

Effect of Vermiwash and Plant Growth Regulators on Anatomical Changes of *Abelmoschus esculentus* (Linn) Moench

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Abstract: The role of vermiwash, as an organic foliar spray on plant's growth and its impact in comparison with the plant growth regulators on the exo-morphological and anatomical characters of *Abelmoschus esculentus* were investigated. The results of the study showed that 15% vermiwash exhibited growth promoting effects on the exo-morphological characters such as plant height, length and diameter of the internode, number of leaves, leaf surface area, root length, wet and dry weight of the shoot and root and internode anatomical features of *A.esculentus*. Various concentration of vermiwash and synthetic plant growth regulators *i.e.*, 10% vermiwash (V – I), 15% vermiwash (V – II), Naphthalene acetic acid (NAA) and Gibberelic acid (GA) at 100 µg/ml were used as foliar sprays among the various foliar treatments used in the study, 15% vermiwash (V- II) showed growth enhancing effects followed by 10% vermiwash (V- I), Gibberelic acid (100 µg/ml) and Naphthalene acetic acid (100 µg/ml). Maximum root length and plant biomass was recorded in V-II. The gross anatomy of the internode as effected by vermiwash and PGRs revealed that vermiwash treatment increased the width of the cortical zone and the vascular cambion zone. These results clearly indicate that vermiwash can be exploited as a potent organic biofertiliser and foliar spray and also has the potential to increase fiber differentiation.

Key words: Earth Worms • Vermiwash • Pgrs • Anatomical Changes

INTRODUCTION

The role of earthworm in soil formation, soil fertility and plant growth promoting effects is well documented and recognized. An approach towards good soil management, with an emphasis on the role of soil inhabitants like earthworms, in soil fertility, is very important in maintaining ecosystem [1-3]. Application of vermicompost, favourably affects soil pH, microbial population and soil enzyme activities [4].

Apart from this the candidature of earthworms for land restoration, soil pollution bio monitoring, soil fertility maintenance and waste water treatment and plant production program is well proven in publish literature [5-7]. The use of foliar fertilizing in agriculture has been a

popular practice with farmers since the 1950's, when it was learned that foliar fertilization was in promoting plant growth effective [8].

Plant Growth regulators (PGRs) in general are organic compounds which bring about an increase or modification of growth in plants. Growth regulators, a new generation of agrochemicals, when added in small amounts as foliar sprays, modify the natural growth right from seed germination to senescence in crop plants. Among them the use of gibberelic acid (GA) and Naphthalene acetic acid (NAA) is of considerable interest in different fields of agriculture [9].

Growth and development events in plants are controlled by growth regulators and these phytohormones are found naturally in plants.

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Manufacturing and production of synthetic phytohormones is not economically feasible and the optimum conditions under which they can function efficiently is also difficult to ascertain [10]. Due to health and environmental pollution problems and reactions caused by artificial growth regulators and their low biodegradability has urged us to search for new biofertilizers with growth regulating activity.

Though there are several organic fertilizers in the form of vermicompost, farmyard manure, pressmud, coir pith compost have been applied. The need for liquid fertilizers has evoked the production of several such materials to be used as foliar sprays. Vermiwash is a liquid fertilizer used in organic agriculture both as replacement and supplement for solids and for their unique capacity to provide nutrients effectively and quickly. Vermiwash has excellent growth promoting effects besides serving as biopesticide. In recent days the vermiwash is used as liquid manure. Even though much work has been done on vermicomposting, very few reports are available related to vermiwash and its impact on the plant growth [11].

Vermiwash (VW), a foliar spray, is a liquid biofertilizer collected after the passage of water through a column of worm activation. It is a collection of excretory and secretory, products of earthworm, along with other micronutrients. It also contains sugars, amino acids and phenols along with plant growth promoting hormones such as in indole acetic acid and humic acid. The fresh vermiwash houses a large number of beneficial micro organisms which help in plant growth and protects it from a number of infestations [12]. Vermiwash also possesses an inherent property of acting not only as a fertilizer but also as a mild biocide [13,14].

A. esculentus (Linn) Moench commonly called 'Ladies finger' or 'Okra' / 'Bhindi' an annual, 3-7 feet tall, pubescent herb and an important vegetable and fibre crop occupying an area of 35,190 ha with production of 2,72,699 [15]. The increasing trend of abundant use of inorganic fertilizers along with herbicides and pesticides and exploiting available water resources, etc in the present agriculture system poses a great threat to the sustainability of our agro-ecosystem. Under such situation it is essential to look for alternatives which are effective and ecofriendly.

Sachs and Lang [16] reported that GA regulates cell division and elongation, but [17] have shown the growth promoting effects of GA. Takahashi and Wada [18] noted that internode elongation is under the control of photo-sensitive reactions, which interferes with exogenous GA. NAA when applied exogenously affects

linear extension in *Avena* [19]. Digby and Wareing [20] reported that Indole acetic acid, GA and kinetin increase cell division. Fathima and Balasubramanian [21] reported the effect of growth regulators on the exo-morphological characters of

A. esculentus. Various combinations of plant growth regulators when used as a foliar spray on *A. esculentus* plants and compared with the control showed maximum increase in height, internodal length and diameter.

The survey of literature reveals that the comparative studies on the growth promoting effects of organic amendments and PGRs in Okra are fragmentary. In this context, the present investigation was planned to study the effect of vermiwash and PGRs on the exo-morphological and the anatomical characters of *A. esculentus* and to study changes in the gross anatomy of the stem particularly with reference to xylem and phloem vessels with a view to corroborate the observed exomorphological changes as effected by vermiwash and PGRs.

MATERIALS AND METHODS

Vermiwash unit was set up by the method suggested by Ismail [13]. Vermiwash, a biofertilizer is produced by the action of epigeic (*Perionyx excavatus*) and anecic worms (*Lampitoma auritii*) varieties was prepared [22].

The plant species utilised in the present investigation is *A. esculentus* (Linn.) Moench, belongs to the family Malvaceae. Authentic samples of seeds procured from National Seeds Corporation, Ambattur, Chennai were used to raise plants for the experiments.

Experiment: Pot experiments with *A. esculentus* were carried out applying different concentration of vermiwash and plant growth regulators as foliar sprays with deionised water as control to study the differences in the exomorphological characters and anatomy that may develop in response to their applications. To the foliar spray solutions 0.01% of teepol was added to act as a surfactant which enhances adherence of the spray solution to the leaves. The spraying was done using an atomizer until there was run-off of the excess spray solutions. The various concentrations of vermiwash and plant growth regulators (PGRs) that were used as foliar sprays for *A. esculentus* are given in (Table 1).

Seedlings of *A. esculentus* were raised in wide pots of 60cm diameter and transplanted to pots of uniform size of 30cm diameter. The pots were filled with sand, red soil and farm yard manure in the ratio of 1:1:1. The plants were

Table 1: Various concentrations of vermiwash and synthetic plant growth hormones (PGRs)

S.No	Treatment
1	Control – deionised water
2	Vermiwash I (10%)- (V-I)
3	Vermiwash (15%) (V-II)
4	Naphthalene acetic acid (100 µg/ml) (NAA)
5	Gibberelic acid (100 µg/ml) (GA)

maintained under garden land conditions. Three plants were grown in each pot and five pots were maintained for each treatment including controls. Plants were irrigated with well water uniformly throughout the period of experiment. Experiments were started when the plants were 10 days old since it has a life cycle of 45-50 days only. The spraying was done at the end of each week for five consecutive weeks and the following aspects of study were carried out in control and treated plants.

Exomorphological studies: At the end of fifth week of spray the following exo-morphological factors such as height of the plant length of the internode, diameter of internode, number of leaves, leaf surface area were recorded in the control and treated plants. Experiments were repeated thrice in order to make sure that uniform results were obtained. Plant samples were observed and analyzed in each of the studies under taken.

Measurement of Plant Growth Parameters: Plant height (cm) was recorded using a measuring tape. Leaf surface area was estimated graphically by outlining the leaf on a graph paper and counting the number of squares (cm²). Plants were harvested after 45 days and then weighed immediately to determine the wet weight of the shoot and root. They were then dried at 60°C to determine their dry weight.

Anatomical Studies: At the end of the fifth week, plants were harvested and subjected to anatomical study. For anatomical study, two plants per pot and six plants per treatment were selected randomly for sectioning. The fifth internode was chosen uniformly in control and treated plants. The excised internodal segments used for anatomical studies, were fixed in FAA (Formalin-5ml+Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary – Butyl alcohol as per the schedule given by Sass [23]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

The paraffin embedded specimens were sectioned with the help of Rotary microtome. The thickness of the section was 10-12 µm. Dewaxing of the section was by customary procedure [24]. The sections were stained with Toluidine blue as per the method published by O’Berien [25]. Nikon Labophot II research microscope was used for taking photomicrographs pertaining to histological studies. Magnifications of the figures are indicated by scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy books [26].

Statistical Analysis: Data on morphological parameters was subjected to statistical analyses. All data were expressed as mean and standard error. The difference between groups were statistically analysed by analysis of variance (ANOVA). The level of significance was set at $P < 0.05$.

RESULTS

Exo - Morphological Characters: The exo-morphological characters observed at the end of the fifth week in control and treated samples are presented in (Table 2).

All treatments showed significant values for plant height when compared to control with maximum shoot length observed in V-II followed by GA and V-I. Plant height at zero hour *i.e.* at the time of starting experiment was 7cm. The mean plant height consistently increased to a maximum of 32.46 cm in plants treated with V-II and 27.66 cm in plants treated with GA, this was followed by plants treated with V-I (27.43 cm). The mean height was 15.6 cm in the control plants.

The mean length of internode at zero hour was 9 cm. The increase in internodal length was maximum (10.28 cm) at the end of five weeks of spray in plants treated with V-II followed by plants treated with V-I (10.15) and GA. Internodal length was minimum (5.2 cm) in the control plants.

The internodal diameter at zero hour was 9 mm. In all the treatments, the internodal thickness was consistently higher than that of the control plants. Maximum thickness (1.9 mm) was observed in plants treated with V-II. In control plants the internodal diameter was 1.6 mm at the end of five weeks.

The mean surface area of leaf at zero hour was 4.6 cm². Maximum leaf surface area was recorded in V-II (12.16 cm²) followed by V-I (11.58 cm²), NAA (10.62 cm²) and GA (9.96 cm²) and all treatments showed statistically significant values when compared to the control.

Table 2: Comparison of the effect of vermiwash and PGRs on the exo-morphological characters of *Abelmoschus esculentus*

Parameters	Control	V – I	V – II	NAA	GA
Height	15.607 ^{ab} ±0.339	27.433 ^{ab} ± 0.679	32.460 ^{ab} ± 0.296	26.820 ^{ab} ± 0.399	27660 ^{ab} ± 0.792
Length of Internode	5.253 ^{ab} ± 0.117	10.153 ^{ab} ± 0.231	10.280 ^{ab} ± 0.201	8.973 ^{ab} ± 0.246	8.787 ^{ab} ± 0.186
Diameter of Internode	1.660 ^{ab} ± 0.032	1.980 ^{ab} ± 0.033	1.993 ^{ab} ± 0.018	1.780 ^{ab} ± 0.045	1.693 ^{ab} ±0.052
No. of Leaves	6.600 ^{NS} ±0.289	7.000 ^{NS} ± 0.309	7.000 ^{NS} ± 0.338	6.867 ^{NS} ± 0.389	6.000 ± 0.276
Surface area Of Leaves	8.540 ^{ab} ± 0.422	11.583 ^{ab} ± 0.419	12.160 ^{ab} ± 0.223	10.623 ^{ab} ± 0.285	9.960 ^{ab} ± 0.707
Root wet weight (g)	2.753 ± 0.074	3.533± 0.174	4.740± 0.074	2.237 ± 0.123	2.643 ± 0.123
Root dry weight (g)	0.387 ± 0.009	0.913 ± 0.05	1.237 ± 0.086	0.473 ± 0.064	0.560 ± 0.076
Shoot wet weight (g)	16.833 ± 0.060	32.623 ± 0.391	44.470 ± 0.751	24.270± 0.257	3.580 ± 0.230
Shoot dry weight (g)	2.570 ± 0.118	5.653 ± 0.131	10.543 ± 0.298	3.580 ± 0.239	4.777 ± 0.049

V I- Vermiwash-I (10 %); V II- Vermiwash-II (15 %); NAA- Naphthalene acetic acid; GA-Gibberelic acid

Values are mean ± S.E of 15 individual observations

Values in parentheses are percent changes over control

a – Represents significance of variance between treatments

b – Represents significance of variance between periods

NS – represents that values are not significant

Degrees of freedom F < 0.05

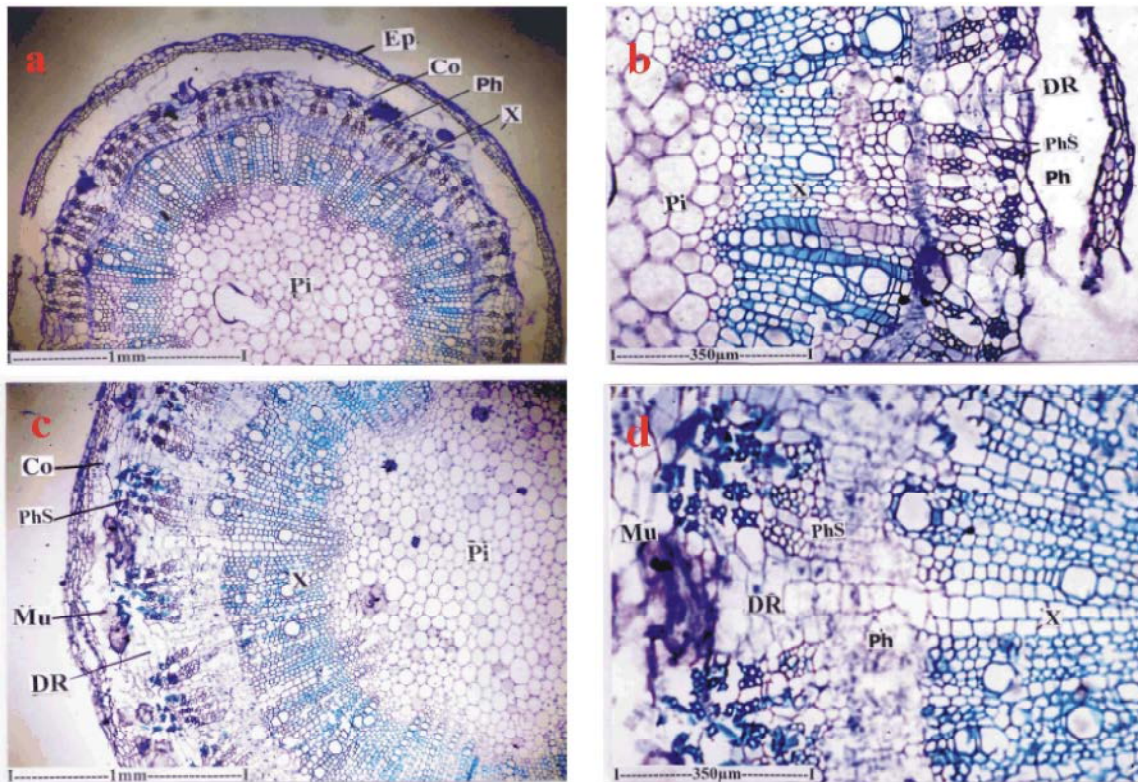


Fig. 1: *Abelmoschus esculentus*: Transactional views of internode showing anatomical features of control and Vermiwash - I treated plants a- T.S. control of stem - half sector, b- T.S. control of stem a sector enlarged, c- T.S. V-I of stem - half sector and d- T.S. V-I of stem a sector enlarged

In control plants the leaf surface area was 8.5 cm² at the end of the fifth week. The average number of leaves at zero hour was 2 in number.

Maximum wet weight of shoot was recorded in V-II (44.47 g) and V-I (32.63 g) and all treatments were statistically significant when compared to the control plants. Shoot dry weight was maximum in V-II (10.54 g)

and all treatments were statistically significant when compared to control. Maximum wet weight of root was recorded as (4.74 g) in V-II and (3.53 g) V-I and all treatments were statistically significant than that of the control. Root dry weight was recorded maximum in V-II (1.237 g) and V-I (0.913 g) followed by GA (0.56 g) and all treatments were significant.

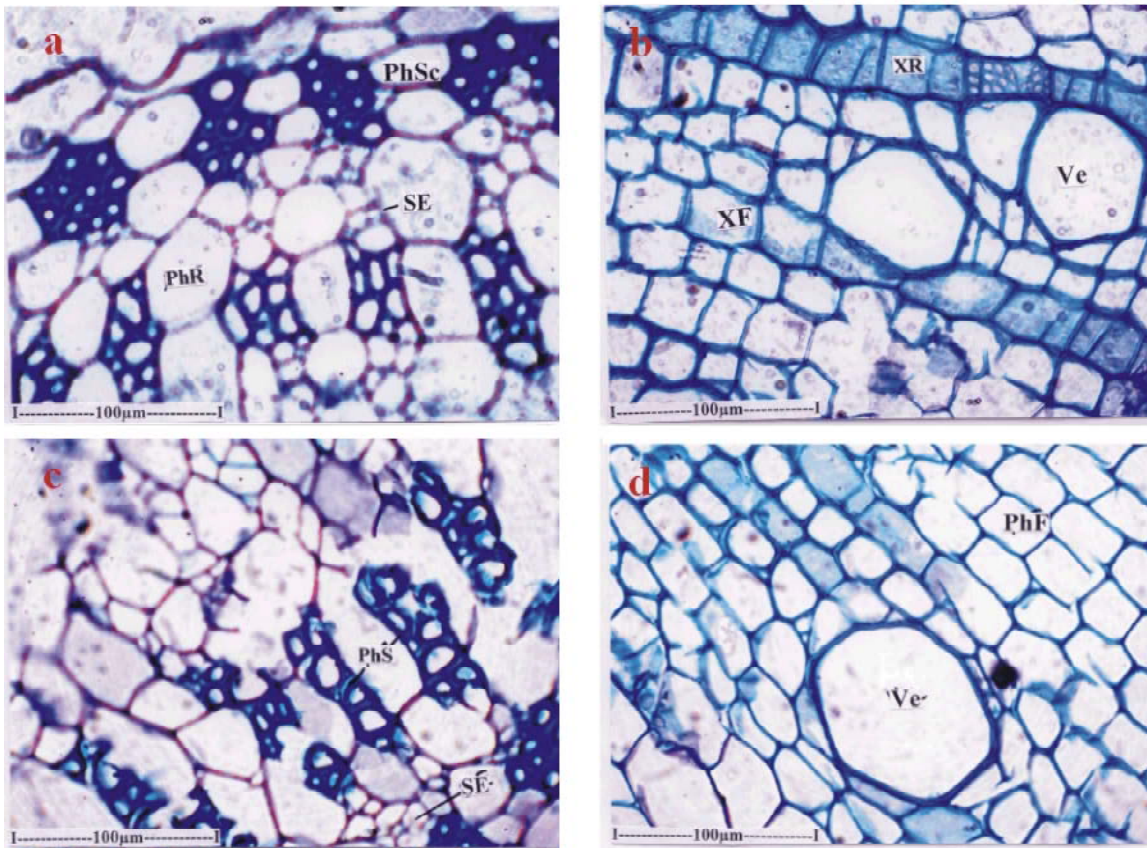


Fig. 2: *Abelmoschus esculentus*: Transactional views of internode showing phloem and xylem elements of control and Vermiwash - I treated plants a- T.S. control of stem showing Phloem elements, b- T.S. control of stem showing xylem elements, c- T.S. V-I of stem showing phloem elements and d- T.S. V-I of stem showing xylem elements

Anatomical Characters of the Internode: The internode is 4mm in thickness in V-II treated plants and 2mm in V-I and control plants. The transactional view of the internode in control plants shows the presence of uniseriate epidermis made up of thin walled cells. The epidermal layer has narrow spindle shaped cells and some of the cells at certain regions are crushed and obliterated (Fig. 1a). The intact cells in the epidermis are 10µm in thickness in vermiwash-I treated plants (Fig. 1c) and the epidermis is intact, well preserved and continuous with tubular thin walled cells measuring 20µm in diameter in vermiwash -II treated plants (Fig. 3a). In GA and NAA treated plants the epidermis is replaced by periderm (Fig. 4a,c). Following the epidermis is the cortex which is made up of 5-6 layers of collenchyma cells followed by 4-5 layers of parenchyma cells in the control (Fig. 1b). In V-II treated cells the cortex is distinct with intact cells and 100µm wide, which is made up of 4 layers of thick walled collenchymas cell and inner zone of two or three layers wide, thin walled parenchyma cells (Fig. 3b). The GA and

NAA treated plants show a crushing up of cells at the innermost region of the cortex. The vascular cambial zone is wide in control plants but did not appear prominent in GA (Fig. 4b) and NAA treated plants (Fig. 4d). The width of this zone is maximum in vermiwash treated plants when compared to the control.

The secondary xylem shows much development in vermiwash treated plants as compared to that of control. Maximum extent of secondary xylem is observed in V-II treated plants (Fig. 3d). Secondary xylem shows the presence of vessels, xylem rays and axial parenchyma in control plants (Fig. 2b). The vessel diameter (100µm) and vessel number is increased in vermiwash treatments. The thickness of the vessels is greatly increased in V-II (700µm) and V-I (500µm) treated plants (Fig. 2d) when compared to the control plants (200µm) (Fig. 2b). Phloem zone is wide, well preserved and 300µm wide in V-II treated plants (Fig. 3c) when compared to the control (Fig. 2a), V-I (Fig. 2c) and PGR treated plants (Fig. 4b,d). Phloem zone shows the presence of dilated and undilated

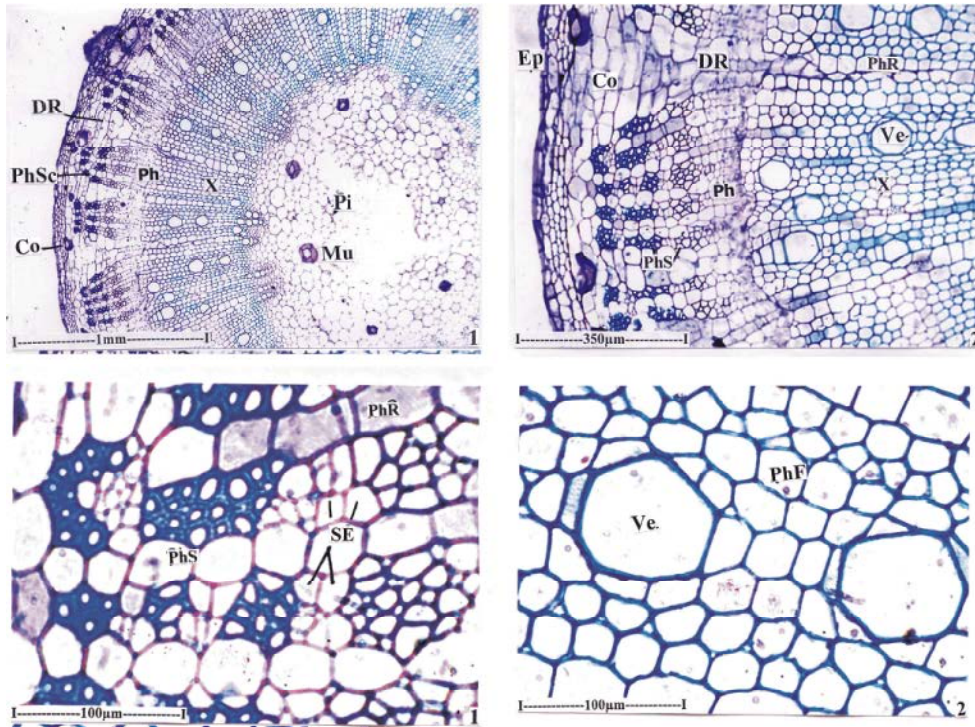


Fig. 3: *Abelmoschus esculentus*: Transactional views of internode showing anatomical features and phloem and xylem elements of Vermiwash - II treated plants a- T.S. V-II of stem - half sector, b- T.S. V-II of stem a sector enlarged c- T.S. V-II of stem showing phloem elements and d- T.S. V-II of stem showing xylem elements

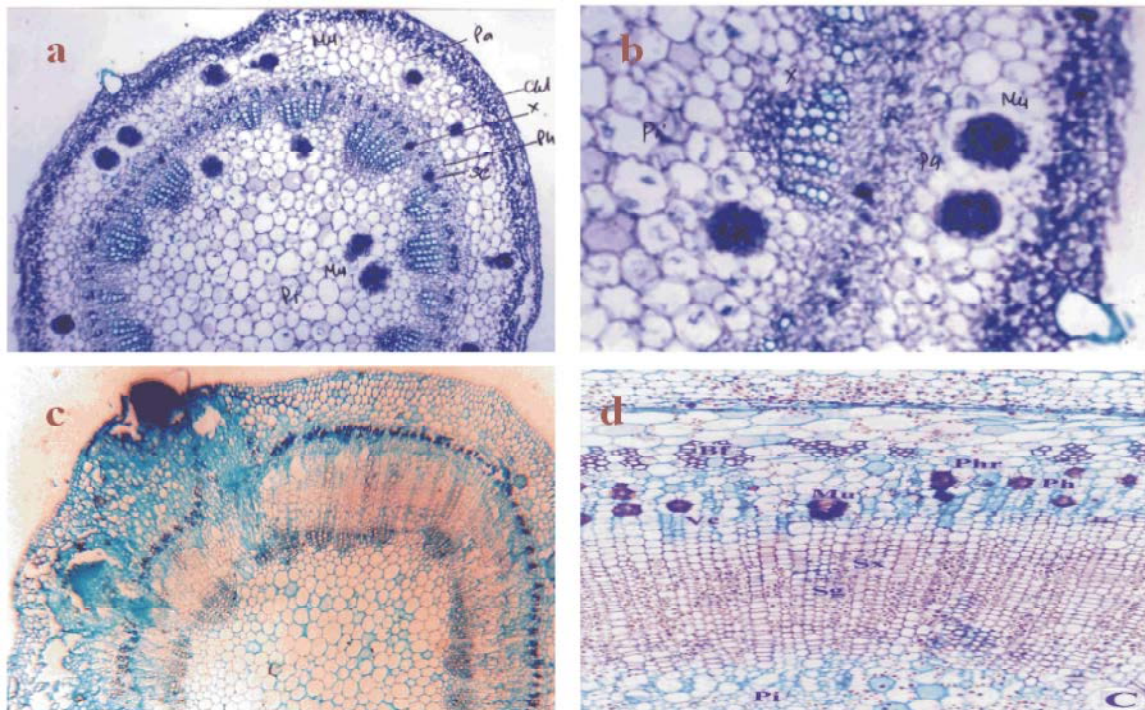


Fig. 4: *Abelmoschus esculentus*: Transactional views of internode showing anatomical features of GA and NAA treated plants a- T.S. GA of stem - half sector b- T.S. GA of stem a sector enlarged c- T.S. NAA of stem - half sector and d- T.S. NAA of stem a sector enlarged

phloem rays, discrete fiber masses alternating with sieve elements and differentiating phloem fibers and phloem elements (Fig. 2a). The phloem rays are 2-3 rows wide in control plants whereas in vermiwash treated plants widening of the phloem rays is observed (Fig. 3c).

In control plants there are two to four rows of radial blocks of fibers alternating with the phloem rays and have small thick walled lignified cells (Fig. 2a). Those fibers that are at the outer end of the segment have thicker walls and narrow lumen. The inner blocks fibers 30 μm wide; the outer cluster of fibers is 40 μm wide. Individual fibers of the inner portion have 10 μm wide lumen; the outer fibers have 3 μm wide lumen.

In vermiwash treated plants the radial layers of fibers have thick, lignified cells. The blocks are 3 to 4 cells thick. The fibers of inner part of the blocks have wider lumen and the blocks are 40 μm wide; the fibers along the terminations of the fiber blocks are circular and 30-60 μm wide with a lumen which is 5 μm wide. The lumen of the inner fibers is 10 μm wide. The fiber elements are located in between the radial blocks of fibers. They are intact and have wide lumen. The dilated rays are 250 μm wide the undilated rays are 30 μm wide (Fig. 3c).

DISCUSSION

PGRs, a new generation of agrochemicals used as foliar fertilizer modifies the natural growth right from seed germination to senescence in crop plants. But the production of these agrochemicals is not economically feasible and the optimum conditions at which they can perform is difficult to ascertain. Moreover due to health and environmental pollution problems, the need for an organic liquid fertilizer arises [27,28].

Among the various foliar treatment used in present investigation, it is obvious from the results that plant height increased at the end of the treatment period. Plant height was maximum in V-II and GA followed by V-I and NAA when compared to control. Maximum plant height was recorded in plants involving vermiwash spray. These observations confirmed early studies on *A.esculentus* Lalitha, [29] and on wheat, paddy, sugar cane and spinach [30, 14]. Feucht and Watson [31] and Kaufman [19] had also reported that growth substances like GA and NAA caused increase in height of the *Phaseolus vulgaris* plant. But maximum shoot length was recorded in vermiwash treated plants, which may be due to increased availability of more exchangeable nutrients in the soil by the applications of vermiwash [32, 33].

The positive effect of vermiwash on plant growth in the present study is in conformity with the studies of Buckerfield [34], who reported that weekly application of vermiwash increased radish growth and yield. Likewise, Thangavel [35] also observed that both growth and yield of paddy increased with the application of vermiwash and vermicast.

Leaf surface area was maximum in V-II and V-I and consistently showed maximum leaf surface area when compared to other treatments as well as control. These observations are in accordance with the earlier studies by Ansari, [14] on the growth enhancing effects of vermiwash and vermicast in the yield parameters of spinach and onion. The growth rate of plants is also increased in the vermiwash treatment then in plant control.

Maximum shoot and root wet and dry weight were recorded in vermiwash treated plants followed by plants treated PGRs. Increase in fresh weight and dry weight is indicative of the increased biomass brought about by vermiwash treatment. Enhanced biomass initiates early flowering and improves the yield parameters. Atiyeh, [36] had reported the increase in root and shoot biomass in tomato plants grown in soil amended with vermicompost. The significant increase in all growth parameters in vermiwash treated plants may be due to the significant increase in the absorption of major plant nutrients such as N, P and K by plants. This clearly indicates that vermiwash is suitable for quick absorption of the major nutrients and provides enhanced nourishment for plants.

The beneficial effects of earthworm on plant growth may due to the presence of macronutrients and micronutrients in vermicast and in their secretions in considerable quantities. There are reports that certain metabolites produced by earthworm may be responsible for stimulating plant growth [37]. It is believed that earthworms release certain vitamins and similar substances into the soil which may be the B group vitamins [38] or some provitamins [39] or free amino acids [40]. Several experiments have proved that wormcasts can promote lush growth of plants, which may be due to the presence of plant growth factors like cytokinins and auxins in the worm cast [41].

The gross anatomy of the internode as affected by vermiwash and PGRs reveals changes in the structure in experimental plants as compared with those of control. Further the observed changes in the internal structure of the internode could be correlated with data on the exo-morphological characters such as internode diameter.

Anatomical studies of vermiwash treated plant internode showed well preserved and intact epidermis, cortex, phloem and xylem when compared to the control. The cortex zone is wide and distinct with intact cells, 700 µm in thickness whereas GA and NAA treated plants showed narrow cortical zone. The wide cortical zone observed in vermiwash treated plants is a factor responsible for the increased biomass recorded in these plants. Moreover the increased diameter recorded is also due to the wider cortical zone observed in the internal structure of the internode. The maximum width of vascular cambium in vermiwash treated plants supports the increased development of xylem and phloem, which in turn accounts for the increased biomass which is observed in the present study.

Unlike the control plants, the phloem zone in V-II is wide, well preserved and 300µm in width. Widening of phloem rays in vermiwash treatments is one of the factors responsible for increased the diameter of the stem. Xylem shows the presence of vessels, parenchyma and rays. The vermiwash treated plants showed increased development of secondary xylem. The enhanced development of secondary xylem with an increased vessel diameter and vessel number also indicates efficient conduction and transport. The frequency and vessel diameter were more pronounced in vermiwash treatments than that of control and PGR treated plants, this probably could be due to the effect of macronutrients, micronutrients and microorganisms that would have produced plant growth promoting hormone like effect. The microflora present in vermiwash makes available inorganic nitrogen, amino acids and inorganic phosphates to plants through aminofication and nitrification processes [42]. Xylem development is among other factors under the control of auxin [43, 44]. Earlier studies have confirmed the effect of auxin on the process of xylem vessel growth and differentiation [45]. Prabhu, [46] also has reported the presence of large number of beneficial microorganism that helps in plant growth. Results in the present study can be correlated with the observations of Wahl [47], who have shown that nutrient supply influences changes in the anatomical structures with larger vessels and increased vessel frequency. Vascular differentiation is vital for the nutrient conduction process for growth and any impaired differentiation might lead to retarded growth and altered morphogenesis [48].

Compared to control, vermiwash treated plants showed an increase in the number of fiber blocks associated with phloem. Each block is 3 to 4 cells thick and the fiber cells in the outer part of the block have a

lumen which is much thicker than that of the control. The increase in number of fiber blocks shows increase fiber differentiation which is manifested as an increase in the fiber yield Fathima and Balasubramanian [21]. Similar results on fiber differentiation as been observed with GA and NAA treatments which proves that vermiwash has effects similar to PGR.

CONCLUSION

The growth promoting effects of vermiwash has been expressed not only on the exo-morphological characters but also brings about favorable changes in the internodal structure as revealed by the anatomical studies. The increase in pith rays, phloem, xylem and fibre differentiation corroborates the increase in girth and increased shoot biomass. Further it is revealed that vermiwash treatment increases the vascular cambium zones which are the meristamatic tissues that differentiate into xylem, phloem and fibres. The maximum width of vascular cambium in vermiwash treated plants supports the increased development of xylem and phloem, which in turn accounts for the increased biomass and diameter observed. Vermiwash, an organic biofertiliser also has the potential to increase fiber differentiation, is the first report of this kind to be documented in this preliminary study, indicating the potential of vermiwash as a biofertilizer in a new perspective.

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