

Germination Behaviour of *Citrullus colocynthis* (L. Schard) with Different Chemical and Physical Seed Treatments

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ABSTRACT: Colocynth (*Citrullus colocynthis* L. Schard.) grows naturally in the Indian Thar desert and other similar drylands of the world and has great survival mechanisms under extreme xeric conditions. It has unsung potential to supply food for human beings, feed and fodder to animals and specific chemical for the industries. Serious constraint to harness the potential benefits from this plant is presence of seed dormancy. In the present investigation, influence of different seed treatments and stages of fruit maturity on seed germination were studied in two experiments. In the first experiment, seeds were treated with different concentrations of gibberelins, auxins, kinetins, ethrel, and inorganic nitrogenous compounds (KNO_3 and thiourea) to break the physiological seed dormancy of colocynth. Pre-treatment of seed with ethrel at 200 ppm recorded highest germination (80.7%) followed by ethrel at 100 ppm (72.7%), KNO_3 at 5000 ppm (46.0%), and thiourea at 1000 ppm (40.7%). Synthetic plant growth regulators (gibberelin, auxins, and kinetin) did not promote seed germination and the performance was at par with control. In the second experiment, physical seed dormancy was studied in intact, decoated and scarified seeds extracted from small, medium, and large sized fruits, each of which was harvested at three different stages of fruit maturity; green, green-yellow, and yellow. Among different physical treatments, decoating and mechanical scarification recorded 66.7% and 35.6% higher germination over the intact seeds (25.94%). Seed germination was significantly affected by the stages of fruit maturity. The decoated seeds collected from small sized fruits harvested at yellow stage of fruit maturity exhibited the highest level of germination (56.4%) over other treatment combinations.

Key words: Colocynth, Germination, Seed treatment, Dormancy, Fruit maturity

Colocynth (*Citrullus colocynthis* L. Schard.) locally known as tumba, has a great survival mechanism under extreme xeric conditions. It grows naturally in the Indian desert and other extreme drylands of the world. It grows with the onset of monsoon (June-July) and produce fruits from August to November in Indian *Thar* desert. The vine of the plant remains green throughout the year except in winter and bears both male and female flowers on a single plant. The aerial part of the plant dries up during the winter season and re-sprouts during spring and monsoon season under favourable conditions. It has yield potential of 20-25 tones ha^{-1} of fresh fruits and 1.0-1.5 tones ha^{-1} of seed under 35-40 mm rainfall and yield of fruits may go up to 40-50 tones ha^{-1} during good rainfall years [1]. Potential use of this plant in dry land farming was reported in several literatures. All parts of the plant are considered valuable for

livelihood security of dry land areas. Cucurbitacin found in different plant parts is employed in many drugs and agro-pesticides [2], bitter fruit pulp could be used in confectionery after suitable chemical treatments [3] and seeds are rich source of oil (26.5%) and protein (13.5%) [4]. Even the powdered flour from roasted seed may be used as rude bread, soup thickener, flavoring agent and as food in famine [5, 6]. Besides, meeting the requirement of desert peasantry, it may enhance rural livelihood through small scale industries of oil, biofuel, soap, bio-pesticide & insecticide.

Like other flora of desert, seeds of colocynth also have dormancy and germination of seeds under field conditions is highly variable, erratic, and very poor [7]. Several reasons have been cited in the literature for the dormancy in colocynth. Published literature

reported presence of hard seed coat [8], covering layer of perisperm over the embryo [9], presence of phytohormone and cucurbitacin in embryo [10, 11] and maturity stage of fruits [12] as principle reasons for the dormancy in colocynth seeds. The inhibitory effect of hard seed coat on germination might be due to mechanical resistance to embryo expansion and impermeability of seed coat to entry of water or other substances inside the seeds. The inner envelope of seed (perisperm) also plays an important role in regulating germination by interfering in the gaseous exchange between embryo and the atmosphere [9]. In earlier studies, it was observed that cucurbitacin B and abscisic acids (ABA) in the cotyledons also have inhibitory effect on the germination of colocynth seeds [10, 11]. In many cucurbits, germination of seeds also correlated positively with age of fruits at harvest and fruit after-ripening [13]. Keeping in view the germination complexity of colocynth seed, an investigation was carried out to evaluate different chemical, physical and physiological treatments to break the seed dormancy.

MATERIALS AND METHODS

The study was conducted at Central Arid Zone Research Institute (CAZRI), Regional Research Station, Jaisalmer, India (26°52'N, 70°55'E and 194.50 m altitude) from 2005 to 2007. The aeolian sands found at top surface of soil profile were alkaline in reaction, shallow in depth and deficient in organic carbon and other plant nutrients except potassium. Two experiments were conducted to study the physiological and physical seed dormancy. In the first experiment, seeds were treated with seven different chemicals separately with an aim to break the physiological dormancy. Seeds were soaked in the solutions of gibberellic acid (GA) at 100 and 200 ppm, indole acetic acid (IAA) at 100 and 200 ppm, naphthalic acetic acid (NAA) at 100 and 200 ppm, kinetin at 20 and 40 ppm, ethrel at 100 and 200 ppm, thiourea at 1000 and 2000 ppm and KNO₃ at 5000 ppm and distilled water for 24 hours.

In the second experiment, three different mechanical treatments were applied with an aim to break the physical dormancy. The seeds of a single colocynth fruit were sown with 2.5 m × 0.75 m spacing on July 15, 2006 with the onset of rain under

rainfed conditions at CAZRI-RRS research farm Jaisalmer. Before sowing, seeds were treated with ethrel 200 ppm, which was found best in the first experiment. Fruits were harvested in the month of November-December, 2006 at three maturity stages: green (developing fruit), green-yellow (half mature fruit) and full yellow (fully matured fruits). Based on the diameter, harvested fruits were sub-grouped into three sizes: small (< 7.5 cm diameter), medium (7.5-9.5 cm diameter) and large (> 9.5 cm diameter) into each maturity stage. The fresh weight of small, medium and large fruits ranged 74.5-190.9 g, 231.5-475.1 g and 495.0-919.7 g, respectively. Seeds were manually extracted from the fruits, washed with water, sun dried and stored in moisture proof glass bottles. Germination study was carried out from the intact seed coat, de-coated seeds and mechanically scarified seeds. Seed coat manually removed from the imbibed seeds and after examining the embryos under magnification, undamaged embryos were used for germination. Under mechanical scarification, seed coat at micropyle end of seed was opened with the help of forceps / pliers and successfully opened seeds were used for germination study.

Seed germination under different chemical and physical seed treatments was studied using the germination chamber (Calton, NSW, India). Colocynth seeds treated with chemicals were soaked in the prepared treatment solutions for 24 hours in dark before being placed for germination in 4 replications of 100 seed each. The treated seeds were kept in between blotter papers and placed inside the germination chamber. The temperature and relative humidity inside the germination chamber was maintained at 30°C and 95%, respectively throughout the germination period. Germination counts (number of germinated seeds per 100 seeds) were recorded at two days interval upto 15 days in each treatment.

Emergence of the radicle was considered as indicator for germinated seeds. Ten normal seedlings from each treatment were randomly selected to study the seedling vigour. Vigour Index (I) and Vigour index (II) were calculated [14] and the data were statistically analyzed using the SPSS 13.0 software package, using Tukey's Multiple Range Test, to determine differences among means.

RESULTS AND DISCUSSION

Experiment I: Effect of chemical seed treatments on germination and seed vigour

The effect of chemical seed treatments on germination of colocynth is presented in Fig 1. The pre-treated seeds with chemicals showed significantly higher germination percentage over control (water-soaked seeds). Pre-treatment of seeds with ethrel at 200 ppm resulted into highest germination (80.7%) followed by Ethrel at 100 ppm (72.7%). Improvements in germination with ethrel have also been reported by other researchers in various crops [15, 16]. It was attributed from the findings that ethylene gas released from the ethrel had countered the negative effects of ABA synthesized in the embryo of most cucurbits during fruit development. Ethylene converts quiescent cells into active cells and thus promotes seeds germination [17]. Though variable response of synthetic plant growth regulators (GA, IAA, NAA and kinetin) in alleviating seed dormancy have been reported for many crops [18], in the present study these growth regulators at different concentrations did not affect germination significantly were at par with control.

Use of nitrogenous compounds (KNO_3 and thiourea) as pre-treatment, also improved germination of colocynth seeds. Pre-treatment of seeds with KNO_3 at 5000 ppm exhibited 46.0% germination whereas thiourea at 1000 ppm recorded 40.67% germination. Plant nutrients especially nitrogenous compounds such as nitric oxide, nitrate, nitrite and thiourea are known to stimulate the germination process in many light sensitive seeds [19]. Nitrogenous compounds (KNO_3 and thiourea) might have enhanced germination by accelerating the respiration rate of the dormant seeds that have physical barrier (seed coat/perisperm) for gaseous exchange between seed and the atmosphere [8, 18, 20]. The pre-treatment of seed with 0.2% KNO_3 improved germination in dormant seeds of *Poa pratensis* with increased rate of respiration [21]. The increased germination with KNO_3 at 1000 ppm in light sensitive seeds of *Dactyloctenium indicum* and *Eragrostis tremula* was also reported earlier [22]. Similarly, the stimulative effect of thiourea on seed germination was also reported in several plant species [23].

The variation in results by different growth hormones may be due to difference in their response

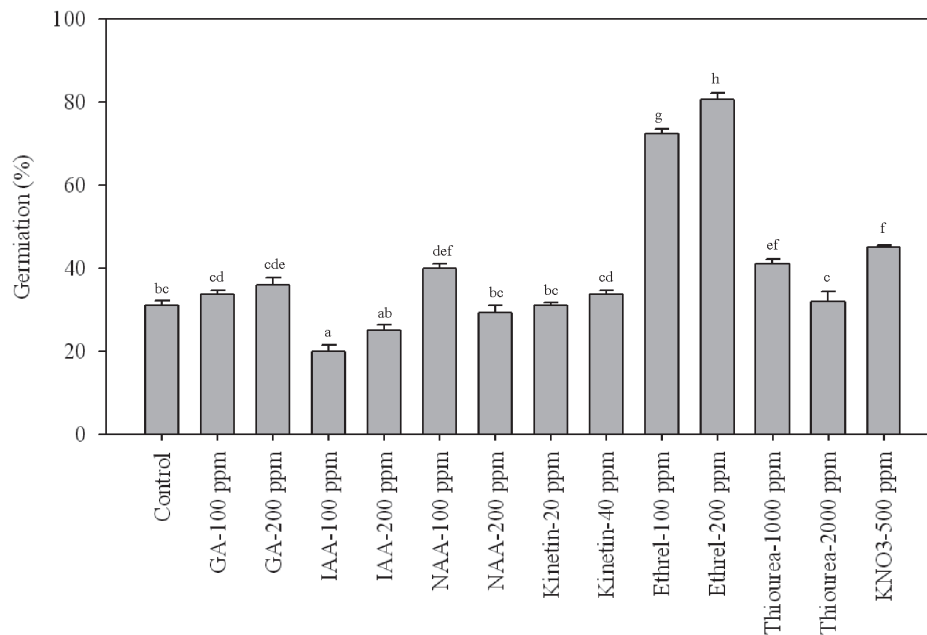


Fig. 1. Effect of different chemical seed treatments on germination of colocynth; control- water soaking; GA-Gibberelic acid; IAA-Indole acetic acid; NAA-Naphthalic acetic acid; Values are mean of 4 replications \pm SE; Columns with the same letters are not significantly different ($P < 0.05$, Tukey test).

to 'Cucurbitacin B', present in colocynth seed. Even the positive effect of 5 ppm GA₃ on germination may be affected by the presence of mere 9 µg ml⁻¹ of 'Cucurbitacin B' resulting in reduction of 50% germination [24] and inhibits seed germination at concentration above 10 µg/ml [11].

The data on germination percentage, seedling length and seedling dry weight was further used to compute vigour index I and II. Maximum root (7.78 cm) and shoot (15.80 cm) lengths of seedlings were recorded from the seeds treated with KNO₃ at 5000 ppm (Table 1). Proportions of root length to total length of seedlings were comparatively lower in GA, IAA, and NAA treatments. Seed treatment with kinetin and ethrel produced shorter root, shoot and total seedling length. Although the seed treatment with ethrel recorded shorter root and shoot length, but exhibited maximum seedling dry weight. This indicated that seedlings obtained from ethrel-treated seeds were thick and stout, which are very important for their survival in extreme arid situations. It is known that ethylene inhibits elongation of root and shoot and thus helps to produce thick and stout seedlings [25]. The thickness of seedling increases the strength of both root and shoots and helps for

better emergence through soil surface. The seeds treated with ethrel also showed highest germination, which was discussed earlier. Therefore, values for vigour indices were highest for seeds treated with ethrel at 200 ppm followed by ethrel at 100 ppm (Fig 2). In the present study, 2-fold increase in vigour index-I and 4-fold increase in vigour index-II was observed with ethrel over control.

Experiment II: Effect of Physical seed treatment on germination and seed attributes

The results of physical seed treatments on seed germination of colocynth is depicted in Fig 3. The results of main effect of three physical seed treatments (two treatments and one control) revealed that the germination in colocynth increased significantly in decoated and scarified seeds over the control (coated seeds). Decoated seeds recorded highest germination (43.49%) followed by scarified seeds (35.43%) which were 66.7% and 35.7% higher than the control, respectively. The interactive effect of fruit size and stages of fruit maturity on germination of physically treated colocynth seed is depicted in Fig. 4. Multiple comparison tests indicated that fruit size and maturity stage of fruits

Table 1. Effect of chemical seed treatments on shoot and root growth of colocynth seedlings

Treatment chemicals	Concentration	Root Length (cm)	Shoot Length (cm)	Dry Weight (g)
Control	water	7.3±0.11 ^f	12.3±0.11 ^d	0.080±0.002 ^{cd}
GA ₃	100 ppm	5.5±0.11 ^{de}	12.4±0.17 ^{de}	0.080±0.001 ^{cd}
	200 ppm	3.4±0.08 ^b	10.3±0.14 ^c	0.090±0.002 ^d
IAA	100 ppm	5.9±0.09 ^e	13.2±0.22 ^e	0.090±0.001 ^d
	200 ppm	7.2±0.14 ^f	14.2±0.11 ^f	0.120±0.002 ^{ef}
NAA	100 ppm	1.0±0.18 ^a	7.1±0.25 ^a	0.050±0.003 ^a
	200 ppm	1.6±0.11 ^a	7.8±0.27 ^{ab}	0.070±0.001 ^{bc}
Kinetin	20 ppm	4.3±0.19 ^c	8.4±0.15 ^b	0.070±0.003 ^{bc}
	40 ppm	5.4±0.20 ^{de}	7.4±0.22 ^a	0.060±0.005 ^{ab}
Ethrel	100 ppm	5.1±0.11 ^{cde}	9.7±0.18 ^c	0.130±0.004 ^f
	200 ppm	7.2±0.18 ^f	9.5±0.03 ^c	0.120±0.008 ^{ef}
Thiourea	1000 ppm	4.9±0.26 ^{cd}	12.9±0.11 ^{de}	0.110±0.003 ^e
	2000 ppm	5.5±0.17 ^{de}	9.7±0.04 ^c	0.090±0.001 ^d
KNO ₃	500 ppm	7.8±0.27 ^f	15.8±0.27 ^g	0.110±0.002 ^e

Values are mean of 4 replications ±SE.

Columns with the same letters are not significantly different (P<0.05, Tukey test).

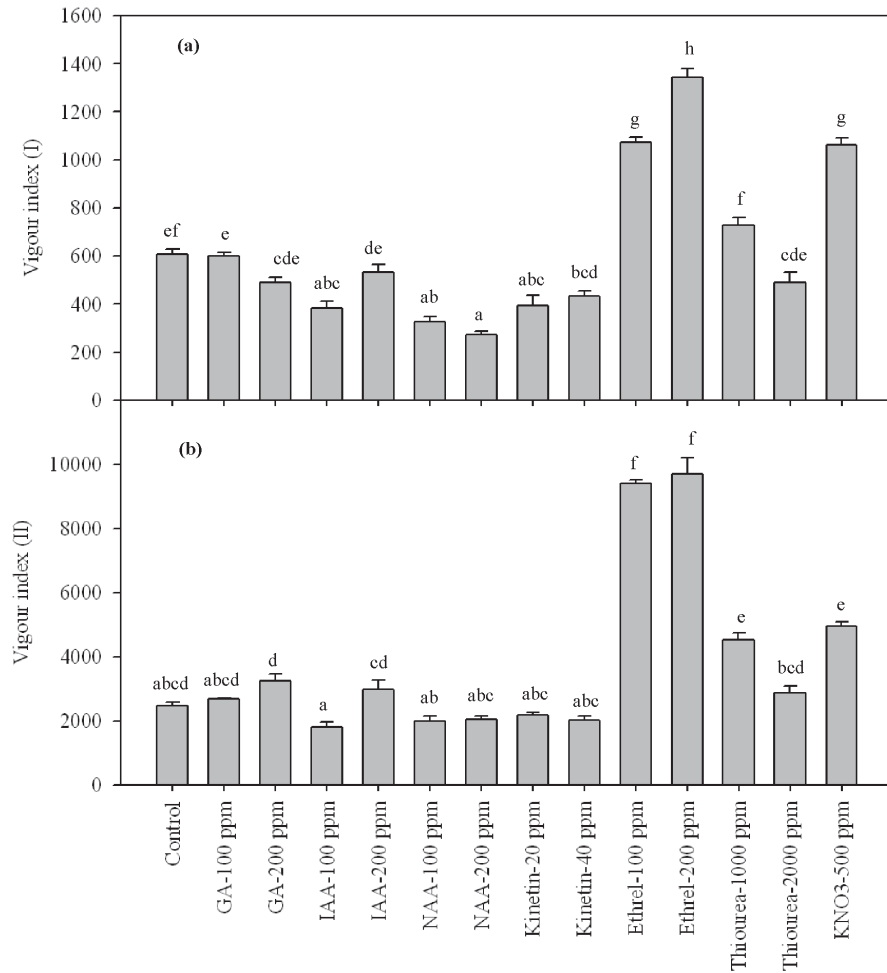


Fig. 2. Effect of different chemical seed treatments on vigour of colocynth seedlings (a) Vigour index (I), (b) Vigour index (II); control- water soaking; GA-Gibberelic acid; IAA-Indole acetic acid; NAA-Napthalic acetic acid; Values are mean of 4 replications \pm SE; Columns with the same letters are not significantly different ($P < 0.05$, Tukey test).

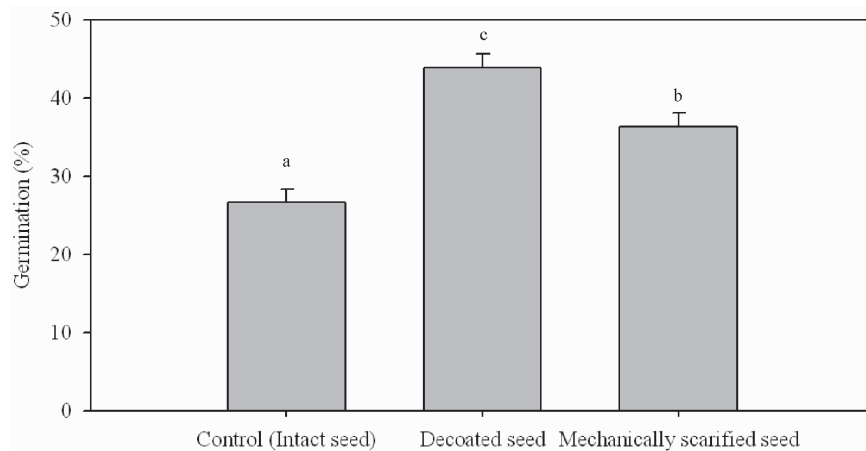


Fig. 3. Effect of physical seed treatments on seed germination of colocynth. Values are mean of 4 replications of each treatment combination: 3 fruit sizes \times 3 fruit maturity stages \pm SE. Columns with the same letters are not significantly different ($P < 0.05$, Tukey test).

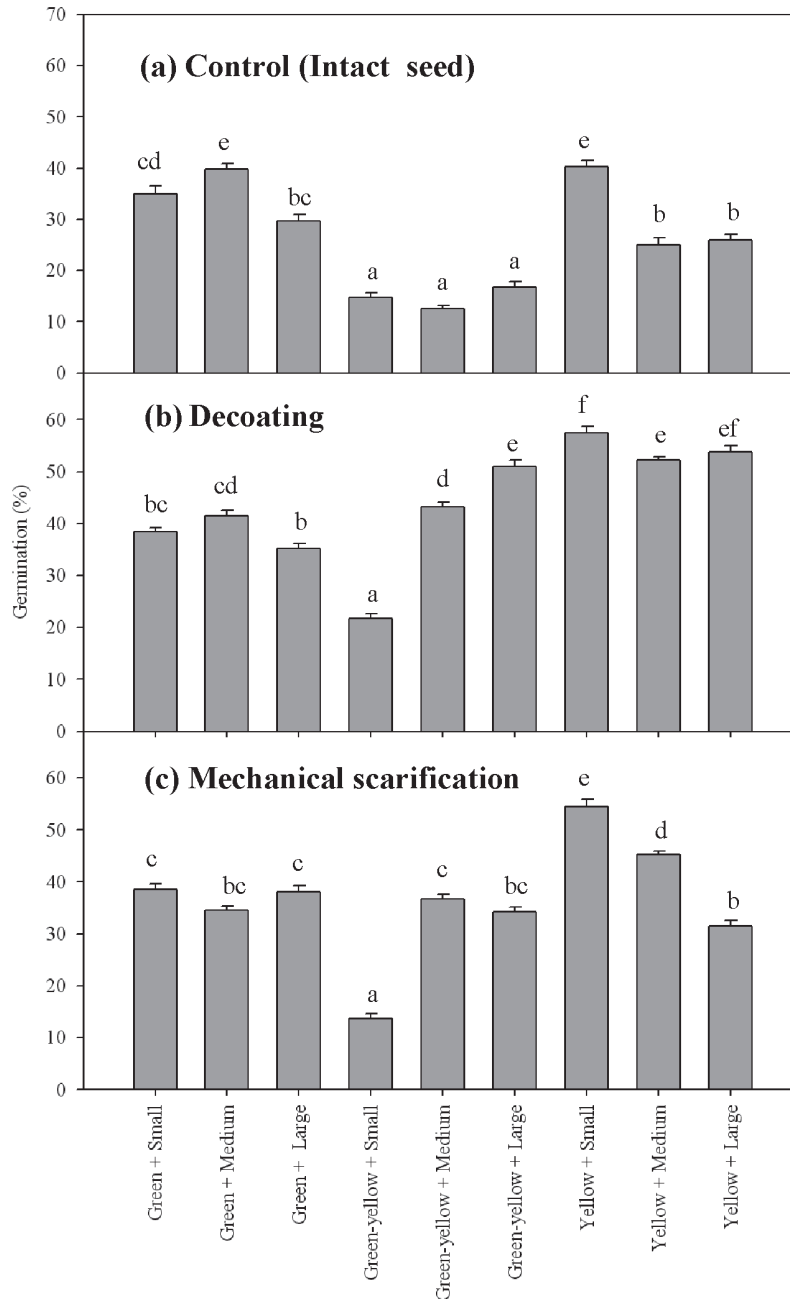


Fig 4: Interactive effects of fruit maturity stage and fruit size on seed germination of colocynth under different physical treatments (a) control (intact seed), (b) decoating, and (c) mechanical scarification. Values are mean of 4 replications \pm SE. Columns with the same letters are not significantly different ($P < 0.05$, Tukey test).

had significant effect on germination of colocynth seeds. Germination was considerably higher in seeds collected from yellow fruits than green and green-yellow fruits in all the three physical seed treatments. Further, seeds collected from small sized fruits at

green and yellow stages recorded higher seed germination and germination percentage decreased with increase in the size of fruits. However, fruits harvested at green-yellow stage showed the reverse trend and seeds collected from large sized fruits

Table 2. Influence of fruit size and stage of maturity on seed weight and seed coat: embryo ratio of colocynth seeds

Fruit size and Maturity stages	100 seed weight (g)	§Proportion in seed weight (%)	
		Seed coat	Embryo
Fruit size			
Small	2.55±0.04 ^a	55.60±0.09 ^a	44.40±0.09 ^c
Medium	2.87±0.03 ^b	56.18±0.18 ^b	43.82±0.18 ^b
Large	3.24±0.02 ^c	57.72±0.10 ^c	42.28±0.10 ^a
Maturity stage			
Green	2.74±0.03 ^a	56.03±0.06 ^a	43.97±0.06 ^b
Green Yellow	2.91±0.03 ^b	56.97±0.17 ^b	43.03±0.17 ^a
Yellow	3.00±0.03 ^b	56.41±0.04 ^a	43.59±0.04 ^b

§proportion of seed coat and embryo was calculated based on total amount of seed coat and embryo of 50 seeds.

Values are mean of 4 replications ±SE.

Columns with the same letters are not significantly different ($P < 0.05$, Tukey test).

recorded higher germination than small sized fruits. The highest level of germination was recorded with seeds collected from small size fruits harvested at yellow stage of maturity.

The size of fruits had significant influence on seed weight and seed coat: seed embryo ratio of colocynth (Table 2). Relatively high seed weight is desirable since it is often correlated with rapid germination and good seedling establishment under a wide range of environments [26 and 27]. Seeds collected from large sized fruits (diameter > 9.5 cm) had higher seed weight (3.24 g per 100 seed) followed by medium (diameter between 7.5 and 9.5 cm) and small (diameter < 7.5 cm) sized fruits. Contribution of seed coat to total seed weight was also higher (57.82%) in seeds collected from large sized fruits and decreased with decrease in fruit size. Reports on synchrony in seeds and fruit maturity and the influence of fruit size on seed content, seed size and vigour have well documented in ash gourd [28, 29], bitter gourd [30] and in cucumber [31]. Further, seed attributes also affected considerably with maturity stages of fruits. Fruits harvested at yellow stages had highest seed weight (3.00 g per 100 seeds) followed by green-yellow stage and green stage. Although the highest seed weight was observed with seeds extracted from yellow fruits, the contribution of seed coat to total seed weight was highest (57.12%) in seeds extracted from green-yellow fruits.

Complete removal of seed coat of colocynth increased seed germination than the intact and/or

mechanically scarified seeds. These results clearly indicate the presence of seed coat dormancy in colocynth seed [8, 32, 33]. Removal of seed coat was effective in lowering germination temperature, shortening the time lag and increasing germination rate [34]. Therefore, the seeds of colocynth may not germinate well in either dark or light until the seed coats were removed [8]. The low germination with intact seeds might also be due to presence of various chemicals like phenols, coumarin etc. that accumulates in the fruit pulp and on seed coat during fruit development and remained on seed surface even after harvest [32, 35].

In the present study it was observed that contribution of seed coat in the total seed weight was higher in seeds extracted from large sized fruits. Therefore, lower germination from seeds of these fruits can be attributed to harder seed coat that might have prevented both gaseous and moisture exchange between seed and atmosphere [8, 36] and/or desiccation in senescence [37]. In case of large sized green-yellow fruits, seed germination was better than small sized fruits. Generally, the increase in germination and vigour is seen with the increase in fruit size [30]. The maturity stage of fruit also has considerable influence on germination of colocynth seeds. The germination of decoated seeds and/or mechanically scarified seed from yellow fruits was higher than that of intact seeds. This indicated that the embryos were fully developed and capable of germination but failed to germinate fully due to seed

coat barrier [8, 38]. The higher germination from green fruits may be due to higher proportion of embryo to total seed weight. Broschat and Donselman [39] also reported better seed germination from green fruits of queen palm (*Syagrus romanzoffiana*) than seeds from half ripe or ripe seeds. The lower germination percentage in the green-yellow fruits might be due to presence of germination inhibitors like ABA in the seeds, concentration of which reaches its peak at the half maturity stage of fruit development and started to decline in the second half of fruit maturity [35, 40, 41]. Thus, the higher germination in the seeds of green and yellow fruits of colocynth might be due to low level of ABA in the seeds.

CONCLUSION

Among seven chemicals tried in the study, ethrel at 200 ppm gave significantly higher germination and vigour over the others. The removal of hard seed coat is found successful in breaking seed dormancy and improvement of seed germination. The role of seed coat and its proportion with total seed weight also had significant role in inducing seed dormancy in colocynth seed. The fruit size and the stage of maturity had also significant influence on seed germination. Seeds extracted from small sized fruits (<7.5 cm in dia) and harvested at yellow stage of maturity exhibited best germination. These findings revealed that the colocynth seeds have both physical as well as physiological seed dormancy. This encourages a systematic approach of research in selecting proper stage of fruit maturity in combination with use of suitable seed pre-treatment for getting desired seed germination for its propagation and cultivation.

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