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# Estimation of Indole Acetic Acid in Panchakavya that is Responsible for the Increase in Mitotic Index in *Allium cepa*

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**Abstract:** ***Panchakavya is a concoction used in traditional Indian system that comprises of five major substances, obtained from cow, which includes cow's urine, dung, milk, ghee and curd. All the five products possess medicinal properties against many disorders and are used in various treatments. Panchakavya is used as organic fertilizer and pesticide. The indiscriminate use of chemical pesticides resulted in environmental pollution. An alternative to the chemical pesticide is panchakavya. Panchakavya is a single organic product that act as a fertilizer, pesticide and growth promoter. The present study focused on the evaluation of growth promoting factor by studying the mitotic index in Allium cepa and also by quantifying the content of indole acetic acid (IAA). Onion bulbs were grown in various concentrations of panchakavya and the results show that at 1% concentration the panchakavya could enhance the rooting up to 2 times. The quantification of IAA proved the growth promoting efficiency. The present study clearly proved that panchakavya has growth promoting activity which was proved from earlier pot experiments and field studies.***

**Keywords:** panchakavya , mitotic index, IAA

## 1. Introduction

Organic farming is quite distinct in the sense that it relies on closed nutrient cycles with less dependence on off-farm inputs. Vedic literature (*Vrikshayurveda*) have clearly outlined a systematized agricultural practice that insisted on the use of 'panchagavya' – a mixture of the five products of cow in a specific ratio to enhance the biological efficiency of crop plants and the quality of fruits and vegetables (Natarajan, 2002). "Panchagavya" are the chief ingredient in ayurvedic medicine. They are used to treat a wide range of health conditions since ancient times. Ancient literatures like Bhav Prakash Nighantu, Sushruta Samhita and Astanga Sangrah described cow urine as the most effective secretion with various therapeutic uses with antimicrobial, antifungal properties. Cow urine acts as an effective agent against a wide range of Gram negative and Gram positive bacteria (Anitha Rao *et al* 2016). Many useful elements have been found in panchakavya like urea, uric acid and minerals, bioactive substances and hormones like urokinase, epithelium growth factor, colony stimulating factor, growth hormone, erythro protein gonadotropins, kallikrin, trypsin inhibitor, Allantoin, etc (Gosavi and Jhon 2012; Sathasivam *et al.*, 2010).

## 2. Material and Methods

**Preparation of Panchakavya:** The ingredients for panchakavya was collected from cow farm (Thiruvallur DT)) using sterile container. Based on the detailed review of literature panchakavya stock solution was prepared by using cow dung (2.5 Kg), cow's urine (1.5 L), cow's milk (1L), cow's curd (1 L) and cow's ghee (0.5kg). tap water (1.5L). In addition, jaggery (1.5 Kg), tender coconut water (1.5 L) and ripe banana (6 Nos.) were also added as modification. The panchakavya stock solution was fermented for 30 days and is covered with a plastic mosquito net to prevent houseflies.

**Effect of panchakavya on Cell division and cell growth:** Cytological studies of root tip of A*llium cepa* was studied for 7 days. Onion bulbs were grown on diluted panchakavya as treatment and tap water as control. A*llium cepa* din (2n=16) (Rank and Nielsen 1994) were used as test system. The outer scale of onion was removed. The dried root present at the base of the onion bulbs were carefully removed, with a sharp razor blade. The onion bulbs were treated with panchakavya at different concentration namely 50%, 25%, 10% and 1%.

The root growth in distilled water was taken as control. The root length was measured for 3,5 and 7 days. The root tip from control and experimental setup were thoroughly washed in distilled water and fixed in Carnoy's fixative ( 6:3:1 ratio v/v/v of ethanol, chloroform and glacial acetic acid) and chromosome studies were done by acetocarmine staining technique.

The fixed root tip were washed in distilled water hydrolyzed in HCL at room temperature for 10 min then treated in 45% acetic acid for 5 min and stained in acetocarmarine stain for 10-15 minutes. After staining the root tips were squashed and mounted on a slide, sealed with DPX and observed under a phase contrast microscope, (Carton type) to study the rate of cell division and observed for any chromosomal aberration.

**Estimation of IAA (Tsavkelova et al.,2007):** The panchakavya was centrifuged and 2ml of supernatant was mixed with 1ml of salkowskis reagent and the O.D was determined at 530nm in a spectrophotometer. The amount of IAA was estimated from standard curve using standard IAA with dilution series of 10,20,30,40,50,60,70,80,90 and 100

µg/ml using the same procedure, the final estimated concentration of IAA was expressed as µg/ml.

## 3. Results and Discussion

The growth of onion roots was observed on panchakavya at the concentrations of, 50%, 25%, 10%, 1%. The onion bulb developed more number of roots in 1% panchakavya on 3,5,and 7 days of treatment (Table 1,2 and 3 , Plate 1,2 and 3). After 7 days in 1 % panchakavya onion bulb produced 48 roots while the control bulbs produced only 26 roots. When control and treatment was compared the rooting was doubled in 1% panchakavya . The root length was 7.5 cm, and the shoot length was 7 cm which was higher when compared to other concentrations. At 1% concentration of panchakavya out of 250 cells in microscopical field (Plate 4), of which 48 of them was in metaphase stage. Whereas in control it was lesser, which showed only 22 cells in metaphase stage out of the 250 cells in the microscopical field. At 1% concentration, panchakavya increased the root initiation in onion bulbs, when compared with control and higher concentration of panchakavya. The effect of panchakavya fortified with *Bauhinia* plant extract showed a positive response as an anthelminthic preparation (Rahul kumar et al. (2014). The plant growth regulator IAA was estimated in Panchakavya, which was 302.0 µg/ml, Similar finding were observed by Xu (2001) reported that effective microorganism cultures in panchakavya could synthesize phytohormones i.e., auxins and other growth regulators that stimulated plant growth. When panchakavya was used as aerial spray, the nutrients are easily transferred to plants through foliar spray and considerable quantities of IAA and GA are present in panchakavya (Kunnal ,1997, Ravikumar,2012). The present study shows that panchakavya has stimulatory effect and growth promoting activity which enhanced the plant growth and yield which was proved from earlier pot experiments and field studies (Ramya and Karpagam 2016; Ramya 2017). Estimation of Growth regulators such as IAA proves that the physiological efficiency including photosynthetic ability can enhance yield of crops (Solaimalai et.al., 2001). Panchakavya contains the beneficial microbes such as *Lactobacillus* which produces antibiotics which are effective against pathogenic bacteria and fungi besides its growth promoting effect.

**Table 1:** Effect of Panchakavya on growth of Onion Roots (3d)

| S.No | Concentration [%] | No. of roots After three days | Root length [cm] | Shoot length [cm] |
|---|---|---|---|---|
| 1 | Control | 16 | 1.5 | Absent |
| 2 | 50 | 27 | 1.9 | 1.2 |
| 3 | 25 | 28 | 2.5 | 2.5 |
| 4 | 10 | 30 | 2.8 | 2.8 |
| 5 | 1 | 38 | 3.2 | 3.5 |

**Table 2:** Effect of Panchakavya on growth of Onion Roots (5d)

| S. No | Concentration [%] | No. of roots After three days | Root length [cm] | Shoot length [cm] |
|---|---|---|---|---|
| 1 | Control | 21 | 2.3 | 1 |
| 2 | 50 | 32 | 2.7 | 3.5 |
| 3 | 25 | 32 | 3. 5 | 4 |
| 4 | 10 | 33 | 3.7 | 4.5 |
| 5 | 1 | 43 | 4.2 | 5 |

**Table 3:** Effect of Panchakavya on growth of Onion Roots (7d)

| S. No | Concentration [%] | No. of roots After seven days | Root length [cm] | Shoot length [cm] |
|---|---|---|---|---|
| 1 | Control | 26 | 5 | 3 |
| 2 | 50 | 37 | 5.5 | 5 |
| 3 | 25 | 37 | 6 | 6 |
| 4 | 10 | 37 | 7 | 6.5 |
| 5 | 1 | 48 | 7.5 | 7 |

**Table 4:** Effect of Panchakavya on cell division and mitotic index

| S. No | Concentration [%] | No.of Metaphase | No. of cells | Mitotic index % |
|---|---|---|---|---|
| 1 | Control | 22 | 250 | 8.8 |
| 2 | 50 | 46 | 250 | 18.4 |
| 3 | 25 | 44 | 250 | 17.6 |
| 4 | 10 | 45 | 250 | 18 |
| 5 | 1 | 48 | 250 | 19.2 |

**Mitotic index formula: I = (Metaphase) / Number of cell × 100** followed by Rudolph;et al.1998 method.

## 4. Conclusion

The greatest challenge of the nation in coming years is providing safe food. Panchakavya is easy to prepare and is cost effective. The present study of Panchagavya shows increased rooting and mitotic index. The plant hormone secreted by microbes promote growth rate. The plant growth substances present in panchakavya help to improve the growth and ultimately improve the productivity of the crops. So panchakavya is a better organic growth promoter, it acts both as an organic fertilizers and pesticide and is effective in any crop.

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