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Extraction and Detection of Zeatin from Moringa Oleifera Leaves and to Check its Crude Extract Effect on Plant Growth

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ABSTRACT

In this study, plant growth hormone zeatin (cytokinin) in Moringa oleifera leaves was extracted and its detection was done. 80% methanolic extract was prepared from leaves of Moringa oleifera (drumstick plant) for the extraction and detection of zeatin (cytokinin). The methanolic extract was further partitioned into Zeatin (cytokinin) fraction using methyl acetate and water saturated n-butanol. The presence of plant growth hormone zeatin in the extracted sample from Moringa oleifera leaves was identified and detected by calculating the Retardation factor (Rf) value using the Thin layer chromatography (TLC) technique. The effect of crude extract of Moringa oleifera leaves was checked on Brassica nigra (black mustard) growth.

Keywords: Zeatin, Moringa oleifera, methanolic extract, Retardation factor, Thin Layer Chromatography (TLC), Brassica nigra.

I. INTRODUCTION

Plant growth hormones are chemicals that regulate plant growth. These are produced within the plant and occur in extremely low concentrations. Hormones regulate cellular processes in targeted cells locally and moved to other locations in other functional parts of plant. Hormones also determine the formation of flower, stems, and leaves, shedding of leaves, development and ripening of fruit. Plant hormones shape the plant affecting seed growth, time of flowering, the sex of flowers, senescence of leaves and fruits. Cytokinins are a class of plant growth hormones that promotes cell division and are involved majorly in development of shoot system. They are involved primarily in cell growth and differentiation but also affect apical dominance axilary bud growth and leaf senescence. Moringa oleifera leaves contain zeatin (a type of cytokinin) and therefore its leaves can be used to promote the growth of plant. The other uses of zeatin are, It promotes growth of apical and lateral buds,It causes auxiliary stems to grow and flower.,It Retards yellowing of vegetables,Also Zeatin has several anti-aging effects on human skin.

II. MATERIALS AND METHODS

To isolate and identify plant growth hormone zeatin from Moringa oleifera leaves Materials and Methods used are as follows.

Eighty percent (80%) methanol extraction

In order to obtain a high concentration of any potential plant growth hormone (cytokinin present in Moringa oleifera) the procedure described by Badr et al. (1971) and modified by Taylor et al. (2004) was adopted. 7.5g of Moringa oleifera leaves powder was weighed and put into a screw cap bottle and 75ml of 80% methanol was added to it. This 75ml of 80% methanolic slurry of Moringa oleifera leaf powder was placed on a mechanical shaker for approximately 24hrs. The slurry was allowed to stand and partitioned into the liquid and solid phases. The supernatant was collected and centrifuged for 6min at 4000rpm,

filtered. Then the extract was reduced to 8ml by evaporation.

Solvent partitioning

The pH of extract was adjusted to 2.5 by adding few drops of 1N sulphuric acid (H_2SO_4). The acidified extract 8ml was transferred into separatory funnel. 63ml of methyl acetate was added, shaken and allowed to partition into the organic and aqueous phases. The organic phase (methyl acetate) was separated from the aqueous phase into a clean conical flask. The pH of remaining aqueous phase was adjusted to 7 by adding a few drops of 1N sodium hydroxide (NaOH). This was then transferred into separatory funnel and the same volume of water saturated n-butanol 7ml was added. The funnel was shaken gently and the solution was allowed to separate into less dense water saturated n-butanol phase (organic) and denser aqueous phase before the two phases were separated. The saturated n-butanol organic phase (which presumed to contain the zeatin) was stored in refrigerator at 4°c for further analysis.

Identification of plant growth hormone (Zeatin)

Identification of Plant growth hormone zeatin was done using thin layer chromatography (TLC) by using ammonia vapours and UV transilluminator.

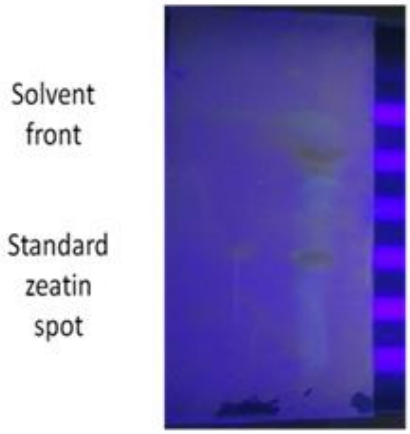
Thin layer chromatography of extract

To detect the presence of Zeatin in the n-butanol phase the spots of the standard zeatin and test sample were developed on TLC plate (of dimension 20x8 cm with 0.25mm thickness of silica gel) by developing the plates in solvent system n-butanol : methyl acetate : water (90:10:10 v/v/v) to about 13cm in vertical direction. The plates were then exposed to ammonia vapours in closed chamber and observed under UV transilluminator for yellow spots.

Detection of zeatin

Data of Measured stem and roots of grown plants

By liquid-liquid extraction of Moringa oleifera leaves, the organic phase presumed to contain cytokinin (zeatin) was obtained. Detection of zeatin was done by TLC method and light yellow coloured spots were observed after separation using suitable solvent system, ammonia vapours and UV transilluminator with Rf value 0.38 identical to the Rf value of standard zeatin.



TLC of organic phase (with zeatin) observed under UV -transilluminator at 254nm.

Effect of the crude extract of Moringa oleifera leaves on Brassica nigra (black mustard) plant

The effect of freshly prepared crude extract of Moringa oleifera leaves was checked on the growth of Brassica nigra plant. 100 grams of soil was added in four small pots with different concentration of crude moringa leaves extract in each pot. Seeds of the plant were sowed in these pots. The plants were grown providing proper sunlight and water and their roots and shoots were measured respectively

Dilution Table

Table 1

Pots with 100 g of soil	Amount of crude extract (g)
1) Control	0
2) Undiluted	5
3) 1:2	2.5
4) 1:4	1.25

Table 2

Dilution	Parts of plant to be measured	1	2	3	4	5
Undiluted	Stem	3.8 cm	2.9 cm	3.9 cm	2.7 cm	2.8 cm
	Roots	6.0 cm	6.5 cm	6.7 cm	8.0 cm	8.0 cm
1:2	Stem	4.0 cm	4.0 cm	3.9 cm	4.0 cm	4.5 cm
	Roots	2.7 cm	2.8 cm	2.9 cm	3.0 cm	3.3 cm
1:4	Stem	3.5 cm	4.0 cm	4.5 cm	4.5 cm	4.8 cm
	Roots	3.5 cm	4.0 cm	4.3 cm	4.0 cm	4.8 cm
Control	Stem	2.5cm	2.5 cm	3.0 cm	2.8 cm	2.8 cm
	Roots	6.0 cm	6.5 cm	6.8 cm	7.5 cm	9.4 cm

III. OBSERVATION

Effect of Crude Moringa Oleifera leaves extract on Brassica nigra (Black mustard) Plant

Figure 2. Effect of Crude Moringa oleifera leaves extract on shoot system of Brassica nigra (Black mustard) plant

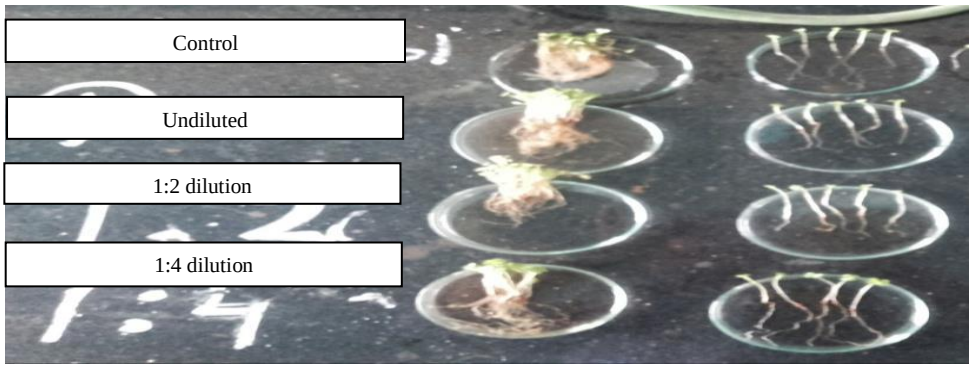


Figure 3

IV. RESULT AND DISCUSSION

In the Project done, plant growth hormone zeatin (cytokinin) was extracted and further detected using TLC (Thin layer Chromatography) technique. Crude extract of Moringa oleifera when added to soil at different dilutions significantly increased the growth of plant. The growth of black mustard plant was better in all the pots with crude Moringa oleifera leaves extract as compared to control. The growth of plant was comparatively better at dilution 1:2 and from this it can be said that, increase in shoot system of black

mustard plant was due to the hormone zeatin present in Moringa oleifera leaves and crude preparation of Moringa oleifera leaves can be used to increase the shoot system of plants.

V. CONCLUSION

From the result obtained it can be concluded that the Moringa oleifera leaves contain plant growth hormone Zeatin that along with other micro and macro nutrients helps in promoting the growth of plants by promoting the shoot system growth of the plant. The crude making of Moringa oleifera leaves in water can be used to get good yield and to improve the shoot system of plants as it contains zeatin.

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