylic acid synthase activity, in turn promoting ethylene biosynthesis, induced an increase in both CAT and SOD activities.

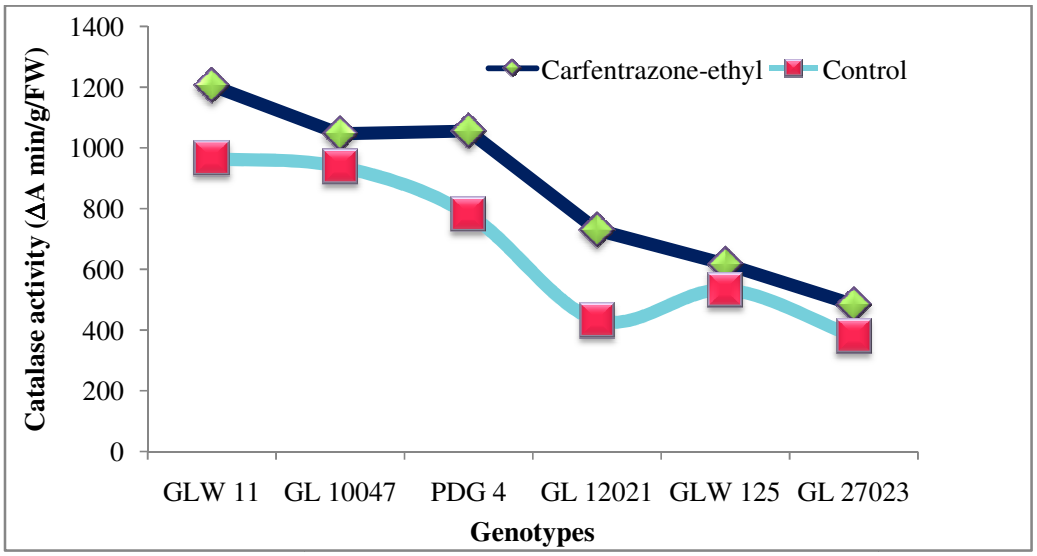

Fig. 4.12 Catalase activity in leaves of chickpea genotypes after carfentrazone-ethyl treatment. Vertical bars represent Standard Error (SE) from mean of replicates

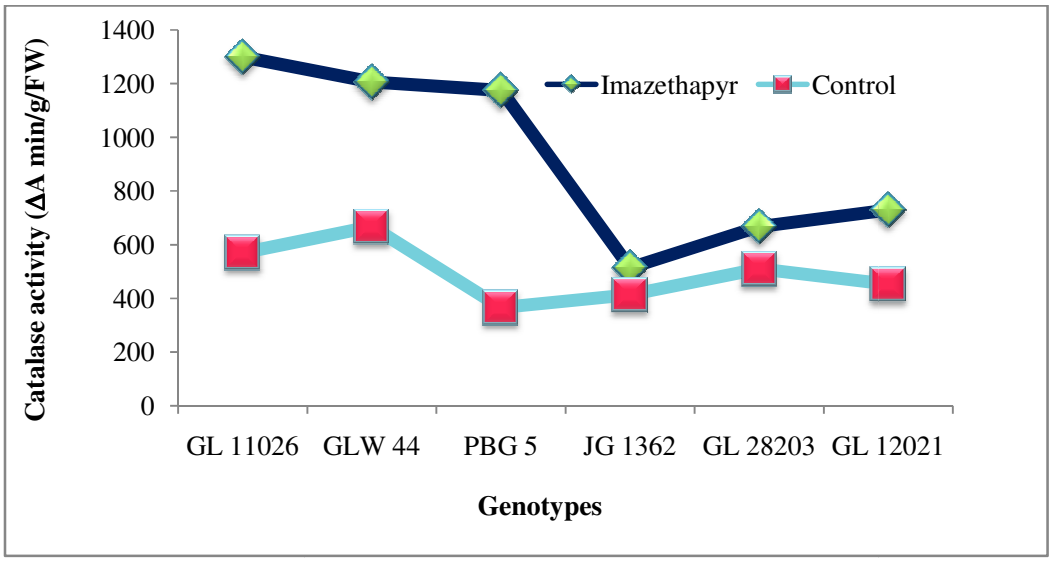

Fig. 4.13: Catalase activity in leaves of chickpea genotypes after imazethapyr treatment. Vertical bars represent Standard Error (SE) from mean of replicates

4.6.3 Peroxidase activity

Higher activity of POX was found in tolerant genotype GLW 11 (267.4 ΔA/min/g FW) and lower in sensitive genotype GLW 125 (79.6 /min/g FW) under Carfentrazone-ethyl treatment (Fig. 4.13), whereas under Imazethapyr treated plants (Fig. 4.14), higher activity was found in tolerant genotype GLW 44 (217.4 ΔA/min/g FW) and lesser in sensitive genotype GL 12021 (94.0 ΔA/min/g FW).

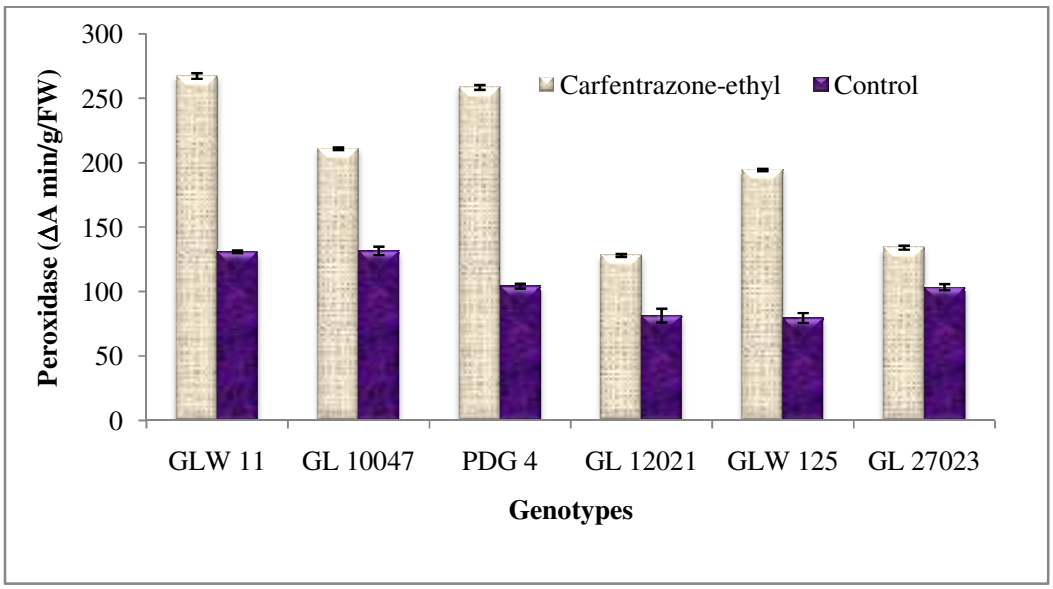

Fig. 4.14: Peroxidase activity in leaves of chickpea genotypes under carfentrazone-ethyl treatment. Vertical bars represent Standard Error (SE) from mean of replicates

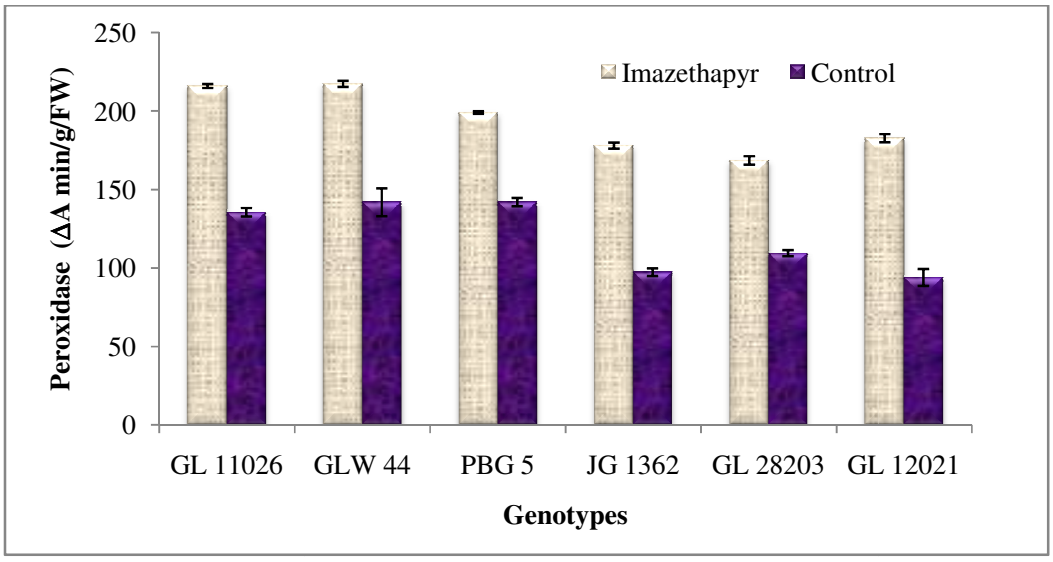

Fig. 4.15: Peroxidase activity in leaves of chickpea genotypes after imazethapyr treatment. Vertical bars represent Standard Error (SE) from mean of replicates

Rajabi *et al* (2012) reported that metribuzin increased peroxidase activity in wheat

indicating an induced oxidative stress in herbicidal treatments.

Physiological and morpho-physiological parameters are one of the important agricultural indices considered for the bifurcation into tolerant and sensitive genotypes. On the former basis, various biochemical trials were carried out in a good correlation with the present study. The integrated network of biochemical responses are one of the factors providing tolerance capacity to genotypes. These herbicide tolerant genotypes may be considered as donor for further breeding programs.

CHAPTER V

SUMMARY

Chickpea (*Cicer arietnum* L.) is a legume of the family *Fabaceae*, subfamily Faboideae. It is grown as winter sown crop in subtropics and tropics and as a spring grown crop in Mediterranean and temperate climates. Chickpea is a poor competitor to weeds due to slow growth rate at early stages of crop growth and establishment (Solh and Pala 1990). Weeds compete with chickpea plants for water, nutrients, sunlight, and space and also harbor insect-pests and diseases. If left uncontrolled, weeds can reduce chickpea yield significantly. The present study entitled, entitled "Physiological variations in herbicide tolerance among Chickpea (*Cicer arietinum* L.) genotypes" was conducted in Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana with the objectives (1) Screening of chickpea genotypes for herbicide tolerance under field conditions. (2) To investigate morpho-physiological and biochemical traits associated with herbicide tolerance.

The study comprised of two experiments viz., Experiment 1 and Experiment 2. The experiment 1 were undertaken in the laboratory and field area of Pulses Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. In the present study, 24 genotypes were taken for screening of herbicide tolerance.

In case of Experiment 1, screening for herbicide tolerance was carried out on the basis of phenological development, visual scoring, physiological development, morphological traits and yield attributes.

The phenological development was recorded for the traits like, days to flower initiation, days to 50% flowering, days to pod initiation and days taken to maturity. Results indicated that herbicides altered the phenological development whereas untreated plants complete its life cycle early in analogy to treated plants. Early flowering and maturation was accounted in GLW 44, PBG 5, GL 26016 under imazethapyr treatment and under carfentrazone-ethyl treatment, GLW 11, GL 10047 and PDG 4 induced early flowering and maturity among all the genotypes. There was significant reduction in morpho-physiological parameters such as plant height, leaf area index, biomass in GL 27023, GLW 125, GLW 12021 and GL 26016 with respect to carfentrazone-ethyl treatment. On the contrary, GL 10047, GL 11026, PDG 4 and GLW 11 lead to less reduction in morpho-physiological traits under carfentrazone-ethyl. The genotypes GL 11026, PBG 5, FLIP-05-93C and GLW 44 showed least reduction and higher reduction was observed in GL 28203, GL 12021 and JG 1362 with imazethapyr treatment.

Herbicides also exhibited significant effect on physiological processes of chickpea genotypes. Physiological parameters like photosynthetic rate, hill reaction activity, leghaemoglobin content, cellular respiration also showed decline with herbicide treatments.

The effect of carfentrazone-ethyl was least in GL 10047 and PDG 4 while more in GL 12021 and GLW 125. In case of imazethapyr treated plants, higher effect of herbicides was found in GL 28203, GL 12021 and DKG 876 while GL 11026, PBG 5 and GLW 44 had least effect of this herbicide on physiological development. Lipid peroxidation increases with increasing herbicide stress but the increment was higher in sensitive genotypes GLW 125 and GLW 28203 under carfentrazone ethyl treatment and instance to imazethapyr treatment, JG 1362 and GL 28203 exhibited higher malondialdehyde content.

Yield and yield attributes were reduced by herbicide stress in both herbicide treatments. Higher yield per plant was observed in PDG 4, GLW 11 and GLW 188 under carfentrazone-ethyl treatment while in case of imazethapyr higher yield was exhibited by PBG 5, GL 11026 and GLW 44. Maximum numbers of pods per plant and 100-seed weight was observed in GL 10047, PDG 4, GLW 11 and FLIP-05-93C under carfentrazone-ethyl treatment and in GL 11026, GLW 44 and PBG 5 among imazethapyr treated plants.

With the exposure of herbicide stress, a number of biochemical changes take place in chickpea. On the basis of all the above observations, six genotypes from each treatment were selected (three tolerant and three sensitive). Proline concentration hiked significantly under herbicide treatments. Proline is an osmoprotectant under any stress conditions, its concentration increases in plants. The capstone genotypes were GLW 11, GL 10047 and PDG 4 which showed a significant increase in proline accumulation and least addendum was found in GLW 125 followed by GL 12021 and GL 27023 with carfentrazone-ethyl effect while with imazethapyr, GLW 44, PBG 5 and GL 11026 showed pronounced effect and JG 1362, GL 28203 and GL 12021 showed less accumulation of proline. Total soluble sugars and free amino acids in leaves accumulated more in tolerant genotypes GL 10047, PDG 4 and GLW 11 and least accumulation in sensitive genotypes GLW 125, GL 12021 and GL 27023 among carfentrazone-ethyl treated plants was observed. In case of imazthapyr treatment, maximum total soluble sugars and total free amino acids in leaves were recorded in PBG 5, GLW 44 and GL 11026 while minimum were found in GL 28203, GL 12021 and JG 1362. Total soluble protein declined in analogy to free amino acids but were higher from untreated plants. A similar trend was exhibited by carfentrazone-ethyl and imazethapyr treated plants for total soluble sugars and free amino acids. Among the antioxidative enzymes, higher activity of CAT, SOD and POD were observed in tolerant genotypes as a result of defence mechanisms induced by plants under stress conditions. SOD activity was found to be higher in GLW 11 and least in GLW 125 with carfentrazone-ethyl treatment while with imazethapyr treatment, higher SOD activity was exhibited by GLW 44. The significant differences in CAT and POD activity was also recorded.

Based on the results of all the parameters undertaken in the present investigation, GL 10047, PDG 4 and GLW 11 were found to be the most tolerant and GLW 125, GL 12021 and

GL 27023 were considered to be sensitive against carfentrazone-ethyl. In case of Imazethapyr, PBG 5, GLW 44 and GL 11026 were found to be tolerant and GL 28203, GL 12021 and JG 1362 were sensitive. GLW 125, GL 12021, GL 27023 GL 28203 and JG 1362 were considered as sensitive to herbicidal stress. GL 12021 was found to be sensitive to both of herbicides.

To summarize, all the plant traits including phenological, morphological, physiological, biochemical and yield attributes pitch in selecting the genotypes for post-emergence herbicide tolerance which can be used as a donor in breeding programme.

REFERENCES

Abbo S, Molina C, Jungma N N R, Grusa K A, Berkovitch Z, Reifen R, Kahl G, Winter P and Reifen R (2005) Quantitative trait loci governing carotenoid concentration and weight in seeds of chickpea (*Cicer arietinum* L.). *Theor Appl Genet* **111**:185-95.

Aboali Z and Saeedipour S (2015) Efficacy Evaluation of some Herbicides for Weed Management and Yield Attributes in Broad Bean (*Vicia faba*). *Res J Environ Sci* **9**: 289-95.

Aggarwal N, Singh G, Ram H and Khanna V (2014) Effect of post-emergence application of imazethapyr on symbiotic activities, growth and yield of blackgram (*Vigna mungo*) cultivars and its efficacy against weeds. *Indian J Agron* **59**: 421-26.

Anderson A, Baldock J A, Rogers S L, Bellotti W and Gill G (2004) Influence of Chlorsulfuron on Rhizobial Growth, Nodule Formation, and Nitrogen Fixation with Chickpea. *Aust J Agric Res* **55**: 1059- 70.

Angiras N N and Rana M C (1995) Dose and time of application of imazethapyr for weed control in soybean (*Glycine max*). *Indian J Agron* **40**: 59-63.

Ansar M, Anwar A, Arif M, Nadeem M and Zahid A (2010) Screening of pre and post-emergence herbicides against chickpea (*Cicer arietinum* L.) weeds under semi rainfed conditions of Pothohar, Pakistan. *Pak J Weed Sci Res* **16**: 421-30.

Arif M, Mukhtar T, Rahman S U, Hussain K, Razaq A and Iqbal R A (2011) Efficacy of different herbicides against weeds in maize (*Zea mays* L.). *Pak J Weed Sci Res* **17**:125–33.

Arnold N R, Murray W M, Gregory J E and Smeal D (1993) Weed Control in Pinto Beans (*Phaseolus vulgaris*) with Imazethapyr Combinations. *Weed Technol* **7**: 361-64.

Ashraf M and Foolad M R (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ Exp Bot* **59**: 206-16.

Ashton F M (1976) Mobilization of storage proteins of seeds. *Ann Rev Plant Physiol* **27**: 95-117.

Baghestani M A, Zand E, Soufizadeh S, Bagherani N and Deihimfard R (2007) Weed control and wheat (*Triticum aestivum* L.) yield under application of 2,4- D plus carfentrazone-ethyl and florasulam plus flumetsulam: Evaluation of the efficacy. *Crop Prot* **26**: 1759-64.

Bahn V M and Kukula S (1987) Weeds and their control in chickpea. *In:* The Chickpea (eds. Saxena M C and Singh K B), pp. 319-28. CAB International, Wallingford.

Basu T K and Sengupta A (2012) Efficacy of new herbicide molecule imazethapyr on weed control in soybean. *J Crop Weed* **8**: 137-40.

Bates L S, Waldren R P and Teare I D (1973) Rapid determination of proline for water stress studies. *Plant Soil* **39**: 205-07.

Benmoussa M, Vezina L P, Page M, Yelle S and Laberge S (2000) Genetic polymorphism in low molecular- weight glutenin genes from *Triticum aestivum* variety Chinese Spring. *Theoret Appl Genet* **100**: 789-93.

Bigot A, Fontaine F, Clement C and Vaillant-Gaveau N (2007) Effect of the herbicide flumioxazin on photosynthetic performance of grapevine (*Vitis vinifera* L.). *Chemosphere* **67**: 1243-51.

Blackshaw R E and Esau R (1991) Control of Annual Broadleaved Weeds in Pinto Beans (*Phaseolus vulgaris*).*Weed Technol* **5**: 532-38.

Bonjoch N P and Tamayo P R (2001) Protein content quantification by Bradford method, in Roger MJR. ed. Handbook of plant ecophysiology techniques. Kluwer Academic Publishers. Pp 283-95.

Chance M and Maehly A C (1955) Assay of catalase and peroxidases. *Methods Enzymol* **2**:764-75.

Cheema M S and Akhtar M (2005) Efficacy of different post-emergence herbicides and their application methods in controlling weeds in wheat. *Pak J Weed Sci Res* **11**: 23-30.

Chen C and Dickman M B (2005) Proline suppresses apoptosis in the fungal pathogen *Colletotrichum trifolii.* PNAS **102**: 3459-64.

Cherry J H (1973) *Molecular biology of plants, a text manual*. pp 1-204. Colombia University Press, New York, USA.

Chin D V and Pandey J (1991) Effect of pre and post-emergence herbicides on weeds and yield of blackgram (*Phaseolus mungo*) *Indian J Agron* **36** (Suppl.): 276-77.

Chodová D and Zemánek J (1971) Effect of the herbicides MCPA and simazine on the respiration rate and content of glycides and nitrogen in bindweed (*Convolvulus arvensis* L.) *Biologia Plantarum* **13**: 234–42.

Choudhary V K, Kumar S P and Bhagawati R (2012) Integrated weed management in blackgram (*Vigna mungo*) under mid hills of Arunachal Pradesh. *Indian J Agron* **57**: 382-85.

Cobb A (1992) Herbicides that inhibits photosynthesis In: Cobb A (ed) *Herbicides and Plant Physiology*. pp 46-80. Chapman and Hall, London.

Dana N, Shenkoru T, Tegegne A (2000) Growth rates and testicular characteristics of Ethiopian high land sheep offered chickpea haulm supplemented with incremental levels of *Leucaena leucocephala* leaf hay. *Livest Prod Sci* 65:209–217.

Datta A, Sinde B, Jessop R, Birchall C and Felton W (2006) Differential response of chickpea genotypes to isoxaflutole. *Commun Agric Appl Biol Sci* **71**:733-42.

Dayan F E, Duke S O, Weete J D and Hancock H G (1997) Selectivity and mode of action of carfentrazone-ethyl, a novel phenyl triazolinone herbicide. *Pestic Sci* **51**:65-73.

Desikin R, Soheila A H, Hancock J T and Neill S J (2001) Regulation of the *Arabidopsis* transcriptome by oxidative stress. *Plant Physiol* **127**:159–72.

Devine M D and Preston C (2000) Herbicides and their Mechanisms of Action. In: Cobb A H and Kirkwood R C (ed) *The molecular basis of herbicide resistance*. pp 72–104. CRC Press, Boca Raton, FL.

Djebali W, Zarrouk M, Brouquisse R, El-Kahoui S, Limam F, Ghorbel M H and Chaibi W (2005) Ultrastructure and lipid alterations induced by cadmium in tomato (*Lycopersicon esculentum*) chloroplast membranes. *Plant Biol* **7**: 258-68.

Du Q P, Jia X S and Yuan B H (2006) Toxic effects of 1,2,4- trichlorobenzene on rice seed germination and seedling growth. *Chinese J Appl Ecol* **17**: 2185-88.

Dubois M, Gilles K A, Hamilton J K, Roberts P A and Smith F (1956) Calorimetric methods for the determination of sugars and related substances. *Analyt Chem* **28**: 350-56.

Dvorak J and Remesova I (2002) Assessment of metribuzin effects on potatoes using a method of very rapid fluorescence induction. *Rostlinna Vyroba* **48**: 107-17.

Egli M A, Low D, White K R and Howard J A (1985) Effect of herbicide and herbicide analogue on C-14 leucine incorporation by suspension cultured *Solanum nigrum* cells. *Pestic Biochem Physiol* **24:** 112-118.

Esterbauer H and Cheeseman K H (1990) Determination of aldehydic lipid peroxidation products: Malonialdehyde and 4-Hydroxynonenal. *Methods Enzymol* **186**: 407-21.

FAOSTAT (2015) http://www.fao.org

Fayed T B, Sabry S R S and Aboul E S H (1998) Effect on wild oat (*Avena fatua*) herbicides on weed density, wheat grain yield and yield components. *Ann Agric Sci* **43**: 173-88.

García N A T, Iribarne C, Palma F and Lluch C (2007) Inhibition of the catalase activity from Phaseolus vulgaris and Medicago sativa by sodium chloride. *Plant Physiol Biochem* **45**: 535–41.

Garg N and Manchanda G (2009) ROS generation in plants: Boon or bane? *Plant Biosystems:* **143**: 81-96.

Garg R, Shankar R, Thakkar B, Kudapa H, Krishnamurthy L, Mantri N, Varshney R K, Bhatia S and Jain M (2016) Transcriptome analyses reveal genotype- and developmental stage-specific molecular responses to drought and salinity stresses in chickpea. Scientific Reports 6, Article number: 19228.

Gaston S, Zabalza A, Gonza´ lez EM, Arrese-Igor C, Aparicio-Tejo P M and Royuela M (2002) Imazethapyr, an inhibitor of the branched-chain amino acid biosynthesis, induces aerobic fermentation in pea plants. *Physiol Plantarum* **114**:524-32.

Gaur P M, Jukanti A K, Samineni S, Chaturvedi S K, Singh S, Tripathi S, Singh I, Singh G, Das T K, Aski M, Mishra N, Nadarajan N and Gowda C L L (2013) Large Genetic Variability in Chickpea for Tolerance to Herbicides Imazethapyr and Metribuzin. *Agron J* **3**: 524-36.

Ghasemi F, Heidari R, Jameii R and Purakbar L (2012) Effects of Ni2þ toxicity on Hill reaction and membrane functionality in maize. *J Stress Physiol Biochem* **8**: 5561.

Ghosh R, Nagavardhini A, Sengupta A and Sharma M (2015) Development of Loop-Mediated Isothermal Amplification (LAMP) assay for rapid detection of *Fusarium oxysporum* f. sp. ciceris - wilt pathogen of chickpea. *BMC Res Notes* **8**:40

Goud V V, Murade N B, Khakre M S and Patel A N (2013) Efficacy of imazethapyr and quizalofop-ethyl herbicides on growth and yield of chickpea. *The Bioscan* **8**:

1015–18.

Hanson B D and Thill D C (2001) Effects of imazethapyr and pendimethalin on lentil (*Lens culinaris*), pea (*Pisum sativum*), and a subsequent winter wheat (*Triticum aestivum*) crop. *Weed Technol* **15**: 190-4.

Hanson B D, Frandrich L, Shaner D L, Westra P and Nissen S J (2007) Recovery of imidazolinone-resistant hard red wheat lines following imazamox application. *Crop Sci* **47:** 2058-66.

Haroun S A (2002) Influence of site of Oxyfluorfen application on growth, pigments, photosynthesis and yield attributes of *Glycine max* plant. *Pakistan J Biol Sci* **5**: 292-96.

Heath R L and Packer L (1968) Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation. *Archiev Biochem Biophys* **125**: 189-98.

Hekmat S, Shropshire C, Soltani N and Sikkema P H (2007) Responses of dry beans (*Phaseolus vulgaris* L.) to sulfentrazone," *Crop Protection* **26**: 525–29.

Holá D, Kočová M, Rothová O, Chodová D and Mikulka J (2004) The effect of low growth temperature on Hill reaction and Photosystem 1 activities in three biotypes of *Kochia scoparia* L. Schrad. with different sensitivity to atrazine and ALS-inhibiting herbicides. *Plant Soil Environ* **50**: 10-17.

Hoseiny-Rad M and Jagannath S (2011) Effect of herbicide Imazethapyr (Imazethapyr) on chickpea seed germination. *Arch Phytopath Plant protec* **3**: 224-30.

Hossain M I, Barma D, Islam M R, Uddin M Z and Rahman M (2008) Effect of newly developed herbicides on the growth and yield of wheat. *Int J Sustainable Crop Prod* **4**(1):1-4.

Johnson B F (2002) Herbicide effects on visible injury, leaf area, and yield of glyphosateresistant soybean. *Weed Technol* **16**:554-66.

Jung S, Lee Y, Yang K, Lee S B, Jang S M, Ha S B and Back S B (2004) Dual targeting of Myxococcus xanthus protoporphyrinogen oxidase into chloroplasts and mitochondria and high level oxyfluorfen resistance. *Plant Cell Environ* **27**: 1436–46.

Khair Eldin A, Galil A and Elhassan G A (1980) Weed Control in Sorghum, Kenana Research Station *Annual Report*, 52-56.Agricultural Research Corporation, Ministry of Agriculture, Sudan.

Khan I A, Hassan G and Ihsanullah (2003) Efficacy of pre-emergence herbicides on the yield and yield components of canola. *Asian J Plant Sci* **2**: 251-53.

Khan I A, Hassan G and Marwat K B (2008) Interaction of wild oats (*Avena fatua* L.) with spring wheat (*Triticum aestivum* L.) seeded at different rates. *Pak J Bot* **40**: 1163-67.

Khan I A, Khan M A, Khan I, Imran M, Idrees M and Bibi S (2013) Effect of different herbicides and plant extracts on yield and yield components of wheat (*Triticum aestivum* L.). *Pak J Bot* **45**: 981-85.

Khan M A and Marwat K B (2006) Impact of crop and weed densities on competition between wheat and *Silybum marianum* gaertn. *Pak J Bot* **38**: 1205-15.

Khan M S, Chaudhry P, Wani P A and Zaidi A (2006) Biotoxic effects of the herbicides on growth, seed yield, and grain protein of greengram. *J Appl Sci Environ Manag* **10**: 141-46.

Khan M S, Zaidi A and Aamil M (2004) Influence of herbicide on chickpea *Mesorhizobium* symbiosis. *Agronomie* **24**:123-27.

Khan N A and Singh S (Eds.) (2008) Abiotic Stress and Plant Responses, IK International, New Delhi.

Khan N U, Khan S U and Hassan G (2004) Herbicides effect on weed flora and grain yield of zero vs. conventional tillage wheat. Paper read at 4th Int. weed *Sci Congr* (IWSC), Durban, South Africa. S13MT17P04, Abstt. No. 300.

Kielak A E, Sempruch B C, Mioduszewska A H, Klocek A J, Leszczyn´ B and Ski B (2011) Phytotoxicity of Roundup Ultra 360 SL in aquatic ecosystems: Biochemical evaluation with duckweed (*Lemna minor* L.) as a model plant. *Pesticide Biochem Physiol* **99**: 237–43.

Knights E (1991) Chickpea. In: Jessop R S and Wright R L (ed) *New Crops: Agronomy and Potential of Alternative Crop Species.* pp 27-38. Intaka Press, Melbourne, Australia.

Kolkman J M, Slabaugh M B, Bruniard J M, Berry S, Bushman B S and Olungu C (2004) Acetohydroxyacid synthase mutations conferring resistance to imidazolinone or sulfonylurea herbicides in sunflower. *Theor Appl Genet* **109**:1147-59.

Kraus T E and Fletcher A (1994) Palclobutrazol protects wheat seedlings from heat and paraquat injury, is detoxification of active oxygen species involved? *Plant Cell Physiol* **35**:45–52.

Kumar N, Hazra K K, Yadav S L and Singh S S (2015) Weed management using post-emergence herbicides in chickpea (*Cicer arietinum*) + mustard (*Brassica juncea*) intercropping system. *Indian J Agric Sci* **85**: 1074-79.

Kumar N, Nandal D P and Punia S S (2014) Weed management in chickpea under irrigated conditions. *Indian J Weed Sci* **46**: 300-01.

Kumar S, Angrias N N and Rana S S (2010) Evaluation of pinoxaden in combination with metsulfuron methyl against complex weed flora in barley. 94p. In: *Proceedings of national biennial conference,* Indian Society of Weed Science, Raipur, February 25

Larik A S, Rajput M, Kakar A A, Bukhari S S and Shaikh M A (1999) Effect of weedicide afaon on character association in *Brassica juncea* and *Eruca sativa*. *Sarh J Agric* **15**: 198-202.

Lee Y P and Takahashi T (1966) An improved colorimetric determination of amino acids with use of ninhydrin. *Anal Biochem* **14**:71-77.

Little D L and Shaner D L (1991) Absorption and translocation of the imidazolinone herbicides. In: Shaner D L and Connor O (ed) *The Imidazolinone Herbicides.* pp 53-69.CRC Press, Boca Raton, FL.

Loreto F and Velikova V (2001) Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiol* **127**: 1781–87.

Lowry O H, Rosebrough N T, Farr A L and Randall R J (1951) Protein measurement with folin phenol reagent. *J Biol Chem* **193**: 265-75.

Lü Z,Min H and Xia Y (2004) The response of *Escherichia coli*, *Bacillus subtilis*, and *Burkholderia cepacia* WZ1 to oxidative stress of exposure to quinclorac. *J Environ Sci Health* **39**: 431-41.

Lukatkin A S (2003) Contribution of oxidative stress to the development of cold-induced damage to leaves of chilling-sensitive plants. Injury of cell membranes by chilling temperatures. *Russian J Plant Physiol* **50**: 243–46.

Lyon D J and Wilson R G (2005) Chemical weed control in dryland and irrigated chickpea. *Weed Technol* **19**: 959-65.

Lyon D J, Kniss A and Miller S D (2007) Carfentrazone Improves Broadleaf Weed Control in Proso and Foxtail Millets. *Weed Technol* **21**:84–87

Malefyt T and Quakenbush L S (1991) Influences of environmental factors on the biological activity of the imidazolinone herbicides. In: Shaner D L and Connor O (ed) *The Imidazolinone Herbicides* pp 103-27.CRC Press, Boca Raton, FL.

Mallikarjuna N, Sharma H C and Upadhyaya H D (2007) Exploitation of wild relatives of pigeonpea and chickpea for resistance to *Helicoverpa armigera. E J SAT Agric Res Crop Improv* **3**:4-7.

Manzoor A A and Goutam K P (2014) Effect of carbofuran on the formation of some biomolecules in brinjal (S*olanum melongena* L.) leaf. *The Bioscan* **9**: 959-63.

Marenco R A (1993) Efeito de herbicidas sobre a fixaçao de nitrogênio, crescimento e partiçao de assimilados em soja (*Glycine max* (L.) Merrill). Viçosa, UFV, 137p.Tese DS.

Marklund S and Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* **47**:469-74.

Marwat K B, Hussain Z and Saeed M (2005) Chemical weed management in wheat at higher altitudes. *Pak J Weed Sci Res* **11**: 102-07.

Marwat K B, Hussain Z, Khan N I and Gul B (2003) Impact of weed management on rapeseed. *Pak J Weed Sci Res* **9**: 207-14.

Masson J A and Webster E P (2001) Use of imazethapyr in water-seeded imidazolinone-tolerant rice (*Oryza sativa*). *Weed Technol* **15**:103-106.

Mateos-Naranjo E, Redondo-Gomez S, Cox L, Cornejo J and Figueroa M E (2009) Effectiveness of glyphosate and imazoamox on the control of the invasive cordgrass Spartina densiflora. *Ecotoxicol Environ Saf* **72**: 1694–1700.

Matters G L and Scandalios J G (1986) Effect of the free radical-generating herbicide paraquat on the expression of the superoxide dismutase (Sod) genes in maize. *Biochem Biophys Acta* **882**:29–38.

Menconi M, Sgherri C L M, Pinzino C and Navari-Izzo F (1995) Activated oxygen production and detoxification in wheat plants subjected to a water deficit programme. *J Exp Bot* **46**: 1123-30.

Mishra J S and Chandrabhanu C (2006) Effect of herbicides on weeds, nodulation and growth of rhizobium in summer black gram (*Vigna mungo*). *Indian J Weed Sci* **38**: 150-53.

Mishra J S, Moorthy B T S and Bhan M (2005) Efficacy of herbicides against field dodder (*Cuscuta campestris*) in lentil, chickpea and linseed. *Indian J Weed Sci* **37**:220-24.

Mishra V, Srivastava G and Prasad S M (2009) Antioxidant response of bitter gourd (*Momordica charantia* L.) seedlings to interactive effect of dimethoate and UVB irradiation. *Sci Hortic* **120**:373-78.

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. *Trends Plant Sci* **9:** 490-98.

Miyazawa Y and Yahata H (2006) Is the parameter electron transport rate useful as apredictor of photosynthetic carbon assimilation rate? *Bull Inst Trop Agric Kyushu Univ* **29**: 23–53.

Mohamed E S, Nourai A H, Mohamed G E, Mohamed M I and Saxena M C (1997) Weeds and weed management in irrigated lentil in Northern Sudan. *Weed Res* **37:** 211-18.

Nandan B, Sharma B C, Kumar A and Sharma V (2011) Efficacy of pre and post-emergence herbicides on weed flora of urdbean under rainfed subtropical Shiwalik foothills of Jammu & Kashmir. *Indian J Weed Sci* **43**:172–74.

Nechet K L, Vieira B S, Barreto R W, Mizubuti E S G and Silva A A (2008) Combination of a mycoherbicide with selected chemical herbicides for control of *Euphorbia heterophylla. XII International Symposium on Biological Control of Weeds*. pp 693-698.

Newsom L J and Shaw D R (1995) Soybean (*Glycine max*) response to AC 263,222 and chlorimuron as influence by soil moisture. *Weed Technol* **9**: 553–60.

Nirala H, Choubey N K and Bhoi S (2012) Performance of postemergence herbicides and hand weedings with respect to their effects on weed dynamics and yields of blackgram (*Vigna mungo* L.). *Int J Agri Stat Sci* **8**: 679-89.

Orcaray L, Zulet A, Zabalza A and Royuela M (2012) Impairment of carbon metabolism induced by the herbicide glyphosate. *J Plant Physiol* **169**: 27–33.

Pandya N, Chouhan G S and Nepalia V (2005) Effect of varieties, crop geometries and weed management on nutrient up take by soybean (*Glycine max*) and associated weeds. *Indian J Agron* **50**(3):218-20.

Papalamprou E M, Doxastakis G I, Biliaderis C G and Kiosseoglou V (2009) Influence of preparation methods on physicochemical and gelation properties of chickpea protein isolates. *Food Hydrocolloids* 23:337-43.

Pasha F (2013) Enzyme Inhibition (AChE) in Brain of *Oreochromis mossambicus* due to Pesticidal Pollution of Herbicide "Imazethapyr". *J Biolog Sci* **1**:91-100.

Peer F A, Hassan B, Lone B A, Qayoom S, Ahmad L, Khanday B A, Singh P S and Singh G (2013) Effect of weed control methods on yield and yield attributes of soybean *Afr J Agril Res* **8**: 6135-41.

Peterson D E, Thompson C R, Al-Khatib K and Regehr D L (2001) Herbicide Mode of Action. Agricultural Experiment Station and Cooperative Extension Service, Kanas State University, Available at internet: http://www.ksre.ksu.edu/ libary/crpsl2 /c715.pdf.

Pike D R, McGlamery M D and Knake E L (1991) A case study of herbicide use. *Weed Technol* **5**:639-46

Polle A (2001) Dissecting the superoxide dismutase–ascorbate peroxidase–glutathione pathway in chloroplasts by metabolic modeling. Computer simulations as a step towards flux analysis. *Plant Physiol* **126**: 445–62.

Prasad K and Singh R S (1995) Influence of weed and nitrogen management on weed growth, nutrient uptake and yield of wheat (*Triticum aestivum*). *Indian J Weed Sci* **65**(2): 117-22.

Qasem J R (2007) Chemical control of wild-oat (*Avena sterilis* L.) and other weeds in wheat (*Triticum durum* Desf.) in Jordan. *Crop Prot* **26**:1315-24.

Quantick H R (1985) Aviation in crop protection pollution and insect control (8th edition). In the Regent University of California, 27-30.

Raj V C, Damame H S, Patel A M and Arvadia M K (2008) Integrated weed management in summer groundnut (*Arachis hypogaea* L.), p.127. In: *Biennial Conference on Weed Management in Modern Agriculture: Emerging Challenges and Opportunities,* at Patna, Bihar during 27-28 February 2008.

Rajabi R, Khavari-Nejad R A, Ghanati F and Najafi F (2012) Effects of stress induced by post-emergence application of metribuzin herbicide on wheat. *Afr J Biotechnol* **11**(16): 3773-78.

Ram H and Singh G (2011) Studies on the bio-efficacy of herbicides for weed control in soybean (*Glycine max* L. Merrill) under different sowing methods. *Indian J Ecol* **38**: 11-13.

Ram H, Singh G, Aggarwal N, Buttar G S and Singh O (2013) Standardization of rate and time of application of imazethapyr weedicide in soybean. *Indian J Plant Prot* **41**: 33–37.

Rao A S and Rao R S N (2003) Bio-efficacy of clodinafoppropargyl on *Echinochloa* spp. in blackgram. *Indian J Weed Sci* **35**: 251-52.

Rathod A D, Solanki R M, Modhavadia J M and Padamani D R (2014) Efficacy of pre-and post-emergence herbicides in onion and their carry over effect on the succeeding crops. *Ann Agric Res New Series* **35**: 209-16.

Renner K A and Powell G E (1992) Responses of Navy Bean (*Phaseolus vulgaris*) and Wheat (*Triticum aestivum*) Grown in Rotation to Clomazone, Imazethapyr, Bentazon, and Acifluorfen. *Weed Sci* **40**: 127-33.

Richburge J S, Wilcut J W and Vencill W K (1996) Imazethapyr systems for peanut (*Arachis hypogaea* L.). *Pean Sci* **23**: 9-14.

Riethmuller-Haage I, Bastiaans L, Kropff M J, Harbinson J and Kempenaar C (2006) Can photosynthesis-related parameters be used to establish activity of acetolactate synthase-inhibiting herbicides on weeds? *Weed Sci* **54**: 974-82.

Royuela M, Gonzalez A, Gonzalez E M, Igor C A, Aparicio-Tejo P M and Gonzalez-Murva C (2000) Physiological consequences of continuous, sub-lethal imazethapyr supply to pea plants. *J Plant Physiol* **157**: 345–54.

Sanders D E, Strahan R E, Linscombe S D and Croughan T P (1998) Control of red rice (*Oryza sativa*) in imidazolinone-tolerant rice. Proc.South. *Weed Sci Soc* **51**:36-37.

Scarponi L, Martinetti L and Alla M M N (1996) Growth response and changes in starch formation as a result of imazethapyr treatment of soybean (*Glycine max* L.). *J Agric Food Chem* **44**: 1572–77.

Scarponi L, Younis M E, Standardi A, Hassan N M and Martinetti L (1997) Effects of chlorimuron-ethyl, imazethapyr, and propachlor on free amino acids and protein formation in *Vicia faba* L. *J Agri Food Chem* **45**: 3652–58.

Scheffer J J C, Douglas J A and Triggs C M (2002) Evaluation Of Some Pre- And Post-Emergence Herbicides For Weed Control In Yacon New Zealand Plant Protection **55**:228-34.

Sestak Z (1991) Leaf ontogeny and photosynthesis. In: Johnson CB(ed), Physiological processes limiting plant productivity. Butterworths, London, pp. 147-58.

Shabana E F, Battah M G, Kobbia I A and Eladel H M (2001). Effect of pendimethalin on growth and photosynthetic activity of *Protosiphon botryoides* in different nutrient states. *Ecotoxicology and Environ Safety* **49***:* 106–10.

Shaddad A A, Radi A F, Zidan M A and Hamada A M (1990) Effects of various concentyrations of 2,4-D and 2,4,5-T on seed germination, dry matter yield, transpiration rate of some economic plants. *J Islamic Acad Sci* **3**: 124-30.

Shaner D L, Anderson P C and Stidham M A (1984) Imidazolinones: potent inhibitors of acetohydroxyacid synthase. *Plant Physiol* **76**: 545-46.

Shannon L M, Kay E and Lew J K (1966) Peroxidase isoenzyme from Horseradish roots. In: Isolation and physical properties. *J Biol Chem* **241**:2166-72.

Shim S I, Lee B M, Ryu E I and Kang B H (2003) Response of leaf acetolactate synthase from different leaf positions and seedling ages to sulfonylurea herbicide. *Pestic Biochem Physiol* **75**:39-46.

Shivay Y S, Kumar S and Singh G (1997) Performance of tralkoxydim and Isoproturon for broad spectrum weed control and wheat growth. *Indian J Agron* **42**: 474-78.

Singh G, Kaur H and Khanna V (2014) Weed management in lentil with post-emergence herbicides. *Indian J Weed Sci* **46**: 187–89.

Singh K, Kumari A, Rinwa R S and Singh S (2010) Effect of Different Temperature Regimes on Persistence of Imazethapyr and Trifluralin. *Indian J Weed Sci* **42**: 88-94.

Singh S, Singh I, Gill RK, Kumar S and Sarkar A (2009) Genetic studies for yield and component characters in large seeded exotic lines of lentil. *J Food Legumes* **22**: 229-32.

Siyuan T, Richard R Evans, Mark L Dahmer, Bijay K Singh and Dale L Shaner (2005) Imidazolinone-tolerant crops: history, current status and future. *Pest Mgmt Sci* **61**: 246-57.

Solh M B and Pala M (1990) Weed control in chickpea. *Options Mediterr Ser Semin* **9**:93-99.

Soltani N, Shropshire C and Sikkema P H (2010) Sensitivity of leguminous crops to saflufenacil. *Weed Technol* **24**: 143–46.

Soltani N, Shropshire C and Sikkema P H (2014) Response of Dry Bean to Sulfentrazone Plus Imazethapyr. *International J Agron*. Article ID: 287908

Steponkus P L and Lanphear F O (1967) Refinement of the Triphenyltetrazolium Chloride method of determining cold injury. *Plant Physiol* **42**: 1423-26

Tang H F, Sheng X M, Xiong L, Luan Y C and Wang Y and Liu X P (2006) The impact of organophosphates pesticide to soluble protein and several oxidant enzymes in vegetable. *J Central China Normal University* (Natural Sciences) 1000-90.

Taran B, Holm F and Banniza S (2013) Response of chickpea cultivars to pre- and post-emergence herbicide applications. *Can J Plant Sci* **93**: 279-286.

Taran B, Warkentin T D, Vandenberg A and Holm F A (2010) Variation in chickpea germplasm for tolerance to imazethapyr and imazamox herbicides. *Can J Plant Sci* **90**:139-42.

Tecle B, Da Cunha A and Shaner D L (1993) Differential routes of metabolism of imidazolinones: Basis for soybean (*Glycine max*) selectivity. *Pestic Biochem Phys* **46**: 120-30.

Tiwari D K, Kewat M L, Khan J A and Khamparia (2007) Evaluation of efficacy of post-emergence herbicides in soybean (*Glycine max*). *Indian J Agron* **52**: 74-76.

Tiwari, A.N., S.N. Tiwari, J.P.S. Rathi, R.N. Verma and A.K. Tripathi.(2001) Crop–weed competition studies in chickpea having *Asphodelus tenuifolius* dominated weed community under rainfed condition. *Indian J Weed Sci* **33**: 198–199.

Toker C, Canci H, Inci N E and Ceylan F O (2012) Improvement in imidazolinone resistance in *Cicer* species by induced mutation. *Plant Breed* **131**: 535-39.

Tranel P J, Wright T R and Heap I M (2003) ALS mutations from herbicide-resistant weeds. . Retreived from http://www.weedscience.com.

Van Eerd L L, McLean M D, Stephenson G R and Hall J C (2004) Resistance to quinclorac and ALS-inhibitor herbicides in *Galium spurium* is conferred by two distinct genes. *Weed Res* **44**:355-65.

VanGessel M J, Monks D W and Johnson Q R (2000) Herbicides for potential use in lima bean (*Phaseolus lunatus*) production. *Weed Tech* **14**: 279-86.

Veeraputhiran and Chinnusamy C (2008) Performance of time and dose of post-emergence herbicide application on relay cropped black gram. *Indian J Weed Sci* **40**: 173-75.

Velini E, Trindade M, Rodrigo L, Barberis M and Duke S (2010) Growth Regulation and Other Secondary Effects of Herbicides. *Weed Sci* **58**: 351-54

Vischetti C (1995) Measured and simulated persistence of imazethapyr in soil. *Bull Environ Contam Toxicol* **54:**420-27.

Vyas M D, Singh S S and Singh P P (2000) Weed management in soybean (*Glycine max*) Merill. *Ann Plant Protec Sci* **8**:76-78.

Wall D A (1996) Lentil (*Lens culinaris*) and faba bean (*Vicia faba*) tolerance to post-emergence applications of Imazethapyr. *Canadian J Plant Sci* **76**: 525-29.

Wang M E and Zhou QX (2006) Effects of herbicide chlorimuron-ethyl on physiological mechanisms in wheat (*Triticum aestivum*). *Ecotoxicol Environ Saf* **64**: 190-97.

Williams P C and Singh U (1987) Nutritional quality and the evaluation of quality in breeding programmes. In: Saxena M C and Singh K B (ed) *The chickpea*. pp 329-90.CAB International, Wallingford.

Willoughby I, Jinks G and Gosling P (1996) Establishing new wood lands by direct sowing. In: Forestry Commission Research Information Note 285, Forestry Commission, Edinburgh.

Wilson D O and Reisenauer H M (1963) Determination of leghaemoglobin in legume nodules. *Ann Biochem* **6**: 27-30

Windley E, White D, Redman L and Short S (1999) Screening broadleaf herbicides for post emergent use in mungbean. *ACIAR-Food-Legume-Newsletter* **30**: 8-11.

Yadav P K, Khan A H and Yadav A S (2007) Effect of herbicides on biochemical and growth parameters of chickpea (*Cicer arietinum* L.). *Indian J Agri Sci* **77**: 542-43.

Ye J, Zhang Q, Zhang A P, Wen Y Z and Liu W P (2009) Enantioselective effects of chiral herbicide diclofop acid on rice XiuShui 63 seedlings. *Bulletin Environ Contam Toxicol* **83**: 85-91.

Young F L, Ogg A G, Papendick R I, Thill D C and Alledredge J R (1994) Tillage and weed management effect on winter wheat yield in an integrated pest management system. *Agron J* **86**:147-54.

Zabalza A, Gaston S and Sandalio L M (2007) Oxidative stress is not related to the mode of action of herbicides that inhibit acetolactate synthase. *Environ Exp Bot* **59**: 150-59.

Zabalza A, Orcaray L and Gaston S (2004) Carbohydrate accumulation in leaves of plants treated with the herbicide chlorsulfuron or imazethapyr is due to a decrease in sink strength. *J Agr Food Chem* **52**: 7601-06.

Zabalza A, Zulet A, Gil-Monreal M, Igal M and Royuela M (2013) Branched-chain amino acid biosynthesis inhibitors: Herbicide efficacy is associated with an induced carbon-nitrogen imbalance. *J Plant Physiol* **170**: 814–21.

Zaccardelli M, Sonnante G, Lupo F, Piergiovanni A R, Leghetti G, Sparvoli F and Lioi L (2013) Characterization of italian chickpea (*Cicer arietinum* L.) germplasm by multidisciplinary approach. *Genet Resour Crop Ev* **60**:865-77.

Zaidi A, Khan M S and Rizvi P Q (2005) Effect of herbicides on growth, nodulation and nitrogen content of greengram. *Agron Sustain Dev* **25**: 497–504.

Zand E, Baghestani M A, Soufizadeh S, PourAzar R, Veysi M, Bagherani N, Barjasteh A, Khayami M M and Nezamabadi N (2007) Broadleaved weed control in winter wheat (*Triticum aestivum* L.) with post-emergence herbicides in Iran. *Crop Prot* **26**:746-52.

Zhang B, Chu G, Wei C, Ye J, Li Z and Liang Y (2011) The growth and antioxidant defense responses of wheat seedlings to omethoate stress. *Pest Biochem Physiol* **100**: 273-79.

Zhu J, Patzoldt L, Radwan W, Tranel O P and Clough S (2009). Effects of Photosystem-II-Interfering Herbicides Atrazine and Bentazon on the Soybean Transcriptome. *Plant Genome* **2**: 191-205.

Zulet A, Gil-Monreal M, Villamor J G, Zabalza A, van der Hoorn R A and Royuela M (2013) Proteolytic pathways induced by herbicides that inhibit amino acid biosynthesis. *PLoS One* **8**: 73847.

VITA

Name of the student	:	Kawaljit Kaur
Father's name	:	S. Amarjit Singh
Mother's name	:	Smt. Raktinder Kaur
Nationality	:	Indian
Date of birth	:	06.03.1993
Permanent home address	:	V.P.O. Hayat Nagar, Gurdaspur

EDUCATIONAL QUALIFICATION

Degree	:	Integrated M.Sc. Botany (Hons.)
University and year of award	:	Punjab Agricultural University, Ludhiana 2016
OCPA	:	7.41/10.00
Title of master's thesis	:	Physiological variations in herbicide tolerance among Chickpea (*Cicer arietinum* L.) genotypes