GERMINATION ECOLOGY OF *Medicago denticulata* Willd. IN RELATION TO SEED COAT COLOUR, PERSISTENCE AND HERBICIDE EXPOSURE

Dissertation

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

DOCTOR OF PHILOSOPHY
in
BOTANY
(Minor Subject: Biochemistry)

By

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(L-2015-BS-66-D)

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2019

CERTIFICATE I

This is to certify that the dissertation entitled, **"Germination Ecology of *Medicago denticulata* Willd. in Relation to Seed Coat Colour, Persistence and Herbicide Exposure"** submitted for the degree of Ph.D., in the subject of **Botany** (Minor subject: **Biochemistry**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Renu Sethi (L-2015-BS-66-D)** under my supervision and that no part of this dissertation has been submitted for any other degree.

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

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CERTIFICATE II

This is to certify that the dissertation entitled, **"Germination Ecology of *Medicago denticulata* Willd. in Relation to Seed Coat Colour, Persistence and Herbicide Exposure"** submitted by **Renu Sethi (L-2015-BS-66-D)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Ph.D.** 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 on the same.

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ACKNOWLEDGEMENT

Words are often less to reveal one's deep regards, I sincerely acknowledge God's blessings in guiding, enriching and giving me sound mind to undertake and accomplish this research project.

With profound reverence, I express my deep sense of gratitude and indebtedness to my Major Advisor, ***Dr. Navjyot Kaur****, Assistant Plant Physiologist, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana being a great mentor, for providing encouragement, guidance and unwavering support academically, professionally and personally throughout my studies which made my Ph.D. programme, a satisfying experience.*

I owe my sincere thanks to members of my Advisory Committee, ***Dr. Namarta Gupta****, Associate Professor, Department of Botany,* ***Dr. Manjeet Kaur Sangha****, Senior Biochemist, Department of Biochemistry,* ***Dr. M.S. Bhullar****, Senior Agronomist, Department of Agronomy,* ***Dr. Manpreet Singh****, Assistant Agronomist, Dr. JC Bakhshi Regional Research Station, Abohar and* ***Dr. Nirmaljit Kaur****, Senior Botanist, Department of Botany and Nominee of Dean, Postgraduate Studies, for their consistent and invaluable inspirations throughout the course of this study.*

I am grateful to ***Dr. Seema Bedi****, Professor-cum-Head, Department of Botany, Punjab Agricultural University, Ludhiana for providing necessary facilities for successful completion of this work. I am also grateful to* ***Dr. Thakar Singh****, Head, Department of Agronomy for providing necessary facilities required for research.*

Language is too inadequate medium for expressing my feelings of indebtedness and gratitude to my beloved parents ***Sh. Vishwanath Sethi*** *and* ***Smt. Jai Sethi*** *for making me stand by my own, showing me the right ways and for their ever available blessings and affection. I duly acknowledge my brother* ***Ajay Sethi*** *whose affection and love during tough hours has helped me a lot.*

I cherish with appreciation the immense love and support of my dear friends ***Shabnam****,* ***Ratnesh****,* ***Priya****,* ***Antul*** *and* ***Gurwinder*** *who helped me a lot in various ways during my entire research work. I am also grateful to the laboratory and field staff* ***Mrs. Jasvir Kaur****,* ***Mr. Bishan Singh*** *and* ***Mr. Beant Singh****, Department of Agronomy for their unhesitant help and co-operation during my research work.*

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Date: ***(Renu Sethi)***

Title of the Dissertation	:	Germination ecology of *Medicago denticulata* Willd. in relation to seed coat colour, persistence and herbicide exposure
Name of the Student and Admission No.	:	Renu Sethi (L-2015-BS-66-D)
Major Subject	:	Botany
Minor Subject	:	Biochemistry
Name and Designation of Major Advisor	:	Dr. (Mrs.) Navjyot Kaur Assistant Plant Physiologist
Degree to be Awarded	:	Ph.D.
Year of award of Degree	:	2019
Total Pages in Dissertation	:	136 + VITA
Name of University	:	Punjab Agricultural University, Ludhiana-141004, Punjab, India

ABSTRACT

The present study entitled, "Germination ecology of *Medicago denticulata* Willd. in relation to seed coat colour, persistence and herbicide exposure" was conducted at Punjab Agricultural University, Ludhiana and Dr. J.C. Bakhshi Regional Research Station, Abohar. Under natural conditions, *M. denticulata* produces heteromorphic seeds differing in seed coat colour - cream and brownish black. The study was aimed to compare the effect of seed coat colour on dormancy and germination behaviour of *M. denticulata* seeds and response of heteromorphic seeds to various environmental variables and herbicide exposure. Brownish black seeds were non-dormant with high moisture content. Cream seeds with low moisture content were dormant and germinated only when subjected to dormancy breaking treatments indicating seed coat imposed dormancy. Germination of both cream and brownish black seeds was independent of light. Cream seeds germinated in the wide temperature range of 15/5 to 30/20°C; while brownish black seeds germinated in narrow temperature range of 15/5 to 25/15°C. Brownish black seeds recorded no germination at 200 mM NaCl; whereas, some cream seeds (10%) were able to germinate at this NaCl concentration. The osmotic potential required for 50% inhibition of maximum germination of cream and brownish black seeds was -0.37 and -0.32 MPa, respectively. Brownish black seeds accelerated aged for ≥ 20 recorded no germination. Whereas, some cream seeds (~13%) aged for 20 days were still able to germinate. Cream seeds placed on soil surface or buried at 2 and 10 cm depth germinated up to 12 months; but no germination was observed in brownish black seeds buried for 12 or more months. Therefore, cream seeds may have longer persistence than brownish black seeds. Herbicide sprays at eight and twelve-leaf stages of *M. denticulata* reduced seed production potential of this weed with concomitant decrease in germination as compared to herbicide sprays done at four-leaf stage of *M. denticulata*. This indicated possibility of herbicide carry-over effect from parent plants.

Key words: Dormancy, herbicide exposure, persistence, salinity, seed

Signature of Major Advisor **Signature of the Student**

ਖੋਜ ਪ੍ਰਬੰਧ ਦਾ ਸਿਰਲੇਖ	:	*ਮੈਡੀਕੈਗੋ ਡੈਂਟੀਕੁਲੇਟਾ* ਵਿਲਡ. ਦੇ ਬੀਜ ਦੇ ਛਿਲਕੇ ਦੇ ਰੰਗ, ਪਰਸਿਸਟੇਂਸ ਅਤੇ ਨਦੀਨਨਾਸ਼ਕ ਸੰਪਰਕ ਦੇ ਸੰਬੰਧ ਵਿੱਚ ਉੱਗਣ ਸਮਰੱਥਾ
ਵਿਦਿਆਰਥੀ ਦਾ ਨਾਂ ਅਤੇ ਦਾਖ਼ਲਾ ਨੰਬਰ	:	ਰੇਨੂ ਸੇਠੀ (ਐੱਲ-2015-ਬੀ.ਐੱਸ-66-ਡੀ)
ਮੁੱਖ ਵਿਸ਼ਾ	:	ਬਨਸਪਤੀ ਵਿਗਿਆਨ
ਨਿਮਨ ਵਿਸ਼ਾ	:	ਜੀਵ-ਰਸਾਇਣ ਵਿਗਿਆਨ
ਡਿਗਰੀ	:	ਪੀ.ਐੱਚ.ਡੀ.
ਮੁੱਖ ਸਲਾਹਕਾਰ ਦਾ ਨਾਂ ਅਤੇ ਅਹੁੱਦਾ	:	ਡਾ. ਨਵਜੋਤ ਕੌਰ ਅਸਿਸਟੈਂਟ ਪਲਾਂਟ ਫੀਜਿਓਲੋਜਿਸਟ
ਡਿਗਰੀ ਮਿਲਣ ਦਾ ਸਾਲ	:	2019
ਖੋਜ ਪ੍ਰਬੰਧ ਦੇ ਕੁੱਲ ਪੰਨੇ	:	136+ ਵੀਟਾ
ਯੂਨੀਵਰਸਿਟੀ ਦਾ ਨਾਮ	:	ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ - 141004, ਪੰਜਾਬ, ਭਾਰਤ ।

ਸਾਰ-ਅੰਸ਼

ਮੌਜੂਦਾ ਅਧਿਐਨ "*ਮੈਡੀਕੈਗੋ ਡੈਂਟੀਕੁਲੇਟਾ* ਵਿਲਡ. ਦੇ ਬੀਜ ਦੇ ਛਿਲਕੇ ਦੇ ਰੰਗ, ਪਰਸਿਸਟੇਂਸ ਅਤੇ ਨਦੀਨਨਾਸ਼ਕ ਸੰਪਰਕ ਦੇ ਸੰਬੰਧ ਵਿੱਚ ਉੱਗਣ ਸਮਰੱਥਾ" ਸਿਰਲੇਖ ਹੇਠ ਪੰਜਾਬ ਐਗਰੀਕਲਚਰਲ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ ਅਤੇ ਡਾ. ਜੇ.ਸੀ. ਬਖਸ਼ੀ ਖੇਤਰੀ ਖੋਜ ਸਟੇਸ਼ਨ ਅਬੋਹਰ ਵਿੱਚ ਕੀਤਾ ਗਿਆ। ਕੁਦਰਤੀ ਹਾਲਤਾਂ ਵਿੱਚ ਮੈਣਾ ਦੋ ਰੰਗਾਂ ਦੇ ਬੀਜ- ਕਰੀਮ ਅਤੇ ਭੂਰੇ ਕਾਲੇ ਪੈਦਾ ਕਰਦਾ ਹੈ। ਇਸ ਅਧਿਐਨ ਦਾ ਉਦੇਸ਼ ਬੀਜ਼ ਦੇ ਛਿਲਕੇ ਦਾ ਡੋਰਮੇਂਸੀ ਅਤੇ ਉੱਗਣ ਦੀ ਸਮਰੱਥਾ ਉੱਪਰ ਅਸਰ ਅਤੇ ਬਹੁਭਾਂਤੀ ਬੀਜ਼ਾਂ ਦਾ ਵੱਖ-ਵੱਖ ਵਾਤਾਵਰਨਾਂ ਅਤੇ ਨਦੀਨਨਾਸ਼ਕ ਸੰਪਰਕ ਨੂੰ ਪ੍ਰਤੀਕਿਰਿਆ ਜਾਨਣ ਦੇ ਉਦੇਸ਼ ਲਈ ਕੀਤਾ ਗਿਆ। ਭੂਰੇ-ਕਾਲੇ ਰੰਗ ਦੇ ਬੀਜ ਡੋਰਮੈਂਟ ਨਹੀਂ ਸਨ ਅਤੇ ਉਹਨਾਂ ਵਿਚ ਨਮੀ ਵਧ ਸੀ। ਕਰੀਮ ਰੰਗ ਦੇ ਬੀਜਾਂ ਵਿਚ ਨਮੀ ਘੱਟ ਸੀ ਅਤੇ ਉਹ ਡੋਰਮੈਂਟ ਸਨ ਅਤੇ ਸਿਰਫ ਡੋਰਮੇਂਸੀ ਤੋੜਨ ਤੋਂ ਬਾਅਦ ਹੀ ਉਗਦੇ ਸਨ ਜੋ ਬੀਜ ਦੇ ਛਿਲਕੇ ਕਾਰਨ ਹੋ ਰਹੀ ਡੋਰਮੇਂਸੀ ਬਾਰੇ ਦੱਸਦੀ ਹੈ। ਕਰੀਮ ਅਤੇ ਭੂਰੇ ਕਾਲੇ ਰੰਗ ਦੇ ਬੀਜਾਂ ਨੂੰ ਉੱਗਣ ਲਈ ਪ੍ਰਕਾਸ਼ ਦੀ ਲੋੜ ਨਹੀਂ ਸੀ। ਕਰੀਮ ਰੰਗ ਦੇ ਬੀਜ 15/5 ਤੋਂ 30/20°C ਤਾਪਮਾਨ ਵਿਚ ਉੱਗ ਪਏ ਸਨ ਜਦਕਿ ਭੂਰੇ ਕਾਲੇ ਰੰਗ ਦੇ ਬੀਜ ਨੂੰ ਉੱਗਣ ਲਈ 15/5 ਤੋਂ 25/15°C ਤਾਪਮਾਨ ਚਾਹੀਦਾ ਸੀ। ਭੂਰੇ ਕਾਲੇ ਰੰਗ ਦੇ ਬੀਜ 200 mM NaCl ਉੱਪਰ ਬਿਲਕੁਲ ਨਹੀਂ ਉੱਗੇ। ਜਦਕਿ ਕੁਝ ਕਰੀਮ ਰੰਗ ਦੇ ਬੀਜ (10%) NaCl ਦੀ ਇਸ ਮਾਤਰਾ ਉੱਪਰ ਉਗ ਪਏ ਸਨ। ਕਰੀਮ ਅਤੇ ਭੂਰੇ ਕਾਲੇ ਰੰਗ ਦੇ ਬੀਜਾਂ ਲਈ ਕ੍ਰਮਵਾਰ -0.37 ਅਤੇ -0.32 MPa ਔਸਮੈਟਿਕ ਸਮਰੱਥਾ ਤੇ 50% ਉੱਗਣ ਦੀ ਸਮਰੱਥਾ ਘੱਟ ਗਈ ਸੀ । ਭੂਰੇ ਰੰਗ ਦੇ ਬੀਜ ਨੂੰ ≥20 ਦਿਨਾਂ ਲਈ ਤੇਜੀ ਨਾਲ ਵਧਾਈ ਉਮਰ ਕਾਰਨ ਕੋਈ ਵੀ ਬੀਜ ਨਹੀਂ ਪੁੰਗਰਿਆ । ਜਦਕਿ ਕਰੀਮ ਰੰਗ ਦੇ ਕੁਝ ਬੀਜ (~13%) 20 ਦਿਨਾਂ ਤੱਕ ਤੇਜੀ ਨਾਲ ਵਧਾਈ ਉਮਰ ਦੇ ਬਾਵਜੂਦ ਉੱਗਣ ਵਿੱਚ ਸਮਰੱਥ ਸਨ। ਕਰੀਮ ਰੰਗ ਦੇ ਬੀਜ ਜੋ ਮਿੱਟੀ ਦੀ ਸਤਹ ਉੱਪਰ ਰੱਖੇ ਸਨ ਜਾਂ 12 ਮਹੀਨੇ ਲਈ 2-10 cm ਡੂੰਘੇ ਦੱਬੇ ਸਨ ਉੱਗ ਪਏ ਸਨ ਜਦਕਿ ਭੂਰੇ ਕਾਲੇ ਰੰਗ ਦੇ ਬੀਜ਼ ਬਿਲਕੁੱਲ ਨਹੀਂ ਉੱਗੇ। ਇਸ ਲਈ ਕਰੀਮ ਰੰਗ ਦੇ ਬੀਜ਼ਾਂ ਵਿੱਚ ਭੂਰੇ ਕਾਲੇ ਰੰਗ ਦੇ ਬੀਜਾਂ ਦੇ ਮੁਕਾਬਲੇ ਵੱਧ ਪਰਸਿਸਟੇਂਸ ਸੀ। ਮੈਣੇ ਦੇ ਅੱਠ ਜਾਂ 12 ਪੱਤਿਆਂ ਦੀ ਅਵਸਥਾ ਉੱਪਰ ਨਦੀਨਨਾਸ਼ਕਾਂ ਦਾ ਛਿੜਕਾਅ ਕਰਨ ਨਾਲ ਇਸ ਨਦੀਨ ਦੇ ਬੀਜ਼ ਉਤਪਾਦਨ ਸਮਰੱਥਾ ਵਿੱਚ ਕਮੀ ਆਉਂਦੀ ਹੈ ਅਤੇ ਉਗਣ ਸ਼ਕਤੀ ਵੀ ਘਟਦੀ ਹੈ। ਇਹ ਦਰਸਾਉਂਦਾ ਹੈ ਕਿ ਨਦੀਨਨਾਸ਼ਕਾਂ ਦੇ ਛਿੜਕਾਅ ਦਾ ਅਸਰ ਨਦੀਨਾਂ ਦੀ ਅਗਲੀ ਪੀੜ੍ਹੀ ਤੱਕ ਜਾਂਦਾ ਹੈ।

ਮੁੱਖ ਸ਼ਬਦ: ਡੋਰਮੇਂਸੀ, ਨਦੀਨਨਾਸ਼ਕ ਸੰਪਰਕ, ਪਰਸਿਸਟੇਂਸ, ਖਾਰਾਪਣ, ਬੀਜ

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER-I

INTRODUCTION

The rice (*Oryza sativa*)-wheat (*Triticum aestivum*) cropping system is the predominant cropping system in India practiced on an estimated area of around 11 million hectares (Mandal *et al* 2018). This system is prevalent in Indo-Gangetic plains (IGP) mainly in Punjab, Uttar Pradesh, Haryana, Bihar and West Bengal. Wheat is the most widely cultivated *rabi* crop of North-Western Plain Zone and Central Zone of India occupying 28.52 million hectares area with a production of 96.64 million tones during 2016-17. In Punjab, it was cultivated over an area of 3.47 million hectares with a total production of 17.7 million tones. Major detriments to sustainable production of wheat are stiff competition from weeds, multiple nutrient deficiencies and incidence of diseases (Ladha *et al* 2003). Among various factors which adversely affect the yield of wheat crop, weed infestation is the most harmful one but less noticeable (Oad *et al* 2007).

Phalaris minor is the major monocotyledonous weed infesting wheat crop in Northern India since 1970s. To control *P. minor*, exclusive use of herbicides like clodinafop and fenoxaprop which are effective against monocotyledonous weeds only caused enormous build-up of dicotyledonous weeds mainly, *Medicago denticulata*, *Coronopus didymus*, *Rumex dentatus* and *Cannabis sativa* (Kaur *et al* 2015). *Medicago denticulata* Willd. is a winter annual weed native to Mediterranean basin but has also infested western and central Asia (Graziano *et al* 2010). In India, *M. denticualata* has invaded many states *viz.,* Punjab, Haryana, Jharkhand, Bihar and West Bengal. Among the various dicotyledonous weeds, *M. denticulata* is the major problematic weed prevalent in wheat fields of Punjab (Chhokar *et al* 2006).

It is an erect annual plant having weak stems which reach up to a height of 60-75 cm. Mature established plant is difficult to pull out because of a deep tap root system. The leaves are trifoliate, clover-like and toothed towards the top. Inflorescences are borne at the end of the stems. The flowers are bright yellow in colour. The fruit (bur) is a prickly, flattened and coiled pod, about 6-7 mm in length. The pods contain several kidney shaped seeds (Walsh *et al* 2013). Reproduction is entirely by seeds which are enclosed in indehiscent coiled pods (burs) covered with hooked spines. Seeds mainly germinate after coming out of pods but germination can also occur within pods. Dormancy is a characteristic attribute of many weeds as it hampers the task of predicting timing and extent of emergence of weeds. Wagner and Spira (1994) reported long term dormancy in seeds of *M. polymorpha* which germinated only after exposure to fluctuating temperature and moisture conditions.

Major weed control methods include mechanical weeding, intercropping, stale seed bed and use of herbicides. Herbicide usage has been escalated during past decades and is still

going up for controlling weeds at farmers' fields due to shortage of labourers and high cost involved in the manual weeding (Gharde *et al* 2018). However, herbicides are able to control the weeds only up to certain time as further flushes of weeds pose new challenges to the farmers during cropping season. *Medicago denticulata* and *Rumex dentatus* are not effectively controlled by 2,4-D but carfentrazone provides effective control against these weed species (Punia *et al* 2006). Howatt (2005) reported that carfentrazone may cause temporary injury to wheat by cosmetic speckling on leaves, but treated plants recover within 2-3 weeks without any reduction in yield. Applying herbicides at a proper time increases the effectiveness of a weed control programme without causing injury to crop. The weed growth strongly influences the uptake and metabolism of herbicides thereby affecting the efficacy of herbicides and herbicide application on bigger weeds increases the rate of herbicide degradation resulting in decreased herbicide efficacy (Singh and Singh 2004). Although herbicides provide cost-effective weed control but over-reliance on herbicides with a similar mode of action can rapidly lead to development of herbicide resistance in weeds (Bhullar *et al* 2017). Therefore, integrated weed management strategies must be adopted to ensure the sustainability of crop production. Detailed knowledge of the environmental factors required for germination of weed seeds is an important prerequisite for the development of integrated and biological weed control strategies.

Seed germination is a critical factor in the establishment of weeds and it mainly depends on weed's interaction with environmental factors *viz*., light, temperature, soil pH, salinity, burial depth and soil moisture (Humphries *et al* 2018). Light or darkness is an important environmental trigger for seed germination. It is an essential requirement for germination of only some seeds where it may help to determine whether seeds are located at soil surface or are buried. Seeds of some species need light to germinate while others germinate equally in light and dark. Exposure of dormant seeds to light triggers the accumulation of active forms of phytochromes which are responsible for breaking seed dormancy in some species (Krenchinski *et al* 2015). Temperature affects both the ability and rate of weed seed germination and is directly related to water absorption and various biochemical reactions occurring in the seeds thereby regulating the germination process (Rana *et al* 2012). Alvarado and Bradford (2002) reported that rate of germination increases linearly along a defined range of temperature and higher temperatures result in the sharp decline of germination. Seed germination and early seedling growth are stages in the life cycle of plant that are more sensitive to water shortage. Moisture stress delays the onset of seed germination, slows the rate of germination and decreases final germination percentage (Patane *et al* 2013).

Salinity is an important abiotic factor which affects germination by decreasing water uptake capacity of seeds. Composition of weed flora is greatly influenced by pH of the field

and pH alteration may change the dominance of weed species infesting crop fields. Seed burial is important environmental factor that impose dormancy on seeds of some species which are shed in a non-dormant condition (Grass *et al* 2016). Burial depth affects germination and emergence of seeds by influencing the availability of light, moisture and temperature. Seed burial at shallow depths can stimulate higher germination as it maintains moist environment around seeds and prevent them from drying. The requirement of light and limited availability of storage reserves are also major constraints for reduced emergence of weeds from deeper soil layers (Bullied *et al* 2012). Seed coat colour is an important attribute which influences ability of seeds to either germinate or remain dormant (Ochuodho and Modi 2013). Seed colour differences among a species results from seeds harvested from fruits having different developmental stages along with some differences at genetic level (Atis *et al* 2011).

Soil seed bank is a reserve of viable seeds present in soil which ensures persistence of weed populations (Anderson *et al* 2012). Weed seed bank of any plant community usually consists of a mixture of transient and persistent species. A species is considered to be transient in the seed bank if its seeds do not persist in the soil in a viable condition for more than a year after being shed. Weeds with transient seed banks germinate when there is any soil disturbance by cultivation or tillage. Weed species with hard seed coats contribute largely to the accumulation of weed seed banks in cultivated fields due to their ability to resist mechanical and environmental stress for longer periods. Moreover, weeds with hard seed coats may result in prolonged emergence periods resulting in multiple flushes throughout the crop growing season. Persistence of seeds in soil is a survival strategy which enables them to disperse effectively throughout time therefore, avoiding unfavorable conditions for germination after attaining maturity on the parent plant (Panetta and Timmins 2004).

Studies on persistence of weeds under field conditions provide reliable information as it incorporates environmental factors- biotic and abiotic. However, increased time consumption and demand for financial resources make these studies ineffective at large-scale (Ishikawa-Goto and Tsuyuzaki 2004). Artificial seed ageing under laboratory conditions constitute a significant ex-situ tool which allows effective prediction of soil seed persistence of weed seeds. Artificial seed ageing is imposed by the high moisture level and temperature. Ageing that occurs over time when stored under natural conditions is called natural ageing. By subjecting the seeds to high relative humidity and high temperature conditions, the accelerated ageing of seeds can be done artificially (Tesnier *et al* 2002). Accelerated ageing helps to predict germination response of a particular weed species over a long time, in a relatively short span of time. Long *et al* (2008) categorized the species into three types depending on half-viability period (P_{50}) for describing seed bank persistence: transient (P_{50} value < 20 days with < 1 year field persistence), short lived (P_{50} value of 20-50 days with 1-3

years field persistence) and extended persistence (P_{50} value of > 50 days with > 3 years field persistence).

Seed production of weeds is a vital factor in success of a weed control programme. While vegetative parts of the weeds determine the impact on crop yield during the current year, seeds added to the seed bank are of great importance for the years ahead. Annual weeds mainly rely on renewable seed production to make sure their persistence; hence, assessment of herbicide sensitivity is more accurately estimated through effects on reproduction. Herbicide application at reproductive stage affects the germination behavior of seeds by reducing the viability of seeds. Increased weed seed bank and late season weed seed rain are mainly prevented by postharvest or late-season herbicide treatments which frequently target the early bloom stage of weed plant or may suppress the initiation of seed set (Jha and Norsworthy 2012). Carpenter and Boutin (2010) reported that herbicide application changes the amount and type of viable seeds present in the soil seed bank thereby, causing long-term consequences on the plant species of arable habitats. Application of selective herbicides to broad-leaved weeds during the reproductive stages of development is effective in reducing seed set (Madafiglio *et al* 2006).

Seed heteromorphism is an important phenomenon which enables a single plant species to produce seeds with different shape, size, color and germination behavior (Gairola *et al* 2018). *M. denticulata* produces heteromorphic seeds that differ in seed coat colour- cream and brownish black. The information on germination ecology of *M. denticulata* in response to seed coat color and various environmental factors is lacking. So, the present study was undertaken to compare the effect of seed coat colour on germination characteristics and dormancy behaviour of *M. denticulata* seeds and the response of these heteromorphic seeds to various environmental variables. Studies on soil seed bank persistence of *M. denticulata* under both field and laboratory conditions may assist in knowing the seed fate under natural conditions and serve as key in understanding of weed seed dynamics in soil for improving weed management strategies. Germination and/or dormancy of weed seeds derived from herbicide treated weed plants will help in understanding the potential of this weed species for its invasiveness and success of future weed generation.

Objectives

1. To study the effect of seed coat colour on germination ecology of *Medicago denticulata*.
2. To access the soil seed bank persistence potential of *Medicago denticulata* under field and laboratory conditions.
3. To investigate germination behaviour of *Medicago denticulata* seeds after herbicide exposure.

CHAPTER-II

REVIEW OF LITERATURE

Wheat (*Triticum aestivum* L.) is the most important *rabi* cereal crop of North-Western Plain Zone and Central Zone of India, occupying about 35.05 lac hectares area with total production of 150.88 lac tonnes and average yield of 97.11 million tonnes in 2017-18. In the North Western states of India, wheat production is under a severe threat due to the increasing intensity of weeds in the crop. Weed infestation is the major cause of low wheat yield in India. Wheat fields are infested with both monocotyledonous and dicotyledonous weeds which may cause the yield loss of 7 to 50% based on the kind of weed flora and their intensity (Fahad *et al* 2015). *Phalaris minor* is the major monocotyledonous weed infesting wheat crop in Northern India since 1970s. To control *P. minor*, exclusive use of herbicides like clodinafop and fenoxaprop in 1990s which are effective against monocotyledonous weeds only caused enormous build-up of dicotyledonous weeds. The major dicotyledonous weeds prevalent in the wheat fields of Northern India in *rabi* season are toothed dock (*Rumex dentatus*), bathua (*Chenopodium album*), maina (*Medicago denticulata*), sengi (*Melilotus indica*), krishn neel (*Anagallis arvensis*) and *Cannabis sativa* (Kaur *et al* 2015). Sardana (2001) reported heavy infestation of *Medicago denticulata* in wheat field of Gurdaspur, Punjab.

Medicago denticulata Willd. is a winter annual weed commonly found in wheat fields of Punjab. It produces inflorescences borne at the end of the stems. The fruits are prickly, flattened coiled pod with 2-6 coils of about 6-7 mm in length. The pods contain 3-5 kidney shaped seeds and are separated by partitions. *M. denticulata* is a prolific seed producer with seed production potential of 3000 seeds per plant, which makes its management very difficult. The literature related to the study on, "Germination ecology of *Medicago denticulata* Willd. in relation to seed coat colour, persistence and herbicide exposure" has been reviewed under the following headings:

2.1 Seed heteromorphism in relation to seed germination

Seed heteromorphism (also termed seed polymorphism or seed dimorphism) is an adaptive strategy where a plant produces seeds of two or more distinct types, which may differ in their external appearance (i.e., size, shape, seed coat color) as well as physiological

characteristics such as dormancy, imbibition and germination behaviour (Mandak and Pysek 2005, Wang *et al* 2010). Various physiological, environmental and genetic factors can contribute to seed heterogeneity which could affect physiological properties of seed dormancy and germination with in a population (Matilla *et al* 2005, Atak *et al* 2008). Seed size directly influences germination (Souza and Fagundes 2014) and seedling vigour, thereby affecting plant distribution and abundance across different habitats (Silveira *et al* 2012). Mandal *et al* (2008) observed that *Hyptis suaveolens* (L.) produces both large and small seeds and reported that large seeds germinated to a higher percentage as compared to smaller seeds. Sallam and Ibrahim (2016) also reported variation in seed coat colour, hilum, seed coat texture and seed shape in *Vigna unguiculata*. Sun *et al* (2009) reported that *Garhadiolus papposus* produces three kinds of achenes with different germination requirements. Fresh central achenes were able to germinate upto 21%, while intermediate and peripheral achenes recorded $< 5\%$ germination. Volis (2016) observed seed heteromorphism in *Triticum dicoccoides* in terms of seed position within a fruit and reported that upper grains in a spikelet are larger and germinate soon after dispersal whereas most of the basal grains with smaller size remain dormant in the soil seed bank for 1 year.

Seed coat occurs as a main modulator facilitating numerous interactions between seed's internal and external environment. Seed coat thickness and composition are important traits related to germination. Development of seed coat is linked to the maternal genotype, but environmental conditions such as photoperiod also influence seed coat colour (De Souza and Marcos-Filho 2001). Seed coat colour is a characteristic attribute associated with absorption of water and has been reported to play a role in seed dormancy and germination (Ochuodho and Modi 2013). Variations in seed colour occur due to presence of differential amounts of colour pigments in the seed coat. Liu and Wei (2007) observed that rate of germination of brown seeds of *Atriplex micrantha* were significantly higher as compared to black seeds at three temperatures (5/15°C, 5/25°C and 15/25°C). Yao *et al* (2010) observed that *Chenopodium album* produces heteromorphic seeds (brown and black) with differential tolerance to salinity stress. Brown seeds were non-dormant and more tolerant of salinity as compared to black seeds which exhibited dormancy and were less tolerant to salinity. Furthermore, the ratio of brown to black seeds produced by the plant increases under saline conditions. Light coloured seeds of radicchio (*Cichorium intybus*) recorded reduced germination and as seed colour became darker, seeds showed higher, faster and more uniform germination (Pimpini *et al* 2002). Wang *et al* (2008) reported that *Saudea aralocaspica* produces dimorphic seeds with brown and black seed coat colour. Brown seeds are non-dormant with greater ability to tolerate salt stress whereas black seeds possessed non-deep physiological dormancy with lesser ability to tolerate salinity stress. Zhang *et al* (2013) reported that black seeds of *Brassica napus* recorded maximum germination and higher

germination index with minimum mean germination time in comparison to dark brown and light brown seeds which indicates that seed coat colour determines seed composition, which further affects seed germination and seedling growth. In contrast to these, Mertz *et al* (2009) observed no difference in germination percentage of soybean genotypes CD-202 and TP with yellow and black seed coat, respectively. Structural mutants of *Arabidopsis* which lacked some seed coat layers and those that showed less pigment impregnation showed better germination than wild types (Debeaujon *et al* 2000). Some studies have showed that coloured seeds absorb water rapidly and consequently have greater germination, compared to light coloured seeds (Atis *et al* 2011). Liu *et al* (2007) reported that black seeds of *Cyamopsis tetragonoloba* showed faster water uptake and higher germination than that of dull-white-coloured seeds.

2.2 Seed micromorphological studies

Scanning electron microscopy (SEM) provides deeper insight where gross morphology proves insufficient to analyse seed coat structure and surface sculpture. Invasive plants have been reported to spread rapidly due to presence of special seed traits (Eslami 2011). Seed coat imposed dormancy as found in legumes, acts as a mechanical barrier for protruding embryo; also the presence of chemical inhibitors interfers with water uptake and/or oxygen exchange can also obstacle the embryo from getting light (Morris *et al* 2000). Physical dormancy (often called hardseededness), involves the development of a water-impermeable seed coat, caused by the presence of phenolics and suberin impregnated layers of palisade cells (Symkal *et al* 2014). Hard seed coats contribute to accumulation of weed seed banks in agricultural areas allowing the reoccurrence of problematic species even after the parent plants have been eliminated. Uzun and Aydin (2004) reported that various ecotypes of *Medicago* species may have hard seededness at a rate of 100%. Germination occurs when embryo growth overcomes the constraints imposed by the seed coat (Steinbrecher and Leubner-Metzger 2016). Buyukkartal *et al* (2013) examined mature seed coat in hard and soft-seeded varieties of *Vicia sativa* L. by scanning and transmission electron microscope and reported that outer layer of both hard and soft-seeded varieties of *Vicia sativa* was composed of macrosclereid (Malpighian) cells. Juan and Crespo (2009) examined seeds of *Medicago* L. sect. *Dendrotelis* under scanning electron microscope and reported that seeds are kidney-shaped having granulate external seed surface. Ochuodho and Modi (2008) studied imbibition behaviour in dimorphic seeds of wild mustard (*Sisymbrium capense*) and reported that light brown seeds absorbed more water and gained weight faster as compared to dark brown seeds. Seed coat pigmentation is correlated with reduced imbibition rates in a number of legume species. Kahn *et al* (1996) observed that dark seeds of proso millet (*Panicum miliaceum*) with heavier seed coats are slow in imbibition of water and take time to germinate, suffer less damage during imbibition and therefore persist for longer time in soil than light-colored

seeds. Bahry *et al* (2007) reported that soybean genotypes with black seed coat exhibit less imbibition damage because of slow water uptake which results in rapid germination. Chachalis and Smith (2000) reported greater rate of imbibition and fast germination in dark soybean cultivars.

2.3 Effect of environmental factors

Detailed information of the environmental factors required for germination of weed seeds is an important prerequisite for the development of integrated and biological weed control strategies. A brief account of various environmental factors influencing seed germination is given below:

2.3.1 Light

Light is an important external factor for germination of weed seeds. Germination response of seeds to light is species-specific. Some species are insensitive to changes in light levels whereas other species show stimulation/inhibition if they are exposed to light during their germination phase (Carta *et al* 2014). Seeds which respond to light for their germination are called photoblastic seeds. The photoblastic seeds may be either positive photoblastic or negative photoblastic. Positive photoblastic seeds (lettuce, tobacco) do not germinate in darkness and require light for their germination. Negative photoblastic seeds (*Amaranthus* spp.) do not germinate in the presence of light and can germinate only under complete darkness. Non-photoblastic seeds germinate irrespective of presesnce and absence of light e.g. *Vicia sativa, Rhyncosia capitata* and *Lathyrus aphaca*. In some species, exposure of dormant seeds to light triggers the accumulation of active forms of phytochromes resulting in breaking of seed dormancy (Krenchinski *et al* 2015).

Weed species belonging to fabaceae family may differ in their requirement of light for germination. Tanveer *et al* (2012) reported that seeds of *Vicia sativa* belonging to fabaceae, germinated equally (90%) under both light and dark conditions which shows that seeds of this species are non-photoblastic. Norsworthy and Oliveira (2006) also reported light independent germination of *Senna obustifolia*. Williams *et al* (2006) reported that germination of *Veronica arvensis*, a dicotyledonous weed in wheat crop was increased upon transfer from darkness to daylight conditions which indicates that darkness prevents germination of seeds that are buried deep in the soil. However, light is not a pre-requisite for germination of other dicotyledonous weeds infesting wheat crop like *Convolvulus arvensis* and *Lathyrus aphaca* (Kumari *et al* 2010). Likewise, germination of toothed dock (*Rumex dentatus*) and common lambsquarters (*Chenopodium abum*) infesting wheat was also effectively stimulated by light which indicates that both species are positively photoblastic and germination in the field will be favoured by presence of seeds at the soil surface (Ghadiri and Niazi 2005).

2.3.2 Temperature

Temperature is an important factor determining seed germination. Alvarado and Bradford (2002) reported that rate of germination usually increases linearly with temperature within a well defined range and then declines sharply at higher temperatures. Weeds infesting wheat crop like *Convolvulus arvensis* and *Chenopodium album* may germinate over wide range of temperature (10-55°C) while others like *Rumex obustifolius*, *Lathyrus aphaca*, *Vicia sativa* and *Raphanus raphanistrum* may germinate in narrow temperature range. Tanveer *et al* (2009) reported that germination of *Chenopodium album* occurred over a range of constant temperatures from 5 to 25°C with maximum germination at 20°C and minimum at 5°C. Tanveer *et al* (2013) studied the effect of constant temperatures on germination of *Convolvulus arvensis* and found that germination occurred over wide range of temperatures from 15 to 40°C, with no germination at 45°C. Benvenuti *et al* (2001) reported that optimum temperature for germination of *Rumex obustifolius* was between 20-25°C and temperature < 15°C or > 30°C resulted in sharp reduction in germination. Tanveer *et al* (2012) observed that *Lathyrus aphaca* and *Vicia sativa*-broadleaf weeds of wheat recorded highest germination at 15 and 20°C, respectively. Steckel *et al* (2004) studied the effect of constant and alternating temperatures on germination of palmer amaranth (*Amaranthus palmeri*) and smooth pigweed (*Amaranthus hybridus* L.) and reported higher germination of both species under alternating temperatures than under constant temperatures of 10, 15 and 30°C; however, similar germination was found at 5, 20 and 35°C.

2.3.3 Moisture stress

Water availability is another important abiotic factor responsible for the germination of plants. Germination and imbibition rate of seeds decrease as the water potential of surrounding environment decreases. Rezvani *et al* (2014) reported that *Capsella bursa-pastoris* seeds were not able to tolerate high drought stress conditions as germination was completely inhibited at an osmotic potential level of -1.0 MPa. Bargali and Bargali (2016) exposed the seeds of *Medicago denticulata* to five water deficit levels and found that germination was decreased from 51 to 10% as the water stress level increased from 0 to -10 bar with complete inhibition at -15 and 20 bar. Ali *et al* (2013) exposed the seeds of *Rhynchosia capitata* to PEG solutions with six levels of osmotic potential (0, -0.2, -0.4, -0.6, -0.8 and -1.0 MPa) and found that *R. capitata* seed germination was completely inhibited at osmotic potential of -1.0 MPa. Germination decreased from 100 to 15% as osmotic potential decreased from 0 to -0.6 MPa. However, more than 10% germination at an osmotic potential of -0.6 MPa shows that some seeds of *R. capitata* can germinate under marginal water-stress conditions. Ghaderi-Far *et al* (2010) reported that *Melilotus officinalis* seeds subjected to moisture stress recorded significant decrease in germination with decrease in osmotic potential. Germination percentage was decreased from 92 to 5% with decrease in osmotic

potential from 0 to -1.0 MPa and no seed was germinated at osmotic potential of -1.2 MPa. Norsworthy and Oliveira (2005) reported that germination of *Cassia occidentalis* seeds incubated at 15°C was completely inhibited at osmotic potential -0.4 MPa, whereas some seeds (1%) were able to germinate at -1.0 MPa when incubated at 30°C**.**

2.3.4 Salinity

Salinity is an important abiotic factor affecting seed germination. It reduces both germination rates as well as root growth of seedlings. Salinity has affected many areas worldwide including parts of USA, Australia, Israel and Mediterranean Basin (Aringhieri 2010). In India, some parts of states *viz*., Punjab, Haryana, Uttar Pradesh, Delhi, Bihar and Rajasthan are affected by salinity. Districts of Punjab affected by salinity are Muktsar, Faridkot, Bathinda and Sangrur (Shakya and Singh 2010). Plants do osmotic adjustment in various ways, including synthesis of compatible solutes (e.g. proline, glycine betaine and raffinose) which help in stabilizing the proteins and cellular structures, maintain cell turgor, remove excess levels of reactive oxygen species (ROS) and re-establish the cellular redox balance (Janska *et al* 2010). Ghaderi-Far *et al* (2010) observed that germination of *Melilotus officinalis* seeds was unaffected by 0-100 mM NaCl concentrations; however, concentrations above 100 mM decreased the germination with complete inhibition at 300 mM NaCl. Kumari *et al* (2010) reported that maximum germination of *Convolvulus arvensis* and *Lathyrus aphaca* was recorded in distilled water. NaCl concentration of 200 mM decreased the germination of *C. arvensis* and *L. aphaca* to 18 and 55%, respectively, which indicates that *L. aphaca* was fairly tolerant to salinity stress. Guan *et al* (2009) reported that germination of *Medicago ruthenica* was maximum in control (100%) at a temperature range of 15-25°C. At NaCl concentration of 50 and 100 mM, germination was more than 80%, however, salinity stress of 200 mM declined germination to 55%.

2.3.5 pH

Composition of weed flora is greatly influenced by pH of the field and pH alteration may change the dominance of weed species infesting crop fields. Singh and Singh (2009) observed that germination of *Amaranthus retroflexus* (redroot pigweed) and *Senna obtusifolia* (sicklepod) was similar in pH range of 5-11 but none of them germinated at pH 3. Thomas *et al* (2006) exposed the seeds of *Amaranthus viridis* to buffer solutions of pH 3 to 10 and observed that seeds germinated best at pH 7 and 8 and germination was declined to 28% at pH 3 and 11. Ghaderi-Far *et al* (2010) exposed *Melilotus officinalis* seeds to buffer solutions of pH 4 to 9 and observed maximum germination (> 92%) at pH 5 and 6. However, germination was declined to 80 and 42% at pH 4 and 9, respectively. Chauhan *et al* (2006) reported that seeds of *Malva parviflora* (little mallow) germinated in broad range of pH from 4 to 10 with 50% germination in pH range 4 to 6.3 with > than 30% germination in pH ranging from 6.4 to 10 which indicates that both acidic and alkaline soils may favour

germination of this weed. Wang *et al* (2009) reported that pH did not affect the germination of *Urena lobata* and maximum germination (88%) occurred at pH 6 and germination was declined to 70% and 67% at pH 4 and 9, respectively. Wang *et al* (2016) reported that germination of *Galium aparine* (catchweed bedstraw) seeds was greater than 60% in pH range of 4 to 10 but the optimum pH for its germination was observed to be near neutral (pH 6 to 7).

2.3.6 Burial depth

Seed burial can enforce dormancy on seeds of some species that are shed in a non-dormant condition (Grass *et al* 2016). The depth of seed burial plays a role in the development of a seed bank by maintaining seed dormancy and adjusting the timing or the prevention of seedling emergence. Germination and emergence of seeds is reduced with increasing depth of planting. The main reason for lack of germination at greater depth is induction of secondary dormancy in seeds which may be caused by higher soil moisture, poor gas exchange, higher CO_2 levels around seeds or exhaustion of seedling reserves (Benvenuti 2003). Weed seeds on the surface are exposed to light, which may promote germination in species requiring light for germination i.e. positively photoblastic. Non-photoblastic seeds germinate irrespective of presence and absence of light and their germination is not affected by burial depth. Chauhan *et al* (2006) reported that maximum emergence of *Malva parviflora* occurred when buried at depths of 0.5 to 2 cm, however, a progressive decline in emergence was observed with further increase in burial depth; and no seed emerged when buried at 8 cm depth or deeper. Benvenuti *et al* (2001) reported that seed burial inhibited germination of *Rumex obtusifolius* in proportion to depth. Maximum seedling emergence occurred when the seeds were placed at 2 cm depth and decreased with increased depth of planting; no seed emerged from depths of 10 cm. Grundy *et al* (2003) observed that ivyleaf speedwell (*Veronica hederifolia*) seed germination gradually increased with decreasing burial depth. Norsworthy and Oliveira (2005) reported that total emergence of *Cassia occidentalis* from sand at depths of 2, 4, 6, 8 and 10 cm was 20, 11, 0.4, 0.4, and 0.6%, respectively; whereas from the sandy loam soil for the same depths, emergence was 36, 4, 6, 2 and 2%, respectively. Gulshan and Dasti (2012) studied the effect of burial depth (2, 4 and 6 cm) on *Medicago denticulata* Willd and *Vicia sativa* L. Maximum emergence was recorded at depth of 2 cm depth with a progressive decline in emergence with increase in depth from 2 to 6 cm.

2.4 Soil seed bank persistence

Weed seed banks are reserves of mature viable seeds present in the soil. Seeds in the soil seed bank may be present in or on the soil but continuity is maintained between seeds placed at the surface or completely buried in soil via means of germination, seed decay and predation (Benvenuti 2007). In annual weed species that reproduce by seed only weed seed bank is the sole source of future weed populations, which gets depleted either by germination,

physiological ageing, decay and predation (Forcella 2003). However, perennial weed species like *Cyperus rotundus*, *Convolvulus arvensis* and *Cirsium arvensis* are more persistent as these may propagate not only by seeds but also vegetatively via rhizomes and tubers. Weeds emerging later or surviving late herbicide applications may not affect yield, but can enrich the weed seed bank through seed production (Gallandt 2006), thereby increasing weed infestation every year. Traditionally, two broad types of soil seed banks have been designated: transient and persistent. Transient seed banks are those where seeds only survive for a short time < 1 year in the seed bank as is the case with kochia (*Kochia scoparia*) and dandelion (*Taraxacum officinale*). Seeds of species such as redroot pigweed (*Amaranthus retroflexus* L.) and lambsquarters (*Chenopodium album* L.) form a persistent seed bank with the ability to remain viable in the soil for > 1 year. Transient seed banks tend to germinate immediately or seasonally, while those that persist for longer periods are dormant until they receive an appropriate trigger (Rokich and Dixon 2007).

Persistent soil seed banks can be subdivided further into short-term persistent for seeds that shows persistence for more than 1 but less than 5 years and long-term persistent seed banks that are present for more than 5 years (Wijayratne and Pyke 2012). Therefore, persistent seed bank formation is one of the essential characteristics in many weed populations for maintaining seed supply in the soil for extended periods which serves as a survival mechanism during unfavorable period (Saatkamp *et al* 2011). Some plants produce both transient and persistent seeds in varying ratios (Tielborger *et al* 2011) and germination of seeds either from transient or persistent seed bank depends on variation in the environmental conditions (Meyer and Allen 2009). Dormancy is a complex mechanism that controls timing of seed germination. However, seed dormancy characteristics and persistence of the seed in the seed bank may or may not be related to each other. Seeds exhibiting physical dormancy where a hard seed coat prevents uptake of water, is an important mechanism for extended persistence in the soil seed bank. Weed families with high levels of seeds with impermeable coats include black medic (*Medicago lupulina* L.), round leaved mallow (*Malva pusilla* L.) and velvetleaf (*Abutilon thoephrasti* L.). Dormancy release and loss of viability through process of ageing results in soil seed bank decline (El-Maarouf-Bouteau *et al* 2011). Depending on their longevity in the soil, seeds can germinate several years after their production and species can thus reappear in a field from which they have been absent for several years. Seeds from these species can readily survive several decades in the soil seed bank. Larger soil seed bank along with consistently higher emergence of a weed population in the field suggests greater seed longevity of the weed seeds than that of the cultivated population (Cavieres and Arroyo 2001).

Presence of some inherent characteristics in seeds determine their persistence in the soil seed bank (Anderson *et al* 2012). Yu *et al* (2007) also reported that soil seed bank

persistence could be predicted based on seed characteristics *viz*., size, shape and weight. Seed size and coat colour are important characteristics to predict seed persistence under soil seed bank conditions, with smaller seeds often showing higher soil seed persistence than larger seeds (Peco *et al* 2003). Ma *et al* (2010) found that seed from persistent seed bank tend to have smaller seeds than species with transient seeds. Variability in weed seed size is responsible for variability in seed germination, persistence and emergence under different environmental factors (Tanveer *et al* 2013). Cao *et al* (2012) reported that black seeds of *Suaeda corniculata* had an annual dormancy/non-dormancy cycle, while brown seeds, which were non-dormant at maturity, remained non-dormant. Brown seeds formed only a transient soil seed bank and black seeds a persistent seed bank. The method most commonly advocated for determining whether a seed bank is transient, short-term persistent or long-term persistent deals with measuring the presence/absence of species (in the vegetation and/or as seeds in the soil) and the abundance of seeds in the upper versus lower soil depths (Walck *et al* 2005). Tsuyuzaki (2006) reported that *Rumex obtusifolius* seeds exhumed after 3 years of burial from a depth of 3 cm showed germination of more than 70% in temperature range of 15-25°C. However, field emergence was completely absent in *R. obstifolius* seeds buried at 3 cm which indicated that presence of light was required for field germination. Mennan and Zandstra (2006) reported that seeds of *Veronica hederifolia* buried for 2 yr at a depth of 0, 5, 10 and 20 cm recorded progressive decrease in viability upon retrieval from field at monthly intervals and seeds placed on the soil surface lost their viability more rapidly than those buried more deeply. Hu *et al* (2017) reported that *Chenopodium hybridium* seeds placed at soil surface and buried at a depth of 5 cm for 22 months recorded 39% and 10%, germination respectively. Gulden and Shirtliffe (2009) reported that common lambsquarters (*Chenopodium album*) form a persistent seed bank with the ability to remain viable in the soil for many decades. Conn *et al* (2006) reported that seed germination and dormancy of common lambsquarters (*Chenopodium album*) did not differ at 2 and 15 cm depth and seeds at both depths recorded 3.0 and 26.7% germination and dormancy after a burial period of 19.7 years.

2.5 Accelerated ageing and seed viability

Long *et al* (2008) assessed the ability of the controlled ageing test (CAT) used in the Millennium Seed Bank Project by the Royal Botanic Gardens, Kew, UK to predict seed persistence in the field. Relative seed persistence was predicted by ranking species based on their CAT P_{50} values, the time in days taken for seed viability to decline by 50%, as originally described by Newton, and by then comparing these to the known field persistence data available from the literature. They described three categories of seedbank persistence: a transient seed bank corresponded to species with a P_{50} value of < 20 days (< 1 year field persistence), a short-lived seed bank corresponded with P_{50} values of 20 to 50 days (1-3 years field persistence) and extended persistence corresponded with P_{50} values of > 50 days (> 3

years field persistence). Long (2007) reported that *Avena sterilis* seeds aged under field conditions differed from those in the accelerated ageing test in terms of membrane integrity and antioxidant capacity which might be attributed to differences in the moisture content of seeds between the two environments. Artificial seed ageing is determined by the seed moisture level and temperature. Long *et al* (2008) reported positive correlation between controlled ageing conditions (45°C and 60% RH) and field seed persistence. Various factors affecting seed vigour include weather conditions during seed producing stage, moisture content, mechanical damages, time of storage and relative humidity of store (Krishnan *et al* 2003, Marshal and Levis 2004). Seed ageing is a function not only of time but also of temperature and moisture (Ellis and Roberts 1981). Biochemical processes which lead to reduction in seed vigour include free radical oxidations, enzymic dehydrogenation and aldehyde oxidation (Ghassemi-Golezani *et al* 2010). The rate of seed deterioration rapidly increases either due to increase in seed moisture content or temperature of storage (Kapoor *et al* 2010). Ghassemi-Golezani *et al* (2010) reported that decrease in germination percentage and germination index is related to physiological and biochemical changes during seed ageing. Seed ageing leads to the loss of vigour and viability and is characterized by the reduced speed of germination, loss of germination and development of poor seedlings (Mosavi *et al* 2011). Bhanuprakash *et al* (2006) reported that accelerated ageing in onion (*Allium cepa* L.) seeds resulted in significant reduction in germination percentage, speed of germination, total seedling dry weight and seedling vigour index. Rastegar *et al* (2011) reported that soybean seeds subjected to rapid ageing at a temperature of 40°C recorded significant decrease in germination and germination index with concomitant increase in mean germination time. Electrolyte leakage is a measure of the loss of semi permeability of the plasma membrane. Kaewnaree *et al* (2011) reported that bell paper (*Capsicum annuum*) seeds aged for a period of 10-30 days recorded increase in electrical conductivity and concentrations of Na^+, Ca^{2+}, K^+ and Mg^{2+} ions in the leachate. Seed germination comprises two distinct metabolic processes *viz*; reserve mobilization by hydrolytic enzymes and utilization of the hydrolysed products for the formation of new structures (Fu *et al* 2005, Soltani *et al* 2006). Accelerated ageing cause significant changes in biochemical composition of seeds. Kapoor *et al* (2010) reported that accelerated ageing in cowpea (*Vigna unguiculata* L.) seeds resulted in decreased rate of germination, seed viability and vigour which was correlated with decreased content of soluble proteins and sugars.

2.6 Herbicide exposure

Weeds pose a major limiting factor in successful crop production and cause huge yield losses depending upon nature and intensity of weed flora. Wheat (*Triticum aestivum* L.) crop is infested with complex weed flora. Chhokar *et al* (2008) reported that average yield losses due to weeds in wheat vary from 20 to 32% across different wheat growing regions in

India. Kaur *et al* (2015) reported that weed flora of wheat in various districts of Punjab included both dicotyledonous weeds (*Cannabis sativa*, *Chenopodium album, Malva neglecta* and *Rumex dentatus*) and monocotyledonous weeds (*Phalaris minor*, *Avena ludoviciana* and *Poa annua*). Herbicides play an important role for weed control in close spaced crops like wheat and barley, where manual or mechanical weeding is difficult (Yaduraju and Das 2002). Herbicides are the key component of weed management program in wheat in India, particularly in North Western states. All types of weeds are not controlled by a single herbicide and the continuous use of a single herbicide results in weed shifts and evolution of herbicide resistance. The presence of mixed weed flora warrants integrated use of chemical control measures. Traditional methods of weed control such as manual hoeing and labour costs have made the use of herbicides popular among Indian farmers. Sulfonylurea herbicides are highly potent against both monocotyledonous and dicotyledonous weeds because of their high potency and low dose requirement. Singh *et al* (2015) reported that post-emergence application (30 DAS) of sulfosulfuron + metsulfuron (32 g/ha) in wheat significantly decreased density and biomass of both dicotyledonous and monocotyledonous weeds with concomitant increase in leaf area index (LAI) and grain yield. For controlling both dicotyledonous and monocotyledonous weeds premix of sulfosulfuron and metsulfuron-methyl and tank mix of isoproturon and 2,4-D are recommended (Pandey *et al* 2006). Pandey and Verma (2004) reported that tank mix application of isoproturon+2,4-D for managing dicotyledonous weeds in wheat crop resulted in maximum reduction in weed population and recorded higher weed control efficiency and maximum grain yield. Singh and Singh (2005) reported that clodinafop and fenoxaprop were used exclusively in 1990s for control of monocotyledonous weeds which resulted in build of dicotyledonous weeds like *Melilotus indica, Medicago denticulata* and *Lathyrus aphaca* which are not even controlled effectively by 2,4-D. Kaur *et al* (2017) reported that pendimethalin and metribuzin used alone provided 33 and 63% control of *Medicago denticulata*, respectively. However, tank-mix application of both herbicides resulted in > 90% control of this weed. Cauchy (2000) reported that carfentrazone-ethyl was active at low dose rates (20 g a.i./ha) and provided better results against young dicotyledonous weeds like, *Gallium aparine* and *Veronica hederifolia* which were controlled within 1 to 2 weeks of herbicide application. Zhu *et al* (2001) reported that application of carfentrazone-ethyl at 15, 22.5, 30 and 45 g/ha for managing dicotyledonous weeds resulted in higher weed control efficacies than that of tribenuron-methyl at 15 g/ha. Singh *et al* (2011) reported that premix of carfentrazone+metsulfuron at 25 g/ha+0.2% surfactant provided effective control of dicotyledonous weeds infesting wheat *viz*., *Malva parviflora*, *Lathyrus aphaca*, *Convolvulus arvensis*, *Rumex dentatus*, *Medicago denticulata* and *Chenopodium album* which were not effectively controlled by these herbicides when applied alone. Brar *et al* (2005) reported that carfentrazone at 25 g/ha produced significantly

higher grain yield, plant height and effective tillers/m row than 2,4-D at 500 g/ha and unweeded control in wheat. Balyan and Panwar (1997) reported that 2,4-D provided good control of some of the non-grassy weeds but caused malformed spikes in many wheat cultivars. Punia *et al* (2005) recorded maximum grain yield under weed free treatment and carfentrazone-ethyl at 25 g/ha which was significantly higher than 2,4-D treatments. Chhokar *et al* (2006) reported that application of flufenacet and metribuzin in *P. minor* at four to five leaf stage resulted in reduced herbicide efficacy. Singh *et al* (2011) reported that fenoxaprop+metribuzin applied at the 2-4 leaf stage provided 90-100 percent control of *P. minor*, whereas its delayed application at 4-6 leaf stage resulted in only 45 to 85 percent control.

The one way to manage weed seed banks is to reduce the input to the soil through reducing weed seed production, which would help in lesser weed population in succeeding years. Emergence of weed seedlings at different times after crop emergence may result in differences in growth and productivity, depending on the conditions during early development of the crop plants (Lindstrom and Kokko 2000). For example, weeds which emerge late, are usually less competitive with lesser biomass and seed production than early-emerging ones (Hartzler *et al* 2004, Chauhan and Johnson 2010). Herbicide application during initiation of flowering or initial seed set could be exploited for decreasing weed seed production (Bennett and Shaw 2000). Steadman *et al* (2006) reported that late-season application of glyphosate resulted in decreased seed viability of sicklepod by 10 to 95%, depending on weed species. Fawcett and Slife (1978) reported that 2,4 D applied at rate of 1.1 kg/ha reduced the seed production of common lambsquarters (*Chenopodium album* L.), redroot pigweed (*Amaranthus retroflexus* L.) and jimsonweed (*Datura stramonium* L.) by 99, 84, 64 and 100%, respectively. El-Ghit (2016) reported that the application of 2,4-D as a post-emergence herbicide even at sub-lethal doses (ranging from 125-500 ppm) effectively controlled three dicotyledonous weeds - *Amaranthus viridis*, *Chenopodium album* and *Xanthium spinosum* associated with maize crop. Application of 2,4-D at 500 ppm significantly decreased number and fresh weight of the three weeds by about 50%. However, in response to higher dose of 2,4-D i.e. 1000 ppm, there was severe reduction in weed number per pot and fresh weight of three weeds. Shuma *et al* (1995) reported that glyphosate application to *Avena fatua* five days after anthesis (DAA) at a rate of 1.76 kg a.i./ha resulted in production of non viable seeds by the surviving weed plants. Wallace *et al* (1998) reported that application of glyphosate (112 g a.i./ha) during early to mid flowering reduced the seed yield of annual medic (*Medicago polymorpha*) by 90% along with reduction in proportion of hard seed. Clay and Griffin (2000) reported that glyphosate application during initial seed set decreased the 100-seed weight of hemp sesbania (*Sesbania herbacea*) and sicklepod (*Senna obustifolia*) by 73 and 46%, respectively. Jha and Norsworthy (2012) studied the effect of glufosinate, 2,4-D and dicamba

on seed production potential of two glyphosate-resistant (GR) palmer amaranth (*Amaranthus palmeri*) biotypes, Mississippi County (MC) and Lincoln County (LC) and reported that late-season applications of glufosinate, 2,4-D and dicamba reduced seed production of LC biotype by 75 to 87% and production of the MC biotype by 94 to 95% compared with nontreated plants.

CHAPTER - III

MATERIALS AND METHODS

3.1 Location and Climate

Experiment on effect of seed coat colour on germination ecology of *Medicago denticulata* was performed in Weed Physiology Laboratory, Department of Agronomy, Punjab Agricultural University, Ludhiana. Seed bank persistence under field conditions was studied at Research Farm, Department of Agronomy, PAU and Dr. J. C. Bakhshi Regional Research Station, Abohar. Experiment on herbicide exposure was conducted at Research Farm, Department of Agronomy, PAU and Regional Research Station, Abohar during *rabi* 2016-17 and 2017-18.

3.2 Experiment No. I

To study the effect of seed coat colour on germination ecology of *Medicago denticulata*.

3.2.1 Plant Material

Pods of *M. denticulata* containing mature seeds were collected from Research Farm, Department of Agronomy during the months of April 2016 and 2017. Seeds were removed from pods immediately before use. Seed lots were prepared by visual inspection of seed coat color and were grouped into categories of two different colours–cream and brownish black (Plate 1).

3.2.2 Germination Protocol

Uniform sized seeds of *M. denticulata* from each seed lot were surface sterilized by soaking them in 0.1% mercuric chloride for two minutes followed by thorough washing with distilled water to avoid any fungal infection. The seed germination was tested by placing 30 seeds of *M. denticulata* from each seed lot in 9 cm Petri dishes lined with Whatman No. 1 filter paper. Seeds were moistened with 5 ml of treatment solution and were incubated at 20°C (optimal temperature) in environmental chamber (Model MAC MSW-127, Delhi, India).

Germination counts were made at 24 hour intervals till 15 days after start of the experiment, with the criterion for germination being visible protrusion of the radicle. Viability of non-germinated seeds was tested using tetrazolium test (Steadman 2004). Data on length, fresh and dry weight was recorded from 15 days old seedlings. Each treatment was replicated thrice and experiments were conducted three times.

3.2.3 Dormancy breaking treatments

Seeds of *M. denticulata* with brownish black seed coat were non-dormant and germinated rapidly after 1 month of initial harvest; however, majority of the seeds with cream coloured seed coat were dormant so following methods were tested to break their dormancy.

a) **Mechanical scarification with sandpaper**: Seed coats were scarified by hand with sandpaper for 1 minute. Later the seeds were washed with distilled H_2O. Scarified seeds

Plate 1: Heteromorphic seeds (cream and brownish black) of *Medicago denticulata* Willd.

were tested for germination.

b) Scarification with H_2SO_4: Seeds were soaked in three concentrations of H_2SO_4 - 50, 70 and 98% for 2, 4, 6, 8 and 10 min. Thereafter, seeds were rinsed several times with distilled water and germinated with protocol described above. Untreated seeds were used as a control.

c) Soaking in hot water: The seeds were transferred to beaker containing distilled H_2O at 50, 70 and 90°C and were kept in water bath for 5, 10, 15 and 30 minutes. At the end of this period, the seeds were cooled, transferred to Petri dishes for germination.

3.2.4 Morphological studies

The morphology of the seed coat was characterized by scanning electron microscope (SEM). For SEM investigation, ten dried seeds of each seed lot (cream and brownish black) were stubbed and sputter coated in Hitachi gold sputter coater (Model E1010) then imaged using a scanning electron microscope (Hitachi S-3400N E1010). Seed size, seed shape and seed coat pattern were recorded as an average of randomly chosen seeds from 5 replicates.

3.2.5 Moisture content

1 g of both cream and brownish black seeds were weighed separately using a digital balance by randomly picking seeds from both the lots. Seed moisture content was determined by drying seeds in an oven at 130°C for 1 h and allowed to cool down in desiccator for 30 minutes before being reweighed (ISTA 2005). The moisture content was calculated as follows:

$$\text{Moisture content (\%)} = \frac{\text{Fresh mass} - \text{Dry mass}}{\text{Fresh mass}} \times 100$$

3.2.6 Imbibition rate (IR)

Three replicates of 200 seeds were selected and weighed for each lot and then soaked in 25 ml of distilled water for 3, 6, 9, 12, 18, 21, 24, 30, 36 and 48 hr at 25°C. The weight of soaked seeds was taken after surface drying them with a blotting paper. The imbibition rate was calculated by following formula

$$\text{Imbibition (\%)} = \frac{\text{Weight of soaked seeds} - \text{weight of unsoaked seeds}}{\text{weight of unsoaked seeds}} \times 100$$

After 24 hrs of imbibition, the electrolyte leakage of both the seeds was recorded using a digital conductivity meter (Systronics Conductivity Meter 304).

3.2.7 α – amylase enzyme activity

α – amylase enzyme activity of cream and brownish black seeds was estimated as per Murata *et al* (1968). 100 mg of non scarified cream and brownish black seeds were homogenized in pestle mortar by adding 5 ml of phosphate buffer (pH=7.0). The homogenate was centrifuged twice at 3000 rpm for 10 minutes. One ml of pooled supernatant was diluted to 10 ml with phosphate buffer. One ml of soluble starch solution and 0.5 ml H_2O were pre incubated at 25°C for 10 min, after which was added 0.5 ml of the enzyme solution to start

the reaction. At various time intervals (0, 5, 10, 15 and 20 min), 1.0 ml of KI-I_2 reagent was added. The mixture was diluted by adding 5.0 ml of H_2O and absorbance was measured at 620 nm. The standard curve was prepared using soluble starch solution (10-60 µg). Enzyme activity was calculated from the analytical data of 5 min incubation and was expressed as µg starch broken down/100 mg seeds.

3.2.8 Experimental treatments

a) Light

To study the effect of light on germination, Petri dishes were kept under three light regimes- continuous light (24 h), light/dark (12/12 hours) using light intensity of 85 m mol m^{-2} s^{-1} and continuous dark (24 h) at 20°C. In latter treatment, Petri dishes were wrapped with double layers of aluminum foil immediately after adding distilled water to completely obstruct penetration of light. The data on germination counts were recorded on 15th day after initiation of the experiment.

b) Temperature

The germination of seeds was tested under fluctuating day/night temperatures (12 h light/12 h dark) *viz*., (15/5, 20/10, 25/15, 30/20 and 30/25°C) and constant temperatures (24 h light) (5, 10, 15, 20, 25, 30 and 35°C).

c) Moisture stress

The ability of seeds to germinate under different levels of moisture stress was tested using solutions of PEG 8000 having water potentials of 0, -0.1, -0.2, -0.4, -0.6, 0.8 and -1.0 MPa (Michel and Kaufmann 1973).

d) Salinity stress

The germination ability of seeds under different levels of salt stress was examined using NaCl solutions of 25, 50, 75, 100, 150, 200, 250 and 300 mM concentrations. The solutions of different concentrations were prepared by diluting stock solution of NaCl having concentration 300 mM (1.753 g in 100 ml).

e) pH

Effect of pH on seed germination was studied using buffered solutions with variable pH ranging from 3 to 10. 0.2 mM potassium hydrogen phthalate was used to prepare buffered solutions of pH 3 and 4 (Chachalis and Reddy 2000). Buffered solutions of pH 5 and 6 were prepared with 2 mM of 2-(N-morpholino) ethanesulfonic acid and 2 mM of 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid was used for preparation of buffered solutions of pH 7 and 8. 2 mM of Tricine was used to prepare buffered solutions of pH 9 and 10. Final adjustments of each buffer solution were made using 0.1 M HCl or 0.1 N NaOH. Unbuffered distilled water having pH 6.6 was used as control.

f) Burial depth

This experiment was conducted using 25-cm diameter plastic pots placed under field

conditions during November-December 2016-17 and 2017-18. Soil filled in these pots was collected from those fields which recorded no previous incidence of this weed. Fifty seeds of both seed lots were sown on the soil surface in pots and covered to a depth of 0, 1, 2, 4, 6, 8 and 10 cm. The pot surface was kept moistened throughout the study period. The emergence was recorded over a period of one month. One set of pots was also kept in which no seeding of this weed was done to eliminate the error. This experiment was conducted using four replications each time.

3.2.9 Observations recorded

a) Per cent germination

Germination counts were daily made for 15 days after start of the experiment. The seeds showing visible protrusion of radicle were considered as germinated. Germination percentage was calculated as:

Percent Germination = [Number of seeds germinated /total number of seeds] × 100

b) Speed of germination

Speed of germination (germination index) was calculated using the following formula given by the Association of Official Seed Analysts (1983).

$$GI = \frac{\text{Number of germinated seeds}}{\text{Days of first count}} + - - - - + \frac{\text{Number of germinated seeds}}{\text{Days of final count}}$$

c) Mean germination time (MGT)

It was calculated using the following equation of Ellis and Roberts (1981):

$$MGT = \frac{\Sigma (Dn)}{\Sigma n}$$

Here *n* is the no. of seeds that had germinated on day *D* and *D* is the no. of days counted from the beginning of germination.

d) Seedling vigour index (SVI)

Seedling vigour index (SVI) was calculated using the following formula (Abdul-Baki and Anderson 1973).

Seedling vigour Index I = seedling length x germination (%)

Seedling vigour Index II = seedling dry weight x germination (%)

e) Growth of seedlings

On 15th day of experiment, shoot and root length were measured with centimeter scale. Seedlings were dried at 70°C for 72 hrs in an oven to determine their dry weight.

3.3 Experiment No. II

To study soil seed bank persistence of *Medicago denticulata* under field and laboratory conditions.

3.3.1 Methodology

Field persistence of cream and brownish black seeds was determined at two locations-

Research farm, Department of Agronomy, PAU, Ludhiana and and Dr. J.C. Bakhshi Regional Research Station, Abohar. For evaluating the persistence behaviour of seeds under field conditions, *M. denticulata* pods were put in permeable nylon bags and were placed on the soil surface (0 cm) and buried in soil at 2 and 10 cm depths. Paper labels were tied with each bag for identification of samples during exhumation. The bags were exhumed from the soil at different intervals (3, 6, 9, 12, 15 and 18 months after burial) followed by removal of seeds from pods. Cream seeds were scarified with sandpaper before germination studies. Brownish black seeds did not receive any scarification treatment. Different components of seed fate (field and laboratory germination and decayed seed) were recorded each time. There were no significant differences between the results of the repeated experiments, so data were pooled.

For studying seed persistence under laboratory conditions, 5 g of freshly harvested seeds from both seed lots were weighed and accelerated ageing of seeds was done by keeping them at 45°C under humid storage (Biabani *et al* 2011). The seeds were contained in a mesh bag which was placed on a sieve suspended over water contained in a desiccator held at 45°C in an oven for 1, 4, 7, 10, 20, 30, 40, 50 and 60 days. Ageing was followed by air drying of seeds at room temperature for restoration of their original weight. Cream seeds after ageing treatment were given scarification treatment. Aged seeds (cream and brownish black) were tested for germination at optimum temperature (20°C) in environmental chamber. Different components of seed fate and storage reserves were recorded each time.

3.3.2 Treatments

a. The bags were exhumed from the soil at different intervals (3, 6, 9, 12 and 18 months after burial).
b. Accelerated ageing of seeds was done at 45°C under humid storage conditions for 10, 20, 30, 40, 50 and 60 days.

3.3.3 Observations recorded

a) Field germination (%)

The seeds that germinated in the field were found with their intact plumule and radicle tissues with in the nylon bag.

b) Seed decay (%)

The seed crush test or pressure test method was used to test viability by visual inspection and the application of pressure with forceps (Borza *et al* 2007). Seeds that appeared intact but collapsed when crush test was performed with forceps were classified as decayed. The crush test categorizes non-viable seeds as those that collapse under gentle pressure, whereas viable seeds remain firm following this pressure.

d) Laboratory germination (%)

Seeds recovered after exhuming nylon bags were washed free of soil and germination was tested by placing 30 seeds of *M. denticulata* from each seed lot in 9 cm Petri dishes using

standard germination protocol as mentioned in experiment 1. The germinated seeds were then counted and removed.

e) Membrane leakage

Membrane leakage of seeds was determined as described by Fletcher and Drexlure (1980). 0.5 g of seeds were immersed in twenty five ml of deionized water contained in glass vials and placed at 25°C. Electrical Conductivity (EC) of leachate was recorded after 24 hrs using Sanco Digital Conductivity Meter. The samples were then boiled for 30 minutes and conductivity was measured again. Membrane leakage was calculated using the following formula:

Membrane leakage % = (Conductivity after boiling – Conductivity before boiling/Conductivity after boiling) x 100

3.4 Experiment No. III

To study germination ecology of *Medicago denticulata* seeds after herbicide exposure.

3.4.1 Methodology

Herbicides *viz*., 2,4-D, carfentrazone and metsulfuron plus sulfosulfuron at 0.5 and 1x recommended rates along with water were sprayed at 4, 8 and 12 leaf stages of *M. denticulata* growing in wheat crop under field conditions during *rabi* 2016-17 and 2017-18. Seeds collected from plants of *M. denticulata* which survived herbicide application were tested for various storage reserves and germination behaviour.

3.4.2 Treatments:

a) Main plots: Weed growth stage: (3)

W_1: 4 Leaf stage

W_2: 8 Leaf stage

W_3: 12 Leaf stage

b) Sub plots: Herbicide spray: (7)

S_1: Control (No spray)

S_2: 2,4-D 0.25 kg/ha (0.5x recommended rate)

S_3: 2,4-D 0.5 kg/ha (1x recommended rate)

S_4: carfentrazone 0.01 kg/ha (0.5x recommended rate)

S_5: carfentrazone 0.02 kg/ha (1x recommended rate)

S_6: metsulfuron+sulfosulfuron 0.015 kg/ha (0.5x recommended rate)

S_7: metsulfuron+sulfosulfuron 0.03 kg/ha (1x recommended rate)

3.4.3 Layout details:

Design	: Split plot design
Number of treatment combinations	: 3 x 7=21
Number of replications	: 3

Total number of plots : 21 x 3 = 63

Gross plot size : 2.5 m x 4.0 m = 10 m^2

3.4.4 Brief description of herbicides used in the study

3.4.4.1 2,4 D

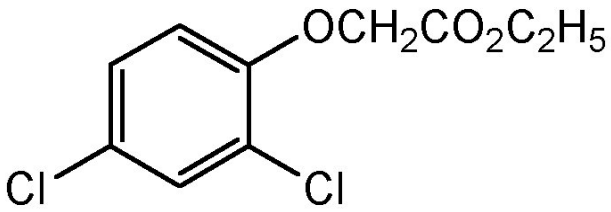
Chemical group Phenoxy-carboxylic-acetic acid

Molecular formula $C_{10}H_{10}Cl_2O_3$

IUPAC name 2,4-dichloro phenoxy acetic acid

Mode of action 2,4-D is a synthetic auxin with hormone like activity. It is mainly absorbed through roots and foliage. 2,4-D binds with ABP-1 protein and therefore do not allow natural auxin to bind with ABP-1 protein. Translocation occurs through phloem and xylem to the meristematic tissues leading to abnormal cell division. Uncontrolled plant growth leads to cell death.

Chemical structure



3.4.4.2 Carfentrazone ethyl

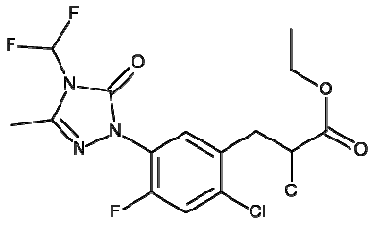
Chemical group Triazolinone

Molecular formula $C_{15}H_{14}N_3O_3F_3Cl_2$

IUPAC name Ethyl 2-chloro-3-{2-chloro-5-[4-(difluoromethyl)-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]-4-fluorophenyl}propanoate

Mode of action Carfentrazone is a post-emergence herbicide used to control broadleaf weeds in cereals. It is applied as a foliar spray and is absorbed through the leaves. The mode of action of carfentrazone is the disruption of membranes by inhibiting the action of protoporphyrinogen oxidase (PPO), causing cell death.

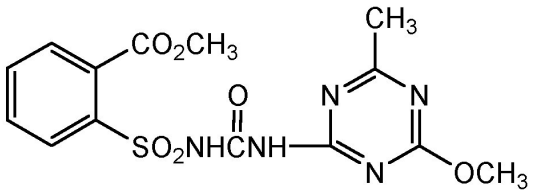
Chemical structure



3.4.4.3 Metsulfuron-methyl

Chemical group	Sulfonylurea
Molecular formula	$C_{16}H_{19}N_5O_9S_2$
IUPAC name	Methyl 2-[(4,6-dimethoxyprimidin-2-yl) carbamoylsulfamoyl]-4(methanesulfonamidomethyl) benzoate
Mode of action	It is a selective post-emergence herbicide with foliar and soil activity and it works rapidly after it is taken up by the plant. Its mode of action is by inhibition of acetolactate synthase (acetohydroxy acid synthase), the first enzyme in the synthetic pathways for valine, leucine and isoleucine.

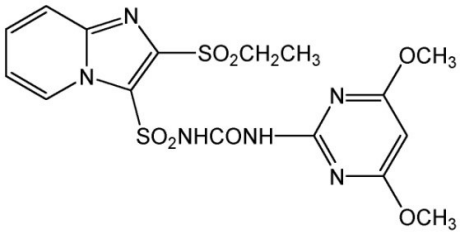
Chemical structure



3.4.4.4 Sulfosulfuron

Chemical group	Sulfonylurea
Molecular formula	$C_{16}H_{18}N_6O_7S_2$
IUPAC name	1-(4,6-dimethoxypyrimidin-2-yl)-3-(2-ethylsulfonylimidazo[1,2-a]pyridin-3-yl)sulfonylurea
Mode of action	It is a selective post-emergence herbicide with foliar and soil activity and it works rapidly after it is taken up by the plant. Its mode of action is by inhibition of acetolactate synthase (acetohydroxy acid synthase), the first enzyme in the synthetic pathways for valine, leucine and isoleucine.

Chemical structure



3.4.5 Cropping history

The experimental field had been under following cropping systems (Table 3.1) for the last three years:

Table 3.1 Cropping history of experimental field

Year	Punjab Agricultural University		Regional Research Station, Abohar	
	Kharif	***Rabi***	***Kharif***	***Rabi***
2015-16	Rice	Wheat	Cotton	Wheat
2016-17	Rice	Wheat (Experimental)	Cotton	Wheat (Experimental)
2017-18	Rice	Wheat (Experimental)	Cotton	Wheat (Experimental)

3.4.6 Crop management: Agronomic practices for raising the wheat crop are given below:

3.4.6.1 Field preparation

A heavy pre-sowing irrigation was applied to ensure adequate moisture in the soil at the time of sowing. On the attainment of proper moisture condition, a fine seedbed was prepared by ploughing the field with a disc harrow, followed by two passes with a field cultivator and two plankings. The seeds of *M. denticulata* were broadcasted uniformly in the field before sowing of wheat crop.

3.4.6.2 Sowing

Sowing of wheat cv. PBW 677 was undertaken on 8th November, 2016 and 13th November, 2017 using seed rate of 100 kg/ha, in 22.5 cm spaced rows. The wheat seed was treated with Dursban (chlorpyriphos 20 EC) @ 4 ml per kg seed and Raxil Easy (tebuconazole) @ 13 ml per 40 kg seed before sowing. Seeds were inoculated using mixture of consortium biofertilizer and water on *pucca* floor.

3.4.6.3 Fertilizer application

The crop was fertilized by using urea as a source of nitrogen @ 125 kg N in two splits; half as basal and half with first irrigation. Phosphorous was applied @ 62.5 kg P_2O_5 ha^{-1} through single super phosphate and potassium @ 30 kg K_2O ha^{-1} through muriate of potash as basal dose.

3.4.6.4 Weed control

Weeding was carried out manually to keep the crop free from weeds other than *Medicago denticulata* which appeared in the field throughout the growth period. Uniform density of *M. denticulata* with fifteen plants per plot was maintained in each experimental plot.

3.4.6.5 Irrigation

Besides one pre-sowing irrigation, the irrigation was applied as per crop requirement at 23, 56, 84 and 115 days after sowing (DAS); at 25, 58, 80 and 120 DAS during 2016-17 and 2017-18, respectively.

3.4.6.6 Plant protection measures

The crop was sprayed with Tilt (propiconazole 25 EC) @ 500 ml ha^{-1} to control yellow rust and Rogor (dimethoate 30 EC) @ 375 ml for the control of aphid at the ear head stage of wheat.

3.4.6.7 Harvesting and threshing

The crop was harvested manually on 17th April, 2017 and 21st April, 2018 from a net area of 4.5 m^2 after excluding non-experimental borders when the grain had matured. The harvested crop was tied in labeled bundles, kept for sun drying and then threshed with a thresher on 22nd April, 2017 and 27th April, 2018.

3.4.7 Physiological parameters of crop and weed

3.4.7.1 Chlorophyll content index

Chlorophyll content index was recorded from fully expanded apical leaves of ten tagged plants in each plot at 1, 4, 7, 14 and 28 days after spray and then at flag leaf stage using a portable chlorophyll content meter (Model – CCM-200, Opti-Sciences, Inc.).

3.4.7.2 Chlorophyll fluorescence

The data of chlorophyll fluorescence was recorded from tagged plants at 1, 4, 7, 14 and 28 days after spray and then at flag leaf stage using chlorophyll fluorometer (Model - OS-30p, Opti-Sciences, Inc.). The portion of the leaf from which data was recorded was dark-adapted for ten minutes by using plastic clips and then exposed to the light of given wavelength emitted by the fluorometer. The fluorescence readings were expressed as F_v/F_m (variable fluorescence/maximum fluorescence).

3.4.7.3 Herbicide toxicity rating

The observation on visual crop and weed toxicity was recorded at 1, 4, 7, 14, 28 days after spray (DAS) by comparing with water sprayed control plants. Toxicity rating was done on visual scale of 0-10 (0 = no phytotoxicity and 10 = complete mortality) (Table 3.2).

Table 3.2: Qualitative description of treatment effects on weeds and crop in the visual scoring scale of 0 to 10 (Rao 1986)

Effect or damage observed	Rating	Grading for leaf injury (%)	Weed	Crop
No effect	0	0	No weed control	No injury
Slight effect	1	1-10	Very poor control	Slight leaf discolouration
	2	11-20	Poor control	Some crop stand loss, stunting and/or discolouration
	3	21-30	Poor-deficient control	Injury is more pronounced, recovery is possible
Moderate	4	31-40	Deficient control	Moderate injury, but recovery is possible
	5	41-50	Deficient-moderate control	Injury is persistent and recovery doubtful
	6	51-60	Moderate control	Injury is severe, recovery not possible
Severe	7	61-70	Satisfactory control	Severe injury and stand loss
	8	71-80	Good control	Almost destroyed, a few plants surviving
	9	81-90	Good to excellent control	Very few plants alive
Complete	10	91-100	Complete mortality	Complete crop destruction/failure

3.4.7.4 Weed density and biomass

Weed count was recorded at 20 DAS (days after spray) from each plot and density was expressed as numbers of plants per m^2. For recording biomass, three plants from each plot were uprooted; dried in sunlight and then placed in the paper bags for oven drying at 60^{o}C for 48 hours. Dry weight was taken till constant weight was achieved. The data was later expressed in g m^{-2}.

3.4.8 Yield and yield attributing parameters of crop

3.4.8.1 Length of spike (cm)

Ten spikes were randomly selected from each plot and their length was measured excluding awns. The average length was calculated and then expressed in cm.

3.4.8.2 Number of grains per spike (g)

Ten randomly selected spikes were taken from each plot and the number of grains was counted, averaged and expressed as number of grains per spike.

3.4.8.3 Grain weight per spike (g)

The total number of grains obtained from ten spikes was weighed and the average was worked out and reported as grain weight per spike in grams**.**

3.4.8.4 Biological yield (q/ha)

Biological yield was recorded by weighing the bundles from each net plot using spring balance and expressed as q/ha.

3.4.8.5 Grain yield (q/ha)

Grain yield was obtained from each net plot after threshing of crop and expressed as q/ha.

3.4.8.6 Straw yield (q/ha)

Straw yield of each plot was computed by deducting the grain yield of net plot from the net plot biological yield and expressed as q/ha.

3.4.8.7 1000-grain weight (g)

Grain samples collected separately at threshing from each plot were dried properly. 1000 grains from each of these samples were taken and their weights were recorded and expressed in grams.

3.4.9 Seed production and seed size parameters of weed

3.4.9.1 Fruit number plant^{-1}

Five plants were taken from each plot at maturity and the number of fruits per plant was counted, averaged and expressed as fruit number plant^{-1}.

3.4.9.2 Seed number plant^{-1}

At the maturity stage, five plants were selected randomly from each plot and pods containing seeds were collected and opened carefully to count number of seeds.

3.4.10 Germination parameters of *M. denticulata* seeds immediately after harvesting

3.4.11 Storage reserves: Following storage reserves were determined in *M. denticulata* seeds after herbicide exposure

3.4.11.1 Total soluble sugars

Total soluble sugars were estimated by method given by Dubois *et al* (1956).

Reagents:

1. 5% phenol
2. Chilled conc. H_2SO_4

Extraction: 100 mg of seeds were homogenized in 5 ml of 80% ethyl alcohol, followed by centrifugation at 5000 rpm. The supernatant was retained; residue was crushed again and centrifuged with 3 ml of 80% ethyl alcohol. The final volume of pooled supernatants was adjusted to 10 ml with extraction medium.

Estimation: In a test tube, 1 ml of 5% phenol was added to the 1 ml of sugar extract. Test tubes were kept for 10 minutes followed by addition of 5 ml of concentrated H_2SO_4. The sulphuric acid was poured directly in the middle of the test tube to ensure proper mixing of the solutions. The tubes were cooled to room temperature under running water after 10 minutes. After 20 minutes, the absorbance was measured at 490 nm against reagent blank. The concentration of total sugars was calculated from the glucose standards (10-60 µg) run simultaneously. The total soluble sugars content was expressed as mg g^{-1} fresh weight.

3.4.11.2 Total soluble proteins

Total soluble proteins were estimated by the method given of Lowry *et al* (1951).

Reagents:

A. 2% sodium carbonate in 0.1 N NaOH

B. 0.5% copper sulphate in 1% sodium potassium tartarate

C. Fifty ml of reagent A mixed with 1 ml of reagent B

D. Folin and Ciocalteau's reagent (2 N) diluted with water in 1:1 ratio

Extraction: 100 mg seeds were ground in 5 ml of 0.1 N Sodium hydroxide followed by centrifugation at 5000 rpm for 10 minutes. The extraction procedure was repeated twice and total volume was made to 10 ml. To 2 ml of the aliquot of protein extract, 2 ml of 20% trichloroacetic acid (TCA) was added and kept at 4°C for 24 hrs. This extract was later centrifuged for 20 minutes at 5000 rpm and precipitates so obtained were dissolved in 0.1 N NaOH.

Estimation: 5 ml of reagent C was added to 1 ml of protein extract. The mixture was constantly shaken and kept at room temperature for 10 minutes. To this 0.5 ml of reagent D was added and kept at 37°C for 30 minutes. Absorbance of the blue colour developed was measured at 520 nm against reagent blank. Proteins were quantitized from the standard curve prepared by using bovine serum albumin (BSA) standards (20-100 µg). The content of protein

was expressed as mg g^{-1} fresh weight.

3.4.11.3 Total free amino acids

Total free amino acids were estimated by method given by Lee and Takahashi (1966).

Reagents:

1. Ethanol: 70%
2. 0.5 M citrate buffer (pH 5.5)
3. 1% ninhydrin in 0.5 M citrate buffer (pH 5.5)
4. Pure glycerol
5. Ninhydrin reagent {1% ninhydrin (5ml): pure glycerol (12ml): 0.5M citrate buffer (2ml)}

Extraction: 100 mg of seeds were homogenized in 5 ml of 70% ethyl alcohol, followed by centrifugation at 5000 rpm. The supernatant was retained and residue was again centrifuged with 3 ml of 70% ethyl alcohol. The final volume of pooled supernatants was adjusted to 10 ml with extraction medium. The combined extract was evaporated and left small amount was dissolved in 0.5 M citrate buffer to make the total volume 25 ml.

Estimation: To 1 ml of extract, 5ml of ninhydrin reagent was added and the contents were mixed. The solution was heated in boiling water followed by cooling at room temperature. Absorbance of the blue purple colour developed was measured at 570 nm against reagent blank. The amino acids were quantitized from the standard curve prepared by using glycine standards (10-50 µg). The amino acid content was expressed as mg g^{-1} fresh weight.

3.4.11.4 Starch content

Starch content was estimated by method given by Clegg (1956).

Reagents:

1. Perchloric acid
2. 5% phenol
3. Conc. H_2SO_4

Extraction: To the residue left after extraction of total soluble sugars, 5 ml of distilled water was added followed by 6.5 ml of perchloric acid. It was stirred for 30 minutes followed by centrifugation for 15 minutes at 3000 rpm. Supernatant was removed and retained. The process was repeated with the remaining residue. Total 25 ml volume was made by adding distilled water. The aliquot was filtered using whatman filter paper, discarding the first 5 ml of filtrate.

Estimation: 5% phenol was added to 1 ml of the extract. To this 5ml of conc. H_2SO_4 was added and the contents were mixed. The test tubes were allowed to cool under running tap water. The absorbance was measured at 490 nm against reagent blank. The starch was quantitized from the standard curve prepared by using glucose standards (10-50 µg). The starch content was expressed as mg g^{-1} fresh weight.

3.4.12 Statistical Analysis

Data in experiment I and II was subjected to ANOVA in completely randomized block design using CPCS1, software based on the procedure outlined by Cochran and Cox (1966). Experiment III was conducted in split plot design.

CHAPTER - IV

RESULTS AND DISCUSSION

The results of present investigation 'GERMINATION ECOLOGY OF *Medicago denticulata* Willd. IN RELATION TO SEED COAT COLOUR, PERSISTENCE AND HERBICIDE EXPOSURE' are presented here under:

4.1 Experiment 1

To study the effect of seed coat colour on germination ecology of *Medicago denticulata* Willd.

4.1.1 Germination, biochemical composition and moisture content of *M. denticulata* seeds

Seeds of *M. denticulata* with brownish black seed coat were non-dormant and recorded 80% germination after 1 month of harvest; however majority of the seeds with cream coloured seed coat were dormant exhibiting ≤ 5% germination. Initial levels of principle seed storage reserves were similar in both cream and brownish black seeds (Table 4.1). Cream seeds exhibited higher α-amylase activity as compared to brownish black seeds. Greater α-amylase activity in cream seeds resulted in better germination as compared to brownish black seeds. There was considerable difference in the moisture content of these heteromorphic seeds with 6.31 and 9.56 % moisture content in cream and brownish black seeds, respectively.

Table 4.1: Physiological, morphological and biochemical characterization of heteromorphic *Medicago denticulata* Willd. seeds

	Parameter	Cream	Brownish black
Morphological	Length of seed (mm)	3.28[a]	3.31[a]
	Width of seed (mm)	1.43[a]	1.61[b]
	1000 seed weight (g)	3.56[a]	3.65[a]
Physiological	Moisture content (%)	6.31[a]	9.56[b]
	Germination (%) in non-scarified seeds	4.4[a]	80.0[b]
	Time to start germination in non-scarified seeds (days)	7[a]	3[b]
Biochemical	Total soluble sugar (mg g^{-1} FW)	20.26[a]	23.56[a]
	Starch (mg g^{-1} FW)	17.65[a]	18.76[a]
	Total soluble proteins (mg g^{-1} FW)	35.36[a]	36.57[a]
	Total free amino acids (mg g^{-1} FW)	33.40[a]	35.85[a]
	α-amylase (µg starch broken down/100 mg seeds)	0.15[a]	0.06[a]

Differences between means were compared using Paired t-test. Means followed by same letter do not differ significantly

α-amylase in the aleurone layer plays an important role during seed germination by hydrolyzing the starch into metabolizable sugars, which provide the energy for seedling growth (Kaneko *et al* 2002). Non-scarified cream seeds with higher α-amylase activity indicate absence of any other intrinsic factor causing seed dormancy. Thus, presence of hard seed coat is only factor responsible for dormancy in cream seeds. Farashah *et al* (2011) reported decreased α-amylase activity in dormant seeds than non dormant seeds of *Origanum*

vulgare. Moisture content of seed plays an important role in the formation of impermeable seed coat. As the seeds become more dry, the percentage of impermeable seeds is increased. Hard seed formation also appears to be associated with the dehydration processes which may take place independently of the plant.

4.1.2 Scanning electron microscopy, imbibition and electrical conductivity of *M. denticulata* seeds

Morphology of the cream and brownish black seeds varied in terms of size and colour. Both seeds were characteristically kidney or bean shaped. Mature cream seeds were smaller in size as compared to brownish black seeds and 1000 seed weight of cream and brownish black seeds was 3.56 and 3.65 g respectively (Table 4.1). Scanning electron micrographs of the mature seed coat of both cream and brownish black seeds showed a papillose surface pattern covered with cell wall caps (Plate 2). But, cream seeds differed from brownish black seeds by having a smooth surface. The roughness of brownish black seeds was due to surface bumps (usually wrinkled, always with rounded sides). Brownish black seeds showed the presence of conspicuous small cracks scattered all over the surface along with some depositions (Plate 3). Cream seeds did not show any such kinds of cracks and exhibited smooth surface.

Imbibition studies demonstrated significantly higher water uptake by brownish black seeds than that of cream seeds (Fig. 4.1). Apparently, presence of small cracks on surface of brownish black seeds assisted in the rapid initial water uptake with more than 80% of the water uptake occurring during the first 3 h of imbibition. Whereas, water uptake in cream seeds started after 3 h with only 10% water uptake occurring during first 3 h and took 21 h for water uptake to reach 90% indicating the resistance offered by hard impermeable seed coat to water uptake. Maximum imbibition or saturation point was attained at 12 h in brownish black seeds and at 21 h in cream seeds.

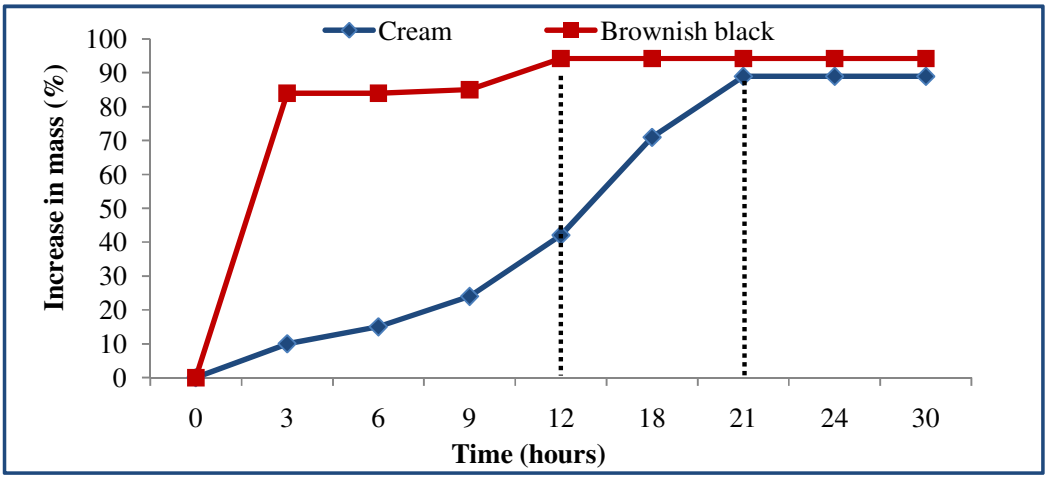


Figure 4.1: Imbibition curve for seeds of *Medicago denticulata* Willd.

Brownish black seeds with small cracks on the seed coat and higher initial water uptake exhibited greater electrolyte leakage after 24 h of imbibition. Whereas cream seeds with low imbibition rate and intact seed coat recorded comparatively less electrolyte leakage after 24 h (Fig. 4.2). Degradation of membrane integrity is one of the primary causes for low seed viability. The seeds with lower viability leave a greater amount of electrolytes while high viability seeds re-establish the membranes at the faster rate with subsequent less leakage. The factors affecting the degree of electrolyte leakage of seeds are size, rate of water uptake and seed coat integrity. The results of present study indicate that brownish black seeds with greater electrolyte leakage had low membrane stability along with weak seed coats as compared to cream seeds.

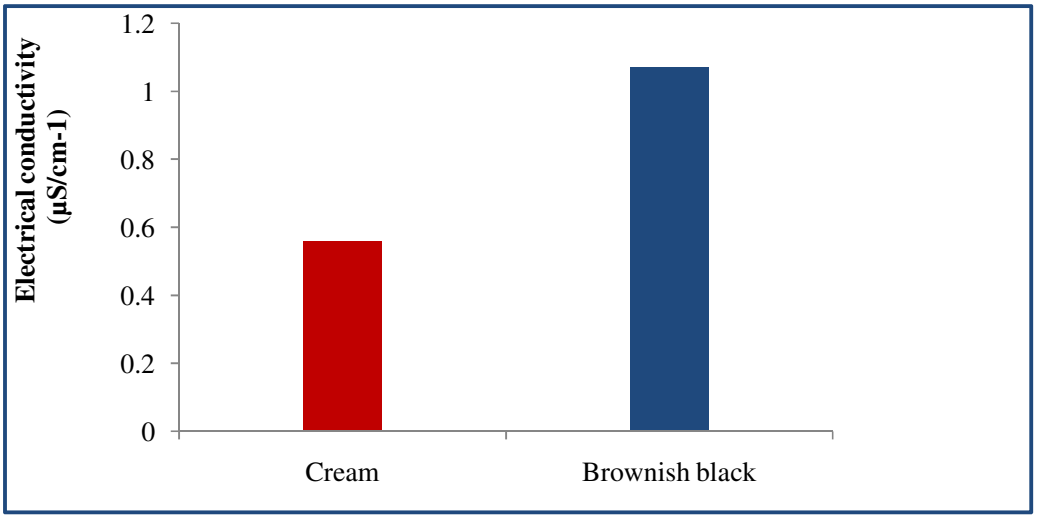


Figure 4.2: Effect of imbibition period (24 h) on electrical conductivity of *Medicago denticulata* Willd. seeds

Presence of small cuticular cracks on seed surface of brownish black seeds is a feature that can be correlated with higher seed coat permeability to water as exhibited by higher imbibition rate. The intact seed coat of cream seeds appears to be an efficient barrier to water imbibition and entry of water into the seed. Shao *et al* (2007) reported that initial imbibition by soft soybean seeds was correlated with the presence of small cracks in the outermost cuticle that covers the seed coat. Lamichaney *et al* (2016) studied the pattern of water uptake in two *Cicer arietinum* ecotypes (BG-1088 and G-229) and reported that presence of cracks on seed surface of BG-1088 resulted in highest water uptake in comparison to G-229 in which cracks were absent.

4.1.3 Dormancy breaking treatments

(i) Mechanical scarification: Table 4.2 depicts that mechanical scarification with sand paper significantly improved the germination of cream seeds in comparison to their control seeds. Scarified seeds recorded increase in germination up to 95% as compared to control. The

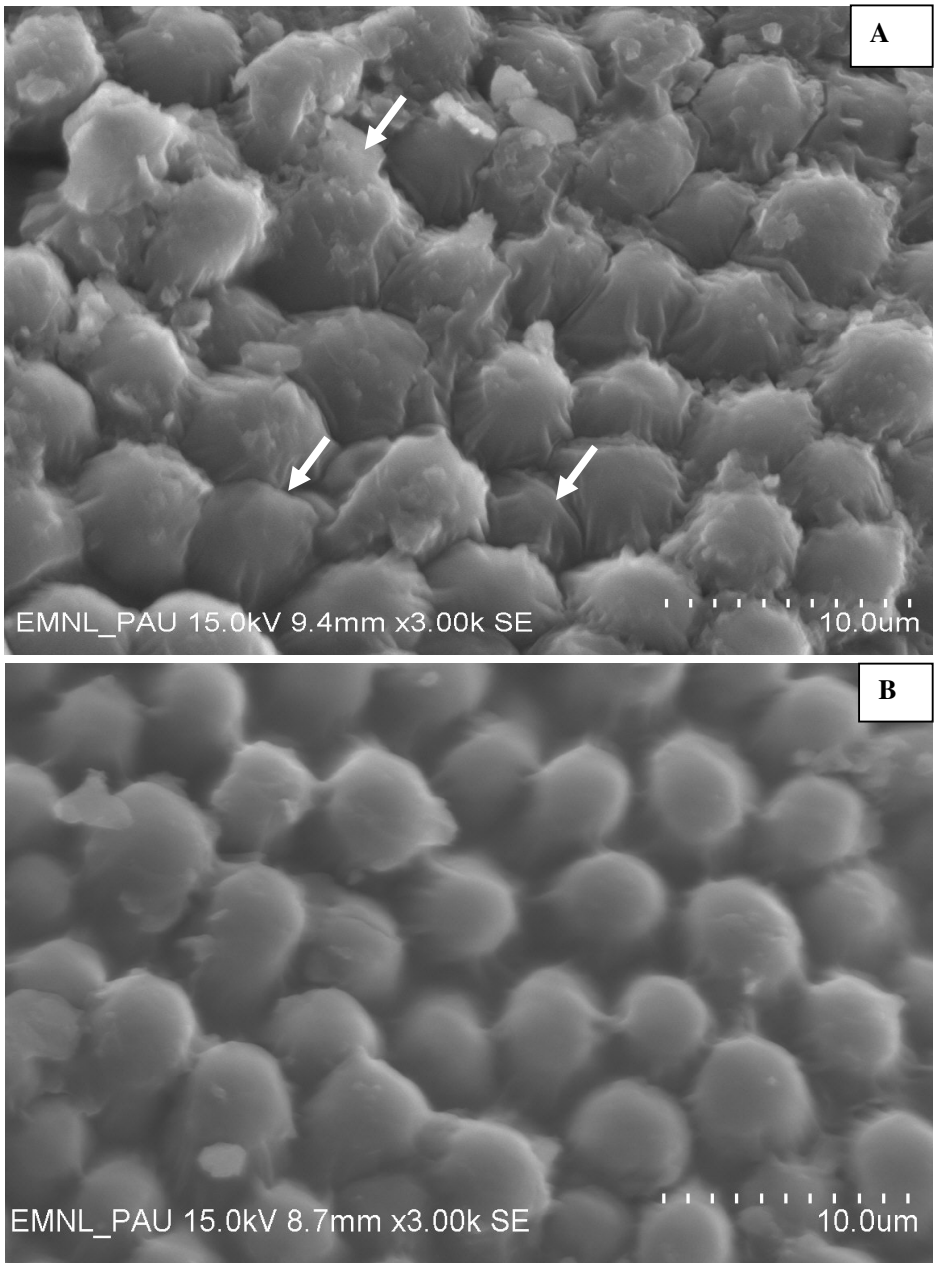


Plate 2: Scanning electron micrograph showing seed coat pattern (A) seeds with browinish black seed coat (B) seeds with cream seed coat. Arrows in (A) show depositions on seed surface.

increase in total germination was accompanied by onset of early germination on 2nd day along with increased germination speed. Whereas, non-scarified seeds took seven days for germination to start along with reduced speed of germination, increased mean germination time and decreased final germination percentage.

Table 4.2: Effect of mechanical scarification on germination of cream seeds of *Medicago denticulata* Willd.

Treatments	Germination (%)	Time to start germination (days)	Germination speed	Mean germination time (days)
Control	4.40	7.00	0.32	8.44
Sandpaper	95.0	2.00	10.52	3.05
SEm±	1.04	1.6×10^{-6}	0.03	0.34
LSD (p=0.05)	4.04	1.5×10^{-5}	0.09	0.82

LSD- Least significant difference; SEm-Standard error of mean

Physical dormancy in many legumes such as *Trifolium subterraneum, Cassia occidentalis*, *Vicia sativa* etc. has been reported to be the result of an impermeable seed coat due to the presence of one or more palisade layers of lignified cells (macrosclereids) (Baskin and Baskin 2014). Mechanical scarification is a technique to physically create scars on seed surface to increase water imbibition of the seed. Uzun and Aydin (2004) reported that various ecotypes of *Medicago* species may have hard seededness at a rate of 100%. Alane *et al* (2016) reported that scarification with sandpaper increased the germination of *M. trunculata* and *M. polymorpha* up to 69.8 and 90.6% respectively. However, Martin and De la Cuadra (2004) reported that scarification with sandpaper damaged the *M. polymorpha* seeds leading to germination of abnormal seedlings. As in present study also, brownish black seeds when scarified with sandpaper were damaged resulting in poor germination indicating the poor mechanical strength of these seeds; while significant improvement in germination was achieved in cream seeds.

(ii) Chemical scarification: Data in Table 4.3 shows the effect of different concentrations of sulphuric acid on germination of cream seeds. Chemical scarification of cream seeds with 50 and 70% sulphuric acid for different time intervals significantly improved the germination. However, sulphuric acid even at 50% concentration was proved to be very harsh to brownish black seeds as it resulted in complete seed mortality. There was progressive increase in germination of cream seeds with increased soaking time in 50% sulphuric acid. Maximum germination of cream seeds was recorded when seeds were soaked for 10 min in 50% sulphuric acid. Time to start germination and mean germination time was minimum with maximum speed of germination in above treatment. Germination was also improved when seeds were soaked for 2-8 min in 70% sulphuric acid. However, no germination was recorded

in seeds soaked for 10 or more minutes in 70% sulphuric acid. Soaking cream seeds in 98% sulphuric acid for any time interval completely damaged the seeds leading to charring.

Table 4.3: Effect of chemical scarification on germination of cream seeds of *Medicago denticulata* Willd.

Treatments (H_2SO_4)	Germination (%)	Time to start germination (days)	Germination speed	Mean germination time
Control	4.4	7.00	0.32	8.44
50 % × 2 min	12.2	5.00	0.52	7.62
50 % × 4 min	18.8	5.00	0.83	7.30
50 % × 6 min	27.7	4.00	1.65	5.32
50 % × 8 min	36.6	4.00	2.25	5.06
50 % × 10 min	42.2	3.00	3.16	4.36
50 % × 20 min	NG	NG	NG	NG
70 % × 2 min	10.0	5.00	0.45	7.95
70 % × 4 min	15.5	5.00	0.57	6.83
70 % × 6 min	24.4	5.00	1.16	6.63
70 % × 8 min	28.8	5.00	1.44	6.26
70 % × 10 min	NG	NG	NG	NG
98 % × 2 min	NG	NG	NG	NG
SEm±	0.12	1.5×10^{-6}	0.01	0.02
LSD (p=0.05)	3.01	1.4×10^{-6}	0.05	0.06

LSD- Least significant difference; SEm-Standard error of mean; NG-No germination

Acid treatments are often used to break thick impermeable seed coats. Since seeds placed in concentrated sulphuric acid can become charred in time, therefore concentration of the acid and the length of time the seeds are soaked are very important. Sulphuric acid is involved in disruption of the seed coat leading to exposure of lumens of the macrosclereids cells which permits imbibition of water thus, triggers germination. The acid treatment has been reported as an efficient method to improve seed germination of species possessing hard impermeable seed coats (MacDonald and Omoruyi 2003). However, time of exposure is critical and needs to be quantified for each species since seeds exposed for a long period can damage the embryo. As in present study, germination was decreased when seeds were allowed to stand for prolonged time suggesting embryo damage due to acid treatment. Positive influence of sulphuric acid has also been reported in other species of *Medicago* such as *M. polymorpha* (Can *et al* 2009), *M. rigidula* (Balouchi and Sanavy 2006) and *M. orbicularis* (Patane and Gresta 2006).

(iii) Soaking in hot water: Hot water scarification for different time intervals caused a significant improvement in the germination of cream seeds of *M. denticulata* (Table 4.4). However, brownish black seeds soaked in hot water at temperature 50°C or above resulted in high number of damaged seeds. Germination of cream seeds was significantly increased with increase in soaking time from 5-30 minutes at 50 and 70°C than control (Table 4.4). However, germination was completely inhibited when seeds were soaked in hot water at 90°C

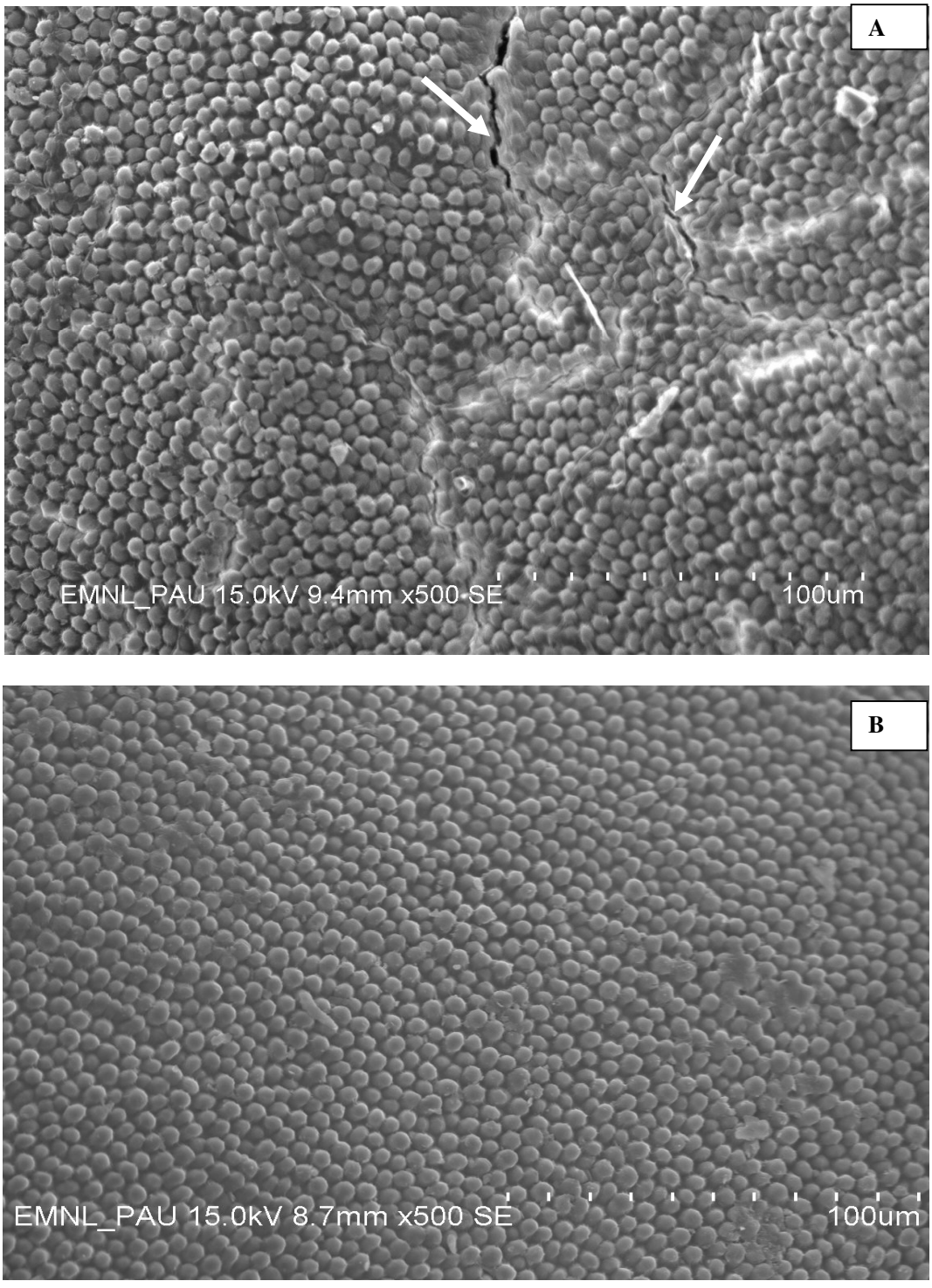


Plate 3: Scanning electron micrograph of mature seed coat (A) seeds with brownish black seed coat (B) seeds with cream seed coat. Arrows in (A) show cracks on seed surface.

for 15 or more minutes. Maximum germination of cream seeds was recorded with hot water incubation at 50°C for 30 min with minimum time to start germination and mean germination time as well as maximum speed of germination in this treatment.

Table 4.4: Effect of hot water scarification on germination of cream seeds of *Medicago denticulata* Willd.

Treatments (Temperature of water)	Germination (%)	Time to start germination (days)	Germination speed	Mean germination time (days)
Control	4.4	7.00	0.38	8.38
50°C × 5 min	34.4	4.00	2.22	5.26
50°C × 10 min	38.8	4.00	2.35	5.04
50°C × 15 min	42.2	4.00	3.05	4.45
50°C × 30 min	80.0	2.00	8.04	3.06
70°C × 5 min	40.0	4.00	3.00	4.62
70°C × 10 min	43.3	4.00	3.36	4.40
70°C × 15 min	50.0	3.00	4.13	4.18
70°C × 30 min	63.3	2.00	5.26	3.90
90°C × 5 min	26.6	5.00	1.75	7.08
90°C × 10 min	36.6	5.00	2.36	6.45
90°C × 15 min	NG	NG	NG	NG
SEm±	1.20	1.5×10^{-6}	0.04	0.02
LSD (p=0.05)	4.98	1.4×10^{-5}	0.12	0.09

LSD- Least significant difference; SEm-Standard error of mean; NG-No germination

Immersion of seeds in hot water can either lead to the rupturing of seed coat wall or softening of barrier imposed by the hard seed coat thereby allowing water to penetrate the seed tissues. The ability to withstand hot water may vary from species to species. In contrast to our study, increased germination (%) and speed of *M. arborea* seeds was recorded with hot water immersion at 100°C for 4 minutes (Travlos and Economou 2006). Khaef *et al* (2011) also reported higher germination of *M. polymorpha* (65.0%) at 90°C for 5 min followed by 56.0% germination at 80°C for 15 min and 44.0% germination at 100°C for 2 minutes.

Weed control is a crucial part of an efficient crop production system, which can be facilitated by new methods to break weed seed dormancy. Seed dormancy, an attribute common to nearly all weed species, influences the persistence of seeds in soil and affects germination patterns in natural ecosystems. The results of various dormancy breaking treatments in our study confirmed that cream seeds of *M. denticulata* exhibit dormancy due to hard seed coat. These studies reflect that build up of *M. denticulata* weed seed bank may be largely attributed to the presence of cream seeds which will allow emergence of multiple flushes of this weed and also enable them to persist for longer periods in soil to escape the weed control measures. Whereas non-dormant brownish black seeds will quickly emerge from the soil under field conditions and can be controlled by stale seed bed and/or application of pre-and post- emergence herbicides.

4.1.4 Temperature

***M. denticulata*:** Cream seeds possessed ability to germinate in the temperature range of 15/5 to 30/20°C with highest germination at 25/15°C (Table 4.5). Maximum germination speed and minimum germination time was observed at day/night temperature of 25/15°C. Cream seeds germinated at day/night temperature of 30/20°C took one extra day for initiation of germination along with reduced speed of germination and increased mean germination time. No germination of cream seeds was observed at day/night temperature of 35/25°C.

Germination of brownish black seeds occurred in narrow temperature range of 15/5 to 25/15°C with maximum germination at 20/10°C; however, germination was completely inhibited at 30/20°C. Seeds also exhibited maximum germination speed and minimum mean germination time at temperature 20/10°C. With increase in temperature above 20/10°C, there was increase in mean germination time along with reduction in germination (%) and speed of germination.

Table 4.5: Effect of day/night temperature regimes on germination of cream and brownish black seeds of *Medicago denticulata* Willd.

Temperature (°C) (12 h light/12 h dark)	Germination (%)		Time to start germination (days)		Germination speed		Mean germination time (days)	
	C	B	C	B	C	B	C	B
15/5	65.5	54.4	2.0	2	4.42	4.21	6.23	6.42
20/10	88.7	80.0	2.0	2	10.09	9.02	3.31	3.40
25/15	96.6	72.2	2.0	2	10.38	7.24	3.08	4.58
30/20	54.4	NG	3.0	NG	2.81	NG	6.58	NG
35/25	NG	NG	NG	NG	NG	NG	NG	NG
SEm±	3.20	3.08	3.5×10^{-6}	2.5×10^{-6}	0.09	0.05	0.09	0.05
LSD (p=0.05)	7.67	7.52	3.4×10^{-6}	2.4×10^{-6}	0.17	0.13	0.11	0.12

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; LSD- Least significant difference; NG-No germination

Data in Table 4.6 and 4.7 shows effect of day/night temperature regime on seedling growth of *M. denticulata*. Increasing day/night temperature from 15/5 to 25/15°C significantly increased the growth of cream seedlings. However, temperature above 25/15°C caused a reduction in seedling growth with a more pronounced effect on root length (Table 4.6). The highest growth of cream seedlings was recorded at day/night temperature of 25/15°C. At 30/20°C, root and shoot length of seedlings emerging from cream seeds was decreased by about 37.5 and 42.2% as compared to seedlings growing at 25/15°C. There was progressive increase in fresh and dry weight of cream seedlings with increase in temperature from 15/5 to 25/15°C followed by a decrease thereafter (Table 4.7). Cream seedlings grown at

25/15°C exhibited greatest seedling vigor indices indicating their higher competitiveness than seedlings grown at other temperatures.

Maximum growth of brownish black seedlings in terms of root shoot length and fresh and dry weight was recorded at day/night temperature of 20/10°C. Highest vigour indices of seedlings emerging from brownish black seeds was recorded at 20/10°C temperature with a significant decrease in vigour index with further increase in temperature (Figs. 4.3 and 4.4).

Table 4.6: Effect of day/night temperature regimes on seedling length and seedling vigour index I of *Medicago denticulata* Willd.

Temperature (^{o}C) (12 h light/12 h dark)	Shoot length (cm)		Root length (cm)		Total seedling length (cm)		Seedling vigour index I	
	C	B	C	B	C	B	C	B
15/5	4.0	3.8	3.2	3.0	7.2	6.8	471	369
20/10	4.6	4.3	3.6	4.0	8.2	8.3	727	664
25/15	4.8	4.0	4.2	3.6	9.0	7.6	869	548
30/20	3.0	NG	2.3	NG	5.3	NG	331	NG
35/25	NG	NG	NG	NG	NG	NG	NG	NG
SEm±	0.14	0.20	0.12	0.16	0.20	0.18	12.45	15.04
LSD (p=0.05)	0.34	0.45	0.26	0.38	0.56	0.44	90.45	103.40

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; LSD- Least significant difference; NG-No germination

Table 4.7: Effect of day/night temperature regimes on biomass accumulation and seedling vigour index II of *Medicago denticulata* Willd.

Temperature (^{o}C) (12 h light/12 h dark)	Fresh weight (mg/seedling)		Dry weight (mg/seedling)		Seedling vigour index II	
	C	B	C	B	C	B
15/5	32.09	31.27	20.41	20.13	1336	1095
20/10	33.25	33.71	20.91	20.85	1854	1668
25/15	34.09	28.01	24.04	18.11	2322	1307
30/20	30.41	NG	18.01	NG	979	NG
35/25	NG	NG	NG	NG	NG	NG
SEm±	1.08	0.87	1.06	0.86	10.66	10.21
LSD (p=0.05)	3.89	3.51	3.72	2.36	87.04	76.52

C-Seeds with cream seed coat; B- Seeds with brownish black seed coat; LSD- Least significant difference; NG-No germination

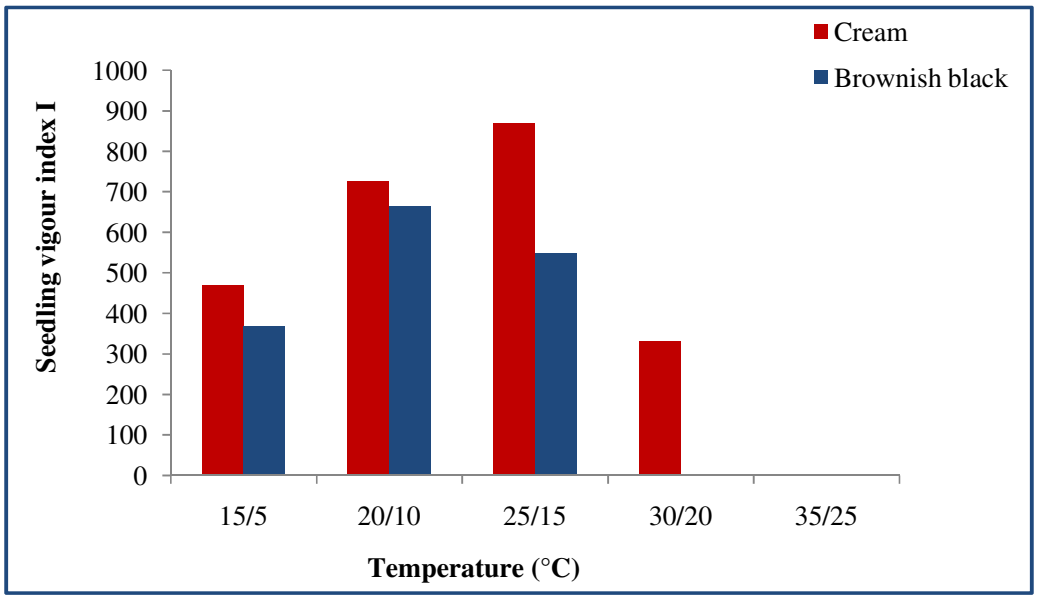


Figure 4.3: Effect of day/night temperature regimes on seedling vigour index I of *Medicago denticulata* Willd.

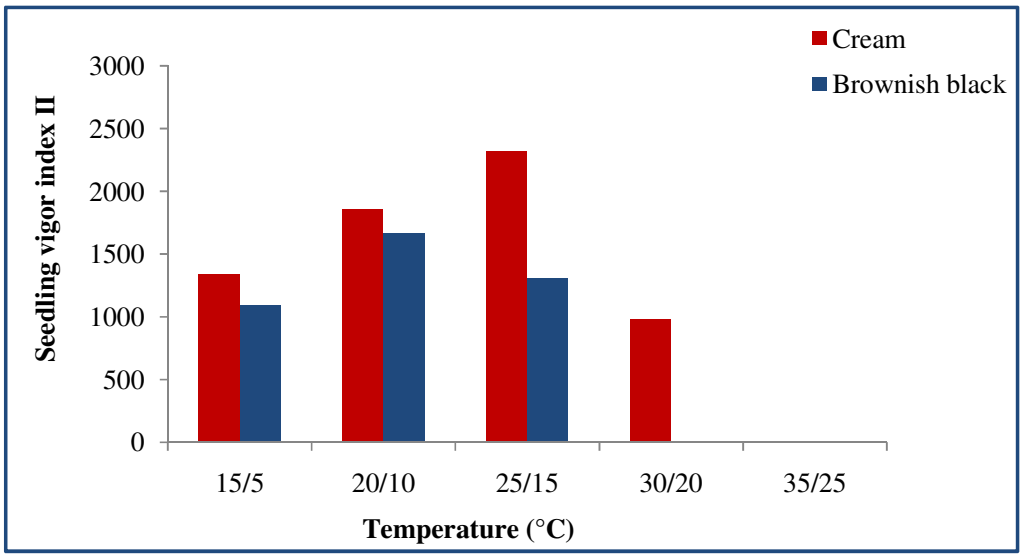


Figure 4.4: Effect of day/night temperature regimes on seedling vigour index II of *Medicago denticulata* Willd.

T. aestivum: The data in Table 4.8 depicts the response of *T. aestivum* seeds to day/night temperature regimes. Day/night temperature regime of 25/15°C (12 h light/12 h dark) recorded highest germination of *T. aestivum* seeds and it was statistically at par with germination observed at 15/5 and 20/10°C. Minimum germination was recorded at day and night temperature regime of 30/20°C with complete inhibition at 35/25°C. Highest germination index was recorded at day and night temperature of 25/15°C, while lowest at 30/20°C.

Table 4.8: Effect of day/night temperature regimes on germination of *Triticum aestivum* L.

Temperature (^oC) (12 h light/12 h dark)	Germination (%)	Time to start germination (days)	Germination speed	Mean germination time (days)
15/5	90.0	2	8.36	2.42
20/10	93.3	2	8.40	2.40
25/15	96.6	2	8.56	2.36
30/20	66.6	3	6.35	4.24
35/25	NG	NG	NG	NG
SEm±	3.54	1.6×10^{-7}	0.23	0.08
LSD (p=0.05)	6.46	1.8×10^{-6}	0.45	0.24

LSD-Least significant difference; NG-No germination

Tables 4.9 and 4.10 show the effect of day/night temperature regimes on seedling growth of *T. aestivum*. Maximum seedling length was observed at day/night temperature regime of 25/15°C. Seedling length and dry weight were decreased by 54.5 and 24.6% respectively at day/night temperature regime of 30/20°C as compared to their respective values at 25/15°C. Likewise, seedling vigour index I and II recorded maximum values at day/night temperature regime of 25/15°C (Fig. 4.5).

Table 4.9: Effect of day/night temperature regimes on seedling length and seedling vigour index I of *Triticum aestivum* L.

Temperature (^oC) (12 h light/12 h dark)	Shoot length (cm)	Root length (cm)	Total seedling length (cm)	Seedling vigour index I
15/5	5.8	6.3	12.1	1089
20/10	8.2	9.4	17.6	1642
25/15	10.2	11.8	22.0	2125
30/20	4.8	5.2	10.0	660
35/25	NG	NG	NG	NG
SEm±	0.35	0.24	0.66	23.45
LSD (p=0.05)	0.67	0.45	1.24	87.34

LSD-Least significant difference; NG-No germination

Table 4.10: Effect of day/night temperature regimes on biomass accumulation and seedling vigour index II of *Triticum aestivum* L.

Temperature (^oC) (12 h light/12 h dark)	Fresh weight (mg/seedling)	Dry weight (mg/seedling)	Seedling vigour index II
15/5	80.50	62.25	5602
20/10	96.28	78.08	7284
25/15	122.35	90.54	8746
30/20	90.06	68.28	4547
35/25	NG	NG	NG
SEm±	1.20	1.34	34.56
LSD (p=0.05)	3.23	3.76	176.23

LSD-Least significant difference; NG-No germination

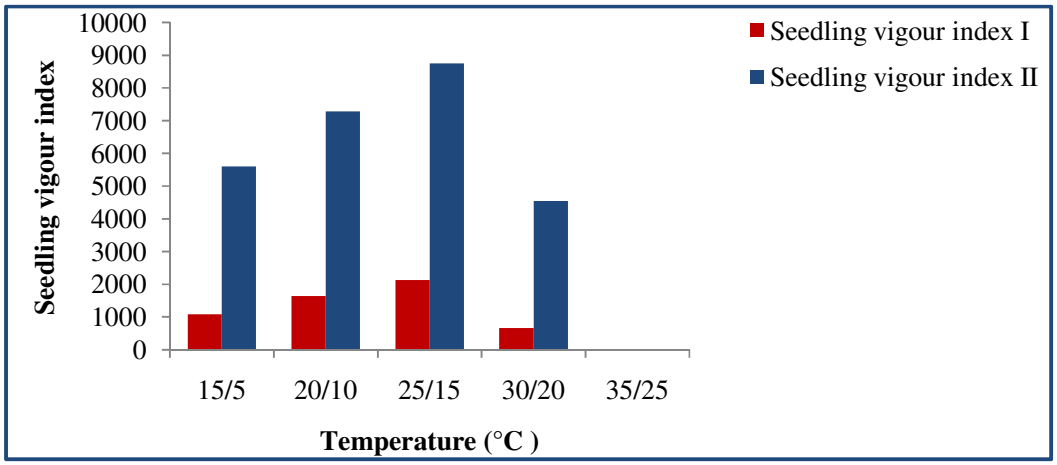


Figure 4.5: Effect of day/night temperature regimes on seedling vigour index I and II of *Triticum aestivum* L.

Table 4.11 shows the effect of constant temperature on germination of *M. denticulata.* Highest germination of both cream and brownish black seeds was recorded at 20°C. Time to start germination and mean germination time was minimum with maximum speed of germination at 20°C. Increasing temperature from 5 to 20°C increased the germination of both cream and brownish black seeds with concomitant reduction in mean germination time. However, temperature above 20°C caused a reduction in germination along with a decrease in germination speed. At 25°C, germination of cream and brownish black seeds was decreased by 9 and 26% along with 1.1 and 1.5 fold reductions in germination speed respectively. Germination of brownish black seeds was completely inhibited at 30°C in contrast to cream seeds which exhibited more than 40% germination at 30°C. Germination of both cream and brownish black seeds was completely inhibited at 35°C.

Table 4.11: Effect of constant temperature on germination of cream and brownish black seeds of *Medicago denticulata* Willd.

Temperature (°C)	Germination (%)		Time to start germination (days)		Germination speed		Mean Germination Time (days)	
	C	B	C	B	C	B	C	B
5	55.5	40.0	4	5	4.65	4.02	6.65	7.62
10	76.6	55.5	3	3	8.00	4.84	4.60	5.60
15	83.3	70.0	2	2	9.18	7.40	3.65	4.30
20	95.5	85.5	2	2	10.48	9.46	3.10	4.02
25	86.6	63.3	2	2	9.35	6.36	4.58	5.35
30	46.6	NG	5	NG	3.32	NG	6.45	NG
35	NG	NG	NG	NG	NG	NG	NG	NG
SEm±	1.34	1.12	1.5×10^{-6}	1.5×10^{-6}	0.02	0.05	0.04	0.06
LSD (p=0.05)	5.10	4.94	1.4×10^{-5}	1.4×10^{-5}	0.08	0.12	0.11	0.15

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference; NG-No germination

Table 4.12 and 4.13 shows the effect of constant temperatures on seedling growth of

M. denticulata. Maximum growth of seedlings emerging from cream and brownish black seeds was recorded at 20°C. With increase in temperature from 5 to 20°C, there was progressive increase in root length, shoot length, fresh weight, dry weight and vigour indices of seedlings emerging from cream and brownish black seeds. At 20°C, seedlings emerging from cream seeds recorded 46.6 and 33.3% increase in root and shoot length respectively than seedlings grown at 5°C. Whereas, brownish black seeds incubated at 20°C produced seedlings with 43 and 29.4% increase in root and shoot length than seedlings grown at 5°C. Temperature above 20°C resulted in significant decline in growth attributes of cream and brownish black seeds. There was 19.3 and 29.8% reduction in seedling vigour I and II of seedlings emerging from cream seeds grown at 25°C than seedlings grown at 20°C. Likewise, brownish black seeds incubated at 25°C produced seedlings with 31 and 48.1% reduction in SVI and SVII respectively than seedlings grown at 20°C (Figs. 4.6 and 4.7).

Table 4.12: Effect of constant temperature on seedling length and seedling vigour index I of *Medicago denticulata* Willd.

Temperature (^{o}C)	Shoot length (cm)		Root length (cm)		Total seedling length (cm)		Seedling vigour index I	
	C	B	C	B	C	B	C	B
5	3.6	3.4	3.0	2.8	6.6	6.2	366	248
10	3.8	3.4	3.3	3.0	7.1	6.4	543	355
15	4.5	4.0	4.0	3.6	8.5	7.6	708	532
20	4.8	4.4	4.4	4.0	9.1	8.8	869	752
25	4.3	4.0	3.8	3.6	8.1	8.2	701	519
30	4.0	NG	3.8	NG	7.8	NG	363	NG
35	NG	NG	NG	NG	NG	NG	NG	NG
SEm±	0.11	0.08	0.09	0.08	0.09	0.11	20.40	18.56
LSD (p=0.05)	0.32	0.26	0.30	0.26	0.30	0.33	40.45	40.12

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference; NG-No germination

Table 4.13: Effect of constant temperature on biomass accumulation and seedling vigour index II of *M. denticulata* Willd.

Temperature (^{o}C)	Fresh weight (mg/seedling)		Dry weight (mg/seedling)		Seedling vigour index II	
	C	B	C	B	C	B
5	34.00	32.05	18.06	16.08	1002	643
10	34.62	33.31	19.22	16.73	1472	928
15	35.29	34.17	20.05	18.08	1670	1265
20	36.05	35.56	22.11	20.17	2111	1724
25	30.61	28.03	17.11	14.13	1481	894
30	29.66	NG	15.24	NG	710	NG
35	NG	NG	NG	NG	NG	NG
SEm±	0.23	0.15	0.15	0.13	23.32	20.66
LSD (p=0.05)	0.70	0.50	0.47	0.38	50.67	46.75

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference; NG-No germination

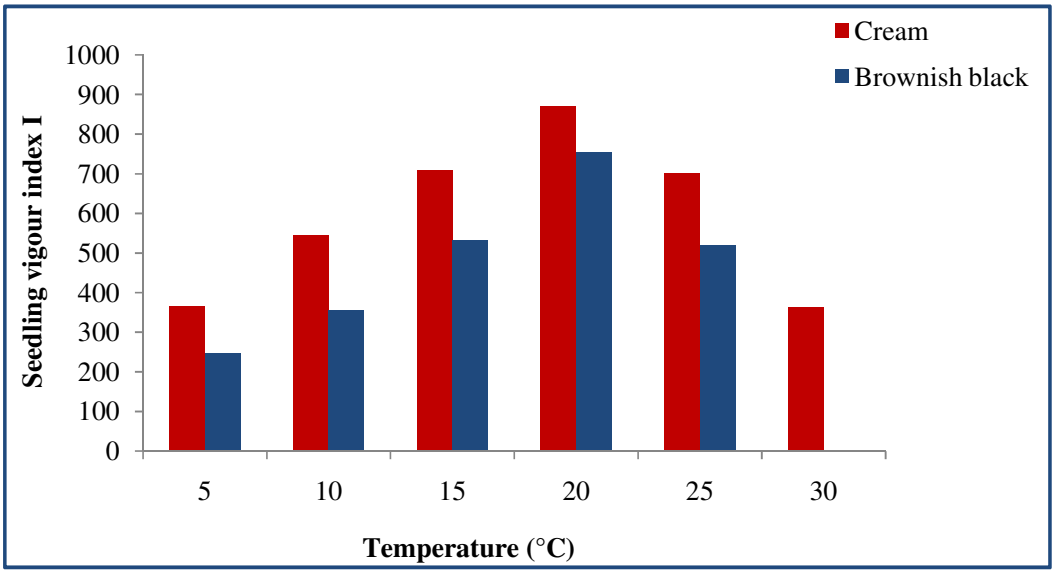


Figure 4.6: Effect of constant temperatures on seedling vigour index I of *Medicago denticulata* Willd.

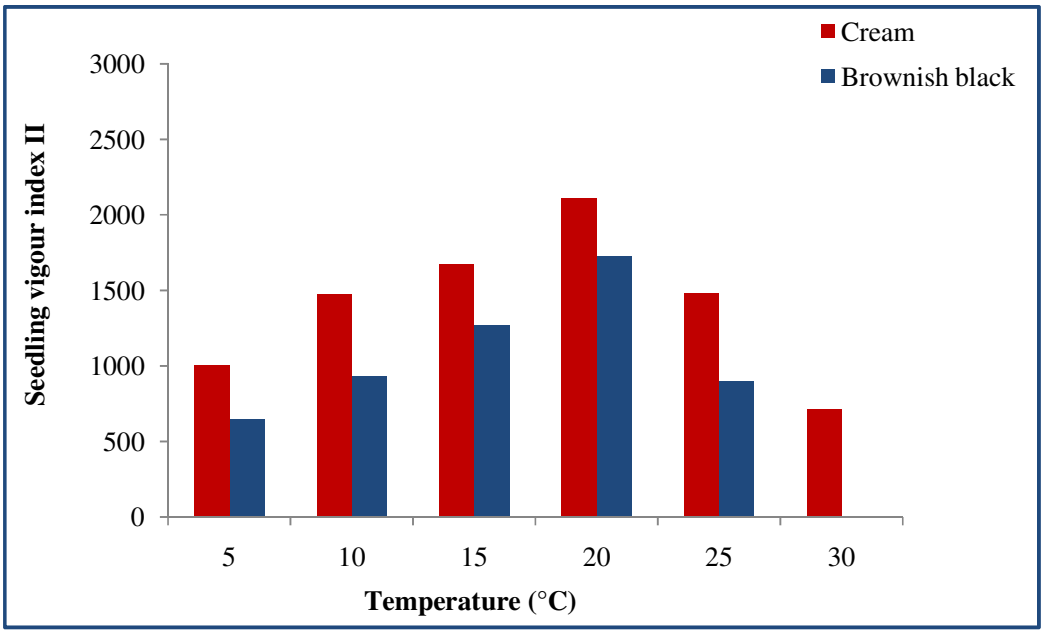


Figure 4.7: Effect of constant temperatures on seedling vigour index II of *Medicago denticulata* Willd.

Data in Table 4.14 shows that maximum germination of *T. aestivum* was recorded at 20°C and minimum at 5°C. Germination did not differ significantly in the temperature range of 10 to 25°C but significant reduction was observed with increase in temperature beyond 25°C. At 30°C, there was 26.7 per cent point reduction in germination with 1.4 fold reduction in germination speed as compared to 20°C. *T. aestivum* seeds incubated at 5°C took maximum time to start germination along with maximum mean germination time than seeds incubated at 20°C.

Table 4.14: Effect of constant temperature on germination of *Triticum aestivum* L.

Temperature (^oC)	Germination (%)	Time to start germination (days)	Germination speed	Mean germination time (days)
5	60.0	5	5.08	4.14
10	85.5	2	7.43	2.65
15	93.3	2	7.84	2.46
20	96.6	2	8.62	2.20
25	90.0	2	8.18	2.48
30	66.6	3	6.23	3.38
SEm±	3.06	1.9×10^{-6}	0.38	0.09
LSD (p=0.05)	9.52	1.8×10^{-5}	1.52	0.30

LSD-Least significant difference; SEm-Standard error of mean; NG-No germination

Increasing temperature from 5 to 20°C resulted in progressive increase in root length, shoot length, fresh weight, dry weight and seedling vigour indices of *T. aestivum* (Table 4.15-4.16 and Fig. 4.8). The highest fresh weight, dry weight and seedling vigour was recorded at 20^{o}C beyond which significant decline was observed in growth and vigour of seedlings. At 30^{o}C, seedling vigour index I and II were decreased by about 43.3 and 47.7% than seedlings growing at 20°C.

Table 4.15: Effect of constant temperature on seedling length and seedling vigour index I of *Triticum aestivum* L.

Temperature (^oC)	Shoot length (cm)	Root length (cm)	Total seedling length (cm)	Seedling vigour index I
5	4.8	5.7	10.5	630
10	6.6	8.8	15.4	1316
15	9.0	10.5	19.5	1819
20	10.7	12.3	23.0	2221
25	10.4	11.8	22.2	1998
30	9.3	10.2	19.5	1298
SEm±	0.32	0.34	0.56	20.43
LSD (p=0.05)	0.91	0.98	1.02	52.23

LSD-Least significant difference; SEm-Standard error of mean; NG-No germination

Table 4.16: Effect of constant temperature on biomass accumulation and seedling vigour index II of *Triticum aestivum* L.

Temperature (^oC)	Fresh weight (mg/seedling)	Dry weight (mg/seedling)	Seedling vigour index II
5	83.40	60.05	3603
10	92.50	65.52	5601
15	115.20	81.57	7610
20	120.60	85.40	8249
25	113.50	78.23	7040
30	98.30	68.12	4536
SEm±	2.23	1.23	24.42
LSD (p=0.05)	8.82	5.64	70.13

LSD-Least significant difference; SEm-Standard error of mean; NG-No germination

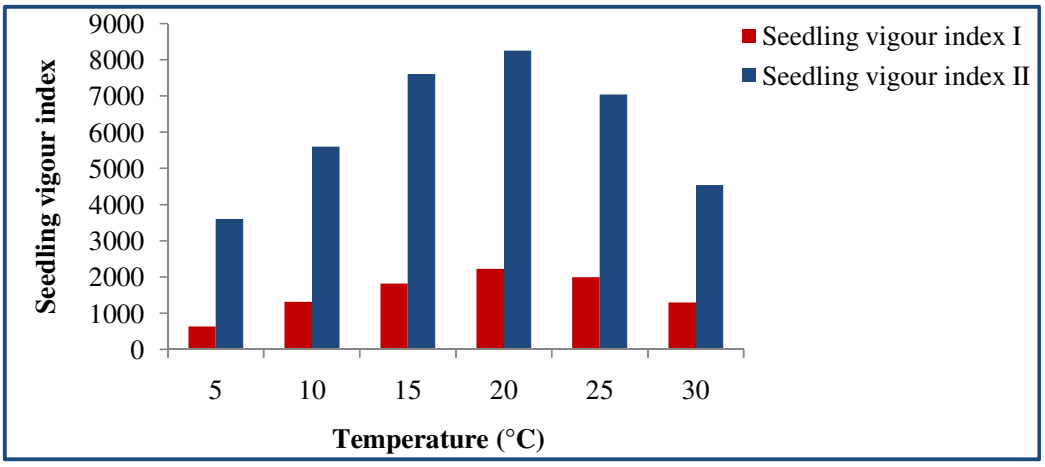


Figure 4.8: Effect of constant temperatures on seedling vigour index I and II of *Triticum aestivum* L.

Like *M. denticulata*, other dicotyledonous weeds infesting wheat crop in Northern India such as *Chenopodium album* and *Rumex dentatus* have been reported to germinate under a wide range of temperature from 5-25°C (Tanveer *et al* 2009, Benvenuti *et al* 2001). This flexible germination habit of weeds provide opportunities for weed proliferation, leading to abundant seed production. The results of present study indicate that cream and brownish black seeds responded positively to day/night and constant temperatures. Cream seeds can germinate in broad range of constant (5-30°C) and day/night temperatures (15/5-30/20°C) whereas germination of brownish black seeds can occur comparatively in narrow range of constant (5-20°C) and day/night temperatures (15/5-20/10°C). This implies robust germination characteristics of cream seeds as compared to brownish black seeds. It is also important to mention here that cream seeds exhibited germination only after scarification indicating seed coat imposed dormancy and greater mechanical strength which could contribute to the perpetuation of seeds under adverse environmental conditions. Cream seeds may also be able to emerge in multiple flushes as and when their seed coat imposed dormancy is relieved under natural conditions. .

Temperature is an environmental factor that can influence emergence patterns and timing of seed germination (Derakhshan and Gherekhloo 2013). The ability of weeds to germinate across a wide range of temperature suggests their ability to emerge throughout the cropping season making weed management difficult. The monthly data of maximum, minimum and mean temperature at Ludhiana of year 2016-17 and 2017-18 recorded by School of Climate Change and Agricultural Meteorology, Punjab Agricultural University is depicted in Table 4.17. Temperature throughout the period of wheat growth (November-March) is favourable for the emergence of *Medicago denticulata* as results of present study indicate that germination of cream and brownish black seeds can occur in day/night

temperature range of 15/5-30/20°C and 15/5-20/10°C, respectively; and constant temperature range of 5-30°C and 5-25°C is also favourable for germination of cream and brownish black seeds respectively. The mean temperature during sowing of *T. aestivum* in Punjab is about 20-25°C. Therefore, *M. denticulata* may emerge in multiple flushes during wheat growth period especially providing competition to wheat during its early establishment period.

Table 4.17: Weather conditions at Ludhiana during *rabi* 2016-17 and 2017-18

Month	Temperature					
	Maximum (°C)		Minimum (°C)		Mean (°C)	
	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18
October	32.7	32.3	19.0	20.5	25.9	26.4
November	27.7	24.7	12.0	11.5	19.9	18.1
December	22.3	20.9	8.5	7.5	15.4	14.2
January	17.2	18.7	7.4	6.2	12.3	12.5
February	23.0	22.8	9.0	9.1	16.0	16.0
March	28.0	29.3	14.6	13.1	21.3	21.6
April	36.6	35.8	19.6	19.9	28.1	27.8

4.1.5 Light

***M. denticulata*:** Germination of both cream and brownish black seeds did not differ significantly under light and dark conditions, which indicates that seeds of this species are non-photoblastic (Table 4.18 and 4.19). Dark grown seedlings of both had pale yellow cotyledonary leaves. There was significant reduction in fresh and dry weight of both seedlings under darkness. Cream and brownish black seedlings incubated under complete darkness showed 28.8 and 34.9% reduction in dry weight respectively as compared to seedlings grown under 24 h light. However, root and shoot length of dark grown seedlings was statistically at par with seedlings growing under light. There was significant reduction in vigour index II but vigour index I did not differ significantly among light and dark grown cream and brownish black seedlings (Figs. 4.9 and 4.10).

Table 4.18: Effect of light on germination and seedling growth of *Medicago denticulata* Willd.

Photo-Period (hours)	Germination (%)		Shoot length (cm)		Root length (cm)		Total seedling length (cm)	
	C	B	C	B	C	B	C	B
24	95.5	86.6	4.3	4.0	3.6	3.3	7.9	7.3
0	93.3	83.3	4.8	4.3	3.4	3.0	8.2	7.3
12/12	90.0	83.3	4.6	4.4	3.6	3.2	8.2	7.6
SEm±	0.45	0.34	0.23	0.30	0.27	0.22	0.24	0.32
LSD (p=0.05)	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; LSD-Least significant difference; NS-Non significant

Table 4.19: Effect of light on biomass accumulation and seedling vigour index of *Medicago denticulata* Willd.

Photo-Period (hours)	Fresh weight (mg/seedling)		Dry weight (mg/seedling)		Seedling vigour index I		Seedling vigour index II	
	C	B	C	B	C	B	C	B
24	34.05	33.62	22.48	21.66	754	632	2146	1875
0	29.51	28.05	16.01	14.09	765	608	1493	1173
12/12	33.27	32.01	22.03	21.24	738	633	1982	1769
SEm±	0.56	1.03	1.12	0.67	0.33	0.22	25.4	28.3
LSD (p=0.05)	2.54	3.16	3.40	2.16	N.S	N.S	90	105

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; LSD-Least significant difference

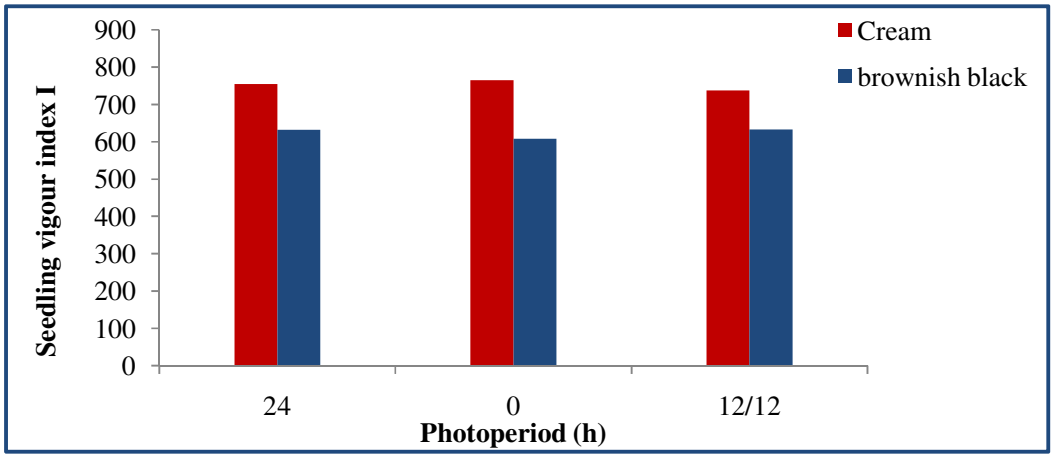


Figure 4.9: Effect of light on seedling vigour index I of *Medicago denticulata* Willd.

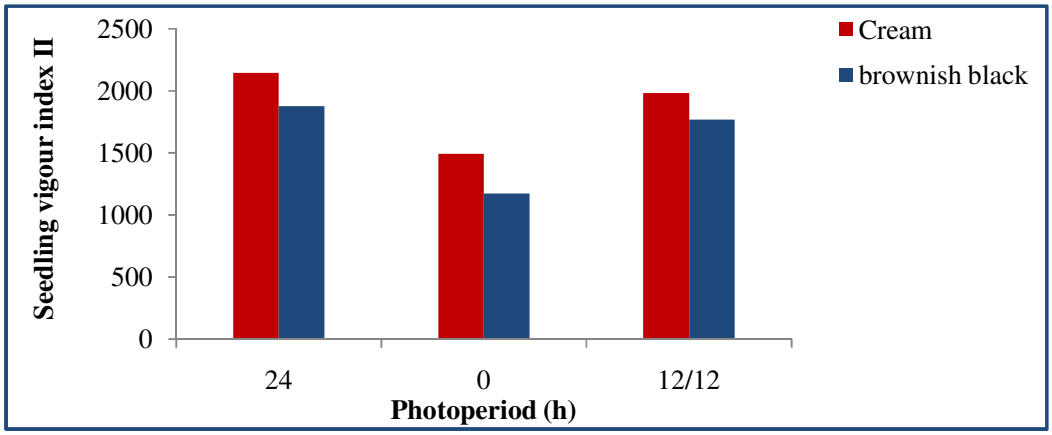


Figure 4.10: Effect of light on seedling vigour index II of *Medicago denticulata* Willd.

T. aestivum: The data in Table 4.20 and 4.21 shows that germination of *T. aestivum* is independent of light. There was no significant effect of light on root length, shoot length, fresh weight, dry weight and vigour indices of seedlings (Fig. 4.11). However, dark grown seedlings were chlorotic having pale yellow colour.

Table 4.20: Effect of light on germination, seedling length and seedling vigour index I of *Triticum aestivum* L.

Photo-period (h)	Germination (%)	Root length (cm)	Shoot length (cm)	Total seedling length (cm)	Seedling vigour index I
24	93.3	11.2	12.5	23.7	2211
0	90.0	11.0	12.1	23.1	2079
12/12	95.5	11.8	13.0	24.8	2368
SEm±	1.23	0.45	0.65	1.20	23.66
LSD (p=0.05)	N.S	N.S	N.S	N.S	N.S

LSD-Least significant difference; N.S-Non-significant

Table 4.21: Effect of light on biomass accumulation and seedling vigour index II of *Triticum aestivum* L.

Photo-period (h)	Fresh weight (mg/seedling)	Dry weight (mg/seedling)	Seedling vigour index II
24	118.23	83.45	7785
0	115.60	80.24	7221
12/12	120.53	83.66	7989
SEm±	12.66	10.65	26.97
LSD (p=0.05)	N.S	N.S	N.S

LSD-Least significant difference; N.S-Non-significant

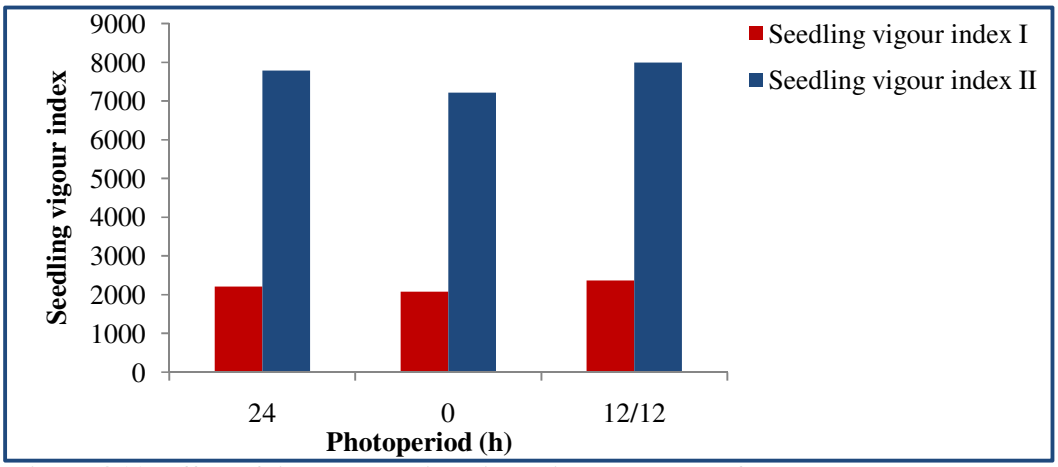


Figure 4.11: Effect of light on seedling vigour index I and II of *Triticum aestivum* L.

The results of present study indicate that germination of both *Triticum aestivum* and *Medicago denticulata* was independent of light; however, dark-grown seedlings were chlorotic. Light is an important ecological determinant for germination and the absence of light acts as a soil depth indicator that prevents germination of many weed species (Crisraudo *et al* 2007). Seed germination response to light may vary considerably from species to species. Ghadiri and Niazi (2005) reported that light effectively stimulated germination of two dicotyledonous winter weeds toothed dock (*Rumex dentatus*) and common lambsquarters (*Chenopodium album*) which indicates that both species are positively photoblastic and germination in the field will be favoured by presence of seeds at the soil surface. Unlike these winter weeds, both cream and brownish black seeds of *M. denticulata* germinated equally under light and dark conditions. These results imply that seeds of this weed may germinate

when buried at shallow depths in the soil or after canopy closure in wheat crop thereby leading to its increased invasiveness. Therefore, weed control practices like seed burial, straw mulch or the presence of closed crop canopies, which prevent seeds to receive light signals, would not reduce germination of dispersed seeds of this weed species. On the other hand, germination in the presence of light suggests that this species could be a problematic weed in no-till systems also, wherein much of the weed seed bank remains on the soil surface.

4.1.6 Moisture stress

***M. denticulata*:** The data in Table 4.22 depicts the effect of moisture stress on germination of *M. denticulata*. Both cream and brownish black seeds were sensitive to moisture stress as evident from progressive decrease in germination and increase in mean germination time as the osmotic potential was decreased from 0 to -0.4 MPa (Table 4.22). Maximum germination in both the seeds was recorded in control with complete inhibition at osmotic potential of ≥ -0.6 MPa. At -0.4 MPa, brownish black seeds took six days to start germination in comparison to cream seeds in which germination started on 3rd day of incubation. Osmotic potential of -0.4 MPa reduced the germination of cream seeds by 57 per cent points with 2.2 fold increase in mean germination time than control. Whereas brownish black seeds recorded 62 per cent point reduction in germination with 3 fold increase in mean germination time as compared to control. The osmotic potential required for 50% inhibition of the maximum germination of cream and brownish black seeds was -0.37 and -0.32 MPa, respectively (Figs. 12 and 13). This indicates that moisture stress exhibited more pronounced effect on brownish black seeds than cream seeds (Plate 4).

Table 4.22: Effect of moisture stress on germination of cream and brownish black seeds of *Medicago denticulata* Willd.

Osmotic potential (MPa)	Germination (%)		Time to start germination (days)		Germination speed		Mean germination time (days)	
	C	B	C	B	C	B	C	B
0 (Control)	95.5	85.5	2	3	10.89	9.86	3.00	3.10
-0.1	84.4	80.0	2	3	9.38	8.76	3.26	3.35
-0.2	78.8	73.3	2	3	8.43	7.78	3.45	3.56
-0.4	38.8	23.3	3	6	3.56	2.77	6.55	9.30
-0.6	NG	NG	NG	NG	NG	NG	NG	NG
-0.8	NG	NG	NG	NG	NG	NG	NG	NG
-1.0	NG	NG	NG	NG	NG	NG	NG	NG
SEm±	1.97	2.10	1.5×10^{-6}	1.5×10^{-6}	0.05	0.09	0.08	0.05
LSD (p=0.05)	5.98	6.38	1.4×10^{-5}	1.4×10^{-5}	0.09	0.17	0.14	0.09

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference; NG-No germination

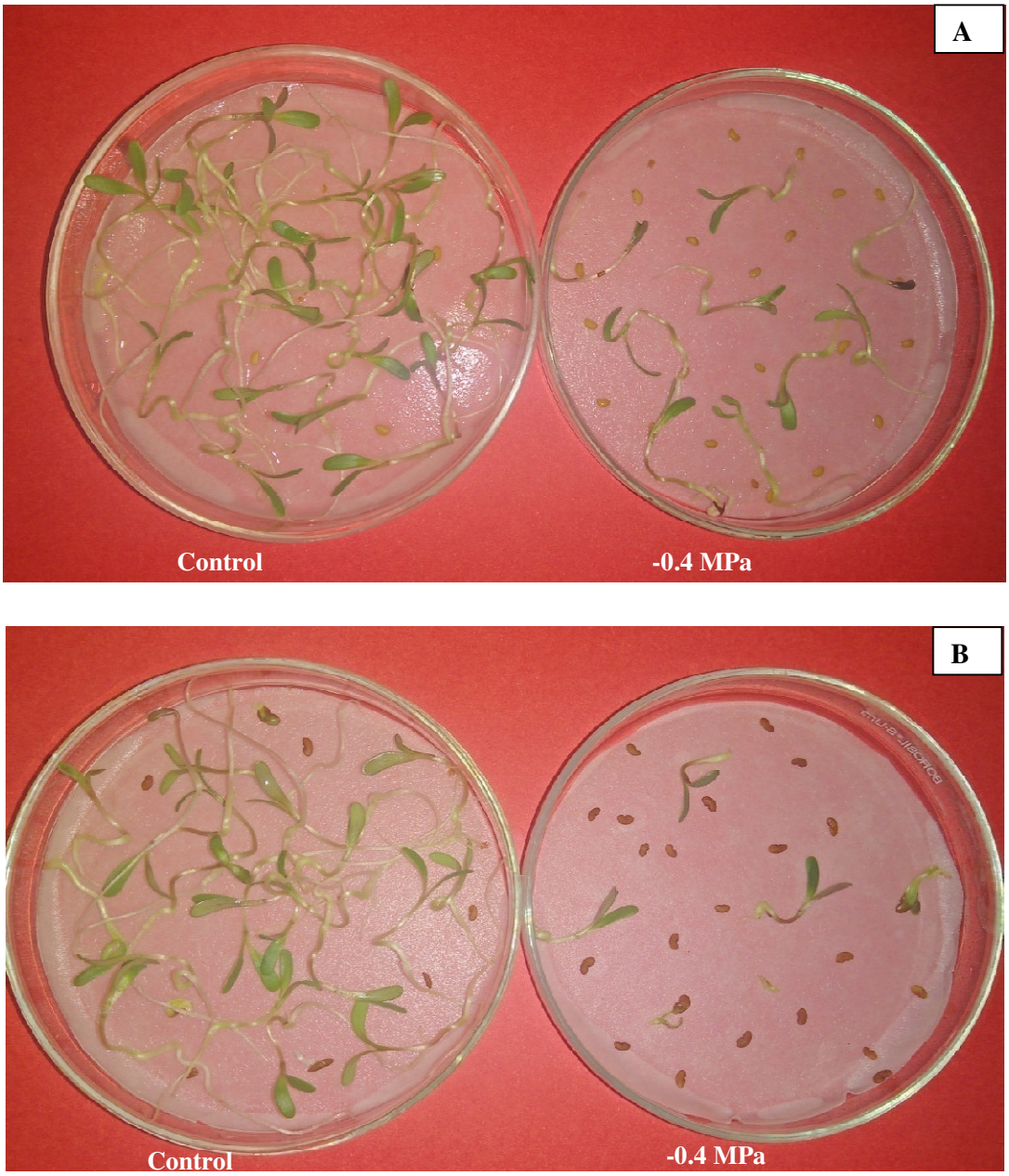


Plate 4: Effect of moisture stress on germination of (A) cream and (B) brownish black seeds of *Medicago denticulata*Willd.

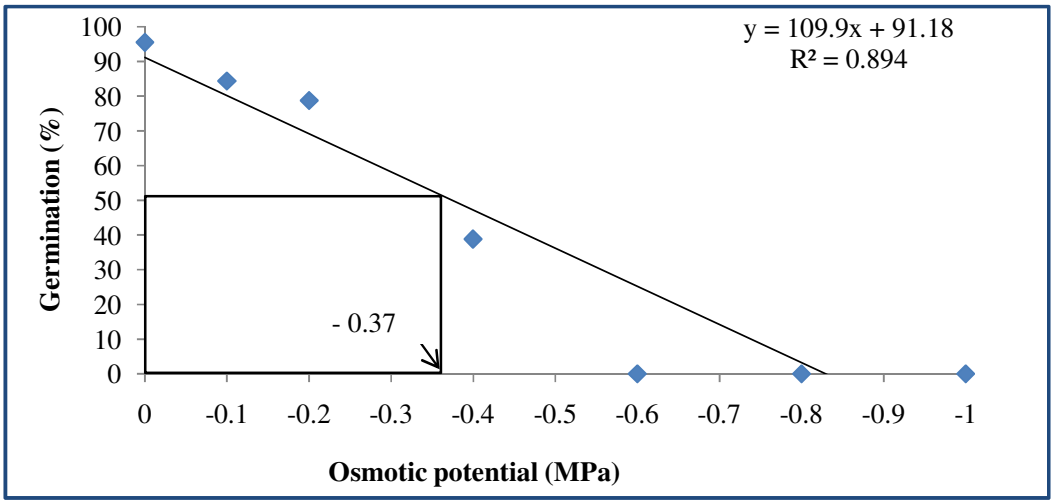


Figure 4.12: **Osmotic potential required for 50% inhibition of maximum germination in cream seeds of *Medicago denticulata* Willd.**

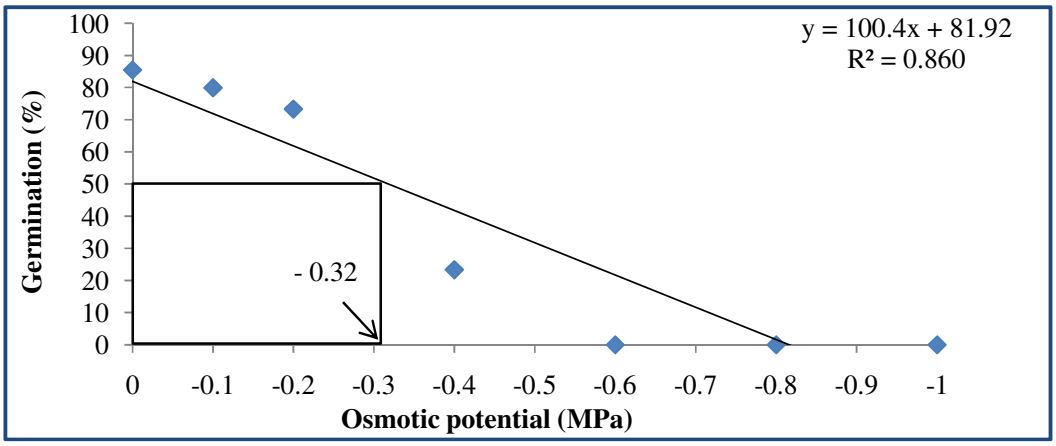


Figure 4.13: **Osmotic potential required for 50% inhibition of maximum germination in brownish black seeds of *Medicago denticulata* Willd.**

Data presented in Tables 4.23 and 4.24 show the effect of moisture stress on seedling growth of *M. denticulata*. Moisture stress significantly reduced root and shoot length of cream and brownish black seedlings. However, seedlings emerging from brownish black seeds were affected to a greater extent than seedlings emerged from cream seeds. At osmotic potential of -0.4 MPa, inhibition in root and shoot length of cream seedlings was 53.5 and 37.5% as compared to their respective controls (Table 4.23). Whereas, seedlings emerging from brownish black seeds exhibited 60 and 47.7% inhibition in root and shoot length than their respective controls.

Increasing moisture stress caused significant reduction in fresh and dry weight of both cream and brownish black seedlings. Osmotic potential of -0.4 MPa decreased the fresh weight of seedlings emerging from cream and brownish black seeds by about 45.1 and 52.4% as compared to their respective controls (Table 4.24). Seedlings emerging from cream and

brownish black seeds recorded 46.2 and 54.2% reduction in dry weight at osmotic potential of -0.4 MPa, respectively, as compared to their controls. Vigour index I and II for both seedlings was maximum in control and moisture stress of -0.4 MPa recorded the least values of both the indices (Figs. 4.14 and 4.15).

Table 4.23: Effect of moisture stress on seedling length and seedling vigour index I of *Medicago denticulata* Willd.

Osmotic potential (MPa)	Shoot length (cm)		Root length (cm)		Total seedling length (cm)		Seedling vigour index I	
	C	B	C	B	C	B	C	B
0 (Control)	4.8	4.4	4.3	4.0	9.1	8.4	869	718
-0.1	4.5	4.0	3.8	3.6	8.3	7.6	700	608
-0.2	3.8	3.4	3.0	3.0	6.8	6.4	535	498
-0.4	3.0	2.3	2.0	1.6	5.0	3.9	194	90
-0.6	NG	NG	NG	NG	NG	NG	NG	NG
-0.8	NG	NG	NG	NG	NG	NG	NG	NG
-1.0	NG	NG	NG	NG	NG	NG	NG	NG
SEm±	0.08	0.10	0.11	0.09	0.11	0.13	15.27	13.24
LSD (p=0.05)	0.27	0.29	0.30	0.28	0.33	0.41	46.35	44.87

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference; NG-No germination

Table 4.24: Effect of moisture stress on biomass accumulation and seedling vigour index II of *Medicago denticulata* Willd.

Osmotic potential (MPa)	Fresh weight (mg/seedling)		Dry weight (mg/seedling)		Seedling vigour index II	
	C	B	C	B	C	B
0 (Control)	35.02	33.71	22.34	20.12	2133	1720
-0.1	32.40	30.23	19.85	17.33	1675	1386
-0.2	29.12	25.74	17.24	14.60	1358	1070
-0.4	20.23	16.06	12.03	9.21	466	260
-0.6	NG	NG	NG	NG	NG	NG
-0.8	NG	NG	NG	NG	NG	NG
-1.0	NG	NG	NG	NG	NG	NG
SEm±	0.87	0.92	0.45	0.87	26.34	28.09
LSD (p=0.05)	2.32	2.65	1.86	2.02	78.50	87.3

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference; NG-No germination

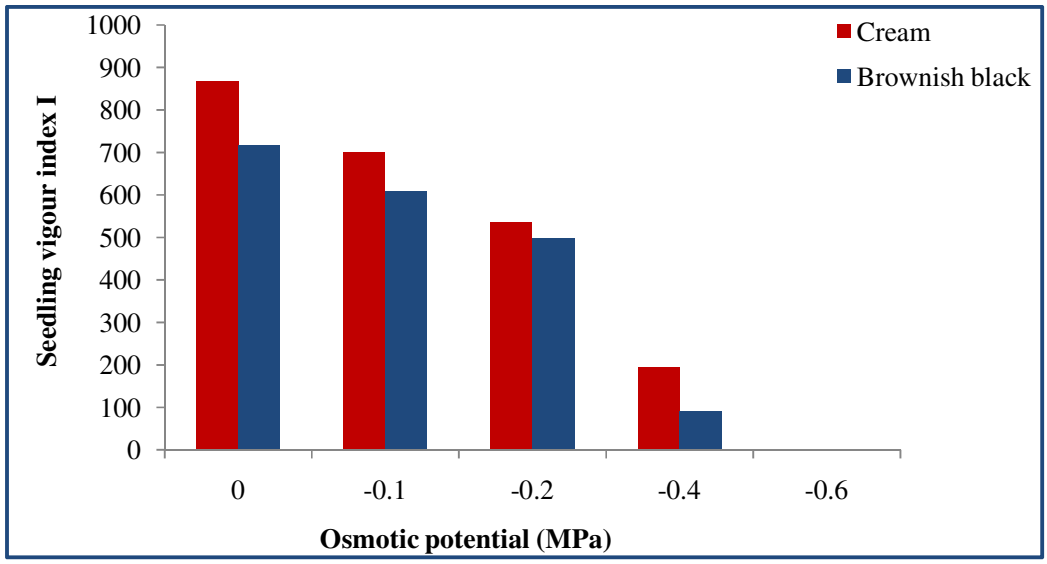


Figure 4.14: Effect of moisture stress on seedling vigour index I of *Medicago denticulata* Willd.

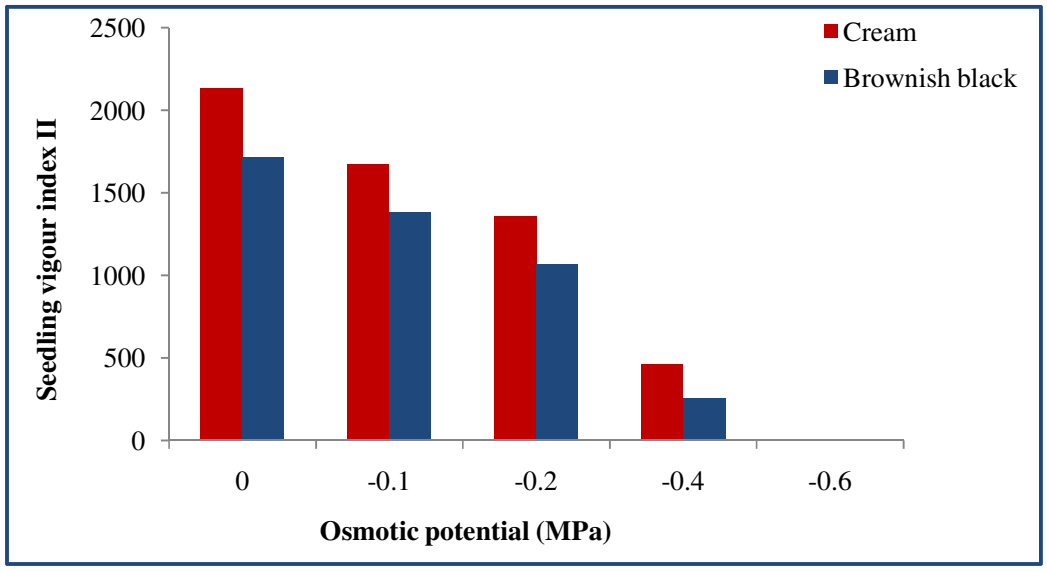


Figure 4.15: Effect of moisture stress on seedling vigour index II of *Medicago denticulata* Willd.

***T. aestivum*:** Data in Table 4.25 indicate that seeds of *T. aestivum* could tolerate moisture stress upto -0.4 MPa but no germination was observed at osmotic potential of -0.6, -0.8 and -1.0 MPa. Time to start germination increased from 2nd day in control to 4th day under moisture stress of -0.4 MPa. Moisture stress significantly decreased the germination speed and increased the mean germination time. The highest germination speed was recorded in control and moisture stress of -0.4 MPa reduced the germination speed and mean germination time by about 2.8 and 2.1 fold as compared to control.

Table 4.25: Effect of moisture stress on germination of *Triticum aestivum* L.

Osmotic potential (MPa)	Germination (%)	Time to start germination (days)	Germination speed	Mean germination time (days)
0 (Control)	93.3	2	8.68	2.34
-0.1	68.3	2	6.25	3.72
-0.2	50.0	3	5.04	4.09
-0.4	28.3	4	3.12	4.87
-0.6	NG	NG	NG	NG
-0.8	NG	NG	NG	NG
-1.0	NG	NG	NG	NG
SEm±	1.95	1.5×10^{-6}	0.04	0.07
LSD (p=0.05)	4.68	1.4×10^{-5}	0.16	0.19

LSD- Least significant difference; SEm-Standard error of mean; NG-No germination

The highest growth of *T. aestivum* seedlings was recorded in control (Table 4.26 and 4.27). Decreasing osmotic potential from 0 to -0.4 MPa significantly decreased the growth of seedlings. Seedlings grown under osmotic potential of -0.4 MPa recorded 60.6 and 38.1% reduction in root and shoot length as compared to their respective controls. Fresh and dry weight of seedlings was reduced by 28.1 and 31.2% at osmotic potential -0.4 MPa as compared to their respective controls (Table 4.27). There was 84.9 and 79.1% reduction in seedling vigour index I and II at osmotic potential of -0.4 MPa as compared to their respective controls (Fig. 4.16).

Table 4.26: Effect of moisture stress on seedling length and seedling vigour index I of *Triticum aestivum* L.

Osmotic Potential (MPa)	Shoot length (cm)	Root length (cm)	Total seedling length (cm)	Seedling vigour index I
0 (Control)	10.5	12.2	22.7	2117
-0.1	9.8	10.9	20.7	1413
-0.2	9.0	8.2	17.2	860
-0.4	6.5	4.8	11.3	319
-0.6	NG	NG	NG	NG
-0.8	NG	NG	NG	NG
-1.0	NG	NG	NG	NG
SEm±	0.20	0.13	0.83	16.34
LSD (p=0.05)	0.66	0.41	2.02	54.07

LSD- Least significant difference; SEm-Standard error of mean; NG-No germination

Table 4.27: Effect of moisture stress on biomass accumulation and seedling vigour index II of *Triticum aestivum* L.

Osmotic potential (MPa)	Fresh weight (mg/seedling)	Dry weight (mg/seedling)	Seedling vigour index II
0 (Control)	120.24	85.20	7949
-0.1	116.52	82.24	5616
-0.2	103.32	76.30	3815
-0.4	86.40	58.61	1658
-0.6	NG	NG	NG
-0.8	NG	NG	NG
-1.0	NG	NG	NG
SEm±	0.34	0.20	21.06
LSD (p=0.05)	1.31	0.60	76.23

LSD- Least significant difference; SEm- Standard error of mean; NG- No germination

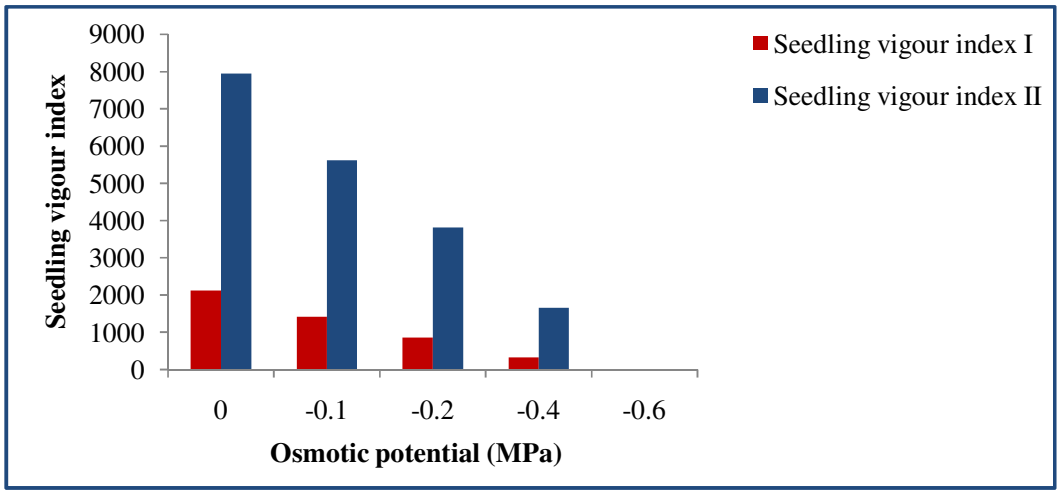


Figure 4.16: Effect of moisture stress on seedling vigour index I and II of *Triticum aestivum* L.

The results of present study show that reduction in water potential significantly decreased the seed germination of both *M. denticulata* and *T. aestivum*. The inhibition of seed germination and reduction in the elongation of roots and shoots of seedlings due to low water availability is related to the reduced activities of hydrolytic enzymes required for mobilisation of reserve substances along with reduced translocation of metabolized products to growing embryo axis (Ayele *et al* 2006). Bargali and Bargali (2016) reported that population of *Medicago denticulata* from Himalayan region of India recorded decrease in germination from 51 to 10% as the water stress level increased from 0 to -1.0 MPa with complete inhibition at -1.5 and - 2.0 MPa. In contrast to this, germination of this North Indian population was completely inhibited even at osmotic potential of -0.6 MPa indicating differential tolerance of

different biotypes of same weed to moisture stress. The results of present study also suggest that cream seeds are less sensitive to moisture stress than brownish black seeds as osmotic potential required for 50% inhibition of maximum germination was higher for brownish black seeds (-0.32 MPa) as compared to cream seeds (-0.37 MPa). Tolerance of cream seeds to moisture stress is consistent with its dormancy characteristics. The differential response of cream and brownish black seeds to moisture stress conditions might be a survival mechanism in *M. denticulata* under adverse environmental conditions. Moisture stress affects the ability of seedlings to develop a root system after germination which is critical for successful establishment and growth. This was demonstrated by higher inhibition of root growth as compared to shoot growth both in *Medicago denticulata* and *Triticum aestivum*.

4.1.7 Salinity stress

***M. denticulata*:** Salinity stress caused a significant decrease in germination and speed of germination with concomitant increase in mean germination time in both cream and brownish black seeds (Table 4.28). Increasing NaCl concentrations above 50 mM was more detrimental to germination of brownish black seeds than cream seeds. At 150 mM NaCl concentration, germination of cream seeds was decreased by 76.8% with 2.5 fold increase in mean germination time than control. Whereas brownish black seeds recorded 92.3% reduction in germination with 2.8 fold increase in mean germination time as compared to control. The NaCl concentration required for 50% inhibition of maximum germination for cream and brownish black seeds was 108.2 and 70.6 mM NaCl, respectively (Figs. 4.17 and 4.18). Cream seeds were fairly tolerant to salinity stress as some seeds (10%) were able to germinate up to NaCl concentration of 200 mM in contrast to brownish black seeds whose germination was completely inhibited at 200 mM NaCl (Plate 5).

Table 4.28: Effect of NaCl on germination of cream and brownish black seeds of *Medicago denticulata* Willd.

NaCl (mM)	Germination (%)		Time to start germination (days)		Germination speed		Mean germination time (days)	
	C	B	C	B	C	B	C	B
0 (Control)	95.5	85.5	2	2	10.70	9.75	3.06	3.22
25	80.0	73.3	2	2	9.82	8.60	3.50	3.72
50	72.2	62.2	3	3	8.56	7.34	3.85	4.28
75	63.3	46.6	3	4	7.20	6.20	5.22	4.85
100	46.6	20.0	4	4	3.80	4.38	9.14	6.50
150	22.2	6.6	5	5	2.30	3.38	7.56	9.10
200	10.0	NG	6	NG	0.34	NG	10.06	NG
250	NG	NG	NG	NG	NG	NG	NG	NG
SEm±	1.66	2.00	2.5×10^{-6}	2.5×10^{-6}	0.06	0.03	0.07	0.04
LSD (p=0.05)	5.61	5.92	1.3×10^{-5}	1.3×10^{-5}	0.16	0.10	0.19	0.15

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference; NG-No germination

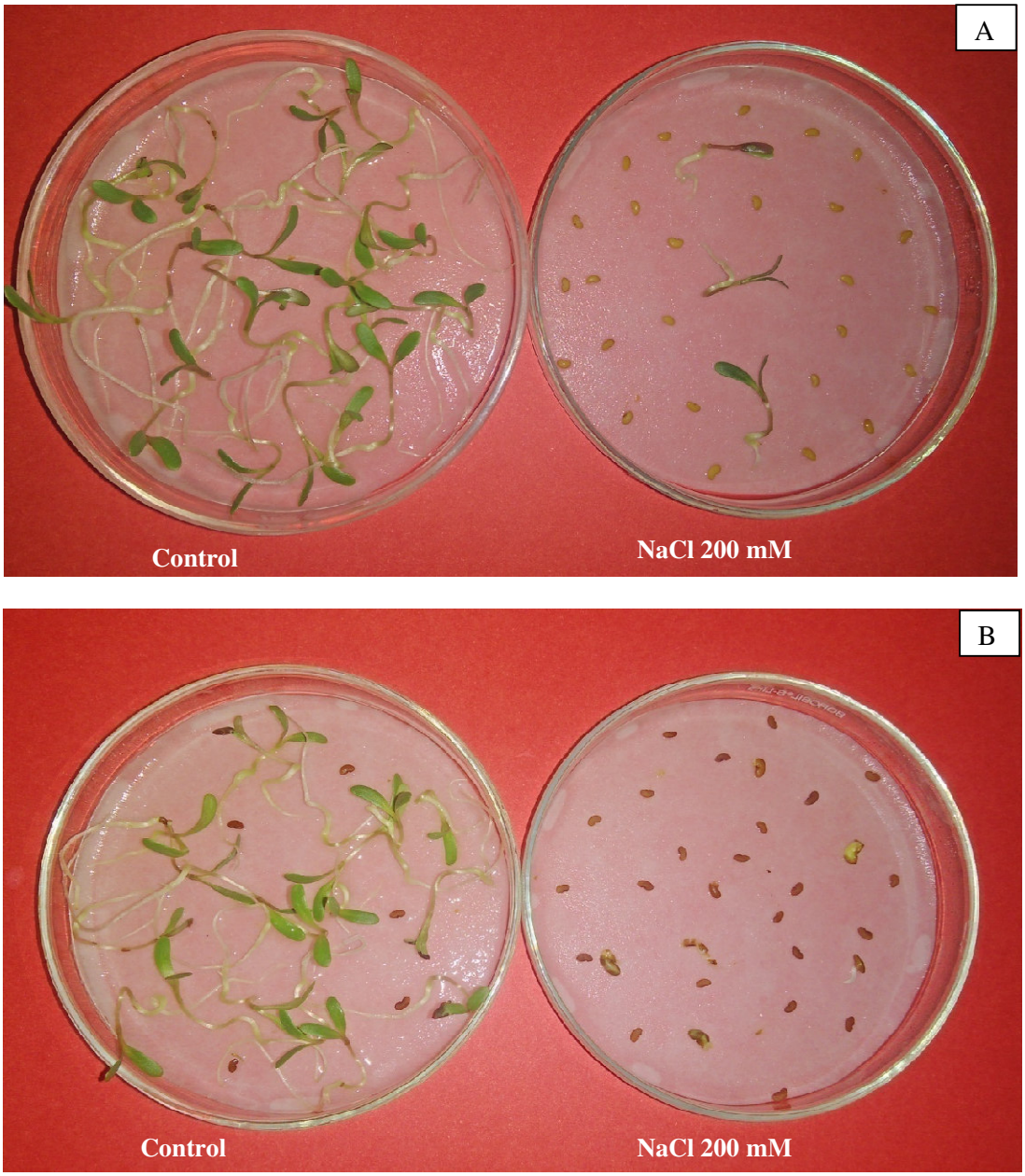


Plate 5: Effect of NaCl on germination of (A) cream and (B) brownish black seeds of*Medicago denticulata* Willd.

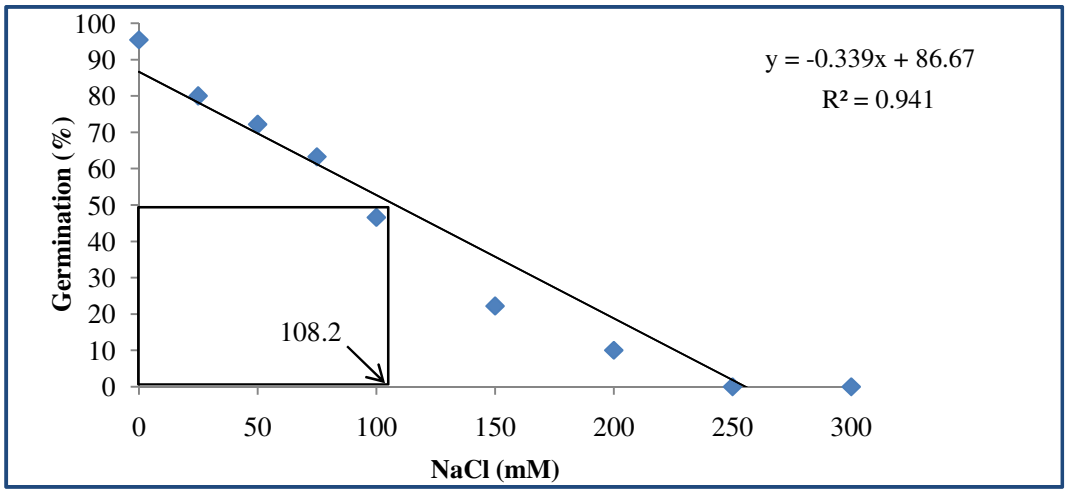


Figure 4.17: **NaCl concentration required for 50% inhibition of maximum germination of cream seeds of *Medicago denticulata* Willd.**

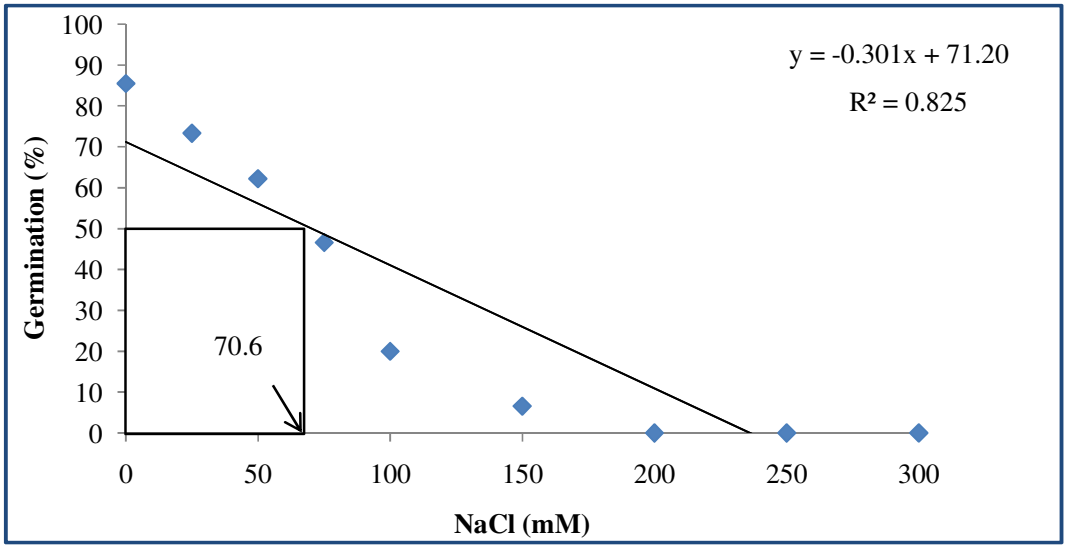


Figure 4.18: **NaCl concentration required for 50% inhibition of maximum germination of brownish black seeds of *Medicago denticulata* Willd.**

Data in Tables 4.29 and 4.30 shows the effect of salinity stress on seedling growth of *M. denticulata*. Salinity stress significantly reduced the growth of both cream and brownish black seedlings. Notably, salinity stress was more detrimental to root growth of both seedlings as compared to shoot growth. At 100 mM NaCl concentration, root and shoot length of seedlings emerging from cream seeds were reduced by 72.1 and 36.2% as compared to their respective controls (Table 4.29). The seedlings emerged from brownish black seeds recorded 80.4 and 54.5% reduction in root and shoot length at 100 mM NaCl as compared to their controls. Cream and brownish black seedlings recorded complete inhibition in root growth at 200 and 150 mM NaCl concentration, respectively. There was significant reduction in fresh and dry weight of cream and brownish black seedlings with increased salinity levels. At 150

mM NaCl concentration, 35.8 and 43.1% reduction was recorded in fresh weight of cream and brownish black seedlings as compared to their respective controls (Table 4.30). Salinity stress significantly declined the vigour index I and II of both cream and brownish black seedlings (Fig. 4.19 and 4.20).

Table 4.29: Effect of NaCl on seedling length and seedling vigour index I of *M. denticulata* Willd.

NaCl (mM)	Shoot length (cm)		Root length (cm)		Total seedling length (cm)		Seedling vigour index I	
	C	B	C	B	C	B	C	B
0 (Control)	4.7	4.4	4.3	4.1	9.0	8.5	859	726
25	4.3	4.0	3.8	3.0	8.1	7.0	648	513
50	3.8	3.4	3.3	2.5	7.1	5.9	512	366
75	3.3	2.8	2.0	1.6	5.3	4.4	335	246
100	3.0	2.0	1.2	0.8	4.2	2.8	195	56
150	2.5	1.5	0.5	-	3.0	1.5	66	9
200	2.0	NG	-	NG	2.0	NG	20	NG
250	NG	NG	NG	NG	NG	NG	NG	NG
SEm±	0.12	0.14	0.10	0.08	0.11	0.09	17.45	15.08
LSD (p=0.05)	0.43	0.40	0.37	0.38	0.54	0.50	54.23	50.34

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference; NG-No germination

Table 4.30: Effect of NaCl on biomass accumulation and seedling vigour index II of *Medicago denticulata* Willd.

NaCl (mM)	Fresh weight (mg/seedling)		Dry weight (mg/seedling)		Seedling vigour index II	
	C	B	C	B	C	B
0 (Control)	36.22	34.54	22.34	20.12	2133	1720
25	33.45	30.26	19.36	18.70	1548	1370
50	30.32	28.90	17.22	15.36	1243	955
75	27.66	24.52	15.28	12.24	967	570
100	23.25	19.58	12.85	7.08	598	141
150	18.43	14.24	6.40	4.40	142	29
200	10.33	NG	3.23	NG	32	NG
250	NG	NG	NG	NG	NG	NG
SEm±	0.68	0.76	0.80	0.56	26.32	29.05
LSD (p=0.05)	2.31	2.67	2.82	2.54	86.03	90.23

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD- Least significant difference; NG-No germination

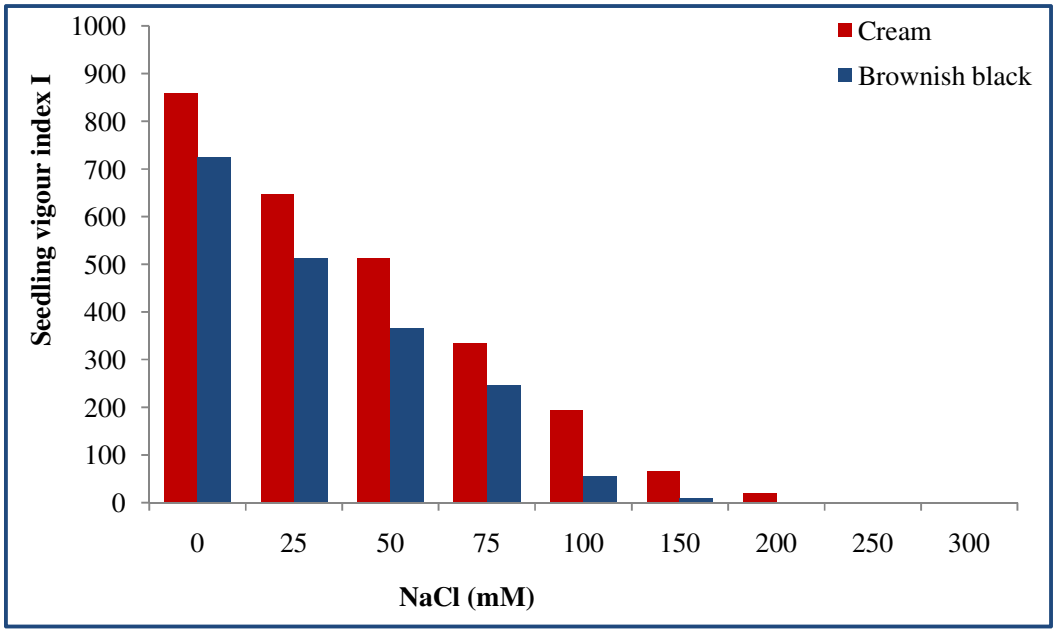


Figure 4.19: Effect of NaCl on seedling vigour index I of *Medicago denticulata* Willd.

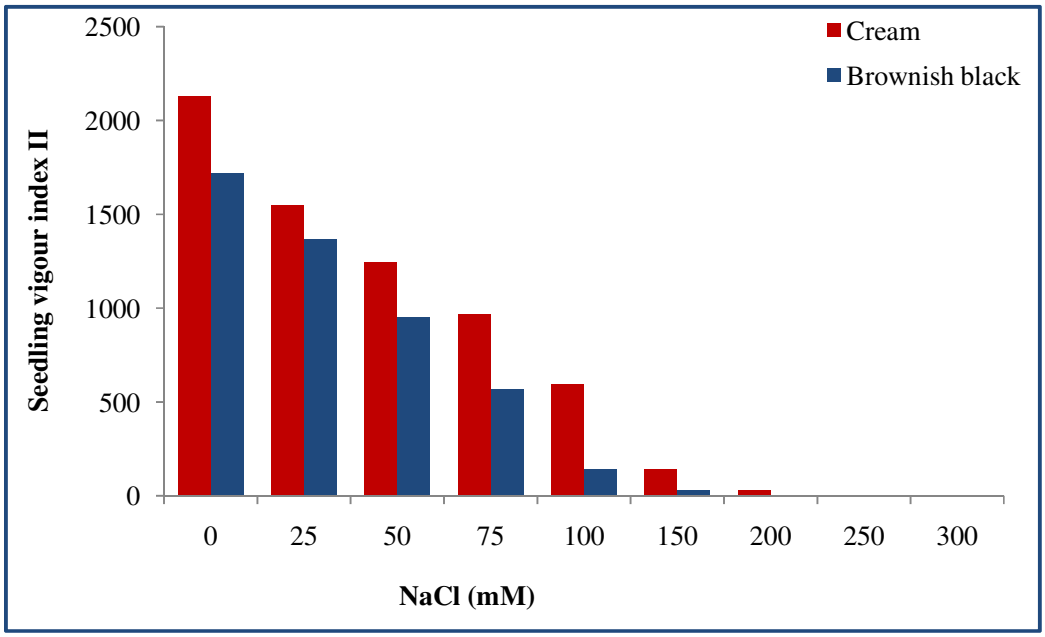


Figure 4.20: Effect of NaCl on seedling vigour index II of *Medicago denticulata* Willd.

***T. aestivum*:** Maximum germination of *T. aestivum* seeds was recorded in control. Increasing NaCl concentration caused significant reduction in germination (%) of *T. aestivum* seeds (Table 4.31). Seed germination was reduced to only 10% at 250 mM NaCl concentration and no seed germinated at 300 mM concentration of NaCl. Time to start germination and mean germination time was increased with concomitant reduction in speed of germination due to salinity stress.

Table 4.31: Effect of NaCl on germination of *Triticum aestivum* L.

NaCl (mM)	Germination (%)	Time to start germination (days)	Germination speed	Mean germination time (days)
0 (Control)	90.0	2	8.70	2.35
25	85.5	2	8.58	2.50
50	80.0	2	8.45	2.65
75	78.8	3	8.32	2.88
100	68.8	3	8.20	3.08
150	62.2	3	7.90	3.26
200	38.8	4	5.65	5.62
250	10.0	5	2.41	7.72
300	NG	NG	NG	NG
SEm±	1.78	1.2×10^{-7}	0.12	0.15
LSD (p=0.05)	4.93	1.1×10^{-6}	0.30	0.45

LSD- Least significant difference; SEm-Standard error of mean; NG- No germination

Salinity stress caused significant decrease in seedling growth and vigour of *T. aestivum*. Maximum root length, shoot length, fresh weight, dry weight as well as vigour indices were recorded in control (Table 4.32 and 4.33). There was 54.2, 78.6 and 87.8% reduction in root length at 100, 150 and 200 mM NaCl concentrations respectively and radicle failed to emerge at 250 mM NaCl. Shoot length of seedlings was inhibited by 67.8% at 200 mM concentration of NaCl as compared to control. Fresh and dry weight of seedlings was reduced by about 63.4 and 77.3% at 200 mM NaCl as compared to their respective controls. Seedling vigour index I and II were reduced by 90.7 and 90.2% as compared to their respective controls due to 200 mM NaCl concentration (Fig. 4.21).

Table 4.32: Effect of NaCl on seedling length and seedling vigour index I of *Triticum aestivum* L.

NaCl (mM)	Shoot length (cm)	Root length (cm)	Total seedling length (cm)	Seedling vigour index I
0 (Control)	11.8	13.1	24.9	2241
25	10.5	12.8	23.3	1992
50	8.8	9.3	18.1	1448
75	6.6	7.5	14.1	1111
100	5.2	6.0	11.2	770
150	4.9	2.8	7.7	478
200	3.8	1.6	5.4	209
250	2.3	0.6	2.9	NG
300	NG	NG	NG	NG
SEm±	0.27	0.20	0.38	23.34
LSD (p=0.05)	0.85	0.70	1.07	80.45

LSD- Least significant difference; SEm-Standard error of mean; NG-No germination

Table 4.33: Effect of NaCl on biomass accumulation and seedling vigour index II of *Triticum aestivum* L.

NaCl (mM)	Fresh weight (mg/seedling)	Dry weight (mg/seedling)	Seedling vigour index II
0 (Control)	118.20	82.25	7402
25	108.32	75.07	6418
50	95.43	68.12	5449
75	89.25	57.17	4504
100	78.00	54.32	3737
150	69.05	47.23	2937
200	43.23	18.66	724
250	25.25	10.25	102
300	NG	NG	NG
SEm±	0.40	0.37	26.12
LSD (p=0.05)	1.30	1.23	80.34

LSD- Least significant difference; SEm-Standard error of mean; NG- No germination

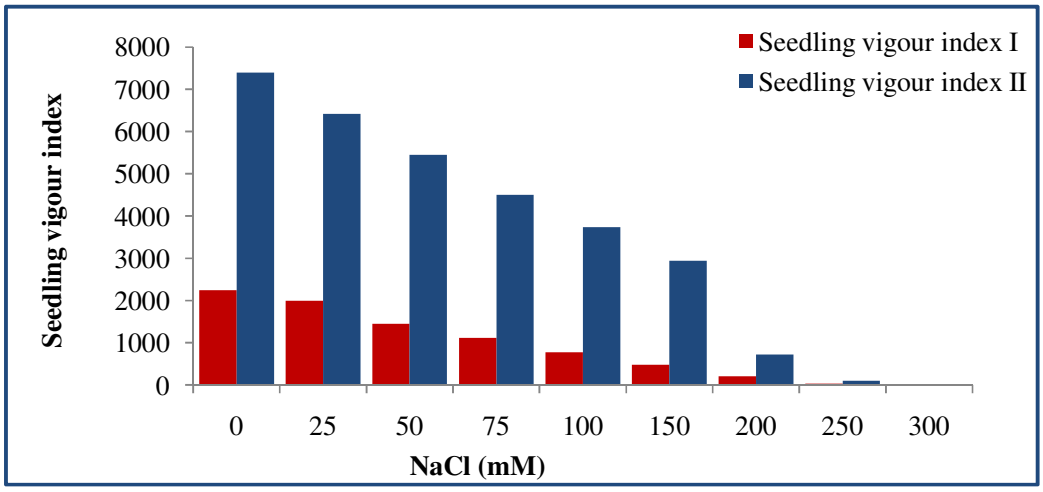


Figure 4.21: Effect of NaCl on seedling vigour index I and II of *Triticum aestivum* L.

Salinity is an important abiotic factor affecting seed germination. It reduces both germination rates as well as root growth of seedlings. The ability to withstand saline conditions may vary from species to species. The results of present study demonstrate that *M. denticulata* seeds possessed the ability to germinate under very high concentrations of NaCl (200 mM), but growth of seedlings was severely hampered due to salinity stress and no radicle emergence was observed at 200 mM NaCl. *T. aestivum* seeds exhibited 62.2% germination at 150 mM NaCl in contrast to cream and brownish black seeds whose germination was reduced to 22.2 and 6.6% respectively. Also, *T. aestivum* seedlings recorded radicle emergence at 200 and 150 mM NaCl. Whereas radicle emergence in cream and brownish black seedlings was completely inhibited at 200 and 150 mM NaCl concentration respectively. This shows that seed germination of *M. denticulata* is more sensitive to salinity stress than *T. aestivum*. Thus, there are lesser chances of this weed to be a problematic weed

in salt affected soils and this weed may be expected to be less competitive against *T. aestivum* particularly in salt affected areas.

Guan *et al* (2009) reported that germination of *Medicago ruthenica* was maximum in control (100%) with > 80% germination at NaCl concentration of 50 and 100 mM. However, salinity stress of 200 mM declined the germination to 55%. In contrast to this, the results of present study depict that germination of both cream and brownish black seeds of *M. denticulata* was < 50% at 100 mM with complete inhibition of germination at NaCl concentration of 250 and 200 mM respectively. These results clearly show that seeds of *M. denticulata* are more sensitive to salinity stress as compared to *M. ruthenica*. Heteromorphic seeds (brown and black) of *Chenopodium album* also showed differential response to salinity stress with brown seeds being more tolerant of salinity as compared to black seeds (Yao *et al* 2010).

4.1.8 pH

M. denticulata: Highest germination of both cream and brownish black seeds was recorded in control having pH 6.6 and lowest at pH 3 (Table 4.34). However, both seeds were able to germinate under both acidic and alkaline pH with > 40% germination in pH range 3-10 which implies that germination of both seeds is not likely to be limited by soil pH. However, germination of brownish black seeds was inhibited to a greater extent as compared to cream seeds in the pH range of 3-10 indicating more vigour of cream seeds. Maximum time to start germination in both cream and brownish black seeds was recorded at pH 3 and 4 indicating that acidic conditions delayed the onset of germination. At pH 3, germination speed of both cream and brownish black seeds was minimum and mean germination time was longest.

Table 4.34: Effect of pH on germination of cream and brownish black seeds of *Medicago denticulata* Willd.

pH	Germination (%)		Time to start germination (days)		Germination speed		Mean germination Time (days)	
	C	B	C	B	C	B	C	B
Control (6.6)	92.2	83.3	2	2	10.80	9.75	3.00	3.26
3	55.5	46.6	4	4	6.02	5.15	6.08	7.20
4	60.0	53.3	4	4	6.43	5.93	5.70	6.15
5	63.3	58.8	2	2	8.48	8.85	3.67	4.68
6	83.3	76.6	2	2	10.20	9.25	3.30	3.48
7	86.6	78.8	2	2	10.52	9.52	3.08	3.36
8	80.0	74.4	2	2	10.36	9.28	3.53	3.52
9	80.0	72.2	2	2	10.30	9.08	3.40	3.60
10	76.6	68.8	2	2	9.80	8.42	3.62	4.05
SEm±	1.57	1.52	1.3×10^{-6}	1.3×10^{-6}	0.08	0.08	0.06	0.08
LSD (p=0.05)	4.66	4.54	1.3×10^{-5}	1.3×10^{-5}	0.18	0.24	0.26	0.25

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference

Data presented in Table 4.35 and 4.36 shows the effect of pH on seedling growth of *M. denticulata*. Maximum growth of both cream and brownish black seedlings was recorded in control and minimum at pH 3. Seedlings emerging from cream seeds grown at pH 3 exhibited 37.5 and 41.2% reduction in root and shoot length as compared to their respective controls. At pH 3, seedlings emerging from brownish black seeds recorded 47.5 and 48% reduction in root and shoot length than seedlings grown in distilled water.

Seedlings emerging from cream and brownish black seeds at pH 3 recorded 28.6 and 31.2% decrease in fresh weight than seedlings grown in distilled water. Dry weight of seedlings emerging from cream and brownish black seeds was decreased by 32.4 and 37% at pH 3 than their respective controls. Increase in pH from 3 to 7 resulted in progressive increase in root length, shoot length, fresh and dry weight of the seedlings with a decrease thereafter. Vigour index of both cream and brownish black seedlings was also increased with increase in pH from 3 to 7 with a decrease thereafter (Figs. 4.22 and 4.23). The highest values of seedling vigor index I and II for both cream and brownish black seeds was recorded in control and lowest at pH 3.

Table 4.35: Effect of pH on seedling length and seedling vigour index I of *Medicago denticulata* Willd.

pH	Shoot length (cm)		Root length (cm)		Total seedling length (cm)		Seedling vigour index I	
	C	B	C	B	C	B	C	B
Control (6.6)	5.1	4.8	4.0	3.8	9.1	8.6	839	716
3	3.0	2.5	2.5	2.0	5.5	4.5	305	209
4	3.0	3.0	2.7	2.4	5.7	5.4	342	287
5	3.5	3.0	3.0	2.6	6.5	5.6	411	329
6	4.5	4.3	3.4	3.3	7.9	7.6	658	582
7	4.7	4.5	3.6	3.6	8.3	8.1	718	638
8	4.2	4.0	3.3	3.2	7.5	7.2	600	535
9	4.0	3.8	3.1	3.0	7.1	6.8	568	490
10	3.8	3.4	3.0	3.0	6.8	6.4	520	440
SEm±	0.12	0.13	0.10	0.12	0.10	0.11	15.65	13.24
LSD (p=0.05)	0.34	0.33	0.31	0.35	0.30	0.30	46.35	44.87

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference

Table 4.36: Effect of pH on biomass accumulation and seedling vigour index II of *Medicago denticulata* Willd.

pH	Fresh weight (mg/seedling)		Dry weight (mg/seedling)		Seedling vigour index II	
	C	B	C	B	C	B
Control (6.6)	36.02	33.74	21.04	20.42	1939	1700
3	25.70	23.06	14.23	12.86	789	599
4	28.85	26.22	18.24	15.05	1094	802
5	29.25	28.13	18.54	16.55	1173	973
6	32.44	30.44	20.55	18.40	1711	1619
7	34.12	32.23	21.21	19.56	1836	1541
8	31.55	28.70	19.32	17.06	1545	1269
9	28.05	25.43	17.66	14.04	1412	1013
10	27.85	24.05	17.02	13.23	1303	910
SEm±	0.85	0.93	0.56	0.78	23.33	20.07
LSD (p=09.05)	2.36	2.45	1.54	2.32	88.43	84.36

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference

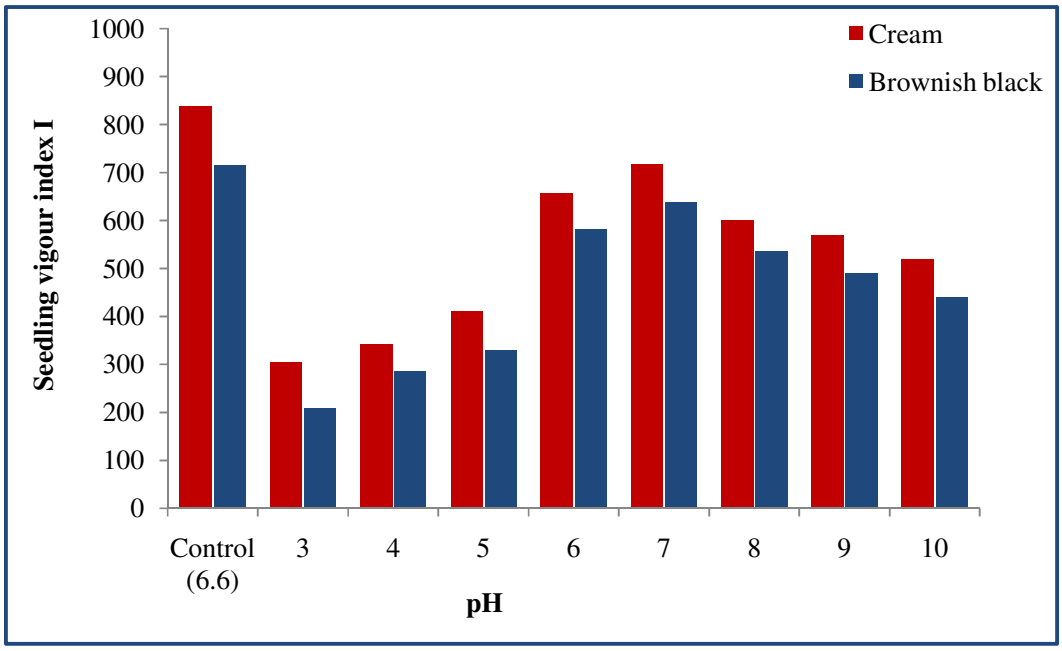


Figure 4.22: Effect of pH on seedling vigour index I of *Medicago denticulata* Willd.

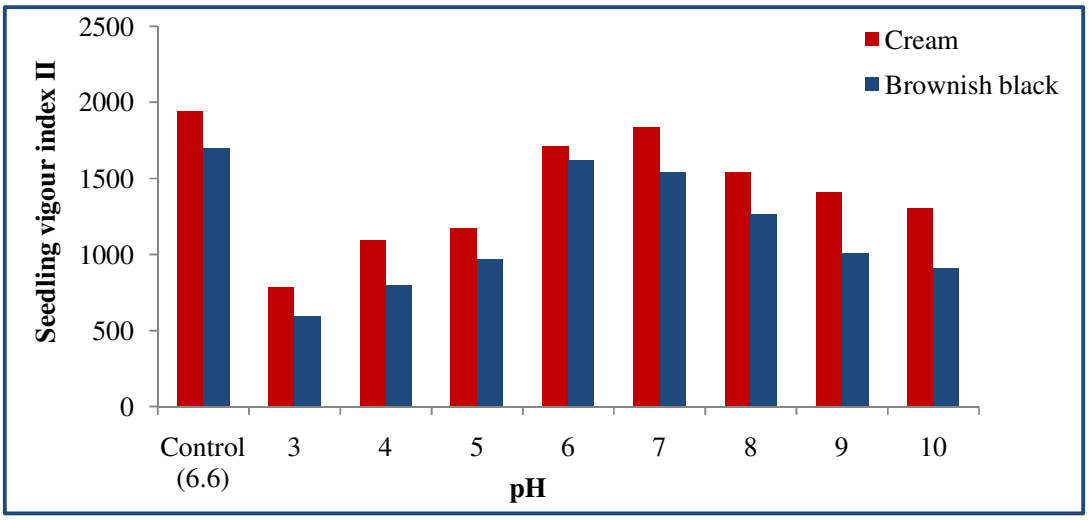


Figure 4.23: Effect of pH on seedling vigour index II of *Medicago denticulata* Willd.

***T. aestivum*:** The data in Table 4.37 indicate that the highest germination of *T. aestivum* seeds was recorded in control having pH 6.6. Highly acidic (pH < 5) and alkaline pH (pH > 8) conditions completely inhibited the germination of *T. aestivum* seeds. The minimum germination (50%) was recorded at pH 5 where seeds also required longest time to start germination. Increasing pH from 5 to 7 increased the germination (%) and speed with concomitant reduction in mean germination time.

Table 4.37: Effect of pH on germination of *Triticum aestivum* L.

pH	Germination (%)	Time to start germination (days)	Germination speed	Mean germination time (days)
Control (6.6)	93.3	2	8.68	2.48
3	NG	NG	NG	NG
4	NG	NG	NG	NG
5	50.0	4	3.85	5.65
6	76.6	2	6.03	3.42
7	80.0	2	7.05	2.98
8	71.6	2	6.68	3.20
9	NG	NG	NG	NG
10	NG	NG	NG	NG
SEm±	1.23	1.2×10^{-6}	0.12	0.07
LSD (p=0.05)	4.36	1.2×10^{-5}	0.25	0.19

LSD-Least significant difference; SEm-Standard error of mean; NG-No germination

There was significant increase in root length, shoot length, fresh and dry weight of *T. aestivum* seedlings when pH was increased from 5 to 7 (Table 4.38). Maximum growth of seedlings was recorded in distilled water (control). At pH 5, root and shoot length was reduced by 64.8 and 49.1% as compared to their respective controls. Fresh and dry weights

were decreased by 51.2 and 73.5% at pH 5 as compared to their respective controls (Table 4.39). The highest values of seedling vigour index I and II were recorded in control followed by seedlings grown under pH 7, 8, 6 and 5, respectively (Fig. 4.24).

Table 4.38: Effect of pH on seedling length and seedling vigour index I of *Triticum aestivum* L.

pH	Shoot length (cm)	Root length (cm)	Total seedling length (cm)	Seedling vigour index I
Control (6.6)	11.2	12.8	24.0	2239
3	NG	NG	NG	NG
4	NG	NG	NG	NG
5	5.7	4.5	10.2	220
6	6.6	6.8	13.4	781
7	10.8	10.5	21.3	1704
8	9.6	8.8	18.4	1317
9	NG	NG	NG	NG
10	NG	NG	NG	NG
SEm±	0.20	0.18	0.78	18.77
LSD (p=0.05)	0.47	0.46	3.16	60.24

LSD-Least significant difference; SEm-Standard error of mean; NG-No germination

Table 4.39: Effect of pH on biomass accumulation and seedling vigour index II of *Tritiicum aestivum* L.

pH	Fresh weight (mg/seedling)	Dry weight (mg/seedling)	Seedling vigour index II
Control (6.6)	115.30	80.21	7483
3	NG	NG	NG
4	NG	NG	NG
5	56.21	21.21	458
6	76.52	40.75	2375
7	108.5	76.42	6113
8	80.50	48.95	3504
9	NG	NG	NG
10	NG	NG	NG
SEm±	1.02	0.94	20.23
LSD (p=0.05)	3.16	3.45	56.74

LSD-Least significant difference; SEm-Standard error of mean; NG-No germination

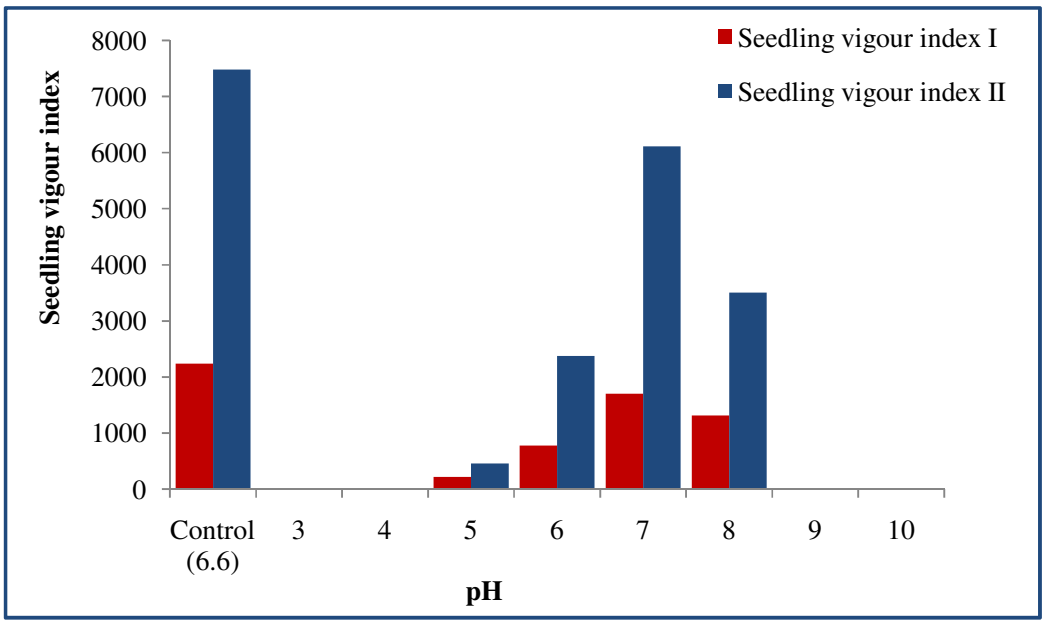


Figure 4.24: Effect of pH on seedling vigour index I and II of *Triticum aestivum* L.

Soil pH is an important factor governing the germination of seeds. The response of plants to different pH values varies among species. Many plants can only germinate within a narrow pH range while others possess ability to germinate within a broad pH range. Soil pH affects development and competitiveness of crop and weeds by affecting availability of essential micronutrients and solubility of toxic elements. Most of the nutrients (micronutrients especially) are less available when soil pH is above 7.5 and are optimally available at a slightly acidic pH of 6.5 to 6.8. Acidic conditions (below pH 5.5) may trigger the release of toxic form of aluminium or manganese, while alkaline conditions (above pH 8.0) impairs the root uptake of scarce trace element cations, such as copper, iron, manganese and zinc thereby affecting germination. Some weed species can grow only on particular soil environment conditions and attention to amend these conditions has a major function in weed management. Weed distribution pattern can be influenced by the ability of weed to tolerate pH of the soil.

The germination of *T. aestivum* was completely inhibited when pH was either less than 5 or more than 8. However, germination of both cream and brownish black seeds of *M. denticulata* occurred in wide pH range of 3-10. Bullitta *et al* (1994) reported that favorable growth of *M. polymorpha* is restricted to soils with a pH of 4.7-8. However, the results of present study reveal that *M. denticulata* has not only ability to grow on extremely acidic soils but also alkaline soils. Graziano *et al* (2010) reported that *M. polymorpha* is well adapted to alkaline soils. The pH of agricultural lands in Punjab varies from 7 to 8. In this pH range *M. denticulata* possessed 80-86% germination, indicating that pH is not likely to be a limiting factor for germination of this weed.

4.1.9 Burial depth

Data in Table 4.40 indicates that maximum emergence of both cream and brownish black seeds was recorded from surface placed seeds. There was progressive decline in emergence of both seeds with increase in burial depth from 1-4 cm. At 4 cm depth, emergence of cream and brownish black seeds was reduced by 52 and 60 per cent points as compared to surface placed seeds. Emergence of cream and brownish black seeds was 13.3 and 6.6% respectively at burial depth of 6 cm. No emergence was observed when seeds were placed at a depth of 8 cm or deeper.

Table 4.40: Effect of burial depth on germination of heteromorphic *Medicago denticulata* Willd. seeds

Burial depth (cm)	Germination (%)	
	Cream	Brownish black
0	94.4	83.3
1	90.0	80.0
2	86.6	74.4
4	44.4	23.3
6	13.3	6.6
8	NG	NG
10	NG	NG
SEm±	2.47	1.82
LSD (p=0.05)	7.50	5.53

LSD-Least significant difference; SEm-Standard error of mean; NG-No germination

Burial depth affects germination and emergence of seeds by influencing the availability of light, moisture and temperature. Seed burial at shallow depths can stimulate higher germination as it maintains moist environment around seeds and prevent them from drying. The requirement of light and limited availability of storage reserves are also major constraints for reduced emergence of weeds from deeper soil layers (Bullied *et al* 2012). Results of our study indicate that light independent germination and bigger seed size of heteromorphic seeds of *M. denticulata* (1000 seed weight of cream and brownish black seeds was 3.56 and 3.65 g respectively); may have favoured the emergence of this weed from soil depths of 6 cm. However, small-seeded winter weed species like, *Poa annua* (1000 seed weight = 0.3 g) and *Rumex dentatus* (1000 seed weight = 2.33 g) may not have enough energy reserves to support their emergence from deeper soil depths along with their absolute requirement of light for germination may be the factors responsible for their emergence only from soil depths of less than 3 cm (Ohadi *et al* 2010, Benvenuti *et al* 2001).

4.2 Experiment 2: To study the effect of accelerated ageing and soil seedbank persistence on germination ecology of *Medicago denticulata* Willd.

4.2.1 Accelerated ageing

Data in Table 4.41 reveals that accelerated ageing caused greater and rapid loss of germination in brownish black seeds than cream seeds. With increase in days of ageing, both seeds recorded considerable reduction in germination (%) along with increased mean germination time and decreased germination speed as compared to their respective controls. Maximum germination of both cream and brownish black seeds was observed in control. Germination of cream seeds was reduced by 61 per cent points with 3 fold increase in mean germination time than control at 10^{th} day of ageing. Whereas brownish black seeds at 10^{th} day of ageing recorded 66 per cent point reduction in germination with 4 fold increase in mean germination time as compared to control. When ageing was done for 20 days, some of the cream seeds (13%) were able to germinate whereas no germination was recorded in brownish black seeds. The P_{50} value for 50% inhibition of maximum germination in cream and brownish black seeds due to accelerated ageing was 9.2 and 5.1 days respectively (Figs. 4.25 and 4.26).

Table 4.41: Effect of accelerated ageing (at 45°C and 60% RH) on germination of *Medicago denticulata* Willd. seeds

Accelerated ageing (days)	Germination (%)		Time to start germination (days)		Germination speed		Mean germination time (days)	
	C	B	C	B	C	B	C	B
0 (Control)	94.4	82.2	2.00	2.00	10.52	9.25	3.05	3.16
1	84.4	68.3	2.00	3.00	10.10	7.43	3.36	4.11
4	75.0	50.0	3.00	4.00	9.17	4.09	4.41	7.06
7	53.3	36.6	4.00	5.00	4.34	1.15	6.56	10.28
10	33.3	16.6	5.00	7.00	1.34	0.42	9.66	13.00
20	13.3	NG	7.00	NG	0.37	NG	12.77	NG
30	NG	NG	NG	NG	NG	NG	NG	NG
40	NG	NG	NG	NG	NG	NG	NG	NG
50	NG	NG	NG	NG	NG	NG	NG	NG
SEm±	1.33	1.05	1.2×10^{-6}	1.2×10^{-6}	0.05	0.04	0.08	0.06
LSD (p=0.05)	3.97	3.13	1.2×10^{-5}	1.2×10^{-5}	0.16	0.12	0.25	0.20

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference; NG-No germination

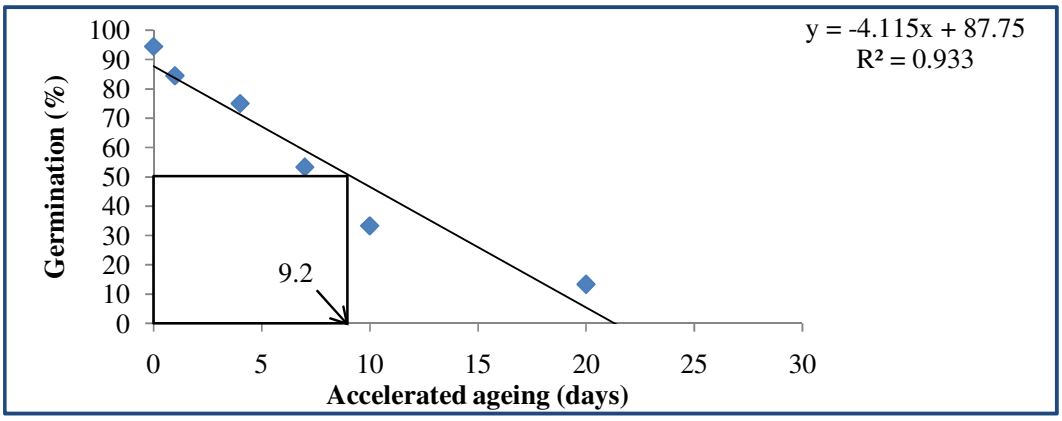


Figure 4.25: Half viability period (P_{50} value) for 50% inhibition of maximum germination due to accelerated ageing in cream seeds of *Medicago denticulata* Willd. The P_{50} value is shown by an arrow.

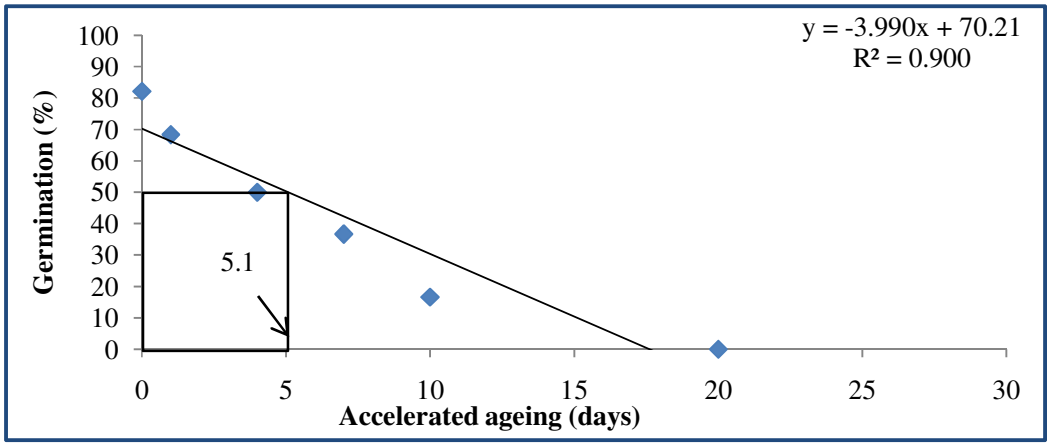


Figure 4.26: Half viability period (P_{50} value) for 50% inhibition of maximum germination in brownish black seeds of *Medicago denticulata* Willd. due to accelerated ageing. The P_{50} value is shown by an arrow.

Table 4.42 and 4.43 shows the effect of accelerated ageing on seedling growth and vigour of *M. denticulata*. Seedlings emerged from unaged cream and brownish black seeds attained maximum growth in terms of seedling length and vigour. With increase in days of ageing, there was progressive reduction in root length, shoot length, fresh weight, dry weight and vigour indices of growth in terms of seedling length and vigour. With increase in days of ageing, there was progressive reduction in root length, shoot length, fresh weight, dry weight and vigour indices of seedlings emerged from cream and brownish black seeds. On 10^{th} day of ageing, seedlings emerging from cream seeds recorded 44 and 54.5% reduction in shoot and root length as compared to their respective controls (Table 4.42). Whereas, seedling emerging from brownish black seeds aged for 10 days recorded 66 and 75% decrease in shoot and root length than their respective controls. Ageing of 10 days reduced fresh weight of seedlings

emerging from cream and brownish black seeds by 45.9 and 67.4%, respectively, than controls (Table 4.43). Likewise, cream and brownish black seeds aged for 10 days produced seedlings with 45.2 and 70% reduction in dry weight as compared to their respective controls. There was progressive reduction in the seedling vigour indices of both cream and brownish black seeds with increase in duration of ageing (Figs. 4.27 and 4.28). On 10^{th} day of ageing, cream seeds recorded 82.7 and 80.7% decrease in seedling vigor index I and II as compared to their respective controls. Whereas, brownish black seeds had 93.8 and 93.9% decrease in seedling vigor index I and II than their respective controls.

Table 4.42: Effect of accelerated ageing (at 45°C and 60% RH) on seedling length and seedling vigour index I of *Medicago denticulata* Willd.

Accelerated ageing (days)	Shoot length (cm)		Root length (cm)		Total seedling length (cm)		Seedling vigour index I	
	C	B	C	B	C	B	C	B
0 (Control)	5.0	4.7	4.4	4.0	9.4	8.4	887	690
1	4.8	4.3	4.2	3.7	9.0	8.0	759	546
4	4.4	3.5	3.6	2.9	8.0	6.4	600	320
7	3.8	2.7	3.0	2.1	6.8	4.8	362	175
10	2.8	1.6	2.0	1.0	4.8	2.6	153	43
20	1.7	NG	1.0	NG	2.7	NG	35	NG
30	NG	NG	NG	NG	NG	NG	NG	NG
40	NG	NG	NG	NG	NG	NG	NG	NG
50	NG	NG	NG	NG	NG	NG	NG	NG
SEm±	0.08	0.06	0.07	0.08	0.09	0.11	15.09	12.33
LSD (p=0.05)	0.25	0.21	0.23	0.24	0.27	0.33	48.67	45.34

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference; NG-No germination

Table 4.43: Effect of accelerated ageing (at 45°C and 60% RH) on biomass accumulation and seedling vigour index II of *Medicago denticulata* Willd.

Accelerated ageing (days)	Fresh weight (mg/seedling)		Dry weight (mg/seedling)		Seedling vigour index II	
	C	B	C	B	C	B
0 (Control)	36.54	34.38	23.08	20.65	2178	1697
1	36.04	33.58	22.75	19.84	1920	1355
4	32.14	26.65	20.40	15.08	1530	754
7	27.43	18.23	17.68	10.23	942	374
10	19.75	11.20	12.64	6.25	420	103
20	11.25	NG	7.50	NG	99	NG
30	NG	NG	NG	NG	NG	NG
40	NG	NG	NG	NG	NG	NG
50	NG	NG	NG	NG	NG	NG
SEm±	0.33	0.25	0.16	0.11	23.56	20.23
LSD (p=0.05)	1.01	0.76	0.50	0.34	60.21	56.67

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference; NG-No germination

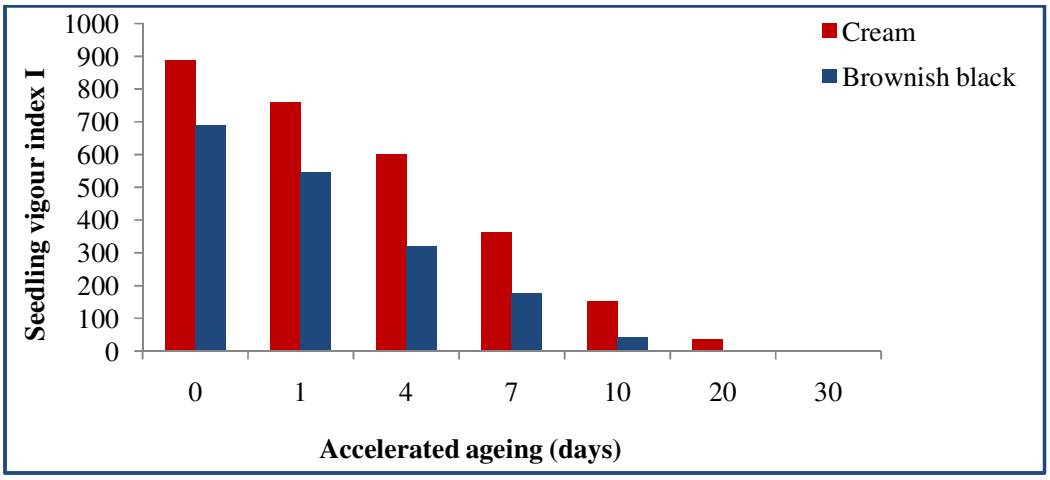


Figure 4.27: Effect of accelerated ageing on seedling vigour index I of *Medicago denticulata* Willd.

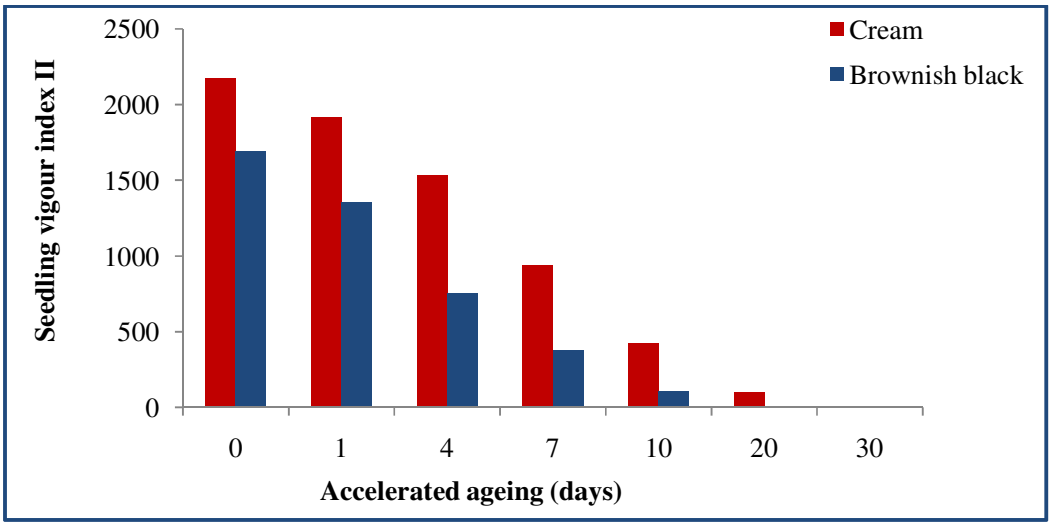


Figure 4.28: Effect of accelerated ageing on seedling vigour index II of *Medicago denticulata* Willd.

Data in Table 4.44 shows the effect of accelerated ageing on seed storage reserves of cream and brownish black seeds. Both cream and brownish black seeds recorded reduction in the starch and total soluble proteins with concomitant increase in the amount of total soluble sugars and total free amino acids. However, seed storage reserves of brownish black seeds were affected to a greater extent than cream seeds. On 50th day of ageing, starch and total soluble proteins of cream seeds were decreased by 83.7 and 64.6% respectively as compared to their respective controls. Whereas, brownish black seeds exhibited 90.5 and 87.6% reduction in starch and total soluble proteins on 50th day of ageing than their respective controls. Accelerated ageing of both cream and brownish black seeds resulted in membrane damage as evident by progressive and significant increase in membrane leakage. On 50th day of ageing, membrane leakage of cream and brownish black seeds was increased by 48 and 57

per cent points as compared to their respective controls (Fig. 4.29).

Table 4.44: Effect of accelerated ageing on contents of various storage reserves in the seeds of *Medicago denticulata* Willd.

Accelerated ageing (days)	Starch content (mg g^{-1} FW)		Total soluble sugars (mg g^{-1} FW)		Total soluble proteins (mg g^{-1} FW)		Total free amino acids (mg g^{-1} FW)	
	C	B	C	B	C	B	C	B
0 (Control)	17.65	18.76	20.26	23.56	35.36	36.57	33.40	35.85
1	17.05	17.60	20.65	24.23	35.02	33.25	33.65	37.12
4	16.56	13.07	23.09	27.34	33.65	29.14	35.43	40.34
7	13.78	10.54	25.07	31.10	30.45	23.52	37.06	42.65
10	10.67	8.34	28.06	36.02	28.63	18.04	39.64	44.72
20	8.52	6.03	32.32	38.78	24.52	15.16	42.08	46.78
30	6.24	5.34	34.65	40.45	21.17	12.38	45.44	50.33
40	4.18	3.56	36.13	43.12	17.64	8.23	48.24	54.05
50	2.87	1.78	38.04	46.08	12.52	4.54	52.13	57.16
SEm±	0.14	0.66	0.63	0.48	1.03	0.68	1.06	1.03
LSD (p=0.05)	0.40	1.50	1.89	1.40	2.78	1.65	2.78	1.87

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference

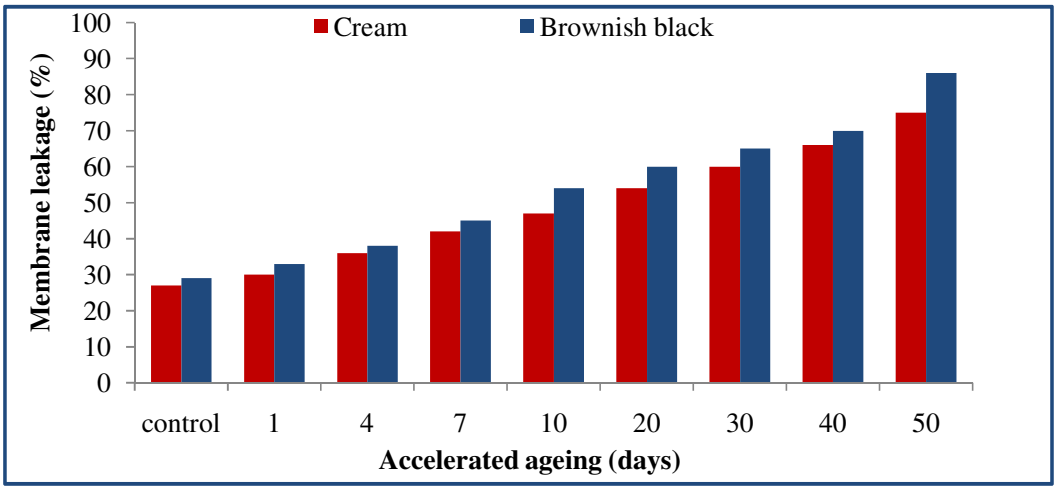


Figure 4.29: Effect of accelerated ageing on membrane leakage of heteromorphic seeds of *Medicago denticulata* Willd.

Accelerated ageing is a process in which seeds are subjected to elevated temperature and moisture conditions under controlled conditions in the laboratory (Mavi and Demir 2007). Field trials are time consuming with high labour costs whereas accelerated ageing is a rapid method for estimating seed fate under field conditions. Therefore, this technique is employed to determine the long term ageing effects within a shorter time. Accelerated ageing slows down the germination process by disturbing the seed metabolism leading to high mean germination time and decreased germination (Nigam *et al* 2019). Increase in the mean germination time is the earliest symptom of ageing (Eski and Demir 2011). In the present

study also, with increased durations of ageing, both cream and brownish black seeds recorded decrease in germination with an overall increase in mean germination time. High temperature and moisture content increases the respiration of seeds that leads to deterioration of seeds (Goel *et al* 2003).

The accelerated ageing of *M. denticulata* seeds caused significant changes in seed germination ability and biochemical composition of seeds. Rapid loss in germination and greater mobilization of complex storage reserves (starch and total soluble proteins) in brownish black seeds can be correlated with their 3% higher moisture content as compared to cream seeds. Jain *et al* (2006) reported that *Raphanus raphanistrum* seeds recorded significant reduction in total protein content with increase in duration of accelerated ageing than unaged seeds. Similarly, Ayyappan *et al* (2006) also reported that total protein content of cucumber seeds was reduced to half of the initial content when aged for 8 days. Significant increase in total soluble sugar content was recorded in *Brassica campestris* seeds with increase in accelerated ageing (Verma *et al* 2003). Seed moisture content is an important criteria which determines seed viability during storage. For every decrease of 1% in seed moisture content, the life of seed is doubled (Harrington 1972). High seed moisture content results in more rapid loss of viability and seed quality. This indicates that cream seeds can remain viable for a longer period as compared to brownish black seeds. Long *et al* (2008) developed three categories to describe seed bank persistence: a transient seed bank corresponded to species with a P_{50} value of < 20 days (< 1 year field persistence), a short-lived seed bank corresponded with P_{50} values of 20 to 50 days (1-3 years field persistence) and extended persistence corresponded with P_{50} values of > 50 days (> 3 years field persistence). According to this, cream seeds of *M. denticulata* may be expected to have persistence of 1-3 years under field condition as some seeds (~13%) were able to germinate after ageing of 20 days. While brownish black seeds may be expected to persist for less than 1 year.

4.2.2 Soil seed bank persistence

The effect of burial depth and time of burial on *M. denticulata* seeds was studied at PAU, Ludhiana and Dr. J. C. Bakhshi Regional Research Station, Abohar. Cream seeds exhumed at monthly intervals from different burial depths at both locations recorded higher germination than brownish black seeds (Table 4.45 and 4.46). There was significant decline in germination of both the seeds with increase in depth and time of burial. Maximum germination in both the seeds was recorded from surface placed seeds. Cream and brownish black seeds buried at 10 cm depth for 6 months recorded 16.7 and 43.3% decrease in germination as compared to their surface placed seeds (Table 4.45). Cream seeds placed on soil surface or buried at a depth of 2 and 10 cm were able to germinate up to 12 months; but no germination was recorded after 15 months even after scarification at both locations. In

contrast, brownish black seeds buried for 12 or more months recorded no germination from any burial depth. Cream seeds exhumed from any depth after 3 or more months recorded no field germination whereas brownish black seeds exhumed from each depth after 3 and 6 months produced seedlings with intact plumule and radicle. However, no seedling was recovered from brownish black seeds exhumed after a period of 6 or more months.

Table 4.45: Effect of burial depth and time of burial on persistence of *Medicago denticulata* Willd. seeds at PAU, Ludhiana.

Burial depth (cm)	Time of Burial									
	3 months		6 months		9 months		12 months		15 months	
	Germination (%)									
	C	B	C	B	C	B	C	B	C	B
0	86.6	76.6	80.0	64.4	76.6	53.3	68.3	NG	NG	NG
2	86.6	72.2	75.5	57.0	65.5	40.0	35.5	NG	NG	NG
10	70.0	60.0	66.6	36.5	47.3	23.3	20.0	NG	NG	NG
SEm±	5.34	3.23	2.28	3.40	3.67	3.17	3.28	-	-	-
LSD (p=0.05)	11.51	7.23	5.89	7.56	8.41	6.56	7.29	-	-	-
	Seed decay (%)									
Burial depth (cm)	C	B	C	B	C	B	C	B	C	B
0	-	4.4	-	16.6	-	33.3	-	80.0	-	90.0
2	-	4.4	-	24.4	-	48.8	-	93.3	-	96.6
10	-	7.7	-	48.8	-	63.3	-	96.6	-	96.6
SEm±		1.05		1.23		1.05		3.08		3.12
LSD (p=0.05)		3.23		5.03		4.34		6.30		6.00
	Field germination (%)									
Burial depth (cm)	C	B	C	B	C	B	C	B	C	B
0	-	4.33	-	3.33	-	-	-	-	-	-
2	-	3.66	-	2.00	-	-	-	-	-	-
10	-	2.33	-	1.33	-	-	-	-	-	-
SEm±		0.23		0.20						
LSD (p=0.05)		0.86		0.78						

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference; NG-No germination

Cream seeds did not show any seed decay whereas brownish black seeds buried for 12 or more months recorded more than 80% seed decay when buried at 2 and 10 cm or placed

on soil surface. High rate of seed decay and decreased germination of brownish black seeds from deeper soil layers could be attributed to their high moisture content and presence of permeable seed coat which allows high rates of water imbibition as discussed earlier in imbibition studies. Therefore, contribution of brownish black seeds to the build up of soil seed bank will be less as they are more prone to damage under field conditions. Whereas cream seeds with hard impermeable seed coat were able to resist damage to seed coat. This indicates that cream seeds will have an advantage over brownish black seeds under harsh and unpredictable environmental conditions which will help them to form a short lived seed bank where seeds can survive for 1-3 years. Brownish black seeds will form only a transient seed bank having persistence of less than 1 year.

Table 4.46: Effect of burial depth and time of burial on persistence of *Medicago denticulata* Willd. seeds at Regional Research Station, Abohar.

Burial depth (cm)	Time of Burial									
	3 months		6 months		9 months		12 months		15 months	
	Germination (%)									
	C	B	C	B	C	B	C	B	C	B
0	80.0	72.2	76.6	63.3	70.0	50.0	60.0	NG	NG	NG
2	80.0	72.2	70.0	60.0	63.3	43.3	40.0	NG	NG	NG
10	73.3	55.3	66.6	34.0	53.3	20.0	26.6	NG	NG	NG
SEm±	4.30	4.18	2.04	2.16	2.60	2.15	3.12	-	-	-
LSD (p=0.05)	10.54	8.15	4.67	6.16	7.43	5.50	6.20	-	-	-
	Seed decay (%)									
Burial depth (cm)	C	B	C	B	C	B	C	B	C	B
0	-	6.6	-	20.0	-	40.0	-	76.6	-	93.3
2	-	6.3	-	26.6	-	50.0	-	90.0	-	96.6
10	-	8.5	-	53.3	-	62.2	-	96.6	-	98.0
SEm±		1.10		1.40		1.28		3.36		3.45
LSD (p=0.05)		3.30		5.14		4.46		6.48		6.25
	Field germination (%)									
Burial depth (cm)	C	B	C	B	C	B	C	B	C	B
0	-	3.00	-	5.33	-	-	-	-	-	-
2	-	2.66	-	4.66	-	-	-	-	-	-
10	-	2.00	-	2.66	-	-	-	-	-	-
SEm±		0.20		0.46						
LSD (p=0.05)		0.78		0.90						

C-Seeds with cream seed coat; B-Seeds with brownish black seed coat; SEm-Standard error of mean; LSD-Least significant difference; NG-No germination

Seed size and coat colour are important characteristics for predicting seed persistence under field conditions, as smaller seeds have been reported to persist for longer period than larger seeds (Peco *et al* 2003). Like in present study, cream seeds with relatively smaller size have recorded higher persistence than brownish black seeds under field conditions. Cao *et al* (2012) reported that *Suaeda corniculata* seeds with black seed coat exhibited dormancy which was improved by mechanical scarification and GA_3, while seeds with brown seed coat were non-dormant and germinated to a high percentage when buried in the soil. Black seeds of *Suaeda corniculata* due to seed coat imposed dormancy formed a persistent seed bank, while brown seeds because of their ability to germinate rapidly formed only a transient soil seed bank. In present study also, cream seeds with seed coat imposed dormancy recorded no field germination when buried in the soil; whereas, non-dormant brownish black seeds produced seedlings with intact radicle and plumule. Field emergence at 2 and 10 cm depth also indicates light independent germination of this weed.

Tsuyuzaki (2006) reported that *Rumex obtusifolius* seeds exhumed after 3 years of burial from a depth of 3 cm showed germination of more than 70% in temperature range of 15-25°C. However, field emergence was completely absent in *R. obstifolius* seeds buried at 3 cm which indicated that presence of light was required for field germination. Mennan and Zandstra (2006) reported that seeds of *Veronica hederifolia* buried for 2 yr at a depth of 0, 5, 10 and 20 cm recorded progressive decrease in viability upon retrieval from field at monthly intervals and seeds placed on the soil surface lost their viability more rapidly than those buried more deeply. Gulden and Shirtliffe (2009) reported that common lambsquarters (*Chenopodium album*) form a persistent seed bank with the ability to remain viable in the soil for many decades. Conn *et al* (2006) reported that seed germination and dormancy of common lambsquarters (*Chenopodium album*) did not differ at 2 and 15 cm depth and seeds at both depths recorded 3.0 and 26.7% germination and dormancy after a burial period of 19.7 years. Unlike *C. album*, germination of both cream and brownish black seeds exhumed from different soil depths differed significantly. The present study indicated that viability of cream seeds buried for 12 months was decreased from 35 to 20% with increase in burial depth from 2 to10 cm.

Persistent seed bank formation is one of the essential characteristics in many weed populations for the maintenance of seed supply in the soil for extended periods which serves as a survival mechanism during unfavorable period (Pakeman *et al* 2012). Soil seed banks are typically characterized by their longevity and are determined by how long an individual seed may reside within it in a viable state (Hossain and Begum 2015). In the present study, results obtained from field persistence studies differed from that of accelerated ageing. The slight disparities like quick loss of seed viability under accelerated ageing conditions than under field conditions can be attributed to differences in the moisture content and temperature of

seeds between the two environments (El-Abady *et al* 2015). During accelerated ageing, high temperature and moisture content results in an increase in respiration of seeds, causing seeds to deteriorate more rapidly (Torres and Filho 2003). However, seed deterioration under natural condition takes a long time to affect the seed qualities. Temperature and moisture content are the two primary factors which determine seed longevity in *ex situ* storage. Under field conditions, seeds experience constant fluctuations in moisture and temperature conditions mainly in the upper centimetres of soil (Merritt *et al* 2007). With increase in soil depth, light is excluded and temperature and moisture conditions become more stable, which slows down the processes of dormancy release and ageing (Saatkamp *et al* 2011).

The depth at which seeds are buried also influences seed persistence, with seeds typically persisting for longer duration at greater depths. However, this is in contrast to results of present study which indicated that there was rapid loss in germination of both cream and brownish black seeds with increase in soil depth. Decreased emergence with increased depth of burial may be caused by higher soil moisture, poor gas exchange, higher CO_2 levels around seeds or exhaustion of seedling reserves (Benvenuti 2003). Benvenuti and Mazzoncini (2018) reported that emergence of *Polygonum convolvulus* L. and *Portulaca oleracea* L. was greatly reduced with increase in soil depth. *P. convolvulus* L. and *P. oleracea* L. seeds buried at a depth of 2 and 4 cm in compacted clay soil recorded emergence of 15 and 7% and 5 and 3% respectively. Similarly, Vasileiadis *et al* (2016) reported reduction in jimsonweed (*Datura stramonium* L.) emergence with increase in burial depth which might be linked to poor gas exchange in the environment surrounding the buried seeds.

Seeds under natural conditions have the ability to resist ageing. For example, in many physically dormant species, such as *Cuscuta*, *Trifolium* and *Rhyncosia*, a densely packed layer of palisade cells with water-repellent properties shields the embryo from the environmental fluctuations in moisture that can influence longevity (Finch-Savage and Leubner-Metzger 2006). Hard-seeded species, such as *Amaranthus* and *Cassia* spp., have been reported to persist in the soil for up to 1300 years, apparently protected from microorganisms and imbibition by their hard coat (Baskin and Baskin 2003). Like in present study also, cream seeds have recorded higher persistence than brownish black seeds under field conditions due to presence of hard seed coat.

4.3 Experiment 3

To study germination ecology of *Medicago denticulata* seeds after herbicide exposure.

This experiment was conducted at two locations- PAU, Ludhiana and Dr. J.C. Bakhshi Regional Research Station, Abohar.

4.3.1 Visual phytotoxicity rating (0-10) of herbicides in *Triticum aestivum* and *Medicago denticulata*

4.3.1.1 *Triticum aestivum*

Different herbicides were sprayed at 4, 8 and 12 leaf stages of *M. denticulata* which corresponded to 35, 50 and 60 days after sowing of crop. The herbicide toxicity on crop stand and growth was recorded at 1, 4, 7, 14 and 28 days after herbicide application by rating scale 0-10 (where 0 = no injury and 10 = complete mortality). Post-emergence application of 2,4-D and metsulfuron plus sulfosulfuron at both 0.5X and 1X doses to 35, 50 and 60 days old crop did not show any phytotoxic effect (visual phytotoxicity rating= 0.0) during both years which indicates that both herbicides were safe to wheat. Carfentrazone at 10 and 20 g/ha did not show any visual phytotoxic symptoms in wheat crop when sprayed at 50 and 60 days after sowing. However, application of carfentrazone-ethyl at 10 and 20 g/ha to 35 days old crop during both years resulted in some minor phytotoxicity symptoms which included appearance of white necrotic spots observed at 7 days after treatment (DAT); however, injury was no longer visible at 14 DAT and wheat yield was not affected by carfentrazone-ethyl injury (Table 4.47).

Howatt (2005) reported that application of carfentrazone-ethyl at 18 g/ha caused 21% wheat injury at 3 days after treatment (DAT), but it was no longer visible 3 weeks after treatment and there was no yield loss. Wheat crop is more tolerant to 2,4-D when sprayed between the tillering stage and early stem elongation, while very early applications may cause morphological deformation, such as defective ears, curled leaves and reduced stature (Agostinetto *et al* 2016). Biswas *et al* (2016) reported no crop phytotoxicity symptoms by application of 2,4-D ethyl ester applied at four different doses - 0.225, 0.450, 0.675 and 0.900 kg a.i. ha^{-1} in wheat crop. In contrast to this, Kumar and Singh (2010) reported that 2,4-D sprayed on the wheat plant at jointing stage reduced the overall growth of the crop thereby decreasing productivity. Sulfonylureas are being widely used by the farmers because of better efficacy but these herbicides have to be sprayed with caution with respect to crops being grown, varieties and cropping system being followed. Further, double spray and more moisture in the soil at the time of spray can lead to slight yellowing in wheat crop which recovers later on and no adverse effect on grain yield have been reported. Lair and Redente (2004) reported that application of metsulfuron and chlorsulfuron caused > 70% reduction in grass weeds *viz*., *Bouteloua gracilis*, *Bouteloua curtipendula*, *Sporobolus cryptandrus* and *Panicum virgatum* L. with an increase in crop biomass up to > 40% as compared to 2,4-D and dicamba which were not effective against these weeds.

4.3.1.2 *Medicago denticulata*

M. denticulata plants exhibited variable phytotoxicity symptoms depending on the stage at the time of herbicide spray and herbicide used (Table 4.48). All the herbicides when

sprayed at four-leaf stage of *M. denticulata* resulted in phytotoxic effects. However, herbicide sprays done at either eight or twelve-leaf stage of *M. denticulata* caused no visible toxic effects. Application of carfentrazone-ethyl at 0.5X and 1X dose to four-leaf stage plants of *M. denticulata* caused white necrotic spots on leaves 4 DAT along with leaf desiccation resulting in complete mortality 7 DAT (visual phytotoxicity rating= 10). 2,4-D applied at 500 g/ha on four-leaf stage of *M. denticulata* resulted in phytotoxic effects to the level of scale 6 (moderate control) at 7 and 14 days after spraying which aggravated to scale 7 (satisfactory control) at 28 days after spraying. However, 250 g/ha 2,4-D sprayed at four-leaf stage of *M. denticulata* resulted in poor control with slight twisting of petioles only. The toxicity symptoms, like epinasty (stem twists outward and downward), curling and narrowing of leaves and chlorosis were observed with 500 g/ha of 2,4-D applied at four-leaf stage. However, symptoms were not evident when 2,4-D was applied at either eight or twelve leaf stage of *M. denticulata*. Metsulfuron plus sulfosulfuron sprayed at both 0.5X and 1X on four-leaf stage of *M. denticulata* recorded satisfactory control (7 on 10 point scale) at 4 days after spray which aggravated to scale 9 (good control) at 7 days after spray with toxicity symptoms like bleaching and malformation of leaves.

Herbicide application is one of the most widely used weed management tools. The herbicides are effective in killing weeds only when sprayed at younger stages of weeds but their late application may not result in complete killing therefore providing variable levels of control. The effectiveness of herbicides is typically assessed using visual estimations of the level of damage present, either to individual leaves or whole plants. The herbicide selectivity can be visually assessed by means of the phytotoxicity symptoms in crop plants. Visual scoring scale is helpful in cases where weed is not killed by the herbicide application and thus information is needed on how the weed responded being damaged, but not killed. Sulfonylurea herbicides are active both via leaf and soil and after absorption; they are quickly transferred to active growth areas such as meristems where they inhibit growth in sensitive plants (Felisberto *et al* 2017). Singh *et al* (2004a) reported that 2,4-D, isoproturon and metsulfuron were not able to control *M. denticulata* effectively whereas carfentrazone provided effective control against this weed. The results of present study also indicate poor efficacy of 2,4-D for controlling *M. denticulata*. In present study, visual toxicity symptoms of three herbicides *viz*., 2,4-D, carfentrazone-ethyl and metsulfuron plus sulfosulfuron applied at different stages of both *T. aestivum* and *M. denticulata* were observed. All the herbicides applied at 35, 50 or 60 days after sowing were safe for use in wheat crop except carfentrazone-ethyl which resulted in some injury to wheat plants when applied 35 days after sowing only up to 7 DAT after which injury symptoms were no longer visible. All the herbicides resulted in greater toxicity to *M. denticulata* plants when sprayed at four-leaf stage as compared to sprays done at eight and twelve-leaf stage.

Table 4.47: Visual phytotoxicity rating (0-10) of different herbicides sprayed at 35 days old *Triticum aestivum* L. during *rabi* 2016-17 and 2017-18 at PAU, Ludhiana

Treatments	2016-17					2017-18				
	1 DAT	4 DAT	7 DAT	14 DAT	28 DAT	1 DAT	4 DAT	7 DAT	14 DAT	28 DAT
Untreated Control	0	0	0	0	0	0	0	0	0	0
2,4 D sodium salt @ 250 g/ha	0	0	0	0	0	0	0	0	0	0
2,4 D sodium salt @ 500 g/ha	0	0	0	0	0	0	0	0	0	0
Carfentrazone-ethyl @ 10 g/ha	0	0	3	0	0	0	0	3	1	0
Carfentrazone-ethyl @ 20 g/ha	0	0	3	1	0	0	0	3	1	0
Metsulfuron + Sulfosulfuron @ 15 g/ha	0	0	0	0	0	0	0	0	0	0
Metsulfuron + Sulfosulfuron @ 30 g/ha	0	0	0	0	0	0	0	0	0	0

*** No crop toxicity was observed when herbicide sprays were done at 50 and 60 days old crop**

Table 4.48: Visual phytotoxicity rating (0-10) of different herbicides sprayed at four-leaf stage of *Medicago denticulata* Willd. during *rabi* 2016-17 and 2017-18 at PAU, Ludhiana

Treatments	2016-17					2017-18				
	1 DAT	4 DAT	7 DAT	14 DAT	28 DAT	1 DAT	4 DAT	7 DAT	14 DAT	28 DAT
Untreated Control	0	0	0	0	0	0	0	0	0	0
2,4 D sodium salt @ 250 g/ha	0	0	2	2	2	0	0	1	0	0
2,4 D sodium salt @ 500 g/ha	0	0	6	5	5	0	0	6	6	5
Carfentrazone-ethyl @ 10 g/ha	0	7	9	9	10	0	7	8	9	10
Carfentrazone-ethyl @ 20 g/ha	0	8	10	10	10	0	7	9	10	10
Metsulfuron + Sulfosulfuron @ 15 g/ha	0	7	8	9	10	0	7	8	8	9
Metsulfuron + Sulfosulfuron @ 30 g/ha	0	7	9	9	10	0	7	9	9	9

No phytotoxicity was observed when herbicide sprays were done at eight and twelve-leaf stages of *M. denticulata

4.3.2 Chlorophyll content index (CCI) studies

Leaf chlorophyll content is an important indicator of plant physiological state due to its role in photosynthesis. Chlorophyll meters are typically used to determine chlorophyll concentration for plant nutrient status assessment, environmental stress impact and fertilizer requirement (Magare and Deshmukh 2016). Effect of weed and crop growth stage at the time of herbicide spray and different herbicides *viz.,* 2,4-D, carfentrazone-ethyl and metsulfuron-methyl plus sulfosulfuron was studied on chlorophyll content index of *T. aestivum* and *M. denticulata*.

4.3.2.1 *Triticum aestivum*

All the herbicides were applied at 35, 50 and 60 days old wheat crop with *M. denticulata* plants at four, eight and twelve-leaf stage, respectively. Data pertaining to the chlorophyll content index (CCI) of *T. aestivum* as affected by stage of crop and weed at time of herbicide application and different herbicides is presented in Table 4.49. From 1 DAT to 70 DAT, wheat crop exhibited highest and lowest values of chlorophyll content index when herbicides were sprayed at 60 and 35 days old crop, respectively. CCI values recorded 7 DAT during both years were statistically similar to each other when herbicide spray was done at either 50 or 60 days old crop.

There was a constant increase in chlorophyll content index (CCI) from 1 DAT to 70 DAT (flag-leaf stage) in the unsprayed control as well as with 0.5X and 1X doses of 2,4-D and metsulfuron plus sulfosulfuron during both years. However, with 10 and 20 g/ha of carfentrazone-ethyl, a decreasing trend in chlorophyll content index was observed up to 7 DAT during both the years, after which CCI readings began to increase constantly in response to both 0.5X and 1X dose of carfentrazone-ethyl. From 1 DAT to 14 DAT, plants treated with 2,4-D and metsulfuron plus sulfosulfuron applied at 0.5X and 1X dose recorded CCI values statistically similar to that of untreated control. Whereas, with carfentrazone-ethyl at 10 and 20 g/ha, CCI values were significantly lower in comparison to untreated control and other herbicide treatments when observed at 1 DAT to 14 DAT. All the herbicide treatments had CCI values at par with untreated control at 28 DAT and flag leaf stage.

4.3.2.2 *Medicago denticulata*

Data presented in Tables 4.50 and 4.51 shows the interaction effect of weed growth stage at the time of herbicide spray and different herbicides on chlorophyll content index of *M. denticulata* recorded at different stages during 2016-17 and 2017-18. *M. denticulata* plants exhibited lowest CCI values when herbicide sprays were done at four-leaf stage. Whereas, plants sprayed at eight and twelve-leaf stages recorded higher chlorophyll content index due to advanced growth stage of this weed. Low chlorophyll content index values when herbicide application was done at four-leaf stage indicate higher herbicide sensitivity of younger growth stages of weed.

Table 4.49: Effect of crop and weed growth stage at the time of herbicide spray and different herbicides on chlorophyll content index (CCI) of *Triticum aestivum* L. during *rabi* 2016-17 and 2017-18 at PAU, Ludhiana

Treatments	2016-17						2017-18					
	1 DAT	4 DAT	7 DAT	14 DAT	28 DAT	Flag leaf stage	1 DAT	4 DAT	7 DAT	14 DAT	28 DAT	Flag leaf stage
Weed growth stage at time of herbicide spray												
4 leaf stage (35 days)	22.4	21.2	20.4	30.0	40.2	50.2	20.4	19.5	18.0	27.5	38.4	48.3
8 leaf stage (50 days)	30.3	33.4	37.6	41.3	47.5	50.4	28.2	31.7	34.3	38.7	45.6	48.6
12 leaf stage (60 days)	32.2	35.8	38.0	41.5	48.5	51.2	31.2	34.6	36.4	40.6	46.2	49.2
SEm±	0.33	0.70	0.52	0.48	0.68	0.82	0.38	0.34	0.80	0.70	0.56	0.74
LSD (p=0.05)	1.27	1.93	1.58	1.50	1.85	NS	1.22	1.30	2.14	1.87	2.02	NS
Herbicide treatment												
Untreated Control	28.3	30.4	33.5	37.4	44.2	48.5	26.4	28.4	31.2	35.4	42.3	47.1
2,4 D sodium salt @ 250 g/ha	28.5	31.5	34.4	38.6	45.8	50.4	26.8	29.7	32.4	36.8	43.8	48.6
2,4 D sodium salt @ 500 g/ha	28.8	31.8	34.6	39.2	46.0	51.2	27.2	30.3	32.8	37.2	44.4	49.0
Carfentrazone-ethyl @ 10 g/ha	27.7	26.3	25.7	34.8	44.6	48.8	26.2	25.3	24.6	32.3	42.8	48.8
Carfentrazone-ethyl @ 20 g/ha	27.4	26.0	25.2	34.5	44.3	48.6	26.0	25.0	24.2	32.0	42.5	48.6
Metsulfuron + Sulfosulfuron @ 15 g/ha	28.6	32.3	35.1	39.3	46.5	51.3	26.7	30.6	33.3	37.7	44.3	49.5
Metsulfuron + Sulfosulfuron @ 30 g/ha	28.8	32.7	35.8	39.6	46.7	51.5	27.0	30.8	33.8	38.0	44.7	49.7
SEm±	0.22	0.67	0.60	0.56	0.88	0.90	0.54	0.90	0.85	0.90	0.78	0.82
LSD (p=0.05)	0.83	2.42	2.37	2.30	2.6	3.1	1.67	2.65	3.02	2.7	2.5	2.6
Interaction LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Values given in parentheses are crop growth stages at the time of herbicide spray.

Table 4.50: Interaction effect of weed growth stage at the time of herbicide spray and different herbicide treatments on chlorophyll content index (CCI) of *Medicago denticulata* Willd. at 7 and 14 DAT during *rabi* 2016-17 and 2017-18 at PAU, Ludhiana

Treatments	Weed growth stage at the time of herbicide spray					
	CCI (7 DAT)					
	2016-17			2017-18		
Herbicide treatment	4 leaf	8 leaf	12 leaf	4 leaf	8 leaf	12 leaf
Untreated control	4.0	10.5	11.0	4.4	10.8	11.4
2,4 D sodium salt 250 g/ha	3.8	10.4	10.8	4.0	10.6	11.2
2,4 D sodium salt 500 g/ha	3.5	10.2	10.6	3.8	10.5	11.1
Carfentrazone-ethyl 10 g/ha	2.0	9.8	10.4	2.4	10.3	10.8
Carfentrazone-ethyl 20 g/ha	1.8	9.6	10.3	2.0	10.0	10.6
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	3.2	10.2	10.4	3.6	10.4	10.8
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	3.0	10.0	10.2	3.3	10.1	10.5
Interaction LSD (p=0.05)	0.30			0.35		
CCI (14 DAT)						
Untreated control	5.0	11.2	12.3	5.7	11.6	12.5
2,4 D sodium salt 250 g/ha	4.0	10.8	11.8	5.3	11.3	12.2
2,4 D sodium salt 500 g/ha	3.8	10.6	11.6	5.0	11.0	12.0
Carfentrazone-ethyl 10 g/ha	0.0*	10.4	11.4	0.0*	10.8	11.7
Carfentrazone-ethyl 20 g/ha	0.0*	10.2	11.2	0.0*	10.5	11.6
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	2.6	10.5	11.5	3.3	10.7	11.8
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	2.3	10.3	11.3	3.0	10.5	11.6
Interaction LSD (p=0.05)	0.18			0.25		

*** No CCI values recorded due to complete mortality of plants.**

Table 4.51: Interaction effect of weed growth stage at the time of herbicide spray and different herbicide treatments on chlorophyll content index (CCI) of *Medicago denticulata* Willd. at 21 days after treatment (DAT) and at flowering stage during *rabi* 2016-17 and 2017-18 at PAU, Ludhiana

Treatments	Weed growth stage at the time of herbicide spray					
	CCI (21 DAT)					
	2016-17			2017-18		
Herbicide treatment	4 leaf	8 leaf	12 leaf	4 leaf	8 leaf	12 leaf
Untreated control	5.6	11.8	12.7	6.0	12.3	13.0
2,4 D sodium salt 250 g/ha	5.3	11.6	12.6	4.8	12.3	12.8
2,4 D sodium salt 500 g/ha	5.0	11.6	12.5	4.4	12.1	12.7
Carfentrazone-ethyl 10 g/ha	0.0*	11.4	12.6	0.0*	12.0	12.8
Carfentrazone-ethyl 20 g/ha	0.0*	11.3	12.4	0.0*	12.0	12.7
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	1.8	11.7	12.5	2.6	12.2	13.0
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	1.5	11.5	12.4	2.2	12.1	12.9
Interaction LSD (p=0.05)	0.34			0.41		
CCI (Flowering stage)						
Untreated control	7.4	12.4	13.3	7.7	12.8	13.6
2,4 D sodium salt 250 g/ha	7.2	12.3	13.3	7.6	12.6	13.5
2,4 D sodium salt 500 g/ha	7.0	12.2	13.2	7.3	12.6	13.6
Carfentrazone-ethyl 10 g/ha	0.0*	12.2	13.0	0.0*	12.7	13.4
Carfentrazone-ethyl 20 g/ha	0.0*	12.1	13.0	0.0*	12.5	13.2
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	1.3	12.3	13.2	1.4	12.7	13.5
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	0.9	12.2	13.2	1.0	12.6	13.5
Interaction LSD (p=0.05)	0.27			0.36		

*** No CCI values recorded due to complete mortality of plants**

At 7 DAT, carfentrazone-ethyl applied at 20 g/ha to twelve-leaf stage of *M. denticulata* did not cause any decline in CCI and plants recorded > 5 fold increase in CCI during both years as compared to CCI recorded at four-leaf stage. Likewise, 30 g/ha of metsulfuron plus sulfosulfuron applied at twelve-leaf stage of *M. denticulata* was ineffective in decreasing CCI and plants exhibited > 3 fold increase in CCI than recorded at four-leaf stage at 7 DAT.

Chlorophylls are the essential photosynthetic pigments in plants and the amount of chlorophyll per unit leaf area indicates the overall condition of plants (Silla *et al* 2010). There is a direct relation between chlorophyll content and light transformation in photosynthesis. Determination of chlorophyll content can be useful to identify and study photosynthetic activity, stress conditions, nutritional status and physiological changes in crops and weeds over time. The decrease in chlorophyll content due to herbicide application may be due to an increase of chlorophyll degradation or by reduction in chlorophyll synthesis (Santos 2004). It has also been reported that herbicide stress may induce reduction in the number of chloroplasts (Cakmak *et al* 2009). In present study also, lower CCI values exhibited by wheat crop when herbicides were sprayed at 35 days after sowing (DAS) indicate stress imposed by herbicide application at earlier crop growth stages. All the herbicide treatments resulted in an increase in chlorophyll content index at all stages of observation except, carfentrazone-ethyl which declined the CCI values up to 7 DAT, after which the effect of carfentrazone was subsided without causing any adverse effects on crop yield.

The reduction in chlorophyll content can be a good indicator for monitoring the damage to the plants growth and development (Song *et al* 2007). Wang and Zhou (2006) reported that chlorimuron-ethyl, a sulfonylurea herbicide when applied at 300 µg/kg decreased the chlorophyll content of *T. aestivum*. Unlike this, in present study application of metsulfuron plus sulfosulfuron (also a sulfonylurea herbicide) recorded no decrease in chlorophyll content index of *T. aestivum* at all stages of observation. Sulfonylurea herbicides inhibit the activity of acetolactate synthase (ALS) enzyme which is the first enzyme involved in chloroplastidic biosynthesis of essential branched-chain amino acids (valine, leucine and isoleucine). They are easily absorbed by both roots and foliage and translocated rapidly in xylem and phloem to the site of action at growing points (Yu *et al* 2017) causing death of meristematic cells and consequently, plant becomes dead. Riethmuller-Haage *et al* (2006) reported that application of 16 g/ha metsulfuron-methyl at three-leaf stage of *Solanum nigrum* caused reduction in leaf chlorophyll content. In present study also, 15 and 30 g/ha of metsulfuron-methyl plus sulfosulfuron applied at four-leaf stage of *M. denticulata* decreased the chlorophyll content. Application of 2,4-D results in uncontrolled growth of plants, including curling and cupping of leaves, brittleness, twisting of stem (epinasty) and petioles, bending of meristem, change in shape of leaves, swelling of basal part of stem and general

abnormal growth (Grossmann 2010). 2,4-D mainly kills plants in three ways: changing the plasticity of cell walls, altering the amount of protein production, and increasing the production of ethylene. When applied to dicotyledonous weeds at high doses, 2,4-D is absorbed through roots, stems and leaves and is translocated to the meristems of the plant (Goggin *et al* 2016). Uncontrolled, unsustainable growth ensues, resulting in stem curl-over, leaf withering, and eventually plant death (Song 2014). 2,4-D application in *Hibiscus cannabinus* Linn at seedling stage resulted in change of colour from green to yellow which was found to be due to inhibition of chlorophyll development by herbicide. It was concluded that the inhibitory action of 2,4-D may be due to its phytotoxicity, interfering the metabolic pathway which may help in effective growth and development (Kamble 2006). El-Gehit (2016) reported that 2,4-D applied two-weeks after sowing at 500 ppm concentration caused decline in chlorophyll content of dicotyledonous weeds *viz*., *Amaranthus viridis*, *Chenopodium album* and *Xanthium strumarium*.

Carfentrazone-ethyl is a diphenyl ether herbicide which is readily absorbed by foliage but has limited translocation. The herbicidal action on susceptible plants involves inhibition of enzyme protoporphyrinogen oxidase (PROTOX) which is involved in chlorophyll biosynthesis pathway. Inhibition of protoporphyrinogen oxidase results in abnormal buildup of chlorophyll precursors which are extremely light sensitive. Upon exposure to light, these unstable precursors undergo photoreactions with the formation of free radicals causing lipid peroxidation and membrane disruption. Initial symptoms appear as quickly as one day after treatment and plant mortality generally occurs within seven days of application. In present study also, carfentrazone-ethyl at 10 and 20 g/ha resulted in complete killing of *M. denticulata* within 7 days when sprayed at four-leaf stage; whereas no phytotoxicity was observed when herbicide sprays were done at eight or twelve leaf stages of this weed.

4.3.3 Chlorophyll fluorescence (Fv/Fm) studies

Chlorophyll fluorescence (Fv/Fm) (variable fluorescence/maximum fluorescence) is a non-invasive and quick tool to detect and study the effects of environmental stress on plants at subcellular and plant canopy level (Murchie and Lawson 2013). Chlorophyll fluorescence can be used for an early detection of stress before a visual damage appears as well as for tracking the plant defense reactions (Nedbal and Whitmarsh 2004). Effect of weed growth stage at time of herbicide application and different herbicides was studied on chlorophyll fluorescence of *T. aestivum* and *M. denticulata* at different intervals.

4.3.3.1 *Triticum aestivum*

The data presented in Table 4.52 depicted the variation in chlorophyll fluorescence (Fv/Fm) of *T. aestivum* as influenced by crop and weed growth stage at the time of herbicide spray and different herbicides. The highest and lowest values of F_v/F_m were recorded when herbicide sprays were done at 60 and 35 days old crop, respectively. Herbicide application at

35 days old crop resulted in constant decline in F_v/F_m values from 1 DAT to 7 DAT during both cropping seasons followed by progressive increase in F_v/F_m from 14 DAT to 70 DAT. Among different herbicide treatments, carfentrazone-ethyl at 10 and 20 g/ha recorded significant decline in F_v/F_m values from 4 DAT to 7 DAT as compared to untreated control and other herbicides after which constant increase in F_v/F_m values was observed from 14 DAT to 70 DAT. Other herbicide treatments recorded F_v/F_m statistically similar to untreated control with progressive increase in F_v/F_m during all days of observation. F_v/F_m recorded at flag leaf stage was statistically similar in all the herbicide treatments and control irrespective of growth stage and herbicide applied.

4.3.3.2 *Medicago denticulata*

Chlorophyll fluorescence of *M. denticulata* was significantly influenced by both growth stage of *M. denticulata* at the time of herbicide spray and different herbicide treatments at all dates of observations. The interaction effect of weed growth stage at the time of herbicide spray and different herbicides was found significant on chlorophyll fluorescence of *M. denticulata* and data is presented in Table 4.53 and 4.54. During both years of study, highest and lowest chlorophyll fluorescence at all stages of observation was recorded when herbicides were applied at twelve and four-leaf stages, respectively. There was significant increase in F_v/F_m values with delay in herbicide application from four to eight-leaf stage. *M. denticulata* plants treated with different herbicides at four-leaf stage recorded least Fv/Fm values at all dates of observations. Whereas, maximum chlorophyll fluorescence was recorded in plants treated with herbicides at eight and twelve-leaf stage. At 7 DAT, Fv/Fm values recorded in metsulfuron-methyl plus sulfosulfuron and 2,4-D treated plants at eight and twelve-leaf stage remained at par with each other during both the years. Plants treated with carfentrazone at four-leaf stage recorded least Fv/Fm values as compared to Fv/Fm values recorded in plants treated at eight and twelve-leaf stages during all dates of observation.

Chlorophyll fluorescence (Fv/Fm) provides a measure of PSII photochemical efficiency and reflects the potential photochemical capacity of PSII. High values of Fv/Fm indicate high light transformation rate, providing more energy for CO_2 assimilation in dark reaction of photosynthesis. The herbicide application may block synthesis/cause degradation of photosynthesis related intermediate metabolites and affect fluorescence emission (Varshney *et al* 2015). A lower value of Fv/Fm indicates that a proportion of PSII reaction centers are damaged, a phenomenon called photoinhibition, often observed in plants under stress conditions (Hess 2000, Hiraki *et al* 2003). Yordanova *et al* (2001) reported that the Fv/Fm values were significantly reduced with the increase in fenoxaprop-p-ethyl concentration from 1.33 to 7.98 ml L^{-1} indicating that PSII was impaired in leaves of *Perilla frutescens* due to herbicide stress and the actual quantum yield of photosynthetic reaction centers was lowered.

Table 4.52: Effect of crop and weed growth stage at the time of herbicide spray and different herbicides on chlorophyll fluorescence (Fv/Fm) of *Triticum aestivum* L. during *rabi* 2016-17 and 2017-18 at PAU, Ludhiana

Treatments	2016-17						2017-18					
	1 DAT	4 DAT	7 DAT	14 DAT	28 DAT	Flag leaf stage	1 DAT	4 DAT	7 DAT	14 DAT	28 DAT	Flag leaf stage
Weed growth stage at time of herbicide spray												
4 leaf stage (35 days)	0.727	0.720	0.716	0.746	0.753	0.760	0.724	0.718	0.711	0.742	0.750	0.756
8 leaf stage (50 days)	0.740	0.740	0.743	0.750	0.757	0.770	0.738	0.740	0.742	0.748	0.754	0.767
12 leaf stage (60 days)	0.750	0.752	0.755	0.760	0.764	0.774	0.746	0.750	0.752	0.757	0.761	0.770
SEm±	0.008	0.005	0.010	0.009	0.011	0.007	0.008	0.005	0.004	0.010	0.002	0.006
LSD (p=0.05)	0.025	0.013	0.026	0.022	0.031	NS	0.020	0.016	0.014	0.021	0.011	NS
Herbicide treatment												
Untreated Control	0.737	0.739	0.744	0.748	0.755	0.766	0.734	0.737	0.740	0.745	0.752	0.762
2,4 D sodium salt @ 250 g/ha	0.740	0.743	0.750	0.758	0.763	0.770	0.738	0.740	0.747	0.755	0.760	0.767
2,4 D sodium salt @ 500 g/ha	0.740	0.742	0.746	0.755	0.760	0.768	0.737	0.739	0.743	0.752	0.757	0.764
Carfentrazone-ethyl @ 10 g/ha	0.738	0.722	0.720	0.746	0.753	0.766	0.736	0.720	0.717	0.742	0.750	0.762
Carfentrazone-ethyl @ 20 g/ha	0.736	0.720	0.710	0.742	0.750	0.765	0.732	0.717	0.708	0.738	0.748	0.760
Metsulfuron + Sulfosulfuron @ 15 g/ha	0.743	0.747	0.754	0.760	0.766	0.773	0.739	0.742	0.750	0.757	0.762	0.770
Metsulfuron + Sulfosulfuron @ 30 g/ha	0.742	0.745	0.748	0.758	0.763	0.770	0.738	0.743	0.746	0.754	0.760	0.766
SEm±	0.002	0.003	0.008	0.007	0.006	0.004	0.005	0.003	0.004	0.011	0.009	0.007
LSD (p=0.05)	0.010	0.014	0.022	0.021	0.017	NS	0.016	0.010	0.015	0.032	0.024	NS
Interaction LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Values given in parentheses are crop growth stages at the time of herbicide spray

Table 4.53: Interaction effect of weed growth stage at the time of herbicide spray and different herbicide treatments on chlorophyll fluorescence (Fv/Fm) of *Medicago denticulata* Willd. at 7 and 14 DAT during *rabi* 2016-17 and 2017-18 at PAU, Ludhiana

Treatments	Weed growth stage at the time of herbicide spray					
	Chlorophyll fluorescence (7 DAT)					
	2016-17			2017-18		
Herbicide treatment	4 leaf	8 leaf	12 leaf	4 leaf	8 leaf	12 leaf
Untreated control	0.678	0.730	0.740	0.682	0.735	0.742
2,4 D sodium salt 250 g/ha	0.675	0.728	0.739	0.678	0.732	0.742
2,4 D sodium salt 500 g/ha	0.660	0.727	0.739	0.664	0.730	0.741
Carfentrazone-ethyl 10 g/ha	0.456	0.724	0.737	0.604	0.729	0.741
Carfentrazone-ethyl 20 g/ha	0.440	0.722	0.735	0.596	0.727	0.739
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	0.592	0.726	0.738	0.625	0.733	0.740
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	0.580	0.724	0.736	0.620	0.730	0.740
Interaction LSD (p=0.05)	0.012			0.013		
Chlorophyll fluorescence (14 DAT)						
Untreated control	0.680	0.733	0.746	0.683	0.738	0.748
2,4 D sodium salt 250 g/ha	0.677	0.732	0.744	0.680	0.737	0.746
2,4 D sodium salt 500 g/ha	0.650	0.730	0.743	0.653	0.735	0.746
Carfentrazone-ethyl 10 g/ha	0.00*	0.730	0.745	0.594	0.734	0.744
Carfentrazone-ethyl 20 g/ha	0.00*	0.728	0.744	0.589	0.733	0.742
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	0.450	0.730	0.745	0.616	0.733	0.743
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	0.442	0.728	0.743	0.607	0.731	0.741
Interaction LSD (p=0.05)	0.014			0.011		

*** No Fv/Fm values recorded due to complete mortality of plants**

Table 4.54: Interaction effect of weed growth stage at the time of herbicide spray and different herbicide treatments on chlorophyll fluorescence (Fv/Fm) of *Medicago denticulata* Willd. at 21 DAT and at flowering stage during *rabi* 2016-17 and 2017-18 at PAU, Ludhiana

Treatments	Weed growth stage at the time of herbicide spray					
	Chlorophyll fluorescence (21 DAT)					
	2016-17			2017-18		
Herbicide treatment	4 leaf	8 leaf	12 leaf	4 leaf	8 leaf	12 leaf
Untreated control	0.687	0.740	0.750	0.690	0.744	0.753
2,4 D sodium salt 250 g/ha	0.685	0.738	0.748	0.688	0.743	0.754
2,4 D sodium salt 500 g/ha	0.685	0.736	0.749	0.686	0.744	0.752
Carfentrazone-ethyl 10 g/ha	0.00*	0.735	0.749	0.00*	0.742	0.753
Carfentrazone-ethyl 20 g/ha	0.00*	0.736	0.747	0.00*	0.740	0.752
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	0.383	0.738	0.750	0.400	0.743	0.754
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	0.372	0.735	0.748	0.386	0.700	0.751
Interaction LSD (p=0.05)	0.013			0.013		
Chlorophyll fluorescence (Flowering stage)						
Untreated control	0.700	0.748	0.755	0.706	0.750	0.760
2,4 D sodium salt 250 g/ha	0.698	0.746	0.754	0.702	0.751	0.760
2,4 D sodium salt 500 g/ha	0.696	0.747	0.752	0.700	0.750	0.758
Carfentrazone-ethyl 10 g/ha	0.00*	0.748	0.753	0.00*	0.748	0.757
Carfentrazone-ethyl 20 g/ha	0.00*	0.748	0.755	0.00*	0.747	0.757
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	0.352	0.746	0.753	0.360	0.746	0.755
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	0.343	0.745	0.754	0.350	0.746	0.754
Interaction LSD (p=0.05)	0.012			0.011		

*** No Fv/Fm values recorded due to complete mortality of plants**

In present study, reduction in F_v/F_m with herbicide application at 35 days old crop may be due to more sensitivity of the physiological processes towards herbicide stress at younger stage of plant growth, as indicated by visual injury caused due to carfentrazone-ethyl at earlier growth stages of wheat. At flag leaf stage, statistically similar values of chlorophyll fluorescence in control and herbicide treated wheat plants indicates absence of any stress.

4.3.4 Yield attributes of wheat

Yield attributes are the most important parameters directly influencing the ultimate yield of a crop. Among the yield components number of grains per spike is essential parameter for assessment of the impact of weed control treatments on yield. Increasing the number of grains per spike will increase the weight of the spike which in turn definitely improves the final yield (Hussain *et al* 2013). Data represented in Table 4.55 and 4.57 showed that these parameters differ significantly when herbicides were sprayed at different growth stages of *M. denticulata* during both years. Wheat plants produced longer spikes with more number of grains having higher 1000 grain weight when herbicides were sprayed at four-leaf stage of *M. denticulata*, while minimum values of these parameters were recorded in plants in which herbicide sprays were done at eight and twelve-leaf stage.

The herbicide treatments showed significant differences in yield attributes in both the cropping seasons. Longer spikes with highest number of grains and 1000-grain weight were produced with carfentrazone-ethyl at 20 g/ha followed by carfentrazone-ethyl at 10 g/ha and 30 g/ha of metsulfuron-methyl plus sulfosulfuron during both years. Other than 250 g/ha of 2,4-D, all the herbicide treatments caused significant increase in yield attributes of wheat over untreated control.

4.3.5 Wheat yield

Grain yield being the economic component of the crop, reflects the resultant impact of all crop growth parameters and yield attributes that are affected by various treatments. It is evident from results that the grain and straw yield of wheat during both years was significantly affected by stage of *M. denticulata* at the time of herbicide spray (Table 4.56 and 4.58). Highest and lowest grain and straw yield of wheat were recorded when herbicides were sprayed at four and twelve-leaf stages of *M. denticulata,* respectively. There was significant decline in grain and straw yield when herbicides were sprayed at advanced growth stages of *M. denticulata*. Herbicide sprays at twelve-leaf stage during both years caused > 7 and 4% reduction in grain and straw yield respectively, than sprays done at four-leaf stage.

As compared to weedy control, all the herbicides during both years resulted in significant improvement in wheat yield except 2,4-D at 250 g/ha (Table 4.56 and 4.58). Highest grain and straw yield during both cropping seasons was recorded in plots treated with 20 g/ha of carfentrazone-ethyl which remained at par with other herbicide treatments *viz*., carfentrazone-ethyl 10 g/ha, metsulfuron-methyl plus sulfosulfuron at 15 and 30 g/ha and 2,4-

D at 500 g/ha but significantly superior to 2,4-D at 250 g/ha and unweeded control. Biological yield was affected by the treatments in the similar manner as that of grain and straw yield, with the highest biological yield being produced by 20 g/ha of carfentrazone during both years. The interaction effect of weed growth stage at the time of herbicide spray and different herbicides on yield and yield attributing parameters of wheat was not significant. Similar results were reported at Regional Research Station, Abohar.

The results of present study revealed that carfentrazone-ethyl and metsulfuron methyl plus sulfosulfuron at both 0.5X and 1X dose were equally effective in increasing yield and yield attributes of wheat over untreated control. However, 0.5X dose of 2,4-D was ineffective in improving productivity of wheat. Similar to present study, Punia *et al* (2005) recorded maximum grain yield under carfentrazone-ethyl at 25 g/ha but was at par with carfentrazone-ethyl at 15 and 20 g/ha and significantly higher than 2,4-D. Kaur *et al* (2015) tested the efficacy of Markpower @ 0.03 kg a.i./ha (new brand of sulfosulfuron+metsulfuron) against Total @ 0.03 kg a.i./ha (standard check) in wheat and reported that both herbicides recorded significantly higher grain yield than untreated control but remained at par to each other.

Pal *et al* (2016) studied the effect of different herbicides on productivity of wheat and reported that application of metsulfuron-methyl plus sulfosulfuron at 25 g/ha resulted in maximum grain yield over untreated control. Jat *et al* (2003) found that application of 2,4-D at 400 g/ha recorded significantly higher yield attributes (grains/ear and test weight) and grain yield, compared to weedy check but remained at par with tank mix application of metsulfuron-methyl + isoproturon (4 + 750 g/ha). Singh *et al* (2004b) observed that the grain yield of wheat recorded with the application of carfentrazone-ethyl at 15 and 25 g/ha was similar to that of weed free treatments. Brar *et al* (2005) found that carfentrazone-ethyl at 25 g/ha effectively controlled dicotyledonous weeds including *Medicago denticulata*, *Malva parviflora* and *Coronopus didymus* in wheat and produced significantly higher grain yield than 2,4-D at 500 g/ha and unweeded control. However, in present study, 2,4-D at 500 g/ha recorded statistically similar grain yield as that of carfentrazone-ethyl. Patel *et al* (2005) reported that grain yield recorded with carfentrazone at 30 g/ha was similar to weed free treatment but reported significantly less wheat yield when carfentrazone ethyl was sprayed at 15 and 20 g/ha.

Table 4.55: Effect of weed and crop growth stage at the time of herbicide spray and different herbicides on yield attributing parameters of *Triticum aestivum* L. during *rabi* 2016-17 and 2017-18 at PAU, Ludhiana

Treatment	2016-17				2017-18			
	Spike length (cm)	Number of grains per spike (g)	Grain weight per spike (g)	1000-grain weight (g)	Spike length (cm)	Number of grains per spike (g)	Grain weight per spike (g)	1000-grain weight (g)
Weed growth stage at the time of herbicide spray								
4 leaf stage (35 days)	11.8	55	2.62	44.30	11.6	53	2.54	44.05
8 leaf stage (50 days)	11.6	51	2.20	41.30	11.3	50	2.06	41.11
12 leaf stage (60 days)	11.4	50	2.04	40.54	11.2	48	1.91	40.34
SEm±	0.05	0.58	0.03	0.31	0.04	0.30	0.02	0.14
LSD (p=0.05)	0.21	2.27	0.14	1.23	0.14	1.18	0.08	0.54
Herbicide treatment								
Untreated control	11.3	49	2.02	41.10	10.9	47	1.84	40.94
2,4 D sodium salt @ 250 g/ha	11.4	50	2.20	41.30	11.1	49	1.91	41.12
2,4 D sodium salt @ 500 g/ha	11.5	52	2.30	42.29	11.2	50	2.13	42.24
Carfentrazone-ethyl @ 10 g/ha	11.7	54	2.37	43.00	11.5	52	2.32	42.72
Carfentrazone-ethyl @ 20 g/ha	11.9	55	2.44	43.00	11.6	54	2.43	42.80
Metsulfuron + Sulfosulfuron @ 15 g/ha	11.6	52	2.32	42.60	11.3	50	2.28	42.26
Metsulfuron + Sulfosulfuron @ 30 g/ha	11.7	53	2.35	42.75	11.3	51	2.31	42.27
SEm±	0.06	0.72	0.04	0.41	0.07	0.67	0.05	0.44
LSD (p=0.05)	0.21	2.06	0.11	1.17	0.20	1.92	0.08	1.28
Interaction LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS

Values given in parentheses are crop growth stages at the time of herbicide spray.

Table 4.56: Effect of weed and crop growth stage at the time of herbicide spray and different herbicides on yield of *Triticum aestivum* L. during *rabi* 2016-17 and 2017-18 at PAU, Ludhiana

Treatment	2016-17			2017-18		
	Biological yield (q/ha)	Grain yield (q/ha)	Straw yield (q/ha)	Biological yield (q/ha)	Grain yield (q/ha)	Straw yield (q/ha)
Weed growth stage at the time of herbicide spray						
4 leaf stage (35 days)	135.58	56.48	79.10	128.87	53.06	75.80
8 leaf stage (50 days)	128.26	52.48	75.78	122.80	50.10	72.71
12 leaf stage (60 days)	124.62	50.85	72.78	121.35	49.00	72.34
SEm±	0.84	0.33	0.46	0.41	0.22	0.21
LSD (p=0.05)	3.32	1.29	1.79	1.59	0.88	0.84
Herbicide treatment						
Untreated control	126.94	52.16	74.78	121.34	49.26	72.08
2,4 D sodium salt @ 250 g/ha	127.50	52.53	74.97	122.19	49.75	72.44
2,4 D sodium salt @ 500 g/ha	129.43	53.33	76.10	124.78	51.02	73.76
Carfentrazone-ethyl @ 10 g/ha	130.24	53.90	76.34	125.92	51.55	74.37
Carfentrazone-ethyl @ 20 g/ha	131.29	54.10	77.19	126.86	52.06	74.80
Metsulfuron + Sulfosulfuron @ 15 g/ha	129.58	53.35	76.23	124.92	51.05	73.87
Metsulfuron + Sulfosulfuron @ 30 g/ha	130.18	53.88	76.30	125.22	51.15	74.07
SEm±	0.83	0.30	0.51	0.71	0.44	0.38
LSD (p=0.05)	2.37	1.16	1.48	2.09	1.26	1.09
Interaction LSD (p=0.05)	NS	NS	NS	NS	NS	NS

Values given in parantheses are crop growth stages at the time of herbicide spray.

Table 4.57: Effect of weed and crop growth stage at the time of herbicide spray and different herbicides on yield attributing parameters of *Triticum aestivum* L. during *rabi* 2016-17 and 2017-18 at Regional Research Station, Abohar

Treatment	2016-17				2017-18			
	Spike length (cm)	Number of grains per spike (g)	Grain weight per spike (g)	1000-grain weight (g)	Spike length (cm)	Number of grains per spike (g)	Grain weight per spike (g)	1000-grain weight (g)
Weed growth stage at the time of herbicide spray								
4 leaf stage (35 days)	11.7	54	2.48	42.10	11.3	51	2.36	40.34
8 leaf stage (50 days)	11.4	50	2.20	40.11	11.0	49	2.10	38.12
12 leaf stage (60 days)	11.3	49	2.10	37.24	10.9	47	1.90	36.53
SEm±	0.06	0.46	0.05	0.33	0.07	0.42	0.04	0.24
LSD (p=0.05)	0.23	2.20	0.20	1.34	0.26	1.90	0.16	1.08
Herbicide treatment								
Untreated control	11.3	47	1.94	38.55	10.8	45	1.80	37.04
2,4 D sodium salt @ 250 g/ha	11.4	48	2.00	38.80	10.9	46	1.84	38.13
2,4 D sodium salt @ 500 g/ha	11.5	50	2.25	39.78	11.1	49	2.18	38.54
Carfentrazone-ethyl @ 10 g/ha	11.7	52	2.32	40.24	11.4	51	2.25	38.78
Carfentrazone-ethyl @ 20 g/ha	11.8	56	2.40	40.56	11.5	54	2.33	39.08
Metsulfuron + Sulfosulfuron @ 15 g/ha	11.5	53	2.30	40.18	11.4	50	2.24	38.56
Metsulfuron + Sulfosulfuron @ 30 g/ha	11.6	54	2.34	40.20	11.5	52	2.30	38.63
SEm±	0.08	0.80	0.03	0.32	0.09	0.72	0.04	0.37
LSD (p=0.05)	0.24	2.15	0.08	1.05	0.22	2.08	0.06	1.05
Interaction LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS

Values given in parentheses are crop growth stages at the time of herbicide spray.

Table 4.58: Effect of weed and crop growth stage at the time of herbicide spray and different herbicides on yield of *Triticum aestivum* L. during *rabi* 2016-17 and 2017-18 at Regional Research Station, Abohar

Treatment	2016-17			2017-18		
	Biological yield (q/ha)	Grain yield (q/ha)	Straw yield (q/ha)	Biological yield (q/ha)	Grain yield (q/ha)	Straw yield (q/ha)
Weed growth stage at the time of herbicide spray						
4 leaf stage (35 days)	129.51	54.23	75.28	124.49	52.12	72.37
8 leaf stage (50 days)	123.47	50.05	73.42	118.66	48.30	70.36
12 leaf stage (60 days)	119.05	48.72	70.33	118.26	48.08	70.18
SEm±	0.52	0.35	0.53	0.32	0.27	0.28
LSD (p=0.05)	3.05	1.33	1.90	1.40	1.05	1.10
Herbicide treatment						
Untreated control	119.71	49.08	70.63	115.66	47.22	68.44
2,4 D sodium salt @ 250 g/ha	121.25	50.13	71.12	117.55	48.30	69.25
2,4 D sodium salt @ 500 g/ha	124.41	51.18	73.23	121.03	49.58	71.45
Carfentrazone-ethyl @ 10 g/ha	125.48	51.34	74.14	122.65	50.55	72.10
Carfentrazone-ethyl @ 20 g/ha	126.48	52.18	74.30	123.06	50.74	72.32
Metsulfuron + Sulfosulfuron @ 15 g/ha	124.78	51.11	73.67	121.34	49.88	71.46
Metsulfuron + Sulfosulfuron @ 30 g/ha	126.07	52.03	74.04	122.01	50.23	71.78
SEm±	0.92	0.24	0.60	0.78	0.40	0.44
LSD (p=0.05)	2.34	1.08	1.53	2.20	1.20	1.25
Interaction LSD (p=0.05)	NS	NS	NS	NS	NS	NS

Values given in parentheses are crop growth stages at the time of herbicide spray.

4.3.6 Weed density, biomass and weed control efficiency

Weed density per unit area is an important and key parameter in figuring out the impact of treatments on weed growth. The more the weeds the more is the nutrients depletion from the soil and the more is their competition with crop plants. Data pertaining to effect of weed growth stage at the time of herbicide spray and different herbicides on *M. denticulata* density, biomass and weed control efficiency during 2016-17 and 2017-18 is presented in Table 4.59 and 4.61. Weed density and biomass of *M. denticulata* varied significantly under different growth stages during both years. Minimum and maximum density and biomass were observed when herbicides were sprayed at four and twelve-leaf stages, respectively. There was significant increase in weed density and biomass as the herbicides were sprayed at advanced growth stages of *M. denticulata*. At eight leaf stage, population of *M. denticulata* per plot during 2016-17 and 2017-18 was increased by 88.9 and 90.9% as compared to number of plants per plot at four-leaf stage, respectively. However, increase in density of *M. denticulata* per plot during 2016-17 and 2017-18 was only < 10 and 6% in plots sprayed at twelve-leaf stage as compared to eight-leaf stage. There was > 6 fold increase in weed biomass when herbicides were sprayed at twelve-leaf stage than sprays done at four-leaf stage of *M. denticulata* during both years. Highest and lowest weed control efficiency during both years was recorded when herbicides were sprayed at four and twelve-leaf stages, respectively. Application of herbicides at four-leaf stage recorded > 70% weed control efficiency which was declined to < 5% when herbicides were sprayed at twelve-leaf stage during both the years. Results recorded at Regional Research Station, Abohar followed similar trend.

The interaction effect of weed growth stage at the time of herbicide spray and different herbicides on weed density, biomass and weed control efficiency during both the years was found significant and data is presented in Table 4.60 and 4.62. Delayed application of all the herbicides at eight leaf stage resulted in significant increase in number of surviving plants of *M. denticulata* with concomitant increase in weed biomass than herbicide sprays done at four-leaf stage during both years. Carfentrazone-ethyl application at 10 and 20 g/ha to four-leaf stage of *M. denticulata* resulted in complete mortality with minimum biomass and > 95% weed control efficiency, whereas its delayed application at eight and twelve-leaf stages resulted in significant increase in number of *M. denticulata* plants with increased biomass leading to reduced efficiency. Similarly, metsulfuron-methyl plus sulfosulfuron was also effective in reducing weed density and biomass of *M. denticulata* when applied at four-leaf stage only. Density of *M. denticulata* was statistically similar in response to 2,4-D application at either eight or twelve-leaf stage. Whereas, carfentrazone-ethyl and metsulfuron plus sulfosulfuron sprayed at twelve-leaf stage recorded significant increase in number of surviving plants of *M. denticulata* as compared to sprays done at eight-leaf stage.

Table 4.59: Effect of weed growth stage at the time of herbicide spray and different herbicides on *Medicago denticulata* Willd. density, biomass and weed control efficiency during *rabi* 2016-17 and 2017-18 at PAU, Ludhiana

Treatment	2016-17			2017-18		
	Weed density (No. per plot)	Weed biomass (g/plant)	Weed control efficiency (%)	Weed density (No. per plot)	Weed biomass (g/plant)	Weed control efficiency (%)
Weed growth stage at the time of herbicide spray						
4 leaf stage (35 days)	6.90	1.01	77.12	7.33	1.08	75.70
8 leaf stage (50 days)	13.04	3.82	11.04	14.00	3.92	10.30
12 leaf stage (60 days)	14.14	6.81	3.94	14.71	6.96	3.59
SEm±	0.17	0.03	0.63	0.11	0.04	1.33
LSD (p=0.05)	0.70	0.15	2.47	0.43	0.17	5.24
Herbicide treatment						
Untreated control	15.00	4.70	0	15.00	4.85	0
2,4 D sodium salt @ 250 g/ha	14.11	4.36	10.11	14.55	4.54	8.87
2,4 D sodium salt @ 500 g/ha	11.44	4.06	24.72	12.44	4.04	24.15
Carfentrazone-ethyl @ 10 g/ha	8.55	3.48	38.30	9.22	3.61	37.00
Carfentrazone-ethyl @ 20 g/ha	8.33	3.38	40.93	8.88	3.46	40.36
Metsulfuron + Sulfosulfuron @ 15 g/ha	11.66	3.61	34.56	12.55	3.71	34.03
Metsulfuron + Sulfosulfuron @ 30 g/ha	10.44	3.57	35.60	11.22	3.68	34.76
SEm±	0.18	0.06	0.72	0.14	0.03	0.58
LSD (p=0.05)	0.52	0.20	2.06	0.40	0.11	1.67
Interaction LSD (p=0.05)	S	S	S	S	S	S

Table 4.60: Interaction effect of weed growth stage at the time of herbicide spray and different herbicide treatments on *Medicago denticulata* Willd. density, biomass and weed control efficiency during *rabi* 2016-17 and 2017-18 at PAU, Ludhiana

Weed growth stage at the time of herbicide spray						
Treatments	**Weed density (No. per plot)**					
	2016-17			**2017-18**		
Herbicide treatment	4 leaf	8 leaf	12 leaf	4 leaf	8 leaf	12 leaf
Untreated control	15.00	15.00	15.00	15.00	15.00	15.00
2,4 D sodium salt 250 g/ha	13.00	14.66	14.66	13.66	15.00	15.00
2,4 D sodium salt 500 g/ha	7.33	13.66	13.33	8.00	14.66	14.67
Carfentrazone-ethyl 10 g/ha	0.00	11.66	14.00	0.00	12.66	15.00
Carfentrazone-ethyl 20 g/ha	0.00	11.33	13.66	0.00	12.33	14.33
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	8.00	12.67	14.33	9.00	14.00	14.66
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	5.00	12.33	14.00	5.66	13.66	14.33
Interaction LSD (p=0.05)	0.89			0.70		
Weed biomass (g/plant)						
Untreated control	2.82	4.22	7.05	3.08	4.30	7.19
2,4 D sodium salt 250 g/ha	2.12	4.10	6.86	2.40	4.20	7.02
2,4 D sodium salt 500 g/ha	1.35	4.05	6.80	1.08	4.15	6.89
Carfentrazone-ethyl 10 g/ha	0.13	3.56	6.75	0.15	3.76	6.94
Carfentrazone-ethyl 20 g/ha	0.05	3.38	6.72	0.10	3.45	6.85
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	0.35	3.72	6.76	0.40	3.80	6.93
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	0.28	3.70	6.74	0.36	3.78	6.90
Interaction LSD (p=0.05)	0.34			0.18		
Weed control efficiency (%)						
Untreated control	0	0	0	0	0	0
2,4 D sodium salt 250 g/ha	24.80	2.84	2.69	22.07	2.32	2.23
2,4 D sodium salt 500 g/ha	66.60	4.03	3.54	64.93	3.48	4.04
Carfentrazone-ethyl 10 g/ha	95.30	15.36	4.25	95.13	12.55	3.34
Carfentrazone-ethyl 20 g/ha	98.22	19.9	4.68	96.75	19.76	4.59
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	87.73	11.84	4.11	87.01	11.62	3.48
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	90.10	12.32	4.39	88.31	12.09	3.89
Interaction LSD (p=0.05)	3.58			2.90		

Table 4.61: Effect of weed growth stage at the time of herbicide spray and different herbicides on *Medicago denticulata* Willd. density, biomass and weed control efficiency during *rabi* 2016-17 and 2017-18 at Regional Research Station, Abohar

Treatment	2016-17			2017-18		
	Weed density (No. per plot)	Weed biomass (g/plant)	Weed control efficiency (%)	Weed density (No. per plot)	Weed biomass (g/plant)	Weed control efficiency (%)
Weed growth stage at the time of herbicide spray						
4 leaf stage (35 days)	7.61	1.07	73.40	7.90	1.18	71.31
8 leaf stage (50 days)	14.23	3.87	9.87	14.42	3.98	8.91
12 leaf stage (60 days)	14.56	6.89	3.66	14.85	7.00	3.24
SEm±	0.17	0.05	0.64	0.11	0.08	0.94
LSD (p=0.05)	0.70	0.18	2.50	0.47	0.20	3.43
Herbicide treatment						
Untreated control	15.00	4.73	0	15.00	4.89	0
2,4 D sodium salt @ 250 g/ha	14.66	4.45	8.26	14.66	4.62	7.77
2,4 D sodium salt @ 500 g/ha	12.77	4.16	22.28	13.22	4.15	21.28
Carfentrazone-ethyl @ 10 g/ha	9.33	3.54	36.58	9.66	3.67	35.34
Carfentrazone-ethyl @ 20 g/ha	9.00	3.44	38.98	9.22	3.55	37.64
Metsulfuron + Sulfosulfuron @ 15 g/ha	12.88	3.68	33.40	13.22	3.76	32.10
Metsulfuron + Sulfosulfuron @ 30 g/ha	11.33	3.62	34.37	11.77	3.71	32.79
SEm±	0.20	0.08	0.66	0.16	0.05	0.53
LSD (p=0.05)	0.55	0.26	2.02	0.46	0.13	1.70
Interaction LSD (p=0.05)	S	S	S	S	S	S

Table 4.62: Interaction effect of weed growth stage at the time of herbicide spray and different herbicide treatments on *Medicago denticulata* Willd. density, biomass and weed control efficiency during *rabi* 2016-17 and 2017-18 at Regional Research Station, Abohar

Weed growth stage at the time of herbicide spray						
Treatments	**Weed density (No. per plot)**					
	2016-17			**2017-18**		
Herbicide treatment	4 leaf	8 leaf	12 leaf	4 leaf	8 leaf	12 leaf
Untreated control	15.00	15.00	15.00	15.00	15.00	15.00
2,4 D sodium salt 250 g/ha	14.00	15.00	15.00	14.00	15.00	15.00
2,4 D sodium salt 500 g/ha	9.00	14.66	14.66	9.66	15.00	15.00
Carfentrazone-ethyl 10 g/ha	0.00	13.66	14.33	0.00	14.00	15.00
Carfentrazone-ethyl 20 g/ha	0.00	13.00	14.00	0.00	13.00	14.66
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	9.33	14.66	14.66	10.00	15.00	14.66
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	6.00	13.66	14.33	6.66	14.00	14.66
Interaction LSD (p=0.05)	0.84			0.76		
Weed biomass (g/plant)						
Untreated control	2.85	4.25	7.10	3.15	4.33	7.19
2,4 D sodium salt 250 g/ha	2.20	4.15	7.00	2.51	4.26	7.11
2,4 D sodium salt 500 g/ha	1.43	4.16	6.89	1.21	4.23	7.02
Carfentrazone-ethyl 10 g/ha	0.18	3.60	6.84	0.23	3.80	7.00
Carfentrazone-ethyl 20 g/ha	0.12	3.43	6.78	0.18	3.52	6.95
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	0.43	3.78	6.84	0.52	3.88	6.90
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	0.33	3.74	6.80	0.46	3.84	6.85
Interaction LSD (p=0.05)	0.45			0.30		
Weed control efficiency (%)						
Untreated control	0	0	0	0	0	0
2,4 D sodium salt 250 g/ha	20.60	2.16	2.02	19.54	2.04	1.75
2,4 D sodium salt 500 g/ha	60.00	3.64	3.21	58.42	3.32	2.12
Carfentrazone-ethyl 10 g/ha	92.20	13.45	4.10	90.11	12.14	3.78
Carfentrazone-ethyl 20 g/ha	94.44	18.20	4.30	92.55	16.34	4.03
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	85.56	10.60	4.06	83.21	9.43	3.67
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	87.63	11.21	4.27	84.04	10.20	4.14
Interaction LSD (p=0.05)	4.02			3.07		

Likewise, weed biomass of herbicide treated *M. denticulata* plants differed significantly when herbicide sprays were done at eight and twelve-leaf stage. Weed control efficiency of all the herbicides also recorded significant decrease with delay in herbicide application from eight to twelve-leaf stage. Chhokar *et al* (2011) studied the effect of different herbicides for control of dicotyledonous weeds infesting wheat crop and reported that sulfosulfuron + metsulfuron and carfentrazone-ethyl were effective in reducing weed biomass of *M. denticulata* as compared to unsprayed check. Punia *et al* (2006) reported greater effectiveness of carfentrazone against dicotyledenous weeds like, *Malva parviflora* and *Convolvulus arvensis* which were not effectively controlled by application of 2,4-D or metsulfuron. In contrast to this, Singh *et al* (2008) reported poor weed control of dicotyledonous weeds infesting wheat crop *viz*., *Lathyrus aphaca*, *Fumaria parviflora* and *Coronopus didymus* with carfentrazone or metsulfuron when applied alone as compared to tank mix application of carfentrazone plus metsulfuron. Singh *et al* (2004b) reported weed control efficiency of 10.1 and 91.6% against dicotyledonous weeds with 2,4-D at 500 g/ha and carfentrazone-ethyl at 20 g/ha, respectively. Bhullar *et al* (2013) reported similar efficacies of 2,4-D sodium salt, carfentrazone-ethyl and metsulfuron-methyl in reducing density of *Medicago polymorpha* in barley.

Weed species have been reported to exhibit differential sensitivity to herbicides depending upon active ingredient of an herbicide, dose of herbicide and weed growth stage at the time of spray. The growth stage of weed affects uptake and metabolism of herbicide thereby influencing herbicide efficacy. Young weeds when their growth stage does not exceed 4-6 leaves are most sensitive to herbicides (Barros *et al* 2007). Greater susceptibility of weeds at earlier growth stages as compared to later growth stages is because of rapid herbicide translocation via plasmodesmata during earlier stages (Kieloch and Domaradzki 2011). Size exclusion limit is a major factor which determines the size of molecules that can pass through plasmodesmata and therefore allows only restrictive macromolecular transport (Yadav *et al* 2014). Size exclusion limit of plasmodesmata in older plants is reduced to > 50 times as compared to younger plants suggesting it to be one of the major reasons for reduced susceptibility of older plants to herbicides due to reduced translocation of herbicides (Concenco *et al* 2007). Therefore, weed control efficiency of herbicides could be increased by spraying herbicides at early weed growth stages as mature plants have reduced sensitivity for herbicides. Carfentrazone-ethyl is a post-emergence contact herbicide and is less effective on weeds at their advanced stages of development. Further, lack of residual activity (half life of carfentrazone is 2-5 days) in soil leads to decreased efficiency to control subsequent weeds which emerge later after herbicide application (Lyon *et al* 2007, Willis *et al* 2007). In present study, carfentrazone-ethyl applied at 10 and 20 g/ha to four-leaf stage of *M. denticulata* resulted in complete mortality with minimum biomass and > 95% weed control efficiency;

whereas its delayed application at eight and twelve-leaf stage resulted in significant increase in number of *M. denticulata* plants which survived herbicide application with increased biomass leading to reduced herbicide efficacy. Results of present study are in agreement with Cauchy (2000) who reported that carfentrazone-ethyl was active at low dose rates (20 g a.i./ha) and provided outstanding efficacy on a wider range of weeds with better results against young weeds, which were controlled within 1 to 2 weeks of herbicide application. Rizzardi and Fleck (2004) reported that application of bentazon + acifluorfen, at rate of 1.25 L ha^{-1} at 2-leaf stage of *Sida rhombifolia* resulted in 95% control. However, control was decreased to 87% when herbicide was applied at 6-leaf stage even with an increased dose of 2.0 L $ha^{-1.}$ Holm *et al* (2000) studied the effect of different herbicides for control of wild oat (*Avena fatua*) in wheat crop and reported that imazamethabenz applied at two and four-leaf stage decreased the fresh weight of *A. fatua*. Whereas, application of imazamethabenz at six-leaf stage increased the fresh weight of *A. fatua* indicating decreased weed control efficacy of imazamethabenz for advanced stages of *A. fatua*. Efficacy of auxinic herbicides has been reported to be reduced with delay in herbicide application (Eure *et al* 2013). For example, Sellers *et al* (2009) reported that control of dogfennel (*Eupatorium capillifolium*) was dramatically reduced when 2,4-D plus dicamba were applied to 154 cm tall plants as compared to 38 cm tall plants. In present study also 2,4-D was more effective when sprayed at four-leaf stage of *M. denticulata* than at eight and twelve-leaf stage. Chafin *et al* (2010) reported that glufosinate applied to 5 cm tall palmer amaranth (*Amaranthus palmeri*) plants resulted in > 90% control. Whereas, application of glufosinate to *A. palmeri* plants with > 18 cm height declined the control to 73%. Chauhan *et al* (2012) reported that application of bispyribac-sodium, fenoxaprop + ethoxysulfuron and penoxsulam + cyhalofop gave > 80% control of barnyardgrass (*Echinochloa crus-galli*) when applied at the four-leaf stage. However, delayed application from the four-leaf to the six-leaf stages reduced the control to < 50%. Tironi *et al* (2012) reported that *Brachiaria brizantha* at two to four-leaf stages was effectively controlled with 2 kg/ha of diuron + hexazinone. Whereas, for control of *B. brizantha* at the stage of one to four tillers, increase in herbicide rate to 3.5 kg/ha was required which indicates reduced efficacy of this herbicide at advanced weed growth stages.

4.3.7 Seed production potential

The seeds of *M. denticulata* are enclosed in coiled pods called burs (fruit) with seed number varying from 3-5 seeds per pod. Growth stage of *M. denticulata* at the time of herbicide spray caused significant effect on fruit and seed number per plant during both years of study. The interaction effect of weed growth stage at the time of herbicide spray and different herbicides on seed production of *M. denticulata* was significant (Table 4.63 and 4.64). Maximum and minimum fruit and seed number per plant of *M. denticulata* was recorded with herbicide application at four and twelve-leaf stages, respectively during both

the years. All the herbicides when applied at eight and twelve-leaf stages of *M. denticulata* caused significant decline in fruit and seed number than herbicide sprays done at four-leaf stage during both the years. During 2016-17, application of 500 g/ha 2,4-D at eight and twelve-leaf stages of *M. denticulata* resulted in 31 and 50% reduction in fruit number per plant respectively, as compared to plants treated with 2,4-D at four-leaf stage. Similarly, in 2017-18 also 2,4-D application at eight and twelve-leaf stages declined the fruit number by 34.6 and 51.6% respectively, than plants sprayed at four-leaf stage. In response to 30 g/ha of metsulfuron plus sulfosulfuron sprayed at eight and twelve-leaf stages of *M. denticulata*, plants produced 17.7 and 26.3% lesser seeds plant^{-1} respectively, as compared to plants treated at four-leaf stage during 2016-17. Application of carfentrazone-ethyl at four-leaf stage of *M. denticulata* during both years resulted in complete mortality of plants thereby completely inhibiting fruit and seed set. However, *M. denticulata* plants treated with carfentrazone-ethyl at eight and twelve-leaf stage were able to set seeds.

Targeting weed seed production provides an effective tool for reducing the spread of herbicide-resistant weeds by preventing their establishment, spatial distribution and build up of seed reservoirs in the soil seedbank (Bagavathiannan and Norsworthy 2012). Herbicide application at or near flowering or seed set has the advantage of decreasing weed seed production, eventually allowing the addition of lesser seeds in the soil seedbank in the next cropping seasons (Jha and Norsworthy 2012, Walker and Oliver 2008). Ganie *et al* (2018) reported that single or sequential applications of 2,4-D or dicamba resulted in ≥ 96% inflorescence injury and reduction in seed production of giant ragweed (*Ambrosia trifida*) in the field as well as in greenhouse studies. The results indicated that 2,4-D or dicamba are effective options for reducing seed production of glyphosate-resistant *A. trifida* even if applied late in the season. Goroee and Saeedipour (2015) reported that metsulfuron plus sulfosulfuron at 30 g/ha was effective in suppressing seed formation in *Malva parviflora*.

Table 4.63: Interaction effect of weed growth stage at the time of herbicide spray and different herbicide treatments on *Medicago denticulata* Willd. fruit number and seed number per plant during *rabi* 2016-17 and 2017-18 at PAU, Ludhiana

	Weed growth stage at the time of herbicide spray					
Treatments	**Fruit number plant^{-1}**					
	2016-17			**2017-18**		
Herbicide treatment	4 leaf	8 leaf	12 leaf	4 leaf	8 leaf	12 leaf
Untreated control	176	180	180	177	182	178
2,4 D sodium salt 250 g/ha	164	128	95	168	136	125
2,4 D sodium salt 500 g/ha	145	100	72	153	100	74
Carfentrazone-ethyl 10 g/ha	0*	145	125	0*	152	133
Carfentrazone-ethyl 20 g/ha	0*	116	86	0*	120	93
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	163	134	120	168	130	96
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	153	108	79	160	114	88
Interaction LSD (p=0.05)	12.75			12.24		
	Seed number plant^{-1}					
Untreated control	704	720	720	708	728	712
2,4 D sodium salt 250 g/ha	656	512	380	672	544	500
2,4 D sodium salt 500 g/ha	580	400	288	612	400	296
Carfentrazone-ethyl 10 g/ha	0*	580	500	0*	608	532
Carfentrazone-ethyl 20 g/ha	0*	464	344	0*	480	372
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	652	536	480	672	520	384
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	612	432	316	640	456	352
Interaction LSD (p=0.05)	53.13			42.37		

*** No fruit and seed set due to complete mortality of plants**

Table 4.64: Interaction effect of weed growth stage at the time of herbicide spray and different herbicide treatments on *Medicago denticulata* Willd. fruit number and seed number per plant during *rabi* 2016-17 and 2017-18 at Regional Research Station, Abohar

Weed growth stage at the time of herbicide spray						
Treatments	**Fruit number plant^{-1}**					
	2016-17			**2017-18**		
Herbicide treatment	4 leaf	8 leaf	12 leaf	4 leaf	8 leaf	12 leaf
Untreated control	170	178	175	175	180	177
2,4 D sodium salt 250 g/ha	161	120	90	164	133	120
2,4 D sodium salt 500 g/ha	140	90	65	148	96	68
Carfentrazone-ethyl 10 g/ha	0*	140	120	0*	143	127
Carfentrazone-ethyl 20 g/ha	0*	108	80	0*	115	85
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	158	128	115	164	124	92
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	150	100	74	153	108	80
Interaction LSD (p=0.05)	12.53			10.44		
Seed number plant^{-1}						
Untreated control	680	712	700	700	720	708
2,4 D sodium salt 250 g/ha	644	480	360	656	496	368
2,4 D sodium salt 500 g/ha	561	360	260	592	384	272
Carfentrazone-ethyl 10 g/ha	0*	560	480	0*	572	508
Carfentrazone-ethyl 20 g/ha	0*	432	320	0*	460	340
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	632	512	460	656	532	480
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	600	400	296	612	432	320
Interaction LSD (p=0.05)	50.12			41.79		

*** No fruit and seed set due to complete mortality of plants**

4.3.8 Effect of herbicide exposure on germination potential of *Medicago denticulata* seeds

Data presented in Table 4.65 shows the interaction effect of weed growth stage at the time of herbicide spray and different herbicides on germination of cream and brownish black seeds collected from herbicide treated plants. Cream and brownish black seeds collected from unsprayed plots (control) recorded higher germination as compared to seeds treated with 2,4-D, carfentrazone-ethyl and metsulfuron methyl plus sulfosulfuron (Table 4.65). Application of herbicides at eight and twelve-leaf stage of *M. denticulata* produced seeds with decreased germination as compared to plants sprayed at four-leaf stage. However, time to start germination and speed of germination was not affected. Wu *et al* (2016) reported that application of glyphosate and paraquat at late budding stage did not stop the growth of fleabane (*Conyza bonariensis*) plants which continued to develop, flower and set seeds. However, significant effects on seed viability and dormancy was recorded. Qi *et al* (2017) reported that application of atrazine and tribenuron-methyl at different growth stages influenced percent germination of *Amaranthus retroflexus*. Mondal *et al* (2017) reported that germination of *Pisum sativum* seeds treated with 3 and 4 mg/L glyphosate was reduced to 55 and 40%, respectively as compared to 100% germination in untreated control. Subedi *et al* (2017) reported that the application of glyphosate alone or as a tank mix with other herbicides (except diquat + glyphosate) significantly reduced germination percentage in red lentil (*Lens culinaris* L.) seeds compared to the untreated control.

Herbicide application at different growth stages of *M. denticulata* produced seeds with storage reserves statistically similar to each other (Table 4.66). Vital *et al* (2017) reported that sunflower plants treated with trinexapac-ethyl did not show any changes in the total soluble sugars, reducing and non-reducing sugars and starch content in leaves. In contrast, glyphosate application to sunflower significantly reduced the total soluble sugars and starch content in leaves by 72 and 92% as compared to control.

The results of present study revealed that wheat crop treated with 0.5X and 1X doses of 2,4-D and metsulfuron plus sulfosulfuron at 35, 50 and 60 days after sowing did not show any visual toxicity symptoms during both years which indicates that both herbicides are safe for use in wheat crop. Carfentrazone application at 10 and 20 g/ha to 50 and 60 days old wheat crop was not phytotoxic. However, some minor injury was observed with carfentrazone application at early crop growth stages (35 days) which subsided as the crop attained maturity without leaving any negative impact on yield. *M. denticulata* plants treated with different herbicides at four-leaf stage exhibited different phytotoxicity symptoms thereby providing effective weed control. 2,4-D application at four-leaf stage of *M. denticulata* resulted in visual toxicity symptoms like epinasty (stem twists outward and downward), curling and narrowing of leaves. *M. denticulata* plants treated with carfentrazone at four-leaf stage showed presence of white necrotic spots on leaves accompanied with leaf desiccation

and complete mortality. None of the herbicides caused any phytotoxicity when applied at eight and twelve-leaf stage of *M. denticulata* resulting in poor weed control efficiencies. At flag leaf stage, wheat crop recorded Fv/Fm and CCI values statistically similar in all treatments. *M. denticulata* plants treated with different herbicides at four-leaf stage recorded least values of Fv/Fm and CCI; whereas significantly higher values of Fv/Fm and CCI were observed when herbicide sprays were done at eight and twelve-leaf stages of this weed. All the herbicides sprayed at eight-leaf stage of *M. denticulata* resulted in an increase in number of surviving plants with high weed biomass and decreased weed control efficiency than herbicide sprays done at four-leaf stage. Significant increase in number of surviving plants of *M. denticulata* was observed with carfentrazone-ethyl and metsulfuron plus sulfosulfuron when sprayed at twelve-leaf stage than sprays done at eight-leaf stage. Similarly, weed control efficiency of all the herbicides also recorded significant decrease with delayed herbicide application from eight to twelve-leaf stage. Poor weed control at advanced weed stages indicates importance of early herbicide application for effective control of *M. denticulata*. Seed production potential of *M. denticulata* was significantly influenced by both stage of herbicide application and herbicide treatments. *M. denticulata* plants sprayed at eight and twelve-leaf stage survived the herbicide application; but great reduction in seed production was recorded as compared to herbicide sprays done at four-leaf stage. Seeds collected from plants sprayed at eight and twelve-leaf stages recorded decrease in germination as compared to seeds collected from plants sprayed at four-leaf stage. This indicates possibility of herbicide carry-over effect from parent plants.

Table 4.65: Interaction effect of weed growth stage at the time of herbicide spray and different herbicide treatments on germination potential of *Medicago denticulata* Willd. during *rabi* 2016-17 and 2017-18 at PAU, Ludhiana

	Weed growth stage at the time of herbicide spray					
Treatments	**Seeds with cream seed coat**					
	2016-17			**2017-18**		
Herbicide treatment	4 leaf	8 leaf	12 leaf	4 leaf	8 leaf	12 leaf
Untreated control	95.5	97.73	94.40	95.0	96.6	95.5
2,4 D sodium salt 250 g/ha	92.2	92.20	89.96	93.3	92.0	90.0
2,4 D sodium salt 500 g/ha	90.0	88.86	82.20	92.2	90	80.0
Carfentrazone-ethyl 10 g/ha	0.00*	87.73	85.5	0.00*	88.8	86.6
Carfentrazone-ethyl 20 g/ha	0.00*	81.10	78.80	0.00*	82.2	80.0
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	89.96	88.86	84.40	90.0	93.3	86.6
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	86.63	83.30	76.63	86.6	84.0	78.3
Interaction LSD (p=0.05)	4.21			4.56		
Seeds with brownish black seed coat						
Untreated control	86.63	85.50	85.50	86.6	85.5	85.5
2,4 D sodium salt 250 g/ha	83.30	77.73	73.3	84.0	78.8	74.0
2,4 D sodium salt 500 g/ha	80.00	74.40	67.76	83.3	75.0	68.3
Carfentrazone-ethyl 10 g/ha	0.00*	76.63	67.73	0.00*	77.0	68.8
Carfentrazone-ethyl 20 g/ha	0.00*	70.00	63.30	0.00*	72.2	64.0
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	82.20	73.30	62.20	83.3	74.4	60.0
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	81.10	72.20	60.00	82.2	73.3	63.3
Interaction LSD (p=0.05)	4.05			4.18		

***No seed set was recorded due to complete mortality of plants**

Table 4.66: Effect of weed growth stage at the time of herbicide spray and different herbicides on seed storage reserves of *Medicago denticulata* Willd. during *rabi* 2016-17 and 2017-18 at PAU, Ludhiana

	2016-17				2017-18			
Treatments	**Starch content (mg g-1 FW)**	**Total soluble sugars (mg g-1 FW)**	**Total soluble proteins (mg g-1 FW)**	**Total free amino acids (mg g-1 FW)**	**Starch content (mg g-1 FW)**	**Total soluble sugars (mg g-1 FW)**	**Total soluble proteins (mg g-1 FW)**	**Total free amino acids (mg g-1 FW)**
Weed growth stage at the time of herbicide spray								
4 leaf stage	17.30	20.00	33.30	33.08	17.27	20.30	34.16	33.15
8 leaf stage	17.00	20.10	34.20	32.86	17.22	20.28	34.11	33.11
12 leaf stage	17.24	20.17	34.00	32.90	17.26	20.26	34.12	33.10
SEm±	0.14	0.18	0.24	0.15	0.11	0.14	0.21	0.17
LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS
Herbicide treatments								
Untreated control	17.50	20.30	34.11	33.45	17.53	20.48	34.30	33.50
2,4 D sodium salt 250 g/ha	17.42	20.27	34.14	33.47	17.46	20.46	34.31	33.50
2,4 D sodium salt 500 g/ha	17.40	20.34	34.07	33.41	17.42	20.42	34.11	33.18
Carfentrazone-ethyl 10 g/ha	16.67	20.32	33.10	32.57	16.80	20.38	34.06	33.03
Carfentrazone-ethyl 20 g/ha	16.60	19.37	33.21	32.52	16.76	19.56	33.78	33.24
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	17.35	20.07	34.22	32.66	17.40	20.43	34.15	32.73
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	17.32	20.10	34.12	32.60	17.38	20.24	34.20	32.66
SEm±	0.23	0.25	0.20	0.17	0.42	0.50	0.40	0.20
LSD (p=0.05)	1.06	1.18	1.05	1.03	0.88	0.96	0.84	1.04
Interaction LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS

CHAPTER V

SUMMARY

The present investigation entitled, "Germination ecology of *Medicago denticulata* Willd. in relation to seed coat colour, persistence and herbicide exposure" was conducted at Research Farm and Weed Physiology Laboratory, Department of Agronomy, Punjab Agricultural University, Ludhiana and Regional Research Station, Abohar.

Experiment I: To study the effect of seed coat colour on germination ecology of *Medicago denticulata*.

A) Physiological, morphological and biochemical characterization of heteromorphic *M. denticulata* Willd. seeds

- Brownish black coloured seeds were large, non-dormant and germinated rapidly to a high percentage. However, cream coloured seeds were small and dormant exhibiting lesser germination. Moisture content of cream seeds was lesser (6.31%) as compared to brownish black seeds (9.56%). Initial levels of principle seed storage reserves were similar in both cream and brownish black seeds.

B) Scanning electron microscopy, imbibition and electrical conductivity of *M. denticulata* seeds

- Scanning electron microscope studies revealed that both seeds exhibited a papillose surface pattern. However, brownish black seeds differed from cream seeds by having small cracks scattered all over the surface. Maximum imbibition was achieved in cream and brownish black seeds at 12 and 21 h respectively. Brownish black seeds with small cracks on the seed coat and higher initial water uptake exhibited greater electrolyte leakage after 24 h of imbibition than cream seeds with low imbibition rate**.**

C) Dormancy breaking treatments

- Mechanical scarification with sand paper significantly improved the germination of cream seeds in comparison to their control seeds. The increase in total germination was accompanied by onset of early germination on 2nd day along with increased germination speed. Whereas, non-scarified seeds took seven days for germination to start along with reduced speed of germination, increased mean germination time and decreased final germination percentage.
- Chemical scarification of cream seeds with 50 and 70% sulphuric acid for different time intervals significantly improved the germination. Maximum germination of cream seeds was recorded when seeds were soaked for 10 min in 50% sulphuric acid. However, sulphuric acid even at 50% concentration proved to be very damaging to brownish black seeds as it resulted in complete seed mortality.
- Hot water scarification for different time intervals caused a significant improvement in

the germination of cream seeds of *M. denticulata*. However, brownish black seeds soaked in hot water at temperature 50°C or above resulted in high number of damaged seeds. Maximum germination of cream seeds was recorded with hot water incubation at 50°C for 30 min with minimum time to start germination and mean germination time as well as maximum speed of germination in this treatment.

D) Effect of environmental variables on germination of cream and brownish black seeds

i) Temperature

- Optimum day/night temperature regime for germination of cream and brownish black seeds was 25/15 and 20/10°C (12 h light/12 h dark), respectively. However, cream seeds possessed ability to germinate in the broad temperature range of 15/5 to 30/20°C; whereas, germination of brownish black seeds occurred in narrow temperature range of 15/5 to 25/15°C.
- Day/night temperature regime of 25/15°C recorded maximum germination in *T. aestivum* seeds. Minimum germination of *T. aestivum* seeds was recorded at day/night temperature regime of 30/20°C and at this temperature seeds took maximum time to start germination along with reduced speed of germination and increased mean germination time.

ii) Light

Germination of cream and brownish black seeds did not differ significantly under light and dark conditions, which indicates that light is not required for germination of heteromorphic seeds of *M. denticulata*. However, dark grown seedlings were chlorotic having pale yellow colour with reduced fresh and dry weight as compared to seedlings grown under light. Germination and seedling growth of *T. aestivum* was also independent of light.

iii) Moisture

- Reduction in osmotic potential from 0 to -0.4 MPa significantly decreased the seed germination of cream and brownish black seeds. Maximum germination of both seeds was observed in control and minimum at osmotic potential of -0.4 MPa. Germination of seeds was completely inhibited at osmotic potentials of -0.6, -0.8 and -1.0 MPa.
- At -0.4 MPa, brownish black seeds took six days to start germination in comparison to cream seeds in which germination started on 3rd day of incubation. The osmotic potential required for 50% inhibition of the maximum germination of cream and brownish black seeds was -0.37 and -0.32 MPa, respectively. This indicates that moisture stress exhibited more pronounced effect on brownish black seeds than cream seeds**.**
- Decreasing osmotic potential from 0 to -0.4 MPa significantly reduced the root and

shoot length of both types of seedlings. However, seedlings emerging from brownish black seeds were affected to a greater extent than seedlings emerged from cream seeds.

- *T. aestivum* could tolerate moisture stress upto -0.4 MPa but no germination was observed at osmotic potential of ≥ -0.6 MPa. Moisture stress significantly decreased the germination speed and increased the mean germination time. Decreasing osmotic potential from 0 to -0.4 MPa significantly decreased the growth of seedlings.

iv) Salinity

- Salinity stress significantly decreased the germination (%) and speed of germination with concomitant increase in mean germination time in both cream and brownish black seeds. Cream seeds were fairly tolerant to salinity stress as some seeds (10%) were able to germinate up to NaCl concentration of 200 mM in contrast to brownish black seeds whose germination was completely inhibited at 200 mM NaCl. Salinity stress significantly reduced the growth of both cream and brownish black seedlings. Notably, salinity stress was more detrimental to root growth as compared to shoot growth.
- Increasing NaCl concentrations caused significant reduction in germination (%) of *T. aestivum* seeds. At, 250 mM NaCl concentration, only 10% seeds were able to germinate with complete inhibition of germination at 300 mM concentration of NaCl.

v) pH

- Cream and brownish black seeds recorded highest germination and seedling growth in control having pH 6.6 and lowest at pH 3. However, both seeds were able to germinate under both acidic and alkaline pH with > 40% germination in pH range 3-10.
- The germination of *T. aestivum* was completely inhibited when pH was either less than 5 or more than 8 and increasing pH from 5 to 7 increased their germination and growth.

vi) Burial depth

Maximum emergence of both cream and brownish black seeds was recorded from surface placed seeds. There was progressive decline in emergence of both seeds with increase in burial depth from 1-4 cm with no emergence when seeds were placed at a depth of 8 cm or deeper.

Experiment 2: To study the effect of accelerated ageing and soil seedbank persistence on germination ecology of *Medicago denticulata* Willd.

A) Accelerated ageing

- Accelerated ageing studies revealed that cream and brownish black seeds recorded decrease in germination (%) and speed along with increased mean germination time as compared to their respective controls with increase in days of ageing. However, effect of accelerated ageing was more pronounced on brownish black seeds than

cream seeds as indicated by rapid loss of germination in brownish black seeds than cream seeds.

- Accelerated ageing of brownish black seeds for 20 or more days resulted in complete inhibition of germination. Whereas, cream seeds aged for 20 days were still able to germinate (13%). Accelerated ageing of both cream and brownish black seeds resulted in membrane damage as evident by progressive and significant increase in membrane leakage. With accelerated ageing, both seeds recorded decrease in starch and total soluble proteins with concomitant increase in membrane leakage, total soluble sugars and free amino acids.

B) Soil seed persistence

- Persistence studies under field conditions revealed that cream seeds were able to germinate up to 40% when buried at a depth of 2 cm after 12 months of burial; brownish black seeds buried for 12 or more months recorded no germination.
- Results of accelerated ageing studies and field persistence suggest longer persistence for cream seeds as compared to brownish black seeds.

Experiment 3: To study germination ecology of *Medicago denticulata* seeds after herbicide exposure

- 2,4-D and metsulfuron plus sulfosulfuron were safe to wheat crop when sprayed at either 35, 50 or 60 days after sowing. Carfentrazone sprayed at 35 days old crop resulted in minor toxicity which subsided at maturity.
- Herbicide sprays done at four-leaf stage of *M. denticulata* provided effective control whereas delayed application resulted in poor control with no visual injury.
- Wheat crop at flag leaf stage recorded statistically similar values of chlorophyll fluorescence and chlorophyll content index irrespective of crop growth stage at the time of herbicide application.
- Higher yield and yield attributing parameters of crop were recorded with herbicide sprays done at four-leaf stage of *M. denticulata*, while minimum values of these parameters were recorded in plants when herbicide sprays were done at eight and twelve-leaf stage. Plants treated with carfentrazone-ethyl @ 20 g/ha gave maximum yield and yield attributing parameters as compared to untreated control.
- Minimum and maximum density and biomass were observed when herbicides were sprayed at four and twelve-leaf stages, respectively. There was significant increase in weed density and biomass as the herbicides were sprayed at advanced growth stages of *M. denticulata*.
- Delayed application of all the herbicides at eight and twelve-leaf stage caused decrease in weed control efficiency than herbicide sprays done at four-leaf stage.

- Application of herbicides at eight and twelve-leaf stages caused significant reduction in seed production potential of *M. denticulata* as compared to herbicide sprays done at four-leaf stage.
- Carfentrazone-ethyl had more pronounced effect on seed production potential of *M. denticulata* than 2,4-D. Whereas, metsulfuron-methyl plus sulfosulfuron was least effective in reducing fruit and seed number of *M. denticulata.*
- Seeds produced by *M. denticulata* plants after herbicide exposure had decreased germination. No differences in seed storage reserves were recorded.

Following important conclusions can be drawn from this study:

1. Optimum temperature for germination of *M. denticulata* is 20°C and mean temperature during sowing of *T. aestivum* in Punjab is about 20-25°C. Therefore, *M. denticulata* may emerge along with wheat providing competition to wheat especially during its early establishment period.
2. Seeds of this weed may germinate when buried at shallow depths in the soil or after canopy closure in wheat crop thereby leading to its increased invasiveness.
3. The differential response of cream and brownish black seeds to moisture stress conditions might be a survival mechanism in *M. denticulata* under adverse environmental conditions.
4. Seed germination of *M. denticulata* is more sensitive to salinity stress than *T. aestivum.* Thus, there are lesser chances of this weed to be a problematic weed in salt affected soils
5. Build up of *M. denticulata* weed seed bank may be largely attributed to the presence of cream seeds which are able to persist for longer periods in soil to escape the weed control measures. Whereas non-dormant brownish black seeds will quickly emerge from the soil and can be easily decayed under field conditions. Therefore can be controlled by stale seed bed and/or application of pre-and post- emergence herbicides.
6. Yield and yield attributing parameters of wheat were maximum when herbicides were sprayed at four-leaf stage and minimum when spray was done at twelve-leaf stage of *M. denticulata.* This demonstrates the importance of early herbicide application in controlling the weeds as effectiveness of herbicides was low on *M. denticulata* when applied at their eight and twelve-leaf stage. The herbicide degradation rate or metabolism could be faster in mature plants, thus herbicide rates may need to be increased to achieve the same level of control.
7. Herbicide sprays at eight and twelve-leaf stages of *M. denticulata* produced seeds which recorded significant decrease in germination as compared to seeds collected from four-leaf stage plants of *M. denticulata* which indicates possibility of herbicide carry-over effect from parent plants.

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LIST OF PUBLISHED/ SUBMITTED RESEARCH PAPERS

Sr. No.	Title	Journal	Score	Remarks
1	Germination Ecology of Heteromorphic seeds of *Medicago denticulata* Willd.	Weed Research	NAAS 7.77	Submitted
2	Morphological and physiological characterization of seed heteromorphism in *Medicago denticulata* Willd.	Seed Science Research	NAAS 8.03	Submitted

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OCPA	:	8.06/10.00
Title of Doctoral Dissertation	:	Germination ecology of *Medicago denticulata* Willd. in relation to seed coat colour, persistence and herbicide exposure