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Research Article

Study the Synergistic Effect of Agro-Waste Compost and Bio-Enzymes on Seed Germination and Plant Growth

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Abstract: In the current scenario, organic farming is a major research area the production of nutrients rich crops with high yield. Bio-composite, manure, fertilizer and enzymes synthesized from agro-waste and animal execratory waste are widely used for sustainable and eco-friendly cultivation. In this study, we evaluate the synergistic effect of bio-composite and bio-enzymes for better seed germination and growth. The seeds were germinated under different composition and measured their growth parameter at a regular time interval. In test sample having a combination of coconut husk fibres fine powder, egg-shell powder, carbon ash shows better seed germination, plantlet growth and pigment synthesis as a comparison to the control sample containing only cow dung and soil. However, the best outcome was observed in the test samples having all these constituents along with mocktail of bio-enzymes isolated from pineapple pulp. The results showed coconut fibre helps in better entrapment of minerals and the enzymes in better degradation of bio-composite, for the uptake nutrients and metabolic activities of growing embryo and plant.

Keywords: *Agro-waste, biofertilizer, bioenzyme, seed, germination.*

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INTRODUCTION

In today's world of overpopulation, deforestation and environmental pollution drastically affects ecological balance. These consequences have detrimental effects on soil quality, water supply, as well as the agriculture production. The challenges to make cost-effective and eco-friendly products with desired properties and long life is major concern raised by society (Allen *et al.*, 1991). Therefore, the researchers and manufactures has been working and research is going on to produce compatible, degradable and sustainable source.

Currently to overcome the huge demand of soil nutrients and to fill the gap of approximately 10 million tons of nutrients supply from soil due to eco-logical disturbances have been supplied through chemical supplements such a urea, potash (Mohanty et al, 2005). Although the application of chemical fertilizer and supplements accomplish the plants or crop needs but they have drawbacks like affects the pH of soil and ground water, kills eco-friendly microflora etc (Savci, 2012). Therefore, research has been going on to synthesis eco-friendly, sustainable sources and growth supplements isolated from microbial culture.

A bio-composite consist of natural fibers matrix (resin) for reinforcement along with the inorganic and non-polymeric materials to protect from the environmental degradation and improve the mechanical strength (Biswas *et al.*, 2000; Gilani and Bharose, 2004). It contains various living microorganism having symbiotic association with plants helps in their efficient growth by the increasing plant enhancements (Faruk *et al.*, 2012). Thus, the bio-composite maintains the prolonged soil fertility, sustainably fix the atmospheric nitrogen and entrapped nutrients for organic farming.

Bio-composites are cost-effective materials having potential to improve the soil texture, inhibits the growth of pathogens, enriched the nutrients quality of soil and enhance the plant yield (Bhat, 2000; Jawadi *et al.*, 2016). Bio-composite protects the environment from pollutants since they are natural fertilizers.

There are several natural products used to improve soil fertility, such as animal excretory waste, egg shell, carbon ash, plant fibers and agro-waste (Bahadur *et al.*, 2014; Anna, 2019). These sources are

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Carbon, Nitrogen, Phosphorus and minerals rich constituents. The agro-waste and animal excretory or biomass are rich sources of high minerals and elements i.e, C, N, K, Na, Zn, Ca, Mg, and Fe) in debris produces the yield from numerous rural harvests (Ramamoorthy *et al.,* 2015; Sapuan *et al.,* 2017).

Plant fibers like banana tree bark, pineapple peel, hemp, rice husk, coconut coir etc., are sustainable and renewable biomaterial mainly composed of cellulose and its derivatives having good mechanical strength (Dungani et al, 2016; Petrasek *et al.,* 2017;). The coir of coconut husk is one of the mostly used natural fibre having large amount of lignin used for the fabrication of composite (Samasudin *et al.,* 2014).

Therefore, the natural fibers having low-weight, high strength, fine dimension, faster degradability are advantageous material for agriculture sector. But bio-fibers have some limitations like anisotropic and extra-moisture absorption properties. They limitations of bio-fibers can be overcome by mixing them with ceramics or inorganic components like Egg shell powder, carbon ash and used in form of bio-composite. Eggshell powder enriched with Ca, K with little amount of sodium is used to grow crops and induces plant production in the most fluid way as compared with standard techniques. Similarly Carbon ash contains carbon nano particles, which can be easily uptake by plants roots (Singh, 2012; Yunusa *et al.,* 2012). It has been found that compost having C: N ration upto 20:1 shows better growth rate of plants and yield (Pan *et al.,* 2012).

Other than bio-composite, enzymes as bio-catalyst isolated from agro-waste used to enhance the degradation of composts, release of micro-nutrients, cell metabolism, cell division and crops yield (Gupta *et al.,* 2000; Peng *et al.,* 2002; Mia et al. (2000). Thus for the proper dissolution of nutrients present in fiber or biomass and for the uptake bio-enzymes helps and results faster growth. Therefore, here, we study the synergistic effect of bio-enzymes and bio-compost synthesized from agro-waste on the seed germination and its growth rate.

EXPERIMENTAL SECTION

Materials:

Agro-waste like fruits peels (coconut husk fibers and pineapple pulp) were collected for the preparation of bio-fertilizer and bio-enzyme. Egg shells were collected from the kitchen waste.

METHODOLOGY

Preparation of Raw Materials:

Coconut husk fibers:

A green coconut is taken and its water is removed. Then it is washed properly and is dried out in sunlight for ~3 days, or in a hot air oven at 115ºC for 24 hours. This is to ensure that the coconut is completely dried out and no moisture is left. The above process is repeated so that the coconut gets converted to its dry jute form. It is ground and converted to a powdered form. This is done so that it mixes with other components easily and the composite formed has good resistivity. This composite will also help to retain water ensuring proper hydration of the plant root.

Egg Shell powder:

Egg shells are collected preferably as a waste material from shops. They are washed with lukewarm water and dried in sunlight for ~6 days. The dried shells are ground and converted to a powdered form. This is done so that it mixes with other components easily and the composite formed has good resistivity. This composite will also help to provide the plant & seedling with nutrition, mainly calcium.

Carbon Ash:

The residue of burning of wood/animal waste is called carbon ash. Carbon ash is collected and sieved to remove unwanted particles. It is then used in the composite as a fertilizer. It also acts in neutralizing the pH of the soil, provision of nutrients and as a pesticide.

Cowdung:

Cowdung is composted properly and converted to manure. It is used as a nutrient-rich fertilizer. It is also used as a pesticide.

Soil:

Good quality soil is chosen such that it provides a good environment to the plant, and makes a good composite. Soil is chosen on the basis of nutrient value, porosity, colour, water absorbing capacity etc. It is properly sieved to remove unwanted particles and used.

- **Preparation of Bio-Enzyme:** 10 liter of water and 900 g of the remains of fruit and vegetables is taken for the preparation of bio-enzyme. Water is poured in a dish which is then mixed with fruits and vegetables waste into the dish. The enzyme extract is firmly sealed and placed in a dark and cool place. After 3 months of fermentation the solution will be ready for use.

- **Preparation of Bio-Fertilizer:** A small amount of coconut husk and egg shell is taken and kept in dry condition. After dying the samples were grinded into fine powder. Five samples (1 control + 4 test samples) were prepared (as shown in Table 1) by mixing prepared powder samples with cow dung, carbon in a definite proportion. Thereafter mixed them properly and 10 ml bio-enzymes solution in each samples to study their synergistic effect on seed germination rate.

Table 1: Composition of prepared bio-fertilizer

Components Sample	Cow dung: Soil (1:1) mixture (gm)	Coconut fiber (gm)	Egg shell powder (gm)	Carbon ash (gm)	Bioenzyme (ml)
Negative control	100	-	-	-	-
Positive control	50	40	5	5	-
Test sample 1	90	-	5	5	10
Test sample 2	50	40	5	5	10

- **Seed Germination Study:** Prepared sample were properly mixed with the soil in ratio of 1:1 and filled them in small pots. After that 10 chick pea seeds will be planted in the pots was grown under controlled condition. The following treatment seed were observe daily and recorded the data for 4 weeks to record the growth and yield of the crops.

- **Metabolic Analysis:** Formation of pigments synthesized by photosynthesis i.e., chlorophyll a, b & carotenoids were determined by following the protocol given in the literature (Faheed and Abd-El Fattah, 2008). Simple leaves or plant parts collected and make fine powder of it for chemical analysis. For the analysis of hydrophilic sugars samples were hydrolyzed by incubating in a boiling (100ºC) water-bath. After 2 hr samples were cooled, filter the hydrolyzed samples and determined sugar content by the anthronesulphuric acid method.

Similarly, the free soluble protein content was estimated by treating the distilled hydrolyzed powdered tissue samples with NaOH. Lowery Method was used to determine the plant protein content by using bovine serum albumin as standard.

Statistical Analysis:

Data analysis and measurement was done by calculating Mean and Standard deviation.

RESULT AND DISCUSSION

Seed Germination Rate:

The effect of prepared sample fertilizer and bio-enzyme on seed germination was measured at regular time interval (Table 2). The seed germination is measured in term of embryonic tissue cell division, formation of plumule and radical, and small plantlet. Here, we observed that among all the samples the percentage of seed germination and its rate is significantly better than the negative control samples. In previous study, coconut coir composite showed upto 70% seed vaibilty, due to proper aeration and water sorption rate by the fiber s (Samasudin *et al.*, 2014). The synergistic effect of enzymatic extract with agro-waste composite enhances the germination quality as well quality of implanted seed. The compost was reported good matrix for seed germination and the enzymes help for the germination of embryo by metabolizing the stored food like proteins inside the seeds into simpler monomers for and transfer the same to growing embryo (Paradelo *et al.*, 2012 and Joshi, 2018). Thus, in test sample 2, faster germination of seed shown as compared to the other one.

Table 2: Effect of the bio-enzyme and bio-fertilizer on seed germination rate

	Day 1	Day 2	Day 3	Day 4	Day 5
Negative Control	-	-	10%	30%	40%
Positive Control	-	20%	50%	100%	-
Test Sample 1	-	20%	70%	100%	-
Test Sample 2	10%	20%	80%	100%	-

Plant Height:

The plant growth is greatly affected by the nutrient supply uptake from the soil. Here, we found that the plant growth rate of bio-composite samples is significantly better than the negative control sample. In case of positive control plant height increase constantly but relatively less than the other samples. In previous study, growth media containing 50% coir showed reduced plant growth (Arenas *et al.*, 2002). However, the combination of all mandatory nutrient in a composite supplying the growth of plant (Reeve *et al.*, 2010; Samsuddin *et al.*, 2014). Therefore, in test samples both have faster plant growth but sample 2 containing bio-enzymes along with the other components shows much better growth constantly with the progression of time. These physiological changes and vegetative growth are related to seedling survival rate that is affected by the metabolism and uptake of nutrient (Ali *et al.*, 2017). Thus, bio-enzyme helps to degrade the bio-composite component and induces their uptake by the growth plants or germinated seedlings more rapidly.

Table 3: Effect of the bio-enzyme and bio-fertilizer on Plant growth or height(cm)

	Day 2	Day 5	Day 7
Negative Control	0	1±0.45	3±0.23
Positive Control	0	2±0.35	4.9±0.2
Test Sample 1	0	2.4±0.56	6.8±0.1
Test Sample 2	0.65±0.002	2.5±0.23	7.8±0.12

Plant Weight:

In the table given below, the fresh and dry weight of plants taken after one week of germination was measured. Here, we observed that we the progression of plant growth, the metabolic activity enhances, results morphological changes and formation of more leave with good size. Besides that the fresh/dry weight ratio represents the good water retention of capacity and vascularization. Among the control samples, positive control sample have good fresh and dry weight due to the presence of fiber matrix of better water holding and uptake by the plants. Similarly, in case of Test sample 2 having fibrous matrix with enzymes showed the largest fresh/dry weight ratio indicating superior water retention capacity as compared to the other samples. This could be the beneficial for better photosynthesis and water osmotic regulation of plant. In earlier study the compost with fibrous material have better water sorption activity, deep roots formation that helps for faster and better uptake of nutrient, water and other solubilized growth supplement to the other upper parts of the plants (Uyenco and Ochoa, 1984, Harris 1992). Thereby the whole mass of the growing plants is increases.

Table 4: Fresh weight and dry weight of the germinated plants in different samples

	Fresh weight (gm)	Dry weight (gm)
Negative Control	5.4±0.02	1.4±0.01
Positive Control	7.8±0.01	4.6±0.02
Test Sample 1	10.2±0.03	5.3±0.02
Test Sample 2	12.6±0.05	7.8±0.01

Metabolic Activity:

The table 6, shows the photosynthetic pigment composition in the growing plant samples at regular time interval. The measurements that are taken of Chlorophyll A, Chlorophyll B, and Carotenoids, to represent the better rate of transpiration and photosynthesis (Wahyudi, 2009; Wulandari and Susanti, 2012). Here, we found that the pigment synthesis in all samples subsequently increase with the time, indicates formation more leaves, increase in biomass and chloroplast numbers. Among all these samples negative control samples shows lesser amount of pigments formation as compared to the others, due to the lack of fibrous and enzymes. Other researcher also observed that the positive effect of coconut fibers in a bio-compost to improve biomass production of growing plants (Pill and Ridley 1998). Test Sample 1 and sample 2, there is no significant difference but the sample 2 show slightly more amount of pigment formation, due to the synergistic effect of bio-enzymes in terms of quantity in the long term. , which indicates better and faster growth of any plant which uses bio-fertilizers instead of a natural growth period. In previous research, coconut shell charcoal increases the soil fertility and with lime shows faster growth rate with increases biomass (Wasis *et al.*, 2019).

Table 5: Total photosynthetic pigment (ug/ml) (Chlorophyll a, Chlorophyll b and cartenoid) present in germinated plants at different time interval

	Day 2				Day 5				Day 7			
	Chla	Chlb	Cart	Total	Chla	Chlb	Cart	Total	Chla	Chlb	Cart	Total
Negative Control	2.4	1.2	0.8	4.4	2.6	2.4	11.0	16	7.0	15.0	1.8	23.8
Positive Control	5.8	4.6	2.2	12.6	9.2	7.4	4.6	21.2	18.2	20.45	5.2	43.85
Test Sample 1	6.3	8.2	2.8	17.3	9.0	13.4	5.44	27.84	17.2	22	6.33	45.53
Test Sample 2	6.2	7.8	3.6	17.6	10.1	14.2	4.82	29.12	18.2	20.0	8.2	46.4

CONCLUSION

In this study we concluded for better seed germination and plant growth, composition of raw material and matrix for the implantation of seed greatly affects the plant growth. The coconut fiber of fine size i.e., micro to nanometer along with proper carbon particle and inorganic minerals enhances the growth rate. Besides that the bio-enzymes mocktail isolated from agro-waste helps to enhance the metabolic activity and germination of developing embryo, by degrading the outer seed coating. Thus, the seed dormant period reduces and faster growth rate occur. The synergistic effect of coconut husk fiber, carbon ash, egg shell component and bio-enzymes activates the plant molecular machinery and rapid for seed growth with increase biomass production. This could be the potential, eco-friendly and cost-effective nutrient mocktail for better plant growth and yield.

Conflict Of Interest

First and Second author have equal contribution in this work. The authors have no conflict of interest.

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