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ORIGINAL ARTICLE

Fusarium sp. isolated from agro waste modulates the phytohormones and enhances growth of *Brassica juncea*

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ABSTRACT
General aim of any agriculture domain is to take high yield. Providing of nutrients and fertilizer alone are not able to supply high yield. There are various components which are answerable for low yield in unstable climate conditions. To increase the yield there are various approaches are used in transmitted modified crops but in India it is aggressive gain and another gain is the use of several function plant hormone like GA3. This research mainly required the production of GA3 from fusarium species and to apply it on crop plant. fusarium species were isolated from spoiled corn seed protect for GA3 production under submerged fermentation .And to study the effect of various parameter on GA3production like incubation time, initial PH ,carbon and nitrogen sources. The high amount of GA3 was observed in agro waste and dried cow dung and panchagavya with glucose and ammonium sulphate and carbon source and nitrogen sources, then optimization GA3 production was observed .The GA3 production was confirmed by the spectrophotometer. The GA3thick extract obtained using submerged fermentation was used to study in action on germination and growth. It was observed comparison of the GA3 taken from cow dried, panchagavya and two agro waste substrate that GA3 treats crop shows the growth and they were taller than non-treated plants, suggested its application in increasing the crop plant harvests.
KEYWORDS – *Phytohormones, Agro waste, Dried cow dung, Panchagavya, Gibberellic acid.*

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INTRODUCTION

Gibberellic acid is a phytohormones present in plant. Gibberellins made up of a family of diterpenoid acids an significance group of phytohormones that workout variant harvest on growth and flowering of plant such as germination, cell elongation, enlargement of leaves and flowers [1-2]. They are enzymatically known as a diterpenoid acid having molecular formula (C19 H22 O6)[3, 4]. Gibberellic acid (GA3) can also be beneficial in nursery, agriculture, beverage, and superficial industry and sometimes used to induce germinationin seeds that would otherwise remain dormant in laboratory. It is also widely used as a hormone in the grape growing industry to induce the development of larger bundles and bigger grapes, especially seedless grapes from Thompson. It is also used as a growth replicator in the cherry sector in the Okanagan and Creston valleys. These plant phytohormones are extra produced by higher plants at the small acute, leaves, buds, seeds, root tips, in addition to some microorganisms, where they are improve produced in from of as growth factor in culture media [5]. In microorganisms namely bacteria and fungi, gibberellic acid is the standard product of gibberellins and show as secondary metabolite. Unto now, 136 gibberellic acids were protected from various plants and between that gibberellic acid shows high biological activity. The use of GA3 take place certified by Food and Drug Administration (FDA) being of its tremendous application and controlled properties and its safety for environment and human was believed by Material Safety Data Sheet (MSDS) [6]..In developing countries where the economy relies mainly on agriculture, the farmers must use fertilizers and plant hormone to increase production. Since most fertilizers are associated with environmental pollution, plant growth hormones such as gibberellic acid must be produced cost-effectively in large quantities in order to

increase the number of agricultural products [7]. Gibberellic acid used directly on the blossoms as a spray, it allows for clementine's to produce a full crop of fruit without seeds.

Agro-Waste

Residues from the production and processing of row agricultural products such as fruits,vegetables, meat,poultry,dairy products and crops are known as agricultural waste. It is the non-product output of the production and processing of agricultural products,which may contain material that may benefit man but whose economic values are lower than the cost of collection, transportation and production for favorable use.Their composition may depend on the farming system and type of activities and may be in the form of liquids, slurries, or solids. Agricultural waste better known as agro-waste.Agro-waste consist of Animal waste(cow dung), Foodwaste (mosambipeel, lemon peel etc.) and crop waste (saw dust,coir pith dust,groundnut shells etc.), with the increase in the agricultural production resulted in increase in agricultural crop residues and agro- industrial by-product. [2]

Mosambi Peel Powder

(*Citrus limetta*) is also known bionomically as sweet lime,sweet lemon,and sweet limetta. It commonly referred to as mosambi [7]. Mosambi peel had large amount of crude fiber in addition to water and oil holding capability [8]. This study looks at the use of fruit peels for the successful plant growth and higher yields by focusing primarily on Nitrogen, phosphorus,potassium.In general, fruit peels are thrownin the garbageand dumping site of solid waste [9].

Lemon Peel Powder

Lemon is one of the most commercial fruit crops grown in all over the world [10]. Botanical name of lemon is *citrus Limon*. L.Citrus fruits are primarily used by juice processing industries, while the peels are usually wasted;because citrus juice yield are less than half of the fruit weight quite of the fruit, large quantities of by-product waste such as peels are produce year after year [11]. Peels waste is extremely persistent and seasonal there is always increased exposure to viable waste products and citrus waste is no exceptions. Citrus(lemon)peel can be screened for antibacterial properties and can be used as food preservatives in various food industries [12]. Lemon peel can be used to acidify two soils. Sprinkle this on soil and mix it together. It is perfect and natural way to increase soil acidity [13].

Saw Dust

Saw dust is a voluminous waste created by the wood-processing industries. The treatment of this solid waste is an economic and environmental problem due to its limited biodegradable quality and low bulk density. Although saw polyester is used as fuel, particularly in developing countries [14]. The low bulk density and high surface area make energy recovery weak and incomplete combustion leading to volatile pollutants [15]. Fungus treated saw dust provided beneficial attributes to different types of soil to make them suitable for forming purposes [16].

Coco Peat

Processed coconut fibers are the by-product of the coconut industry that would otherwise be disposed of without their utility to gardeners. Coco peat, also known as coir or coir dust, as a growing medium similar to sphagnum peat, provides an alternative to potting soil with high water retention, suitable aeration, and antifungal benefits. Coco peat is not only a natural product, mostly organic, but also a green product with a slightly acidic PH in which many plants prefer to grow [17].

Groundnut Shell Powder

Ground nut cultivation is common in many famous countries as an important food crop. According to the World Food and Agriculture Organization, India,China,the USA, Indonesia are the major groundnuts producing countries. Groundnut producing worldwide occupies approximately 22.2 million hectares of land, which is 16.3 million hectares in Asia, 7.39 million hectares in Africa and 0.7 million hectares in south and central America [18]. Use of groundnut shell was previously reported as an organic fertilizer for growth of ornamental plants [19].Compost can also be used as a component of low-cost peat substitute which acts as a potential source against various soils borne disease and help the plant achieve high yields [20].

Dried Cow Dung Cake

Cows dung is a major source of organic fertilizer but, at the same time, cow's urine, cow's horn and a dead body of a cow can be used to make effective bio-fertilizer. In our region as per the conventional age –old system, forming and forming are used with cow dung serving as manure among others, there are a number of cow dung and cow urine product that can be used in agricultural practices as fertilizer and pest repellent. Therefore, products are very popular and used every day [21].

Panchagavya

Organic farming is a comprehensive system of production management that encourages and improves agro-ecosystem health, including biodiversity, soil biological activity and biological cycles. It places

particular emphasis on the use of management practices using off-farm inputs, taking into account that regional conditions require, taking into account that regional conditions require locally adapted systems. Panchagavya is a special made from five cow by-products to gather with some other ingredients, has the potential to play the role of promoting growth and providing immunity in plant system. Panchagavya plays an important part in organic agriculture. Ingredient used to make panchagavya is fresh milk, cow urine, cow milk, cow curd, cow ghee, ripened banana, sugarcane juice.Panchagavya increases the health and fertility of the soil. It used against pathogens and pests. It increases production yield and quality. There are no chemical used. The cost of preparation is smaller. There is no need for special strategies. It provides multiple applications [22].

Standard Gibberllic acid

Gibberellic acid -3 (GA-3) is a naturally occurring plant growth regulator that can in some cases cause a variety of effects including seed germination stimulation. GA-3 occurs naturally in the seeds of many species and is produced commercially by growing fungus cultures of *Gibberellicfujikuroi* in vats, then extracting and purifying the GA-3. In many cases, pre-soaking seeds in GA-3 solution can induce the rapid germination of many forms of highly dormant seeds that would otherwise require cold treatment, post-ripening, aging, or other prolonged pre-treatments. Gibberellins are used for various purposes in agriculture. To increase the size and yield of the grapes, GA-3 is sprayed on seedless grapes and used on novel oranges, lemons, blueberries, tart cherries, sweet and artichokes and other crops low or high fruit set, delay rind aging etc. Such results are highly dependent upon plant concentration and growth level. [23].

Submerged Fermentation

Submerged fermentation is the techniques where microorganisms are grown in the liquid medium which is vigorously aerated and mostly agitated as opposed to solid media. To achieve a fairly fast fermentation process, it uses free flowing substrates such as molasses and broths. This fermentation process is ideal for micro- organisms that require a high level of humidity to develop during fermentation [25]. Substrates selection is extremely important because each organisms responds in a different way to each substrates and so does affects productivity. Agro-waste, fruit, vegetables juices, molasses, soluble sugar, and sewage / waste water are the most common substrates used for submerged fermentation [18]. During the fermentation process, various medium ingredients and different conditions of submerged culture, such as temperature, pH, oxygen supply, incubation period and inoculation rate, affect the output of fermented drinks [24].

Advantages of *Fusarium species:*

Fusarium spp were mentioned that produce gibberellic acid which are the group of diterpenoic acid which acts as plant growth regulators influencing the range of developmental process in higher plants including elongation of stem, germination, flowering, fruit and leaf growth. Shotarohori described that symptoms were induced by infection of fungus belongs to *Fusarium*, culture filtrate was prepared from dried rice seedlings cause elongation in rice plants.

MATERIAL AND METHODS

Samples

Six different substrate such as Coco peat, saw dust, Panchagavya, Dried cow dung cake, readymade gibberellic acid powder, and water were used for plant growth phytohormones analaysis. The contaminated corn seed was collected from Local vegetable market for the isolation of the candidate microorganism.

Sample G1: Coco Peat

Coco peat sample was prepared by allowing the Coco Husk to be shade dried and grounded in the mixer blender. The fermentation media inoculated with fungi culture containing glucose, magnesium sulphate, potassium hydrogen sulphate, ammonium sulphate and incorporated with substrate that incubated at28 °C for 7 days on a rotary shaker at150 rpm.

Sample G2: Saw Dust

Saw dust sample was collected from the wood manufacturing industries. And prepare the fermentation media inoculated with fungi culture containing glucose, magnesium sulphate, potassium hydrogensulphate, ammonium sulphate and incorporated with substrate that incubated at 28°C for 7 days on a rotary shaker at150 rpm.

Sample G3: Panchagavya

Panchagavya was prepared by five ingredients include cow urine, fresh cow dung, cow milk, cow curd, and cow ghee. These are mixed in proper ratio and then allowed to kept for fermentation in room temperature up to15 days mix it daily with wooden stick.

Sample G4: Dried Cow Dung Cake

Collect the fresh cow dung mixed with water to make a thick paste. Make flat cake and leave it to dry for 3-5 days. After that dried cow dung cake soaked in water for fermentation in room temperature up to 7 days.

Sample G5: StandardGibberillic Acid

Commercially available Gibberellic acid powder was used for the study. About 1g of Gibberellic acid powder was dissolved in 10 ml of sterile distilled water and stirred until it was completely dissolved to and stored at room temperature.

Sample G6: Water

Water is kept for control to knowing the variation of these five different substrates.

Isolation of *Fusarium species*

The spoiled corn seeds samples were diluted in sterile distilled waterCzapek Dox agar medium was prepared and sterilized at 121°C for about 15 min in autoclave. The medium was cooled and about 20 ml poured onto sterile plate spread plate technique was performed on plate using respective sample and was noted. These plates were then incubated at room temperature for about4 days. The distinct colonies so obtained were then plated separately and these sub cultures were preserved at 4°C.

Identification of *Fusarium species*

Macroscopic Identification

The morphology of colonies on agar plate was observed for size, shape, texture, elevation, pigmentation, etc.

Microscopic Observation

It was observed by performing fungal staining of (LPCB) Lacto phenol cotton blue.

Fermentation

Gibberellic acid production under submerged fermentation (SAMPLE G1 AND G2)

Two Erlenmeyer flask were taken and noted as sample G1 and G2. Cezapack Dox media was prepared and pH was adjusted to 4 using buffer standards. (1 ml of *Fusarium* culture, isolated from sample was inoculated into respective Erlenmeyer flask containing glucose 5g, agro waste 6g, magnesium sulphate 0.4g, potassium hydrogensulphate 0.2g, ammonium sulphate 1.5g, 100ml distilled water. These Erlenmeyer flasks were then incubated for7 days in rotary shaker at 150rpm. [29].

Gibberlic Acid Production Under Submerged Fermentation (Sample G3)

Take about 500 g of fresh cow dung in the clean earthen pot and add with500ml of cow's ghee mixed well using a wooden stick and allowed for incubation for (two)2 days. And add half litre of cow's milk and again mixed well using the wooden stick. Add half litre of cow's urine mixed well using wooden stick. Then add 500 g of curd mix well and also add 2 ripened bananas. The whole mixture is fermented for 14 days in the shadow place. In this time period daily2-time whole mixture should be mixed for 2 times per day in the direction of clockwise and anticlockwise direction using wooden stick [30].

SUBMERGED FERMENTAION (SAMPLE G4)

Collect the fresh 500gm of cow dung mixed with water to make a thick paste. On the wall of home make 7 small flat cakes and leave it to dry in the shadow place for 3-5 days and take 7 dried cow dung cake soaked in sterilized half bucket of normal water and allowed for fermentation in room temperature up to 7 days. [31].

Optimization Study

The optimization of inoculated culture conditions for maximum GA3 production by *Fusariumspecies* were conducted varying the following parameter including effect of carbonsource (coco peat and saw dust) and effect of different pH, temperature on gibberellic acid production investigated by incubation at four different temperatures (20 °C, 30 °C, 40 °C, 50°C). Incubation time(24h, 48h, 72h, 96h, 120h, 144h, and 168h). The effect of nitrogen sources such as magnesium sulphate, potassium hydrogensulphate, ammoniumsulphate and inoculated at 30 °C for 7 days.[32].

Extraction and Qualification of the Gibberellic acid

The five fermented broth (G1, G2, G3, G4, G5) was taken and filtered at what man no-1 filter paper and centrifuged at 1300 rpm for 10 min. After centrifugation of fermented broth take a supernatant and discard the pellet. In supernatant acidified to pH. 2.0 using HCL. The supernatant was extracted that was determined by the spectrophotometer at 254 nm in UV-VIS spectrophotometer. [33].

Confirmatory Test for Gibberellic Acid

The slurry of silica gel was pouring on a TLC plates air fired and the matrix was activated by keeping the plates on hot air oven at 80°C for 1 h. Gibberellic acid of sample coco peat and saw dust) dissolved in ethanol was added as spot and plate was run using isopropanol, ammonia, water for 2 h. (10:1:1) The plate was covered sprayed with sulphuric acid containing 50 mg and $FeCl_3$ of heated oven at 80 °C for 10

minute the gibberellic acid appeared under UV light as greenish black / spot fluorescence. The position of each substance and RF values is defined as RF=distance travelled by the solute /distance travelled by the solvent. [8].

Gibberellic Acid on Plant Growth

There are six plant named as (G1, G2, G3, G4, G5, C), five plants will be treated with different substrate: (G1 and G2), plant will be treated with coco peat and saw dust, one with panchagavya (G3), one with dried cow dung sample and one plant will be treated with just gibberellic acid, one plant will be a treated with water it kept as a control (G6). The gibberellic acid was sprayed once a day on the plants and noted the height of the grown plants and leaf length and width of the grown plant observed in 60 days at regular interval

RESULTS AND DISCUSSION

Isolation of the Microorganism

The spoiled corn seeds was collected Grained and subjected to serial dilution technique. Czapek Dox agar medium was prepared, spread plate technique was performed on plate using respective sample and was noted. *Fusarium species* identified by the LPCB (lacto phenol cotton blue) stain.

Table 1: Enumeration of organisms from the sample

S. NO	SAMPLE	ENUMERATED ORGANISMS
1.	SPOILED CORN SEED	*FUSARIUM* SPS.

Table 2: Morphological Characterization

Colony Morphology	*Fusarium species*
Color	White
Appearance	Cottony
Surface	Smooth

Foot Notes: The morphology of colonies on agar plate was observed as colour in white, Appearance in Cottony, surface in smooth these are the *Fusarium species* Morphology identification in the microscope.

Table 3: MICROSCOPIC OBSERVATION

MICROSCOPIC OBSERVATION	*Fusarium species*
Shape	Sickle
Arrangement	Arranged in balls (Occasionally occurring in chains)

Foot Notes: Fungi *Fusarium species* the Shape is sickle in shape, Arrangement of *Fusarium species* is (occasionally occurring in chains) Microscopic observation.

COLLECTION OF AGRO-WASTE SUBSTRATE:

Table 4: AGRO-WASTE SUBSTRATE

S. NO	AGRO-WASTE SUBSTRATE
A	**LEMON SKIN**
B	**COIR PITH**
C	**MOSAMBI SKIN**
D	**GROUNDNUT SHELL**
E	**SAW DUST**

Foot Notes: Agro waste substrate such as Lemon Skin, Coir pith, Mosambi peel, Groundnut Shell, Sawdust. Is used to increase in the agricultural production resulted in increase in agricultural crop residues and agro- industrial by-product. [2]

Table: 5: ABSORBANCE RATE

AGROWASTE SUBSTRATE SAMPLE	READING OF ABSORBANCE
LEMON PEEL	0.290
MOSAMBI PEEL	0.219
SAW DUST	0.367
GROUNDNUT SHEEL	0.183
COCOPEAT	0.818

Foot Notes: Agro waste substrate sample their absorbance rate measured in the spectrophotometer in 620 manometers hey are; lemon is 0.290, Mosambi peel is 0.219, Saw dust is 0.367, Groundnut Shell is 0.183, Coco peat is 0.818. These absorbent rate is representation in a graph.

Table:6 pH value of saw dust and coco peat.

DAY	SAW DUST	COCOPEAT
1st day	2.25	2.57
2nd day	2.64	3.39
3rd day	2.71	3.45
4th day	2.83	3.52
5th day	2.87	3.59
6th day	2.91	3.63
7th day	2.97	3.68

Foot Notes:
Measure the pH Value of Saw dust and coco peat, in 1st day of Saw dust is 2.25 and Coco peat of the pH Value is 2.57. In 2 nd day of Saw dust is 2.64 and Coco peat of the pH 3.39 and the 3rd day of the pH Value is 2.71 and the Coco peat of the pH Value is 3.45. 4th day Saw dust of the pH Value is 2.83 and the coco peat is 3.52. And the 5th day of the pH Value is 2.87 and the coco Peat 3.59. In6th day pH Value of saw dust 2.91 and coco peat of the pH Value is 3.63. In 7th day saw dust is 2.97 and the coco peat of the pH Value is 3.68. These are the Value of pH Measured in the pH Meter.

EFFECT OF INCUBATION TIME OF *Fusarium species* ON GIBBERLIC ACID PRODUCTION:

Table: 7: INCUBATION RATE

INCUBATION TIME	SAW DUST	COCOPEAT
24 hours	0.12	0.14
48 hours	0.23	0.20
72 hours	0.28	0.29
96 hours	0.37	0.31
120 hours	0.41	0.32
144 hours	0.43	0.35
168 hours	0.45	0.38

Foot Notes: The Highest absorbance range of saw dust and coco peat that can be incubated in 168 hours to procedure the Gibberellic acid in the Metabolic Shaker by the Method of Submerged Fermentation.

CALCULATION OF RF VALUE:

Table:8 Showing RF values of sample (coco peat and saw dust)

SAMPLE	DISTANCE MOVED BY SOLUTE	DISTANCE MOVED BY SOLVENT	RF VALUE	RESULT
DRIED COW DUNG	7.9	0.8	0.73	GIBBERELLIN
PANCHAKAVYA	6.9	10.8	0.63	GIBBERELLIN

$$\text{RF value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}} \text{ In (cm)}$$

SAMPLE: 1(COCO PEAT)
Distance travelled by the solute =7.9
Distance travelled by the solvent =10.8
RF value =7.9/10.8 = 0.73
SAMPLE: 2 (SAW DUST)
Distance travelled by the solute =6.9
Distance travelled by the solvent =10.8
RF value= 6.9/ = 10.8 =0.63
From this sample spot detected =69 and 0.73. The value was closing approximate of the Gibberellic acid standard value range is similarly an approximate RF value was recorded for the GA3 extracted from

Fusarium monilifome. The TLC was to confirm the GA3 produced by isolated from spoiled corn seed *Fusarium species*

PREPARATION OF PANCHAGAVYA:

The five ingredients of Panchagavya preparation such as;

Table: 9 PREPARATION OF PANCHAGAVYA

S.NO	INGREDIENTS
1.	COW DUNG
2.	COW GHEE
3.	COW MILK
4.	COW URINE
5.	COW CURD
6.	RIPENED BANANA

Foot Notes: Panchagavya was prepared by five ingredients include cow urine, fresh cow dung, cow milk, cow curd, and cow ghee. These are mixed in proper ratio and then allowed to kept for fermentation in room temperature up to (fifteen) 15 days mix it daily with wooden stick. This Method Know as Submerged Fermentation.

PLANT GROWTH OBSERVED AT REGULAR INTERVAL [Fig 1 to 6]

INFORMATION ABOUT THE FULLY GROWNPLANTS

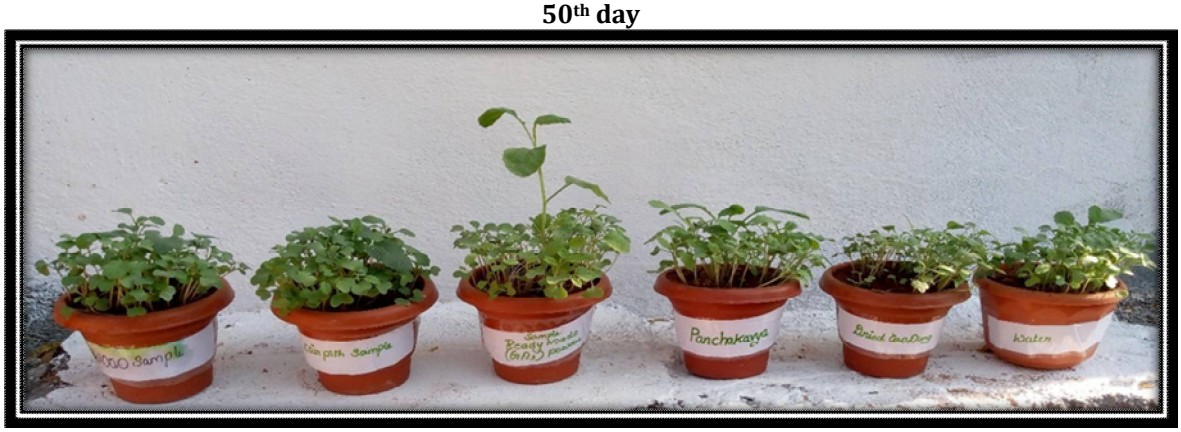
10thday

20thday

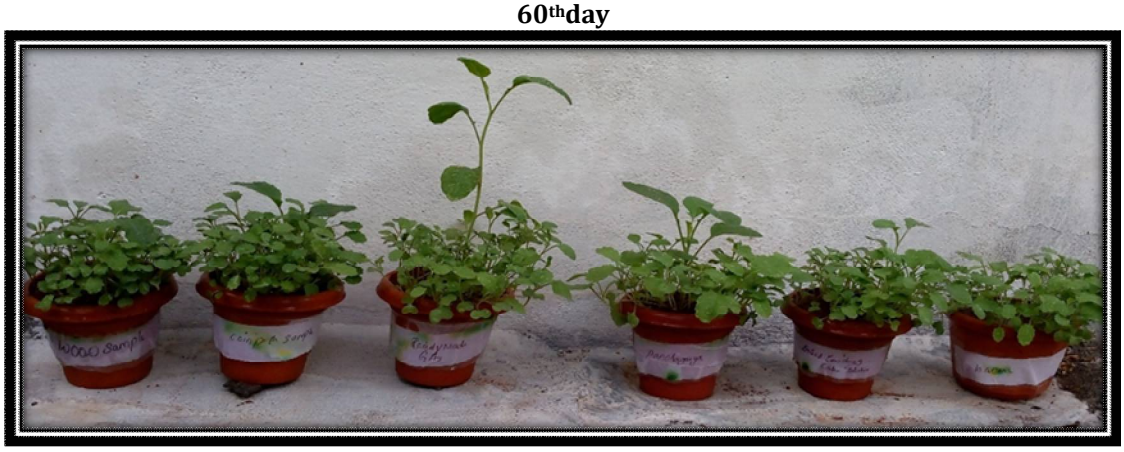
30th day

40th day

50th day



60thday



HEIGHT (FROM TOP OF TALLEST LEAF TO THE SOIL LEVEL OF MUSTARD PLANT

Table: 10 Height Of The Plants

S NO	SAMPLE	SUBSTRATE (GA3)	HIGHT VALUES
1.	G1	COCOPEAT (GA3)	16.2 CM
2.	G2	SAW DUST (GA3)	14.5 CM
3.	G3	PANCHAGAVYA (GA3)	25.2CM
4.	G4	READY MADE (GA3)	25.2CM
5.	G5	DRIED COW DUNG (GA3)	27.6CM
6.	G6	WATER (CONTROL)	13.2 CM

In above table shows the top of the tallest leaf to the Soil level of Mustard plant that can be Measured in the Centimetre scale due to the adding of Nutrition using the Agro waste by the activation of Fungi species.

LEAF LENGTH (FROM STALK OF PLANT TO LEAF TIP)

Table: 11 LEAF LENGTH OF THE PLANTS

NO	SAMPLE	SUBSTRATE (GA3)	LENGTH VALUES
1.	G1	COCOPEAT (GA3)	6.5 CM
2.	G2	SAW DUST (GA3)	6.2 CM
3.	G3	PANCHAGAVYA (GA3)	10.8 CM
4.	G4	READY MADE (GA3)	7.2 CM
5.	G5	DRIED COW DUNG (GA3)	9.2 CM
6.	G6	WATER (CONTROL)	4.6 CM

Foot Notes: In above table shows the leaf length from the stalk of plant to leaf of Mustard plant that can be Measured in the Centimetre scale due to the adding of Nutrition using the Agro waste by the activation of Fungi species

LEAF WIDTH (WIDTH ON WIDEST LEAF:

Table:12 LEAF WIDTH OF THE PLANTS

NO	SAMPLE	SUBSTRATE (GA3)	WIDTH VALUES
1	G1	COCOPEAT (GA3)	6.4 CM
2.	G2	SAW DUST (GA3)	6.2 CM
3.	G3	PANCHAGAVYA (GA3)	10.2 CM
4.	G4	READY MADE (GA3)	7.5 CM
5.	G5	DRIED COW DUNG (GA3)	8 CM
6.	G6	WATER (CONTROL)	4.6 CM

Foot Notes: In above table shows the leaf Width from the stalk of plant to leaf of Mustard plant that can be Measured in the Centimetre scale due to the adding of Nutrition using the Agro waste by the activation of Fungi species

CONCLUSION

From the above work, I have compared the improvement in the growth of plants by various substrates as plant growth promoters. It has been found out that dried cow dung and panchagavya turned out to be the best plant growth promoters in equivalent with the readymade gibberellic acid.

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