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# Effectiveness of Ultra-high Dilution of *Thuja occidentalis* in the Management of *Rhizoctonia Leaf* Blight *in Amaranthus Tricolor*

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**ABSTRACT**

**Background:** Amaranthus is one of the most important leafy vegetable consumed and cultivated in Southern India. *Rhizoctonia solani Kuhn* is known to be the most devastating fungal disease threatening the cultivation of this plant. The control of this disease with fungicides is likely to cause serious health hazards. **Objective:** To obtain an effective Agrohomoeopathic management of the leaf blight disease in *Amaranthus tricolor* with ultra-high dilution of Thuja, to compare different methods of medicinal applications by soaking, drenching and foliar spray methods, to compare the eco-friendly management with that of fungicides and to compare the growth parameters of various sets of experimental treatment. **Methods:** The methodology comprises of different methods of medicinal applications in four sets of plants, out of which one set is treated with Copper oxychloride by foliar spray method, second set (seeds germinated by soaking in *Thuja* 30) and third set (seeds germinated without soaking) by foliar spray, drenching and combined methods and the fourth set by drenching and foliar spray with spirit. **Result:** From the study it was observed that the branching index, average number of leaves, average plant height, and quality of leaves per plant was more in those treated with ultra-high dilution of Thuja by all the three methods and the newly formed leaves are edible and free from the blight disease.In the analysis report it was noted that the Ascorbic acid was found more in those old unaffected leaves treated by all the three methods and Carotenoids was observed to be more in the set treated with spirit. **Conclusion:** The commercial use of ultra-high dilution of *Thuja occidentalis* which can effectively manage the leaf blight disease in *Amaranthus* cultivation will prove to be cost effective and can revolutionize agricultural production in large scale.

**Keywords:** Ultra-high dilution of *Thuja occidentalis*, Agrohomoeopathy, Rhizoctonia leaf blight, *Amaranthus tricolor*

## INTRODUCTION

*Amaranthus* (*Amaranthus tricolor L.*), widely known as the 'poor man's spinach' is one of the cheapest, most accepted and commercially cultivated leafy vegetable. Among the different diseases affecting Amaranth, the leaf blight caused by *Rhizoctonia solaniKuhn* is known to be the most devastating disease threatening the cultivation of this plant seen especially after monsoon rains. The disease is characterised by light cream colored spots on the foliage which rapidly spreads causing extensive damage leading to destruction of crop and thus heavy economic loss to the farmers. On the underneath surface of the infected leaves, white powdery masses of basidiospores of the telemorph of the causative fungus are seen. The humid weather conditions in Southern India make

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the disease serious constraint for Amaranth cultivation. The chemical control of the disease through the use of fungicides could cause serious health hazards**.** Rhizoctonia solani Kuhn is a soil-borne fungus that causes disease on many economically important crops worldwide. The pathogen over winters as soil-borne sclerotia and mycelium in plant debris these constitute the primary inoculums. Control of pathogen is difficult because of its ecological behavior, extreme broad host range, and high survival rate of sclerotic under various environmental conditions [1]. The pathogen infects more than 90% of plants in the field and causing considerable economic loss owing to reduction in the marketability of the product [2]. Therefore, for the management of this devastating disease, Homoeopathic remedies can be used. Agrohomoeopathy makes the plant resistant to disease by strengthening them inside out. In nature it is the weakest of organisms that are attacked and destroyed. Agrohomoeopathy helps build up the plants basic structure and gives it optimum health, thus reducing and sometimes eliminating its susceptibility [3]. Dr. Samuel Hahnemann states that 'if the laws of nature I proclaimed are true, then it can be applied in all life beings'. This is the major warranty given by the idealizer of homoeopathy for the utilization of homoeopathic science in all living organisms, inclusive of plants [4]. Various studies show that Leaf Blight can be effectively controlled by *Thuja occidentalis* in ultra-high dilution and by this study the effectiveness of this remedy in Leaf Blight of *Amaranthus tricolor* could be analysed [5-8].

**OBJECTIVES**

a. To show the effectiveness of ultra-high dilution of Thuja in the leaf blight of *Amaranthus tricolor*.
b. To compare the effectiveness in different modes of medicinal administration.
c. To compare the effect of eco-friendly management of leaf blight of *Amaranths tricolor* with ultra-high dilution of Thuja with that of fungicides.
d. To compare the growth parameters in the four sets of experimental treatment**.**

**Materials Used for the study**

**Seeds:** Pure *Amaranthus* seeds obtained from the Agricultural University, Vellayini, Kerala, India, is used for the study.

**Medicine:** *Thuja occidentalis* 30 (Homoeopathic ultra-high dilution in 30th potency) will be called Thuja 30 in the context, which is used in different methods for treating the plant by soaking method, foliar spray and drenching methods respectively [9]. Fungal Culture of *Rhizoctonia Solani Kuhn*: 100mL Culture of *Rhizoctonia solani Kuhn* is obtained from the Biogenix Research Center, Thiruvananthapurm, Kerala, India [10] (Figure 1 and Figure 2).

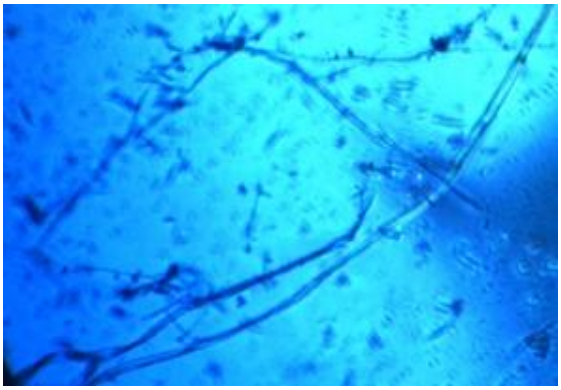

**Figure 1: Microscopic structure Rhizotonia mycelium on Lactophenol cotton blue staining**

**Figure 2: Fungal Culture of *Rhizoctonia Solani Kuhn culture in Potato Dextrose Agar medium***



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**Seed Tray:** Two Seed trays are used for the germination of *Amaranthus* seeds. One seed tray was used for the germination of seeds after soaking in *Thuja* 30 for 12 hours and the other for seeds not soaked in *Thuja* 30.

**Soil:** Normal loamy soil is used for growing the plants Amaranth is a commonly grown leafy vegetable in Kerala. Normal sandy loamy soil would help in the growth of this plant [11,12].

**Spirit:** Spirit obtained from a pharmaceutical shop is used for this study for the purpose of negative control in SET 4. Spirit is diluted in water in the ratio of 1:20 i.e., 5 drops in 100ml.

**Fungicide:** For this study, the fungicide used is Copper oxychloride. This is utilized in positive control set of plants (SET1). Copper oxychloride is obtained in the powder form which is diluted in water before its use. 25g of Copper oxychloride is mixed in 200ml of water before it is sprayed over the leaves of Amaranth. It is the recommended fungicide for the leaf blight in Amaranthus [13,14].

**Methods Adopted for Medicine Application**

**Soaking Method:** In this method some of the Amaranthus tricolor seeds (SET 2) are soaked in 5 drops of Thuja occidentalis 30, diluted in 100mL of water. This is then left for 12 hours before they are sowed [15].

**Foliar Spray Method:** In this method 5 drops of Thuja occidentalis 30 is diluted in 100ml of water and sprayed over leaves affected with the blight (done in Sub-set 1,3,4, and 6). This method is also used in the positive and negative control set of plants (SET 1 and SET4).

**Drenching Method:** In this method 5 drops of Thuja occidentalis 30 is diluted in 5000ml of water and poured over the soil planted with the Amaranth (done in Sub-sets 2,3,5 and 6). This method is also used for the positive control with spirit (in SET 4).

**METHODOLOGY**

Seeds of Amaranthus tricolor soaked in Thuja 30 (5 drops in 100mL water) for 12 hours (Figure 3).

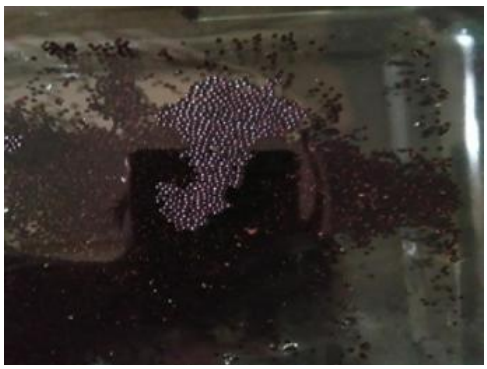

**Figure 3: 3Overnight soaking of seeds of A.tricolor in Thuja dilution (5 drops of 30CH in 100 ml of distilled water)**

Seeds after soaking in *Thuja occidentalis* 30 and those not soaked are transferred to the seed tray as separate sets for germination (Figure 4).



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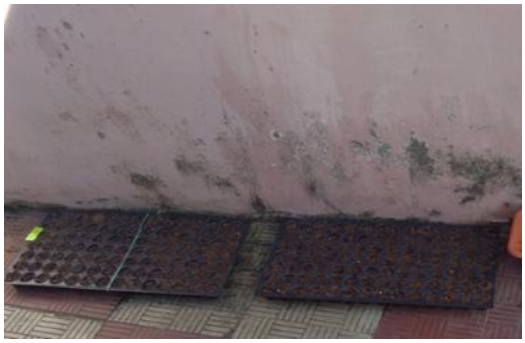

**Figure 4: 4 Seeds transferred to separate seed trays**

Seeds germinated in the seed trays

**Figure 5: Seeds germinated in the seed trays**

Amaranthus plants are transferred to the field and grown (Figure 6,7,8)

**Figure 6,7,8: Seedlings were transferred to Field which was separated into blocks**

The 61-day old Amaranth was later inoculated with the fungal culture (Figure 9,10,11)



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**Figure 9,10,11: Amarathus plants ready for inoculation of pathogen (61days grown plants)**

Amaranth leaves are pricked with thumb pins (Figure 12)

**Figure 12: Leaves pricked with Thumb pins**

The culture is inoculated by wiping the leaves with cotton (Figure 13)

**Figure 13: Leaves inoculated with fungal culture**

Inoculated leaves are left with cotton for humidification for the fungal growth (Figure 14)

**Figure 14: Leaves are left with cotton for humidification for the fungal growth**

Amaranthus leaves infected with leaf blight disease (Figure 15)



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**Figure 15: Amaranthus leaves infected with leaf blight disease**

Amaranthus experimental field in which the plants are divided into different sets (Figure 16)

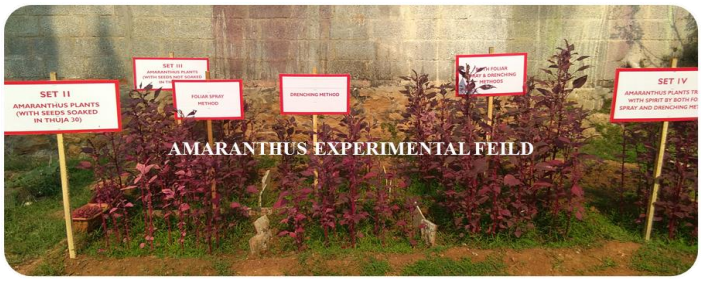


**Figure 16: Amaranthus experimental field in which the plants are divided into different sets**

The infected Amaranthus are treated in the following manner

**SET 1 (**plants treated with Copper oxychloride by foliar spray method) (Figure 17)


**Figure 17: Control set treated with Copper oxychloride by foliar spray method**

**SET 2 (**seeds germinated by soaking method) and **SET 3** (seeds not soaked in Thuja 30), each having three sub-sets viz., foliar spray, drenching and foliar spray & drenching methods (combined method). SET 2 comprises of Sub-sets 1,2 &3 and SET 3 comprises of Sub-sets 3,4 & 6.The medicine was administered twice a week (Figure 18-Figure20)


**Figure 18: Foliar spray method (Sub-sets 1 &4)**


**Figure 19: Drenching method (Sub-sets 2 &5)**


**Figure 20: Combined method (Sub-sets 3&6)**



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**SET 4** plants treated with Spirit by both foliar spray and drenching Figure 21

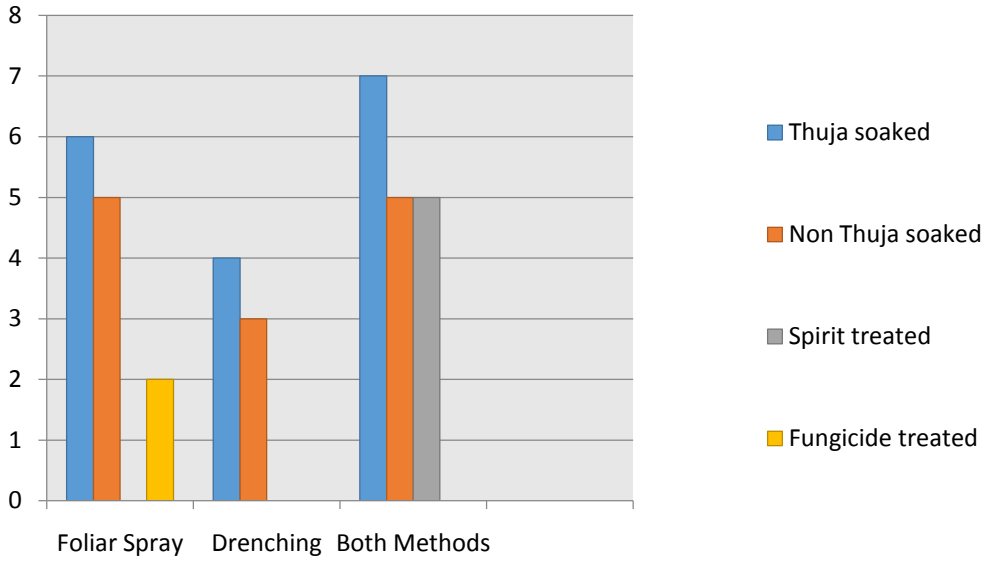

**Figure 21: Experimental set treated with both foliar spray and drenching method with spirit**

## RESULTS

### I. Branching Index (average no: of branches in each set)

**Table 1: Comparison of Branching Index between different sets**

| SL NO | METHOD | THUJA SOAKED SEEDS | NON THUJA SOAKED SEEDS | SPIRIT TREATED PLANTS | FUNGICIDE TREATED PLANTS |
|---|---|---|---|---|---|
| 1. | FOLIAR SPARY | 6 | 5 | - | 2 |
| 2. | DRENCHING | 4 | 3 | - | - |
| 3. | BOTH FOLIAR SPARY & DRENCHING | 7 | 5 | 5 | - |




**Graph 1: Comparison of Branching Index between sets**



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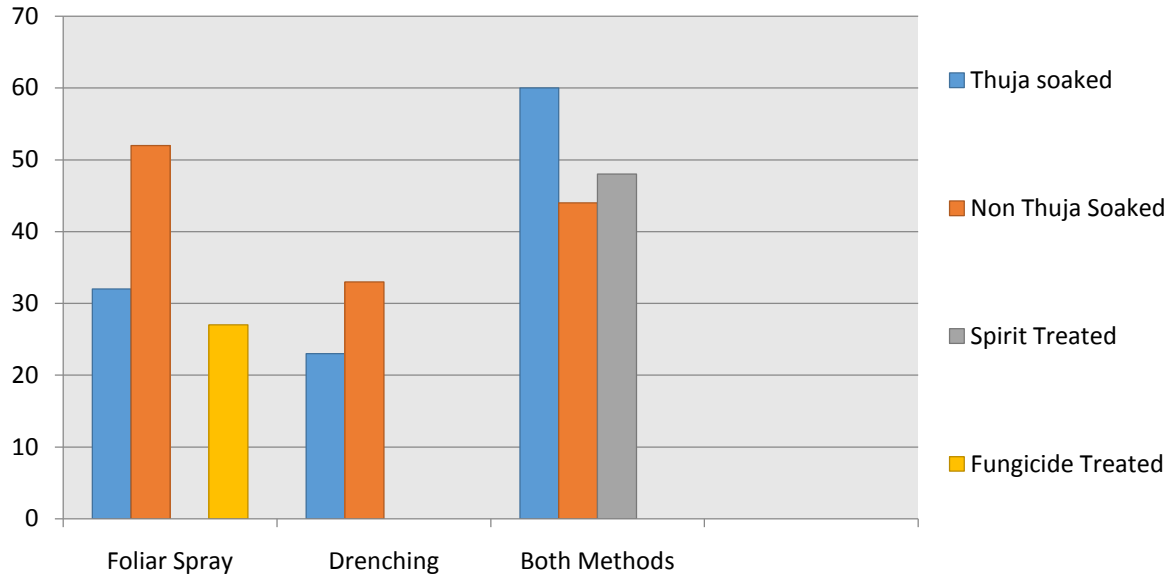
It has been observed that the number of branches in the Amaranth treated by all the three methods (soaking, foliar spray and drenching methods i.e., **Sub-set 6**) is more compared with non *Thuja* soaked, spirit treated and fungicide treated plants.

**II. Average no. of leaves per plant in each set**

**Table 2: Comparison of average number of leaves in different sets**

| SL NO. | METHOD | THUJA SOAKED | NON THUJA SOAKED | TREATED WITH SPIRIT | TREAED WITH COPPER OXYCHLORIDE |
|---|---|---|---|---|---|
| 1. | FOLIAR SPRAY | 32 | 52 | - | 27 |
| 2 | DRENCHING | 23 | 33 | - | - |
| 3. | BOTH FOLIAR SPARY AND DRENCHING | 60 | 44 | 48 | - |




**Graph 2: Number of leaves (mean) between sets**

It is observed that there are more number of leaves in plants treated by seed soaking, foliar spray and drenching methods (**Sub-set 6**) following that the next maximum number of leaves is observed in the foliar spray method in the set of plants with seeds not prepared by soaking method (**Sub set 1)**.



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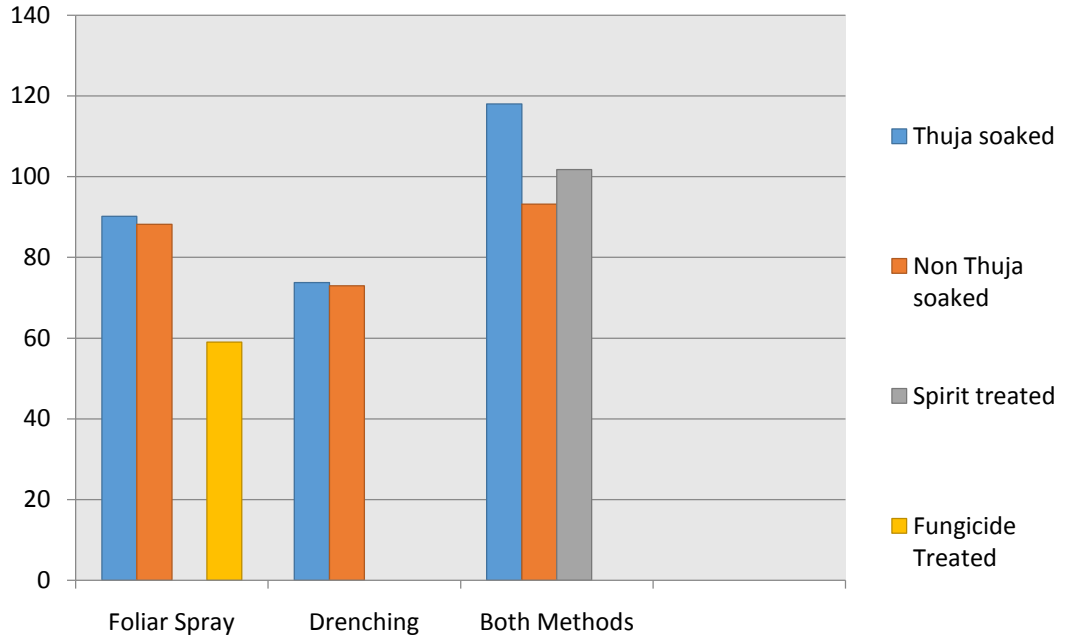
**II. Average height per plant in each set in cm**




**Graph 3: Comparison of Height (cm) among sets**

**Table 3: Comparison of Height (cm) among sets**

| SL NO. | METHOD | THUJA SOAKED | NON THUJA SOAKED | TREATED WITH SPIRIT | TREAED WITH COPPER OXYCHLORIDE |
|---|---|---|---|---|---|
| 1. | FOLIAR SPRAY | 90.2 | 88.2 | - | 59 |
| 2. | DRENCHING | 73.8 | 73 | - | - |
| 3 | BOTH FOLIAR SPRAY AND DRENCHING | 118 | 93.2 | 101.8 | - |

The height of Amaranth plants in different sets and methods of medicinal application. In this, those plants treated by all the three methods with *Thuja occidentalis* 30 (**Sub-set 6)** shows the greatest height compared with the other sets and methods.

**IV.Quality of leaves per plant in each set (A – A**verage no. of old unaffected leaves **B -** Average no. of new unaffected leaves**)**



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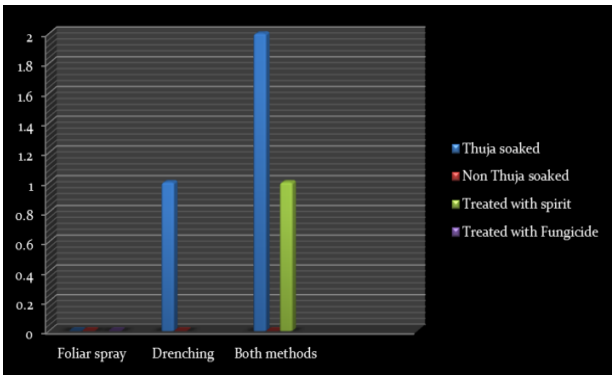
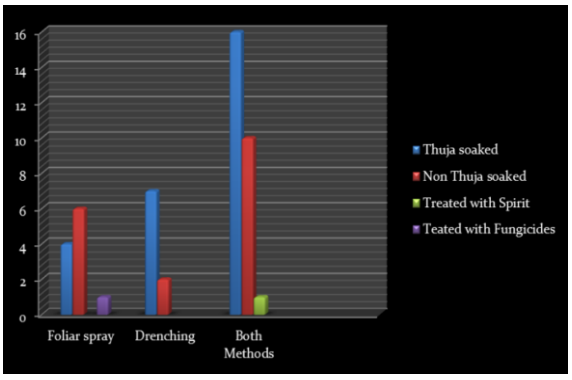
**Table 4: Average number of Healthy leaves**

| SL. No: | METHODS | THUJA SOAKED | | NON THUJA SOAKED | | TREATED WITH SPIRIT | | TREATED WITH COPPER OXCHLORIDE | |
|---|---|---|---|---|---|---|---|---|---|
| | | A | B | A | B | A | B | A | B |
| 1. | FOLIAR SPRAY | 0 | 4 | 0 | 6 | - | - | 0 | 1 |
| 2. | DRENHING | 1 | 7 | 0 | 2 | - | - | - | - |
| 3. | BOTH FOLIAR SPRAY AND DRENCHING | 2 | 16 | 0 | 10 | 1 | 1 | - | - |

The incidence of the disease is observed by noting the leaves unaffected with blight in both the old and new leaves. The old leaves unaffected with the blight are observed in an average of five plants in each Set and Sub-sets and the mean value is calculated and recorded. Similarly the new unaffected leaves are calculated by considering an average of five plants in each Set and Sub-sets and the mean values are calculated and recorded in Table 4.




**Graph 4a – Average No. of Unaffected Old leaves**




**Graph 4b – Average No. of New Unaffected leaves**

Graph 4a represents the average number of old leaves unaffected with leaf blight, from which it is observed that the plants treated with Thuja occidentalis 30 by the seed soaking, foliar spray and drenching methods have at least a few leaves unaffected.

Graph 4b represents the average number of new leaves unaffected with leaf blight, from which it is observed that the plants treated with Thuja occidentalis 30 by the seed soaking, foliar spray and drenching methods have many leaves unaffected.



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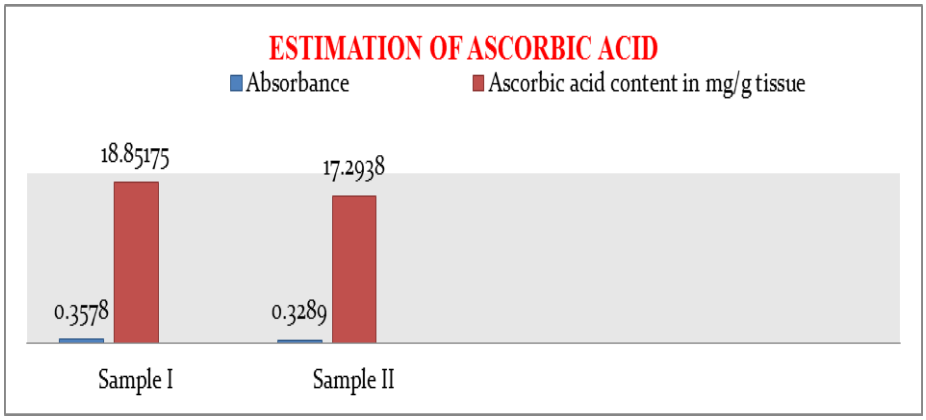
## V.ANALYSIS REPORT

### i. Estimation of Ascorbic acid

**Table 5: Biochemical estimation of Ascorbic acid**

| Sample code | Absorbance | Ascorbic acid content in mg/g tissue |
|---|---|---|
| Sample I | 0.3578 | 18.85175 |
| Sample II | 0.3289 | 17.2938 |




**Graph 5: Biochemical estimation of Ascorbic acid**

**Sample I** – Old unaffected leaves of **Sub-set 3** of **SET 2** (ie., *Amaranthus* treated by all the three methods)
**Sample II** – Old unaffected leaves of **SET 4** (ie., *Amaranthus* treated with spirit)

### ii. Estimation of Carotenoids

**Table 6: Comparison of Pigments among sets**

| Sample | OD at 470nm | OD at 645nm | OD at 663nm | mg chlorophyll a/g tissue | mg chlorophyll b/g tissue | mg Carotenoid/g tissue |
|---|---|---|---|---|---|---|
| Sample I | 0.2083 | 0.0796 | 0.2178 | 0.2551936 | 0.0803536 | 0.203034227 |
| Sample II | 0.2753 | 0.0902 | 0.2437 | 0.2844732 | 0.0927872 | 0.269589385 |

It is observed that the leaves of amaranth in **Sample I** shows 1.55295 mg/g more quantity of ascorbic acid which indicates better nutritive value [12].



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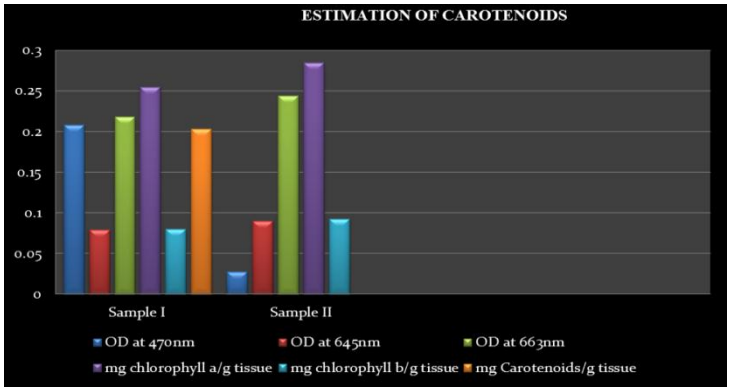


**Graph 6: Comparison of Pigments**

**Sample I** – Old unaffected leaves of **Sub-set 3** of **SET 2** (i.e., Amaranthus treated by all the three methods)
**Sample II** – Old unaffected leaves of **SET 4** (ie.,Amaranthus treated with spirit)

The sample II shows presence of more carotenoids than in sample I

New leaves formed are unaffected in plants treated with *Thuja occidentalis* 30 in SET 2 (Sub-set 3) and SET 3(Sub-set 6) as in Fig.22, treated by both foliar spray method and drenching method.

**Figure 22: New unaffected leaves after medication**

**DISCUSSION**

The Amaranthus plants germinated from seeds soaked in *Thuja occidentalis* 30(SET 2) showed better growth when branching index, no. of leaves, height of plants from all Sets were compared. The methods of foliar spray and drenching with Thuja 30 showed the most beneficial action in growth of the Amaranthus plants as well as in managing the disease especially when it is combined with the seed soaking method. Thus from this whole study the best results were obtained in SET 2 for its resistance against *Rhizoctonia* leaf blight disease in the newly formed leaves.

The plants treated with fungicides (SET 1) as a positive control for the study by foliar spray method did not show much resistance against the fungal infection and the growth parameters when compared with the other sets of plants were not so pronounced. Even though the chemical control is promising under intensive cropping progarammes, their use poses certain problems of residue left over on crops, especially in a leafy vegetable like amaranth which seriously pose many health hazards [13,14].



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Chemicals used to control soil borne diseases are un-economical, less effective and leave residue in soil and plants [16]. As a negative control measure Spirit is used in SET 4 plants. This was considered in the study to rule out the placebo effect of Homoeopathic medicines. It was observed that the growth parameters were not as good as those treated with *Thuja occidentalis* 30. The incidence of the fungal infection was noticed in the newly formed leaves as well. The SET 3 plants which were grown from un-socked method and treated with Thuja 30 by foliar spray and drenching methods showed better growth patterns and the newly formed leaves showed resistance to the fungal infection when compared to SET 1 and SET 4. Spirit has better effects on plant growth rather than the disease resistance. Foliar sprays of spirit causes growth stimulation in plants and increases stem growth. The root applications are said to cause severe damage to plants [17]. In this study it was observed that the drenching method did not show any harm to the Amaranth as the spirit is treated after being properly diluted in water. Chlorophyll and Carotenoid pigments are markers for the quality of food intake. The leaves of *Amaranthus tricolor* also have considerable quantities of Ascorbic acid and Carotenoids (0.03±0.02mg/100g). The variations in the values of genotypic coefficient are noted in successive cuttings. The highest expected genetic advance was noticed for Ascorbic acid followed by foliage yield and leaf size [18].

## CONCLUSION

From the above study it is proved beyond doubt that the Ultra high dilution of Thuja used as Homoeopathic medicine (*Thuja occidentalis* 30C) is highly effective in treating the *Rhizoctonia* leaf blight in *Amaranthus tricolor* plants. This ensures eco-friendly management of this fungal disease in an effective manner. The disease management is economical and most suited to the Agricultural sector. Since the dynamic homoeopathic medicine shows its subtler level action in the plants it makes the newly formed leaves edible [19]. The commercial use of *Thuja occidentalis* 30 C which effectively manage the leaf blight disease in Amaranthus cultivation will prove to be cost effective and can revolutionize its agricultural production in a large scale.

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