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EFFECT OF VERMIWASH AND PLANT GROWTH REGULATORS ON GROWTH AND STEM ANATOMY OF *HIBISCUS CANNABINUS* L.

K. Vadamalaikrishnan and Mujeera Fathima *

PG and Research Department of Botany, Government Arts College for Men (Autonomous), Nandanam, Chennai - 600035, Tamil Nadu, India.

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Correspondence to Author:
Dr. Mujeera Fathima

Associate Professor and Head, PG and Research Department of Botany, Government Arts College for Men (Autonomous), Nandanam, Chennai - 600035, Tamil Nadu, India.

E-mail: mujeera2011@gmail.com

ABSTRACT: Growth and development events in plants are controlled by growth regulators and Phytohormones. These are found naturally in plants. Environmental Pollution is one of the major problems and reactions caused by artificial growth regulators and their low biodegradability has urged us to search for new biofertilizers with growth-regulating activity. Vermiwash is a liquid fertilizer that has been shown to mimic phytohormones in bringing about growth-promoting effects. In the present study vermiwash at 100% and 50% concentration and Gibberellic acid treatment along with a control treatment with distilled water were given to *Hibiscus cannabinus* L.,a conventional fiber crop in order to determine the growth-promoting effect of vermiwash in comparison to Gibberellic acid. The results showed that vermiwash at lower concentration was able to bring about enhanced growth which has been observed as an increase in the height of the plant, length and width of internode and has been substantiated by an increase in the bast fiber zone as seen from the histological studies. It is thus revealed that vermiwash could be used to increase the fiber content and thus can be employed to exploit eco-friendly sources of fibers as a substitute for plastic.

INTRODUCTION: In recent years, the use of liquid fertilizers given in the form of foliar sprays has gained importance. Most of the foliar sprays used so far have been effective in bringing about growth improvement in most of the vegetable crops studied. “Sustainable agriculture” can be ensured in the future with the help of organic farming systems, which includes various processes of biological origin such as compost and vermin compost [1] earthworms known as vermicompost and the technology of using such local species of earthworms for crop production or composting is called vermitech [2].

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Vermiwash is a liquid fertilizer used in organic agriculture both as a replacement and supplement for solids and for their unique capacity to provide nutrients effectively and quickly. It is generally collected by the passage of water through a column of worm activation. There have been several reports on the use of Vermiwash and its growth-promoting effects. A new problem in today’s world is the use of plastics, which are non-biodegradable and lead to environmental pollution. The search is on for biodegradable fibers that could replace plastic, so a number of vegetable fiber crops have been considered. An interesting fact is to explore the possibility of cultivating these crops on fallow land and attempting to bring about growth enhancement by using a cost-effective liquid fertilizer like vermiwash. The study is based on the earlier reports of [3, 4]. Kenaf (*Hibiscus cannabinus* L.) is a common wild plant that grows in the tropics [5].

Hibiscus cannabinus is largely cultivated for its fiber, which is extensively employed by the natives in the manufacture of rope, coarse sacking, and other articles required for agricultural purposes [6]. The role of earthworms in soil formation and soil fertility has thus been well documented and recognized. Application of vermicompost favorably affects soil pH, microbial population, and soil enzyme activities, Growth and development events in plants are controlled by growth regulators, and these phytohormones are found naturally in plants [7].

Earlier studies done on *Hibiscus sabdariffa* also have revealed that plant growth regulators like Naphthalene acetic acid (NAA) could bring about growth improvement and increase in fiber content [8]. Fertilizers enhance the growth of the plants due to the presence of nutrients. Overuse of these fertilizers has made the soil less fertile in recent times due to the unnecessary addition of salts that form a part of chemical fertilizers [9].

MATERIALS AND METHODS: The plant species utilised in the present investigation is *H. cannabinus* Linn. Belonging to the family Malvaceae which is commonly used as a fiber crop. The leaves are used as a salad and for pickles. Authentic samples of seeds procured from National Seeds Corporation, Ambattur, Chennai, were used to raise plants for the experiments. The plant was authenticated and identified with the Voucher number PARC/2020/4369 deposited at the Plant Anatomy Research Centre, Mudichur, Chennai, India. The studies were conducted in pots of uniform height and diameter. Gibberellic acid for the experiment was prepared as a 1000 µg/ml stock solution. Vermiwash unit was set up by the method suggested by [10]. Vermiwash, a biofertilizer, is produced by the action of epigeic (*Perionyx excavatus*) and anecic worms (*Lampito mauritii*) varieties [11].

The Vermiwash was collected from the vermi-compost unit. A total of ten liters of vermiwash was collected and used for the experiment. Vermiwash was prepared and used in two dilutions VW I- which was the concentrated filtrate collected from the unit is 100% and used directly, while the other one is VW II that was diluted by adding an equal volume of distilled water and is at a concentration of 50%. The plants were reared by the pot culture method. The experiment has been conducted using garden soil having red soil, sand, and humus in the ratio of 1:1:1. The seeds were sown in 30 cm wide pots and then were transplanted after ten days to less wide pots having a uniform diameter of 15 cm and a height of 30cm. The plants were irrigated uniformly and assessed for their exo - morphological characters which were recorded on the field.

The plants under the experimental setup received a foliar spray with vermiwash at an interval of three days up to about five times. The control plants were given distilled water as foliar spray. There were four different sets of experimental plant groups, which were designated as shown in Table 1. Each set had five pots, each with five plants, and so a total of 25 plants were analyzed for the exo-morphological characters, biomass, and chlorophyll content. The experimental groups were maintained as shown in Table1to facilitate comparison of the effects of the Vermiwash I and II and the effects of GA_3 when used alone in comparison to the control. The test solutions were given as foliar sprays using an atomizer. The plants were sprayed till run-off. Fresh weight and dry weight of the experimental and control plants in pot culture was also calculated to determine the biomass.

Histological studies were done following standard methods [12], to study the effect of vermiwash on the extent of the bast fiber zone development. Chlorophyll estimation was done to elucidate the growth-promoting effect of vermiwash along with GA_3 in comparison to the control. The values for Chlorophyll a, Chlorophyll b, and total chlorophyll were determined. Biochemical parameters such as Total proteins and carbohydrates were estimated by standard methods. The results obtained were statistically analyzed according to [13]. The standard error of means was determined for all the parameters studied.

RESULTS AND DISCUSSION:

Exo-Morphological Characters: Observations on exo-morphological characters revealed that plant height was maximum in GA treated plants followed by VW I and VW II treated plants and were minimum in the control **Table 1**, **Fig. 1**.

TABLE 1: CONCENTRATIONS OF GA AND VERMIWASH USED IN THE STUDIES ON *H. CANNABINUS*

S. no.	Treatment	Combinations of GA and Vermiwash
1	Control	Distilled water
2	GA	Gibberellic acid 100 ug/ml
3	VW I	100 % vermiwash
4	VW II	50% Vermiwash diluted with distilled water

The internode length and diameter was found to be maximum in VW II treatment followed by GA, control, and then VW I treatment **Fig. 2**. The leaf number and leaf area were high in vermiwash treated plants followed by GA-treated ones and was minimal in control.

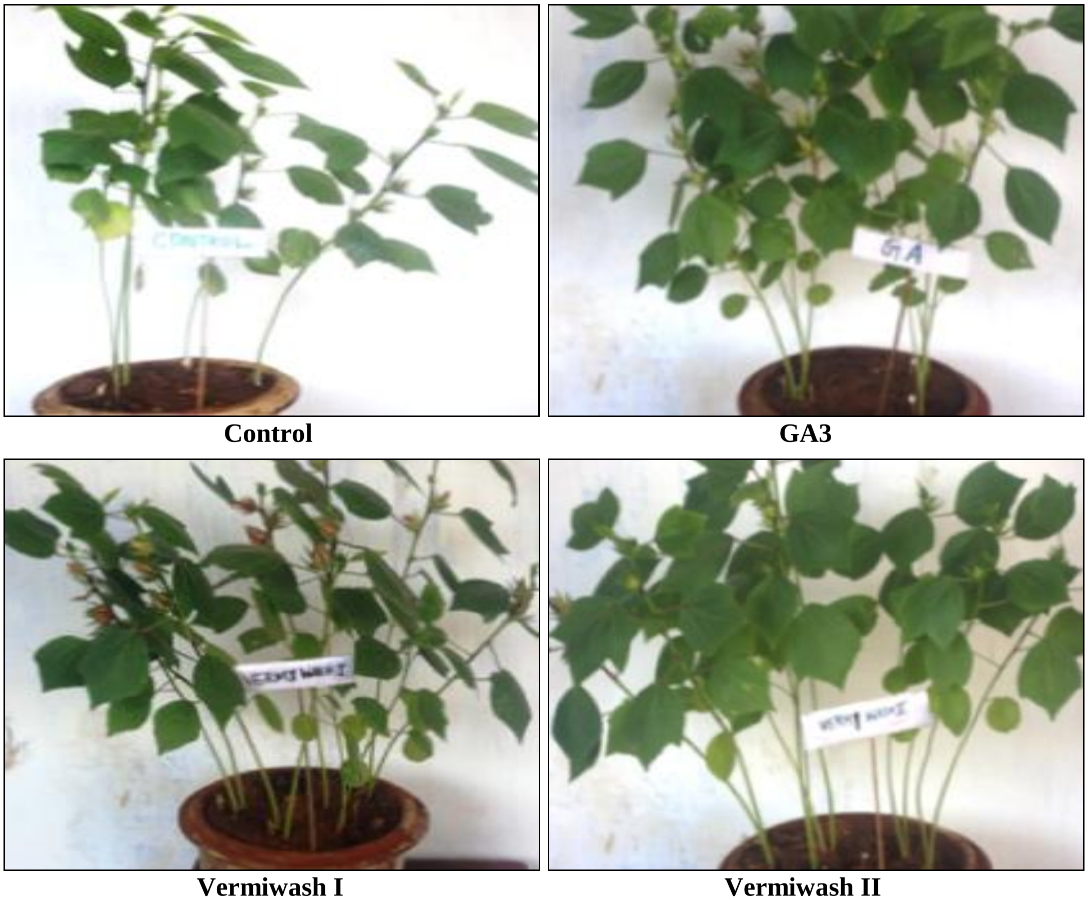

FIG. 1: EXO-MORPHOLOGICAL CHARACTERS OF *H. CANNABINUS*

TABLE 2: EFFECT OF VERMIWASH ON EXO-MORPHOLOGICAL CHARACTERS OF *H.CANNABINUS*

S. no.	Treatment	Height (cm)	No. of leaves	Leaf area (cm^2)
1	Control	21.68(0.58)	7	13.62(0.042)
2	GA	25.38(0.56)	8	17.25(0.051)
3	VW I	23.52(0.64)	9	19.26(0.068)
4	VW II	23.84(0.68)	8	17.83(0.054)

The values of standard error of means is given in brackets

Table 2, **Fig. 1** Number of flowers and fruits and percentage biomass were also seen to be high in VW II treated plants **Table 3**.

These observations are in accordance with earlier studies done by [14] on *Abelmoschus esculentus* given foliar sprays of vermiwash and those of [8] on *Hibiscus sabdariffa* treated with various combinations of plant growth regulators.

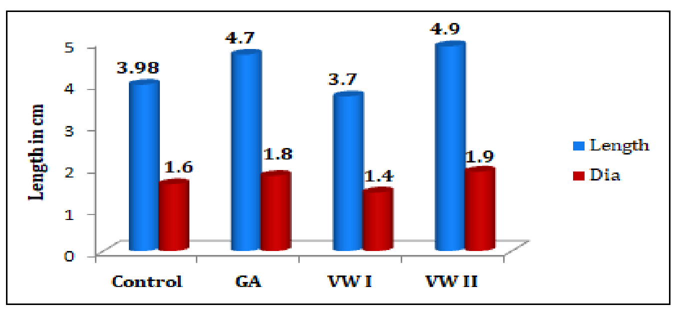

FIG. 2: EFFECT OF VERMIWASH ON INTERNODAL LENGTH AND DIAMETER IN *H. CANABINUS*

TABLE 3: EFFECT OF VERMIWASH ON FLOWERS, FRUITS AND BIOMASS OF *H. CANNABINUS*

S. no.	Treatment	No. of Flowers	No. of fruits	Biomass%
1	Control	21	21	12.4
2	GA	20	20	13.6
3	VW I	26	24	12.03
4	VW II	21	21	14.8

These results revealed that vermiwash has a beneficial effect on growth parameters but 100 % concentration of vermiwash is not able to bring about significant enhancement in growth because the effects of vermiwash are similar to that of phytohormones, which are effective at low concentrations. Biochemical studies to estimate the chlorophyll content, protein and carbohydrates showed a high value for VW II treated plants followed by GA treatment, VW I, and was seen to be minimum in the control plants **Table 4**. The reports of [15, 16] very well corroborate the results of this study. An increase in carbohydrate level shows increased growth and metabolism because vermi-wash contains an optimal content of nutrients as observed by [17], in their studies on the effects of industrial wastewater on cyanobacterial growth.

TABLE 4: EFFECT OF VERMIWASH ON BIOCHEMICAL CONSTITUENTS OF *H. CANNABINUS*

S. no.	Treatment	Chlorophyll (mg/g)	Protein (mg/g)	Carbohydrate(mg/g)
1	Control	5.23(0.021)	11.26 (0.048)	7.84 (0.063)
2	GA	4.98 (0.037)	12.17(0.045)	9.42(0.078)
3	VW I	5.41 (0.024)	11.46 (0.052)	9.27 (0.064)
4	VW II	5.98 (0.029)	13.89(0.076)	10.24 (0.071)

(Standard error of means given in brackets)

Histological Studies: Internodal cross-sections of the treated and control plants were taken to analyze, the changes in the anatomical features, the increment in the vascular tissues and the bastfibre zone. The control plants showed a wide cortex and its inner boundary is demarcated by a dis-continuous cylinder of small patches of fibers. The fibre masses are separated by dilated phloem rays. The fiber masses are 20-30 µm thick, and fibers are 10-20 µm wide. The cortical fibers show poor lignification. In the case of GA-treated plants, the internodal sections showed a narrow cortex and scattered small masses of fibers, which are about 60 µm thick and have thick secondary walls with the narrow lumen. The lignification is moderate.

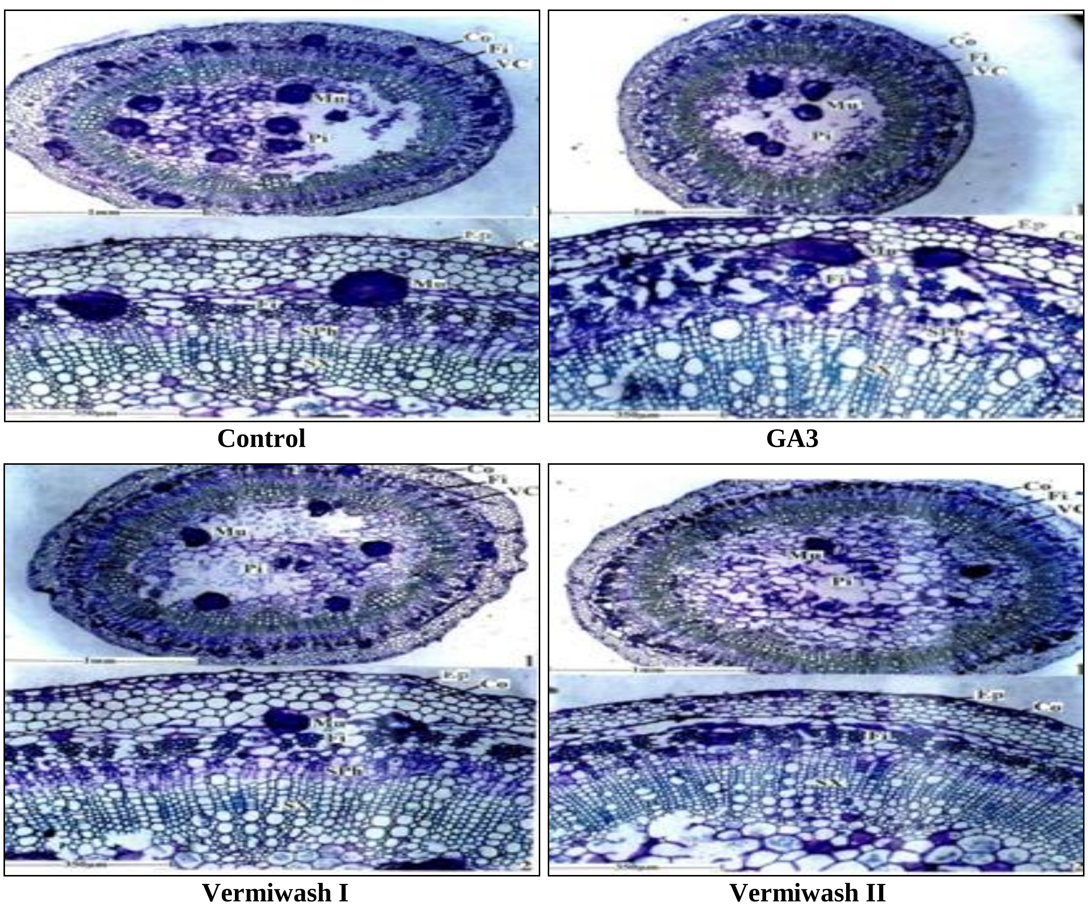

FIG. 3: ANATOMICAL CHANGES IN THE INTERNODAL SECTIONS OF *H. CANNABINUS*
1-Overall view; 2 –A portion of the cortex + bast fiber zone enlarged

The vermiwash I (VW I) treated plants show a thick cortex, several discrete prominent masses of fibers about 20-70 µm wide. The individual fibers were 50- 110µm wide and had well-lignified walls which were about 20µm in thickness. The cross-sections of the internode of Vermiwash II (VW II) treated plants showed a narrow cortex and several discrete masses of cortical fibers, which were about 30-50 µm thick. The individual fibers had a diameter of about 30µm, and the walls were well lignified and had a thickness of about 5µm. The increase in girth and biomass has been proved by the observations on intermodal cross-sections (B).

The results of the present investigation are in accordance with the earlier studies on *Abelmoschus esculentus* and *Hibiscus sabdariffa* [8], the studies of *Cannabis sativa* all of which are fiber crops. In these studies, Gibberellic acid was used, and it brought about an increased level of fiber development. The results of anatomical studies reveal that vermiwash treatment was able to bring about an increase in tissue differentiation in terms of fibre development, which in evident from the increase in intermodal diameter. These findings are similar to those observed in Gibberellic acid treatment and hence growth promoting effects of vermiwash can be confirmed.

SUMMARY AND CONCLUSION: The present study on *Hibiscus cannabinus* has shown that Vermiwash at a concentration of 50% is able to bring about an enhancement in growth as seen from the exomorphological characters, biomass increase and changes in the histology. The effects of vermiwash are similar to those of the plant growth regulator gibberellic acid that has been used in this study to compare the effects caused by vermiwash. The results have thus established that vermiwash could be an effective biofertilizer that could be used in the liquid form and can bring about the effects at very low concentration and allows ease of use for the farmer. The objectives of the present investigation have been fulfilled, as can be observed from the results obtained for the various morphological and Biochemical parameters, including those for flowers and fruits. Setting up a vermiwash unit is very simple and easy, and it can be easily taught to the farmer, and the organic fertilizer may be conveniently collected and used. This could be applied to other non-conventional fiber sources also. In this way, we would be able to find and exploit new biodegradable vegetable fibers that can be used as substitutes for plastic. Since these are hardy, they could be grown almost anywhere and the fibers can be easily harvested by simple methods.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest regarding the publication of this paper.

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