Study of seed germination responses under stress conditions in accessions of genus *Mollugo* Linn. of Western Rajasthan

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Abstract

The genera of Molluginaceae have great herbal medicinal value having vermicidal, insecticidal, anthelmintic and antibacterial properties. The present study was aimed to investigate and compare the seed germination requirements in terms of temperature and salt stress in *Mollugo cerviana* Linn. and *Mollugo nudicaulis* Lamk. Interspecific variability in germination responses under various treatments (temperature, sodium salts, nitrogenous salts, thiourea and sucrose) for both the species of *Mollugo* Linn. were studied. Seeds were scarified in 95% Sulphuric acid (5, 10, 15, 20, 25, 30, 45 and 60 min. duration) in addition to mechanical rubbing for breaking the seed coat dormancy and subjected to temperature stress by treating with dry heat in preheated oven (70^0C for 1,2 and 4 hrs.; 70^0C for 1,2,3 and 4 days and 100^0C for 5,10,15,30 and 45 min. incubation), hot water (5, 15, 30, 60, 90, 120 and 150 min. incubation) and cold treatment by storing in refrigerator at a temperature of 2-5^0c for 5, 10, 30 and 60 min.; 3, 6 and 12 hours and 1,2,4,8,15 and 30 days. Seeds were also given salinity stress by treatment with 5, 10, 25, 50, 100, 200 and 300 mM of various sodium salt (NaCl, Na_2CO_3, Na_2SO_4 and $NaHCO_3$) and 10, 25, 50, 75 and 100 mM of various nitrogenous salts (KNO_3, $NaNO_3$, NH_4Cl and NH_4NO_3). Some treatments of germination promoting chemicals (thiourea and sucrose) were also given to seeds. The germination response was significantly different between different level of treatments applied ($P< 0.05$). It was observed that seeds of *Mollugo nudicaulis* Lamk. were more responsive in comparison to the seeds of *Mollugo cerviana* Linn. in all of the salt stress treatments. Seed treatments with dry heat, hot water and cold treatment hardly showed any response on breaking of seed dormancy.

Keywords: *Mollugo* Linn., Germination responses, Salt stress, Temperature stress, Thiourea, Sucrose.

1. Introduction

Plants inhabiting Western Rajasthan are adapted to a variety of environmental stresses (i.e. extreme aridity, high salinity, extreme temperature and deficiencies of nutrients in soil) and seed germination is one of the most critical events subjected to environmental control in plant life.

The family Molluginaceae, also called as carpetweed family, includes several herbal weed species that are well represented in Western Rajasthan. The genus *Mollugo* Linn. has a great herbal and medicinal importance and there is very little work on genetic diversity in relation to multiple stressors reported so far. The species are

normally adapted to either high or low temperatures in anatomical, biochemical, and physiological features (Kennedy et al. 1980; Arora et al. 2017).

In phylogenetically related species such as congeneric species, it is well known that several environmental factors (e.g., light, temperature, moisture, salt and soil composition) have direct impact on germination (Ellison 2001; Sivasankarmoorthy et al. 2010). Several studies have highlighted the presence of inter and intra- specific variations in seed germination and dormancy (Andersson and Milberg 1998; Murru et al. 2015; Santo et al. 2015a, 2015b), attributing these phenomena to environmental differences and genetic variations or both (Degreef et al. 2002; Cruz et al. 2003; Yao et al. 2010). The germination responses of plant species can be suggestive of relationship among plants and support the assignment of taxa in respective groups based on phenotypic characters.

In particular, salt may inhibit seed germination either by creating a low osmotic potential, which prevents water uptake or through the toxic effect of Na^+ and Cl^- ions on metabolic process (Khajesh et al. 2003; Kaya et al. 2006; Murru et al. 2015). The annual plants that produce seeds only once, seed germination response to environmental condition is crucial for recruitment of taxa (Tobe et al. 2005). Salt stress can cause change in the germination regulating mechanism, thereby inducing a physiological secondary dormancy (Ungar 1995; Gulzar et al. 2001; El-Keblawy 2004).

Temperature is also critical in determining successful establishment of plants because it has significant effect on onset, potential and rate of germination of various species (Gorai and Neffati 2007; Zehra et al. 2013; Baskin and Baskin 2014). Seed germination occurs between minimum and ceiling threshold temperatures and the highest germination percentage is at the optimal temperature (Huang et al. 2003; Dürr et al. 2015). It has a dual effect on seed germination and regulation on seed dormancy. The germination percentage and rates increases when temperature lies within ambient range (Fenner and Thompson 2005; Lai et al. 2016).

Therefore, this study was conducted to investigate the interspecific variability by comparing seed germination responses under various treatments of temperature, nitrogenous salts, sodium salts, thiourea and sucrose for both the species of *Mollugo* Linn. i.e. *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn.

2. Material and methods

2.1 Acquisition and collection of germplasm

The mature plants of the *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn. were collected from various sites of the Western Rajasthan (Plate 1 and Table 1).

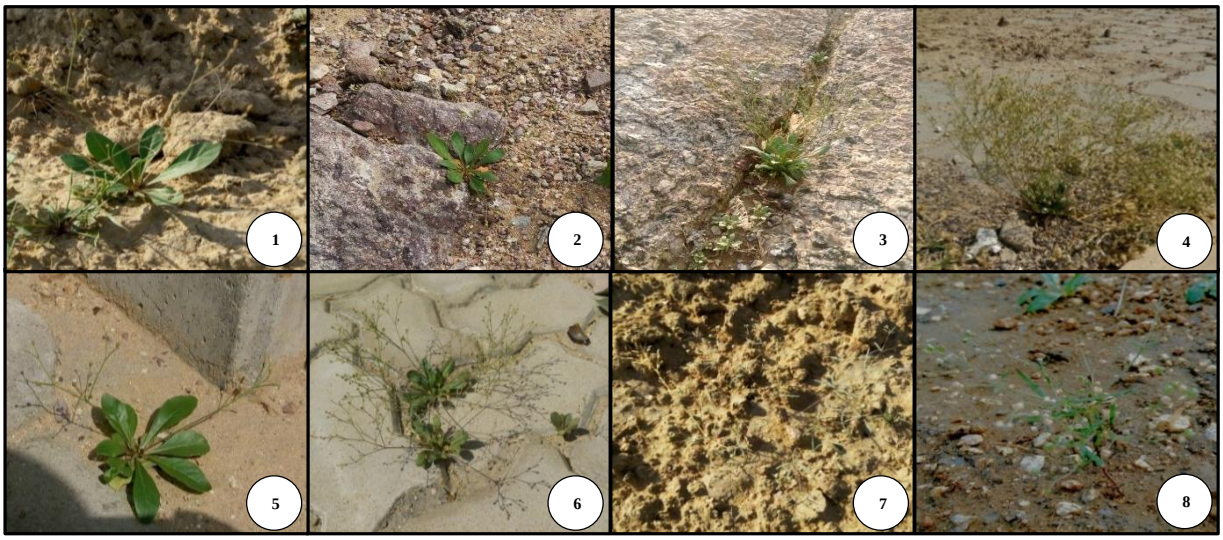

Plate 1: Various natural habitats and collection sites of *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn.

1. ***Mollugo nudicaulis* Lamk. – Agriculture Region, Mandore, Jodhpur.**
2. ***Mollugo nudicaulis* Lamk. – Hilly Region, Siddhanath Mahadev, Jodhpur.**
3. ***Mollugo nudicaulis* Lamk. – Hilly Region, Village- Sena, Distt.-Pali.**
4. ***Mollugo nudicaulis* Lamk. – Gravel Region, Machia Biological Park, Jodhpur.**
5. ***Mollugo nudicaulis* Lamk. – Gravels along to the pedestrian path, Masuria, Jodhpur.**
6. ***Mollugo nudicaulis* Lamk. – Cracks among cemented tiles on pedestrian path, Shastri Nagar, Jodhpur.**
7. ***Mollugo cerviana* Linn. – Agriculture field, Mandore, Jodhpur.**
8. ***Mollugo cerviana* Linn. – Hilly Region, Village- Sena, Distt.-Pali.**

2.2 Screening of seed sample

After the collection of plants, seeds were separated and the undesired material including unripe/damaged seeds was separated. After screening only ripened, undamaged and apparently healthy seeds were selected and stored in polythene bags under controlled conditions (28^0c ±2) for a period of 7 days. Now each seed sample was cleaned by rinsing with distilled water and then surface sterilized by 0.1% $HgCl_2$ solution and again washed 5 times with distilled water.

Viability of seeds was confirmed by tetrazolium viability test using 0.1% solution of 2, 3, 5- tri-phenyl tetrazolium chloride (Porter et al. 1947; Wharton 1955; Verma et al. 2013) (Plate 2 and 3). For better visualization, seeds were bleached with commercial bleach ($NaOCl_2$+Triton X- 100) before T.Z. test and cleaning agent lacto phenol after T.Z. test (Verma et al. 2013) (Plate 4). Only viable, mature, healthy and uniform in size seeds were selected for germination experimental set up.

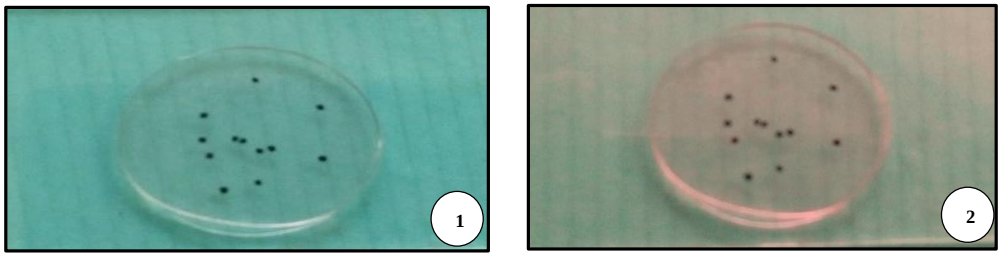

Plate 2: Seeds of *Mollugo nudicaulis Lamk.* : 1. Before viability test 2. After viability test

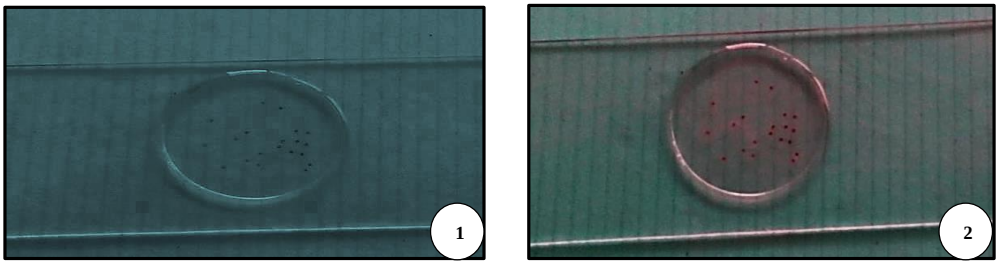

Plate 3: Seeds of *Mollugo cerviana Linn*. : 1. Before viability test 2. After viability test

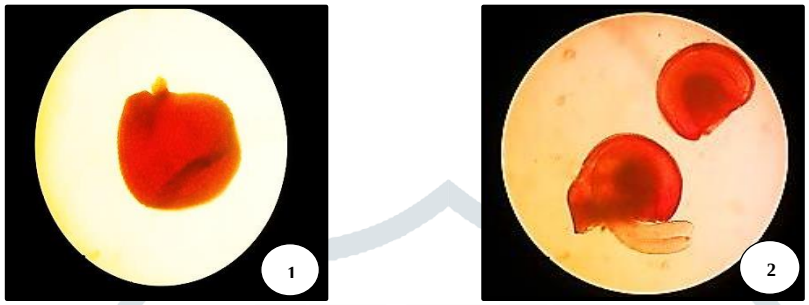

Plate 4: Microscopic view of seeds after viability test: 1. *Mollugo nudicaulis Lamk.* 2. *Mollugo cerviana Linn.*

2.3 Seed germination experiments

Seed germination studies were performed in sterilized petri dishes (5 cm. diameter) lined with a single layer of Whatman no.10 filter paper moistened with 3 ml of distilled water. The experiments were carried out at 28±2^{0}C., arranged in a completely randomized design being in triplicate and 30 seeds per replicate (petri dish). Germination counts were recorded every day and the emergence of the radicle was taken as a criterion for germination.

2.4 Acid scarification

Seeds of *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn. were soaked in 95% Sulphuric acid (H_2SO_4) for 5, 10, 15, 20, 25, 30, 45 and 60 minutes at room temperature in addition to mechanical rubbing with sand paper. After the duration of incubation in acid, seeds were removed and rinsed several times with distilled water and placed in germination set up.

2.5 Temperature stress

2.5.1 Dry heat treatment

Seeds of *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn. were taken in petri-dishes in a preheated oven for exposure to set temperature and duration, i.e. 70^{0}C for 1,2 and 4 hrs.; 70^{0}C for 1,2,3 and 4 days and 100^{0}C for 5,10,15,30 and 45 min. After incubation in each treatment, the seeds were immediately cooled and then placed for germination.

2.5.2 Hot water treatment

Seeds of *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn. were placed in boiling water for 5, 15, 30, 60, 90, 120 and 150 min. After incubation, seeds were taken out from boiling water and kept at room temperature (28±2^{0}c) to cool down and placed for germination.

2.5.3 Cold treatment

Seeds of *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn. were stored in a refrigerator at a temperature of 2-5^{0}c for 5, 10, 30 and 60 min.; 3, 6 and 12 hours and 1, 2, 4,8,15 and 30 days.

2.6 Salt stress

2.6.1 Sodium salt treatment

Seeds of *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn. were treated with various concentrations i.e. 5, 10, 25, 50, 100, 200 and 300 mM of different sodium salts (NaCl, Na_2CO_3, Na_2SO_4 and $NaHCO_3$) at room temperature till seeds show germination.

2.6.2 Nitrogenous salts treatment

Seeds of *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn. were treated with four nitrogenous salts i.e. potassium nitrate (KNO_3), sodium nitrate ($NaNO_3$), ammonium chloride (NH_4Cl) and ammonium nitrate (NH_4NO_3) with various concentrations i.e. 10, 25, 50, 75 and 100 mM of each at room temperature to observe the germination response.

2.7 Germination promoting chemicals

2.7.1 Thiourea treatment

Seeds of *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn. were treated with thiourea alone and thiourea in combination with potassium nitrate (KNO_3) of 50 mM concentration of each at room temperature to observe the germination response.

2.7.2 Sucrose

Seeds of both the species of *Mollugo nudicaulis* Linn. were treated with various molar concentrations i.e. 0.1M, 0.5M and 1.0M of sucrose at room temperature till initiation of germination.

Observations were made on germination percentage and median germination time (T_{50}) for both species of *Mollugo* Linn. The germination percentage was computed using formula given by international seed testing association (ISTA 2015) which is mentioned as follows-

$$\mathbf{Gp = (n_g/n_t) \times 100}$$

Where $\mathbf{n_g}$ is the number of germinated seeds and $\mathbf{n_t}$ is the total number of seeds. Similarly median germination time (T_{50}) is calculated using formula given by Coolbear et al. (1984) and modified by Farooq et al. (2005) as follows-

$$\mathbf{T_{50} = t_i + [(n/2 - n_i)(t_j - t_i)] / n_j - n_i}$$

Where, $\mathbf{T_{50}}$ is the median germination time, **n** is the final number of germinated seeds and $\mathbf{n_i}$ and $\mathbf{n_j}$ are the total number of seeds germinated in adjacent count at time $\mathbf{t_i}$ and $\mathbf{t_j}$ respectively, when $\mathbf{n_i < n/2 < n_j}$ (Aravind et al. 2019). All observed data regarding to germination response are subjected to one-way analysis of variance (ANOVA) and Tukey's post-hoc test is used to determine significant differences among treatments. All statistical analysis were performed using the data analysis tool of MS Excel (Microsoft Office Professional Plus 2010).

3. Result and discussion

The seeds of both *Mollugo* Linn. species are very minute with hard seed coat and they took almost one month to germinate in case of *Mollugo nudicaulis* Lamk. while correspondingly 45±2 days were needed to break dormancy in case of *Mollugo cerviana* Linn. at room temperature in normal conditions. The germination responses were significantly variable between all stress treatments showing P value less than 0.05 in both *Mollugo* Linn. species. It is found that *Mollugo cerviana* Linn. is less responsive in comparison to *Mollugo nudicaulis* Lamk. during all stress experiments.

3.1 Effect of acid scarification

Scarification and low pH treatment is the effective method to remove dormancy caused by hard seed coat (Munawar et al. 2015; Chaves et al. 2017; Kumari and Gehlot 2018). In our study, seeds of *Mollugo* Linn. species were treated with 95% H_2SO_4 for 5,15,30,45 and 60 minutes. During this acid scarification treatment, it is found that one hour treatment is most effective period for both *Mollugo* Linn. species than other durations. These acid scarified seeds had maximum germination percentage as well as T_{50} to respond in both species as compared to control. According to germination data shown in Table 2, it is recorded that the seed samples of *Mollugo nudicaulis* Lamk. responded earlier than the seed samples of *Mollugo cerviana* Linn. Germination response is significantly different between various treatments of 95% sulphuric acid showing P value less than 0.05 for different time intervals (Fig. 1 and 2)

Species	Treatments (min.)	Germination (%)	T_{50} (d)
***Mollugo nudicaulis* Lamk.**	Control	00.00	16.03
	5	00.00	15.00
	15	20.00	12.50
	30	40.00	11.00
	45	80.00	10.00
	60	96.67	07.50
***Mollugo cerviana* Linn.**	Control	00.00	21.42
	5	00.00	20.00
	15	23.33	16.50
	30	33.33	15.00
	45	80.00	12.50
	60	93.33	10.00

Table 2: Effect of acidic scarification with 95% H_2SO_4 on seed germination in *Mollugo* Linn. species

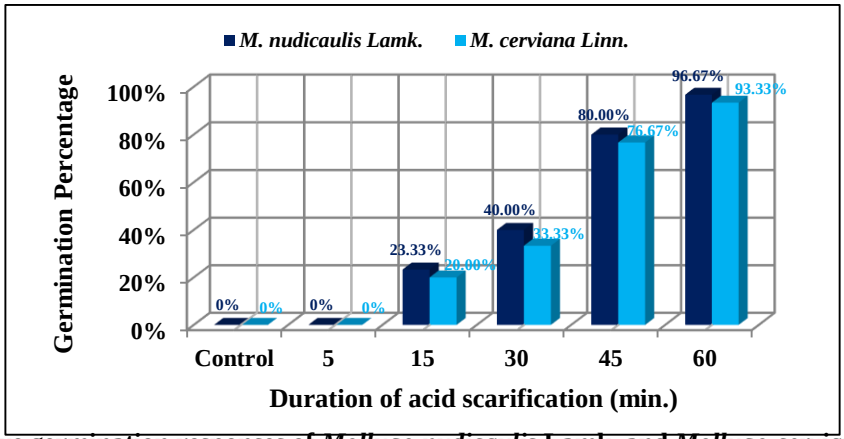

Fig. 1: Comparative germination responses of *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn. after scarification with 95% sulphuric acid

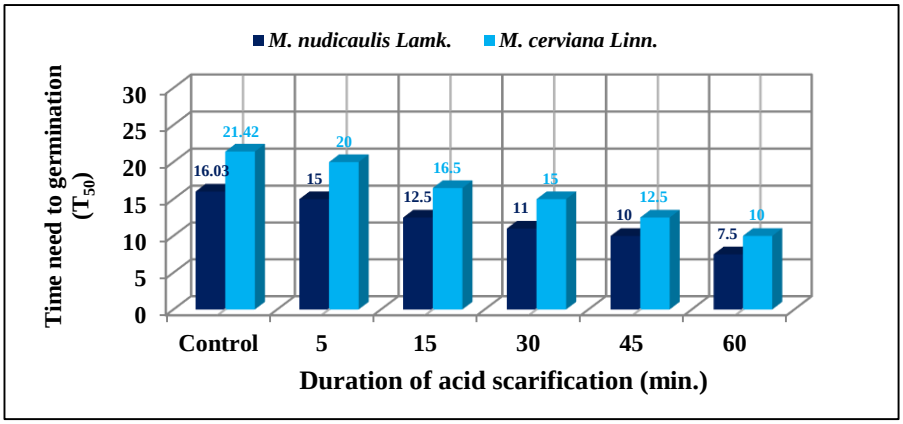

Fig. 2: Comparative duration of median germination time (T_{50}) of *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn. after scarification with 95% sulphuric acid

3.2 Effect of temperature stress

Seed treatments with dry heat, hot water and cold treatment barely showed any response on germination in both *Mollugo* Linn. species. All of them successfully responded after acidic and mechanical scarification.

3.3 Effect of sodium salt stress

Seed germination is the most critical and sensitive stage to salinity/sodicity stresses. Singh and Prasad (2009) explained that the growth inhibition in plant under salt stress was due to disturbed balance of hormones through alteration in osmotic system. The inhibitory effect of high salinity on germination of desert plants has been reported in various studies (Duan et al. 2007; Wei et al. 2008; Guma et al. 2010; El-Keblawy et al. 2011).

In present investigation the replicates of seed samples of both *Mollugo* Linn. species were separately treated with solution of various molar concentrations of various sodium salts (NaCl, Na_2CO_3, Na_2SO_4 and $NaHCO_3$) in increasing order. The used concentrations of various sodium salts are 5,10,25,50,100,200 and 300 mM respectively. Salinity revealed the significant inhibitory effect on seed germination of both species of *Mollugo* Linn.

The interpretation of recorded data shown in Table 3, reveal that germination response reduces with increase in sodium salt concentration in both species of *Mollugo* Linn. Germination response is significantly different between various treatments of sodium salts showing P value less than 0.05 (Fig. 3 and 4). There is the significant inhibitory effect found on seed germination of both species of *Mollugo* Linn. with increase in salinity stress. Germination inhibition was in following order $NaHCO_3 > Na_2SO_4 > Na_2CO_3 > NaCl$ (Fig. 5 and 6).

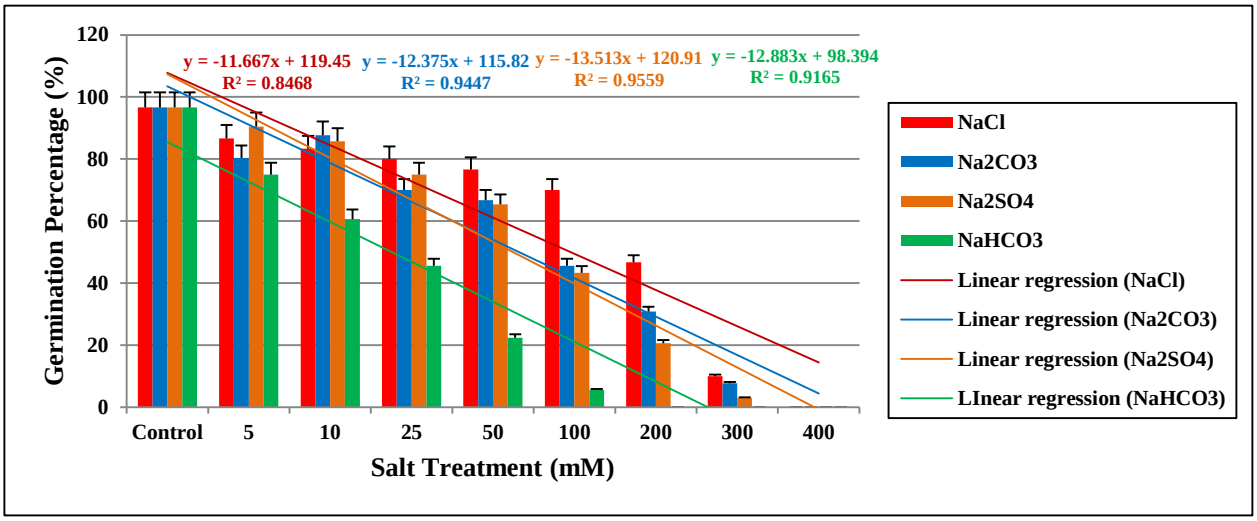

Fig. 3: Comparative germination responses of *Mollugo nudicaulis* Lamk. against different molar concentrations of various sodium salts with their R^2 values calculated by linear regression

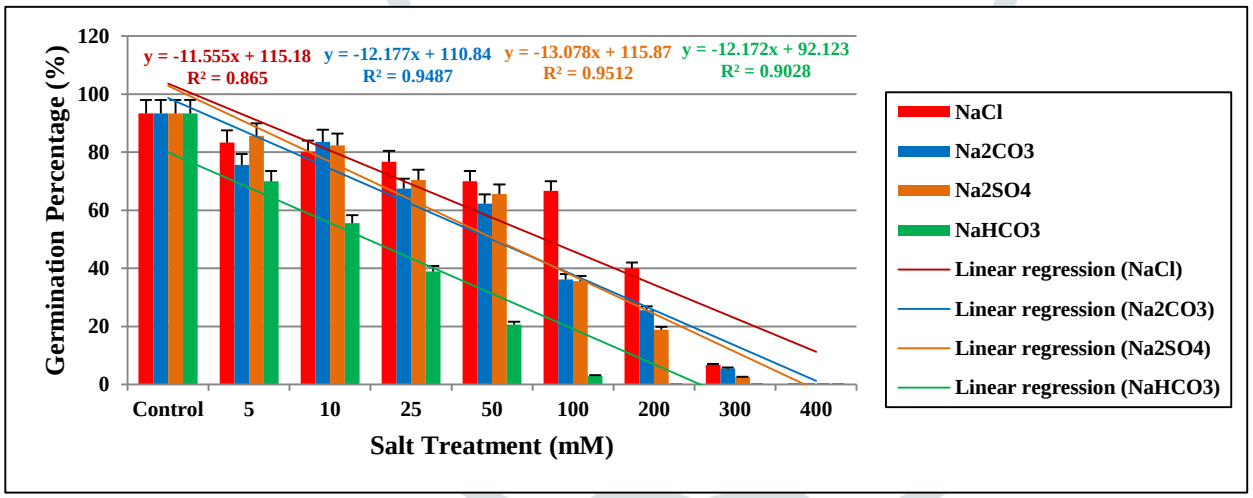

Fig. 4: Comparative germination responses of *Mollugo cerviana* Linn. against different molar concentrations of various sodium salts with their R^2 values calculated by linear regression

Species	Treatments (conc. in mM)	Seed Germination Percentage (%)				Time need to germination in days (T_{50})			
		NaCl	Na_2CO_3	Na_2SO_4	$NaHCO_3$	NaCl	Na_2CO_3	Na_2SO_4	$NaHCO_3$
Mollugo nudicaulis Lamk.	Control	96.67	96.67	96.67	96.67	16.03	16.03	16.03	16.03
	5	86.67	80.33	90.47	75.00	16.15	16.25	16.01	16.28
	10	83.33	87.67	85.67	60.66	16.20	16.17	16.15	16.52
	25	80.00	70.00	75.00	45.57	16.25	16.43	16.28	17.14
	50	76.67	66.67	65.33	22.33	16.30	15.00	14.90	20.50
	100	70.00	45.55	43.33	05.57	16.43	16.25	16.00	28.00
	200	46.67	30.85	20.65	00.00	17.14	25.50	21.25	30.00
	300	10.34	07.75	03.00	00.00	25.00	27.00	29.00	30.00
	400	00.00	00.00	00.00	00.00	30.00	30.00	30.00	30.00

Mollugo cerviana Linn.	Control	93.33	93.33	93.33	93.33	21.42	21.42	21.42	21.42
	5	83.33	75.57	85.67	70.00	21.60	21.72	21.50	21.90
	10	80.00	83.55	82.33	55.57	21.66	21.60	21.60	22.85
	25	76.67	67.47	70.47	38.88	21.73	21.95	21.90	23.30
	50	70.00	62.33	65.57	20.57	21.90	22.15	22.00	30.00
	100	66.67	36.17	35.55	03.00	22.00	22.75	22.75	43.00
	200	40.00	25.57	18.87	00.00	23.33	28.25	33.75	45.00
	300	06.67	05.55	02.50	00.00	40.00	40.25	43.50	45.00
	400	00.00	00.00	00.00	00.00	45.00	45.00	45.00	45.00

Table 3: Effect of various Sodium salt treatments on seed germination in *Mollugo* Linn. species

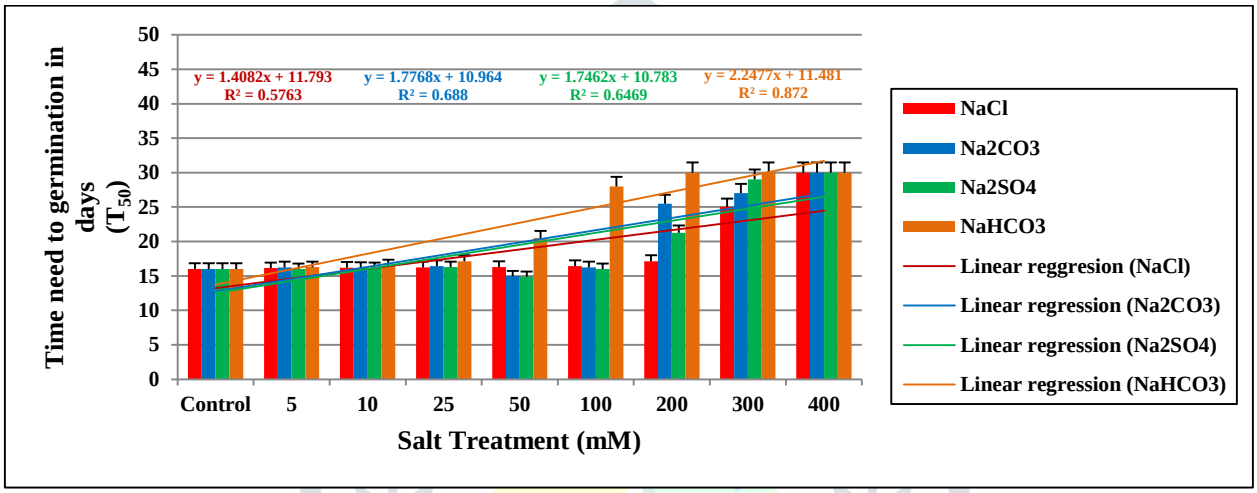

Fig. 5: Comparative duration of median germination time (T_{50}) of *Mollugo nudicaulis* Lamk. at different molar concentrations of various sodium salts with their R^2 values calculated by linear regression

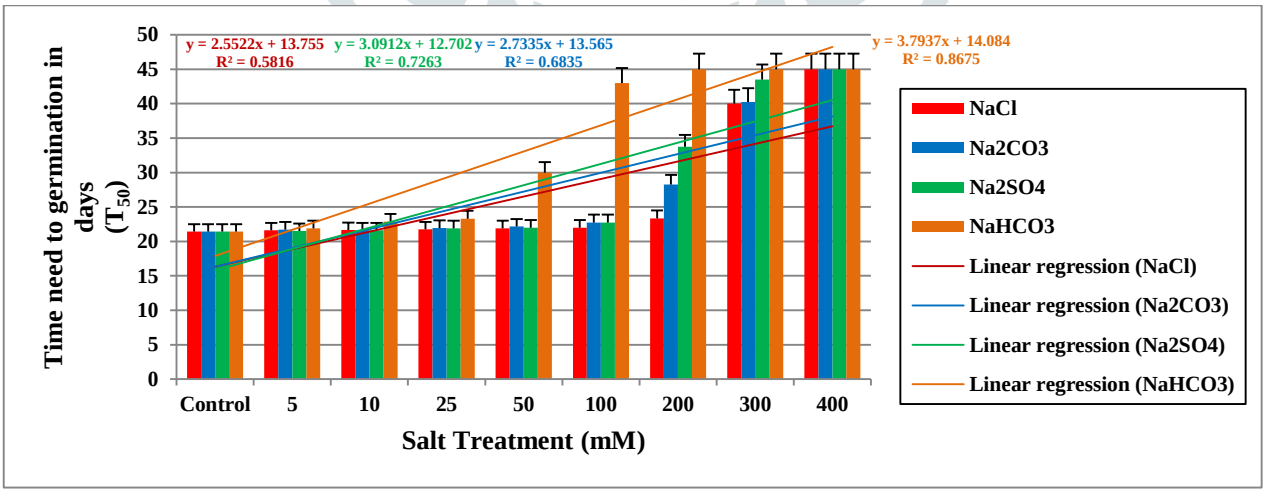

Fig. 6: Comparative duration of median germination time (T_{50}) of *Mollugo cerviana* Linn. Lamk. at different molar concentrations of various sodium salts with their R^2 values calculated by linear regression

In case of *Mollugo nudicaulis* Lamk., only 10.34% germination occurred at 300mM concentration of NaCl and consumed nine days more germination time as compare to control. Similarly in case of *Mollugo cerviana*

Linn., only 06.67% germination found at 300mM concentration of NaCl and consumed nineteen days more germination time as compare to control. These facts indicate that seeds of *Mollugo cerviana* Linn. are more sensitive against salt stress and remained less reactive as compare to *Mollugo nudicaulis* Lamk. seeds.

3.4 Effect of nitrogenous salts

The nitrogenous compounds, and more specifically potassium nitrate (KNO_3), are reported to promote seed germination (Khan 2003; Atia et al. 2009; Zehra et al. 2013).

Species	Treatments (conc. in mM)	Seed Germination Percentage (%)				Time need to germination in days (T_{50})			
		KNO_3	$NaNO_3$	NH_4Cl	NH_4NO_3	KNO_3	$NaNO_3$	NH_4Cl	NH_4NO_3
***Mollugo nudicaulis* Lamk.**	Control	00.00	00.00	00.00	00.00	16.03	16.03	16.03	16.03
	10	87.67	69.33	65.25	38.75	7.43	7.55	7.98	12.43
	25	92.55	76.67	70.00	42.66	6.42	6.5	6.8	10.52
	50	96.67	80.00	76.67	46.67	5.34	5.42	5.43	8.57
	75	90.00	75.55	68.25	40.00	7.55	7.88	8.03	13.5
	100	85.25	63.33	58.88	35.55	12.52	12.66	12.88	15.55
***Mollugo cerviana* Linn.**	Control	00.00	00.00	00.00	00.00	21.42	21.42	21.42	21.42
	10	75.50	58.25	55.55	20.00	14.75	18.67	18.95	20.22
	25	84.25	65.50	62.25	25.50	12.25	17.5	17.75	19
	50	90.00	70.00	66.67	33.33	10.74	16.42	16.5	18
	75	80.00	60.00	58.88	22.25	13.42	17.25	17	19.5
	100	70.50	55.55	50.00	15.75	15.5	19.75	20	21.25

Table 4: Effect of several nitrogenous salts on seed germination in *Mollugo* Linn. species

The data shown in Table 4 indicates that there is a significant variation found in germination response of the species of *Mollugo* Linn. when the seeds are treated with various concentrations i.e. 10, 25, 50, 75 and 100 mM of each solution of different nitrogenous salts (KNO_3, $NaNO_3$, NH_4Cl and NH_4NO_3). *Mollugo nudicaulis* Lamk. took just half or less time and 6-18% more germination as compared to *Mollugo cerviana* Linn. The treatment with KNO_3 shows that there is rapid response in germination in both species of *Mollugo* Linn. In case of *Mollugo nudicaulis* Lamk. the response was three times early as compare to control while *Mollugo cerviana* Linn. responded two times early than control (Fig.7 and 8).

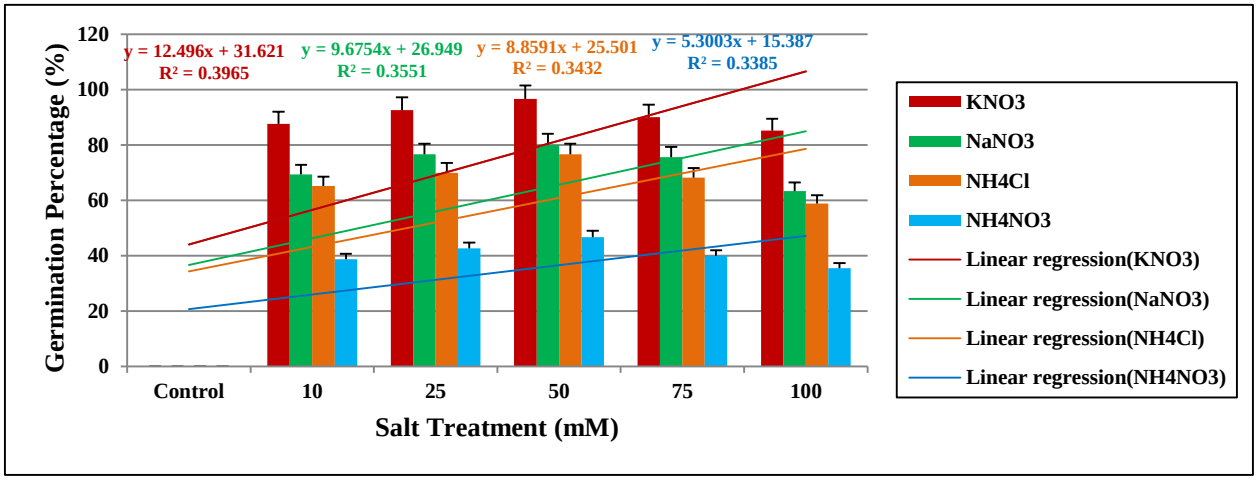

Fig. 7: Comparative germination responses of *Mollugo nudicaulis* Lamk. against different nitrogenous salt with their R^2 values calculated by linear regression.

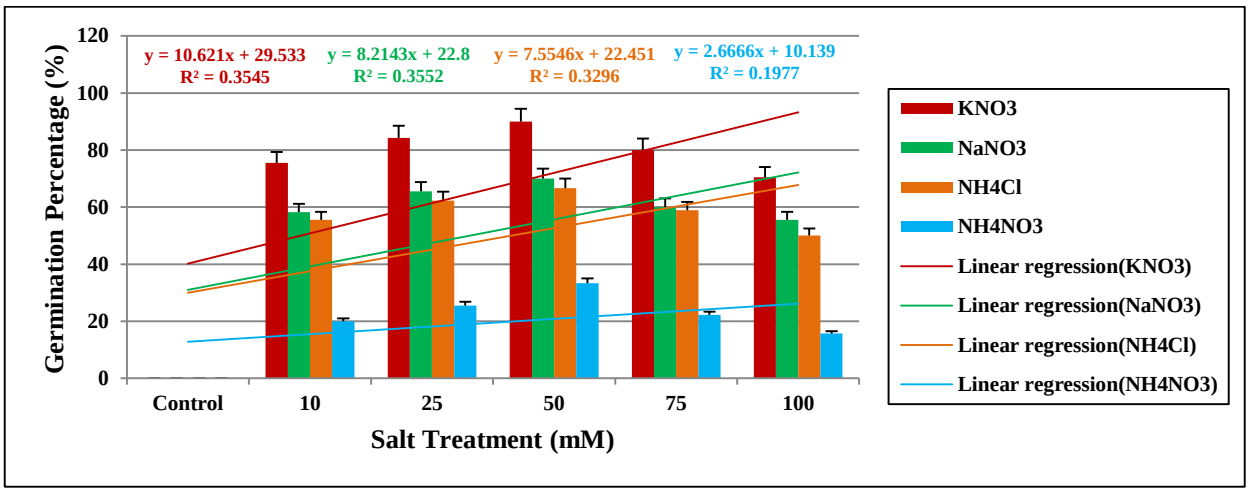

Fig. 8: Comparative germination responses of *Mollugo cerviana* Linn. against different nitrogenous salt with their R^2 values calculated by linear regression

It is observed that potassium nitrate (KNO_3) gave maximum germination response with lowest median germination time (t_{50}) value as compare to sodium nitrate ($NaNO_3$) that means potassium is more effective for germination than sodium with combination of nitrate in both species of *Mollugo* Linn. (Fig. 9 and 10). The ammonium nitrate (NH_4NO_3) salt expressed slower response in both species whereas treatment with ammonium chloride (NH_4Cl) gave two times faster response.

The germination percentages are significantly different with different treatments of nitrogenous compounds showing P value less than 0.05. The maximum enhanced germination response was observed at 50 mM concentration of treatments and the germination enhancement was in the order KNO_3>$NaNO_3$>NH_4Cl>NH_4NO_3 but when the concentration of nitrogenous salts increased the germination response was found in decreased manner (Table 4).

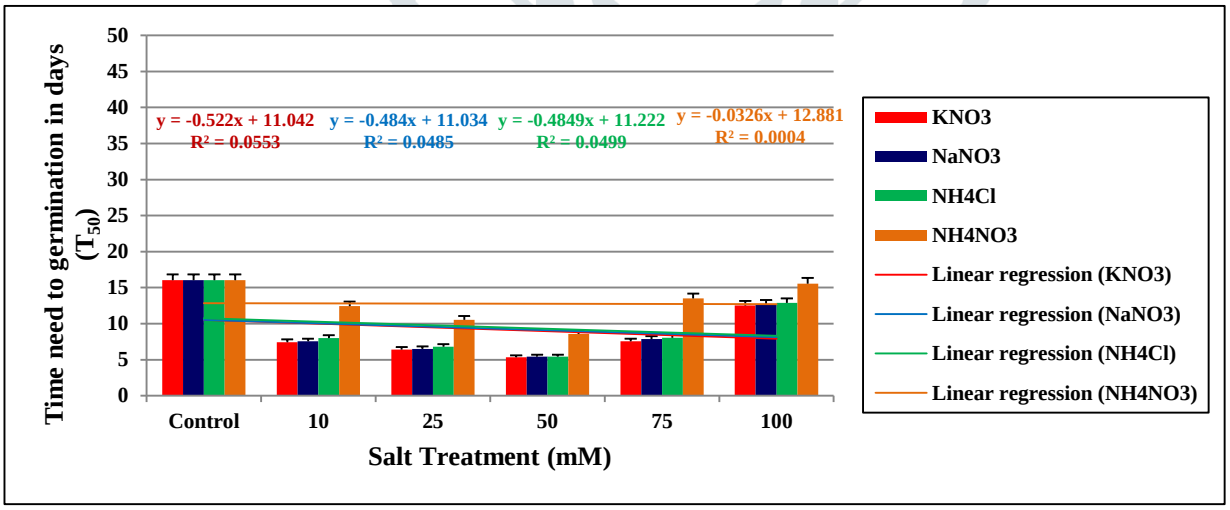

Fig. 9: Comparative duration of median germination time (T_{50}) of *Mollugo nudicaulis* Lamk. against different nitrogenous salt with their R^2 values calculated by linear regression

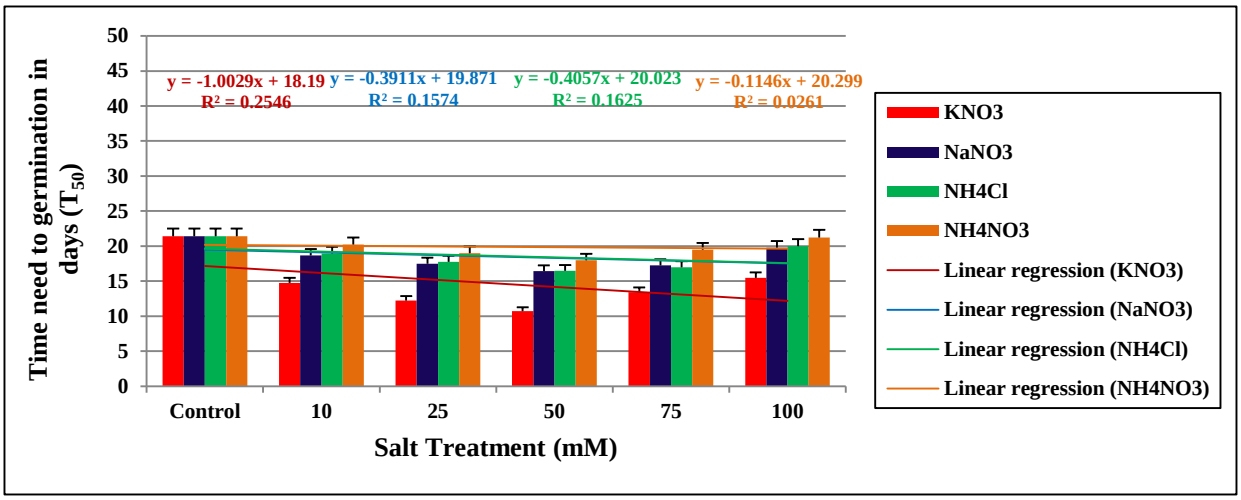

Fig. 10: Comparative duration of median germination time (T_{50}) of *Mollugo cerviana* Linn. against different nitrogenous salt with their R^2 values calculated by linear regression

3.5 Effect of Thiourea

In plants thiourea is known for its dormancy breaking action (Poljakoff-Mayber and Mayer 1960; Garg et al. 2006; Mathur et al. 2006; Naruka et al. 2013). In present study, the enhanced germination was shown by seeds of both the species of *Mollugo* Linn. after thiourea [$(NH_2)_2CS$] treatment but it is recorded that germination percentage is observed less as compared to treatment of KNO_3 (Table 5).

Species	Treatments (10 mM each)	Seed Germination Percentage (%)	Time need to germination in days (T_{50})
Mollugo nudicaulis Lamk.	Control	00.00	16.03
	Thiourea	76.67	07.57
	KNO_3+Thiourea	86.67	07.34
Mollugo cerviana Linn.	Control	00.00	21.42
	Thiourea	73.33	12.74
	KNO_3+Thiourea	76.67	12.42

Table 5: Effect of thiourea on seed germination in *Mollugo Linn.* species

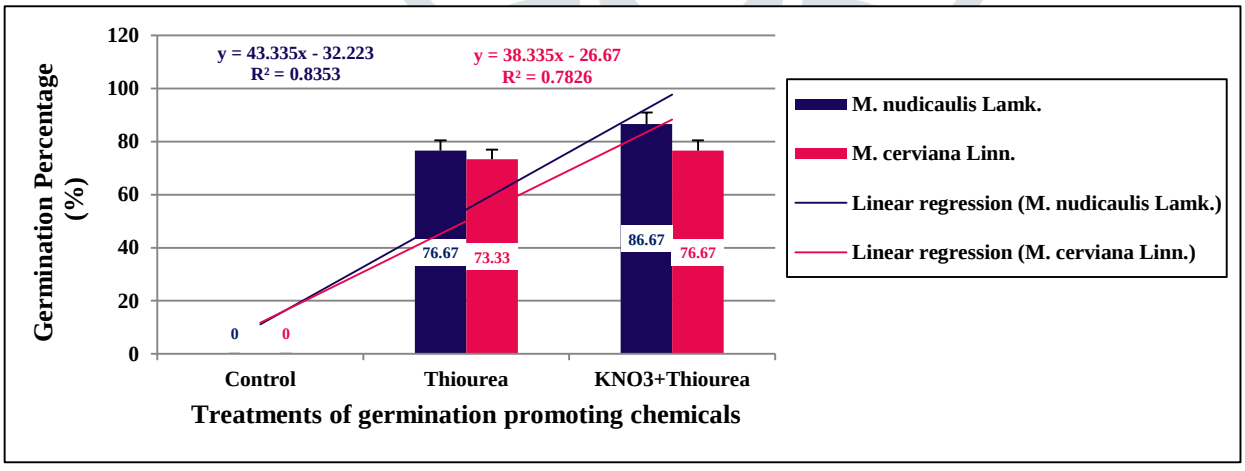

Fig. 11: Comparative germination responses of *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn. against thiourea.

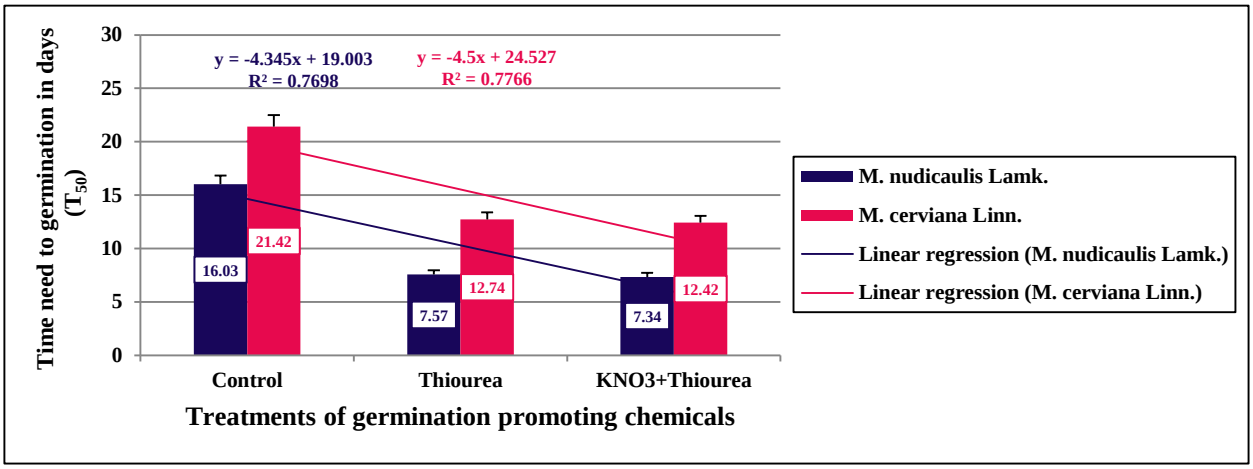

Fig. 12: Comparative duration of median germination time (T_{50}) of *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn. against thiourea.

It is found that germination percentage is increased when seeds were treated with thiourea combined with KNO_3 instead of thiourea alone. The germination percentage is significantly variable with treatments of thiourea showing P value less than 0.05 (Fig.11 and 12).

3.6 Effect of sucrose

Salt stress responses are generally correlated with higher levels of various osmolytes, such as fructose, sucrose, complex sugars, malate, and proline (Gong et al. 2005). Sucrose is an important carbohydrate that functions as a key signaling molecule in the regulation of germination and seedling development, although sucrose is readily converted into fructose and glucose, and the combined treatments of glucose and fructose are less effective than sucrose treatments (Francisco-Arenas-Huertero et al. 2000; Teixeira et al. 2005; Xu et al. 2010).

Species	Treatments (Conc. in M)	Seed Germination Percentage (%)	Time need to germination in days (T_{50})
Mollugo nudicaulis Lamk.	Control	00.00	16.03
	0.1	60.00	16.67
	0.5	73.33	16.36
	1.0	93.33	16.07
Mollugo cerviana Linn.	Control	00.00	21.42
	0.1	56.67	20.00
	0.5	66.67	22.00
	1.0	90.00	21.48

Table 6: Effect of different molar concentrations of sucrose on seed germination in *Mollugo* Linn. species

In the present study, germination response is significantly different with different concentrations of sucrose treatments showing P value less than 0.05. The data shown in Table 6 indicate that increase in the molar concentration of sucrose increases the germination response in both *Mollugo* Linn. species. In 1M sucrose solution, *Mollugo nudicaulis* Lamk. respond 3% more than *Mollugo cerviana* Linn. but the median time need to germinate is similar to control in case of both species (Fig.13 and 14).

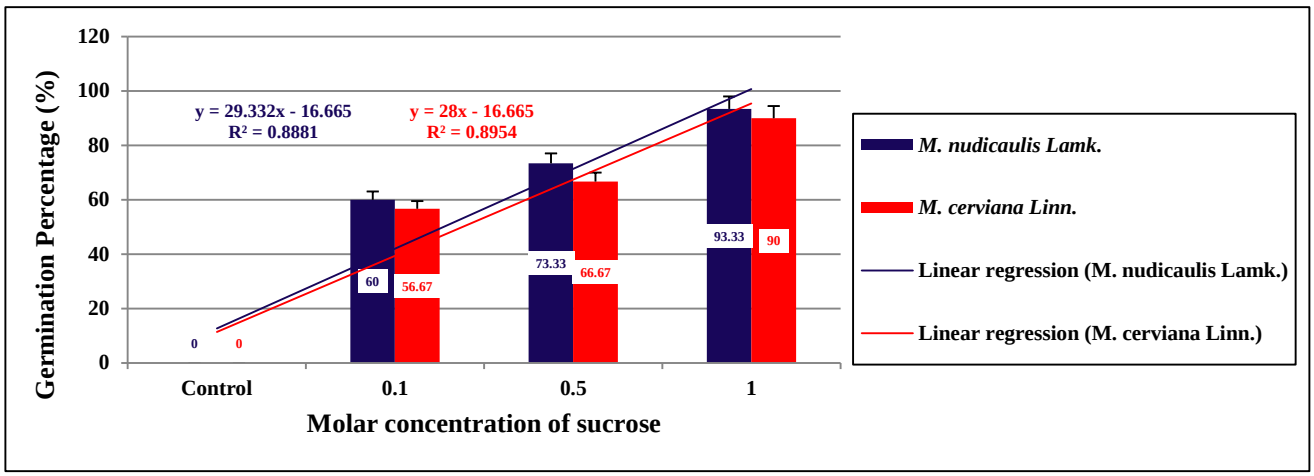

Fig. 13: Comparative germination responses of *Mollugo nudicaulis* Lamk. and *Mollugo cerviana* Linn. in various sucrose concentrations

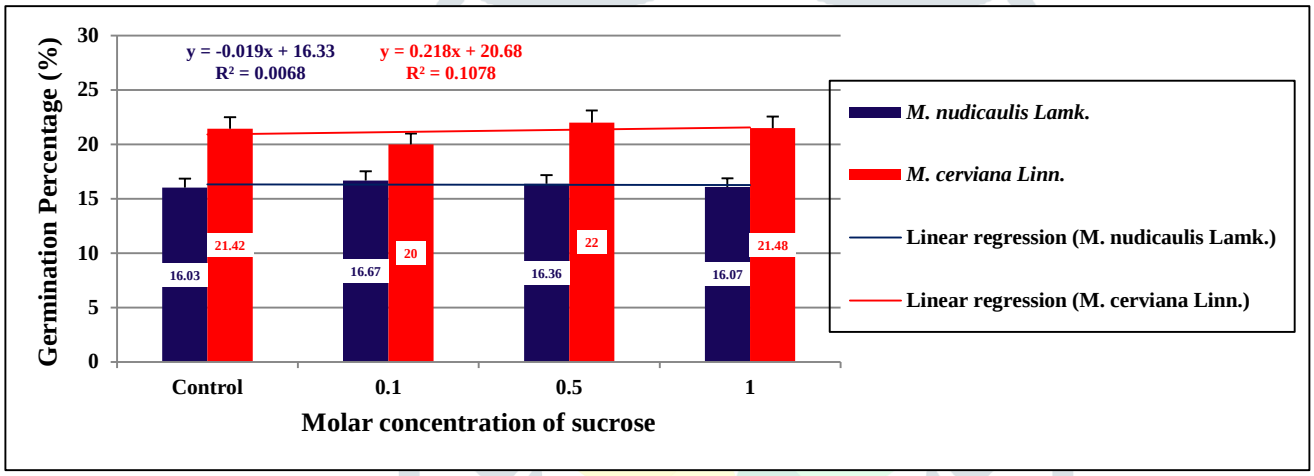

Fig. 14: Comparative duration of median germination time (T_{50}) of *Mollugo nudicaulis Lamk.* and *Mollugo cerviana Linn.* in various sucrose concentrations

4. Conclusions

The seeds are very minute with hard seed coat and they took almost one month to germinate in case of *Mollugo nudicaulis* Lamk. and 45±2 days in case of *Mollugo cerviana* Linn. to break dormancy at room temperature in normal conditions. On the basis of observations of present study it is found that temperature (dry heat and hot water) apparently has no effect on germination response in *Mollugo* Linn. species.

It is also evident that different salts show both osmotic and ionic effects on germination. Effects of salt stress indicate that both the species are able to establish themselves in variable habitats. The seeds remained viable but with less germination percentage when higher concentrations of different salts were used.

Therefore it can be concluded that salt levels used in present investigation for both species show relationship in their germination responses. *Mollugo cerviana* seeds are less responsive as compared to *Mollugo nudicaulis* when treated with different salts that are the case in normal condition also.

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