

Studies of Biodegradation of *Ipomea Carnea* Weed from Kavinadu

Big Tank in Pudukkottai District (India)

Jeridah Moindi^{1*}, Enock Onyambu², Sabella J. Kiprono³, Suge Titus K⁴, Wilda Onyancha⁵

1. School of Health sciences, Kampala international University, P.O Box 9790, Dar Es Salaam
2. School of Pharmacy, Nyanchwa Adventist College, P. O Box, 1020, Kisii
3. Department of Medical Laboratory Science, Masinde Muliro University of science and Technology, P.O Box 190-50100, Kakamega
4. School of Health Sciences, Mount Kenya University, Kigali Campus. Rwanda
5. Department of Biological Science, Laikipia University college.

* E-mail of the corresponding author: jeryderkem@yahoo.com

Abstract

Introduction: The adverse effect of aquatic vegetation on the environment is an increasingly serious world-wide problem. Challenging the international community. The development of control method will require innovative thinking and creative research. The rapid growth rate, spread, and adaptability from aquatic to xerophytic habitats indicate this plant may potentially become another ecological disaster in India like water hyacinth and *Salvinia* spp.

Methods: Sterile bag samplers were used to collect the water from various sampling site and were processed after collection. Water sample was aseptically passed through 0.22 μ M pore size filters and placed on nutrient Agar plates and incubated. After incubation the isolated bacterial colonies were picked with sterile toothpicks and stabbed into nutrient agar contained in screw capped vials for further process. Sediment samples were collected in polypropylene tube with a hole drilled in the bottom and serially diluted samples were spread on the nutrient agar plates. Then the plates were incubated for 24 hrs. After incubation the isolated bacterial colonies were picked with sterile toothpicks and stabbed into nutrient agar contained in screw capped vials for further process.

Results: The predominant microbial load was isolated from the samples and they were identified as *Pseudomonas* sp. and *Bacillus* sp. By biochemical characterization and selective media. Bacteria, Actinomycetes and fungal growth in aquatic biocompost were gradually increased. The organic content of biocompost also increased. The pH of *Ipomea carnea* compost was 7.61. Highest number of thermophilic bacteria (43×10^6) was observed at 50°C Biocompost of 30th day, 26×10^6 was observed at 60 °C. Thermophilic fungal growth was not observed in other compost

Conclusion: Composting is one of the most promising ways to recycle the wastes generated from power plants, as the process reduces the volume and stabilizes the waste. The high organic matter content in the compost product also preserves soil fertility. A large variety of thermophilic micro-organisms have been reported in composting and other self-heating organic materials. Such information is of particular interest because these bacteria may be the major active organisms in the thermophilic stages of composting.

Keywords: *Ipomea carnea*, Vermicompost, biodegradation, thermophilic

Introduction

Ipomea carnea (Besharam) which grows wild in India has been identified as a useful material for several applications including housing. Problems associated with this material for housing are listed and a study of the structure, properties, mechanical and thermal behavior has been conducted. *Ipomea carnea* is a lingo-cellulosic plant material with a measured tensile strength of the order of 15-25MN/m² and high flexure strength. (Zink and Allen, 1998). The habit of the plant is vine like but stems can grow up wards to a height of 5-6m on land, while the aquatic plants are shorter, growing not only near the margins in shallow water but also as rooted emergent plants 1 to 2 m above the water. The branches are found mostly at the base of the stem, which is short and stout, but firmly rooted in the soil. The powder of *Ipomea carnea* is stable up to nearly 200°C, above which it shows signs of degradation, *Ipomea carnea* chips, and its powder mix and bond reactively with polyester resin, cement and mud, coating of creosote, polyester, cashew-nut shell liquid and copper were successfully applied on the surface of *Ipomea carnea*. The tensile strength of polyester containing 3.68% vol. *Ipomea carnea* powder was of the order of 22 MN/m². The bond strength of *Ipomea carnea* with mud plaster +2.5% cement was good. The ultimate load of bearing capacity of 2.5% cement stabilised mud plastered panel having 14% vol. *Ipomea carnea* gave a factor of safety of 3 for normal wind loading indicating that it is a promising material for housing.

Ipomoea carnea woody stems were pyrolyzed in a laboratory-scale reactor in the temperatures ranging from 350°to600°c and at constant heating rate of 5°c/min. yield, density, ash content, volatile matter, fixed carbon content and calorific value of the charcoal samples produced were evaluated (Parr and Hornick, 1992).

Efforts were also made to control; different aquatic plants biologically using different specific bio-agents, like weevils (*Neochetina elehhorniae* and *Neochetina crassipes*) herbivores fish, for example grass carp (*Ctenopharyngodon idella*) for hydrilla (*Hydrilla verticillata*) and duck weed (*Lemna minor*), fresh water snail (marine cornuaries) and tropical snail (*Pila globosa*) for water fern (*Salvinia molest*) (Gupta, 1987). Majid (1986) clearly mentioned that complete eradication of aquatic weeds was not possible by chemical, mechanical or biological means (Parr and Hornick, 1992). Vermicompost are finely-divided mature peat-like materials with a high porosity, aeration, drainage and water holding capacity and microbial activity which are stabilized by interactions between earthworms and microorganisms in a non-thermophilic process (Edward and Burrows, 1988). Vermicompost contains most nutrients in the plant available from such as nitrates, phosphates and exchangeable calcium and soluble potassium. Vermicompost have large particulate surface areas that provide many micro sites for microbial activity and for the strong retention of nutrients (Domini et.al, 2000). Vermicompost are rich in microbial populations and diversity, particularly fungi, bacteria and actinomycetes (Tomati et al., 1987). Vermicomposts consistently promote biological activity which can cause plants to germinate flower and grow and yield better than in commercial container media, independent of nutrient availability (Atiyeh et al., 2000). Vermicompost contains plant growth regulators and other plant growth influencing materials produced by microorganisms. Krishnamurthy and Vajrabhiah (1986) reported the production of cytokinins and auxins in organic wastes that were processed by earthworms. (Dees and Ghiorse, 2001).

The earthworms have beneficial physical, chemical and biological effects on soil and many researchers have documented that these effects can increase the plant growth and crop yield (Edwards and Bohlen 1996). The chemical fertilizer is substituted by compost, the well –decomposed organic manure prepared from crop residues, weeds, lawn mowing, tree leaves, kitchen refuges, animal excreta and city garbage's (Sannigrahi and Chakraborty, 2000). Recently, the use of natural materials such as manure is used as a substitute of the chemical fertilizer. Remediation of the soil with organic manure improves their physical, chemical and biological properties of the status of essential nutrients and soil fertility (Khalil et al., 1999). In the vermicomposting processes were the organic waste are converted into valuable fertilizers, leucaena is a 'conflict tree' being widely promoted for tropical forage production and reforestation, at the same time, it is spreading naturally and widely reported as a weed. Vermicompost is homogenous, with desirable aesthetics, plant growth hormones and high levels of soil enzymes, while enhancing microbial populations and tending to hold more nutrients over longer periods without adverse impacts on the environment (Sandhu et.al).Organic matter plays a key role to achieve sustainability in agricultural production because it possesses many desirable properties such as high water holding capacity, cation exchange capacity (CEC) ability to sequester contaminants (both organic and inorganic) and biological characteristics of soil. The organic degradable refuse of plant and animal origin provides a good source of nutrients to improve soil productivity. (Dees and Ghiorse, 2001).

During the composting process the organic material is decomposed and progressively transformed into mineral material (Adholeya and Parkash, 2004). The compost process could be defined as a biotechnology where microorganism, worms and insects participate to produce an innocuous product, chemically stable and utilizable to improve soil fertility and crop production. Compost had shown to increase crop production at green houses and under field conditions (Krishnamurthy and Vajrabhiah, 1986)). The utilization of composts improved the porosity, aeration and water retention in the soil and the high nitrate content of composts produced an increased uptake of nitrogen by the plant tissues, resulting in increased plant growth (Atiyeh et al., 2001). Composting is a natural biological process in which soil-inhabiting organisms break down various organic materials, such as leaves, grass clippings and food wastes. When decomposition is complete, a dark, brown, powdery material called humus has been produced. As you can tell by its rich earthy aroma, the finished compost is full of nutrients essential for the healthy growth of plants and crops. Composting is a viable means of transforming various organic wastes into products that can be used safely and beneficially as biofertilizer and soil conditioners. A number of problems associated with the use of raw and unstable organic wastes as soil amendments can be resolved through composting, such as malodors, human pathogens and undesirable physical and chemical properties. During the composting process, organic wastes are decomposed. (Parr and Hornick, 1992)

Research methods

Isolation of biodegradation bacteria from water samples: Sterile bag samplers were used to collect the water from various sampling site. the samples were processed after collection. water sample was aseptically passed through 0.22 µm pore size filters (47 mm diameter, millipore corp) is filtered and 20 to 200 ml of water per filter

depending on bacterial population densities in the sample. The filters were placed on nutrient agar plates. Then the plates were incubated at 37°C for 24 to 36 hrs. The isolated bacterial colonies were picked with sterile toothpicks and stabbed into nutrient agar contained in screw capped vials for further process.

Isolation of biodegradation bacteria from water sediment samples: Sediment samples were collected in polypropylene tube with a hole drilled in the bottom. The sample was immediately transferred to a sterile glass jar. Serially diluted samples were spread on the nutrient agar plates. Then the plates were incubated for 24 hrs. The isolated bacterial colonies were picked with sterile toothpicks and stabbed into nutrient agar contained in screw capped vials for further process.

Biochemical characterization : Several tests were carried out for various test organism this are gram staining, motility test (hanging drop method), imvic test, indole test, methyl red (MR) test, voges-proskauer (VP) test , citrate utilization test and triple sugar iron (TSI) test

Preparation of biocompost & analysis: Biocompost was prepared by using aquatic weed *Ipomoea carnea* and the Produced compost was air dried, sieved uniformed and stored for use in the pot experiments. A separate sample was oven dried and analyzed for various physico chemico parameters and microbial (bacterial, Actinomycetes, Thermophiles bacteria and fungi) analysis was carried out.

Isolation and enumeration of bacteria from compost: Seven ependroff tubes was taken, 900µl of water was transferred into each tubes. 0.1g of compost was weighed and transferred into conical flask containing 900 µl of distilled water. It given the dilution 10⁻¹. The flask was shaken gently for 10 minutes. Similarly the serial dilution was carried out up to 10⁻⁷ dilution. 0.1 ml of suspension from 10⁻⁴ to 10⁻⁷ dilutions was transferred into nutrient agar plates and incubated at 37 °C for 24-48 hours.

Isolation of actinomycetes from compost: Starch agar medium was prepared and sterilized. The compost sample was collected, and dried at 50 °C for 15 minutes so that most of bacteria and fungi may be killed. Serial dilution was prepared (10⁻¹ to 10⁻³). Finally, 0.1ml of suspension 10⁻¹ and 10⁻³ dilution was transferred to starch agar medium. The plates were incubated at 30 °C-35 °C for 7-14.

Isolation and enumeration of fungi from compost: Seven Ependroff tubes were taken, 900 µl of distilled water was transferred to each tube. Small amount of compost was collected. 0.1g of compost was weighed and transferred into conical flask containing 900 µl of distilled water. The flask was shaken gently for 10 minutes. 0.1ml of suspension from 10⁻¹ dilution was transferred into first ependroff tube containing 900 µl of sterilized water to get the dilution 10⁻². The suspension was mixed gently. Similarly 0.1 ml of suspension from 10⁻² dilution was transferred into second ependroff tube containing 900 µl of sterilized water to get final dilution 10⁻³. The suspension was mixed gently. Aseptically, 0.1ml suspension from 10⁻² and 10⁻³ dilutions was transferred into sabourauds dextrose agar plates (supplemented with vancomycin at 30mg/l). Gently the plates were rotated by using L-rod to spread the suspension on medium. The plates were incubated at 51° for 4-5 days. After incubation bacteria were counted.

Isolation and enumeration of thermophilic bacteria and fungi from compost: Seven ependroff tubes was taken, 900µl of water was transferred into each tubes. 0.1g of compost was weighed and transferred into conical flask containing 900 µl of distilled water. It given the dilution 10⁻¹. The flask was shaken gently for 10 minutes. Similarly the serial dilution was carried out up to 10⁻⁷ dilution. 0.1 ml of suspension from 10⁻⁴ to 10⁻⁷ dilutions was transferred into nutrient agar plates for Thermophiles bacteria. 0.1 ml of suspension from 10⁻² to 10⁻⁴ dilutions was transferred into SD agar plates for Thermophiles fungi. The suspension was spread uniformly on the medium by using sterile L-rod. The plates were incubated at 50°C and 60°C for 24-48 hours for bacteria and 5 days incubation for fungi. CFUs of bacteria and fungi were counted.

Analysis of Results

Water and sediment samples were collected from the Kavinadu big tank located in Pudukkottai town. The predominant microbial load was isolated from the samples and they were identified as *Pseudomonas* sp. and *Bacillus* sp. By biochemical characterization and selective media. The isolates were stored for further processing of biodegradation (Table 1) .

Aquatic weed (*Ipomoea carnea*) samples were chopped and mixed with cow dung and *Bacillus* sp. and *Pseudomonas* sp. for microbial degradation (Plate 1). Organic content and microbial load were analyzed periodically. Bacteria, Actinomycetes and fungal growth in aquatic biocompost were gradually increased in the 10th, 20th and 30th days. Number of bacteria present in the aquatic biocompost was too higher (32x10⁷) than Actinomycetes (24x10⁴) and fungi (53x10⁴) (table 2)

The organic content of biocompost also increased (Table 3)

Physicochemical properties of *Ipomoea carnea* compost: The pH of *Ipomoea carnea* compost was 7.61. The percentage of EC, Nitrogen, Phosphorus, Potassium and mg of Iron, Manganese. Zinc, Copper per kg of *Ipomoea carnea* compost (Table 4). Highest number of thermophilic bacteria (43x10⁶) was observed at 50°C

Biocompost of 30th day, 26x10⁶ was observed at 60 °C . 23x10⁴ thermophilic fungi 32x10⁴ were observed at 50 °C and 60 °C respectively. Thermophilic fungal growth was not observed in other compost (Table 5). Ration of different (Conventional compost, Vermicompost, Azospirillum and Phosphobacterium) compost prepared by soil volume according to the treatment plan (Table 6).

Germination of different seeds in different days: In groundnut, Ipomoea compost mixture showed highest (83%) percentage of germination when compared to OS (49%) in 5th day. In 15th day maximum percentage (100%) observed in Bio+Azo+Pho compost (Fig 1).

In cowpea 5th day, 70% of germination was in all the compost mixture, 50% was observed in OS. In 15th day, 90% of germination observed in Bio+Azo+Pho (Fig 2)

Number of leaves developed in different plants in different days: In groundnut, 14.5 number of leaves present in 15th day of growth in the compost of Azo-Pho mixture. In biocompost, more than 9 leaves observed in 10th day. In cowpea, Five number of leaves observed in all compost combination at 15th day but 5th day 2 number of leaves observed.

Total plant height of different seeds in different days: In cowpea, 34 cm plant height was observed in BC1 than OS (26.2 cm). Slight variation was observed in OS and CC1, maximum 0.94g dry weight observed in CC1 (Fig 4).

Maximum 19.4 cm plant height observed in BC1 in ground nut than OS (18.4cm) at 15th day (Table 8b, Plate 4, Fig 3). No major variation was observed in 10th and 15th day. In ground nut, maximum 2g of fresh plant weight observed in BC in 15th day. 1.4 g of dry weight observed in same sample (Fig 5).

Discussion

At present composting is one of the best opportunities for managing organic wastes. It is an important option for both sustainable waste management and sustainable agriculture. Compost has numerous benefits and nearly all crop farmers like to apply good quality compost to their fields. Its benefits include improving soil properties, maintaining stable soil moisture content, preventing soil-borne diseases and acting as buffer to facilitate gradual release of plant nutrients. Organic matter is generally derived from the remains of plant roots, leaves, stubble and animal manure and from soil bacteria, fungi and earthworms as well. It acts as a sole source of plant nutrients, providing energy for micro organisms and seat for chemical changes and biochemical reactions, organic matter influences a number of physical characteristics, as it helps to create friable consistency, improves soil structure and increase water holding capacity.

The increase of microbial population may be caused by congenial condition for the growth of microbes within the worm digestive tract and by the ingestion of nutrient rich organic wastes which provide energy and also act as a substrate for the growth of micro organisms are reported by Tiwari et al (1989). Fungi and actinomycetes in general are of importance in composting, especially during the later curing stage, but it is likely that bacterium predominant during the earlier thermophilic stage. The reasons for this predominance are not entirely clear, but undoubtedly involve interactions between temperature, pH, moisture content oxygen concentration, available carbon sources, and inherent maximum rates of proliferation.

Application of compost and bio-fertilizers to improve plant growth, soil structure, fertility, and consequently development. Nitrogenase enzyme activity in the soil of Marjoram plants increased as a result of treatments. With aqueous extract of compost at 15 and 30 % nitrogen fixers strains, *B. circulans* alone or in combination compared with control (NPK fertilizers) during three cuttings highest nitrogenase enzyme activity was obtained with aqueous extract of compost at 15% inoculation with both nitrogen fixers and *B. circulans* recording 9.0, 10.5 and 12.27 $\mu\text{mole C}_2\text{H}_4\text{ G}^{-1}\text{ dry root h}^{-1}$ compared with 0.44, 1.17, 1.41 $\mu\text{mole C}_2\text{H}_4\text{ G}^{-1}\text{ dry root h}^{-1}$ for control plants at first, second and third cuts, respectively. Similar, in maize and sorghum grown in the field, acetylene reduction was higher during the reproductive stage. This can be explained by an increase in the number of mature roots associated with *Azospirillum* at the beginning of reproductive growth. at this stage, these was an increase in uptake of available nitrogen by the plant that, together with leaching and denitrification processes, may deplete the soil of combined nitrogen, thus derepressing the nitrogenase of *Azospirillum* (Neyra and Dobereiner, 1977).

Authors' contributions

Conceived and designed the experiment; Jerida M. Performed the experiment, contributed reagents/ materials/ analysis tools; EO, SJB, SKT, WO

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Competing interests: The authors declare that they have no competing interests

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Tables and figures

Table 1: biochemical characterization

S.No.	Biochemical tests	Bacillus sp.	Pseudomonas sp.
1.	Gram Staining	-	-
2.	Catalase	-	+
3.	Oxidase	+	+
4.	Indole	-	-
5.	Methyl Red	-	-
6.	Voges Proskauer	±	-
7.	Citrate Utilization	-	+
8.	Urease\	-	-

+ → Positive Result

- → Negative Result

Table 2: total microbial counts in composting period

S.No	No. Of Days	Bacterial count Cfū/gm	Actinomycetes count Cfū/gm	Fungal count Cfū/gm
1	10 th	14 X 10 ⁷	7×10 ³	34×10 ⁴
2	20 th	26 X 10 ⁷	17×10 ⁴	46×10 ⁴
3	30 th	32 X 10 ⁷	24×10 ⁴	53×10 ⁴

Table 3: total organic content in compost

Days	A	B	C	Loss of ignition
20 th	40.42	40.92	40.59	66%
30 th	40.42	40.92	40.53	78%

Loss of ignition: $(B-C) / (B-A) \times 100$

Table 4: physico chemical properties of *ipomoea carnea* compost

S.No	Parameters	Results
1	pH	7.61
2	EC %	0.03
3	Nitrogen %	3.84
4	Phosphorus %	8.16
5	Potassium %	6.10
6	Iron as Fe mg/kg	8.24
7	Manganese as Mn mg/kg	7.61
8	Zinc as Zn mg/kg	2.14
9	Copper as Cu mg/kg	2.42

Table 5: thermophiles bacterial count in composting period

S.No	No. of Days	Temperature					
		50°C			60°C		
		Bc	Vc	Cc	Bc	Vc	Cc
1	10 th	26×10 ⁶	4×10 ⁴	9×10 ⁴	16×10 ⁶	9×10 ⁴	2×10 ⁴
2	20 th	32×10 ⁶	8×10 ⁴	16×10 ⁴	21×10 ⁶	14×10 ⁴	6×10 ⁴
3	30 th	43×10 ⁶	14×10 ⁴	22×10 ⁴	26×10 ⁶	12×10 ⁴	7×10 ⁴

- Bc→ Biocompost (Ipomoea)
- Vc→ Vermicompost
- Cc→ Conventional compost

Table 6: thermophiles fungal count in composting period

S.No	No. of Days	Temperature					
		50°C			60°C		
		Bc	Vc	Cc	Bc	Vc	Cc
1	10th	16×10 ⁴	Nil	Nil	24×10 ⁴	Nil	Nil
2	20th	22×10 ⁴	Nil	Nil	18×10 ⁴	Nil	Nil
3	30 th	32×10 ⁴	Nil	Nil	23×10 ⁴	Nil	Nil

- Bc→ Biocompost (Ipomoea)
- Vc→ Vermicompost
- Cc→ Conventional compost

Fig 1: Germination of ground nuts seed

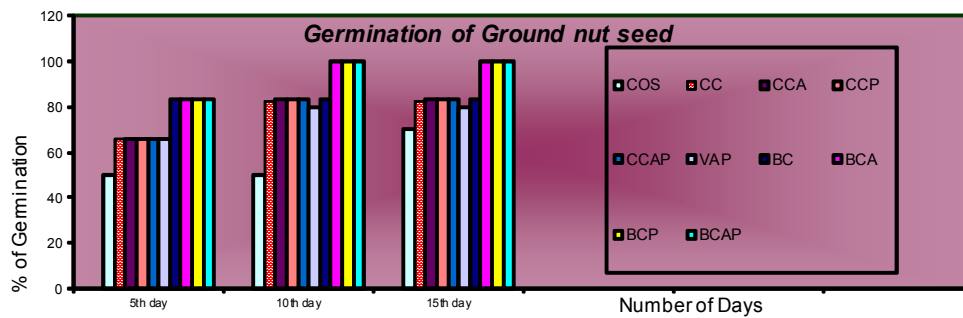


Fig 2: Germination of cowpea seed

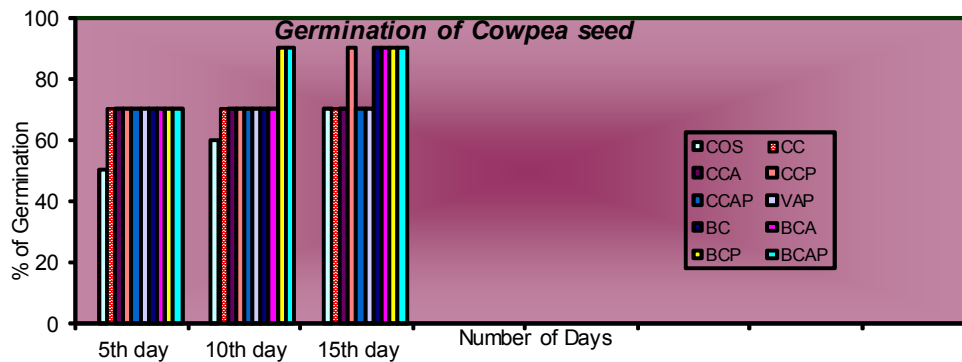


Fig 3: Number of leaves developed in cowpea

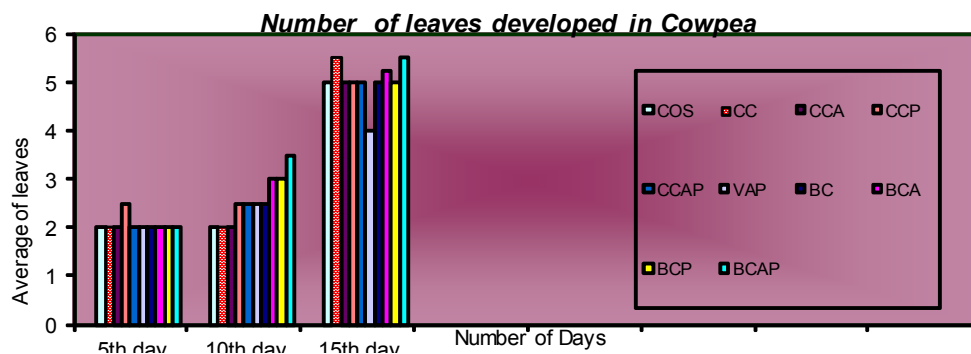


Figure 4: Plant height of cow pea

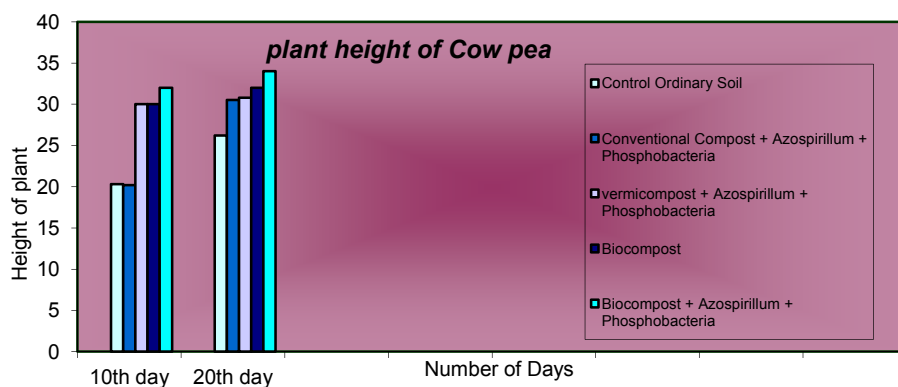
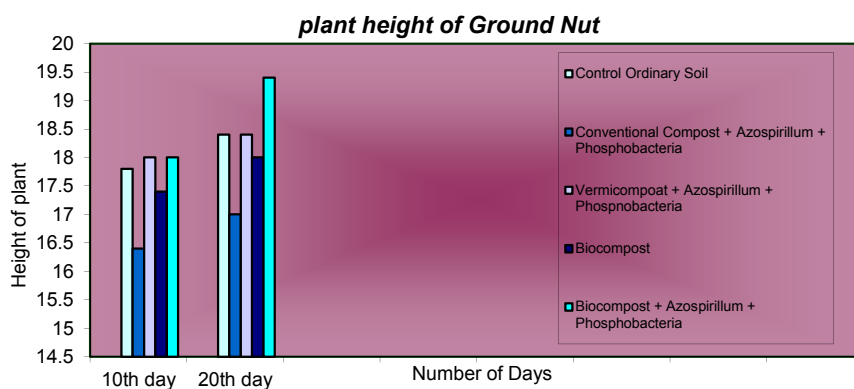


Figure 5: Plant height of ground nut



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