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Bio efficacy of different aqueous leaf extraction of *Morinda tinctoria* against seed germination and its related attributes in camel crop sorghum (*Sorghum bicolor* (L). Moench)

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Abstract
In Today's agriculture trees and plants plays vital role in ecosystem and it is an essential part to lead healthy human life. Various aqueous leaves extracts of *Morinda tinctoria* were tested for the potential exhibitory and inhibitory effects on sorghum. There were four extraction methods *viz.,* i) Fresh leaf extract ii) alcoholic extracts iii) decoction at (1:4) and (1:6) each at 5 and 10 percent concentrations and distilled water served as control. Seed germination of sorghum was significantly exhibited by fresh leaf extracts at 5 per cent. Radicle, plumule growth, seedling vigor index I and II and other germination attributes were also increased by fresh leaf extract. It is assumed that optimum pH, EC of fresh leaf aqueous extract and also the presence of some growth promoting phytochemicals. It was at par with decoction (1:6) at 5 percent. The highest inhibition was noticed with alcoholic extract at both concentrations. It is found to have better efficiency in extracting the bioactive compound present in the tree leaves of *Morinda*. Further, instead of using an alcohol, it is natural way of extraction and it could be adopted easily and followed without any extra input or instrumentation or tool.

Keywords: Sorghum, germination percentage germination attributes, leaf extraction method, *Morinda tinctoria*

Introduction
Sorghum (*Sorghum bicolour* (L.) Moench) enjoys the status of fifth most important coarse cereal and multiuse crop used as a food, feed and bioenergy crop. Due to its wider adaptations, it can be cultivated in numerous environments. This crop has more tolerant to biotic and abiotic stresses including drought, heat, and flooding It is also considered as a camel crop (Ali *et al*., 2011) [1]. Trees and plants play an indispensable role for the ecosystem and it is the pool of numerous organic compounds they have long been used as sources of medicines, seasonings, beverages, cosmetics and dye. The kingdom Plantae contains about 300–315 thousands of plant species and the genus *Morinda* is one of the ethnic plants of the Rubiaceae family and its eleven species were found in India (Bharat Singh and Sharma, 2020) [2]. This includes *Morinda citrifolia* Linn. *Morinda elliptica Ridley. Morinda pubescence, Morinda trimera* Hillebr, *Morinda umbellate and Morinda tinctoria Roxb.* etc of which *Morinda tinctoria Roxb.* commonly known as Nuna, Aal and Indian mulberry is an evergreen shrub native to southern Asia, upper and lower Burma, Bengal, Bihar, central provinces and in the Deccan westwards to the eastern slopes of the Ghats in India. Species *Morinda tinctoria* broadly cultivated in south India and traditionally used as a medicine in India and China (Kirtikar and Basu, 1935) [3] and scattered throughout Tamil Nadu and in some parts of Kerala (Anuradha *et al.,* 2013) [4].
Predominantly this tree has grown in high clay soil considered as a menace in South East Asia and a weed tree in vacant lands (Narayanasamy *et al.,* 2006 and Praveena *et al.,* 2012) [5, 6]. Botanicals and pharmaceuticals are a perfect fit for prophylactic measures to prevent disease and for normal well-being. Among this *Morinda tinctoria* is very well known for its theraupeutic benefit in Indian system of medicine including Ayurveda and siddha and in other forms of traditional medicineworldwidee for the treatment of several ailments. Natural extracts and their segregated compounds from plant materials have spectrum of biological activities.
Some researchers hypothesized that *Morinda tinctoria* may also distinguished with some chemical and medicinal properties that may also be useful for crop production.

(Swaminathan and Nandakumar, 2017) [7] reported tree leaves extracts offer potential for development of new bio-formulations, which would be used as growth stimulants, as well as growth tonics for compatible environmental crop production. Bioactive components with commercial value extracted from aerial parts of plant like leaves and roots used as astringents and treat gout (Thirupathy and Saravanan, 2009) [8] also acts as bibiosorbentsn waste water management Suneetha and Ravindranath, 2012[9]. Leaf juice is used for many medicinal purposes (Rex Jeya Rajkumar *et al*., 2018) [10] anti-fungal and antibacterial properties (Jayasinghe *et al*., 2002) [11] and anticonvulsant and anti-microbial activity (Deepti *et al.*, 2011) [12] and also for seed treatment (Kumar *et al*., 2014) [13]. Since, widely it has many therapeutic purposes had been used in human and animal health care but with very limited attempts in agriculture. Utilization of natural tree leaf extracts, other parts as botanicals which was surplus in bio-active constituents known as secondary metabolites used as prophylactic in organic farming and biological control to rectify ills. It does not have any special functions in plant physiology but it can able to achieve and ensure good crop health and becomes really important in today's agriculture.
Based on these views, the present study was done in sorghum crop for studying the efficacy of *Morinda tinctoria* by various aqueous extracts as seed fortification and assessing the best extraction method suitable for enhancing germination and foliar spray for crop growth.

Materials and Methods

The lab experiment was conducted at the Agricultural College and Research Institute, Department of Agronomy, Madurai, to found out the efficacy of aqueous leaf extracts of *Morinda tinctoria* against seed germination and its attributes in sorghum.

Collection of plant materials

Fresh and clean leaves of *Morinda tinctoria* were collected from the experimental farm of Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, India. From freshly collected leaves, different aqueous and alcoholic extracts were prepared. *viz*., Fresh leaf extracts Alcoholic extract, Decoction method (1:4) and (1:6) ratio. From the distinct stock solution, different strengths *viz*., 5 and 10% concentrations were prepared. Distilled water served as a control and was subjected to pre-seed treatment each.

Methods of preparation of extracts

1. Fresh leaf extract

The fresh leaf extract was prepared by grinding fresh leaves and distilled water at 1:1 proposition. The extract was filtered and this served as stock solution. From the stock solution 5 and 10% solutions were made and subjected to seed fortification**.** (Swaminathan and Nandakumar, 2017) [7].

2. Alcoholic extract

The fresh leaves were collected and cut in intoall pieces, soaked in alcohol and water at 1:1 proportion and kept for overnight. After 12 hours of soaking, soaked leaves were ground using electric mixer-grinder. From the grounded leaf paste, the leaf extract was prepared which served as stock solution (Sripunitha, 2009) [14] and then 5 and 10% concentrations were prepared and subjected to seed fortification treatment.

3. Decoction method with (1:4) and (1:6) ratio

This method is suitable for extracting water soluble/ heat-stable constituents and this method typically used in the preparation of Ayurvedic extracts quath/kawath. Fresh leaves were collected and boiled with distilled water at (1:4) and (1:6) ratio for 15 to 20 minutes to get a specified volume of distillate and distillate was filtered and served as stock solution (Handi, 2008) [15].

pH and EC of the spray solution

pH indicates negative logarithm of hydrogen ions it was measured by pH meter and EC indicates the Electrical conductivity measured in EC meter. Both EC and pH are important parameters in nutrient management (Samarakoon and Palmer, 2020) [16] and foliar sprays require an acidic pH for proper absorption (Ace, 2016) [17].pH affects the solubility of nutrients and their interaction with other components in the water. The pH of the treated solution presented in Table 1.

Table 1: pH and EC of the different extracted solution of *Morinda tinctoria*

Treatments	pH	EC (dsm^{-1})
Fresh extract at 5%	5.27	0.03
Fresh extract at10%	5.39	0.07
Alcoholic extract at 5%	1.64	1.58
Alcoholic extract at10%	1.43	1.0
Decoction (1:4) at 5%	5.27	0.03
Decoction (1:4) at 10%	5.58	0.06
Decoction (1:6) at 5%	5.41	0.57
Decoction (1:6) at 10%	6.26	0.57
Control (water)	7.0	0

Setup for the investigation of the experiment

The Germination test was performed by using between-paper (BP) techniques (Fig. 1) prescribed by ISTA, 2006 [18]. Germination was counted daily and total seedling length was recorded at the end of the study. The data recorded on the daily count of Germinants were used to calculate various germination indices as given below.

Measurement and analysis of data

The treated seeds were evaluated for the following seed and seed quality characteristics. Number of germinants was counted daily from the onset of germination up to 10 days thereafter. From the daily count of germinants the following parameters were computed: Germination percentage, shoot length, root length and Vigour index I {Germination% X total seedling length (cm)} and vigour index II {Germination percent% X total dry weight of seedling} Abdul-Baki and Anderson, 1973 [19].

Fig 1: Seeds were placed in between paper technique bottom filled with different aqueous solution

The other germination parameters were i) Germination Energy (GE), ii) Germination Value (GV), iii) Emergence Energy Value (EEV) and iv) Germination Relative Index (GRI).

1. Germination Energy (Maguire, 1962) [20]
Germination energy (GE) was calculated by the formula

$$GE = \frac{X1}{Y1} + \frac{X2-X1}{Y2} + \ldots\ldots\ldots\ldots + \frac{Xn-Xn-1}{Yn} \quad \ldots\ldots\ldots \text{Eqn. 1}$$

Where Xn = number of germinants on the n^{th} counting date; Yn = number of days from sowing to the n^{th} count.

2. Germination Value
Germination value is the integral of final mean daily germination percentage (MDG) and peak value (PV). Final MDG MDG is mean daily germination calculated as the percentage of full-seed germination at the end of the test divided by the number of days to the end of the test. PV is peak value, or the maximum quotient derived from all of the cumulative full-seed germination percentages on any day divided by the number of days to reach these percentages (Czabator, 1962) [21].

3. Germination value
Germination value (GV) is calculated by the given formula, Djavanshir and Pourbeik, 1976 [22].

$$GV = (\sum DGS / N) \times \frac{GP}{10} \quad \ldots\ldots\ldots \text{Eqn. 2}$$

Where GV = germination value, GP = Germination per cent at the end of the test, DGS = Daily germination speed obtained by dividing the cumulative germination per cent by the number of days since sowing, ∑ DGS = Summation of all DGS figures, N= Number of daily counts effective from the date of first germination.

4. Emergence Energy Value (EEV)
Emergence Energy Value (EEV) is the highest value obtained when the germination percentage on a day is divided by the number of days since test begun, when that germination percentage was reached (Bahugana *et al.*, 1987) [23].

5. Germination Relative Index (Sreevatsava and Sareen, 1972) [24]
(GRI) was computed by the given formula

$$GRI = [\sum Xn\,(h - n)] \quad \ldots\ldots\ldots \text{Eqn. 3}$$

Where, Xn = number of germinants at n^{th} count, h = the total number of counts and n = count number.

6. Response Index
The magnitude of inhibition versus simulation in bioassay was compared through response index (RI) (Richardson and Williamson, 1988) [25] determined as follows:

If T > C, RI=1-(C/T)
If T = C, then RI = 0
If T < C, then RI= (T/C) -1 ……. Eqn. 4

Where T is the treatment mean and C is the control mean. A negative RI reflects the proportional disparity in output (germination, radicle length or plumule growth) of test crop in the treatment relative to output in the control.

Statistical analysis
All the data on observations were recorded and subjected to ANOVA with 5% level of significance (Panse and Sukhatme, 1967) [26] white prints. Supply the best quality illustrations or pictures possible.

Results and Discussion
pH and EC of various aqueous extracts on germination
Plants vary in their response to pH and EC of the irrigation water and also spray solution. Variation in the response of plant to pH and EC is even reflected during the germination of seeds (Table 1). In this study, 90-100 per cent seed germination occurs in 5.0-7.0 pH and 0.03-0.5 dsm^{-1} EC range. The pH of foliar sprays should be slightly acidic (~5.8) to allow the solution to easily penetrate the cuticle (due to the complex electrostatic repulsion and attraction phenomena, which is regulated by pH within the cuticle) and be absorbed by the leaves (Ace, 2016) [17]. But in the present investigation, pH of spray solutions was less than 3.0 and EC more than 1.0 dsm^{-1} in the alcoholic extract which restricted the germination rate and seedling growth. This is in accordance with Perez-Fernandez *et al.*, 2006 **(27)** showed that pH lower or higher than seven reduced seed germination of some species.

Aqueous extracts on germination per cent, shoot length, root length and vigour index
The present study explored the potential of aqueous leaf extracts of *Morinda tinctoria* on germination and its related parameters in sorghum (Tables 2 & 3). Germination and seedling growth of sorghum were influenced and inhibited by various leaf extracts of *Morinda tinctoria* and showed mixed values for response index (Fig. 2) when compared to control. While, fresh leaf extracts at 5% showed positive responses in all germination attributes. This showed similarity with the findings of (Kathiravan and Marimuthu, 2019) [28] When maize seeds were treated with 1% *noni* (*Morinda citrifolia*) leaf extract.
The promotion of germination by these botanical leaf extracts is due to active principle involved in it. It is supposed that these botanicals may contain micro-nutrients which are conducive for seed invigouration (Manimekalai, 2006) [29]; presence of biochemical properties *viz*., phenolic compounds, organic acids, proteins and alkaloids Senthilkumar *et al.*, 2016 [30] provided support for the present findings. Little inhibition effect of sorghum was observed when seeds treated with decoction (1:6) at 5% this was at par with fresh leaf extract at 5%. The highest hindrance effect was shown when alcoholic extracts at both the concentrations were used. These results are in similarity, while using leaves and fruit extracts of *Morinda citrifolia* the presence of allelopathic substances inhibited the germination of *Senna occidentalis* and *Senna obtusifolia* (Gonclaves *et al.*,2016) [31]. In other hand, other extraction methods showed the inhibitory effects when compared to fresh leaf extract at 5% and control. It may be due to extracts at higher concentration inhibiting the germination intensity and development were noticed in lettuce (Pereira *et al.*, 2003) [32].

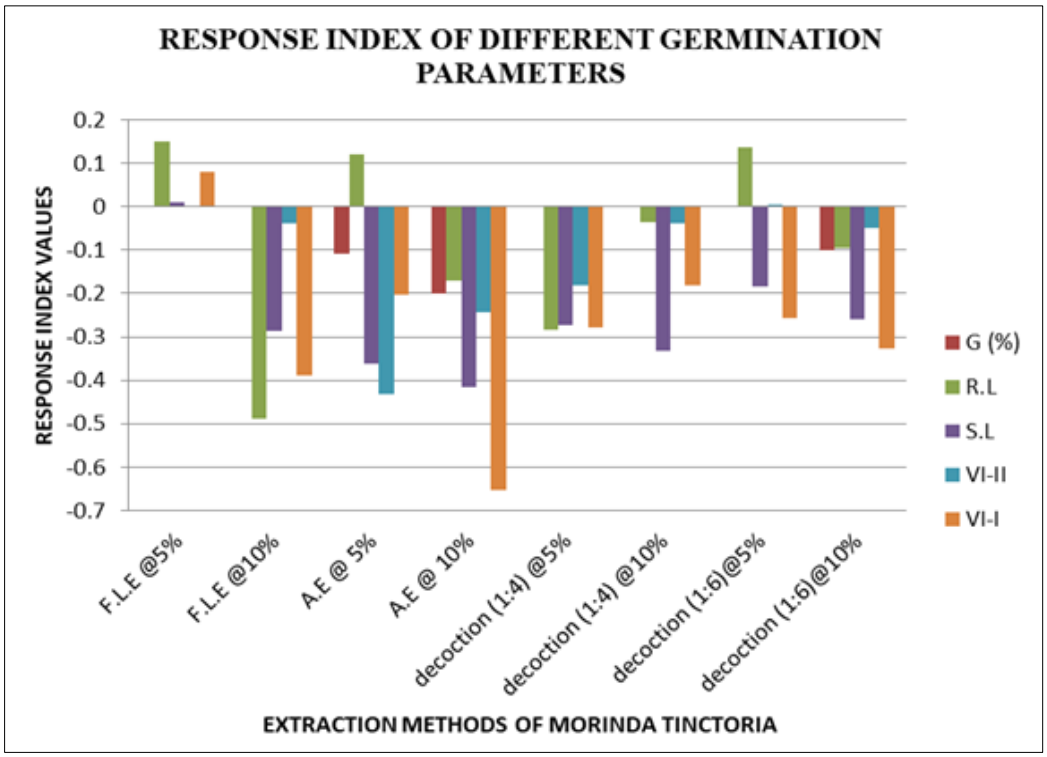

Fig 2: Response index values of different *Morinda tinctoria* leaf extracts on sorghum

Influence of concentration of spray solution on germination and germination-related attributes

Among the different aqueous solutions concentrations at 5 per cent registered better germination and germination parameters which enhanced the germination up to cent per cent. When compared to 10 per cent. Instead of 5 percent concentration 10 per cent has recorded more inhibition. This may be due to the presence of phytotoxins instead of osmotic inhibition which ensures low osmolality Richardson and Williamson, 1988 [25].

Table 2: Effect of extraction methods on germination percentage, root length, shoot length and vigour index in sorghum

Treatments	G.P (%)	Root Length (cm)	Shoot Length (cm)	VI (I)	VI (II)
F.E at 5%	100	7.08	5.92	1300	18.20
F.E at 10%	100	3.11	4.18	729	17.50
A.E at 5%	90	6.84	3.74	952.2	10.35
A.E at 10%	80	1.76	3.42	414.4	13.76
Decoction (1:4) at 5%	100	4.35	4.27	862	14.90
Decoction (1:4) at 10%	100	5.86	3.92	978	17.50
Decoction (1:6) at 5%	100	7.04	4.78	1182	18.10
Decoction (1:6) at 10%	90	5.50	4.34	885.6	17.28
Control (Water)	100	6.08	5.87	1195	18.20
C.D. Value (0.05)	-	0.54	0.49	127.27	0.50
S.Ed Value	-	0.25	0.23	60.58	0.24

Aqueous extracts of *Morinda tinctoria* on germination energy, germination value, germination relative index and emergence energy value

Germination energy is the number expressing the percentage of fast germinating seeds and also determines the viability and vigour of the seedlings (ISTA, 2006) [18] and it was presented in (Fig. 3) Germination value is an index of combining speed and completeness of seed germination Czabator, 1962 [21] and is related to survival of seedlings Djavanshir and Pourbeik, 1976 [22].

Table 3: Response index values for aqueous extracts of *Morinda tinctoria* leaf extracts on sorghum

Treatments	G.P	Root Length (cm)	Shoot Length (cm)	VI (I)	VI (II)
F.E at 5%	+0.000	+0.150	+0.009	+0.081	+0.000
F.E at 10%	+0.000	-0.488	-0.287	-0.389	-0.038
A.E at 5%	-0.110	+0.120	-0.362	-0.203	-0.431
A.E at 10%	-0.200	-0.170	-0.417	-0.653	-0.243
Decoction (1:4) at 5%	+0.000	-0.284	-0.272	-0.278	-0.181
Decoction (1:4) at 10%	+0.000	-0.036	-0.332	-0.181	-0.038
Decoction (1:6) at 5%	+0.000	+0.136	-0.185	-0.258	-0.005
Decoction (1:6) at 10%	-0.100	-0.095	-0.260	-0.327	-0.050
Control (Water)	0.000	0.000	0.000	0.000	0.000

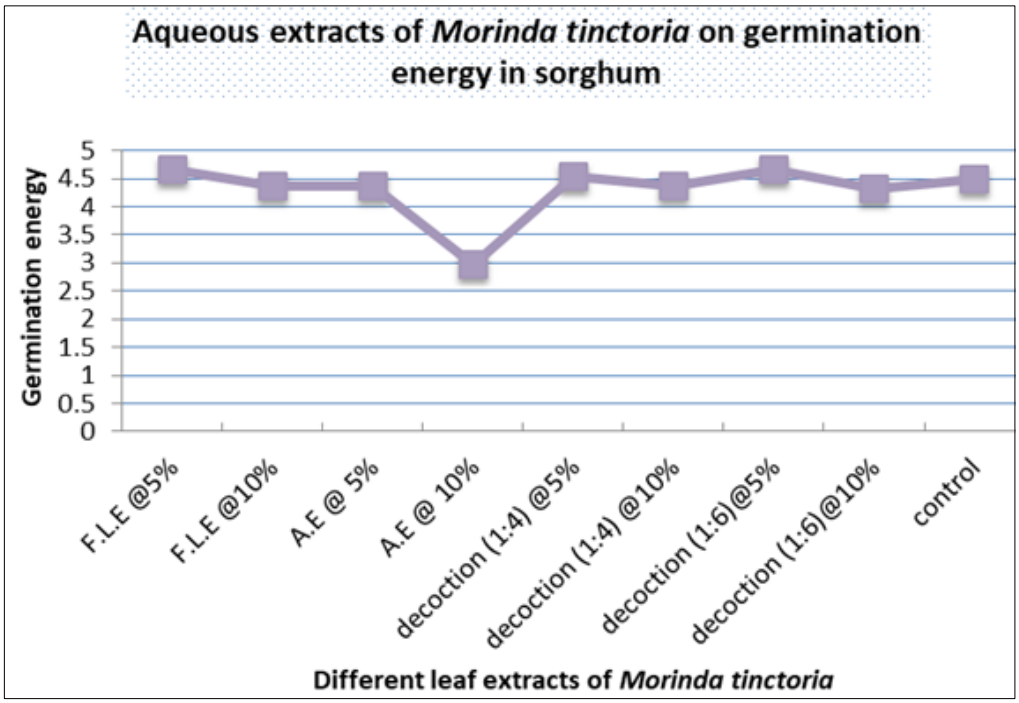

Fig 3: Different aqueous leaf extracts of *Morinda tinctoria* on germination energy

In the present study, both the parameters were positively influenced by various aqueous extracts of *Morinda tinctoria*. Among the different aqueous leaf extracts of *Morinda tinctoria* seeds subjected with 5% fresh leaf extract and 5% as decoction method has a positive influence and maximised the germination energy of 4.66 and also stipulated the germination values of both Czabator (333.33) and Djavanshir and Pourbeik method (46.67) Table 4.

This may be due to presence of several phytochemicals in water extracts of *Morinda tinctoria* containing glycosides, phytosterols, essential oils, saponins, phenols and flavonoids (Kumar, 2016) [33] which acts as precursor of GA_3 due to presence of saponins Li *et al*., 2010 [34] and presence of terpenoids, phytosteroids, fatty acids and glycosides (Takashima *et al.*, 2007) [35] However, it was statistically at par with 5% decoction (1:4) and distilled water (control). The least germination energy and germination values were recorded when seeds were treated with 10% alcoholic extract. Likewise, germination relative index and emergence energy value were also found to be greater (3.33) with treatment of fresh leaf extract at 5%, decoction (1:6) at 5% and distilled water (control). However, it was at par with decoction (1:4). The least germination energy and germination values were registered with 10% alcoholic extract. The alcoholic (methanolic) extracts of *Morinda tinctoria* leaves contain high content of phenolic acids compared to other extracts (Kolli *et al*., 2015) [36] This might be the reason to inhibition or reduce the speed of germination due to the higher concentration and presence of phytotoxic substances in plant extracts.

Table 4: Different aqueous extracts of *Morinda tinctoria* on germination-related attributes in sorghum

Treatments	(GE)	(GRI)	(GV) I	(GV) II	(EEV)
F.E at 5%	4.66	78	46.67	333.33	3.33
F.E at 10%	4.37	75	43.67	200.00	2.00
A.E at 5%	4.16	70	37.50	270.00	3.00
A.E at 10%	2.97	52	23.82	71.12	0.88
Decoction (1:4) at 5%	4.53	76	45.33	200.00	2.00
Decoction (1:4) at 10%	4.37	75	43.67	200.00	2.00
Decoction (1:6) at 5%	4.66	78	46.67	333.33	3.33
Decoction (1:6) at 10%	4.33	71	38.99	270.00	3.00
Control (Water)	4.50	77	45.00	333.33	3.33
C.D. Value (0.05)	0.28	3.79	4.24	31.52	0.31
S.Ed Value	0.13	3.05	2.01	15.00	0.15

Conclusion

From the investigation made with different methods of extraction and tree species used for the study, two conclusions can be drawn for further research studies.

1. Regarding preparation of leaf extracts for seed fortification and foliar spray the best method is fresh leaf extract at 5%
2. The species *Morinda tinctoria* which is notified as a weed in vacant lands in India and but research shows that can be used as an agent for enhancing seed germination in crops especially sorghum, maize and millets and also for foliar spray for plant development

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have

appeared to influence the work reported in this paper.

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