



Influence of environmental factors on germination and seedling emergence of *Melilotus indicus*

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ABSTRACT

To find out the effect of temperature, osmotic stress, light, pH, salt stress, burial depth, mulching and flooding duration on seed germination and seedling emergence of *Melilotus indicus*, laboratory and screen house experiments were conducted at CCSHAU, Hisar, during *rabi* seasons of 2015-16 and 2016-17. All the experiments were conducted in a completely randomized design with each treatment having four replications. Based on research results, it was observed that the optimum temperature range for the germination of *M. indicus* was 15-30°C day temperature and 10-20°C night temperature. It was more sensitive to moisture stress, as its seed germination decreased from 95% to 38% with increasing moisture stress (from 0 to -1.2 MPa), whereas it was less sensitive to salt stress: as more than 50% seed germination occurred at 125 mM NaCl salt concentration. The optimum pH for its seed germination (90%) was neutral (pH 7). It was non-photoblastic in nature, as dark and light period had no significant effect on its seed germination. The optimum burial depth for higher seedling emergence was 1 cm (>90%), but its emergence decreased as burial depth increased further. The seedling emergence was reduced by the application of rice straw as mulch, though a high amount (4 to 10 t/ha) of straw was required to suppress its emergence significantly. Continuous flooding for different durations significantly reduced the seedling emergence of *M. indicus* over no-flooding condition, and was not able to emerge after 32 days of continuous flooding. The information gained from this study would help in developing effective weed management strategy against *M. indicus*.

Key words: Burial depth, Flooding, Light, Mulching, Osmotic and Salt stress, pH, Temperature

In agricultural ecosystem, weeds are major biotic constraints which have a bearing on the quality and quantity of crop produce, as weeds compete with the crop plants for resources such as soil nutrients, space, moisture and light (Devi *et al.* 2018a). As weeds cause major production losses to agricultural system, is a serious threat that could have global nutritional food security implications. Therefore, their effective management is very important (Devi *et al.* 2017).

There are many methods like preventive, cultural (agronomical), mechanical and chemical methods, used to control the weeds throughout the world. Among weed control methods, the farmers rely on herbicides for effective weed control in different crops. Due to injudicious and indiscriminate use, continuous use of single herbicide or same or different chemical class with similar mechanism of action, herbicide resistance is increasing in weeds throughout

the world (Singh *et al.* 1999). There are currently at least 500 unique cases of herbicide-resistant weeds globally, with 256 species (149 dicots and 107 monocots) (Heap 2019).

A check on emergence of herbicide resistance can be achieved by reducing selection pressure through diversification of weed control techniques. In this respect better understanding of biology and ecology of weeds prevalent in agricultural fields is of prime importance. Weed biology is concerned with the study of weeds in relation to their geographical distribution, habitat, growth and reproduction, establishment, population dynamics and communities (Hakansson 2003). On the other hand, ecology of weed is concerned with the development of a single species within a population and interrelationship between weed and its environment at a given site. The better understanding of weed ecology and biology can play an important role in formulating sustainable weed management strategies.

M. indicus is a dicotyledonous leguminous winter annual herb belonging to the *Fabaceae* family; known by many common names in different languages such as sweet clover, Indian sweet clover, annual yellow sweet clover, small flowered sweet clover, small flowered melilot, senji, janglimethi and banmethi. *M. indicus* resembles *M. alba* and *M. officinalis* in growth pattern except for the flower

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colour. Sweet clover is an important forage crop in many regions of USA and African regions (Al Sherif 2009). It is considered a noxious weed in grain, vegetable and oilseed crop's fields like wheat, mustard, pea, chickpea, fenugreek, berseem, lucerne, potato, oat *etc.* (Devi *et al.* 2018b). Along with other winter weeds, *Melilotus* species caused more than 45% yield losses in winter season crops. Its seed has contained leachable allelochemicals, which have negative impact on seed germination and seedling growth of rice crop.

In India, it emerges in the months of November-December when day/night temperature is about 18/5°C and set seeds in March-April at a day/night temperature of about 35/18°C (Singh *et al.* 1995). Its seeds possess hard seed coat and remain viable in the soil seed bank for at least 20 years.

A study on weed biology and ecology of a particular weed generates information on the growth and development behavior which can be exploited while working out an effective weed management programme. To date, there is a little information on seed germination and seedling emergence of *M. indicus*, especially under Indian conditions. Better understanding of seed germination and emergence of *M. indicus* in relation to environmental variable would help to predict its potential spread and developing effective weed control measures with minimal environmental damages. Therefore, the objective of this study was to ascertain the effect of environmental factors such as light, temperature, salinity, moisture stress, pH, seed burial depth, flooding duration and mulching on seed germination and seedling emergence of *M. indicus*.

MATERIALS AND METHODS

General protocol and considerations

The experiments to find out the effect of temperature, osmotic stress, light, pH and salt stress on germination of *M. indicus* were conducted under laboratory conditions, while the experiments to study the effect of burial depth, mulching and flooding duration on seedling emergence were carried out in the screen house at CCS Haryana Agricultural University, Hisar, during *rabi* seasons of 2015-16 and 2016-17.

For this study, seeds of *M. indicus* were collected from Research Farm of CCS HAU, Hisar, for two consecutive years 2014-15 and 2015-16. Seed of mature plants were hand-harvested, cleaned, sieved and, stored in paper bags at room temperature until used for the experimental trials.

In screen house experiments, plastic pots (15 cm top diameter), with 10 kg soil capacity, were used to study the effect of burial depth and flooding durations, while, to study effect of mulching on seedling emergence, large sized plastic pots (30 cm diameter) with 15 kg soil capacity were used. The soil used for filling the pots was in the ratio of 3:1:1 with field soil, dunal sand and vermicompost. The soil was collected from fields where no herbicides were used for the last 4-5 years. For each treatment, 10 seeds of *M. indicus* were planted uniformly in plastic pots at 0.5-1 cm

depth, unless otherwise specified. The seedling emergence was considered, when, cotyledons started to appear above soil surface.

In the laboratory experiments, 10 seeds (per Petri dish) of *M. indicus* were placed uniformly in 90mm diameter Petri dish with double layered filter paper (Whatman No. 1). Seven milliliters of de-ionized water or a treatment solution (for pH, salinity and osmotic potential experiments) used to moisten the filter paper at the initiation of experiments. After that, the Petri dish were incubated at 30/20°C (except for the temperature experiment) and 12-hour light and dark period (except for light experiment). Distilled water (1-2 ml) was added to maintain adequate moisture of filter paper, when required. Petri dishes were wrapped in a plastic film to reduce loss of water. The seed germination was observed daily (except in the light experiment), and radical protrusion was the criterion for germination of seed. Germination percentages were determined on the basis of number of viable seeds.

M. indicus is reported to possess physical dormancy due to hard seed coat (Dhawan 2009). To break the seed dormancy, seeds were mechanically scarified by mild rubbing with a sand paper.

All experiments were conducted in completely randomized design (CRD) with four replications and all experiments were repeated over time. The experimental data was analyzed using SPSS v. 17.0. Standard error of means was calculated and regression trends were plotted in graphs.

Effect of temperature on seed germination

The Petri dish were placed in growth cabinets already set to 15/10, 20/10, 25/15, 30/20, 35/25, 40/30 and 45/35°C day/night temperatures.

Effect of osmotic stress on seed germination

Osmotic stress studies were carried out at -0.1, -0.2, -0.4, -0.8, -1.0 and -1.2 MPa at 30/20°C. Aqueous solutions with these osmotic potentials were prepared by dissolving 85.4, 127.8, 188.2, 274, 308.5 and 339.75 g of polyethylene glycol 8000 (PEG 8000) powder in 1 kg distilled water (Michel and Kaufmann 1973). The osmotic potentials were selected to mimic the possible osmotic stress conditions that the species might encounter due to salinity and aridity problems in the soil.

Effect of light on seed germination

Seeds were exposed to light for 0 (dark), 0.5, 1, 2, 4, 8 and 16 hr and after treatment, Petri dish were wrapped in double layers of aluminum foil to ensure no light penetration. The Petri dish were kept in growth cabinets which was already set at 30/20°C temperature. Wrapped Petri dish were kept for seven days undisturbed and then were unwrapped in dim light to observe germination.

Effect of pH on seed germination

Germination of *M. indicus* was investigated under pH range of 5.0, 7.0, 9.0 and 11.0 at 30/20°C temperature.

Potassium hydroxide pellets and citric acid were used for preparing different pH solutions.

Effect of salt stress on seed germination

The seeds of *M. indicus* were incubated in 25, 50, 75, 100 and 125mM sodium chloride solution at 30/20°C temperature along with control (deionized water only). Formula used was $X \text{ mM NaCl} = (\text{Molecular wt. of NaCl} \times X \times 10^{-3}) / \text{litre of water}$, where, X = concentration of salt in mM, Molecular weight of NaCl = 58.45.

Effect of burial depth on seedling emergence

For this, seeds were placed at a depth of 0 (surface), 0.5, 1.0, 2.0, 4.0 and 8.0 cm in plastic pots.

Effect of flooding duration on seedling emergence

Five flooding durations were maintained for 0 (no flooding), 4, 8, 16 and 32 days. Flooding levels were maintained continuously by keeping 5-7 cm water layer in the pots. After specified flooding durations, holes were made at the bottom of the pots to drain out the excess water.

Effect of mulching levels on seedling emergence

For this, large sized plastic pots were used and 0 (no mulch), 2, 4, 6, 8 and 10 t/ha chopped paddy straw mulching levels were applied.

RESULTS AND DISCUSSION

Laboratory experiments

Effect of temperature: Seed germination of *M. indicus* gradually increased from 15/10 to 30/20°C (Fig 1); and with further increase in temperature, its germination decreased sharply with maximum germination at 30/20°C (95%). At 45/35°C, only 25% seeds were able to germinate. The germination percentage as a function of temperature was found to follow the equation ($y = -3.86x^2 + 21.64x + 68.98$, $R^2 = 0.955$) where, y is germination percentage and x is temperature. Ghaderi-Far et al. (2010) also reported maximum germination (80-97%) of *M. officinalis* between 15 and 30°C and a marked decrease in germination occurred outside this range and germination reached zero at 0 and 35°C.

Effect of osmotic stress: Seed germination of *M. indicus* get reduced as osmotic potential decreased from 0 to -1.2 MPa, with maximum germination (95%) under control condition (Fig 2). Seed germination is a growth process of previously quiescent or dormant seed, beginning with imbibition of water, till the water content of embryo is adequate, which trigger biochemical events and lead to cell expansion. Therefore, lack of adequate moisture can inhibit germination process of seed. More than 80%

seed germination of *M. indicus* was recorded up to -0.4 MPa and <40% seed germination occurred at -1.2 MPa; suggesting that *M. indicus* prefers a moist environment for its optimal germination, but can tolerate moisture stress up to some extent. The germination percentage of *M. indicus* varied with osmotic potential according to relation, where y is germination percentage and x is osmotic potential. Some other weed species that reported tolerant to low osmotic potential for seed germination are *Solanum sarrachoides* (Zhou et al. 2005), *M. officinalis* (Ghaderi-Far et al. 2010).

Effect of salt stress: The highest mean germination of *M. indicus* (95%) occurred under the control treatment (no stress), and its germination reduced with increasing concentrations of NaCl from 25 mM (93 %) to 125 mM (60 %) (Fig 3). There were about 2, 11, 18, 26 and 37% reduction in germination percentage of *M. indicus* at 25, 50, 75, 100 and 125 mM NaCl with respect to control treatment, respectively. The germination percentage of *M. indicus* varies exponentially with salt stress, according to equation, where y is germination percentage and x is salt concentration. The salinity condition around the seeds exerts higher osmotic pressure like drought; in addition to this, sodium chloride

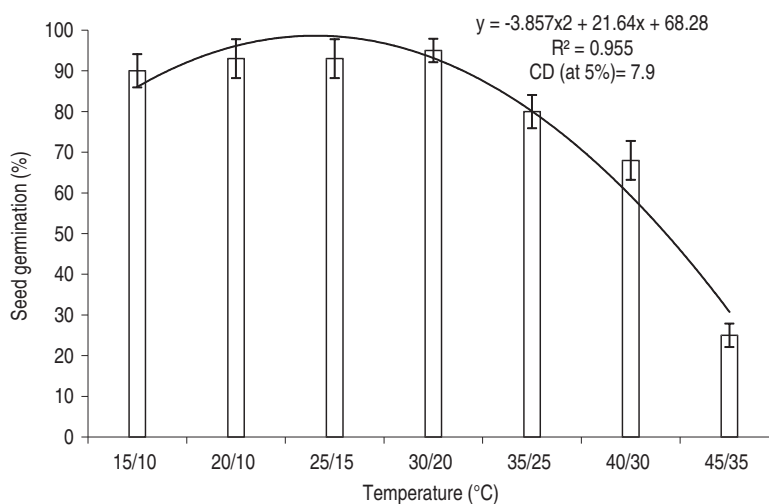


Fig 1 Effect of alternating day/night temperature on seed germination of *M. indicus* at 4WAT (weeks after treatment).

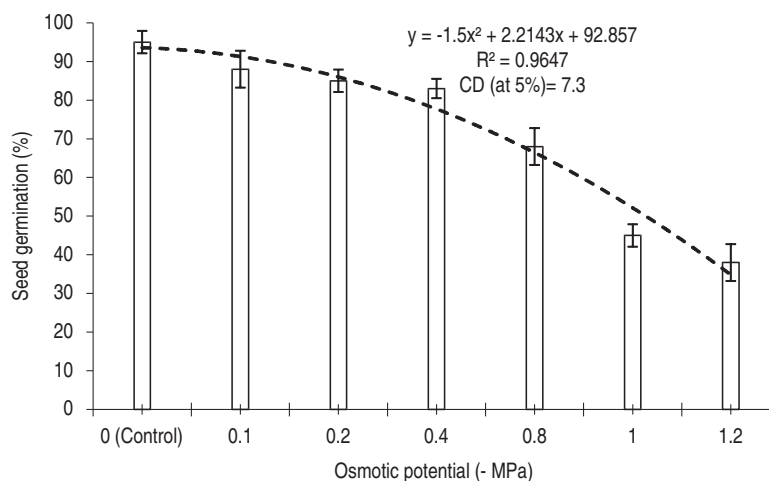


Fig 2 Effect of osmotic stress on seed germination of *M. indicus* at 4 WAT.

toxicity is also associated with a significant decrease in seed potassium (K^+) content which triggers activation of enzymes, essential for production of adenosine triphosphate (ATP) etc. (Kaymakanova 2009) and might have a dormancy inducing effects in seed. As reported by Al Sherif (2009), *M. indicus* germination percentage was not significantly affected up to 200 mM NaCl (salinity levels), while, only 25% seeds germinated at 300 mM NaCl. *Melilotus officinalis* was also found to be less sensitive to salinity up to 90 mM NaCl, but its germination decreased drastically as the salt concentration is increased further (Ghaderi-Far *et al.* 2010).

Effect of pH: The maximum seed germination (90%) of *M. indicus* occurred at pH 7 (neutral). As pH reduced to 5 from 7, about 32% reduction in seed germination was recorded (Fig 4). Seed germination of *M. indicus* at pH 9 and 11, was 65 and 50%, respectively, which was about 28 and 45% lower than at pH 7. The hydrogen-ion concentration in soil may not be a limiting factor for seed germination of this weed species in most soils. Their distribution is probably dependent on a combination of several ecological factors rather than on pH of soil alone. The results indicate that seeds of *M. indicus* can germinate in wide range of pH, i.e 5 to 11, but *M. indicus* prefers near neutral pH 7. These results are similar to the results reported by Ghaderi-Far *et al.* (2010) on *M. officinalis*. The variation in germination percentage of *M. indicus* with pH can be reported by the equation, where x is germination percentage and x is pH value.

Effect of light period: Light did not show any significant effect on germination *M. indicus* with respect to variations in light and dark period exposure (Table 1). These results indicate that light is not a prerequisite for germination of this *rabi* season weed; therefore, it may be considered that this weed is non-photoblastic in nature. These results are similar to the finding of Kumari (2010) on *M. denticulata*, *V. sativa*, *L. aphaca* and *C. arvensis*, whose germination was also not influenced by different light periods, whereas, germination of Brazil pusley was stimulated by light (Singh and Singh 2009) and *M. nudiflora* germination was completely inhibited by darkness (Ahmed *et al.* 2015). The effect of light on germination is quite helpful in understanding whether seed can germinate at deeper depths, hence, this weed can germinate at deeper depths where light can not reach.

Screen house experiment

Effect of burial depth: The seedling emergence of *M. indicus* increased firstly with increasing burial depth from zero (surface) to favorable depth (i.e. 1 cm), and then its emergence decreased remarkably. Maximum seedling emergence (>90 per cent) of *M. indicus* was recorded

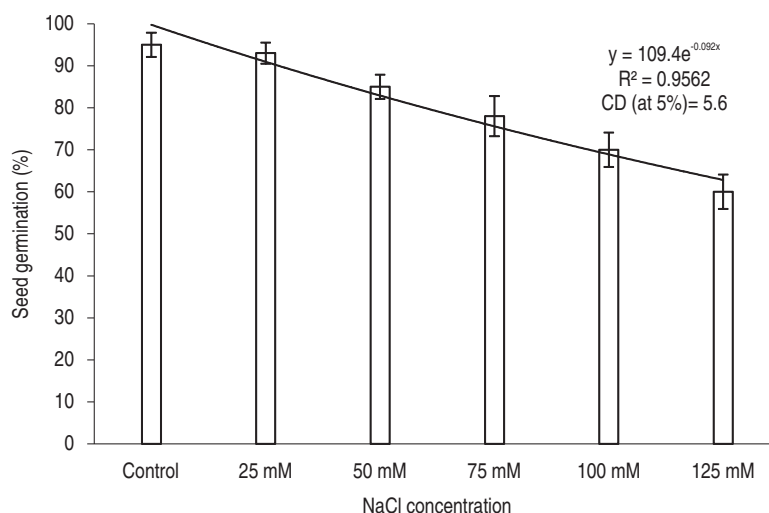


Fig 3 Effect of salt stress on seed germination of *M. indicus* at 4 WAT.

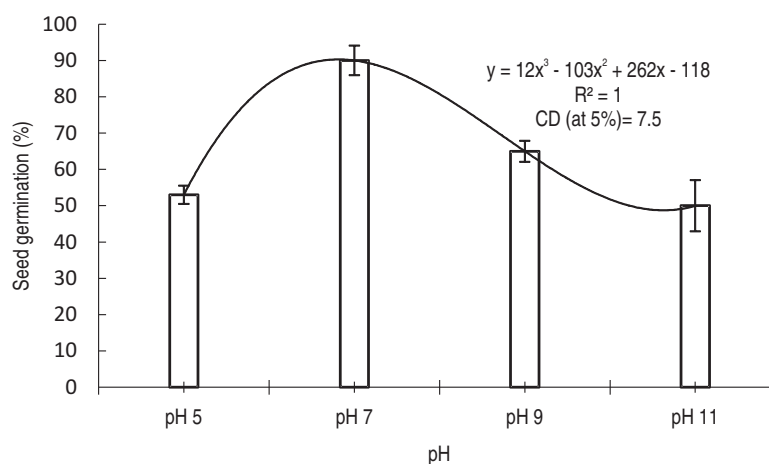


Fig 4 Effect of pH on seed germination of *M. indicus* at 4 WAT.

Table 1 Effect of light duration on seed germination of *M. indicus* at 4 WAT

Light duration (hour)	Germination
0 (dark)	72 (90)
0.5 h	74 (93)
1 h	72 (90)
2 h	70 (88)
4 h	70 (88)
8 h	70 (88)
16 h	68 (85)
Mean	71 (89)
LSD (P=0.05)	NS

at 1 cm burial depth (Fig 5). Seeds placed on the soil surface had lower emergence compared with seeds placed at 1 cm. This may be due to limited contact of seed with soil, which might influence moisture availability to seeds; however, seeds placed just below the soil surface might receive adequate water for their germination initiation and emergence. Increasing burial depth beyond 4 cm resulted

in drastic reduction in its seedling emergence and <20% seedlings of *M. indicus* were able to emerge from 8 cm depth. This might be related to food reserves of the weed seed, as extra energy (food reserve) is required to emerge from deeper depth. Another reason for emergence reduction with increasing depth may be poor gaseous exchange as with increasing depth oxygen availability decreases around germinating seeds (Benvenuti 2003). Effect of burial depth on seedling emergence is reported by Chauhan *et al.* (2006) on *B. tournefortii* as seedling emergence of these weeds was influenced by different burial (seedling) depth. The variation in emergence percentage of *M. indicus* with burial depth can be modeled by equation, where y is emergence percentage and x is seed burial depth.

Effect of mulching: *M. indicus* was able to emerge from different mulching levels, viz. 2, 4, 6, 8 and 10 t/ha (Fig 6), with maximum seedling emergence (>85%) from no mulch or mulch free condition. Application of 10 t/ha chopped rice straw decreased about 56% seedling emergence of *M. indicus* over control treatment. The impact of mulch on weed emergence, however, depends on quantity, position relative to weed seeds (below or above), and allelopathic potential of mulch residue with the weed species (Chauhan *et al.* 2006). Emergence suppression of weed by mulch can be attributed to various physical and chemical factors; and the former include lower soil temperature, shading and physical obstruction provided by mulch itself. Mulch block access of sunlight to weeds, so, weed would not have enough energy to push through the mulch again. In a pot experiments in screen house, Chauhan and Johnson (2008) reported that the seedling emergence of *Eleusine indica* declined markedly with the addition of crop residue on the soil surface @ 4 to 6 t/ha. The variation in emergence percentage of *M. indicus* with mulching can be modeled by equation, where y is emergence percentage and x is mulching levels.

Effect of flooding duration: The seedling emergence of *M. indicus* decreased dramatically with increase in flooding duration from zero to 32 days (Fig 7) with maximum seedling emergence (>95 per cent) under normal (no flooding) condition. Continuous flooding for 8 days reduced more than 50% seedling emergence of *M. indicus*, whereas it completely failed to emerge after continuous flooding up to 32 days. It might be due to reduced oxygen level and accumulation of certain toxic substances

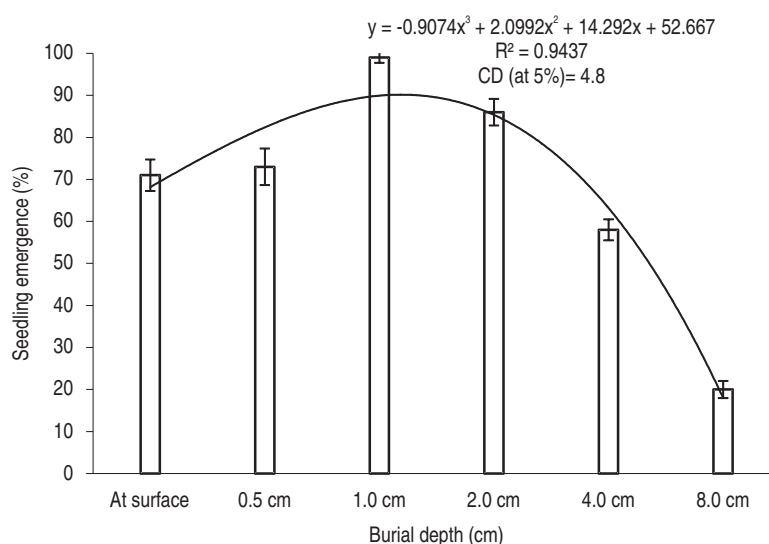


Fig 5 Effect of burial depth on seedling emergence of *M. indicus* at 4 WAS (weeks after sowing).

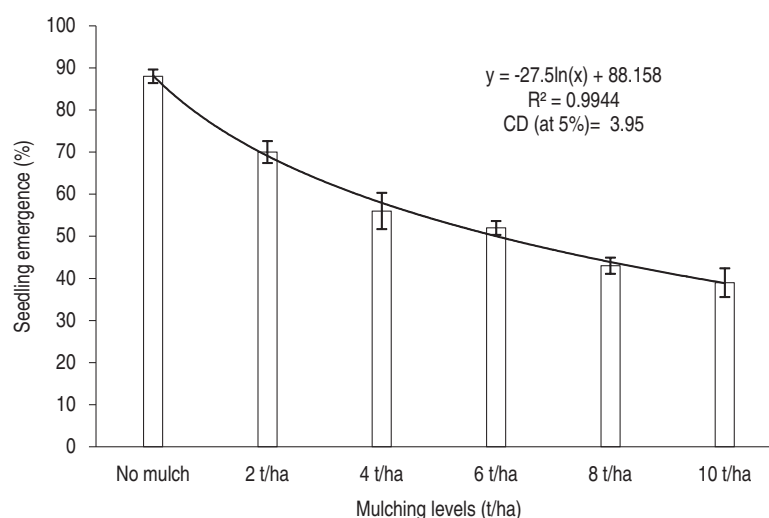


Fig 6 Effect of mulching on seedling emergence of *M. indicus* at 4 WAS.

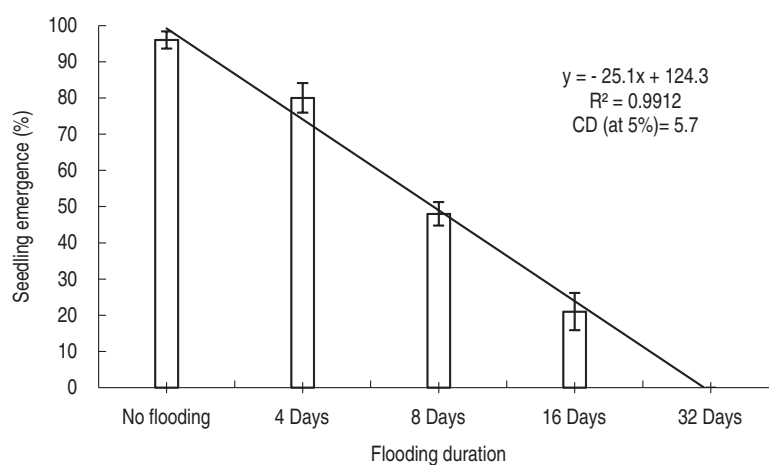


Fig 7 Effect of flooding duration on seedling emergence of *M. indicus* at 4 WAD (weeks after drainage).

around the seed by anaerobic microbes. Similar results were reported by Aulakh *et al.* (2006) that *Leptochloa chinensis* seeds under continuous submergence failed to emerge but after the termination of submergence, some seedlings of this species were able to emerge. The variation in emergence percentage of *M. indicus* with flooding duration can be modeled by equation ($y = -25.1x + 124.3$, $R^2 = 0.991$) where, x is emergence percentage and x is flooding duration.

The results of present study conclusively showed that the optimum temperature range for germination of *M. indicus* was 15-30°C and it was found to be sensitive to moisture and salt stress. The optimum pH for its germination was pH 7 and this weed was found non-photoblastic in nature, as, dark and light period had no effect on its germination. The optimum burial depth for higher emergence of *M. indicus* was 1 cm and its emergence decreased with increasing burial depth. The application of rice straw mulching decreased the emergence of *M. indicus*. The continuous flooding for different periods significantly reduced its seedling emergence, whereas continuous flooding for 32 days completely inhibited its emergence. The results of this study may lead to a better understanding of the requirements for *M. indicus* germination and emergence and would provide information for better management strategy of this weed.

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