

ASSESSING PHYTOTOXIC IMPACT OF *MELILOTUS INDICUS* L. ON THE GERMINATION AND SEEDLING EMERGENCE OF *SORGHUM BICOLOR* L. UNDER VARIOUS SALINE CONDITIONS

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Abstract

Sorghum is a very important cereal crop over the world and its average production is very low as compared to its potential yield due to the weed infestation and salinity. Therefore, a lab and greenhouse oriented study was carried out to assess the allelopathic impact of yellow sweet clover on the germination and seedling growth of sorghum at different saline conditions. In laboratory experiment, the aqueous extract of four different plant parts of *Melilotus indicus* (leaves, stem, root and whole plant) at different concentrations (5, 10, 15 and 20%). While in greenhouse soil bioassay, three different factors such as residue concentrations (0, 2 and 4%), salt concentrations (0, 4 and 8%) and decomposition period (2 and 4 weeks) were studied with completely randomized design with factorial arrangement having five replicates in lab experiment. Results indicated maximum inhibitory effect on seed germination was recorded when 20% extract of *M. indicus* was applied and maximum seedling growth was noted at control (distilled water). In the case of *M. indicus* parts, highest inhibition was noted when whole plant part water extract was applied at 20% concentration. In second greenhouse experiment, the results showed that *M. indicus* plant materials mixed with the soil and left for the period of two weeks for the fully decomposition caused non-significant impact on the seed germination of sorghum. 19.72%, 5.15% and 4.23% increase in germination, root growth and shoot length was noticed with the *M. indicus* root extract as compared to the whole plant extract. However, saline conditions showed inhibitory effect on germination when its level was increased. Germination and seedling emergence of sorghum significantly affected by the allelopathic potential of *M. indicus* and it was declined by the gradually increased of salt contractions in the soil.

Key words: Allelopathy, Inhibition, *Melilotus indicus*, Plant extracts, *Sorghum bicolor*.

Introduction

Sorghum (*Sorghum bicolor* L.) is an important summer cereal crop; grown worldwide in arid and some semi-arid regions, including Pakistan. Among five cereals, it is considered as a vital crop contributing to annual production across the globe with sixty two million tons (Anon., 2020; Iqbal *et al.*, 2017). In African countries, it is staple food for people as well as used for feeding animals. The millions of humans from Africa, Central America and South Asia utilize it as a major ingredient for protein, energy and minerals (Kimber, 2000). The 10-12%, 3% and 70%, protein, fat and carbohydrate contents, respectively have been noticed in its grains, and it can substitute with other grains for feeding poultry and dairy cattle's cattles (Ullah *et al.*, 2007). Micronutrient malnourishment can be minimized by using its grain and it has strong capacity to be acclimatized under wide range of climates. Average forage yield per hectare is extremely lower besides having favorable soil and climate conditions in Pakistan (Bhatti, 1996; Ahmad *et al.*, 2012). Koca *et al.*, (2007)

reported that imperfect cultivation methods, nutrients imbalance, scarcity of good varieties and salinity are the main problems which extremely affect plants growth, development and production in semi-arid and arid areas. Unusual biological, morphological, physiological and biochemical functions of plants cause unfavorable effects of abiotic stresses. Reduction of photosynthetic electron chain is due to the reactive oxygen species (ROS) production under induced soil salinity (Waśkiewicz *et al.*, 2013; Lalarukh & Shahbaz, 2020) and these ROS adversely affected on the plant growth and yield. Under salinity and drought stress, secondary metabolites synthesis showed elevation. It helps to improve the mechanism of defense and some biochemical pathways assisting water and nutrient accession, ions uptake, ions balancing, chloroplast functions, specific proteins and synthesis of active osmotically metabolites as well as production of various metabolites acting as osmo protectant and detoxifying radicals like phenolic, proanthocyanidins, flavonoids and phenolic acids (Parida, 2005; Waśkiewicz *et al.*, 2013; Zaheer *et al.*, 2019).

Melilotus indicus L. (yellow sweet clover) is very problematic weed due to its allelochemicals (Devi *et al.*, 2020). Field millet, ribbed millet, official melilot and cornilla real are the alternative names of *M. indicus*. It is a good source of food and shelter for various birds and animals, for example, Elk, deer and antelope intake stems and leaves as a main source of their diet. *M. indicus* is also used for green manuring and reclamation of salt-affected soils can also be done by it, which is grown under various saline regions globally (Evans & Cameron, 1998; Al-Sherif, 2009). Allelochemicals of *M. indicus* also hindered the sprouting and growth of many weed species (Raza *et al.*, 2019). Exogenic application of plant extract exploits mechanism of allelopathy in crop science and can be utilized as mulch, intercropping and befitting the crop rotation for suppressing weeds, boosting growth, managing insect and disease attack (Farooq *et al.*, 2011; Afridi & Khan, 2014). Various researches on allelopathy have been counted the interaction between crops and weeds (Singh *et al.*, 2007, Raza *et al.*, 2019). Numerous allelochemicals have been reported in winter wheat crop (*Triticum aestivum* L.), winter rye (*Secale cereal* L.), sorghum (*S. bicolor*), sunflower (*Helianthus annus* L.), and fine fescues (*Festuca* spp.), barley (*Hordeum vulgare* L.), *Brassica* spp., and rice (*Oryza sativa* L.) (Czarnota *et al.*, 2001; Kato-Noguchi *et al.*, 2003; Bertholdsson, 2005). Many researchers are reported the phytotoxic substances present in the various weeds extracts (Elmore, 1980; Alam *et al.*, 2001). For example, caffeine is present in tea (*Camellia sinensis* L.) that can inhabit the seedling growth and germination of other plants (Suzuki & Waller, 1987). Harmal (*Peganum harmala*) seeds can decrease the germination of Persian clover and mustard (Hussain & Nasrin, 1985). *Bothriochok pertusa* seeds aqueous extract inhabit the germination of mustard, lettuce, tomato, millet and chilli (Hussain *et al.*, 1987). The levels of allelopathic impact mainly rely on degree of stresses, like solar radiation, temperature, soil type, moisture and salinity apart from optimum nutrients and biological features which arise during crop growing period (Putnam, 1988).

Salinity stress can be haltered with allelochemicals and it also helps plants to survive under various stresses. Former studies suggested that several allelopathic crops help to manage weeds and diseases as well as mitigate crops under different stresses such as salinity. The exogenic application of several inorganic compounds and plant growth regulators for reclamation of salt-affected soils are among various approaches to cope with salinity. A fine substitute is to utilize biotic tactics like usage of allelopathy which is way accessible and environmental friendly. The objectives of present study was to examine the allelopathic effect of *M. indicus* plant materials at different concentration levels with various saline soil conditions on the germination and seedling growth of sorghum.

Materials and Methods

Experimental site and planting material: The plants of *M. indicus* were harvested from various farmer fields of District Bahawalpur, Punjab Province, Pakistan (29°23'44" N latitude, 71°41'1" E longitude) during the month of May in 2015. The collected plant materials were

dried for seven days at room temperature (30 ± 5°C). After shaded dry, the extracts of the various plant parts were prepared by the following method and applied in mid-July after conducting the experiments. The soil that was used in the pot have the pH of 7.8 having the 14 mg kg⁻¹ N, 105 mg kg⁻¹ P, 90 mg kg⁻¹ K and only 0.5% organic matter.

Method for preparation of water extracts: The collected *M. indicus* plants were separated in different plant parts including root, leaf, stem and whole plant and each part were chopped down by the help of fodder cutter into the small pieces. The dried small pieces were weighed through the electronic balance and soaked separately into the plastic buckets filled with tap water at the 1:5 (w/v) ratio for about 24 hours at room temperature to prepare the stock solution. After one day, all of the solution was filtered through the sieves of 10, 60 and 100-mesh and gained the plant extracts of different *M. indicus* parts. These water extracts were separately placed in the clean bottles for the use in experiment. Extract of shoot, root, leaf and whole plant were made in 1:5 (w/v) for stock solution (20% concentration) preparation and more diluted to make concentrations of 5, 10 and 15% using the technique of parallel dilution ($C_1V_1 = C_2V_2$). However, the distilled water was used for preparation of different concentrations of extracts.

Experiment no. 1: laboratory trial: The study was performed with completely randomized design (CRD) in factorial arrangement followed by five numbers of replications. The experiment was carried out in petri plates to find out the allelopathic effect of *M. indicus* on the germination of sorghum seeds and its seedling emergence in the laboratory of Department of Agronomy, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan during 2015. Seeds of "Sorghum-2011" sorghum variety were used in the experiments that were obtained from the Fodder Research Institute, Sargodha and this experiment was started on 10th of July, 2015. There were four *M. indicus* plant parts including whole plant, leaves, stem and root, along with control treatment and four water extract concentrations 5%, 10%, 15%, 20% along with distilled water as control. Extracts of various plant parts were applied on the sorghum seeds before sowing. Ten seeds of sorghum were placed on the filter paper in every petri plate with diameter of 9 cm and 5 mL of distilled water or water extract was supplemented in every petri plate as per treatment plan. A para film was used to wrap the petri plates to avoid seeds drying during the whole growing period. All petri plates were inspected on daily basis and germination was noted by counting the number of seeds that were germinated over a period of eight days.

Experiment no. 2: soil bioassay: The study was carried out in completely randomized design (CRD) with factorial arrangement followed by three number of replications to investigate the *M. indicus* allelopathic impact on the germination and sorghum seedling growth in greenhouse of Department of Agronomy, University College of Agriculture and Environmental Sciences, The

Islamia University of Bahawalpur, Pakistan during 2015. The greenhouse was located within the campus receiving natural solar radiations and maintaining an average temperature of 28°C. For this experiment, air dried and sandy loam soil was collected from Agronomic Research Area with no infestation of *M. indicus* seeds or plant parts. To maintain different salt levels such as 0, 4 and 8%, sodium chloride (NaCl) was mixed in dried and well pulverized soil and crushed *M. indicus* plant residues were also added with 0, 2 and 4% in this saline soil. Different concentrations of salt (0, 4 and 8 %) were prepared and placed in plastic pots having diameter of 15 cm × 12 cm and the filled pots were divided into two sets, one set of pots was allowed for two weeks decomposition period of *M. indicus* while other set was allowed for four weeks of decomposition. After that, these pots were placed in the greenhouse and water was applied to enhance decomposition on every 3rd day with hand shower. After 2 and 4 weeks, 10 seeds of sorghum were sown in every set of pots, respectively. 120-60-60 NPK kg/ha are the recommended dose of fertilizers for the maize, we calculate the fertilizers for the pots and applied accordingly. Before seed sowing, the sorghum seeds were sterilized with the solution of 1.5% (v/v) sodium hypochlorite. For priming sorghum seeds were imbibed in the filtered water for about 24 hours. The temperature of the greenhouse varied between 19°C and 29°C during the day and night. After the sowing, water was supplied at regular intervals and after 5 days, the thinning was carried out and 5 plants were kept in each pot. The seedlings were pulled out and washed with water after 15 days. The fresh and dry weight of plant, whole plant length and shoot lengths were noted.

Data recorded: For laboratory and greenhouse experiments, total number of germinated seeds was calculated on daily basis. Percentage emergences were calculated by the ratio of emerged seeds divided by total seeds in the percent. Time for 50% emergence was calculated with the following formula (Coolbear *et al.*, 1984).

$$T_{50} = t_i + \frac{\left(\frac{N-n_i}{2}\right)}{n_j - n_i} (t_j - t_i)$$

where, N is final number of the seed emerged and n_i and n_j are cumulative seeds number emerged by the adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.

Similarly, mean emergence time (MET) was calculated by using the following formula proposed by Ellis and Roberts (1981).

$$MET = \frac{\sum Dn}{\sum n}$$

where, n is the seeds number which were emerged on the day D and D is the number of days counted from germination beginning.

The germination index (GI) was calculated by taking total number of germinated seeds at first to final count / total number of days from first to final count. The seedling vigor index was recorded by the multiplication of

total means of shoot and root lengths with emergence percentage. Under wet condition the survived plants were separated from petri plates and root and shoot length of these plants were measured in centimeter by a measuring scale/tape and then the averaged. By using electric balance, fresh weight and dry weights were measured before and after drying in the oven at 70°C.

Statistical analysis

To analyze the data, Fisher's analysis of variance method was used and least significant difference (LSD) test at the probability level of 5% was used for comparing means of treatments (Steel *et al.*, 1997).

Results

Extracts of different plant parts and different concentrations:

Final seedling emergence percentage is an important trait which revealed potential of crop and helpful in getting target population. Impact of different plant parts of *M. indicus*, their extract concentrations and interaction exhibited significant results on the germination percentage of sorghum seeds (Table 1). Whole plant extracts showed least seed germination percentage of sorghum as compared to other plants part extracts which exhibited maximum germination percentage and seeds were immersed with distilled water followed by root, stem and leave extracts, respectively. The 5% and 20% concentration of *M. indicus* extract used with distilled water gave maximum and minimum germination percentage, respectively. 19.72% increase in germination, 5.15% increase in root growth and 4.23% increase in the shoot length was noticed with the root extract as compared to the whole plant extract of *M. indicus*. Those seeds which completed 50% emergence in earlier are vigorous with better seedling. During the study, time to the 50% emergence of sorghum seeds was significantly influenced by the impact, concentration and interactions of plants parts extracts of *M. indicus* (Table 1). The control treatment showed less time to obtain 50% emergence as compared to other plant parts. Influence of plant parts of *M. indicus* and their concentration significantly affected the mean emergence. It was observed that mean emergence time steadily influenced by plants parts of *M. indicus*.

In the same way, mean emergence time was delayed by plant parts concentration and minimum mean emergence time was recorded (Table 1). Results clearly showed that mean emergence time was increased by the increase in concentration of plant parts extract and it also revealed that emergence index steadily decreased by different plant parts extracts. With the increase in concentration of different plant parts extract; emergence index was reduced. Significant results were shown in the interaction and concentration of various plant parts extracts. Present study also revealed that increasing in concentration of different plant parts extract significantly decreased the vigor index of seedling. Fresh and dry seedlings weight of sorghum was also significantly influenced by the effect of concentration of various plants parts extract (Table 2).

Table 1. Effect of aqueous extracts of different plant parts and their different concentrations on the germination parameters of sorghum.

Treatments	Germination (%)	Mean germination time, days	Germination index	Time to 50% germination, days	Seedling vigor index
Plant extracts (E)					
Root	64.33 b	6.23 d	5.53 b	5.26 d	1147 b
Stem	51.13 c	7.20 c	5.06 b	6.24 c	881 c
Leaf	40.13 d	8.59 b	3.86 c	7.60 b	566 d
Whole plant	30.66 e	8.74 a	3.00 d	7.91 a	355 e
LSD (M)	2.06	0.12	0.72	0.020	168
Concentrations (C)					
0% (Control)	71.53 a	5.94 e	6.60 a	5.04 e	1591a
5%	62.93 b	6.58 d	5.73 b	5.68 d	1250 b
10%	53.53 c	7.36 c	4.86 c	6.40 c	928 c
15%	44.60 d	7.70 b	4.13 d	6.73 b	672 d
20%	35.86 e	7.99 a	3.26 e	7.05 a	455 e
LSD (S)	2.06	0.11	0.71	0.020	168

Mean values sharing different letter are significantly different to each other at 5% level

Table 2. Effect of aqueous extracts of different plant parts and their different concentrations on the growth attributes of sorghum.

Treatments	Root length (cm)	Shoot length (cm)	Plant fresh weight (g)	Plant dry weight (g)	
Plant extracts (E)					
Root	10.45 b	6.56 b	0.690 b	0.173 b	
Stem	10.02 b	6.32 b	0.666 b	0.166 b	
Leaf	7.81 c	4.94 c	0.521 c	0.129 c	
Whole plant	5.98 d	3.78 d	0.399 d	0.100 d	
LSD (M)	1.29	0.82	0.085	0.022	
Concentrations (C)					
0% (Control)	13.06 a	8.12 a	0.855 a	0.214 a	
5%	11.21 b	7.19 b	0.758 b	0.190 b	
10%	9.76 c	6.12 c	0.643 c	0.161c	
15%	8.10 d	5.08 d	0.536 d	0.134 d	
20%	6.28 e	4.00 e	0.423 e	0.105 e	
LSD (S)	1.27	0.81	0.085	0.021	
E × C Interaction					
Root extract	0% (Control)	13.00 cde	8.20 cd	0.863cd	0.216 cd
	5%	11.80 cdef	7.46 cdefg	0.786 cdefg	0.196cdef
	10%	11.00 defg	6.73 defghi	0.706defgh	0.176 defgh
	15%	9.53 fghi	5.80 ghijk	0.613 ghijk	0.153 fghij
	20%	6.93 ijkl	4.60 jklm	0.483 jklm	0.123 ijk
Stem extract	0% (Control)	13.90 bc	8.13 cde	0.860 cde	0.213 cd
	5%	10.86 efg	7.43 cdefg	0.780 cdefg	0.193cdef
	10%	10.06 fgh	6.36 efghij	0.670efghij	0.170 defghi
	15%	8.60 ghijk	5.46 hijkl	0.576 hijkl	0.146 ghij
	20%	6.66 jkl	4.23 klmn	0.446 klmn	0.110 jkl
Leaf extract	0% (Control)	11.26 cdefg	7.13 cdefgh	0.750 defgh	0.186 cdefg
	5%	9.40 fghij	5.93 fghijk	0.630 ghijk	0.156efghi
	10%	7.86 hijk	4.96 ijkl	0.523 ijkl	0.130 hijk
	15%	6.03 kl	3.83 lmn	0.403 lmn	0.100 kl
	20%	4.50 lm	2.83 mno	0.300 mno	0.073 lm
Whole plant extract	0% (Control)	9.50 fghij	6.03 fghijk	0.633 fghijk	0.160 efghi
	5%	7.93 hijk	5.00 ijkl	0.530 ijkl	0.133hijk
	10%	6.06 kl	3.83 lmn	0.403 lmn	0.103 kl
	15%	4.16 lm	2.63 no	0.276 no	0.070 lm
	20%	2.26 m	1.43 o	0.153 o	0.036 m
LSD (E × C)	2.83	1.83	0.954	0.048	

Mean values sharing different letter are significantly different to each other at 5% level

Table 3. Effect of aqueous extracts of different residues concentration, salinity levels and decomposition period on the emergence attributes of sorghum.

Treatments	Germination (%)	Mean germination time, days	Germination index	Time to 50% germination, days	Seedling vigor index
Residues concentration (C)					
0% (Control)	73.39	6.26	6.17	5.28	1825 a
2%	72.66	6.34	6.26	5.35	1559 b
4%	71.88	6.40	6.32	5.40	1279 c
LSD (M)	NS	Ns	Ns	Ns	60
Salinity levels (S)					
0% (Control)	89.66 a	5.23 c	5.17 c	4.42 c	2135 a
4%	78.66 b	5.97 b	5.89 b	5.04 b	1609 b
8%	50.22 c	7.80 a	7.69 a	6.58 a	918 c
LSD (S)	3.22	0.29	0.29	0.24	58

Mean values sharing different letter are significantly different to each other at 5% level; NS- not significant

Table 4. Effect of aqueous extracts of different residues concentration, salinity levels and decomposition period on the growth attributes of sorghum.

Treatments	Root length (cm)	Shoot length (cm)	Plant fresh weight (g)	Plant dry weight (g)
Residues concentration (C)				
0% (Control)	13.31 a	9.44 a	78.40 a	17.04 a
2%	9.80 c	8.27 b	68.65 b	14.92 b
4%	11.65 b	6.95 c	57.75 c	12.55 c
LSD (C)	0.10	0.25	0.62	0.13
Salinity levels (S)				
0% (Control)	13.88 a	9.85 a	81.77 a	17.78 a
4%	11.97 b	8.49 b	70.51 b	15.32 b
8%	8.91 c	6.32 c	52.52 c	11.41 c
LSD (S)	0.10	0.20	0.62	0.11

Note. Mean values sharing different letter are significantly different to each other at 5% level

Residue concentrations of *M. indicus*, salinity levels and decomposition periods: Final germination percentage (%) of sorghum seeds was significantly influenced by soil salinity but the impact of plant parts of *M. indicus* showed no significance results after two weeks of the decomposition (Table 3). Two salinity level such as 4% and 8% showed higher and less final germination percentage of sorghum seeds, respectively. The time taken to 50% emergence of seeds was not significant by effect of *M. indicus* and after decomposition of two weeks in soil, the interactive effect of *M. indicus* and soil salinity level also showed non-significant results. After four weeks of decomposition period, less time taken to 50% emergence was noted in zero soil salinity and where no *M. indicus* residues were incorporated into the soil; however, statistically it was equivalent to 2 and 4% residue concentration which was incorporated for decomposition.

Present study revealed that emergence attributes were not significantly affected from *M. indicus* and no plant residue and 8% salinity level represented maximum value of mean emergence. After decomposition of four weeks, 4% *M. indicus* residue and 8% salinity level exhibited minimum value of the mean emergence time than that of control treatment (Table 3). This is understandable from the results that increased level of salinity and *M. indicus*, when incorporated into the soil improved the emergence time and consequently decreased the unwanted effects of soil salinity and reduced the emergence time when left into soil for four weeks. The incorporation of the *M. indicus* residues into the soil did not significantly affected the seedling vigor index and the maximum seedling index vigor was noted at all

concentrations. Likewise, sorghum seedling vigor index was steadily affected by soil salinity. Maximum and minimum seedling vigor index were noted under no level of salinity and salinity level of 8%, respectively and the growth attributes showed inhibitory trend.

After two weeks of decomposition period, highest and lowest fresh weight of seedling was observed when zero and 4% of *M. indicus* plant residue was incorporated into the soil. Similarly, maximum and minimum seedling dry weight was also noted when zero and 4% of *M. indicus* residues were incorporated into the soil (Table 4). The plant parts of *M. indicus* significantly influenced seedling dry weight and highest value of dry weight was noted after the four weeks decomposition period. Zero percent *M. indicus* incorporation into the soil represented lower seedling dry weight. Similarly, dry weight of seedling was significantly altered by various salinity levels. Zero percent salinity and 8% salt concentration indicated maximum and minimum seedling dry weight, respectively. The interaction of different levels of salinity and *M. indicus* residue percentage incorporated into the soil was significant. The salt level at 0% and no *M. indicus* residue concentration showed maximum shoot length. After decomposition period of two weeks, minimum seedlings shoot length was observed with salinity level 8% and 2 or 4% of *M. indicus* residue concentration (Table 5). Seedling fresh weight was also affected by the concentration of various plants parts and the control treatment showed higher shoot length (8.91 cm). The concentration of various plant parts also influenced seedling shoot length as well as *M. indicus* plant parts and salt concentration steadily affected the seedling root length.

Table 5. Effect of aqueous extracts of different residues concentration (RC), salinity levels and decomposition period (DP) on the growth attributes of sorghum.

Treatments		Root length (cm)	Shoot length (cm)	Plant fresh weight (g)	Plant dry weight (g)	
2 weeks DP	0% RC	0% salinity level	16.25 a	11.53 a	95.73 a	20.81 a
		4% salinity level	14.12c	10.02 c	83.20 c	18.09 c
		8% salinity level	9.22 f	6.54 f	54.33 f	11.81 f
	2% RC	0% salinity level	14.45 b	10.25b	85.13b	18.51 b
		4% salinity level	12.11 d	8.59 d	71.36 d	15.51 d
		8% salinity level	8.72 g	6.19 g	51.40 g	11.17 g
	4% RC	0% salinity level	11.27 e	8.00 e	66.40 e	14.43 e
		4% salinity level	9.34 f	6.63 f	55.03 f	11.96 f
		8% salinity level	8.80 g	6.24 g	51.83 g	11.26 g
4 weeks DP	0% RC	0% salinity level	17.13 d	11.59 d	99.69 d	23.03 d
		4% salinity level	13.87 f	9.38 f	80.73 f	18.65 f
		8% salinity level	9.58 i	6.48 i	55.77 i	12.88 i
	2% RC	0% salinity level	20.82 b	14.08 b	121.15 b	27.99 b
		4% salinity level	15.92 e	10.77 e	92.65 e	21.40 e
		8% salinity level	11.51 h	7.79 h	66.99 h	15.48 h
	4% RC	0% salinity level	23.85 a	16.14 a	138.80 a	32.07 a
		4% salinity level	18.13 c	12.26 c	105.49 c	24.37 c
		8% salinity level	12.56 g	8.50 g	73.10 g	16.89 g
LSD (C × S × T)		0.78	0.53	4.56	1.05	

Mean values sharing different letter are significantly different to each other at 5% level

Discussion

Poor performance of seeds was recorded when the seeds were treated with different concentration of *M. indicus* plants parts, because different allelochemicals present in *M. indicus* hinder beginning process of enzyme and cell expansion process. Germination of various weed species hindered by alfalfa water extract as it had hindering allelopathic matter available in the extract (Sathishkumar *et al.*, 2020). Rise in allelochemicals concentration like coumaric acid, phenolic and flavonoids compounds have the hindering effect on germination features at maximum concentration of weed extract (Leela, 1995; Beres & Kazinczi, 2000; Raza *et al.*, 2019). Seeds treated with distilled water reduced the time to 50% emergence which is because of quicker metabolism of seed stockpile, quicker making of germination metabolites and greater metabolic actions. The existing allelochemicals in *M. indicus* solution overdue the beginning of metabolic actions in germination and seed influences weak membrane integrity which cost in late germination and weak emergence and consequently, seeds taken more time to germinate and emerge. The seeds treated with distilled water reduced the mean time of emergence and greater emergence index which was resulted due to early activation of enzymes, quicker collapse of food stockpile, quicker formation of metabolites mandatory for germination process and greater metabolic action. The greater mean emergence time and lesser emergence index was observed when seeds were treated with the different plant parts of *M. indicus* and this is due to collapse of earlier beginning of hydrolytic enzymes of seeds. Consequently, the greater emergence time and lesser emergence index was shown by the seeds. Allelochemicals are famous for modifying plant growth and development, comprising of germination and earlier seedling growth. The seedlings treated with various concentrations of plant parts of *M. indicus* lowered seedling vigor index which might be due to the availability of allelochemicals in the solution. It also reduced final percentage of germination and lowered shoot

and root length because cell differentiation, cell division, water status, ion and water uptake, phytohormones metabolism, photosynthesis, respiration, signal transduction, gene expression and enzyme function are significantly affected by allelochemicals (Macías *et al.*, 1992; Inderjit & Nielsen, 2003).

Melilotus is a familiar weed found in various crops. Some findings suggest that allelopathic effect of this weed is because of a few secondary metabolites which intermixed with each other and allelochemicals (Olofsdotter *et al.*, 1995; Raza *et al.*, 2019). A few well-known allelochemicals are as followed; ferulic and phenolic acid as well as o-hydroxyphenylacetic acid (Chung *et al.*, 2001). These allelochemicals act as inhibitory agents of seedling growth, seed germination and weeds such as barnyardgrass (Chung *et al.*, 2002). The lower dry and fresh weight and lesser root and shoot length were observed when seedlings were immersed with different plant parts water extract of *M. indicus*. The allelochemicals present in *M. indicus* hinder plant growth; as a result, lower dry and fresh weight, lesser shoot and root length was recorded. More hindering effect was observed by whole plant extract than other plants parts and this significantly decreased shoot length, root length, dry and fresh weight of the seedlings. Our study overlaps the results revealed by Mousavi *et al.*, (2013) who stated that various plant parts of *melilotus* significantly affected the shoot and root length of wheat seedlings by aggregating allelopathic concentrations. The greater shoot length was observed under 10 g/L stem and leaf extracts followed by control than that of concentrations where 30 and 50 g/L *melilotus* stem and leaf extract showed reduction in shoot length. This study revealed that sorghum root and shoot length is highly vulnerable to the whole plant extract as of leaf, root or stem extracts. The root extract of *M. indicus* did not decrease root and shoot length but root length of *Trianthema portulacastrum* was influenced by sorghum plant part extracts and significantly decreased by 75 and 100% (Randhawa *et al.*, 2002). This hindering effect was due to allelopathic matter present in the aqueous extract of sorghum.

The decreasing trend in shoot growth of seedlings was very much comparable to root growth. Leaf extract of *melilotus* was best useful inhibitor for shoot growth of seedlings. The 80% reduction in seedlings shoot length was recorded at the concentration of 50 g/L leaf extract than control (Al-Turki *et al.*, 2003). As a conclusion, whole plant extract of *M. indicus* at concentration of 20% showed greater hindering impact as compared to various plant parts water extract. Likewise, seeds treated with distilled water with no salinity stress showed greater germination percentage, minimum time to achieve 50% emergence, mean emergence time, higher seedling vigor index and emergence index in the second experiment. The reason is might be freely uptake of water by seeds which caused enlargement of the seeds. Seeds showed less time to germinate, mean emergence time and time to 50% emergence because of the quicker metabolites production which are needed for the germination of seeds and greater metabolic actions (Ashraf & Foolad, 2005).

The lower germination percentage, maximum time to 50% emergence, mean emergence time, lower seedling vigor index and emergence index was recorded when seed was treated with different concentrations of salinity. It was because of salinity stress which decreased the capacity of seeds to uptake water and cost in lessening germination rate (Rani, 2011). The seeds and seedlings are highly sensitive to salinity stress and rise in membrane damage and decrease in germination rate is due to reactive oxygen species (ROS). Plant growth and development is noticeably limiting to salinity stress which is a major abiotic stress. The reduction in percentage with higher salt stress is because of external osmotic potential which halts uptake of water due to harming impact of sodium and chloride ions (Tabatabaei & Angholi, 2012).

Higher salinity rates significantly decreased shoot and root length, dry and fresh weight of a few sorghum varieties (Kandil *et al.*, 2012). Salinity level more than 10 dS/m considerably reduced the germination rate and with the increase of salinity level; root and shoot length of a few forage sorghum varieties was also reduced (Tabatabaei & Angholi, 2012). These outcomes are also overlapping to results revealed by Reddy & Vora (1983) that increased NaCl significantly reduced radicle length. The fresh and dry weight of sorghum and millet were also reduced with higher salinity concentration (Al-Hatlani, 1995). The germination percentage and dry weight significantly decreased under water salinity up to 8 dS/m. The same findings were also described by Datta *et al.*, (2009) who stated that wheat seedlings showed reduction in fresh weight and height of wheat when NaCl concentration was increased in Hoagland's solution. Higher salinity level reduced germination percentage because of hindrance in germination rate. It correlated with former findings that seeds germinate good in control treatments and germination percentage reduced with the increase of salinity level (Duan *et al.*, 2007). Sneha *et al.*, (2013) also stated that increase in level of salinity reduced germination percentage in millet as of control treatment.

Conclusion

Different water extracts of *M. indicus* plant parts at different concentrations showed alleopathic potential on the germination and seedling growth of sorghum. More sorghum seeds were germinated than the seeds which

were treated with various concentrations of *M. indicus* plant parts. The extract of whole plant has more suppressive impact against the sorghum germination as compared to other plant parts. In greenhouse experiments only *M. indicus* residue concentration and decomposition period did not affected the seed germination and growth of sorghum. However, soil salinity at different concentration significantly affected the germination as well as growth of sorghum crop while higher salt concentration inhibited both seed germination and seedling growth of sorghum.

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Contribution

H.H. Ali supervised and planned the whole study, M.S. Zaheer and Z. Ismail involve in planning and execution of the experiment and write discussion section, Z. Ismail involve in data collection and analysis, A. Raza write the detailed methodology, M. Aasim write the manuscript, M. Adnan and M. Aasim write discussion and review the paper, K. Ikram improve the technical and English language of the paper, M.A. Bodlah review whole manuscript and improve the paper.

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