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STUDY ON THE CYTOGENETIC EFFECT OF PANCHAGAVYA AND UREA IN *ALLIUM CEPA* (L.) ROOT TIP MITOSIS

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ABSTRACT

Today for improving the crop yield, organic and inorganic fertilizers are applied. Mostly chemical fertilizer are applied in greater extend to get faster result. The negative impact of chemical fertilizers includes chromosomal abnormalities in plants and also to the animals who consumes that crop. Panchagavya is an incredible source of growth promoting substances. Panchagavya is a term used in ayurveda to describe five important substances obtained from cow, which include cow's urine, milk, ghee, curd and dung. In ayurveda all these five products possess medicinal properties against many disorders and are used for the medicinal purpose singly or in combination with some other herbs. Urea is one of the common fertilizer that enriches the soil with nitrogen. As nitrogen is a major component of chromosomes it is quite possible that the residues or metabolites of urea may cause some damage to them. Root initiation in between the panchagavya and urea fertilizer in *Allium cepa* was calculated. Number of roots in panchagavya and urea treatment was calculated. Mitotic index of panchagavya and urea fertilizer was calculated. Cytogenetic effect in panchagavya and urea was studied on onion bulbes. In 5% of panchagavya solution is favorable for root initiation. The mitotic index is higher in panchagavya solution than urea and control. There is no cytogenetic effect in panchagavya treated onion bulbs. But the abnormalities are shown in the urea treated onion bulb root tips. So finally the panchagavya is good eco friendly and good root initiator fertilizer.

KEYWORDS: Panchgavya, urea, *Allium cepa*, mitotic index, aberration.

INTRODUCTION

Today for improving the crop yield, organic and inorganic fertilizers are applied. Mostly chemical fertilizers are applied in greater extend to get faster results. The negative impact of chemical fertilizers includes chromosomal abnormalities in plants and also to the animals who consumes that crop. Both organic and inorganic fertilizers provide the plants with the essential elements required for plant growth and reproduction. Each contains different ingredients and supplies these nutrients in different ways. Organic fertilizers contain only plant and animal material that are either by product or end product of naturally occurring process. The biggest advantage of organic fertilizers is that the danger of over fertilization reduced as the nutrients are released slowly and hence they are available over a large period. It improves the ability of soil to hold water and nutrients, decrease the erosion and soil crusting caused by rain and wind. Inorganic fertilizers are synthetic fertilizers they are made artificially and contain minerals or synthetic chemicals. Synthetic nitrogen fertilizers are typically made from petroleum or natural gas. Urea, phosphorus, potassium and other trace elements in inorganic fertilizers are often mined from the earth. Inorganic fertilizers provide plant nutrients in a readymade form and this lead to over exposure of plants to nutrients. This may cause toxicity to the plants. It is reported that inorganic fertilizers can cause changes in chromosomal number and structure.

Panchagavya is an incredible source of growth promoting substances. From ancient period cow's urine has been used as a medicine. Panchagavya is a term used in Ayurveda to describe five important substances obtained from cow, which include cow's urine, milk, ghee, curd and dung. In Ayurveda all these five products possess medicinal properties against many disorders and are used for the medicinal purpose singly or in combination with some other herbs. This kind of treatment is called 'panchagavya therapy' or 'cowpathy'. Panchagavya is the single organic input that acts as a fertilizer, pesticide, growth promoter and immunity booster. The plant growth substances present in panchagavya may help to improve the growth and ultimately improve the productivity of the crops. Urea is one of the most common fertilizer that enriches the soil with nitrogen. This fertilizer has been found to be present either in residual or some metabolized from among the plants grown over them, thus get access to the body of animals that feed upon these plants. As nitrogen is a major component of chromosomes it is quite possible that the residues or metabolites of urea may cause some damage to them.

Growth and reproduction occur at cellular and organism levels. The growth and reproduction at organismal level take place by the growth and multiplication of cells. There are three kinds of cell division – Amitosis, Mitosis and Meiosis. Mitosis is the peculiar type of cell division, which ensures the biological continuity of organisms maintains the genetic consistency and preserves their somatic chromosome value unchanged through the successive generations. In unicellular organisms, it serves as the fundamental mechanism of asexual reproduction and genetic transmission. But in multi-cellular organisms, it is a means of growth, development, repair, regeneration and revitalization. The daughter cells formed by mitosis, are genetically and metabolically identical to the parent cell. So, Mitosis is described as an equational division. Essentially, mitosis involves two closely related processes, namely nuclear duplication and cytoplasmic cleavage, known as karyokinesis and cytokinesis. The mitotic phase is conventionally divided into five stages- prophase, prometaphase, metaphase, anaphase and telophase. Chromosomes have a definite structure and organization which is normally constant from one mitosis to the next. They, however, sometimes undergo certain structural modification which are known as chromosomal aberrations. Many physical and chemical agents are known to produce chromosomal abnormalities and gene mutation of varies magnitudes in both plant and animal cells. Chromosomal aberration may occur either spontaneously or they can be induced artificially by treatment with chemicals, plant extracts, x rays and atomic radiations. These agents induce breaks in chromosomes or chromatids and the defects are soon corrected by their repair mechanism. If the repair mechanism fails, the aberration is perpetuated. Some of the agent which induces chromosomal aberration have species specificity. Some of them are effective only when the cells are dividing or when the chromosomes are replicating. Depending on the stage of the division cycle at which aberrations are caused, they may include one or both chromatids of the chromosome. Gross structural changes of chromosome are called structural aberration and numerical changes are called numerical aberration. During normal mitosis the chromosome start moving to opposite pole in anaphase, when chemicals, plant extract and radiation induces aberration. Then such chromosomes fail to get separated during anaphase and they are observed to be clumped together. Sometimes anaphase bridges are formed when the chromosomes start to move to opposite poles. Aberrations may also occur in the formation of spindles that arise from different poles i.e. multi polar spindle. Laggards means even if chromosomes start moving to opposite poles, some chromosomes fail to move and are seen at the equatorial plane. The mitotic index and some nuclear abnormalities are used to evaluate cytotoxicity and to variety mutagenicity of different chemicals. Mitotic index is defined as the ratio between the

numbers of cells in a population undergoing mitosis to the number of cells without division. Mitotic index is used to quantify the difference in cell division when environmental parameters are changed.

MATERIALS AND METHOD

The various materials used during the present study was *A. cepa*, panchagavya, urea, acetocarmine, ethyl alchol:acetic acid in (3:1), 1N Hydrochloric acid, water, test tubes, petridishes, slides, and compound microscope.

Healthy young onion bulbs of the same size were used for test. The outer dry scales were removed from bulbs and scrapped at the root to promote the emergence of new root. The panchagavya is collected from Agricultural University, Coimbatore and Urea was purchased from Pattambi, Kerala.

Preparation of panchagavya and urea solution

The panchagavya was prepared in water with different percentages viz., 1,5,10,15,20,25 and 50. Urea was also prepared in the same percentage.

Mitotic squash preparation

A.cepa bulbs were grown in moist cotton placed in a petridish. Various dilutions of panchagavya and urea were made and used for root initiation instead of water. Simultaneously a control kept using distilled water for root initiation. All the experiments were done in triplicates.

Onion roots were allowed to grow for 3 days until approximately 1 cm long root tip was emerged. All the emerged root tips were fixed on 4^{th} day between 10 to 10.30am in Carnoy's fixative containing 3:1 ratio of ethyl alcohol and acetic acid. After fixation, the fixed material was taken on a clean microscopic slide and the growing tip was cut off from rest. The tips were kept and the rest was discarded. Acetocarmine was added to the slide to stain the root tip and gently heated. A cover slip was placed on it. After covering the slide with a few layers of tissue paper. It is gently pressed with the thumb so that the root tip spreads uniformly. The observations were made from these slides**.**

Cytogenetic study

Three slides were prepared for each experimental set up for calculating mitotic index. Mitotic index was calculated as percentage of dividing cells, using the formula.

$$\text{Mitotic index} = \frac{Number\ of\ cells\ dividing}{Total\ number\ of\ cells} \times 100$$

The slides were observed to investigate the different stage of mitosis. The slides were scored for mitotic index and percentage of abnormal cell.

Statistical analysis

Cytotoxicity was evaluated by the parameter of mitotic index. The mitotic index was calculated as a percentage ratio of number of dividing cells to the total number of cells in this field. All the experiments were done in triplicates (n=3).

RESULT AND DISCUSSION

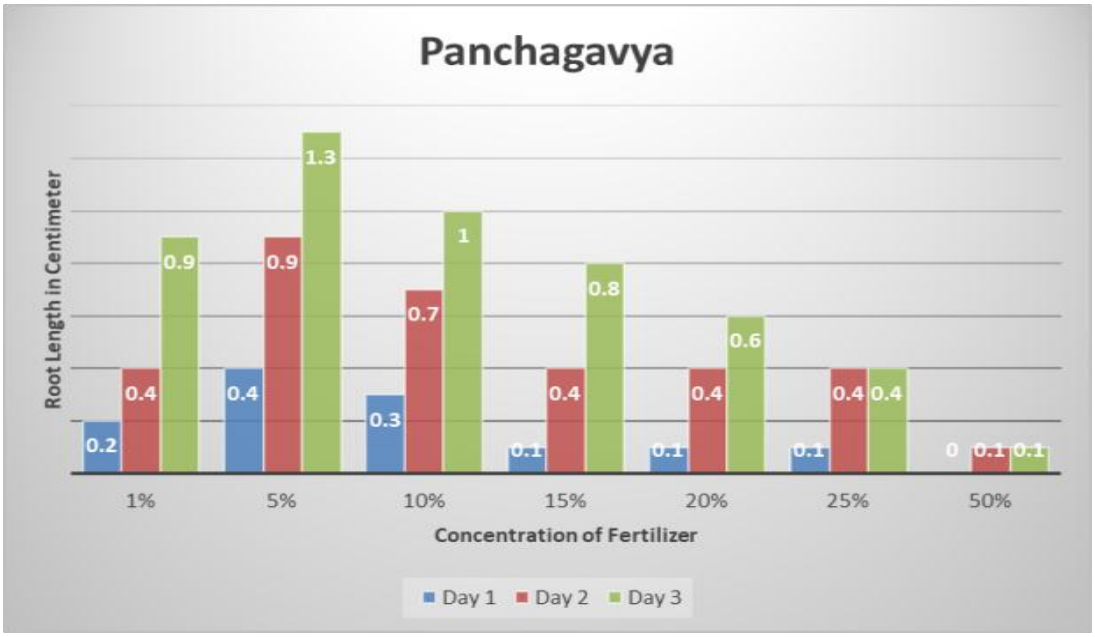
Root initiation in between the Panchagavya and Urea fertilizer treatment in Onion.

When the onion bulbs were treated with different concentration of panchagavya solution (Table 2), maximum number of roots (48) and largest root (1.3 cm) was produced in bulb treated with 5% of panchagavya solution and then in 1% number of roots (39) and in 10% number of roots (37) concentration. Usually, farmers use 3% concentration of panchagavya solution and the experiment proves that growth of onion bulb have a peak value in 5% concentration of solution were the maximum roots and root length is observed, (Graph 1 – 5).

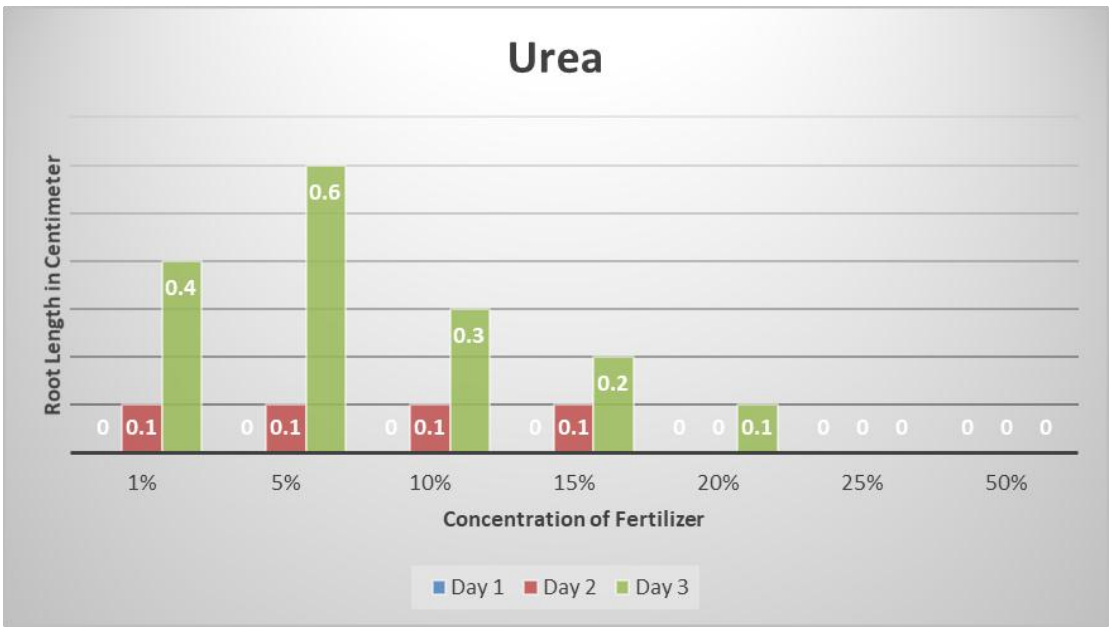
When the onion bulbs were treated with different concentration of urea solution, maximum number of roots (16) and largest root (0.6 cm) was produced in 5%. When this concentration was increased to 10% urea concentration the number of roots got reduced to 12. The experiment proves that growth of onion bulb have a peak value in 5% concentration of urea solution is optimum (Table 1).

Table 1: Depicting the length of onion root initiation in panchagavya and urea.

Concentration	Panchagavya				Urea				Control			
	No of Roots	1Day (cm)	2Day (cm)	3Day (cm)	No of Roots	1Day (cm)	2Day (cm)	3Day (cm)	No of Roots	1Day (cm)	2Day (cm)	3Day (cm)
1%	39	0.2	0.4	0.9	12	-	0.1	0.4	42	0.9	2	2.5
5%	**48**	0.4	0.9	**1.3**	**16**	-	0.1	**0.6**				
10%	37	0.3	0.7	1	10	-	0.1	0.3				
15%	37	0.1	0.4	0.8	5	-	0.1	0.2				
20%	37	0.1	0.4	0.6	3	-	-	0.1				
25%	35	0.1	0.4	0.4	0	-	-	-				
50%	26	-	0.1	0.1	0	-	-	-				



Graph 1: Comparing Root Length Produced By Three Days In Panchagavya.



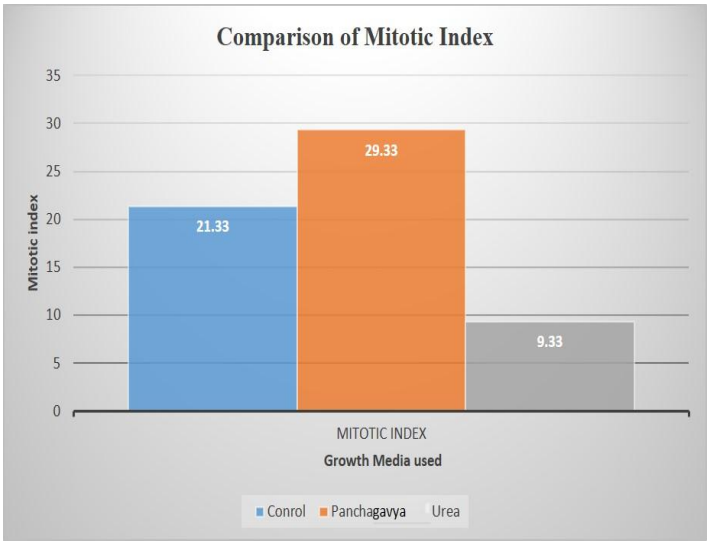
Graph 2: Comparing The Root Length Produced By Three Days In Urea.

Mitotic Index in Panchagavya and Urea Fertilizer Application in Onion Root Tip

The mitotic index was calculated as number of cells containing visible chromosomes (i.e. cells in the division stages) divided by the total number of cells scored. Mitotic index of the treated cells at each dose of panchagavya and urea compared with that of the control. A marked decrease in mitotic index was observed with the increased concentration of panchagavya solutions. In panchakavya the mitotic index was higher than that in control (Table 2, Graph 6).

Table 2: Mitotic index.

Number	Medium	Number of cell dividing	Total number of cells	Mitotic index
1	Control	16	75	16/75*100 = 21.33
2	Panchagavya	**22**	75	22/75*100 = **29.33**
3	Urea	7	75	7/75*100 = 9.33



Graph 6: Mitotic Index of Control, Panchagavya and Urea.

Chromosomal Abnormalities in the Panchagavya and Urea Fertilizer Application in Onion Root Tip

Among the onion bulbs treated with different concentration of urea, the longest root was obtained from the bulb treated with 5% of solution. The experiment proved that high concentration of urea causes abnormality in the shapes of onion bulbs. Bulbs that were grown in high concentration of urea solution (25% and 50%) showed chromosomal abnormalities.

It was also noticed that, as the concentration of urea solution increased, the root length formed decreased also the abnormalities increased. But there were no such effects with panchagavya solution. It took almost two to three days for root initiation in bulbs treated with urea but root initiation occurred within short time in those bulbs were treated with panchagavya solution.

The aberration that were observed include nucleus and chromosomal abnormalities. Abnormalities in nuclear shape may be primarily as an indicator of genetic instability.

Exposure to different concentration of urea fertilizers showed effect on chromosome multiplication in root tips of *A.cepa* and caused a general decline in mitotic index values. A

wide range of abnormal mitotic stages were detected in treated cells when compared to control. Among these frequently observed chromosomal abnormalities were stickiness, disturbed metaphase and anaphase, laggards, chromosome bridges, etc.

Stickiness of chromosomes, Laggarding arrangement of chromosomes, Micro nucleated and biucleated cells in treated cell population, Doubling of chromosome, Chromatin bridge formation, Chromosomal break up are the abnormalities were observed in roots grown in urea. Stickiness of chromosomes is one of the major abnormality noted in this study. Sticky chromosome that fail to condense completely at metaphase. Chromatin masses which are undistinguished as chromosomes are seen as clumps in extreme cases. Cells having lack of spindle fibers. The abnormal chromosome was attributed to abnormal DNA condensation (Osterbeg *et al*., 1984) and the entanglement of inter chromosomal chromatin fibers (Patil and Bhat, 1992). It may be caused by the loss of activity of microtubules in spindle fibers leading to complete inhibition of spindle formation. The mitotic bridge formed by the breakage and fusion of chromosome.

Mitotic cells that lack spindle fibers with unattached whole chromosomes laying scatted through the cell. Laggards are the whole chromosomes that fail to migrate to either pole at anaphase because of possible damage to the kinetochore. Stickiness of chromosomes, Laggarding arrangement of chromosomes, Micro nucleated and biucleated cells in treated cell population, Doubling of chromosome, Chromatin bridge formation, Chromosomal break up this are main aberration which was induced by the urea fertilizer in the present study, (Plate – 1).

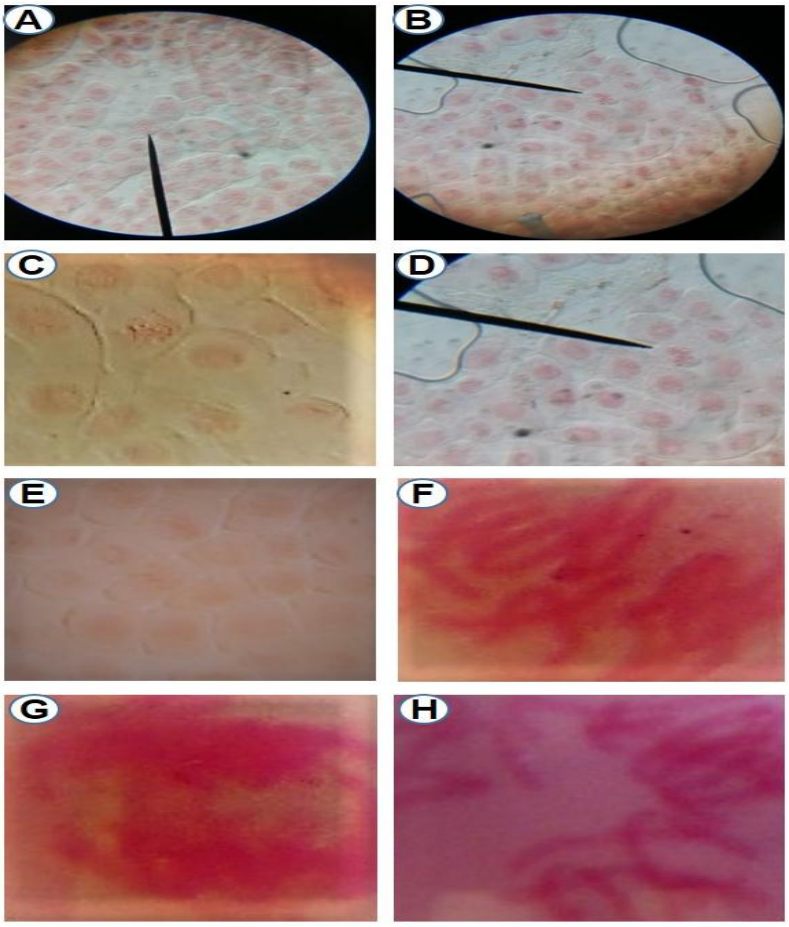


Plate 1: Abnormal Cell Division and Chromosomal Anomalies in Root Grown In Urea.

(A and B) Abnormal cells in microscopic view, (C) Stickiness of chromosomes, (D) Laggarding arrangement of chromosomes, (E) Micro nucleated and biucleated cells in treated cell population, (F) Doubling of chromosome, (G) Chromatin bridge formation, (H) Chromosomal break up.

From this study, it has been revealed that panchagavya, an organic product has the potential to promoting growth and providing proper cell division in plant system. Physico-chemical properties of panchagavya analysed by Selvaraj *et al*., 2007 revealed that it possess almost all macronutrients, micronutrients and growth hormones required for crop growth. Plant when sprayed with panchagavya, produces bigger leaves, thus activating photosynthetic activity, it also branching, profuse and dense rooting is observed when treated with panchagavya.

From the experiment conducted, it was clear that maximum root length can be obtained without causing any aberration in 5% solution of panchagavya. This proves that organic fertilizers are good for plant growth and development of plants.

In previous studies on the panchagavya organic fertilizer and stimulatory effect on the seed germination of *Abelmoscus esculatus* and *Solanum mecongena* (Ramya and Karpagam., 2017) noticed that panchagavya is used in different forms like folilar spray, soil application along with irrigation, seed treatment or seedling treatment etc.

Urea is one of the most common fertilizers that enhances the soil with nitrogen. As nitrogen is major component of chromosome it is quite possible that metabolites of urea may cause some damages to them.

Sangeetha and Thevanathan (2010) studied the potential of panchagavya as biofertilizer for certain pulses by growing in soil amended extract and panchagavya. Experimental seedling recorded higher rate of liner growth of both shoot and root as compared to controls. Maximum lateral root production was always observed in seedling grown in soil amended with seaweed based panchagavya at low concentrations.

The present study reveals that panchagavya is a potential biofertilizer which can be used instead of urea. The current global scenario firmly emphasizes the need to adopt eco-friendly agricultural practices for sustainable agriculture. Chemical input had an adverse impact on the health-care of not only soil but also the beneficial soil microbial communities and the crops. This eventually led to a high demand for organic products. Farmers all over the world realize the need to detoxify the land by switching over to organic farming dispensing with chemical fertilizers, pesticides, fungicides and herbicides. In India, nowadays organic farming is getting more attraction than any other time. Well developed and systematized agricultural practice during the past and this ancient wisdom obtained through Indian knowledge is the use of ‘panchagavya’ in agriculture for the health of soil, plants and humans.

CONCLUSION

Panchagavya is good root initiator and they have active cell division and also they have no abnormalities. The negative impact of urea includes chromosomal abnormalities in plants and also to be animals consumes the crop. The biggest advantages of panchagavya is act as a fertilizer, growth promoter, immunity booster and good active cell division. This organic fertilizer is effective than inorganic fertilizer in agricultural field.

REFERENCES

1. Ankit Samuel singh, Ramteke P.W., Anupriya Paul., Arun A., David, Pradeep kumar shukla and Eugenia P., Lal 2018. Influence of jeevamrutha on seed germination of *Ocimum basilicum* L. under NaCl salinity stress. *Journal of Pharmacognosy and Phytochemistry*, 7(2): 705-703.
2. Boraiah B., Devakumar N., Shubha S. and Palanua K.B. 2017. Effect of panchakavya, jeevamrutha and cow urine on beneficial microorganisms and yield of capsicum(*Capsicum annuum L.var. grossum*). *International Journal of Current Microbiology and Applied Science*, 6(9): 3226-3234.
3. Chauhan, L.K.S. and Sundararaman V. 1990. Effect of substituted ureas on plant cells l. cytological effects of isoproturon on the root meristem cells of *Allium cepa*. *Cytologia*, 91-98.
4. Chaurasia, O.P. and Sinha S.P. 1987. Effect of urea on mitotic chromosomes of mice and onion. *Cytologia*, 877-882.
5. Duygu Akdeniz and Ali Ozmen 2011. Antimitotic effect of the biopesticide oxymatrine. *Research Gate*, 64(1): 117-120.
6. Fernandes, T.C.C., Mazzeo D.E.C. and Marin- Morales M.A. 2007. Mechanism of micronuclei formationin polyploisizated cells of *Allium cepa.*
7. Ramya V. and Karpagam S. 2017. A study of biochemical constituents of panchakavya. *International Journal of Science and Research (JSRI),* 7: 79-57.
8. Ramya V. and Karpagam S. 2017. Estimation of indole acetic acid in panchkavya that is responsible for the increase in mitotic index in *Allium cepa*. *International Journal of Science and Research (IJSR),* 7: 79-57.
9. Ramya V. and Karpagam S. 2017. Panchakavya: Organic Fertilizer and Its Stimulatory Effect on the Seed Germination of *Abelmoschus Esculentus* And *Solanum Melongena*. *Biotech Sustainability*, 525-533.
10. Sailaja V., Naga Rangini N., Kanderi Dileep Kumar, Rajasekhar, Reddy B. and Satyanarayanan S.V. 2014. Effect of foliar application of panchagavya on growth and development of leafy vegetable. *International Journal of Agricultural and Food Science*, 4(4): 119-122.
11. Sharma A. K. and Sharma A. 1990. Chromosome technique, theory and practice, Butter Worth, London.
12. Sharma, C.B., S.R. 1971. Cytological effects of chemicals on mitotic plant cells. *Jour. Scientific and Indu, Res*., 30: 571-587.

13. Sifa Turkoglu 2005. Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. *Mutata Res*, 4(14): 626.
14. Stanislav A., Geraskin, Vladimir G., Dikarev, Yevgenia Ya, Zyablitskaya, Alla A., Oudalova, Vevgeniy V., Spirin 1986. Cytogenetic effect of radiation on agricultural plants observed in the Chernobyl region during the first year after the accident.
15. Swain S.S, Sahu G.S and Mishra N. 2015. Effect of panchagavya on growth and yield of chilli (capsicum annuum L.) c.v Kuchinda local. *Green farming (Internationaljournal of Applied Agricultural and Horticultural Sciences),* 6(2): 338-340.
16. Taylor D., Green N. and Stout G. 1997. Biological science 3rd edition, Cambridge university press, Australia.
17. Veeranan Uthirapandi, Selvam Suriya, Ponnerulan Boomibalagan, Saminathan Eswaran, Subramanian Sivasangari Ramya, Narayanan Vijayanand and Durairaj Kathiresan 2018. Organic Fertilizing Effect of Panchagavya on Growth and Biochemical Parameters of Holy Basil (*Ocimum sanctum* L.). *International Journal of Current Microbiology and Applied Sciences*, 7(6): 2637-2644.