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Evaluation of different concentration of vermiwash on seed germination and biochemical response in *Abelmoschus esculentus* (L.)

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Abstract
Earthworm's role as farmer's friend is well known since time immemorial. In contemporary time, commercial vermin culturists have started promoting a product, called vermiwash. Vermiwash contains enzymes, macro and micronutrients that could promote growth and yield of crops. It can be formed in different ways. In the present study, vermiwash was generated from the earthworm, *Eisenia foetida*, under in laboratory. This vermiwash was employed in different concentrations of seed germination of *(Abelmoschus esculentus)* lady's finger plant. Four combinations were prepared from vermiwash for used in seed germination and biochemical analysis. Unusual concentration of vermiwash like, T1 (0.25%); T2 (0.5%); T3 (0.75%); T4 (1%); control (without vermiwash) as test treatments. Each treatment was made in triplicate to reduce the variations. Experiments stood at 96 hours. The results demonstrate that the maximum shoot length was discovered in T3 (2.1cm) and root length were also recorded in T1 (2.9cm) treated groups. The results were significantly increased ($P<0.05$). The vermiwash treated groups have 100% seed germination when compared with control. The maximum chlorophyll content was found in T2 (4.91), when opposed to other treatment groups. The highest total protein content was noted in T3 (9.50 mg/g) was observed when objected to control. Hence it can be possible to conclude that the effect of vermiwash on the seed germination and biochemical responses of lady's finger plants is more significant in comparison to control.

Keywords: lady's finger plant, vermiwash, seed germination, chlorophyll, protein content

Introduction
Indian agriculture is among the most important sectors in the economy of country where synthetic fertilizers play a key role for enhancing the crop yield. Now-a-days, dependent on the chemical fertilizers for agricultural growth leads to unattainable Burden on the Environment Government of India has been trying to promote environmentally friendly approach for sustainable agriculture among which organic farming is one of the relatively inexpensive and convenient methods.
Nowadays, advent of chemical fertilizers is bringing up in agriculture day by day which ultimately destroys that fertility of soil upon long term use. Organic fertilizers may be employed as an alternate source to control the hazardous effect of chemical fertilizer. In this way, vermicompost and vermiwash may be used for better yielding of crops chemical properties of soil, *viz*., temperature, moisture, regime, pH [1], soil organic content, and litter input [2].
Vermiwash protect the environment from various chemical fertilizers. Vermiwash is a liquid extract of untreated waste material, which is perceived after the passage of water through the different layers of earthworm culture units. Vermiwash is used as a liquid major nutritive and enzymatic element for promoting growth of all green plants. Vermiwash, extracted body fluid of earthworms is further nutrient rich with components promoting good plant-growth [3-5].
This vermiwash contains enzymes, secretions of earthworms which would stimulate the growth and yield of crops and even develop resistance in crops receiving this spray [5, 6]. Vermiwash also has soluble plant nutrients apart from some organic acids and mucus of earthworms and microbes showed the effectiveness of vermiwash on cowpea plant growth by laboratory-scale trial. The effect of vermiwash was respected on the growth and productivity of Marigold [7, 8]. The effect of vermiwash spray significantly increased dry chilli yield [5, 9] indicated that weekly applications of vermiwash increased radish yield by 7.3% [10]. Also showed that both growth and paddy yield increased with the application of vermiwash and vermicast extracts [11]. The present study was carried out to evaluate the effect of vermiwash on seed germination of lady's finger plant on vermiwash extracted by altered concentrations and find out which concentration was more effective in seed germination and survival.

Materials and methods
Collection of wastes
Animal wastes (cow dung) were harvested from sri vittal rukmini samshthan, Govindapuram, near Kumbakonam, Tamilnadu, and different agro kitchen wastes were collected from rural area of Kumbakonam. Partially decomposed mixture of animal, agro/kitchen wastes was used for enhancement of vermiculation efficiency. Animal dung and different agro wastes were exposed to sunlight for 5 to10 days

to remove the numerous harmful organism and noxious gas, before the preparation of vermibeds.

Collection of earth worm
Earthworms *Eisenia foetida* an epigeic species were procured from Periyar Maniyammai University, Vallam, Taminadu. The collected earthworms are produced under laboratory conditions.

Experimental setup for vermicomposting:
Vermicomposting took place on cemented earth surface. There are 35 vermibeds were formed by a combination of separate animal, agro/ kitchen wastes in a 1:1 ratio. The size of each vermibed is 3m ×1m × 9cm. After formation of vermibeds moisten it and inoculated 2kg of cultured *Eisenia foetida* in each bed. The beds were included in the bed by useless jute pockets and moist the bed daily up to 40 to 50 days for maintaining the moisture content. The weds were manually turned over at each week interval up to 3 weeks. After 45 to 50 days granular tea like vermicompost appears on the upper surface of beds. These vermicomposts were used only for the extraction of vermiwash [11].

Extraction of vermiwash
Vermiwash extracted from vermiwash collecting device. The apparatus is made from plastic or metal drum having a capacity of 2 litres and a tap at the bottom of the drum filled with crushed breaks, about 10cm thickened which is followed by a sand layer of 2-3cm thickness, lastly filled with vermicompost with a heavy population of earthworms. Simultaneously added fresh water, in to drum and a container kept below the tap of drum [12]. The watery extract of vermicompost i.e. vermiwash drained out of drum and collected, drop by drop in to the container. The colure of vermiwash ranges from yellowish to black. After 1 to 2 days the process of extraction has been carried out. The different concentrations of collected and kept in a cool place.

Collection of plant seed
Fresh seed of (*Abelmoschus esculentu*) was bought from Mercury Agency. Kumbakonam, Thanjavur District.

Experimental design
Vermiwash was done to 25%, 50%, 75%, and 100% concentration using distilled water. (Panchagavya were recorded in 25, 50, 75, and 100 ml and the volume was composed of 100 ml using 75, 50, 25 ml of distilled water).

Seed germination studies
Abelmoschus esculentus (Lady Finger) seed soaked in distilled water for 24 hr. The seedlings were subsequently transferred to Petri dishes containing filter paper, moistened from below with sterilized cotton pads and treated with 0, 0.1%, 0.25%, 0.5%, and 1% (v/v) vermiwash prepared from a stock solution. After 7 days the experiments ended, seedlings were studied for percent seed germination, and the shoot and root length and their dry weight were identified. Biochemical parameters i.e., chlorophyll, nitrogen and protein content were further analyzed. Five seedlings from each petridishes were taken and weighed to get the fresh weight [13].

Germination percentage

$$\text{Germination percentage} = \frac{\text{Number of normally germinated seeds}}{\text{Total number of seeds}} \times 100$$

Biochemical parameters
Following formula will be used to calculate the amount of chlorophyll (a) and (b):

Chlorophyll a mg/l=12.7 × A663-2.69 × A645
Chlorophyll b mg/l=22.9 × A6454.68 × A663 Chlorophyll (a+b) mg/l=8.02 × A663+20.20 × A645 (2)

The amount of nitrogen will be calculated as follows:

$$\% \text{ Nitrogen} = (T\text{-}B)\ N \times 1.4/S \quad (3)$$

Where T=Sample titration in ml.
B=Blank titration in ml.
N=Normality of titrant (0.01 NHCl).
S=Weight of plant material in g.
The protein content will be calculated as follows %Protein=%nitrogen 6.25

Results
Shoot and Root length
The shoot length of seed germinated plant was recorded in 24, 48, 72 and 96 hours. The maximum shoot length was recognized in T3 treated group (2.1cm) and list length was recorded in T4 and control group (1.9cm) Fig 1. The results were substantially increased ($P<0.05$; table 2). The root length of germinated seeds was important to point out in 24 to 96 hours. The maximum root length was found in T1 (2.9cm) and a minimum root length was found in control and T4 (2.0cm) (Fig 2), considerably increased ($P<0.05$; table 3).

Seed germination index
The seed germination index was entered in various concentrations of vermiwash treated seed for 24 to 96 hours. The highest seed germination was noted in T1 (100%) and least seed germination was found in control (88%) (Fig 3). The consequences were insignificantly ($P>0.05$; table 4).

Chlorophyll a, b and (a+b)
The chlorophyll level was found in 96 hours post vermiwash treatment. The maximum cholorophil a was observed in T2 (4.91) and least cholorophil a was observed in control (3.95) (Fig 4), considerably increased ($P<0.05$; table 5). The maximum chlorophyll b was found in T3 (9.40) and least chlorophyll b, was found in control (8.05). (Fig 5). The results increased substantially ($P<0.05$; table 6). The highest total chlorophyll a+b content was found in T3 (13.80) and least total chlorophyll content a+b was found in control (12) (Fig 6), the results statistically significant ($p<0.05$; table 7).

Total protein content
The highest absolute protein content was to be found in T3 (9.50 mg/g) and least absolute protein content was recorded in T4 (8.321 mg/g) (Fig 7), the results statistically significant ($p<0.05$; table 8).

Discussion

Okra (*Abelmoschus esculentus* (L.) Moench) is generally known as 'bhendi' or lady's finger in India. It is the choicest fruit vegetable grown extensively in the tropical, subtropical and temperate area of the temperate zones of the world. Okra is appreciated for its delicious tender fruit. It is the better source of iodine and calcium. Okra accounts for 60 per cent of export of fresh vegetables excluding potato, onion and garlic [13-15]. The green tender fruits of okra are highly nutritious containing 66 mg of calcium and 0.2 mg of iodine for every 100 g of edible portion and fair amount of vitamins *viz.,* A, B and C. It is equally rich in protein and mineral matter [16]. Recently an attention has been paid to the use of okra seed as a source of protein. Some time, the seeds are roasted and employed as a substitute for coffee. Apart from its nutritive value, the stem and husk of fruit are employed in the manufacture of paper and jaggery as they contain more of crude fibre.

Vermiwash is liquid manure, extracted of vermicomposts riches with more number of earthworms. Its foliar spray dramatically improves the growth and productivity of crop [7, 17] has indicated that it is coelomic fluid extraction contains several enzyme, plant growth hormones like cytokinins, gaberdine and vitamins along with micro and macro nutrients. It improves disease resistant power in crop, [5, 12, 18] have reported that nitrogen in the form of mucus, nitrogenous excretory substance; growth stimulating hormones and enzyme are found in vermiwash [19]. considered the stimulating effect of vermiwash on crinkle red variety of *Andurium andreanum*. Effect of vermiwash on plant growth of black gram reported by [16, 20] and on tea, coconut and horticultural crops by [4, 21, 22] have reported that vermiwash caused momentous effect on the seed germination and development of hatchling [23]. studied that the vermiwash have to yield a good result, especially initiating flowering and long lasting inflorescence of *Anthuriums.* In the present study deals with diverse concentration of vermiwash on seed germination and biochemical responses were significantly increased ($P<0.05$) when compared to control. The optimum concentration of 0.25% to 0.75% of vermiwash shows best for seed germination in Lady's finger plant [24].

[24, 25] have considered the effect of vermiwash on the field grown tomato *(Lycopersicon esculentum*) indicated the late blight suppression and improve the fruit quality [26-28]. The application of vermiwash has been demonstrated to reduce disease caused by necrotrophs as well as biotrophs [24, 29, 30]. It was pointed out that vermiwash have been showed to depress soil borne pathogen and pest [8, 24, 27] also reported that the use of vermiwash is more applicable when the presence of water in the soil is very poor. It was necessary to demonstrate that after treatment of vermiwash showed similar growth pattern due to addition of auxins, gibberellin and cytokinins in the soil [13, 20, 31, 32].

Conclusion

From the present study, it is evident that the nutrients and growth promoting substances present in the vermiwash showed its potentiality in seed germination and biochemical analysis. However, the vermiwash diluted at the ratio of 0.25 to 0.75% produced superior results of vermiwash produced naturally, i.e. without stress. The use and application of vermiwash could play a beneficial role in sustainable agriculture as it is environment friendly, cost effective, reliable and easily available.

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Conflict interest

The authors state that they have no conflict of interest.

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