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INFLUENCE OF VERMIWASH AND GIBBERELLIN ON ANATOMY OF THE STEM IN CROTALARIA JUNCEA LINN. TREATED WITH TANNERY EFFLUENT

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ABSTRACT

The present study was undertaken to study the effect of vermiwash on the growth of *Crotalaria juncea* (Linn.) treated with tannery effluent. Vermiwash is a biofertilizer produced by earthworms degrading organic waste material and plants residues. Randomized block design pot experiments with four replicates were carried out to evaluate the effect of the combination treatment of soil drench with tannery effluent and foliar spray with gibberellin (GA) and vermiwash on the anatomical changes in the stem of *Crotalaria juncea* Linn. The present investigation is able to throw light on the fact that tannery effluent affects the internal organisation causing tissue damage and cellular disintegration which could be reversed by the use of vermiwash and gibberellin.

KEY WORDS: Bark, Cortex, Gelatinous fibre, Phloem, Soil drench, secondary wall.

Introduction

Environmental pollution is one of the major problems of the world and it is increasing day by day due to urbanization and industrialization. Over the last few decades large scale usage of chemicals in various human activities has grown very fast, particularly in our country which has undergone rapid change in industrialization to sustain the problem of over population[1].

Water pollution is a predominant global issue as the oceans are spread throughout the world and changes in one part tend to cause changes in the other regions of the globe[2]. Tanning industries are one of the key sources causing pollution in our environment. The enormous pollution load along with the toxic nature of waste water makes the tanneries a potential threat to the areas in the vicinity of their locations.

In India, leather industry contributes 15% of the world's total leather production[3]. It also provides employment opportunity to about three million people of economically weaker sections and thus occupies an important role in Indian economy. On the other hand, tannery wastes are major pollutants among all the industrial wastes[4]. Tannery clusters of India are common in the states of Tamilnadu, West Bengal, Uttar Pradesh and Punjab. In Tamilnadu, 53% of the total Indian tanneries are functioning and contributing more than 50% of the export of finished leather goods from India[5]. Here, tanneries are mainly concentrated in the districts of Vellore, Trichy, Dindigul and Erode which have caused a great deal of soil pollution laying waste much of the agricultural land. As a result, measures to bring about soil reclamation and cultivation of effluent–tolerant plants becomes the need of the hour and use of an organic liquid fertilizer would be a sustainable measure, as synthetic fertilizers cause environmental pollution[6].

Crotalaria juncea (Sunnhemp), a legume, is an important green manure crop and is also a fibre source for pulp and paper. The soft, lignified fibres produced in the stem could be utilized in the manufacturing of paper pulp and other potential products can also be developed from these fibres[7].

Vermiwash, a liquid manure obtained from earthworms is used as a foliar spray and contains N, P, K, Ca and hormones such as auxin and cytokinin. Vermiwash has excellent growth promoting effects, besides serving as biopesticide[8,9,10]. It can also increase fibre differentiation[11].

Gibberellin is a plant growth regulator that enhances plant growth and development[12].

The present investigation seeks to study the effect of vermiwash and gibberellin (GA) on the anatomical characters of the stem in *Crotalaria juncea* Linn., with reference to fibre differentiation.

Materials and Methods

The plant species used in this investigation is *Crotalaria juncea* Linn., a member of the legume family *Fabaceae*. Matured seeds of *Crotalaria juncea*, procured from National Seeds Corporation Ambattur, Chennai, India were used to raise plants for the experiment.

Vermiwash unit was set up as per standard procedure[9] with some modifications. A plastic can of twenty five litre capacity was taken and was filled with pebbles, sand in the lower 1/3rd of the can and over this cow dung cakes and leaf litter were added. Living healthy adult *Eisenia fetida* earthworms were introduced. Three litre of water was added to it. The setup was left undisturbed for about twenty days. Water was sprayed every day to maintain moisture required for the worm bed. On the twenty first day one litre of distilled water was poured over the worm bed and the liquid was collected by opening the tap at the bottom of the can. The collected vermiwash was stored in tightly stoppered bottle.

The effluent samples were collected from a tannery industry situated at Pammal near Chennai in clean plastic cans. The effluent was directly collected from the outlet of the industry and diluted with water for treatment in the ratio of 1:3.

Experimental design

Seedlings of *Crotalaria juncea* were raised in wide nursery pots of 60 cm diameter and fourteen day old seedlings of similar size were transplanted to pots of uniform size of 30 cm diameter. The pots were filled with sand, red soil and farm yard manure free from pebbles and stones in the ratio of 1:1:1. The experiment was conducted in an open terrace with natural light, temperature and humidity, to keep the plants under conditions similar to those on the field. Three plants were grown in each pot and four pots were maintained for each treatment including controls as per randomized block design (Table 1). Experiments were started when the plants were thirty days old and established in the new pots. The spraying was done at the end of each week for seven consecutive weeks (Table 2).

Anatomical studies

At the end of the seventh week, plants were harvested and subjected to anatomical study for which the fifth internode was chosen uniformly in control and treated plants and three samples were taken in each group. The excised internodal segments were fixed in FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethylalcohol-90ml). After twenty four hours of fixing, the specimens were dehydrated with graded series of tertiary – butyl alcohol (TBA)[13]. After infiltration the specimens were cast into blocks, sectioned, stained and mounted[14,15].

Microscopic descriptions of tissues have been supplemented with photomicrographs wherever necessary. For normal observations bright field was used. Magnifications of the figures have been indicated by scale-bars. Anatomical features have been described as given in the standard Anatomy books[16].

Results and Discussion

Histological studies have revealed the following in the study of influence of tannery effluent, gibberellin, vermiwash singly and in combination on the tissue differentiation of the experimental plant (Table 2).

1. Plants drenched with water (T1 to T3): The epidermal layer (Ep) consists of circular, thick walled darkly stained continuous layer of cells. In Control (T1) the cortical zone including cortex (Co) is 100 µm thick and consists of two to three layers of cortical fibres (CF) in small discrete groups along the outer zone of the cortex. There are two types of fibres (Fi) located in different regions of the cortex. At certain regions the fibres are libriform type having lignified secondary walls.

In plants treated with vermiwash 20% (T2) the total thickness of the bark is 720µm. The cortical fibres are of two types. There are libriform type and gelatinous type. The libriform fibres have thick lignified secondary wall and a wide lumen. Along the outer border of the secondary phloem (SPh) small clusters of phloem fibres (PhF) occur. The phloem fibres are mostly gelatinous type. They

have thick, lignified primary walls and gelatinous, shrunken secondary walls.

When compared to control, the plants treated with GA 100 ppm (T3) show less libriform fibres and rectangular gelatinous fibres (GF) with wide lumen. The bark is about 750µm thick. The cortical zone includes outer zone of scattered cortical fibres and inner zone of sparsely distributed phloem fibres. The cortical fibres are mostly gelatinous type with thin lignified primary walls and thick gelatinous secondary walls. The gelatinous wall (GW) consists of either single shrunken wall layer or two or more circular cylinders of secondary walls. This type of fibre has highly reduced cell lumen. Some of the gelatinous secondary walls have two layers with a small gap in between. (Plate 1: T1 to T3)

The studies revealed that the control showed a thin bark in comparison with all the treated plants which showed a drastic increase in the bark region.

II. Plants drenched with tannery effluent (T4 to T6): In tannery effluent treated plants (T4), the bark measures 700µm in thickness. The epidermal layer is intact and consists of rectangular, fairly thick walled cells. The cortical zone has inner and outer regions. In between the outer and inner cortical zone there is a discontinuous cylinder of large masses of cortical fibres. The cortical fibres are totally gelatinous in nature. They consist of slightly thin lignified primary walls. The secondary wall is gelatinous and has shrunken into an uneven cylinder of gelatinous substances. The secondary phloem includes small, isolated groups of fibres. The phloem fibres are very few and they occur in the form of small clusters alternating with sieve elements and there are gaps seen in this region because of tissue damage which may be due to heavy metals present in the tannery effluent.(Plate 1: T4)

In the plants treated with tannery effluent receiving a foliar spray of vermiwash 20% (T5), the bark consists of a thin discontinuous cylinder of cortical fibres which occurs in the middle zone of the parenchymatous cortex. Secondary phloem includes a few isolated small groups of fibres. The cortical fibres are typical libriform fibres with thick lignified secondary walls and wide lumen. The phloem fibres are in small groups of two to five cells and they are all libriform type. The tissue damage was repaired or in other words reversed, which was similar to the effects of T2 (Plate1). This clearly indicates that vermiwash has growth promoting effects and is quickly absorbed by the plants through foliar spray providing necessary nutrients to the plants.

In the plants treated with tannery effluent receiving a foliar spray of GA 100 ppm (T6), it is observed that the bark is about 750 µm thick. The cortical zone is parenchymatous with scanty, scattered gelatinous fibres. The phloem fibres are more abundant and they include mostly gelatinous fibres (Plate 2). The gelatinous walls may be single or double layered. The phloem fibres occur in large compact groups and most of them have shrunken gelatinous secondary walls. In general tannery effluent induced dislodging of the wall of the gelatinous fibre (Plate 2: T4 to T6) and scattered tissue damage (Plate 1: T4 to T6) are noticed.

The results obtained in the present investigation are seem to corroborate the findings of previous report in *Abelmoschus esculentus* (Linn.) Moench, in which vermiwash and plant growth regulators (PGRs) have brought about an increase in the width of cortical zone and vascular cambium[11]. The results are also similar to the reports of gross anatomy of the internode in *Coleus blumei* as effected by PGRs[17]. The cellular level changes in B*acopa monneiri* due to chromium toxicity were also reported which was correlated with the results of the present study[18].

Conclusion

The present study has attempted to investigate the cellular level changes in *Crotalaria juncea* L. to assess the adverse effects of tannery effluent within the plant system after its uptake, and also the reversible action of vermiwash and gibberellin. The study has indicated that the tannery effluent affects cellular organisation which is remedied by vermiwash and gibberellin.

Table 1. Randomized Block design with treatment groups

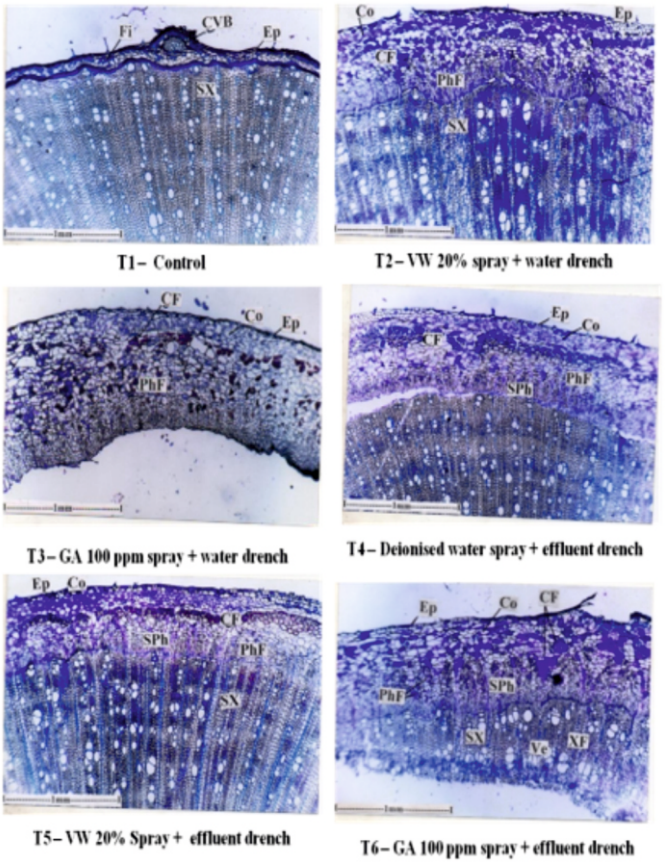
A Block	B Block	C Block	D Block
AT1	BT1	CT1	DT1
AT2	BT2	CT2	DT2
AT3	BT3	CT3	DT3
AT4	BT4	CT4	DT4
AT5	BT5	CT5	DT5
AT6	BT6	CT6	DT6

Table 2. Treatment groups in the experimental design

Treatment group	Water (soil drench)	Tannery effluent (25%) (soil drench)	Foliar spray (once a week)
T1 (control)	750 ml twice a day	---	Deionised water
T2	750 ml twice a day	---	Vermiwash 20%
T3	750 ml twice a day	---	Gibberellin 100 ppm
T4	---	750 ml twice a day	Deionised water
T5	---	750 ml twice a day	Vermiwash 20%
T6	---	750 ml twice a day	Gibberellin 100 ppm

PLATE-1

Transverse Section of Stem - Crotalaria Juncea L.



T1– Control

T2– VW 20% spray + water drench

T3– GA 100 ppm spray + water drench

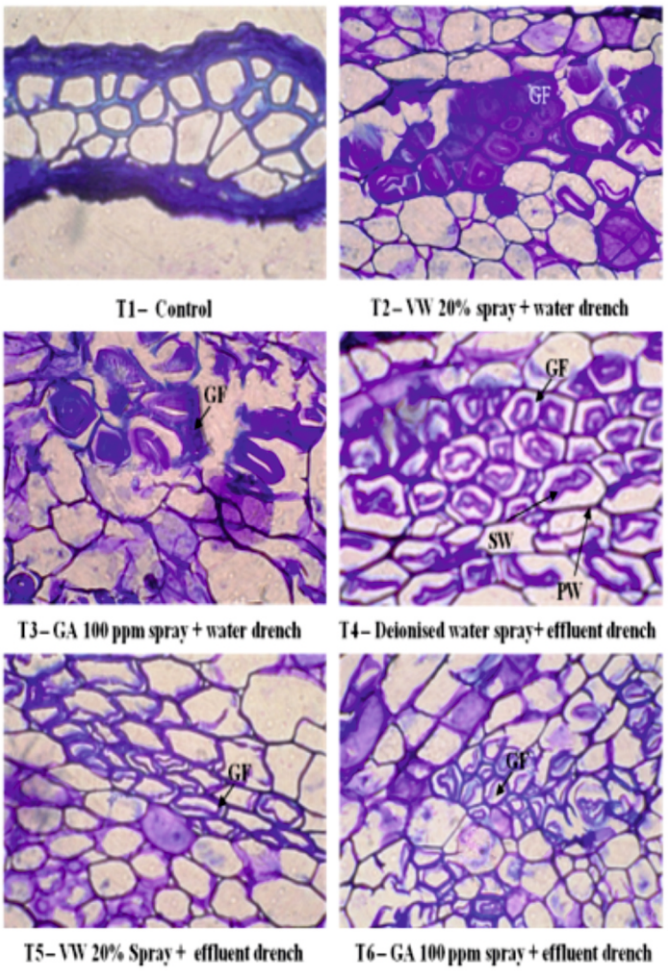
T4– Deionised water spray + effluent drench

T5– VW 20% Spray + effluent drench

T6– GA 100 ppm spray + effluent drench

PLATE – 2

Enlarged view of transverse section of stem showing fibre zone of *Crotalaria Juncea L.*



T1– Control

T2– VW 20% spray + water drench

T3– GA 100 ppm spray + water drench

T4– Deionised water spray+ effluent drench

T5– VW 20% Spray + effluent drench

T6– GA 100 ppm spray + effluent drench

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