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EFFECTS OF PRIMING ON GERMINATION AND SEEDLING VIGOUR OF BIRD'S EYE CHILLI (*CAPSICUM FRUTESCENS* L.) SEEDS COLLECTED FROM EASTERN HIMALAYAN REGION OF INDIA

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ABSTRACT

A study was conducted to find out the efficacy of organic and inorganic priming on germination and seedling vigour of bird's eye chilli (*Capsicum frutescens* L.) seeds collected from eastern Himalayan region of India. Results showed that, all studied traits were affected by priming treatments and there was completely significant difference ($p \leq 0.05$) in germination percentage between primed and non primed seeds. Among all priming treatments, 1% KNO_3 (39.68 % higher) recorded the highest germination percentage as compared to non primed control. Other best treatments recorded were 2% KH_2PO_4 (37.50 % higher than control) followed by 200g/Kg seed neem leaf powder (NLP) (35.12 % higher than control) and 3% KNO_3 (35.12 % higher than control). In addition to this, 200g/Kg seed NLP treatment recorded lowest mean germination time (13.53 days) while control recorded maximum mean germination time (21.63 days). From the study it was concluded that, seed treatment with NLP is essential for pre and post storage treatment of seeds, as it maintained seed viability and seedling vigour optimally among all other treatments.

INTRODUCTION

Bird's eye chilli (*Capsicum frutescens* L.) is a perennial chilli with small sized fruits, blood red in colour, highly pungent and is commonly found in Southeast Asia. In India, it is commonly found in the eastern Himalayan regions like Mizoram and some areas of Manipur. It is extensively used as spice in curried dishes, as an ingredient in curry powder, seasonings and hot sauces, as medicine it is used as a counter irritant in Lumbago, Neuralgia, and Rheumatic disorders. Huge diversity of the crop with respect to fruit size, shape, pungency, colour and growth habit are found in this areas. The crop holds a huge scope and potential in these areas.

Seed is considered as one of the important agricultural inputs for obtaining higher yields. Good quality seed acts as a catalyst for realizing the potential of all other inputs in agriculture. Without good seed, the investment on fertilizers, water, pesticides and other inputs will not pay the desired dividends. But unfortunately, our preliminary study revealed that poor seed germination and weak seedling vigour are the major problems concerning this perennial chilli. Also, seeds of this crop are very difficult to germinate and harbour a huge load of fungi and/or bacteria, resulting in seed deterioration and poor nursery stand. Chandrasenan (1996) observed decline in germination percent, root length, shoot length, seedling vigour index and seedling dry weight as the storage period increased. Roberts (1972) found that seed deterioration during storage was due to the damage in cell membrane and other chemical changes in the seed system such as the protein and nucleic acid accumulation. Some physiological and biochemical changes leading to seed deterioration have been related to increased activity of enzymes (catalase, peroxidase, etc.), lipid autoxidation (Koostra and Harrington, 1969) and accumulation of toxic metabolites, free radicle damage, decreased protein synthesis, breakdown in mechanism triggering germination, reduced respiration, changes in polar lipids, decreased contents of glyco and phospholipids, ultra structural damage to cell and its organelles, accumulation of cytotoxic and mutagenic compounds etc. (Roberts, 1972). Seed treatment with compatible bio-agents (Rai and Basu, 2014) and inorganic chemicals (Chavan *et al.*, 2014) has been reported to be very effective in enhancing the plant growth and yield attributes. The possible mode of action of these seed invigoration treatments was ascribed to leaching of toxic metabolites from the seed during soaking, germination advancement and embryo enlargement during hydration (Basu *et al.*, 1974). Kazem *et al.*, 2012 observed improved field performance of aged chickpea seeds by hydro-priming under water stress.

Maraddi (2002) observed cowpea seeds treated with neem leaf powder @ 5 g per kg of seeds recorded higher germination (39.5%) and vigour index (1072) compared to control (34.2% and 8.64, respectively) at the end of 10 months of storage period. Seed treatment of chilli with thiram improved emergence and untreated seeds showed lowest emergence after 10 months of storage (Koteshwar-Rao *et al.*, 1962). Yogananda *et al.* (2004) noticed that bell pepper seeds invigorated with GA_3 (200 ppm) or KNO_3 (1.0%) recorded higher germination, root and shoot length, seeding dry weight, rate of germination and seedling vigour index over control.

Muthuswamy *et al.* (1983) reported that among all the fungicides tested for chilli, captan (4 g/kg), sulphur dust (4 g/kg), bavistin (2 g/kg), vitavax (2 g/kg) and benlate (benomyl, 4 g/kg) recorded higher germination compared to the untreated seeds. Dhyani *et al.* (1991) revealed that the seed treatment with captafol, thiram, aureofungin, topsin and vitavax each at 0.3 per cent concentration of seed weight improved seed germination and seedling length of chilli. In recent years however, attempts have been made to replace synthetic (inorganic) seed treatment chemicals with organic materials of plant origin which are cheaper, safer and eco-friendly. Among the various methods followed, use of botanicals has been a traditional method and is being given much attention because application of chemical pesticide and nutrients create the pressure on humans as well as environmental for their health hazards and also to think their substitute (Nakkeeran *et al.*, 2005). Consequently, seed priming may be used as an important tool to improve seed performance and stand establishment in the field.

Until now no attention has been paid to promote the seed health of perennial, difficult to germinate bird's eye chilli seeds. The use of botanicals and inorganic compounds as seed pre-treatment is now receiving much attention these days because of their proven advantages. This main aim of the work was to practically and technically investigate some of these earlier works; with reference to the perennial bird eye chilli. The objectives of the present study was to find out suitable organic and inorganic seed treatments to enhance seed germination and seedling vigour of perennial birds eye chilli (*Capsicum frutescens* L.).

MATERIALS AND METHODS

Seed source, treatments and growth conditions

Bird's eye chillis (MZBEC 7) for the study were collected from Kolasib district (24.2304ºN, 92.6761ºE) of Mizoram, India. Seeds were extracted and washed properly with double distilled water and primed with various treatments. Treatments included neem leaf powder (NLP: 100 and 200 g/kg of seeds for 24 hrs), KH_2PO_4 (2 and 6 % for 30 and 15 min), KNO_3 (1 and 3% for 30 and 15 min), K_2SO_4 (1 and 3% for 30 and 15 min), Captan + Imidachloropid (3g each/kg of seed for 24 hrs) and control (considered as non primed) with distilled water (for 24 hrs). Germination test was conducted following the method of ISTA (1999). After priming seeds were washed thoroughly with distilled water and placed in Petri dishes lined with Whatman No.1 filter paper (Whatman International Ltd., Maidstone, England) with 6 replication/ treatment and 30 seeds/replication) under BOD (Caltan, Narang Scientific Works Pvt. Ltd., New Delhi, India) incubated conditions (average temperature: 25.08 $\pm$2.15ºC) and monitored daily. The filter papers were moistened daily using distilled water.

Germination percentage and Mean germination time

Seeds were considered germinated upon radical emergence; the first germination was observed after one day and final germination was obtained on seventh day. Mean germination time (MGT) was calculated according to Labouriau (1983).

$$t = \frac{\sum n_i \times t_i}{\sum n} \text{ (days)}$$

where:

t - mean germination time,

t_i - given time interval,

n_i - number of germinated seeds during a given time interval,

n - total number of germinated seeds.

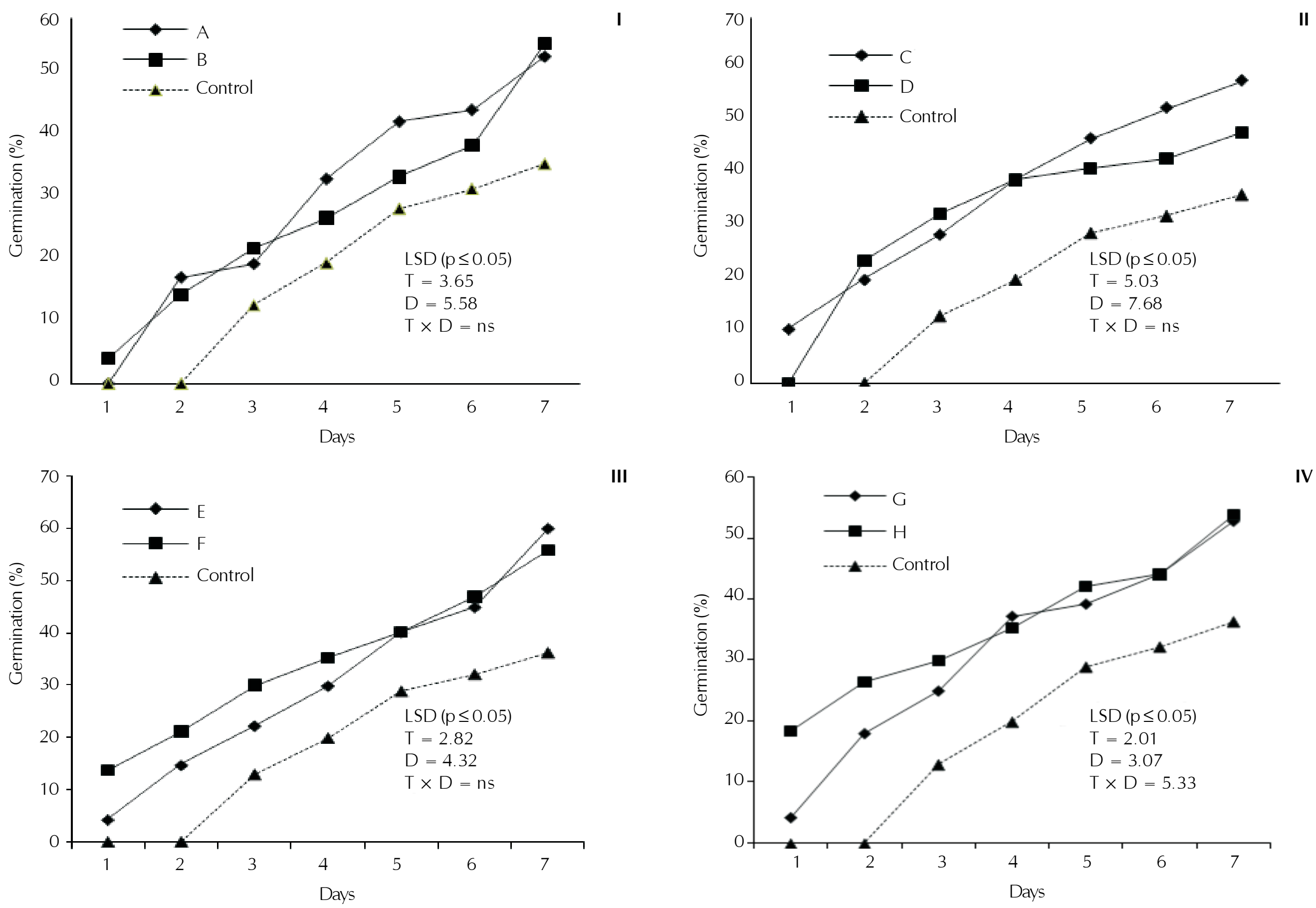
Seedling growth in nursery conditions

All the treatments of *in vitro* germinated seeds were sown (0.5-1 cm depth; distance between seeds 5.0 cm) in plastic (PVP) seedling trays (3 sprouted seeds per well; 4 well per replication; 6 replications per treatment, 50cm^3 well capacity) in a medium consisting mixture of 2:1:1 (v/v) with cocopeat (Saveer biotech pvt. India Ltd.), vermiculite (Saveer biotech pvt. India Ltd.) and perlite (Saveer biotech pvt. India Ltd.). Seedling trays were kept in glasshouse and temperature was maintained at 24.8$\pm$5.228ºC max., 16.5$\pm$ 4.87ºC min.; RH: 87 $\pm$ 1.28 max., 78.5 $\pm$3.62 min., with alternate day watering. After 15 days, seedlings were thinned to single plant to mitigate competition, maintaining 8-10 cm distance. After 30 days, seedling growth was assessed by harvesting five individual plants per treatment. Seedlings were uprooted and shoots and root fresh weight (g) as well as the root length (cm) and the shoot length (cm) were determined.

Table 1: Effect of priming (NLP: Neem leaf powder.:100 and 200 g/kg of seeds; KH_2PO_4: 2 and 6 %; KNO_3:1 and 3%; K_2SO_4: 1 and 3%; Captan + Imidachloropid: 3g each/kg of seed and control treatments on shoot and root fresh weight (g/plant) as well as the root and shoot length (cm/plant) by bird's eye chilli under nursery condition (greenhouse) after 30 days of sowing

	Mean germination time (days)	Shoot length (cm)	Shoot FW (g)	Root length (cm)	Root FW (g)
100 g/kg of seeds NLP	13.88^{c}	9.67^{a}	0.165^{a}	5.67^{a}	0.052^{cde}
200 g/kg of seeds NLP	13.53^{c}	6.80b^{cd}	0.109^{bcd}	4.77^{abc}	0.057^{abc}
2 % KH_2PO_4	17.17^{b}	7.23^{abc}	0.124^{abc}	3.47^{d}	0.049^{cde}
6 % KH_2PO_4	16.74^{bc}	6.20^{bcd}	0.102^{bcd}	4.83^{ab}	0.067^{ab}
1 % KNO_3	15.95^{bc}	6.93^{bcd}	0.145^{ab}	3.90^{bcd}	0.057^{bcd}
3 % KNO_3	18.29^{b}	8.33^{ab}	0.085^{cd}	3.87^{bcd}	0.043^{def}
1 % K_2SO_4	16.34^{bc}	7.07^{abc}	0.114^{bcd}	5.33^{a}	0.071^{a}
3 % K_2SO_4	16.72^{bc}	4.60^{cd}	0.101^{bcd}	3.30^{d}	0.040^{ef}
Captan + Imidachloropid (3g each/kg of seed)	13.82^{c}	8.07^{ab}	0.096^{bcd}	3.83^{cd}	0.049^{cde}
Control	21.63^{a}	4.37^{d}	0.070^{d}	3.47^{d}	0.031^{f}

FW; fresh weight; NLP: Neem leaf powder; in each column, values followed by the same letter did not differ significantly ($p < 0.05$) according to Duncan's multiple range test



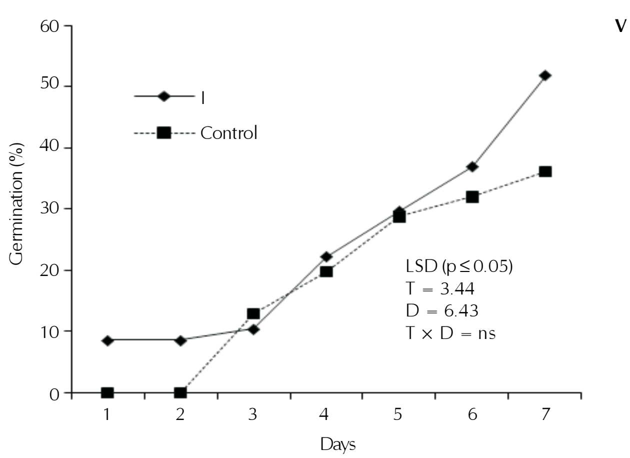


Figure 1: Germination percentage of pre-soaked seeds with I: NLP (A:100g and B:200g /Kg of seed), II: KH_2PO_4 (C:2 and D:6 %), III: KNO_3 (E:1 and F: 3 %), IV: K_2SO_4 (G:1 and H:3 %), V: H:Captan (3g/ Kg seed) + Imidachloropid (3g/Kg seed) and control. NLP: Neem leaf powder. Vertical bars indicate ± standard error, n = 5

Experimental design and statistical analysis

The experiment was carried out in completely randomized design in an unheated glasshouse with a north-south orientation at the ICAR RC NEH Region, Mizoram Centre, Kolasib, India (24.2304ºN, 92.6761ºE). Percentage data were log-transformed before analysis. The growth parameters of seedlings were subjected to one-way analysis of variance (ANOVA) employing Duncan's multiple range test (Duncan's MRT) at $p \leq 0.05$ in SPSS (SPSS, v. 16, Chicago, IL).

RESULTS AND DISCUSSION

Effect of priming on germination and seedling vigour of bird's eye chilli (*Capsicum frutescens* L.) seeds collected from eastern Himalayan regions were examined. Our results showed that, all studied traits were affected by priming treatments and there was completely significant difference ($p \leq 0.05$) in germination percentage between primed and non primed seeds (Fig. 1). Besides, significant differences ($p \leq 0.05$) were reported for the days after priming in all the treatments, but the interaction between priming treatments and days after treatment revealed non-significant difference except for K_2SO_4 treated seeds. Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops, particularly seeds of vegetables and small seeded grasses (Donaldson *et al.*, 2001). Basra *et al.* (2003) reported improvement in germination percent, emergence and seedling stand by using seed priming techniques. Priming induces a range of biochemical changes in the seed that required initiating the germination process *i.e.*, breaking of dormancy, hydrolysis or metabolism of inhibitors, imbibitions and enzymes activation (Ajouri *et al.*, 2004). Some previous researcher indicated that some or all process that precede the germination are triggered by priming and persist following the re-desiccation of the seed (Asgedom and Becker, 2001). Thus upon sowing, primed seed can rapidly imbibe and revive the seed metabolism, resulting in higher germination percentage and a reduction in the inherent physiological heterogeneity in germination (Rowse, 1995).

NLP (100g and 200g /Kg of seed) significantly ($p \leq 0.05$) increased the germination percentage as priming agent. Both the treatments exerted 32.60 and 35.12 % increase in germination percentage over control after seven days of treatment. Our findings were clearly in line with Maraddi (2002) who observed that cowpea seeds treated with neem leaf powder (5g/kg) recorded higher germination (39.5%) and vigour index (1072) compared to control (34.2% and 864, respectively) at the end of 10 months of storage period. Research activities proved that Neem leaves and seeds have the ability to kill some disease causing fungi, viruses and parasites (Khan *et al.*, 1988). The enhanced germination upon seed priming with neem leaf powder may be attributed to its similar activities against biotic stressor reported by researchers like fungi *Spherotheca pannosa var rosac* which causes powdery mildew and *Ampellomyces quisaqualis* (Pasini *et al.*,1997), fungi *Curvularia lunata*, and the germination of some pathogenic spores, retarding the expansion of leaf spotting in barley plants (Paul and Sharma, 2002), inhibited the growth of the fungi *Rhizoctonia solani* and *Fusarium solani* at percentages of 52.4% and 37.5% respectively (Darwish and Shaker, 2005). Srimathi *et al.* (2013) found that, botanical leaf powder seed pelleting not only improve the longevity of seeds through protecting the seeds from fungal and insect attack but also improve the seed and soil relationships through enriching the rhizosphere region of seed to produce better growth and development.

KH_2PO_4 (2 and 6 %) priming increased seed germination by 37.50 and 24.46 % respectively compared to control, thus confirming its stimulatory effect. This was in confirmatory with the previous findings of Gayathri (2001), who reported increase in germination percentage with KH_2PO_4 priming. Sathish *et al.* (2011) soaked fresh and aged seeds of maize hybrid (COH (M) 5) and its parental lines in water, 1% KH_2PO_4, 3% KNO_3 and 2% $CaCl_2$ solution for 6 hrs and found that KH_2PO_4 proved to be more effective, showing higher germination and emergence percentages, shoot length, root length and dry matter production than those primed with other salts and distilled water. Increased germination due to KH_2PO_4 priming might be due to ions absorption by seeds as reported by Alvarado *et al.* (1987). Moreover, the potassium salts had been reported to raise the ambient oxygen level by making less oxygen available for the citric acid cycle (Bewley and Black, 1982).

Priming of seeds with KNO_3 (1 and 3 %) enhanced the seed germination by 39.68 and 35.12% over control possibly through oxidized forms of nitrogen causing a shift in respiratory metabolism to the pentose phosphate pathway (Roberts and Smith, 1977). In a study with salt priming with 3% KNO_3 for 3 days and 1% NaCl for 2 days at 20ºC of cucumber seeds that were harvested 25, 35 and 45 days after anthesis, increased emergence and growth of seedlings. Maximum advantage of priming was observed in seeds harvested at 25 days after flowering and in all cases, KNO_3 priming was more effective than NaCl priming (Ghassemi-Golezani and Esmaeilpour, 2008). A similar increase was reported by Tzortzakis (2009) in endive and chicory. Moreover potassium nitrate solution has long been known as a suitable chemical approach for promoting germination in various plant species and generally as a priming agent or germination media (McDonald, 2000).

K_2SO_4 (1 and 3 %) priming exerted a positive influence via increasing seed germination percentage by 31.29 and 32.60 % compared to non primed seeds. A similar trend has been found by Sundstrom *et al.* (1987). The reason for this increase is still unknown but it may be due to better metabolic activity in seeds primed at higher water potentials. Degree of seed hydration has been found to be correlated with the osmotic potential of the priming solution (Bradford, 1986). Therefore, seeds incubated in solutions with relatively high water potentials have higher moisture contents and potentially greater metabolic activity. Hegarty (1977) has shown that oxygen use is highest in seeds in solutions with the highest osmotic potential.

Treatment with Captan: 3g/Kg seed + Imidachloropid: 3g/Kg seed enhanced 30.05 % seed germination as compared to non primed seeds. Fungicide acts as protective agent against seed deterioration due to fungal invasion and physiological ageing as a result of which the seed viability was maintained for comparatively longer period of time (Savitri *et al.*, 1994) and also phytotonic effect of imidacloprid maintained the seed viability for longer period (Jarande and Dethe, 1994). These findings are in agreement with results obtained by Hunje *et al.* (1990) in cowpea. Therefore among all the priming agents 1 % KNO_3 was found to be the best recording highest germination percentage. These stimulatory treatments may help germinating seedlings early, providing them higher competitive ability (Zhang and Maun 1990) and hence reducing chances of their mortality.

Mean comparison results displayed that MGT was affected by different priming agents. Generally significantly less ($p \leq 0.05$) MGT was attained from seed priming treatments than control (Table 1).The least MGT was obtained from 200g /Kg seed of NLP (13.53 days) whereas non primed seeds recorded highest MGT (21.63 days). The probable reason for early emergence of the primed seed maybe due to the completion of pre-germination metabolic activities making the seed ready for radicle protrusion and the primed seed germinated soon after planting compared with untreated dry seed (Arif, 2005).

Under nursery condition seed priming influenced shoot length significantly ($P \leq 0.05$) as seed priming with 100g /Kg seed of NLP recorded maximum shoot length (54.80 % higher than control) and shoot fresh weight (57.57 % higher than control), while least was recorded in non primed seeds. Root length and root fresh weight were found to be significantly higher ($P \leq 0.05$) in primed seeds as compared to control plants. In comparison to control there was a increase of 38.80 % in root length when primed with 100 g/kg seed of NLP and 56.33 % increase in root fresh weight when primed with 1 % K_2SO_4. In the present study, the increase in shoot length, root length and dry matter production due to priming might be due to earlier start of emergence as evidenced by lesser day's germination and nonexistence of seed borne diseases. Moreover, the stimulation of primed seed comparing with non primed seeds might be due to altered physiology of embryos and activation of enzymes, so that developmental processes occur more rapidly after sowing (Kattimani *et al.*, 1999).

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