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In silico and *in vitro* Assessment of Indigenous Organic Practices on Germination and Seedling Metrics of Compact Cotton using GerminaR R Software Package

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ABSTRACT

Background: Cotton crop is sensitive in its young stages *i.e.* stand establishment. Cotton seeds are fuzzy and messy in handling and field sowing. Delinting is a necessary process done commonly using acids and are comprehensively not protective. Optimal plant population, growth and persistence are strongly influenced by germination and establishment. In line with this, it is necessary and needful of an hour to find an alternative, eco-friendly, ecologically safe and low cost seed treatment method.

Methods: The experiment was conducted in two methods *viz*., a petridish method and roll-towel method with seed treatment methods *viz*., cowdung and red earth mix treated with and without biofertilizer and compared with acid-delinted and fuzzy seeds. The germino-metrics were determined using GerminaR package, an interactive web application and statistical analysis was performed using R software package v. 4.2.1.

Result: The summative effect of seed treatment with fresh cow dung, red earth and biofertilizer (*Azospirillum* and *Phosphobacteria*) per kg of seeds at respective quantities (0.5:0.25:0.12) recorded significantly maximal germination indices and seedling growth determinants. This present study would be a complementary to comprehensively promote the use of indigenously available natural materials as an alternative to acids in cotton seed delinting.

Key words: Germinar, Germination indices, Indigenous, R software, Seedling characters, Two-way ANOVA.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is the world's primary natural fibre crop, third largest oilseed crop (Waseem *et al*., 2017) and a prime agricultural commodity for several global economies. The crop provides the basic raw material (cotton fibre) to textile industry and known as "King of Fibre Crops" and "White Gold" (Walia *et al*., 2022). Globally, 113.11 million bales of 170 kg were produced with an area of 33.48 million hectare whereas India produces 36.5 million bales of 170 kg (32% share of global production) cultivated in 13.48 million hectare (Anonymous, 2022a; Anonymous, 2022b). India is the largest producer, third largest exporter and second largest consumer of quality cotton (Anonymous, 2022a). Hence, cotton is a vitally important crop of Indian agrarian society and economy. Remarkable benefits had been witnessed in recent with organic cotton production, organic clothing demand and employment opportunities (Asif, 2017). Regarding the statistics on organic cotton, 51 per cent of world organic cotton production (2.43 million tonnes) is produced in India (1.23 million tonnes). Around 99 per cent of organic cotton production is concentrated in Madhya Pradesh (0.38 Mt), Odisha (0.11 Mt), Maharashtra (0.19 Mt), Gujarat (0.08 Mt) and Rajasthan (0.06 Mt) (Anonymous, 2022b). Farmers of India are considering organic as a better option meanwhile Indian government through various means promoted organic cotton research under ICAR-CICR and DoA-NFSM and also through a central scheme "*Paramparagat Krishi Vikas Yojana* (PKVY)" (Anonymous, 2022b). A prominent shift in major portion of cotton cultivation to organic cotton will be experienced in short period. Eco-friendly, ecologically safe, low cost technologies, resilient genetics and better agronomic approaches are needed to sustainably enhance cotton production.

Several researchers have reported that germination, seedling emergence and stand establishment are critical in cotton *i.e.* sensitive in young stages. The cotton seeds are

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generally fuzzy (lint <0.5 cm) which makes it messy in handling (due to clinging), sowing and reported to be less effective in stand establishment. Removal of fuzz from the seed (delinting) may promote seed germination (De Groot *et al*., 2018). Delinting using acid (concentrated H_2SO_4) is a common practice and considered to be very effective and inexpensive, however, issues concerning the process include potential seed damage, worker safety, waste disposal and corrosion of acid-exposed equipment (Meada *et al*., 2021). Some of the issues raised by acid delinting could be addressed by using an alternative way of preparing cottonseed for sowing using eco-friendly and ecologically stable substances meanwhile delinting associated with nutrient supply may enhance seed germination, speed, vigour and seedling establishment. In organic cotton production using acids are also not highly encouraged. Therefore, the objective of this study is to explore the conglomerate effect of indigenous natural substances (cow dung and red earth) and microbilization (using biofertilizers) on germination, vigour and seedling characters of cotton and to evaluate whether the alternative method of delinting is feasible to adopt.

MATERIALS AND METHODS

Seed material

The genetically pure compact cotton seed variety CO 17 (Coimbatore 17) was used for this research. The seeds were purchased from Department of Cotton, Tamil Nadu Agricultural University, Coimbatore. The cultivar is early (125-135 days), high yielding, compact (no monopodia) and suitable for high density planting and mechanized cultivation.

The cotton seeds are treated with materials *viz*., conc. H_2SO_4, fresh cow dung, red earth and biofertilizer. The details regarding site of collection, use-ratio and nutrient contents are presented in Table 1 and 2.

Experimental details

The experiment was carried out under partially controlled conditions (16 h/8 h light/dark pattern and temperature maintained at 25°C) in Agronomy Laboratory belonging to Department of Agronomy, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India (Latitude-11°N, Longitude – 76.9°E). The experiment was conducted in two methods *viz*., a petridish method and roll-towel method (Mortensen and Mathur, 1993), both were laid out in completely randomized block design (CRBD) with 4 treatments and 5 replications. The indigenous natural mix seed treatment methods (CR - cotton seed treated with cow dung and red earth and CRB - CR + biofertilizer) were compared with acid-delinted and fuzzy seeds (control) (Fig 1). The indigenous natural materials were mixed with the seed, to remove lint and shade dried for 6 hours. For each treatment, 25 healthy seeds were placed in petri dishes (12 cm diameter) with two layers of seed germination paper and in germination paper (12 inches × 18 inches, 26 lb.psi, 90 gsm). Both

Table 1: Inputs used in the experiment.

Materials used	Site of collection	Proportion used	Reference
Conc. H_2SO_4 (98% AR grade)	Agronomy Laboratory, Department of Agronomy, TNAU, Coimbatore	100 ml acid per kg of seeds	The respective quantities were used as per TNAU crop production guide (CPG) 2021 recommendation
Biofertilizer (*Azophos*)	Department of Agricultural Microbiology, TNAU, Coimbatore	120 g per kg of seeds	
Red earth	Eastern-block farms, TNAU, Coimbatore	0.25 kg per kg of seeds	The quantity of cow dung and red earth were fixed as per the multi-trail method (manual reference). The mixture was added until removal of fuzz (>95%) from the cotton seed
Fresh cow dung	Central Farm Unit, Department of Veterinary and Animal Sciences, TNAU, Coimbatore	0.50 kg per kg of seeds	

were maintained wet throughout the study. The germination counts were recorded at 4 days, 8 days and 12 days interval in petridishes. A seed was considered as germinated when radical had emerged more than 2 mm (Meada *et al*., 2021). The roll-towel method kept undisturbed upto 12 days and was used to record morpho-physiological seedling characters after 12 days.

Statistical analysis

R software package v. 4.2.1, were used for statistical analysis, data processing and plotting figures (Anonymous, 2022c). GerminaR package (Lozano-Isla, 2019) with interactive web application was used to calculate ten germination indices from petridish germination count data and multiple comparisons were made with students-newman keuls test. The seedlings characters, subjected to two-way analysis of variance (ANOVA) and box-violin plots were made using "ggstatsplot" package (Patil, 2021). Family-wise error rate was accounted for with adjusted p-values for multiple comparisons using the "fdr" method at 5% confidence interval ($p<0.05$).

RESULTS AND DISCUSSION

Effect of seed treatments on germination indices of compact cotton seedling characters and quality of cotton

Germination indices were derived using count data recorded from petridish method. Germinability (%) (Table 3), germination seed number (counts), germination speed (%), mean germination time (days), mean germination rate (day^{-1}), synchronization index, uncertainty index, germination variance (day^2), germination standard deviation (day), coefficient of variance (%) were found to be significant at 5% confidence interval (p-value < 0.05) (Fig 2). Among the treatments, maximum germinability (98) and number of seeds germinated (24) were recorded in seeds treated with cow dung, red earth and biofertilizer at respective quantities. Germination speed (11.6) and mean germination rate (0.12) was higher in acid-delinted seeds which was on par with other organic seed treatments. Mean germination time (10.3), synchronization index (0.48), germination variance (68.4), germination standard deviation (8) and coefficient of variance (78) were found to be maximum in fuzzy seeds.

Table 2: Nutrient content of input used.

Parameters	Fresh cow dung	Methods used
Total nitrogen (%)	0.83	Diacid digestion, Micro kjeldhal method. Humphries (1956)
Total phosphorous (%)	0.27	Triple acid digestion with calorimetry method Jackson (1973)
Total potassium (%)	1.09	Triple acid digestion with flame photometry method

Table 3: Seed vigour index and germination percentage of organic compact cotton.

Treatments	Seed vigour index-I	Seed vigour index-II	Germination percentage (%)
Cowdung + Redearth treated seeds (CR)	2428.72	4.511	89.6
Cowdung + Redearth + Biofertilizer treated seeds (CRB)	2987.04	6.26	98.4
Acid-delinted seeds (D)	2268.72	3.496	92
Fuzzy seeds (F)	994.32	1.809	55.2
p-value	<0.001	<0.001	(Not analyzed statistically)

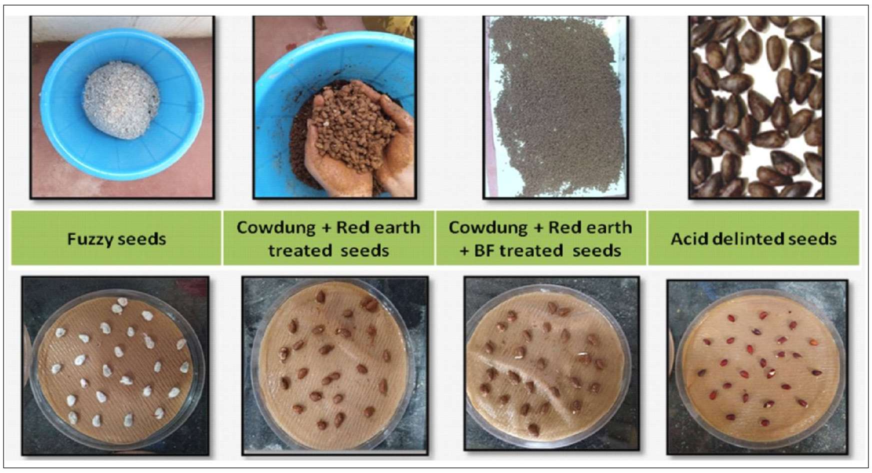
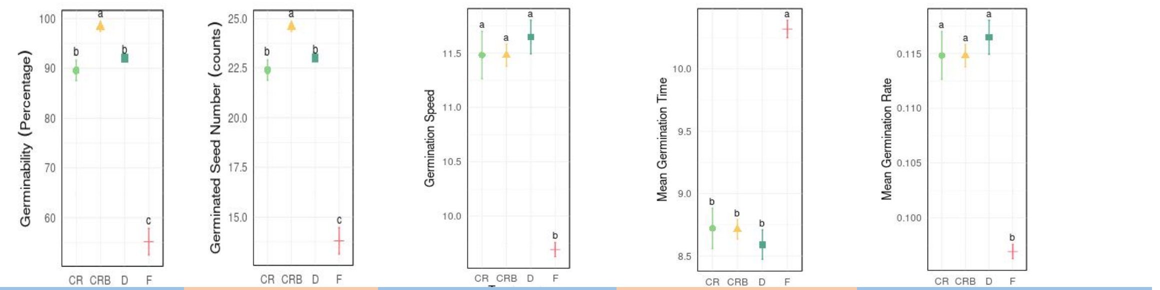
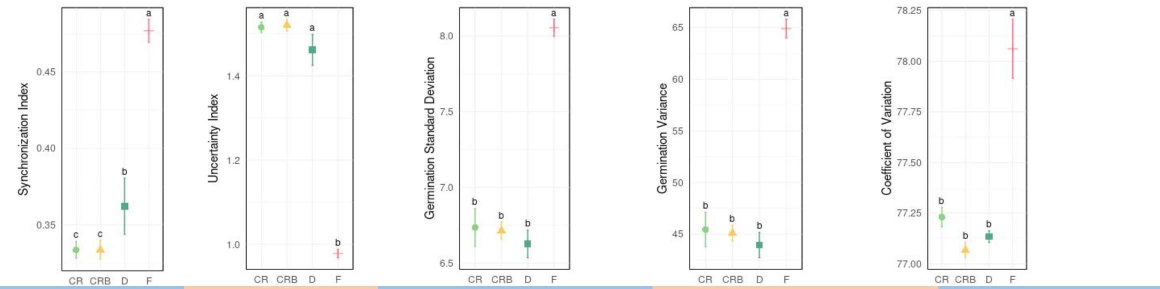


Fig 1: Seed treatment methods.

The uncertainty index (1.52) was higher in indigenous mix and biofertilizer treated seeds which were on par with cow dung and red earth and acid-delinted seeds. The growth promoting substances (auxin), different minerals, including N, K, S, traces of P, Fe, Ca, Mg, Co and Mn in cow dung ominously enhanced the cotton seed germination (Gupta *et al*., 2016). Moreover, cow dung-treated seeds are generally protected from pathogenic fungal and bacterial attack because bacteria, notably *Bacillus spp.* in cow dung microflora, play an important role in preventing the growth of pathogenic microorganisms by colonising the surface area of the seeds (Swain *et al*., 2017). The findings are in



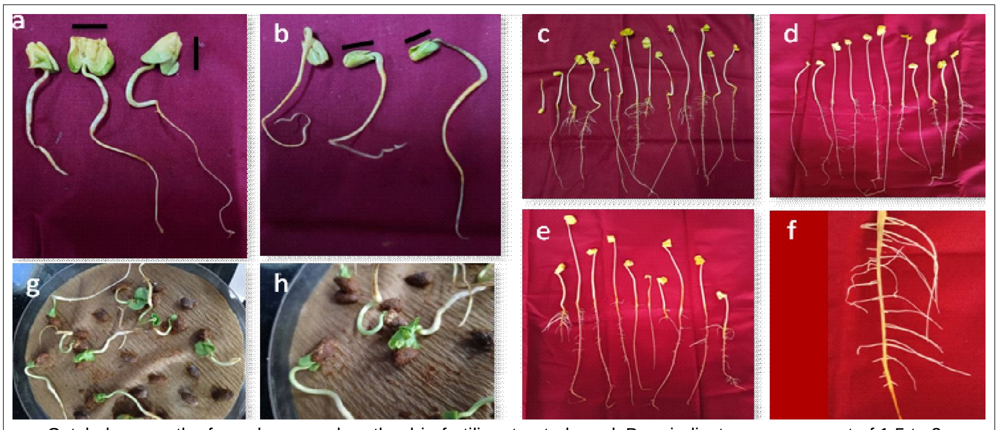
Mean	MSerror	CV	Mean	MSerror	CV	Mean	MSerror	CV	Mean	MSerror	CV	Mean	MSerror	CV
83.8	15.33	4.67	20.95	0.958	4.68	11.076	0.117	3.086	9.086	0.066	2.836	0.111	0.001	3.086



Mean	MSerror	CV	Mean	MSerror	CV	Mean	MSerror	CV	Mean	MSerror	CV	Mean	MSerror	CV
0.377	0.001	5.333	1.369	0.002	3.004	7.003	0.031	0.227	49.84	7.395	5.456	77.37	0.031	0.226

Treatments: F- Fuzzy seeds, D- Acid delinted seeds, CR- Cowdung + Red earth treated seeds, CRB- Cowdung + Red earth + Biofertilizer treated seeds. a, b, c in the graph indicates the Students Newman-Keuls test pair wise comparisons.

Fig 2: Effect of seed treatment on germination indices of organic compact cotton using GerminaR package.



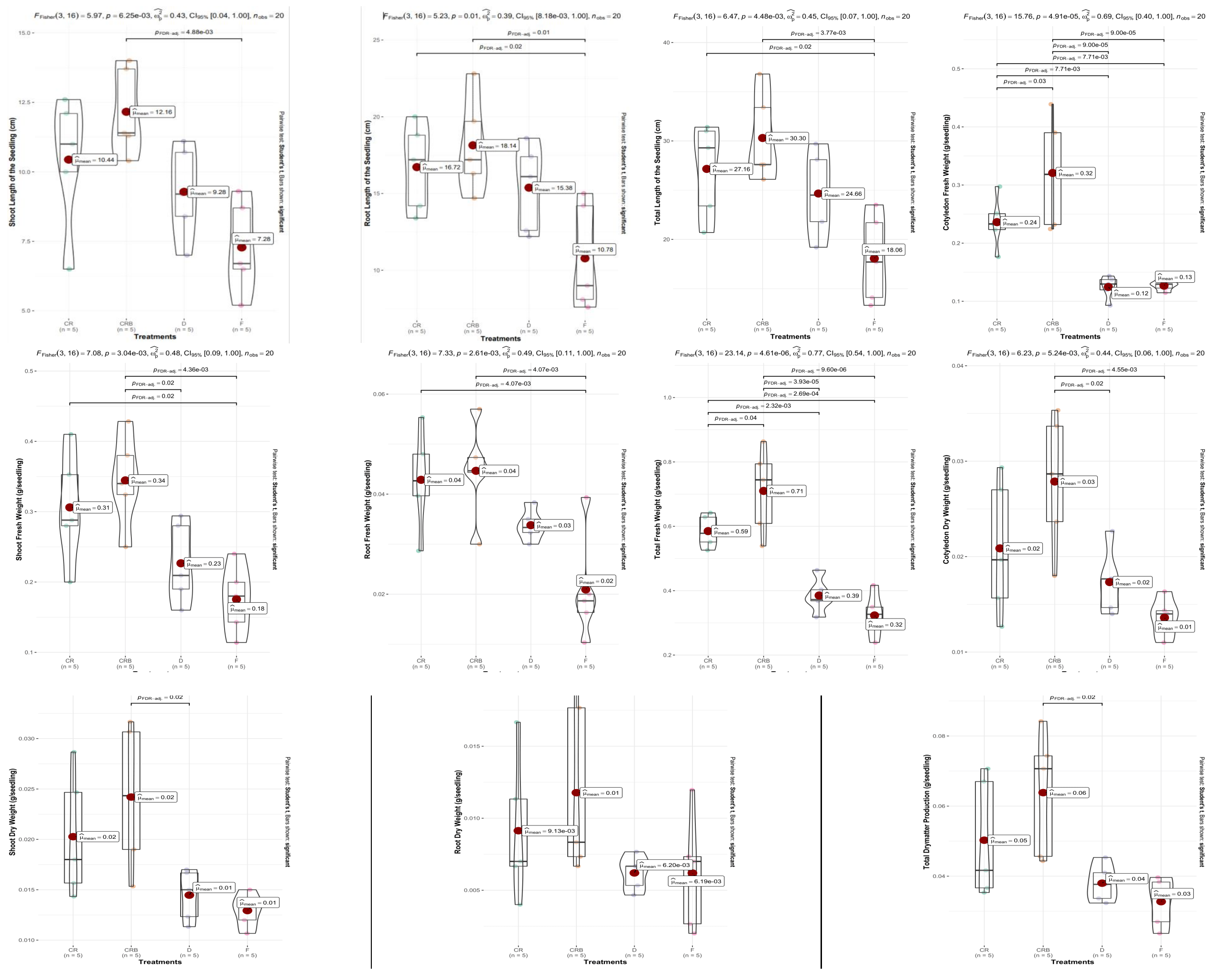
a. Cotyledon growth of cowdung + red earth + bio-fertilizer treated seed. Bars indicate measurement of 1.5 to 2 cm.
b. Cotyledon growth of acid delinted seed. Bars indicate measurement of 0.5 to 1 cm.
c, d and e. seedling characters of CRB, CR and D treatments.
f. Root growth of the Seedling (CRB treated seed).
g and h. Cotyledon growth of CRB and CR treated seeds in petridish with moistened germination paper.

Fig 3: Laboratory results of organic seed treatments on germination and seedling characters of organic compact cotton.

concordance with other researchers (Vachanni *et al*., 2014; Shinde and Malshe, 2015; Raj *et al*., 2016). The texture of red sandy loam soil had dominant proportion of sand particles (>0.2 mm diameter) and fair concentration of clay and silt which may cause abrasion in the seed surface area when rubbed along with cow dung and beneficial (Veeraragavathatham *et al*., 2006) in subsequent removal of fuzz above the cotton seed meanwhile the silt (<0.02 mm diameter) and clay particles (<0.002 mm diameter) could hold good amount of water and amplified the water retention time for imbibition which perhaps enhanced germination.

Effect of seed treatments seedling characters and quality of compact cotton

The seedling characters and vigour were calculated from observation recorded from roll-paper towel method. The shoot length (12.1 cm), root length (18.1 cm), total seedling length (30.3 cm), cotyledon fresh weight (0.32 g/seedling), shoot fresh weight (0.35 g/seedling), root fresh weight (0.05 g/seedling), total fresh weight (0.71 g/seedling), cotyledon dry weight (0.03 g/seedling), shoot dry weight (0.03 g/seedling), total drymatter production (0.064 g/ seedling), seed vigour index I (2987) and II (6.26) (Table 3) were found to be higher and two-way ANOVA was statistically significant at 5% level in cow dung, red earth and biofertilizer treated cotton seed treatment (Fig 3 and 4). The non-significance in root dry weight (0.012 g/seedling) was recorded (Fig 4) due to negligible dry-weight of roots.Minimum values were recorded in fuzzy seeds. Most of the seedling characters were found to be identical with organic treatments and acid-delinted seeds. The productive and protective seed germination process due to seed treatment presumptively led to better stand establishment in young cotton seedlings. Further, microbilizing the seed surface with *Azospirillium* and *Phosphobacteria* through biofertilizers must have attributed the cotton seedling growth



Treatments: F- Fuzzy seeds, D- Acid delinted seeds, CR- Cowdung + Red earth treated seeds, CRB- Cowdung + Red earth + Biofertilizer treated seeds.

Fig 4: Effect of seed treatment on seedling characters of organic compact cotton (two-way ANOVA).

parameters and vigour. *Azospirillium* is one of the formidable plant growth promoting rhizobacteria (PGPR) which produces constructive phytoharmones (including auxin, cytokinin and gibberellins) and elicit changes in root development and root architecture are focally reported outcomes of *Azospirillium* inoculation (Cassan *et al*., 2020). The bacterial strains in *Phosphobacteria* biofertilizer intensified the production of secondary metabolites (polyphenols, flavonoids, terpenes and alkaloids *etc*.) which correspondingly supported the germination, growth and development of the crop. The researchers (De-la-Cruz Chacon *et al*., 2013) reviewed and reported that about 200 secondary metabolites are identified to be accumulated and functioning during the entire period of the plant life cycle.

CONCLUSION

The laboratory screening study concluded that the cumulative effect of seed treatment with fresh cow dung, red earth and biofertilizer (*Azospirillum* and *Phosphobacteria*) at respective quantities recorded significantly maximal germination indices and seedling quality determinants. In most of the parameters, organic seed treatments performed better and commensurate with acid-delinting of cotton seed. Hence, the usage of naturally available indigenous substances in addition with biofertilizer in cottonseed for fuzz removal and nutrient supply by seed dressing can possibly be used as an alternative to acids in organic cotton production.

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Conflict of interest

The authors declare that there is no conflict of interest.

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