UTILIZATION OF ALTERNATIVE HERBS AND MATERIALS FOR THE PRODUCTION OF BIODYNAMIC MANURES AND THEIR EFFICACY ON GROWTH OF SELECTED PLANTS

THESIS

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By

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DECLARATION

I hereby declare that the thesis entitled "Utilization of alternative herbs and materials for the production of biodynamic manures and their efficacy on growth of selected plants" submitted by me for the degree of Doctor of Philosophy is the record of work carried out by me during the period from January 2004 to December 2010 under the guidance of Dr. K. Perumal and has not formed the basis for the award of any degree, diploma, associateship, fellowship, titles in University of Madras or any other University or other similar institution of higher learning.

Signature of the Candidate

(J. Arunkumar)



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1. INTRODUCTION

The agriculture technologies in green revolution paved way for utilization of synthetic fertilizers, herbicides, pesticides, growth regulators and livestock feed additives (Baruah, 2000; Zaller and Kopke, 2004). The over exploitation of green revolution led to significant externalities, affecting natural resources and human health as well as agriculture itself. Increasing consciousness about conservation of environment as well as of health hazards caused by agrochemicals has brought a major shift in consumer preference towards food quality and can be achieved through natural farming or organic agriculture.

Largely excludes the use of synthetic compounded fertilizers, pesticides, growth regulators and livestock feed additives. Organic farming is an age old traditional plant cultivation practice evolved by countless villages and farming communities' debated before more than 10,000 years and recently gaining popularity in developed and developing countries. Our ancient farmers cultivated crops utilizing natural resources such as agricultural waste, animal waste (dung and urine) and green manure available in and around their farms. Literatures like Rig Veda, Ramayana, Mahabharata, Kautilya's Arthasashthra and Holy Quran have described briefly on several organic agricultural inputs for gainful production of crops (Bhattacharyya and Chakraborty, 2005). The organic farming in real sense envisages a comprehensive management approach to improve the health of underlying productivity of the soil.

Agricultural land (67 million hectares) managed under organic practices is rapidly increasing in more than 130 countries. The total production 9,76, 646 Metric

tons (MT) in 4.5 lakh hectares was produced organically in India during 2007-08 (IFOAM, 2008 and 2009).

The foremost challenges of organic farming is labour intensive, requires immense patience and considerable skills for the timely weed control and pest control and do not have off-shelf formulae for fixing up the various farming problems they encounter. Intensive farming to meet food demand of huge population exhausted native soil fertility. Organic farming is more dependent on the (local) environment (soil and weather) and has less powerful tools for instant growth regulation than non-organic farming methods (Vereijken *et al.*, 1997).

Large-scale conversion to organic agriculture would result yield reductions of organic systems relative to conventional agriculture average 10-15 %. A large gap exists between the available potential and utilization of organic wastes. The challenges faced in organic agriculture system for better crop yield and sustainable soil fertility can be achieved by implementing biodynamic agriculture methodologies during crop production.

Biodynamic agriculture (BD) is an unique organic farming system that utilizes specific fermented herbal preparations such as BD 502, BD 503, BD 504, BD 505, BD 506 and BD 507 as compost additives and field sprays such as cow horn manure, horn silica and cow pat pit manures (Boggs *et al.*, 2000). The biodynamic farming is considered as a living energizing biological system or organism that sustains better soil quality (Reeve, 2003 and Reganold *et al.*, 1993). A significant difference in microbial biomass nitrogen and organic carbon content were recorded in BD system than the

conventional farming systems (Fließbach et al., 2007; Perumal and Vatsala, 2002; Droogers and Bouma, 1996). Microbial biomass, community structures and activities, and anthropogenic influences on agricultural soils were recorded in the soil amended with biodynamic manures (Hartmann et al., 2006; Perumal et al., 2001). The biodynamic preparations helped to manure decomposition, increasing soil biology, hormone like activity, crop yield and quality of products like nutritive values (Perumal et al., 2003; Perumal and Stalin, 2006). Recently, there has been an increasing interest in biodynamic farming practices due to mitigating some detrimental effects of chemical-dependent conventional agriculture. However, limited investigation was conducted characterizing the biodynamic manures and their influence on soil quality for better crop growth (Reganold, 1995). Moreover, the herbs such as yarrow (Achillea millefolium), chamomile (Chamomilla officinalis), stinging nettle (Urtica dioca), oak bark (Quercus robur), dandelion (Taraxacum officinale) and valerian (Valeriana officinalis) are grown only in tropical regions and processed for the formulating the herbal preparations namely BD 502 - BD 507. The availability of these herbs is a limiting factor for mass scale production.

Hence, the present study dealt on the following objectives.

- To collect commercially available organic and biodynamic manures and composts from various regions in India.
- To evaluate physico-chemical, biochemical and microbiological properties of commercially available organic and biodynamic manures.

- To prepare biodynamic compost, and BD 500 and periodically evaluate manure maturation, physical, chemical, biochemical and microbiological properties during biotransformation (material into manure.).
- > To identify locally available and suitable alternative herbs and materials for biodynamic manure preparation.
- To study the efficacy of biodynamic manures on the growth of selected crops such as tomato (*Lycopersicon esculentus*), moringa leaves (*Moringa oleifera*) and ground nut (*Arachis hypogea*).

Review of literature

2. REVIEW OF LITERATURE

During the last two decades, there has been a significant sensitization of the global community on environmental conservation, health jeopardy impair the ecological balance associated with agrochemicals. Consumers' preferences to safe and hazard-free food are the major factors that lead to the growing interest in alternate forms of agriculture in the world. Now it has environmental sustainability at its core in addition to the concerns for healthy soil and healthy food. Organic agriculture is one among the broad spectrum of production methods that are supportive of the environment. The demand for organic food is steadily increasing both in the developed and developing countries with an annual average growth rate of 20–25%. Organic agriculture, without doubt, is one of the fastest growing sectors of agricultural production.

2.1. Concept of Organic Farming

Intensive agriculture with the use of agrochemical in large amount has no doubt resulted in manifold increase in the productivity of farm commodities but the adverse effects of these chemicals are clearly visible on the soil structure, soil micro flora and quality of water, food and fodder. The World Health Organization (WHO) has estimated the globally at least 3 million people are poisoned by pesticides every year out of whom 20,000 die. Thus organic farming is certainly an answer for making available safe food and clean environment.

Organic farming means farming in spirit of organic relationship. It is the agriculture system which aims at cultivation of the land is a way so that the soil is kept dynamic with living activities and in good health at the same time keeping the

environment clean, maintaining the ecological balance and providing stability to the production level without polluting soil, water and air. This method of farming helps in keeping agriculture production at higher level and makes it sustainable and also reduces the production cost.

The roots of organic farming in the English-speaking world can be found in India, where two scientists had been working an agricultural scientist, Albert Howard (1873–1947), and a doctor, Robert McCarrison (1878–1960). The importance of soil microorganisms are realized in the various steps during the formation of soil humus, from the decomposition of fresh plant and animal remains and their organic constituents (sugars, starches, pectins, celluloses, proteins, amino acids, lignins, etc.) to the production of available N for crop uptake by the slow oxidation of humus. Importance of humus in soil aggregation and aeration and discusses how the loss of humus through chemical farming influences soil erosion, diseases and pests of crops, livestock and humans, and the production, taste, quality and keeping properties of agricultural products.

2.2. Current status of organic agriculture

Organic agriculture is developing rapidly and growing in many countries around 141 countries of the world. Almost half of the world's organic producers are in Africa and the regions of Oceania, Europe and Latin America are leading to maintain the largest areas of land by organic methods. Large numbers of organic producers are in Uganda, India and Ethiopia. In Asia, nearly 4.1 million hectares of land (230'000 producers) are managing and using for organic produce and it constitutes nine percent

of the world's organic agricultural land. In India, 1 million hectares are maintained and produce crop by organically and wild collection areas play a major role in India and China (IFOAM, 2008 and 2009). Changing public perceptions about environmental issues associated with current manure management practices have forced farmers to examine alternative options for significantly reduce environmental problems associated with manure management process (Johnson *et al.*, 1998; Jongbloed and Lenis, 1998; Carr *et al.*, 1995).

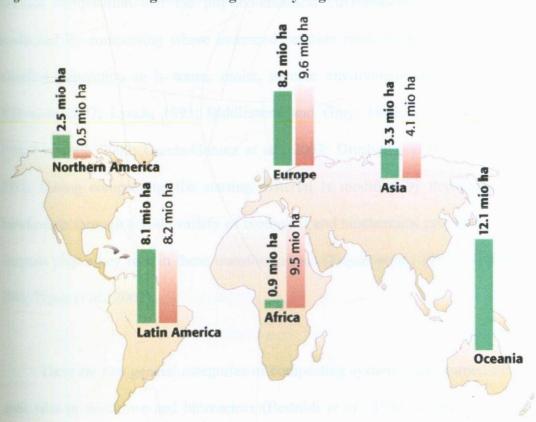


Fig 1.1: Land under organic management by region 2009

Source: IFOAM annual report

2.3. Composting process

Composting can be defined as a process of controlled biological decomposition of biodegradable materials under managed conditions that are predominantly aerobic and achieve effective, inexpensive uniform and stable compost that confers beneficial effects when added to soil and used in conjunction with plants (Litterick et al 2004; Tiquia and Tam, 2000b; Tiquia and Tam, 1998; Tiquia et al., 1998; Tiquia et al., 2002). Organic matter spontaneously undergoes microbial transformation, depending on its chemical composition and the physico-chemical environment and this process is accelerated by composting where increased reaction rates are promoted by a mixed microbial population in a warm, moist, aerobic environment (Singh et al., 2006; Wilkinson, 2007; Lynch, 1993; Biddlestone and Gray, 1985; Schaub and Leonard, 1996; Maynard, 2000; Garcia-Gomez et al., 2003; Dresbøll and Thorup-Kristensen, 2005). During composting, the starting material is modified by decomposition and humification through a wide variety of biological and biochemical processes especially Enzymes play a key role in these transformations (Tiquia et al., 1997c; Tiquia et al., 2001; Tiquia et al., 2002).

There are two general categories of composting systems, open turned/aerated or static piles or windrows and bioreactors (Bertoldi *et al.*, 1985; Schaub and Leonard, 1996; Epstein, 1997). In passive or static systems, the material is undisturbed with aeration by natural convective airflow or assisted by perforated piping (Lynch and Wood, 1985; Epstein, 1997). Anaerobic regions and low activity regions may develop

in these systems resulting in non-uniform processes (Lynch and Cherry, 1996; Maynard, 2000). In aerated piles, air blowers and perforated pipes supply air to the material. Closed mechanical reactors or bioreactors for composting are high cost systems where mechanically turning and mixing may also incorporate air with automated control of moisture, aeration and temperature. These controlled bioreactors can reduce composting times from 3–5 weeks to 10–14 days (Singh *et al.*, 2006).

The composting process in the production of manures may significantly reduce the environmental problems associated with the management of manures by transforming them into a safer and more stabilized material for application to soil. The stability and maturity of the compost are essential for their successful application in high value horticultural crops (Tiquia and Tam, 2002; Yamada and Kawase, 2006; Brandon *et al.*, 2008). Stabilization of manure can accelerate Na⁺ leaching, decrease the exchangeable sodium, electrical conductivity and increase water infiltration, waterholding capacity, aggregate stability, soil microbial biomass and some soil enzymatic urease, alkaline phosphatase, β-glucosidase, arylsulfatase and dehydrogenase) activities (Tejada *et al.*, 2006; Lazcano *et al.*, 2008).

Vermicomposting is a non-thermophilic biodegradation of organic material through interaction between earthworms and microorganisms resulting in production of vermicompost. Earthworms accelerate the mineralization rate and convert the manures into casts with higher nutritional value and degree of humification than traditional method of composting (Albanell *et al.*, 1988; Jeeji Bai and Suriyakumar, 1993; Ismail, 1997; Suriyakumar, 1999). Vermicompost fertilization resulted in highest microbial

biomass, available phosphorus, nitrogen and achieving significantly better plants height, root length, greater biomass, quicker onset of flowering and enhancement of fruit yield (Gajalakshmi and Abbasi, 2004; Paradelo *et al.*, 2007; Gaind and Nain, 2007).

2.3.1. Maturity indices for compost

Composting seems to be one of the most interesting process to biotransformation of wastes by means of this process allows the elimination of potentially dangerous residues and the final product obtained, improves soil quality such as bulk density or total porosity, chemical characteristics as cation exchange capacity, atmosphere or soil pH and biotic factors, mainly microbial growth. Nevertheless, these positive effects take place only when the compost applied shows an adequate state of maturity (Garcia, et al., 2006). Compost maturity is beginning to be more recognized as a significant parameter to evaluate compost. The reason is that immature and poorly stabilized composts pose known problems during storage, marketing and agronomical use (Gaind et al., 2005).

The composting process offers the potential to significantly reduce environmental problems associated with manure management (Carr *et al.*, 1995). Unfortunately, the cost of composting relative to utilization of raw manures can be considerably higher (Rynk, 1992). Therefore, composts of high quality must be produced consistently to offset these production costs. Compost stability is an important aspect of compost quality and it can be assessed with respirometry (Iannotti *et al.*, 1994; Scaglia *et al.*, 2000). It relates to the degree to which the organic matter has been stabilized during the composting process (Chen, 2003).

Many tests have been considered as maturity indices for compost, and most of them focus on the chemical and physical properties of compost. The most common parameters include compost odor; texture; temperature, pH and cation exchange capacity, microbial respiration, dissolved organic C, C/N ratio, gas production, level of ammonium, nitrate, and immobilized nitrogen, humification index and plant growth bioassay (Jimenez and Garcia, 1992; Iannotti *et al.*, 1994; Tiquia *et al.*, 1996a; Sullivan and Miller, 2001; Lopez *et al.*, 2002; Cooperband *et al.*, 2003; Suzuki *et al.*, 2004; Tiquia *et al.*, 2005). Although many methods have been proposed, none of these have been pursued far enough to allow full appreciation of their potential value (He *et al.*, 1995).

Composting is a process resulting in biosynthesis of humic acids. There have been and will continue to be efforts to develop and refine methods which evaluate stability and maturity, but no one universally accepted and applied method exists (Brinton, 2000; Tiquia et al., 1996b). At the same time, a more biological approach to compost quality has emerged even more recently, with a focus on measuring stability and phytotoxicity. Raw materials used as bedding or bulking agents before composting typically inhibit plant growth and stimulate diseases whereas stabilized mature composts tend to stimulate growth and provide disease control (Hoitink and Boehm, 1999).

The activity of microorganisms during composting depends on the availability of nutrients to perform the decomposition of organic wastes. The ratio of carbon to nitrogen (C/N ratio) is an one of important aspects for total nutrient balance and

assessing the suitability of waste as a substrate for composting (Tiquia et al., 1997a; Abu Qdais and Hamoda, 2004).

Chemical analysis of compost which produced poultry litter with yard trimmings showed loss of C. This result was due to mineralization of organic matter during composting, as shown by the decline in water extractable C (Tiquia *et al.*, 1997b; Tiquia *et al.*, 2001).

Firmicutes (especially Bacillus), Actinobacteria and Proteobacteria were reported to play important roles, especially in the degradation of macromolecules in waste material, such as hemicellulose, lignin and cellulose, at the later stages of composting (Priest 1977; Beffa et al., 1996; Apun et al., 2000; Haruta et al., 2002; Ryckeboer et al., 2003; Steger et al., 2007). The process reportedly releases inorganic nutrients and also plays an important role in humus formation (Epstein, 1997; Liew, 2009).

In order to classify compost as non-phytotoxic it must have a germination index higher than 60%. Both stability and maturity are distinct properties in evaluation of compost quality along with several other physico-chemical parameters. Humus content, microbial biomass, phytotoxicity and plant growth study are the parameters of equal importance (Gaind *et al.*, 2005; Hoitink and Boehm, 1999).

Heat output as a result of biological activity in composts can also be monitored on farms if several precautions are taken (Weppen, 2002). Temperature has been widely recognized as the single most important parameter in the composting process, which has the greatest effect on both microbial biomass and microbial activity during composting

of municipal sludge, with an optimal temperature being 30-50°C (McKinley and Vestal, 1985; Tiquia et al., 2001).

Compost maturity, on the other hand, which implies non limited plant growth in compost-amended substrates immediately upon their utilization (Zucconi *et al.*, 1981), still is best assessed with plant growth bioassays. This applies even though numerous chemical and biological tests have been proposed to characterize this aspect of compost quality (Hsu and Lo, 1999; Chen, 2003).

The type of animal bedding used on farms (sawdust, wood, straw, etc.) may affect compost quality through its effect on plant growth, presumably through differential effects on N availability (Fauci *et al.*, 1999; Gagnon and Simard, 1999). Similar effects have been described for composted sewage sludge (Bernal *et al.*, 1998). Raw materials used as bedding or bulking agents before composting typically inhibit plant growth and stimulate diseases whereas stabilized mature composts tend to stimulate growth and provide disease control (Hoitink and Boehm, 1999). For these reasons, maturity is one of the most important aspects of compost quality, particularly for composts used in high-value horticultural applications (Gouin, 1998; De Ceuster and Hoitink, 1999).

Total water soluble organic C (Eggen and Vethe, 2001) and several other stability indices have been proposed more recently (Chefetz *et al.*, 1998; Cooperband and Middleton, 1996; Dinel *et al.*, 1996; Forster *et al.*, 1993; Ouatmane *et al.*, 2000). To develop a better understanding of how organic matter transformations during

composting affect plant growth, various types of spectroscopic analyses, including NMR and IR spectroscopy have been utilized (Chen, 2003).

Total amount of agricultural animal manure were produced in the world between 10^{10} and 10^{11} tons annually (Fayer and Trout, 2005). Manure abundance brings a mixed blessing for humanity, being an important source of plant nutrients and energy if used properly, otherwise causing substantial pollution of water or produce if managed improperly. Pathogenic microorganisms that are found in manure can cause serious illness and death in humans (Cotruvo *et al.*, 2004; Pachepsky *et al.*, 2006).

Composting is a promising technique for management of solid animal waste. This indicates that composting, if carried out effectively, can provide a suitable system for pathogen inactivation (Wilkinson, 2007). Under controlled conditions of aeration, moisture, particle size and carbon–nitrogen ratio of the combustible material, composting temperatures of 55–65°C can be reached which would be sufficient to inactivate pathogen (Duffy, 2003). General disease suppression can be attributed to the general microbial activity of the compost microflora, which in turn is related to the decomposition level of organic matter (Veeken *et al.*, 2005). Various aspects of disease suppressiveness of composts and compost-amended mixes have been documented extensively (Hoitink *et al.*, 2001; Boulter *et al.*, 2000). Composted biowastes has the capability of disease suppressiveness for *Pythium ultimum*, *Phytophthora cinnamomi and Rhizoctonia solan* (Schuler *et al.*, 1989; Tuitert *et al.*, 1998; Erhart *et al.*, 1999; Lievens *et al.*, 2001; Blok *et al.* 2002; Hoitink *et al.*, 2001; Stone *et al.* 2001).

2.3.2. Effect of co composting materials in compost

Recently, coal fly ash and bottom ash Volcanic ash, Coal clinker, Charcoal ash have been used as co-composting materials with paper pulp, Chicken feces, litter manure, Composted material from food garbage, sawdust pine bark, and sewage sludge (Suzuki *et al.*, 2004).

2.4. Organic manure (animal manure)

Cattle manure is a low cost, renewable organic resource available abundantly and provides high contents of primary (Nitrogen, Phosphorus and Potassium), secondary (Ca, Mg and S) and micro-nutrients (Boron, chlorine, Copper, Iron, Manganese, Molybdenum and Zinc) for crop growth. Sheep manure was considered as more readily available nutrients (Nitrogen, Phosphorus, and potassium) than cow or horse manure (Paula, 1995; Agamuthu, 1994). The incorporation of sheep manure in soil during crop growth increases leaf nitrogen and potassium, plant height, number of branches, leaf area, number and weight of fruits of tomato (Ojeniyi *et al.*, 2007).

Animal production is an integral part of crop production and characterized by mixed farming. The practices in such crop animal systems are very complex (Carangal 1995). Large ruminants provide draft power and animal manure for crop production while crop residues and crop by-products are important sources of animal feeds (Paris and Sevilla, 1995). Small farmers recognize the value of buffalo manure as a cheap and natural fertilizer mainly for rice and vegetable crops (Sirivaidyapong *et al.*, 2004).

The treatment of organic wastes before applying them to soil can be aerobic (composting) or anaerobic (fermentation, biogas digestion). However, knowledge about

the stabilization efficiency of waste C is relevant from the viewpoint of C sequestration and ways of maintaining or increasing the humus content of soils and has been scarcely considered (Kirchmann and Bernal, 1997).

2.5. Biodynamic agriculture and manures

The most widely used composting method on farms is conventional composting where manure is deposited in windrows and allowed to pass a phase with increased temperatures for several days in order to sanitize and stabilize the material (Rynk, 1992). Biodynamic composting is a special form of composting where manure is additionally inoculated with pulverized and/or liquid compost preparations aiming to facilitate the decomposition of the material (Koepf *et al.*, 1980). Since biodynamic preparations are added to composting organic material in very low doses of a few grams per ton of compost material, the primary purpose of these preparations is not to add nutrients, but to stimulate the processes of nutrient and energy cycling, hasten decomposition and to improve soil and crop quality (Koepf 1993).

Counting of both the total culturable and antagonistic microorganisms was processed in six different composts, available commercially as biodynamic or BD preparations (different herbs as raw material). A total of 1,443 colonies were observed from the nine samples different composts, available commercially as biodynamic or BD preparations and 11–32% exhibited halos around them. Of these, 67 bacterial colonies showed the largest halo (>2 mm) and 17 colonies out of 67 were suppressed at least one of the four disease causing fungi (*Rhizoctonia bataticola, Sclerotium rolfsii, Fusarium oxysporum* and *Aspergillus flavus*). A high population of antagonists (>1,000 per g

compost) was indeed present in the composts used by organic farmers in Karnataka and Tamil Nadu, India, and may be the reason for the low incidence of diseases and insect pests (Rupela, 2003).

Ahrens (1984) found a significantly larger amount of carbon dioxide mineralized inoculating 1.5-3.0% (dry matter basis) concentrations of bio-dynamic compounds (BD Preparations). As a result of a larger C mineralization, lower C/N ratios and higher total N concentrations were determined in the straw amended with compost compounds after one year.

May cattle manure was used to prepare composting heaps with biodynamic preparation and total ash content increased the compost sample. In compost the ash composition analysed as HCl soluble and insoluble fraction. Whereas the soluble ash increased from May to October by 1.23 times (from 13% to 16% dry matter), the insoluble portion from 18.5% to 53.3%. The higher rise of the HCl-insoluble fraction gives a hint that soil material was mixed into the heaps during composting, as the insoluble fraction originates mainly in soil-borne silicates while the soluble fraction is derived from (plant and animal) organic matter. No such disproportionate changes of the soluble and insoluble ash fractions were observed in another composting study without turning under these conditions (Raupp, 2002).

Plant disease suppression has also been associated with the composition of the microbial community, particularly the populations and activity of antagonistic microorganisms, which naturally colonise compost during the curing phase of the composting process. More of isolated microorganism groups from composted

biodynamic manure exhibited antagonism towards R. solani and P. ulitimum than exhibited antagonism towards the pathogenic fungi Verticillium longisporum and Aphanomyces euteiches. (Arora et al., 2005).

The more frequent cultivation crops in the crop rotation of bio-dynamic farms resulted in denser earthworm populations, slightly better aggregate stability, and a higher soil density (Maidl *et al.*, 1988; Bauchhenss and Herr, 1986). 10-15% higher activity of several enzymes in bio-dynamically treated soils in the spring after fertilization compared with other agricultural soils (Beck, 1986).

The content of the heap such as green materials, dry materials is the major consideration for temperature while using straw consumes a lot more oxygen than green material. The hydrogen peroxide and molasses mixed in water to supply oxygen to bacteria and fungi an energy sources. The bacteria and fungi are very susceptible to 59° C and hold to break down of chemical residues, hormones and antibiotics for 2 to 3 days (Priestley 2003).

Decomposition rates were additionally affected by biodynamic preparations indicating that the qualitative property of biodynamic FYM also favours decomposer organisms in soils. Long-term studies on biodynamic preparations show significant alterations in the soil pH, basal respiration, metabolic quotient, Cmic/Corg, decomposition rate, microbial biomass, dehydrogenase and saccharase activity and composition of earthworm communities, Differences in compost qualities due to the biodynamic preparations stimulated earthworms living in the soil more than those feeding on organic material from the soil surface (Zaller, 2004).

Higher activities of dehydrogenase, protease and phosphatase in biodynamic system were indicating a higher overall microbial activities and capacity to cleave protein and organic phosphorus in soil. Mycorrhizae as member of the soil community ameliorate plant mineral nutrition and contribute to soil aggregation was 40% higher than conventional system. Activity and density of epigaeic arthropods like carabids, staphylinids and spiders (predators and sensitive indicator of soil fertility) in organic plots almost twice than conventional. The organic system shows an efficient resource utilization and enhanced floral and faunal diversity of both above-ground and below ground (Mader *et al.*, 2002).

The VAM colonisation level was significantly higher in the biodynamic soils and did not differ significantly within the three conventional or three biodynamic soils. The relationship between VAM colonisation and shoot P concentrations in conventional and biodynamic soil also suggests that the VAM fungi in conventional soils were not more tolerant of P. The soil biological community to contrasting farm management systems may depend on the types of processes affected. For instance, large additions of organic matter on biodynamic and organic farms have been found to alter the composition of the soil community, resulting in a greater ability to decompose cellulose in organic soil and an increase in organisms antagonistic to pathogens (Ryan and Ash, 1999).

Higher Temperature, Slightly greater dehydrogenase activity and slightly lower CO, respiration (higher ratio of dehydrogenase: CO, release) were revealed in BD compost piles than in control piles. Although all compost piles had moderately high

moisture contents, there was no difference in moisture between treatments. Nitrate content was on average 65% greater in the BD-treated compost and total N, total C, C: N ratio was not statistically different between treatments in the final compost samples. PLFA indications, BD composts did support a larger proportion of anaerobic metabolism. Biodynamically treated composts had lower proportions of 16:1 u) 7c, an indicator of aerobic bacteria, and a larger proportion of 17:1 G>6c, an indicator of sulfate-reducing bacteria (Boggs *et al.*, 2000).

Role of Humus in soil fertility:

Humus is key source of nutrients and also contributes to moisture retention and soil structure (Prescott *et al.*, 2000). Romell (1935) pointed out humus has regarded both as "the very essence of soil fertility and a necessary evil. The formation of different types of humus is a consequence of local ecological conditions (Bernier, 1968), particularly the climate, vegetation and parent material.

Two main types of humus such as mull and mor were distinguished by Muller 1948.

The mull humus has been defined as organic material has been transformed through soil, animal activity and mixed with mineral soil whereas Mor or raw humus forms are surface fresh litter has been transformed into relatively homogenous humus.

Moders are an intermediate humus forms between mulls and mors, but are distinctly different because they encompass characteristics of both main humus (Green et al., 1993).

Classification of humus forms (Green *et al.*, 1993) is useful in estimating the nutritional status and potential productivity of forest sites. Romell (1935) stated the real

index of fertility is not the amount of humus, but the type of humus formation.

Voetman, 1980 stated that it is not the rate but rather the type of decomposition that determines the amount and form of humus.

Melillo *et al.*, (1989) linked the decay process to a filter, through which litter of highly variable chemistry is transformed into a relatively homogenous material, called humus. Humus is made up of organic matter that has been transformed and stabilized by the activity of soil organisms and the amount of humus in the soil is determined by the organic matter added, balanced against the processes of decay that it break down (Merlin Dillon 2002). Humus is defined as a brown to black complex variable of carbon containing compounds not recognized under a light microscope as possessing cellular organization in the form of plant and animal bodies.

Activity of microorganisms in the soil

Survival of microorganism depends firstly on the quality of the inoculant's itself, i.e. purity, strain trueness, viable numbers, the degree of infectivity, and level of contaminants (*Abbott and Robson* 1982; *Kennedy et al.* 2004). Secondly, the establishment and proliferation of inoculants in the soil environment are determined by many edaphic and climatic factors, the presence of host organisms (for symbionts and endophytes) and, most importantly, by competitive interactions with other microorganisms and soil fauna (Stotzky 1997; Slattery *et al.*,2001; McInnes and Haq 2003).

Positive effects of inoculants on the soil microbial biomass may be short-lived (Kim et al., 1997b), and increases in biomass or activity can even be due to the

indigenous population feeding on the newly added microorganism (Bashan 1999). Provided soil conditions are favourable for rhizobia survival (Slattery *et al.* 2001), inoculation can increase microbial C and N in the rhizosphere compared with uninoculated soils (Beigh *et al.*, 1998; Moharram *et al.*, 1999).

Population changes can be limited to the season of inoculation if the newly added organism is not as well adapted to the soil conditions as the indigenous population (McInnes and Haq 2003). Inoculant application research is increasingly focusing on co-inoculation with several strains or mixed cultures enabling combined niche exploitation, cross-feeding, complementary effects, and enhancement of one organism's colonization ability when co-inoculated with a rhizosphere-competent strain (*Goddard et al.*, 2001). An example is the use of phosphorus-solubilising bacteria to increase available phosphorus along with mycorrhizae that enhance phosphorus uptake into the plant (Kim *et al.*, 1997). Saini *et al.*, (2004) achieved maximum yields of sorghum and chickpea at half the recommended rates of inorganic fertilizer when a combination of mycorrhizae, N₂-fixing bacteria, and phosphorus- solubilising bacteria was added. Increases in microbial biomass C, N, and P in soils of inoculated treatments were strongly correlated with N and P uptake of the plants.

Garbaye (1994) suggested that specific 'helper' bacteria may improve the receptivity of the root to the fungus to enhance mycorrhizal colonisation and symbiotic development with plant roots (Founoune *et al.*, 2002). Legume root nodulation can be enhanced by co-inoculation with *Azospirillum*, which increases root production and susceptibility for rhizobium infection and may also increase secretion of flavonoids

from roots that activate nodulation genes in Rhizobium (Burdman *et al.*, 1996). Conn and Franco (2004) found a significant reduction in indigenous actinobacterial endophytes upon inoculation of soil with a commercial multi-organism product, compared with no change in diversity after inoculation with a single species.

Significance of microbial diversity in soil

Diversity of microbial population in soil in relation to various agricultural practices was evaluated. In the soil profile, the microbial population mostly occurs within 40 cm of top soil. Bacteria are predominant followed by actinomycetes and fungi. Diversity index was much higher in Alfisols than Vertisols under different crop management practices. In both the soils, addition of organic manure (FYM) showed greater species diversity over control and inorganic fertilizer application. Continuous monoculture had a negative impact on species diversity as compared to crop rotations. (Venkateswarlu and Srinivasarao 2007)

Microorganisms form a vibrant living community in the soil contributing to a number of nutrient transformations. They are involved in organic matter decomposition, N2-fixation, solubilization and immobilization of several major and minor nutrients (Alexander, 1971). During the last 50 years, many beneficial effects of microbes in soil have been discovered (Alexander, 1971; Subba Rao and Gaur, 2000) for improving productivity in agriculture, industry and pharmaceuticals. Soil microbial biodiversity (SMD) is a vast frontier of potential gold mine for the biotechnology industry as it offers countless new genes and biochemical pathways (Tilak, 2000). One cubic meter of soil may house many hundreds of species of bacteria, Actinomycetes, fungi and algae.

The distribution of microorganisms in a typical soil profile has been described by (Alexander, 1971).

Plant growth regulator (PGR):

PGR can be defined as an organic compound other than nutrient, which is small amount produce, inhibit or otherwise modify any physiological process in plant. Plant growth and development are controlled by extremely low concentration of chemical substances called plant growth substance or plant growth regulators. In early 1900's Went made the profound statement "Without growth substance, no growth".

In addition to replenishing plant nutrients with organic inputs the application of growth hormones such as Indole acetic acid (IAA), Gibberlic acid (GA₃) Cytokinin and Abscisic acid (ABA) are incorporated by the farmers during various stages of crop growth (*Li* - xiu Ju, *et al.*, 1998).

Penicillium chrysosporium ME 446 synthesized the growth substance IAA, GA3, ABA and Zeatin as primary and secondary metabolites. Recovery of IAA, GA3, ABA and Zeatin were respectively 55.5±10%, 74.6±8%, 51.6±10% and56.63±6% (Unyayar et al., 1996). It has been found that the plant growth hormone Indole Acetic Acid (IAA) induces invasive growth in Saccharomyces cerevisiae. Genome expression profiling of cells treated with IAA identified YAP 1, a fungal specific transcription factor as a key mediator for this response. Strains lacking YAP 1 are hypersensitive to growth on IAA because they accumulate more IAA than wild type. The ability of a fungus to perceive plant hormones that cause it to differentiate in to an invasive form has important implication for plant-pathogen interaction (Reetaprusty et al., 2004).

In addition to higher plants, bacteria, fungi, there is a evidence that mosses also synthesize the plant growth regulators Auxin, Gibberlic acid, Abscisic acid and cytokinin. For example, Auxin is produced by sea algae. The production of IAA was also detected in mosses of Phycomitrellapatens and Funaria hygrometrica, Gibberlic acid and ABA are produced by algae, cytokinins are produced by red algae and brown algae. For determination of the level of these plant growth regulators, spectrophotometer was used (Nuray et al., 2002). The content of IAA and cytokinins in soil treat or with different organic manure were analysed in an apple pot trial. From the result it was concluded that organic manure increased the soil content of growth regulators and stimulated the plant growth (Lixiju et al., 1998).

Extraction and purification of growth hormones:

Bio assays of extracts from these galls contained as much as 17 times more auxin activity and as much as 21 time more gibberellins like activity per needle than extracts from normal needles of same age. The highest levels of these plant growth substance occurred during the early stages of gall formation. Extracts of insects' larvae did not contain auxin at detectable level but traces of substances with gibberellins like activities were present. Cellular hypertrophy and hyperplasia also support the idea that plant growth controlling substance such as auxin and gibberellins probably play an important role in gall formation (Byers *et al.*, 1976).

Soybean is an important crop in Brazil. Nonetheless, there are number of reports on the use of plant growth regulator potential in relation to this crop in the National literature. A pot experiment was carried out to study effects of GA₃, and Cytokinin on

the vegetative growth of the soybean. GA₃ (50 mg l⁻¹) was applied as seed treatment; leaving plant with water application as control. GA₃ (100 mg l⁻¹) and cytokinin (30 mg l⁻¹) were sprayed on leaves at the physiological stage V₃/V₄and 15 days after cytokinin (30 mg¹⁻¹) also as foliar spray seed treatment decreased plant emergence and initial soybean root growth, but as the season progressed, difference in root growth disappeared, plant were shorter and presented a decrease in number of nodes, in stem diameter in leaf area in dry matter yield. Conversely foliar application of GA₃ led to an increase in plant height, first node height and stem diameter. Leaf area and dry matter production also increased as a result of GA₃ foliar application. There was no effect of endogenous gibberellin and cytokinin on the number of soybean leaves, number of stem branches and root dry matter. Joint application of gibberellin cytokinin tended to inhibit gibberlines effects. Cytokinin applied to leaves during soybean vegetative growth was not effective in modifying any of the evaluated plant growth variables. (Vagner *et al*, 2003).

A general gas chromatography mass spectrometry (MS) – based screen was performed to identify catabolites and conjugate of Indole -3 buytric acid (IBA) during vegetative growth of *Arabidopsis*. The experiment revealed the existence of two new conjugate; N (indole – 3 – acetyl) alfa – alanine (IA – Ala) and N – (Indole 3 – acetyl – alfa lencine (IA – Leu). A method for quantitative analysis of IAA metabolites in plant extract by liquid chromatography – electro spray tandem MS has been developed. The low detection limits, 0.02 to 0.1 p mol for different metabolites, made it possible to use as little as 50 to 100 mg of tissue for quantitative analysis. The analysis was performed

on different tissues of an *Arabidopsis* plant at stages of development using heavy labeled internal standards of the catabolites 2- oxioindole -3 – acetic acid as well as IAA, conjugated to amino acid; asparatate, glutamate Ala and Leu. Expanding leaves and roots that generally contain the highest levels of IA – aspirate. In – glutamate and 2 onoindole – 3 – acetic acid, supporting their role as irreversible catabolic products. Interestingly, the level of IA – Leu was highest in root and IA – Ala in the aerial tissues.

2.5. Organic manure (animal manure)

Animal production is an integral part of crop production and characterized by mixed farming. The practices in such crop animal systems are very complex (Carangal 1993). Large ruminants provide draft power and animal manure for crop production while crop residues and crop by-products are important sources of animal feeds (Paris and Sevilla, 1995). Small farmers recognize the value of buffalo manure as a cheap and natural fertilizer mainly for rice and vegetable crops (Sirivaidyapong *et al.*, 2004).

Soil Enzyme Microbe Physicochemical

Composting is one of the oldest bio-technological processes used by human beings. It can be defined as the partial decomposition of heterogeneous organic matter by a mixed microbial population in a moist, warm and aerobic environment. In the organic matter, a dense population of various micro-organisms is found. The micro-organisms use organic matter, minerals, water and oxygen for their growth and metabolic activity. the gas permeability decreases as the gas velocity increases. For raw material, the gas permeability decreases with the wetness, whereas for older material there is no clear relationship. For composting material which has been turned once, the gas permeability

is larger than for raw material. The oxygen diffusion coefficient is proportional to the gas-filled volume fraction to the power 1.5. There is no clear relationship between the oxygen delusion coefficient and the age of the material. It is found that at a given temperature and for volume fractions of solid phase of 0.33 or less, the thermal conductivity increases linearly with the volume fraction of the liquid phase. The thermal conductivity is not influenced by the age of the composting material. The thermal conductivity increases with temperature (Ginkel, et. al., 2002).

2.6. Influence of organic amendments on soil properties

Compost application to agricultural soil needs to maximize agronomic benefits while protecting soil and water quality. The main determinant for efficient agronomic use is nitrogen availability. High nitrogen utilization in agriculture from mineral fertilizers is well established and understood, whereas increasing the nitrogen use efficiency of organic fertilizers requires further investigation (Gutser *et al.*, 2005).

2.6.1. Land application of composts/organic amendments

Most existing research has focused on the utilization of animal manure and sewage sludge in agriculture, whereas the investigation for biowaste and vegetable waste compost utilization in agriculture is limited. The paragraphs below give an overview of the pertinent literature concerning compost application to agricultural soil, with particular regards to crop production, soil properties, nitrogen dynamics, and a brief of the economic implications involved.

Soil organic matter (SOM) is one of the most important factors of the soil which positively effects on physical, chemical and biological properties of the soils (Yuksel,

2004; Pimentel *et al.*, 2005; Marriott and Wander, 2006). The addition of organic amendments would enhance the soil environment by increasing the soil fertility and soil tilth. Manure is often applied to soil to increase productivity through the addition of organic matter or carbon. Inputs of organic matter are, however, essential in order to replenish nutrients, improve soil structure and enhance soil disease suppressiveness (Ehaliotis *et al.*, 2005). Plants do not require organic matter for growth or reproduction, however growing conditions is enhanced by their presence in soil. Various organic agricultural technologies have been used for about 6000 years to make agriculture sustainable while conserving soil, water, energy and biological resources. The benefits of organic amendments in the ecological farming are nutrient reservoir with increased biological activity, retention of nutrients before they are leached from the system and crumb-like aggregate formation with increased porosity (Grunthal, 1996).

Animal manure is one of the most common types of organic amendments and fertilizers. It is readily accessible, inexpensive and effective. Nonetheless care must be taken in the application of animal wastes to soil. Salts in manure can accumulate to toxic levels in the soil if excessive amounts are applied or immigration and precipitation are insufficient to leach the accumulated salts from the soil (Chaney *et al.*, 1992). Since the availability of animal manure is not meeting the demand, the search is prevailing for alternative organic sources. Plant based lignocellulosic wastes holds promise to substitute diminishing farm based organic sources.

Composting helps to optimize nutrient management and the land application of compost may contribute to combat soil organic matter decline and soil erosion (Van-

Camp *et al.*, 2004). Compost land application completes a circle whereby nutrients and organic matter which have been removed in the harvested produce are replaced (Diener *et al.*, 1993).

The recycling of compost to land is considered as a way of maintaining or restoring the quality of soils, mainly because of the fertilising or improving properties of the organic matter contained in them. Furthermore, it may contribute to the carbon sequestration, and may partially replace peat and fertilizers (Smith *et al.*, 2001).

Soil organic carbon (SOC) is the most important parameter and an indicator of soil quality and agronomic sustainability because of its impact on the other physical, chemical and biological indicators of soil quality (Reeves, 1997; Nardi *et a.l.* 2004).

The depletion of TOC is also related to tillage techniques which mix and aerate the soil leads to stimulate microbial decomposition and accounts for much of the organic matter loss that follows (Vance, 2000). Reduced tillage, combined with modest inputs of organic residues, maintained SOC content at the initial levels of 15 g kg⁻¹ in the topsoil (0–20 cm) and at 11 g kg⁻¹ in the subsoil (20–40 cm). Increased inputs of effective C only marginally increased SOC content in the topsoil and there is no real prospect or need for increasing SOC contents beyond current levels (Wang *et al.*, 2007).

Soil microbial diversity has a key role in carbon cycling, organic matter decomposition and maintenance of the edaphic fertility. The use of organic amendments such as vermicompost from source-separated household solid waste, vermicompost from horse and rabbit manure chicken manure (Gomez *et al.*, 2008) and farmyard or liquid manure (Nannipieri, 1993; Nardi, 2004) is helping improve soil condition and act

as a source of carbon and other nutrients, improve soil structure which favor to enhance microbial biodiversity and activity in soil (Albiach *et al.*, 2000; Potter and Meyer, 1990; Gomez *et al.*, 2008).

Nutrient cycling in soils involves biochemical, chemical and physiochemical reactions, with biochemical processes being mediated by microorganisms, plant roots, and soil animals. It is well known that all biochemical reactions are catalyzed by enzymes, which are protein with catalytic properties owing to their power of specific activation. Enzymes are catalysts, that is, they are substances that without undergoing permanent alteration cause chemical reactions to proceed at faster rates. In addition, they are specific for the types of chemical reactions in which they participate (Tabatabai, 1994). Enzymes specificity is often dictated by the nature of the groups attached to the susceptible bonds. e.g., maltase hydrolyzes maltose to glucose, but not vice versa. Differences between the two substances seem slight in that maltose is an "βglucoside and cellobiose is a β -glucoside. Both and β -glucosidases are present in soils (Eivazi and Tabatabai, 1988). Physiochemical measurements indicate that enzymecatalyzed reactions in soils have lower activation energies than non enzyme catalyzed reactions and, therefore, have faster reaction rates (Browman and Tabatabai, 1978; Dick and Tabatabai, 1978). Enzymes in soil are similar to enzymes in other systems, in that their reaction rates are markedly dependent on pH, ionic strength, temperature, and the presence or absence of inhibitors (Burns, 1978 and Tabatabai, 1982).

Over 30,000 tons of pesticides is used annually on lawns and gardens in the United States (Aspelin *et al.*,1991; World Health Organization, 1989). Although there

have been many studies on the degradation of pesticides in soils, very little research has been done on the fate of these compounds during the composting of yard trimmings (Aspelin *et al.*, 1991; Lemmon and Pylypiw, 1992; Petruska *et al.*, 1985). Furthermore, non-degraded pesticides could potentially be concentrated during composting, as degradable OM is mineralized to carbon dioxide and water. There are a number of potential fates for pesticides during composting such as mineralization to carbon dioxide, conversion to humic matter, incorporation into microbial biomass, adsorption, biotransformation, volatilization, and leaching (Michel *et al.*, 1995).

2.6.2 Effect on crop production

Iglesias-Jimenez & Alvarez (1993) showed that biowaste compost application at rates up to 50 Mg ha-1 increased ryegrass yield under greenhouse conditions, although not at the same levels as with mineral fertilization. Montemurro *et al.* (2005) demonstrated that the application of relatively lower levels of MSW compost to sunflower production in Italy resulted in similar oil and protein yield performance as mineral fertilization.

Clark et al. (2000) showed in a 3-year field experiment in Florida that the incorporation of MSW compost into sandy soils under drip-irrigation provided improved vegetable growth and yield. Work undertaken by Svensson et al. (2004) during a 4-year field experiment in Sweden indicated that biowaste compost should not be used as a sole fertilizer, but it should be complemented with mineral N for optimum crop yield, unless very high rates of application are used. However, high rates of compost application may increase N loss potential by leaching (Mamo et al., 1999).

The low fertilizer value of composts is reported for both biowaste and vegetable waste composts (Båth and Rämert, 2000; Sikora and Enkiri, 2001; Nevens and Reheul, 2003). The main reason for this is found to be the low release of compost N following compost application and hence the low plant N availability (Svensson *et al.*, 2004). However, the longer-term effects of repeated compost application on crop yield are better. Leroy *et al.* (2007) showed increased maize yields in the last years of a 7-year fruit, vegetable and garden waste compost in Flanders. The increase was attributed to stimulation of the soil food web, through cumulative applications, and thus enhance N turnover. Mamo *et al.* (1999) found that adequate maize grain yield was achieved in the 3rd year of consecutive biowaste compost application, without any mineral fertilizer addition to be necessary, in a field experiment in Minnesota. Compost application also influences soil properties, thus improving the growing conditions for the crop and consequently the crop yield.

Effect on soil properties

Compost application to agricultural soil has been reported to increase soil organic matter, improve soil fertility, conserve soil moisture, control weeds and suppress disease.

Soil chemical properties

The application of biowaste and vegetable waste composts increases soil organic matter and total N content (He *et al.*, 1992; Crecchio *et al.*, 2001; Nevens and Reheul, 2003; Hartl and Erhart, 2005). Hadas *et al.* (2004) showed that the application of MSW compost as mulch at a rate of 43 Mg ha⁻¹ resulted in an increase of soil organic matter at

a rate of 21% of the organic matter applied by the compost in 3 years. Increase in the quantity and quality (humic acids) of soil organic matter were shown in both sandy (Weber *et al.*, 2007) and clay soils (Melero *et al.*, 2007). Biowaste and vegetable compost application can increase plant available P, K (Hartl *et al.*, 2003; Martínez *et al.*, 2003) and Mg (Parkinson *et al.*,1999; Weber *et al.*, 2007) levels of soils, and soil CEC (Bartl *et al.*, 2002; Weber *et al.*, 2007).

Increased levels of soil EC (Stamatiadis *et al.*, 1999; Madejón *et al.*, 2001) due to the application of mixed green and animal waste compost, and also vinasse compost have also been reported. Although the increase of EC was not found capable of causing a sodium hazard to the soil, it indicates potential problems following the repeated application of compost to agricultural soil. Addition of organic matter improves soil nutrient availability and uptake by plants (Marschner, 1995). While with the increase in soil organic matter, N and P availability also increases (Chaudhary *et al.*, 1998; Ewulo *et al.*, 2008)

Compost application can ameliorate soil acidity (Wong *et al.*, 1998; Van De Berghe and Hue, 1999), contribute towards the reclamation of sodic soils (Kochba *et al.*, 2004), and prevent the acidification effect of mineral fertilization (Stamatiadis *et al.*, 1999).

Physical properties

Biowaste and vegetable compost application to agricultural soil is shown to improve soil physical properties. This improvement is mainly a result of the organic matter addition to the soil by the compost application.

Jakobsen (1995) found that after eight years of different types of compost application to sandy clay soil the soil structure was improved, especially when the compost was applied on the soil surface. The surface was thereby protected from the compressing effects of rain drops and the rapid drying. Soil infiltration rate was also found to be higher. Contradictory results were found by Stamatiadis *et al.* (1999), who demonstrated that after one year of compost application to silt clay loam soil, the infiltration rate decreased. Pandey and Shukla (2006) showed that compost addition to sandy soil resulted in higher retention of rainfall, if application levels are sufficiently high.

Urban waste compost application to the soil was shown to increase soil total porosity (Guisquiani *et al.* 1995) and aggregate stability (Aggelides and Londra, 2000; Annabi *et al.*, 2007). Increased soil porosity, field water capacity and the amount of plant available water, but only in the short time after MSW compost application were demonstrated by Weber *et al.* (2007). Johnson *et al.* (2006) showed that surface application of dairy manure compost increases water retention capacity and decreases soil bulk density. These results are in accordance with findings by Aggelides and Londra (2000) who used a composted mixture of MSW, sewage sludge and sawdust. Aggelides and Londra (2000) also showed that compost application can reduce the penetration resistance of the soil.

Biological properties

The application of compost to agricultural soil is shown to reduce the number of parasitic nematodes, and increase both the numbers of micro-arthropods and

earthworms (Leroy *et al.*, 2007). Stimulation of soil biological activity and increase of micro-arthropods were also demonstrated by Petersen *et al.* (2003). Melero *et al.* (2007) showed a clear increase of microbial biomass and enzymatic activities at the fourth year of compost application to clay soils.

Composts have the potential to provide biological control of many soil-borne plant diseases. Foliar, vascular, and root pathogens may be affected by compost application (Hoitink *et al.*, 1997; Noble and Coventry, 2005; Yogev *et al.*, 2006). Reported levels of disease suppression vary, even if similar composted material is used at the same rates. Sterilisation of composts generally results in a loss in the disease suppressing capability of composts, indicating that the mechanism is predominantly biological, although chemical and physical factors have also been implicated. The mechanisms and antagonistic micro-organisms involved in disease suppression need further investigation (Bailey and Lazarovits, 2003; Noble and Coventry, 2005).

The use of compost as a mulch in an orchard ecosystem was shown to be beneficial to management of weed, fungal, and insect pests (Brown and Tworkoski, 2004). Other experiments have shown that compost application can benefit weeds. Blackshaw (2005) demonstrated that the gradual N release from manure and compost over years appeared to have more positive effect on weeds rather on the growing crop. Boyhan *et al.* (2006) found that compost was not very affective for weed control in ones. A study by Law *et al.* (2006) indicated that shallow cultivation following transplanting, combined with midseason compost mulch application, can result in high

yields in an organically managed bell pepper system, which was comparable to yields of most varieties grown under conventional practices.

The acceptability of organically grown products and nutritional quality was found to be significantly better than conventionally grown products (Menaka *et al.*, 2005; Menaka *et al.*, 2006).

Materials and Methods



3. MATERIAL AND METHODS

3.1. Collection of commercially available organic and biodynamic manure

The organic and biodynamic manures such as cow horn manure (BD500), BD500B, BD502, BD503, BD504, BD505, BD506, BD507, CPP, CPP-B, BD500-D, BD500-K, compost-K, compost-KG, compost-KK, CPP-K, compost-OYO, callies manure, coir pith compost, compost-B, cow urine, cow dung cake, FYM-P, goat manure, GP-FYM, jeewamirtham, panchakavya, compost- US, compost- UM, vermicompost-M and vermicompost-R were collected from the following places in India such as Tamil Nadu A). Ratnagiri, Maharastra, B). Gudur, Andhra Pradesh, C). Palani agriclinic, Hosur, Krishnagiri District, D). Badri, Palani, Dindugal district E). Kurinji Organic Foods Pvt. Ltd., Genguvarpatti, Madurai district F). Nadavan farm, Kodaikanal, Dindugal district (Fig.1). The collected manures were transferred to sterile polypropylene bags as described by Wu and Ma, 2002 and transported to the laboratory of Shri AMM MCRC, Chennai. Manure were processed for microbial enumerations such as total bacteria, total fungi, Azospirillium, Azotobacter, Rhizobium like colonies, Actinomycetes by standard microbiological techniques as mentioned in the section 3.2.21 and biochemical properties such as protease, alkaline phosphatase, invertase, cellulase and humic acid were determined by standard analytical methods as mentioned in the section 3.2.16 to 3.2.20. The remaining samples were air dried and preserved in sterile polypropylene bags stored at room temperature. The preserved samples were pulverized in to powder, sieved (30 mesh sizes) before the experiments, by following the modified method of Arora et al., 2005. The physico-chemical pararmeters such as pH, moisture,

electrical conductivity (EC), organic carbon (OC), available nitrogen (N), phosphorus (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) were analysed in the air dried manures.

Figure 2.1: Map indicating the collection sites of manures in India



- Ratnagiri, Maharastra
- B Gudur, Andhra Pradesh
- Palani agriclinic, Hosur, Krishnagiri District
- D Badri, Palani, Dindugal district
- B Kurinji Organic Foods Pvt. Ltd., Genguvarpatti, Madurai district
- Nadavan's farm, Kodaikanal, Dindugal district

3.2. Physico-chemical analysis of manures

3.2.1. Estimation of pH and electrical conductivity (EC)

The pH and EC of the manure samples were determined by following the modified methods of Bernal *et al.* (1998); Tiquia *et al.* (2000) and Taiwo (2004). Five gram manure (5 g) was suspended in distilled water (1:10 w/v) ratio, mixed in a rotary shaker (120 rpm) and filtered through Whatman No. 3 filter paper. The collected filtrates were determined for pH (Ecoscan pH meter, Eutech, Singapore) and EC (Ecoscan EC meter, Eutech, Singapore).

3.2.2. Estimation of moisture

The moisture content of the manure samples were determined by following the methods as described by Factsheets (1996) and Abu Qdais *et al.* (2004). A glass beaker containing 3 g of manure sample was dried in hot air oven at 105 °C (220 °F) for 5 hrs. The percentage of moisture content for the manure sample was recorded from the weight of the sample before and after drying by following the formula as described below.

3.2.3. Estimation of Organic carbon

Organic carbon (OC) content in the manure was estimated by following the methods as described by Nelson-Sommers (1975).

Reagents

- 1. Potassium dichromate solution (1N): Dried potassium dichromate (49.024 g) was dissolved in 800 mL of distilled water and the volume was made up to 1 L.
 - 2. Concentrated sulphuric acid (99 %).
 - 3. Ferrous Ammonium Sulphate (FAS, 0.2M): FAS (78.390 g) was dissolved in 50 mL of Con. sulphuric acid and made up to 1 L of distilled water.
 - 4. Indicator solution: *N*-phenylanthranillic acid (0.1 g) and sodium carbonate (0.1 g) was dissolved in 100 mL of distilled water.

Procedure

In a 100 mL glass beaker, manure (2 g), potassium dichromate (10 mL) and concentrated sulphuric acid (20 mL) were added, mixed and incubated at still condition for 30 min and cooled at 30 °C. To each beaker, ortho phosphoric acid (10 mL) and indicator solution (0.3 mL) were added and this mixture was titrated against 0.2 M FAS reagent. Control was maintained with the addition of all the reagents excluding the samples. The appearance of green colour was observed as the end point and recorded the titrate value. The percentage of organic carbon present in manure sample was recorded by following the formulae as described below.

V_n=FAS (0.2 M) titrant value (mL) against blank

 V_s = FAS (0.2 M) titrant value (mL) against sample.

3.2.4. Estimation of Total nitrogen (N)

Reagents

- 1) Salicylic acid Sulphuric acid mixture (1:30 w/v)
- 2) Sodium thiosulphate, fine ground crystals or Zinc dust.
- 3) Concentrated sulphuric acid (H₂SO₄)
- 4) Standard hydrochloric acid (HCl) solution: N/10
- 5) Sodium hydroxide (NaOH) solution (45 %): NaOH (45 g) was dissolved in 100 mL of distilled water.
- 6) Mixed indicator: Bromocresol green (0.5 g) and methyl red (0.1 g) were dissolved in 100 mL of 95 % ethanol.
- 7) Boric acid (H₃BO₃) 4% (Indicator solution): H₃BO₃ (4 g) was dissolved in 100 mL of distilled water by heating gently, mixed indicator (5 mL) was added to this and pH was adjusted to 4.5 by using NaOH or HCl (Colour turns from blue to slightly pink).
- 8) Granulated Zinc: To Prevent bumping in distillation flask.
- 9) Catalytic mixture: Sodium sulphate (10 g) and copper sulphate (0.3 g) was mixed.

Digestion of sample

Manure sample (0.1 g) was weighed and transferred into a Kjeldahl flask and 30 mL of salicylic acid- sulphuric acid mixture was added. It was shaken thoroughly and kept

for 30 min and sodium thiosulphate (5 g) was added and heated over a low flame till frothing ceases to come out.

Distillation

Few pieces of glass beats were added in the round bottom flask to prevent bumping and about 80 mL of 45 % NaOH was added gently. The round bottom flask was connected to distillation assembly and end of condenser tube was dipped into the conical flask containing 50 mL of 4 % boric acid solution with mixed indicator. The distilled ammonia which was dissolved in boric acid was tested with red litmus paper. The distilled ammonia was titrated against the 0.1 N HCl until the pink colour turned to green colour. The end point was recorded and estimated the total nitrogen by following the formulae given below.

Calculation

Nitrogrn (%) =
$$\frac{0.0014 \text{ X T X } 500}{25} \text{ X } 100$$

Where T=Sample reading -Blank reading

3.2.5. Estimation of Phosphorus

The estimation of phosphorus (P) in manure was analysed by following the method as described by Olsen *et al.*, 1954

Reagents

1.Triple acid mixture: Con. Nitric acid (HNO₃), Con. Sulphuric acid and Perchloric acid (9:2:1)

2.Concentrated nitric acid (71 % w/v)

3. Molybdovanadate reagent:

Molybdate solution - Ammonium molybdate (40 g) was dissolved in 400 mL of hot distilled water and the solution was cooled to room temperature (RT).

Vanadate solution - Ammonium meta-vanadate (2 g) was dissolved in 250mL of hot distilled water and cooled to RT and 250mL of Nitric acid was added to vanadate solution.

Molybdate (400 mL) and vanadate (500 mL) solutions were mixed, stirred and the volume was made up to 2 L with distilled water.

4. Phosphorus stock solution (2 mg P mL⁻¹):

Potassium dihydrogen phosphate (8.788 g) was dissolved in 500mL of distilled water and the volume was made up to 1 L.

5. Phosphorus working solution (0.1 mg P mL⁻¹):

50 mL of phosphorus stock solution was diluted with 1 L of distilled water.

Procedure

Dried manure samples (2 g) were individually weighed in a glass beaker and mixed with triple acid mixture. The glass beaker containing the mixture was kept in a hotplate and heated until the mixture became clear solution (colourless). This solution was allowed to cool at room temperature (30±2) and transferred to 250 mL of volumetric flask. The volume was made up to 200 mL with distilled water and filtered through Whatman No.1 filter paper. Filtrate (5 mL) was pipetted in to 100 mL of volumetric flask. 20 mL of molybdovanadate reagent was added and the volume was

made up to 100 mL with distilled water. The contents were mixed well and allowed to stand for 10 min. The colour developed was read at 420 nm in a Double beam UV visible spectrophotometer (Chemito, India). The amount of total phosphorus in the manure was determined from the standard graph prepared with different concentrations of phosphorus ranging from 0.25 to 1.5 mg mL⁻¹. The amount of phosphorus present in the manure was determined by following the formulae as described below.

Calculation:

Phosphorus (% w/w) = mg phosphorus in aliquot / g manure in aliquot x 100

3.2.6. Estimation of potassium

The estimation of potassium (K) in manure sample was analysed as described by Jackson (1973).

Reagents:

- i. Double distilled water
- ii. Standard potassium chloride (KCl): Dried potassium chloride (dried at 60 °C for 1 h) (1.5851g) was dissolved in 200 mL of distilled water, in one liter volumetric flask and it was made up to the volume of the mark which gave the 1000 ppm concentration of K₂O. Ten mL of 1000 ppm solution was diluted upto 100 mL and obtained the 100 ppm concentration of K₂O.

Procedure

Triple acid digested solution (5 mL) was diluted to 50 mL with distilled water and values were recorded in flame photometer. The total potassium content in the manures was determined using a standard graph plotted with values obtained from

different concentrations of standard KCl solutions and potassium percentage was calculated by following formulae.

Calculation

Weight of the organic manure used in digestion : 1 g

Volume of the digested aliquot taken for K estimation : 5 mL

Final volume was made up to : 50 mL

Concentration of Total Potassium from Flame photometer : a ppm

Therefore the amount of available potassium in organic manure (%)

3.2.7. Estimation of Calcium (Ca) in manures

The estimation of calcium in manure sample was analysed as described by Tandon (1993)

- i. Double distilled water
- ii. Calcium Carbonate (CaCO₃) standard: CaCO₃ (2.49 g) was dissolved in distilled water and 25 mL of 1M HCl was added and the volume was made up to 1 L (1000 ppm of Ca). 10 mL of 1000 ppm solution was diluted to 100 mL and obtained 100 ppm of Ca solution.

Analysis in Atomic Absorption Spectrometer

The blank (distilled water) and standards viz., 5, 15 and 25 ppm were loaded in the auto sampler. The filtrates obtained from dry ashing method were diluted (5 mL to

50 mL) and loaded sequentially in auto sampler. The sample was read at 422.7 nm under the Nitrous Oxide - Acetylene flame. From the above concentrations of sample, the quantity of calcium (%) was calculated in organic manure by following the formulae.

Calculation

Weight of the organic manure used in digestion : 1 g

1 g of digested aliquot volume made up to : 100 mL

Volume of the digested aliquot taken for Ca estimation : 5 mL

Final volume was made up to : 50 mL

Concentration of Total Calcium from AAS : a ppm

Therefore the amount of available calcium in organic manure (%)

3.2.8. Estimation of Magnesium (Mg) in manures

The estimation of magnesium in manure sample was analysed as described by Tandon (1993)

Reagents:

- i. Double distilled water
- ii. 1.0 N Ammonium acetate
- iii. Standard solution: Magnesium metal ribbon (1 g) was dissolved in distilled water and 20 mL of 5 M HCl was added, dissolved and the volume was made up

to 1L with distilled water (1000ppm of Mg). 10mL of stock was diluted to 100mL (100ppm Mg solution).

Analysis in Atomic Absorption Spectrometer

The blank (distilled water) and standards *viz.*, 5, 15 and 25 ppm were loaded in the auto sampler. The filtrates (from dry ashing method) were diluted (5 mL to 50 mL) and loaded sequentially in auto sampler. The samples were scanned at 285.2 nm under the air - acetylene (1.1 - 1.3 l/min⁻¹) flame. From the above concentrations of sample, the quantity of magnesium (%) was calculated in organic manure by following the formulae.

Calculation

Weight of the organic manure used in digestion : 1 g

l g of digested aliquot volume made up to : 100 mL

Volume of the digested aliquot taken for Mg estimation : 5 mL

Final volume was made up to : 50 mL.

Concentration of Total Magnesium from AAS : a ppm

Therefore the amount of available magnesium in organic manure (Y %)

magnesium (%) =
$$-X - X - X 100$$

 $10^6 1 5$

3.2.9. Estimation of Sodium (Na) in manures

The estimation of sodium in manure sample was analysed as described by Tandon (1993)

Reagents:

- i. Double distilled water
- ii. Standard Solution: Sodium chloride (2.5420 g) was dissolved in distilled water, the volume was made up to 1 L and obtained the concentration of 1000ppm of Na. 10mL of 1000ppm solution was diluted to 100mL and to get 100ppm Na solution.

Analysis in Atomic Absorption Spectrometer

The blank (distilled water) and standards *viz.*, 5, 15 and 25 ppm were loaded in the auto sampler. The filtrates (from dry ashing method) were diluted (5mL to 50mL) and loaded sequentially in auto sampler. The sample read at 589 nm under the air – acetylene (1.1 - 1.3 l/min) flame. From above concentrations of sample, the quantity of total sodium (%) was calculated in organic manure by following the formulae.

Calculation

Weight of the organic manure used in digestion : 1 g

1 g of digested aliquot volume made up to : 100 mL

Volume of the digested aliquot taken for Na estimaion : 5 mL

Final volume was made up to : 50 mL

Concentration of Total Sodium from AAS : a ppm

Therefore the amount of available total sodium in Organic manure

$$a 100 50$$
Sodium (%) = -- X ---- X 100
$$10^6 1 5$$

3.2.10. Estimation of micro nutrients in organic manure

The estimation of micro nutrients in manure sample was analysed as described by Tandon (1993)

Reagents:

- i. Iron (Fe) Standard Solution: Iron metal Powder of (1 g) was dissolved in 20 mL of 5 M HCl and 5 mL of concentrated nitric acid (HNO₃) and the volume was made to 1 L (1000 ppm of Iron) with distilled water. For working concentration 10 mL of stock was made upto 100 mL of distilled water and obtained 100 ppm of iron solution.
- **Zinc (Zn) Standard Solution:** Zinc metal chips (1 g) were dissolved in 30 mL of 5 M HCl and the volume was made to 1 L and obtained the concentration of 1000 ppm of Zn. From the stock solution, 100 ppm Zn solution of working concentration was prepared.
- was dissolved in 50 mL of Con. HCl and volume was made to 1L (1000 ppm of Mn). From the stock solution, 100 ppm Mn solution of working concentration was prepared.

- iv. Copper (Cu) Standard Solution: Copper metal foil (1 g) was dissolved in 50 mL of 5M HNO₃ and the volume was made to 1 L (1000 ppm of Cu). From the stock solution, 100 ppm Cu solution of working concentration was prepared.
- v. **Molybdenum (Mo) Standard Solution:** Molybdic acid (1.5003 g) was dissolved in 20 mL of concentrated nitric acid and the volume was made to 1 L (1000 ppm of Mo). From the stock solution, 100 ppm Mo solution of working concentration was prepared.

Procedure

The concentrations of Fe, Mn, Zn, Cu and Mo (ppm) present in the manures were directly measured in the atomic absorption Spectrometer.

Calculation

Weight of the organic manure used in digestion : 1 g

1 g of digested aliquot volume made up to : 100 mL

Concentration of Total Sodium from AAS : a ppm

Therefore the amount of available total Fe, Mn, Zn, Cu and Mo (Y ppm).

in Organic manure = a X 100 = Y

3.2.11. Estimation of humic acid in manures

The estimation of humic acid in manure sample was analyzed by following the methods as described by Welte, 1952.

Sodium oxalate (0.5 g) and sodium hydroxide (0.5 g) were dissolved in 100 mL distilled water and used as the extracting solution. Two gram of manure (2 g) was taken in a clean vial; 50 mL of 0.5 % sodium oxalate and sodium hydroxide solution were

added and incubated in the boiling water bath for 30 min. After the boiling, the mixture was allowed to cooled and centrifuged at 10,000 rpm for 20 min. The supernatants were collected and OD was read at 472 and 664 nm in spectrophotometer.

3.2.12. Estimation of alkaline phosphatase activity in manures

The estimation of alkaline phosphatase in manure was analysed by following the methods as described by Tabatabai and Bremner, 1969.

Modified Universal Buffer (MUB): Tris (hydroxymethyl) amino methane (TRIS buffer) (12.5 g), Maleic acid (11.6 g), Citric acid (14 g) and Boric acid (6.3 g) were dissolved in 500 mL of 1N sodium hydroxide and diluted to 1 L with distilled water.

p-Nitrophenyl phosphate solution (0.05 M): Substrate (80.4 g) was dissolved in 500 mL of MUB Buffer.

Calcium chloride (CaCl₂ (0.5M)): CaCl₂ (73.51 g) was dissolved in 1 L of distilled water.

Sodium hydroxide (NaOH (0.5M)): NaOH (40 g) was dissolved in 1 L of distilled water.

Standard:

Stock: p- Nitrophenol (1 g) was dissolved in 1 L of distilled water .

Working: 1 mL of the stock solution was diluted to 100 mL with distilled water.

Preparation of standards for alkaline phosphatase

The p- Nitrophenol solutions solution such as 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mL were individually taken in the test tubes. These solutions were made up to 5 mL with distilled water in all the test tubes. Which is equivalent to 10, 20,30, 40, 50, 60, 70, 80, 90 µg. Blank was maintained with a tube 5 mL of distilled water. 1 mL

of 0.5 M CaCl₂ and 4 mL of 0.5 M NaOH was added to each test tube including to the blank and uniformly mixed. The development of yellow colour was observed as the indicator and recorded at 420 nm in UV – Vis spectrophotometer and the values were plotted using the standard graph.

Estimation of Alkaline phosphatase in Manure samples

Test tubes containing one gram of manure was added with 4 mL of MUB buffer and 1 mL of buffered substrate. Manure (1 g) was weighed in to a test tube and 1 mL of MUB buffer was added and maintained as control. All the tubes containing the manure and substrate were incubated at 37 °C for 60 min. After incubation, 1 mL of 0.5 M CaCl₂ and 4 mL of 0.5 M NaOH were added and mixed well. All the test tubes containing the manure mixture were then filtered using Whatman No 1 filter paper, and the filtrates were read at 420 nm in a spectrophotometer. The enzyme activity was calculated by following formulae

Calculation



3.2.13. Estimation of Invertase activity in manures

The estimation of invertase in manures were analysed by following the methods as described by Ross, 1966.

Reagents

Acetate Buffer (2M)

Sodium acetate (27.2 g) was dissolved in 100 mL of distilled water. The pH was adjusted to 5.5 with 2 M acetic acid (12 mL of acetic Acid was made upto 100 mL of distilled water).

Sucrose (1.2 %): Sucrose (1.2g) was dissolved in 100 mL of acetate buffer.

Dinitrosacylic acid (DNS) Reagent: DNS (7.5g), NaOH (13.9 g), Sodium Potassium Tartarate (216 g), Phenol (5.3 mL) and Sodium metabisulfite (5 g) were dissolved in 1L of distilled water.

Standard solution: - 100 mg of glucose was dissolved in 100 mL distilled water in a standard flask.

Procedure

The glucose contains such as 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 mL were taken individually in a test tubes. These solutions were made up to 4 mL with distilled water in all the test tubes. Which is equivalent to 100, 200, 300, 400, 500, 600, 700, 800 and 900 µg. Blank was maintained with a tube with 4 mL of distilled water. Three mL of DNS reagent was added to each test tube including the blank and incubated in boiling water bath at 100 °C for 5 min. The solutions were read at 540 nm in a spectrophotometer and a standard graph was drawn.

Substrate Control: Acetate buffer (3 mL) and Sucrose (buffered substrate) (3 mL) were added in a test tube and kept as control.

Sample Control: Manure sample (1 g) and Acetate buffer (6 mL) were added in a test tube and maintained as sample control.

Test: Manure (1 g) was weighed in a test tube and 3 mL of acetate buffer and sucrose (buffered substrate) (3 mL) was added and mixed well the mixture. All the tubes were incubated at 50 °C for 2 h. Then 3 mL of DNS reagent was added in the all tubes and mixed well. The mixture was filtered through Whatman No 1 and filtrates were read at 420 nm in a spectrophotometer. the invertase activity was estimated by following formulae.

Calculation

$$(Test OD- Sample Ctrl) - Substrate Ctrl \quad 0.4 \quad 2$$

$$Invertase \ (\mu g/g) = ---- X --- X \ Std. \ Con.$$

$$Standard \ OD \qquad \qquad 6 \qquad 24$$

3.2.14. Estimation of Cellulase activity in manures

The estimation of cellulase in manure sample was analysed by following the methods as described by Ross, 1966.

Reagent

Acetate Buffer (2M)

Sodium acetate (27.2 g) was dissolved in 100 mL of distilled water. The pH of was adjusted to 5.5 with 2 M acetic acid (12mL of acetic acid was made up to 100 mL distilled water).

Sucrese (1.2%)

Sucrose (1.2 g) was dissolved in 100 mL of acetate buffer.

DNS Reagent

DNS reagent was prepared as like method mentioned in the section 3.2.18

Standard solution: 100 mg of glucose was dissolved in 100 mL distilled water in a standard flask

Estimation of Cellulase activities in Manure Samples

One gram Manure (1 g) was transferred in a test tube containing 3 mL of acetate buffer and 3 mL of sucrose (buffered substrate).

The glucose contains such as 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 mL were taken individually in a test tubes. These solutions were made up to 4 mL with distilled water in all the test tubes. Which is equivalent to 100, 200, 300, 400, 500, 600, 700, 800 and 900 µg. Blank was maintained with a tube with 4 mL of distilled water. DNS reagent (3 mL) was added to each test tube including the blank and the test tubes were incubated in boiling water bath at 100 °C for 5 min. These solutions were read at 540 nm in a spectrophotometer and a standard graph was plotted.

Substrate control: of acetate buffer (3 mL) and sucrose (3 mL) (buffered substrate) were added in a test tube and kept as control.

Sample control: Manure (1 g) and Acetate buffer (6 mL) were added in a test tube and maintained as sample control

Test: Manure (1 g) was weighed in a test tube and 3 mL of acetate buffer and sucrose (buffered substrate) (3 mL) was added and mixed well the mixture. All tubes were incubated at 50 °C for 24h. After incubation, the solution was filter through whatman no 1 and filtrate (0.4ml) was made up to 1 mL with distilled water was added. Then DNS

reagent (3 mL) was added and mixed well. This reaction mixture was filtered through Whatman No. 1 filter paper. The solutions were read at 420 nm in a spectrophotometer and the cellulase activity was estimated by following formulae.

3.2.15. Estimation of Protease activity in Manures

The estimation of Protease in manure sample was analysed by following the methods as described by Ladd and Butler, 1972.

Reagents

THAM Buffer (0.1 M): THAM (TRIS (tris- hydroxymethyl-aminomethane)) (12.2 g) was dissolved in 800 mL of distilled water and the pH was adjusted to 8.1 using 0.1 M HCl and the solution was made up to 1 L with distilled water.

Casein Soluble in Alkali: Substrate (100 mg) was dissolved in 100 mL of 0.1 M THAM Buffer.

TCA (17.5 %): TCA (17.5 g) was dissolved in 100 mL of distilled water.

Sedium Carbonate (1.4 M): Sodium Carbonate (148.39 g) was dissolved in 1 L of distilled water.

Folin's Ciocalteau Reagent: Folin's Ciocalteau reagent was percured from HIMEDIA (P) Ltd, India and diluted with distilled water at 1:1 ratio.

Standard: 100 mg of tyrosine was dissolved in 100 mL of distilled water, was diluted at 1:10 ratio and served as a working concentration.

Estimation of protease activity in manure samples:

Tyrosine solutions such as 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 mL were taken individually in test tubes. These solutions were made up to 2 mL with distilled water in the all test tubes. Which is equivalent to 10, 20, 30, 40, 50, 60, 70, 80 and 90 µg. Distilled water (2 mL) was maintained as the blank. Three mL of 1.4 N Na₂CO₃ and 1 mL of Folin's Ciocalteau reagent was added to all the tubes including the blank. The readings were measured at 660 nm and standard graphs were plotted.

Substrate control: Buffered casein solution (2.5 mL) was taken in a test tube and kept as substrate control.

Sample control: Manure (1g) with THAM buffer (2.5 mL) was taken in a test tube and maintained as sample control

Test: Manure (1 g) was weighed in a test tube and buffered casein (2.5 mL) was added. All tubes were incubated at 45 °C for 1 h. After incubation, 17.5 % TCA (1 mL) was added to stop the reaction and centrifuged at 8000 rpm for 20 min. The supernatant (2 mL) was taken and 1.4 N Na₂CO₃ (3 mL) followed by 1 mL of Folin's Ciocalteau reagent was added. This mixture was read at 660 nm in spectrophotometer and the protease activity was estimated by following formulae..

Calculation:

$$(Test OD- Sample Ctrl) - Substrate Ctrl 3.5$$

$$= ---- X ---- X Std Con.$$

$$Standard OD \qquad 2$$

3.2.16. Enumeration of microbe

The microbial enumeration in manure was analyzed by following the technique as described by Waksman, 1952.

Bacteria and fungi in different manure such as organic and biodynamic were enumerated as the number of total colony-forming units (CFUs) per gram of manure. Total viable count was enumerated in five different media such as nutrient agar (NA) for assessing the bacterial load and Rose Bengal agar (RBA) medium for enumeration of fungi. Yeast extract mannitol agar (YEMA) medium for *Rhizobium*, Burk's medium for *Azotobacter*, and Starch casein agar (SCA) medium for *Actinomycetes* enumeration. The media composition was mentioned below.

Table 1: Media composition for microbial enumeration

Peptone	5
Sodium chloride	5
Beef extract	3
Distilled water	1
Agar	20
Medium for fungi enumeration: Mar 6.5 ± 0.2) (g L ⁻¹)	tin's Rose Bengal chloramphenicol agar (pH
Glucose	10
Peptone	5
K ₂ HPO ₄	1
Magnesium sulphate	0.5
Rose Bengal dye	0.5
Distilled water	Green of the contract of the c
Agar	20
Medium for Rhizobium like colonies Agar (pH 7.0 ± 0.2) (g L^{-1})	s enumeration: Yeast Extract Mannitol Sal
Magnitol	10
Calcium carbonate	were the second 4 and produced
K ₂ HPO ₄	0.5

Yeast extract	partition and 10.4 leads
Sodium chloride	0.2
Distilled water	nts reproduce with J 1 of January
Agar	20
Congo red dye	1mL/100 mL
	ration: Starch casein agar (pH 8.0 ± 0.2) (g L ⁻¹)
Soluble starch	10
K ₂ HPO ₄	using the many of the 2 or many paret.
Sodium chloride	2
Casein	0.3
MgSO ₄ . 7H ₂ O	0.05
Calcium carbonate	0.02
FeSO ₄ . 7H ₂ O	0.01
Distilled water	
Agar	20

Medium for Azotobacter enumeration: Burk's medium (pH 7.8 ± 0.2) (g L⁻¹)

Burk's medium (Himedia) (21.3 g) was dissolved in 1 L

Specific medium for Azosprillium (Himedia)

The specific medium for *Azospirrilum* was procured from Himedia. Dissolved 0.81 g of part A medium in 95 mL of glass distilled water and 0.4 g of part B on 95 mL of glass distilled water (pH - 7.0).

All the microbial media listed in the thesis under various sections were autoclaved at 15 psi for 15 min in a conventional vertical autoclave unless otherwise mentioned differently.

Serial Dilution method:

Manure (1 g) was weighed accurately and suspended in 100 mL of distilled water, which gave the 10⁻² dilution. The flask containing manure suspension was kept in a shaker at 200 rpm for 60 min and it was kept undisturbed for 30 min. 1 mL of supernatant, was pipette out and transfer to a test tube containing 9 mL of sterile

distilled water. Serial dilution was performed till 10⁻⁶ dilution. The petric plates containing the respective medium was transfer with 1 mL diluted solution the petric plates were incubated at 37 °C for 24 h for total heterotrophic bacterial count and 72 h for fungi, Actinomycetes, *Rhizobium* like colonies, *Azospirillum* and *Azotobacter*.

3.3. Production of biodynamic compost and manure preparation

3.3.1. Preparation of biodynamic compost for comparative analysis

The biodynamic compost was prepared by heap method as described by Procter (1997) in MCRC, Taramani, Chennai on July 2004 and extension centre of MCRC in Vadakadambadi on November 2005. The comparative study, both biodynamic and non biodynamic compost were prepared July 2006 by heap method in MCRC, Taramani, Chennai (Plate 1).

The grass and debris were removed from the place selected for compost preparation; the place was measured and marked as rectangle shape and 5′ X 5′ X 2.5′size. An air tunnel with the bundle of coconut thatches was placed in the middle of the rectangle lengthwise at the bottom of the heap for providing sufficient aeration. The drenched dry matter (carbonaceous) was spread in the rectangle as the first layer with 20 cm height. A thick paste of cow dung slurry was applied up to 2 cm thickness to cover the dry matter completely. The second layer was made with the green matter with a height of 15 cm. Then the crushed slaked lime was sprinkled on green layer. The dry matter layer was repeated with cow dung slurry, 3 kg bore-well soil (rock phosphate), wood ash of 500 g and 3 kg old compost. The preceding two layers (first and second layer) were repeated until the height of the compost heap reaches 2.5 feet. The compost

Plate 1: Preparation of Biodynamic and non biodynamic

- A). Layering of compost
- B). Green layer of biodynamic compost
- C). Biodynamic compost heap
- D). Non biodynamic compost heap



heap was plastered with cow dung. Two grams from each of BD preparation BD502 to BD506, placed individually in middle level of the heap along the longitudinal two sides. BD507 (10 mL of BD507 preparation was mixed with 1 liter of clean water and stirred it vigorously in both clockwise and anti clockwise direction for 20 min) was poured equally into the holes and sprinkled evenly on all the four vertically surfaces of the plastered compost heap. The heaps were also maintained without BD preparation for non biodynamic compost. The compost heap was maintained with sufficient moisture content and not allowed to dry up. The compost heap was mixed and turned every 30 days once. After wetting it, rearranged the heap around the original tunnel.

The temperatures of compost piles were taken from four different locations in the pile such as top, middle, bottom, and surface every day. Samples were taken for analysis from these four locations of each pile at day 0 and then 15 days once until day 120th day (Tiquia and Tam, 2002). The compost sample was analyzed for microbial load of culturable colony-forming units (CFU), total nitrogen, potassium, phosphorus, enzyme activities and plant growth regulators by following the procedures outlined under section 3.2.1 and 3.2.16

3.3.1.1. Estimation of protein in water extract of manure

Concentration of protein in water extract of manure was estimated by following the method as described by Lowry *et al.*, 1951.

Reagents

- 1. **Reagent A:** Sodium carbonate (2%) in sodium hydroxide (0.1 N) Sodium hydroxide (0.4 g) was weighed and dissolved in distilled water 100 mL. To this solution sodium carbonate (2 g) was added.
- 2. **Reagent B:** Copper sulphate (0.5%) in potassium sodium tartarate (1%): Potassium sodium tartarate (1 g) was dissolved in of distilled water (100 mL). To this copper sulphate (0.5 g) was added.
- 3. **Reagent C**: Alkaline copper solution: 50 mL of reagent A and 1 mL of reagent B was mixed. It was mixed just before the use.
- 4. **Reagent D:** Folin Ciocalteu reagent (1 N) was prepared by diluting commercially obtained Folin's Ciocalteu reagent (2 N) with equal volume of distilled water.
- 5. Stock solution: 50 mg of BSA was dissolved in 50 mL of distilled water.
- 6. **Working solution:** 5 mL of the stock was made up to 50 mL with distilled water

Method

In clean test tubes, 1 mL of manure water extract, 0.5 mL of alkaline copper solution was added and allowed to stand at room temperature for 10 min. Diluted Folin-ciocalteau reagent (0.5 mL) was added and mixed well by shaking. The solutions were kept uninterrupted for 30 min and absorbance was read at A₅₀₀ in a Double beam UV visible Spectroscan (Chemito, India). The amount of total protein in the manure was determined from the standard graph prepared with different concentrations of Bovine serum Albumin ranging from 40 to 200 μg/mL.

33.1.2. Estimation of Total sugars in manure

The contents of Total sugars in the aqueous extracts were determined by following the anthrone reagent methods (Hofman and Dusek, 2003).

Reagents

Anthrone: Anthrone reagent (0.2 g) was dissolved in ethanol (5 mL) and 95 mL of 75 % sulphuric acid.

Method

In clean test tubes, water extracts (0.5 mL) were treated with anthrone (2.5 mL) solution in an ice-water and cooled to RT. These solutions were kept in boiling water bath for 10 min and cooled at RT (around 28 °C). The mixtures were shaken well using the vortex machine and absorbance was read at 625 nm. Simultaneously, the negative control was processed with water instead of sample. A standard curve was prepared with different concentration of glucose. Results for HSA were expressed in mg 100 g⁻¹.

33.1.3. Estimation of Total Dissolved Solids (TDS)

The total dissolved solids (TDS) were determined by following the method as described by Ehrman (1994).

In a clean 500 mL of glass beaker containing, 150 mL of distilled water was added and 10 g of fresh manure was mixed thoroughly using magnetic stirrer for 30 min. The slurry was filtered through Whatman No.1 filter paper and the filtrates were collected. The collected filtrates was again passed through a syringe filter (0.5 µm), the filtrates were collected and kept in a hot air oven at 105 °C for 48 hrs. The amount of total dissolved solids in manure was calculated using the formula given below.

Total Dissolved Solids (%) =

Wt. of filtered sample - Wt. of cruicible x 100

3.3.1.4. Estimation of Growth Hormone

The estimation of total potassium in manure sample was analysed as described by Unyayar *et al.* (1996)

Reagents

Methanol, Chloroform, Ammonia and Ethyl acetate

StStock solution

The stock solution was prepared by dissolving 10 mg of plant growth regulators (PGR) such as Indole acetic acid (IAA), Gibberellic acid (GA₃), Cytokinin, Abscisic acid (ABA) and Kinetin individually in 10 mL of methanol.

The amount of total growth hormones in the test sample was determined from the standard graph prepared with different concentrations (10 to 100 μ g) of Indole acetic acid (IAA), Kinetin, Abscisic acid (ABA) and Gibberellic acid (GA₃) (Himedia, Bombay, India).

Method

Extraction and estimation of growth hormones such as IAA, GA₃, ABA and Zeatin in the manure samples were determined by following the modified methods as described by Ünyayar *et al.* (1999). Stored manure samples (5 g) (unmodified) were taken and combined with 60 mL of methanol: chloroform: 2N ammonium hydroxide (12:5:3 v/v/v). Combined extract was treated with 25 mL of distilled water. The chloroform phase was discarded. The water-methanol phase was evaporated by room air. The water phase was adjusted to the pH 2.5 using 1 N HCl or 1 N NaOH, 15 mL ethyl acetate was added to this solution and extracted the ethyl acetate phase. This step

as repeated for pH 7.0 - 11.0 levels. The spectro-photometric assay was read at 222 m and 280 nm wave lengths for IAA, 254 nm for GA₃, 263 nm for ABA, and 269 nm or Zeatin, all standard synthetic IAA, GA₃, ABA, Zeatin and isolated samples.

3.2. Cow horn manure (BD 500)

Cow horn manure preparation was done in MCRC, Taramani, Chennai

lorn collection

The lactating cow horns were collected from slaughter house Veeraganur, Salem District and Kurinji organic farms, Battalagundu.

Cow dung collection

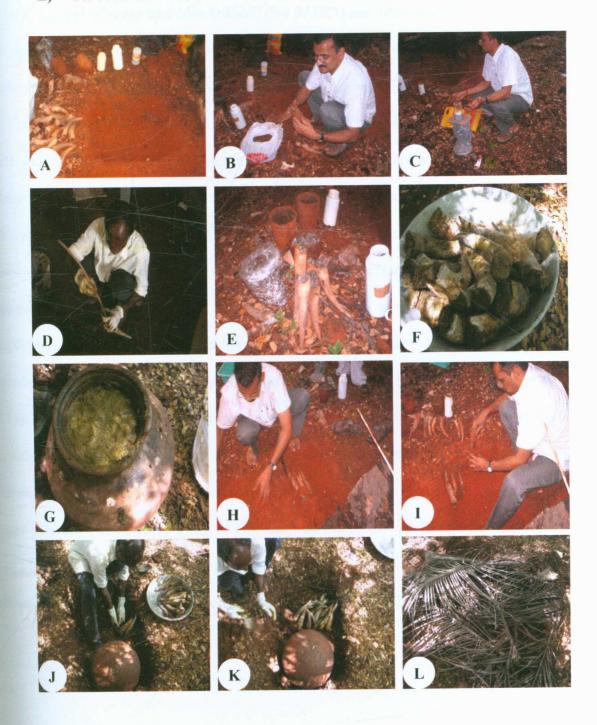
Cow dung, buffalo dung and goat were collected from local farmers. The bedding materials like straw were removed from dung.

Processing of cow horn manure:

The horns were filled completely with the dung. The horn were knocked, its opening at the top, several times against the floor to make sure the manure goes right down into the tip (Plate 2.). The filled horns were buried 40 cm depth in humus-rich arable soil. The wetlands, areas covered by the crowns and roots of trees and shrubs and areas close to walls, roads or ditches were avoided. The pit was making with a size of 2'X2'X1.5'. The base pit was filled with humus-rich topsoil to a depth of about 5 cm. The horns were placed into pit with open end of horn down to ensure drainage. The pit was maintained with moisture condition to provide the suitable condition to microbial tole. The spaces between the rows of horns were filled with loose soil, so that every horn is surrounded. The horn manure sample was analyzed for microbial load of culturable

Plate 2: Preparation of BD 500

- A) Pit, A) to D) Cow, Buffalo, and Goat dung filling in cow horn, mud pot plastic and glass bottles
- E) to G) Filled cow horn, mud pot plastic and glass bottles
- H) to K) Burring cow dung filled horn, bottles (Glass and plastic) and mud pot
- L) Pit covered with coconut leaves



colony-forming units (CFU), total nitrogen, potassium, phosphorus, enzymes activities and plant growth regulators by following the procedures outlined under section 3.2.1. to 3.3.4

3.3.3. Extraction of humic acid and analysis

Air-dried Manure (10 g) was taken and transferred in a conical flask contain 40 mL of 1 MHCl. The pH of setup was adjusted to 2 (0.1 MHCl) and final volume of the liquid was made up to 200 mL with 1 M HCl (liquid/manure ratio = 10:1 V/W). This mixture was kept in a shaker for 60 min and separated the supernatant liquid by centrifugation at 6000 rpm 20⁻¹ min. The pellet was neutralized to pH 7 with 0.1 M NaOH and added with 100 mL of 0.1 MNaOH solution under nitrogen atmosphere. This solution was kept in a shaker for 4 h and allowed to stand overnight. The supernatant was separated by centrifugation at 6000rpm 20 min and acidified the supernatant to pH 1 with 6 M HCl to precipitate the humic acid. The pH adjusted solution was allowed to stand for overnight and centrifuged at 6000rpm 20⁻¹min to separate the fulvic acids fractions. The humic acid fraction was redissolved by adding 0.1 MKOH under nitrogen and then centrifuged at high speed (8000 rpm 20⁻¹ min) to remove the suspended solids. Humic acid was reprecipitated by adding 6 M HCl with constant stirring to pH 1 and Centrifuged. The pellets was transferred to a dialysis tube by slurring with water and dialyzed against distilled water until the dialysis water gives a negative Cl- test with silver nitrate AgNO3. The FT-IR spectra were recorded on pellets obtained by pressing under reduced pressure a mixture of 1 mg of HA and 400 mg of dried KBr, spectrometry grade, using a Nicolet Nexus FT-IR spectrophotometer equipped with a PerkinElmer spectrum one software. Spectra were recorded in the range 4000–400 cm⁻¹ with 2 cm⁻¹ resolution.

3.3.4. Effect of water extract of manure on in vitro growth of spilanthus calva

In vitro grown shoots of spilanthus calva inoculated on water (1 L) supplemented with 20 % water extract of different biodynamic manures (put manure names) and agar (0.8 %) for the evaluation of growth (**Plate 3.**). All cultures were incubated at temperature of 23 ± 1 °C, and 16/8 h of photo periods. The light intensity (cool white fluorescent lamps) was maintained in between 2000-4000 lux.

3.4. Identification of locally available alternative resources

3.4.1. Alternative vessels and dungs for BD500 preparations

The vessels of mud pot, glass bottle and plastic bottle (wide mouth contain) were filled completely with the dung. The vessels were buried in the pit with open end of vessels to touch the ground. The pit was maintained with moisture condition to provide the suitable condition to microbial role. The horn manure sample was analyzed for microbial load of culturable colony-forming units (CFU), nitrogen, potassium, phosphorus, enzymes activities and plant growth regulators by following the procedures are described in the section 3.2 to 3.3.

3.4.2. Identification of locally available alternative herbs from tropical region and production of biodynamic herbal preparations

3.4.2.1. Preparation of BD502 - Yarrow Flowers (Achillea millefolium)

The shade dried flowers (150 g) was placed into a stag's bladder and stitched the cut after filling (Plate 5). The bladder was then hung into a tree for two month from September to October 2006. It was taken down and buried on January 2007 for the duration of six month same way as the cow horns were buried. The spot was marked to make sure to easily the find place again.

Plate 3: Effect of biodynamic manure on *invitro* growth of Spillanthus calva cutting

- A). Control
- B). Treatments -T1(Non-BDC)T2(BDC)T3(BD500CH) T4(BD500CM)T5(BD500CP)T6(BD500GG)
- c). Root measurement

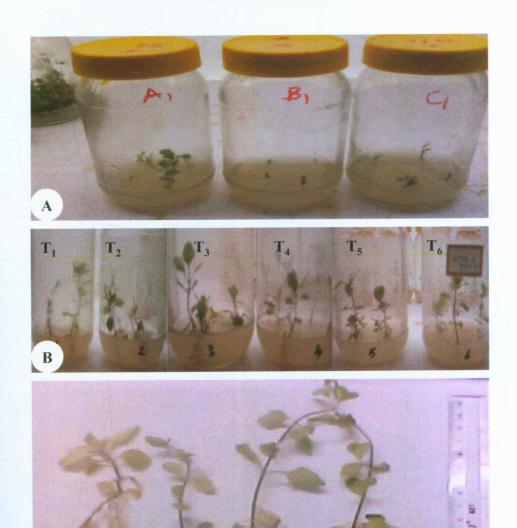
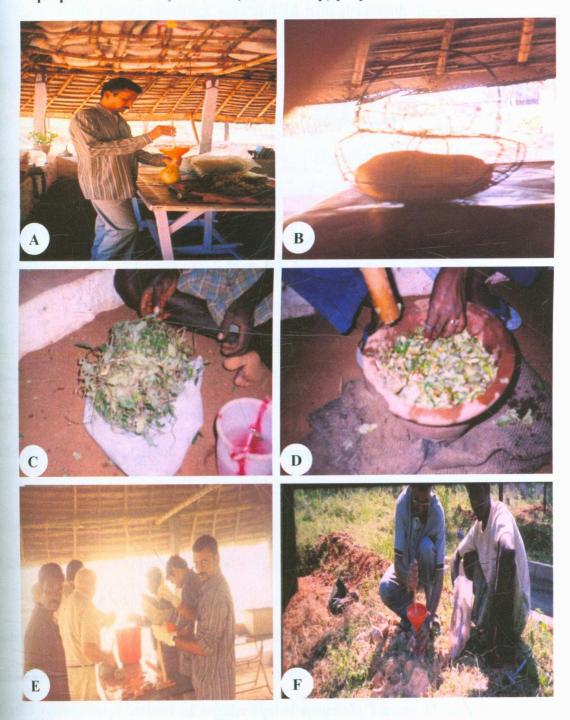


Plate 5: Preparation of of BD prepas' using local herbs

A) and B)BDA502 (Areva lanata) preparation, C) and D) BDA504 (Tragia Involucrata) preparation, E) BDA503 (Tridex procumbens) preparation and D)BDA505 (casuarina sp) preparation



3.4.2.2. Preparation of BD503 - German Chamomile (Matricaria chamomilla)

The cattle (three) intestines were stuffed with chamomile flowers dried under shade. The intestines (Sausages) were kept in a mud pot and cover with fertile sand. The mud pot was buried in soil on September 2006 for 8 months.

3.4.2.3. Preparation of BD504 - Stinging Nettle (*Urtica dioica*)

The plants were harvested just before they flower. The whole plant was dried in shade condition and filled in an unglazed earthenware pot. The pot was buried in healthy soil and left it for a whole year.

3.4.2.4. Preparation of BD505 - Oak Bark (Quercus robur)

Chopped Oak bark was put into the cow skull. The hole of the skull was close with a small stone. The skull was placed in an Earthenware pot and covered the skull with dried plant matter. This pot was placed in a concrete water tank and filled with water on December 2006 for six months.

3.4.2.5. Preparation of BD506 - Dandelion (*Taraxacum officinale*)

The dandelion flowers were packed together and sew in a bovine mesentery (vertebrate membrane). This package was buried in the soil.

3.4.2.6. Biodynamic alternative herbal preparation

The shade dried flowers of Aerva lanata (150 g), Tridax procumbens (150 g), whole plant material of Tragia involucrata (500 g) and Casuarina sp. (150 g) for were used to prepare BD alternative herbal preparation (BDA502, BDA503, BDA504 and BDA505 respectively) instead of regular herbal materials Yarrow Flowers (Achillea milefolium), German Chamomile (Matricaria chamomilla), Stinging Nettle (Urtica

dioica) and Oak Bark (Quercus robur). The preparation methods were followed as described in the session 3.2 to 3.3

3.4.2.7. Estimation of α-cellulose

Reagent

- 17.5 % Sodium hydroxide (NaOH)- NaOH (17.5 g) was dissolved in distilled water of 40 mL and made up to 100 mL with distilled water
- 2. Glacial acetic acid

The α -cellulose content in the plant material was determined gravimetrically by the method as described by Weimer and Zeikus (1977). 1.5 g of plant material was added with 100 mL of NaOH (17.5%), 165 mL of distilled water, stirred the mixture thoroughly and allowed to stand for 60 min. The mixture was filtered through a preweighed cotton cloth using a Buckner funnel. The cotton cloth containing the residue was transferred to a glass beaker, added with 10 mL of acetic acid and kept at 30 °C for 5 min. The residue containing acetic acid was washed with 500 mL of distilled water. The residue was kept in an oven at 105 °C for 12 h. The residue was collected, cooled in the desiccators and weighed. The α -cellulose in the agro waste was calculated based on the formula given below.

Calculation:

Weight of the empty bottle (g) = A

Weight of the cotton cloth (g) = B

Weight of the sample taken (g) = C

Weight of the empty bottle + Weight of the cotton cloth (g) = A + B

Weight of the empty bottle + Weight of the cotton cloth

+ Weight of
$$\alpha$$
 -cellulose after drying (g)
$$= D$$
Weight of α -cellulose (g)
$$= D - (A + B)$$

$$= (D - (A + B))$$

$$= C \times 100$$

3.4.2.8. Estimation of Hemicellulose

Reagent

- 1. Hydrochloric acid (HCL) 12 %
- 2. Phloroglucinol (0.7%)

The hemicellulose content was quantified gravimetrically by following the method as described by Krober's (1901). A round bottom flask of distillating unit containing 1.5 g of each plant material was individually added with 100 mL of HCl (12%), kept on a heating mantle maintained at 100 °C for up to 60 min and collected the distillates, 30 mL of HCl (12%) was used to replenish the boiling process each time. The collected distillate was added with 80 mL of HCl (12%) and 40 mL of phloroglucinol (0.7%) prepared with of HCl (12%) and precipitate was observed. The precipitate was filtered through a muslin cloth and repeatedly washed with distilled water and removed the traces of HCl. The precipitate was kept in an oven at 105 °C for 12 h. The precipitate was collected, cooled in desiccators and weighed. The hemicellulose in the plant material was calculated by applying the formula given below.

Calculation:

Weight of watch glass
$$(g)$$
 = A

3.4.2.9. Estimation of phenolic content in manure

Phenolic compounds (PC) in the aqueous extract of manure were determined by Folin-Ciocalteu method as described by Said-Pullicino, 2007.

Reagents

- 1. Folin-Ciocalteu's reagent
- 2. 2 M sodium carbonate

Procedure

In clean test tubes, water extracts (2.5 mL), Folin-Ciocalteu's reagent (0.2 mL) and 2 M sodium carbonate (0.4 mL) solutions were added and was kept incubated in room temperature for 1 h for the blue colour development and the absorbance was measured at 760 nm. Simultaneously, a negative control was also processed without the addition of the sample. Concentrations were calculated against a calibration curve

prepared with different concentrations of vanillic acid (from 1 to 6 μg mL⁻¹) and expressed in mg Cl⁻¹ vanillic acid-C equivalents.

3.4.3. Chromatogram images of biodynamic manures at various concentrations (Manure alone)

Whatman No 1 filter paper of 1 x1 cm was cut and rolled tightly so as to serve as wick was inserted through the centre hole of the filter paper disc of 15 cm diameter. The filter paper disc was placed on the plastic ring where the filter paper disc containing the wick touches the photo reactive solution. The AgNO₃ solution was allowed to spread up to the point A (3 cm) and the wet wick was removed. The filter paper was left in the box and dried at $28-35\pm 2$ °C for 2 h. Erlenmeyer flask containing 50 mL of NaOH (1 % W/V) was individually added at various concentrations (0.25, 0.5, 1.0, 2.0 and 3.0 g) of biodynamic manures (BD 500). The set up was individually kept in an orbital shaker (120 rpm) for 60 min. The flask was individually removed from the orbital shaker and kept at still condition for 60 min at 30 ± 1 °C. Each extracts (3 mL) was individually dispensed into the watch glass and performed. The chromatograms were exposed for 48 h in daylight and observed for zonation, colour, size, and shape, spikes and radiating spike of image (**Plate 4,6**).

3.4.4. Amendment biodynamic manures in soil sample and development of image through CPC

Erlenmeyer flask containing 50 mL of NaOH (1% W/V) was individually added with 3 g of soil. The flask containing the soil was again amended with various concentrations (0.25, 0.5, 1.0, 2.0 and 3.0 g) of biodynamic manures such as BD 500,

Plate 4: Amendment of BD 500 at different concentration in soil and development of on chromatographic images

- A) BD500
- B) BD500 (0.25g)
- C) BD500 (0.5g)

- D) BD50 (0 1.0g)
- E) BD500 (2.0g)
- F) BD500 (3 g)

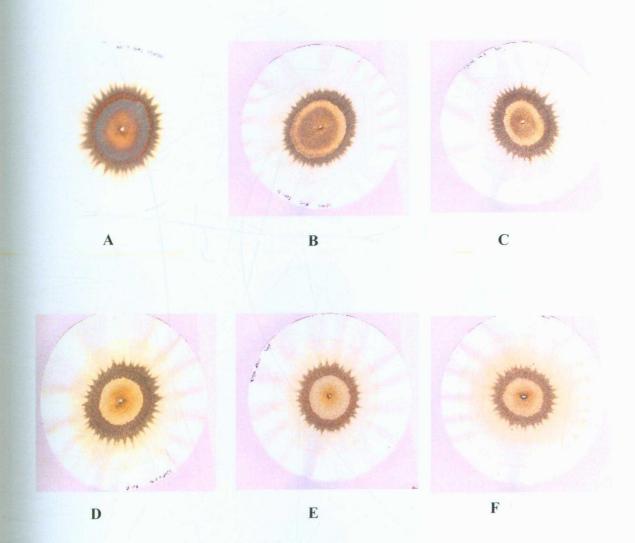
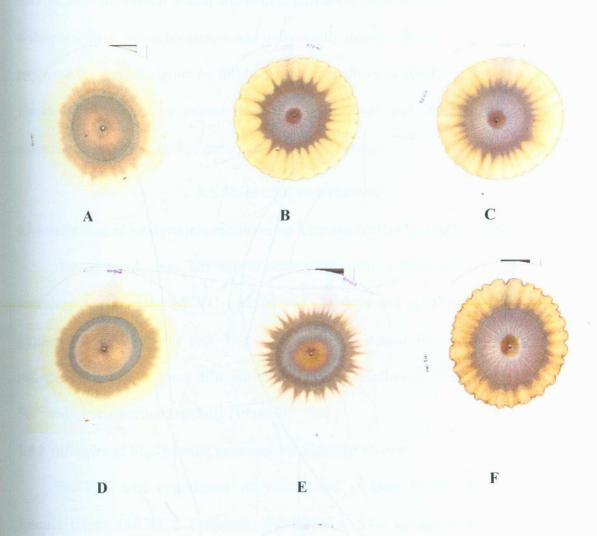


Plate 6: Chromatographic images of different biodynamic manures

- A) BD501 B) BD502 C) BD503 D) BD504
- E) BD505 F) BD506 G) BD507



vermicompost, biodynamic compost and Cow pat pit manure. The set up was individually kept in an orbital shaker (120 rpm) for 1 h. The flask was individually removed from the orbital shaker and kept at still condition for 60 min at 30 ± 1 °C. One to three milliliters of each extracts was individually dispensed into the watch glass and performed the chromatogram by following the procedures as described in section 3.4.3. The chromatograms were exposed for 48 h in daylight and observed for zonation, colour, size, and shape, spikes and radiating spike of image.

3.5. Field trial experiments

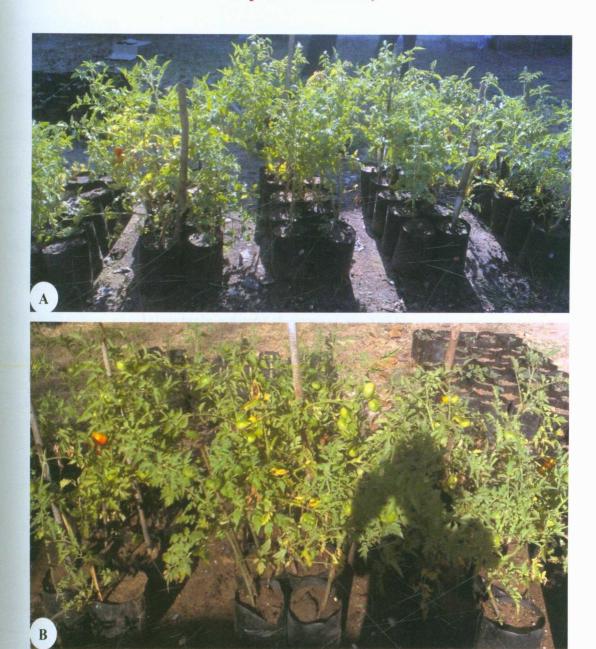
3.5.1. Influence of biodynamic manures on Tomato fruits (Lycopersicon esculentus)

Taramani, Chennai. The tomato seeds were sown in the round dup with soil for developing seedlings in MCRC campus and 25 days old seedling transfer in the polythene bags filled with soil. Two bags were maintained for single treatment as replicate. There twenty three different treatments were followed. The growth of plant, fruit yield, were recorded regularly (**Plate 7**).

3.5.2. Influence of biodynamic manures on Moringa oleifera

The field trial experiment was conducted at Shri AMM Murugappa Chettiar Research Centre (MCRC), Taramani, and Chennai. Moringa seeds were sowed in the polythene bags filled with soil. Two bags were maintained for single treatment as replicate (Plate-8). There twenty three different treatments were followed. The growth of plant, yield of leaves and soil physicochemical properties (pH, EC, N, P, K) were recorded regularly.

Plate 7: A bag experiment of Tomato (A. Tomato plants and B. tomato plant with fruit)



3.5.3. Influence of manures on soil properties and yield attributes of ground nut (Arachis hypogea)

Field experiments were conducted at Shri AMM Murugappa Chettiar Research Centre (MCRC), Taramani, Chennai during February 2006 to June 2006. The temperature during the growth period varied from 28 °C to 37 °C \pm 2 °C. The sowing of seeds was laid out in Randomized block design (RBD) in the experimental plot of 25² mt. There five different treatments were followed. The yield attributes like pod, hulm, dry biomass yield and soil physicochemical properties were recorded regularly.

3.6. Moringa leaves analysis

3.6.1. Estimation of total protein content (Lowry et al., 1951)

Reagents

- 1. Reagent A: Sodium carbonate (2 g) was dissolved in 0.1 N Sodium hydroxide solutions.
- 2. **Reagent B:** Copper sulphate (0.5 g) was dissolved in 100 mL of 1 % Potassium sodium tartrate solution.
- 3. Reagent C: Alkaline copper solution: Mixed 50 mL of A and 1 mL of B prior to use
- 4. Folin Ciocalteau reagent
- 5. Standard protein stock solution: Bovine Serum Albumin (BSA) (50 mg) was dissolved in distilled water and the volume was made up to 50 mL in a standard flask.

Plate 8: A bag experiment of Moringa olifera

- A) Bags with soil
- C) Moringa plant
- E) Dried moringa leaves powder
- B) Germinated seedling
- D) Drying















Plate 9: A field experiment of Ground nut



6. Working Standard: The stock solution (10 mL) was diluted to 50 mL with distilled water in a standard flask. One mL of this solution contained 200μg protein.

Procedure

The standard solution (0.2, 0.4, 0.6, 0.8 and 1 mL) was taken into test tubes. Moringa leaf extract (0.1 mL and 0.2 mL) was taken in two other test tubes and the volume was made up to 1 mL in all the test tubes. A tube with 1 mL of distilled water served as the blank. Reagent C (5 mL) was added to each tube including the blank and mixed well. This setup was allowed to stand for 10 min. Reagent D (0.5 mL) was then added and mixed well. The test tube was incubated at room temperature in dark condition for 30 min till the development of blue colour. The samples were read at 660 mm in spectrophotometer. The total amount of protein was calculated and recorded as g 100⁻¹g of sample.

3.6.2. Estimation of carbohydrates by anthrone method

Reagent

- 1. Perchloric acid (52 %)
- Anthrone reagent Anthrone (200 mg) was dissolved in 100mL of 95 % cold
 Sulphuric acid (freshly prepared)
- 3. Standard Stock Glucose (100mg) was dissolved in 100 mL distilled water. Stock soution (10mL) was diluted to 100 mL with distilled water.

Procedure

Young and old leaves of *Moringa oleifera* (0.5 g each) were added with 2 mL of distilled water and ground in to fine paste by using mortar and pestle. The ground sample was transferred to a test tube and added with 1.5 mL of perchloric acid (52 %). The sample was mixed well and kept for 20 min without disturbing. The mixture was made up to 10 mL with distilled water. The mixture was filtered through a Whatman No. 1 filter paper. The filtrate was collected and the volume was made up to 25 mL with distilled water. From this filtrate, 0.1 mL was taken in a test tube and added with 0.9 mL of water. Anthrone reagent of 4 mL was added and boiled for 8 min in a boiling water bath. The mixture was cooled rapidly at room temperature and read OD at 630 nm in spectrophotometer.

3.6.3. Estimation of β-carotene

Moringa leaves (3g) were ground in a mortar and pestle and 100mL of 12% alcoholic KOH was added. This mixture was kept for 5 min at room temperature. The above mixture was transferred to a separating funnel using alcoholic KOH for rinsing. To this, 40 mL of petroleum ether was added and gently shaken 30 seconds. This content was kept for 30 min to get separate layers. If the alcoholic water layer is still yellow in colour and the layers were not separated, distilled water containing 5 % Na₂SO₄. The petroleum ether layer was collected and allowed to evaporate at RT and the residue volume was finally made up to 5 mL with ethanol. This solution was read at 450 nm. carotenoid was calculated as per the formula given below (Ranganna's cold saponification procedure, 1986).

OD × dilution factor × total extract made up

Carotene (%) =

250

3.7. Analysis of soil physicochemical properties

3.7.1. Estimation of pH and Electrical conductivity (EC) in soil

Soil (25 g) was weighed and mixed with 50 mL of distilled water in the ratio of 1:25 were added. It was stirred well using glass rod and allowed it to stand for 30 min with intermittent stirring. The pH and EC were recorded with pH meter and EC meter (Ecoscan).

3.7.2. Estimation of Organic matter in soil

Reagents:

i. Distilled water

- ii.Standard Potassium dichromate (K₂Cr₂O₇) solution (1 N): K₂Cr₂O₇ (49.04 g) was dissolved (dried at 105°C for 120 min) in 1 L of distilled water.
- iii. Ferrous ammonium sulphate (FeSO₄.(NH₄)₂SO₄.6H₂O) solution (0.5 N): Reagent grade of FeSO₄. (NH₄)₂SO₄.6H₂O (196.1 g) was dissolved 800 mL distilled water and 20 mL Con. H₂SO₄. This was cooled and made up to 1 L with distilled water.
- iv. Diphenylamine indicator: diphenylamine (0.5 g) was dissolved in a mixture of 20 mL distilled water and made upto 100 mL with Con. H₂SO₄.
- v. Orthophosphoric acid (85 %): Orthophosphoric acid (85 mL) was diluted to 100 mL of distilled water.

Procedure

Soil (0.5 g) was weighed (powered and sieved in 0.2 mm sieve) and mixed with 10 mL of 1 N K₂Cr₂O₇ and 20 mL of Con. H₂SO₄ was added and the flask was swirled. The setup was allowed to stand for 30 min and 200 mL of distilled water to arrest further oxidation. 85 % of ortho phosphoric acid (10 mL) was added for stabilizing the oxidation potential of FeSO₄ during titration. Diphenylamine (1 mL) indicator was added in the solution and mixed well. This solution was titrated against ferrous ammonium sulphate (0.5 N) solution till the blue colour turns green. Simultaneously a blank (without soil) was processed. The amount of organic matter prepsent in the soil sample was calculated using the following formulae.

Calculation

Organic Carbon (%) =
$$\begin{array}{c} 10 \text{ (B - T)} & 0.003 \text{ X } 100 \\ & & \text{Wt. of soil (g)} \end{array}$$

Where

B = Volume (mL) of Ferrous or Ferrous ammonium sulphate solution required for blank titration.

T = Volume (mL) of Ferrous or Ferrous ammonium sulphate solution required for soil sample.

Wt. of soil = 1 g.

Organic Matter (%) = Organic Carbon (%) X 1.724.

3.7.3. Estimation of available phosphorous in soil

Reagents:

- i. Distilled water.
 - ii. Sodium Bicarbonate (NaHCO₃₎ 0.5M and pH 8.5 NaHCO₃ (84 g) was dissolved in distilled water and made up to 2 L with distilled water. This solution was mixed thoroughly and pH was adjusted to 8.5 with 1 M NaOH (NaOH (4 g) dissolved in 100 mL) solution.
- iii. Reagent A: Ammonium molybdate (12 g) was dissolved in 250 mL of distilled water in reagent bottle. Antimony potassium tartrate (0.2908 g) was dissolved in 100 mL water separately. These two solutions were mixed with 1 L of 2.5 M H₂SO₄, and made up to 2 L with distilled water. This solution was stored in Pyrex glass bottle in a dark and cool place.
 - iv. Reagent B: ascorbic acid (1.056 g) was dissolved in 200 mL reagent A and mixed. This solution was prepared daily in fresh as required.
 - v. Sulfuric acid 2.5M: Con. H_2SO_4 (140 mL) was diluted to 1 L.
 - vi. P free activated charcoal
 - vii.Standard stock solution: KH₂PO₄ (0.4390 g) was dissolved in distilled water in 1 L volumetric flask. The volume was made up to 1 L. This is 100ppm Phosphate solution.
 - vii. Working standard solution: The required stock was diluted with distilled water to 1:10 ratio (10 ppm concentration).

The series of P₂O₅ standard solution such as 0.5, 1.0, 1.5, 2.0, and 2.5mL was taken in to test tube from the 10 ppm working standard solution. Which was given concentration of 0.2, 0.4, 0.6, 0.8, and 1.00 ppm respectively to this 4.0 mL of reagent B was added. This solution was made up to 25 mL with distilled water.

Estimation of P in the Soil (Rayment and Higginson, 1992)

Soil (5 g) was weighed and mixed with pinch of activated charcoal and 50 mL of 0.5 M NaHCO₃. The solution was shaken in a reciprocating mechanical shaker for 30 min under 180 rpm and filtered through Whatman No.40 filter paper. The filtrate (5 mL) was taken into a 25 mL volumetric flask and acidified with 1 N H₂SO₄ to pH 5.0 using p-nitrophenol indicators. Then, reagent B (4mL) was added and made up to 25 mL with distilled water. It was incubated in room temperature for 10 min.

Calculation

Weight of the soil used in the extraction : 5 g

Volume of 0.5 M NaHCO3 used for extraction : 50 mL

Volume of the extracted solution taken for P estimation : 5 mL

Final volume was made up to 25 mL

Concentration of P2 O5 read in the standard curve

Corresponding to the percent absorbance : a ppm

Therefore the amount of available P in the soil : a X - - - - X - - - = y ppm

3.7.4. Estimation of potassium in soil

Reagents

i. Double distilled water

ii. 1.0 N Ammonium Acetate

iii. Potassium Chloride was dried at 60 °C for 60 min

Preparation of Standard Solution

Potassium chloride (1.5851 g) was dissolved in 1 L of distilled water which

gave 1000 ppm. It was diluted to 1:10 ratio and was taken as working concentration of

100 ppm. Prepared 5, 10 and 15 mL of 100 ppm solution were made up to 50 mL with

distilled water. These solutions contain 10, 20 and 30 ppm of K2O respectively and

were sequentially loaded in flame photometry.

Procedure

Soil (5 g) was taken into the polypropylene bottle and 25 mL of neutral 1 N

ammonium acetate was added. The solution was shaken in a mechanical shaker for 30

min under 180 rpm. The mixture was filtered through Whatman No.1 filter paper and

the filtrate was collected in vials. The filtered extract of soil samples was load in to

flame photometry (Tandon, 1993). The potassium concentration was calculated by

following formulae.

Calculation

Weight of the soil used in the extraction

5 g

Volume of Ammonium acetate extractant used

25 mL

The Concentration of K estimated in AAS

a ppm

Therefore the amount of available K_2O (ppm) in the soil : 25 a X ---- = y 5

3.7.5. Estimation of Calcium (Ca) in soil

Reagents

i.Double distilled water

ii.1.0 N Ammonium Acetate

iii.Calcium Carbonate (CaCO₃)

iv.1M Hydrochloric acid

Standard solution was prepared by dissolving the dried CaCO₃ (2.49 g) in 100 mL of distilled water and 25 mL of 1 M HCl and it was made up the 1 L (1000 ppm of Ca). 1000 ppm solution (10 mL) was diluted to 100 mL with distilled water, which gave 100 ppm Ca solution (1:10 dilution). 5, 15 and 25 ppm standard solution were prepared from 100 ppm solution for drawing standard curve.

Calcium analysis in soil (Tandon, 1993)

Soil sample (5 g) was weighed in the polypropylene shaking bottle and added 1 Nammonium acetate (5 mL). This mixture was shaken in a mechanical shaker for 30 min at 180 rpm. The mixture was filtered through Whatman No.1 filter paper and the filtrate was collected in vials. The blank (distilled water) and standards *viz.*, 5, 15 and 25 ppm were loaded in the auto sampler. After standards, the filtered extract of soil samples was loaded sequentially. The concentration of Ca was estimated in Atomic absorption spectrometer at wavelength of 422.7 nm in Nitrous Oxide – Acetylene flame.

Calculation

Weight of the soil used in the extraction : 5 g

Volume of Ammonium acetate extractant used : 25 mL

The Concentration of Ca estimated in AAS : a ppm

Therefore the amount of available Ca (ppm) in the soil : a X = y

3.7.6. Estimation of Magnesium (Mg) in soil

Reagents:

- i. Double distilled water
- ii. 1.0 N Ammonium Acetate
- iii.Magnesium metal ribbon

iv.5M Hydrochloric acid

Magnesium metal ribbon was used for preparing standard solution by dissolving in 1 g of Magnesium metal ribbon in distilled water and 20mL of 5 M HCl and volume was made up to 1 L (1000 ppm of Mg). For working concentration, this solution was diluted to 1: 10 ratio with distilled water (100 ppm Mg). 5, 15 and 25 ppm standard were prepared from 100 ppm solution.

Magnesium analysis in soil

Soil (5 g) was dissolved in 1 N Ammonium acetate (25 mL). This mixture was shaken in a mechanical shaker for 30 min at 180 rpm. The mixture was filtered through Whatman No.1 filter paper and the filtrate was collected in vials. The blank (distilled water) and standards *viz.*, 5, 15 and 25 ppm were loaded in the auto sampler. Filtered

extract of soil samples and standards were loaded sequentially to the auto sampler. The concentration of Mg was estimated in atomic absorption spectrometer at wavelength of 422.7 nm in Nitrous Oxide – Acetylene flame (Tandon, 1993).

Calculation

Weight of the soil used in the extraction : 5 g

Volume of Ammonium acetate extractant used : 25 mL

The Concentration of Mg estimated in AAS : a ppm

Therefore the amount of available Mg (ppm) in the soil : 25

a X ---- = y

5

3.7.7. Estimation of Sodium (Na) in soil

Reagents

- I. Double distilled water
- II. 1.0 N Ammonium Acetate
- III. Sodium Chloride

The stock was prepared by dissolving 2.542 g of the sodium chloride in one liter distilled water (1000 ppm of Na). For working solution, the stock was diluted to 1:10 ratio (100 ppm Na) with distilled water.

Sodium analysis in soil

gramSoil (5 g) was taken and mixed with 1N Ammonium acetate (25 mL). This mixture was shaken in a mechanical shaker for 30 min at 180 rpm and filtered through Whatman No.1 filter paper, The filtrate was collected in the vials. The concentration of

Na was estimated in atomic absorption spectrometer at wavelength of 589 nm in Airacetylene flame (1.1 - 1.3 L min⁻¹) (Tandon, 1993).

Calculation

Weight of the soil used in the extraction : 5 g

Volume of Ammonium acetate extractant used : 25 mL

The Concentration of Na estimated in AAS : a ppm

Therefore the amount of available Na (ppm) in the soil : 25

5

3.7.8. Estimation of Micronutrients in soil

Reagents:

- I. Double distilled water
- II. 0.0005 M of Diethylene Triamine Pentaacetic Acid (DTPA)
- III. CaCl₂.2H₂O (0.01 M)
- IV. Tri Ethanalammine (0.1 M)

Preparation of Extracting Solution:

DTPA extracting solution (1 liter): Tri Ethanalammine (TEA - 13.1 mL), DTPA (1.967 g) and CaCl₂ (1.47 g) in 100 mL of distilled water and pH was adjusted to 7.3 ± 0.05 with 0.1 *M* HCl. This solution was made up to 1 L with distilled water.

Iron (Fe) Standard Solution: Iron metal powder (1 g) was dissolved in the 5 M HCl (20 mL) and Con.HNO₃ (5 mL). The volume was made in to 1 L with distilled water

(1000 ppm of Fe). This solution was diluted in 1:10 ratio for 100 ppm working standard solution. Various standards were prepared from 100 ppm solution.

Manganese (Mn) Standard Solution: Manganese chloride (MnCl₂.4H₂O) (3.6077 g) was dissolved in 50 mL of Con. HCl and the volume was made up to 1 L (1000 ppm of Mn). This solution was diluted in 1:10 ratio with distilled water for working standard solution (100 ppm Mn solution).

Zinc (Zn) Standard Solution: Zinc metal chips (1 g) were dissolved in 30 mL of 5 M-HCl and the volume was made up to 1 L with distilled water (1000 ppm of Zn). Stock was diluted in 1:10 ratio for working standard solution (100 ppm Zn solution).

Copper (Cu) Standard Solution: Copper metal foil (1 g) was dissolved in 50 mL of 5 M-HNO₃ and the volume was made up to 1 L (1000 ppm of Cu). The stock was diluted in 1:10 ratio for working standard solution (100 ppm Cu solution).

Procedure

Soil sample (10 g) was weighed and dissolved in 20 mL of DTPA extracting solution. It was mixed well by mechanical shaker for 120 min at 180 rpm. This mixture was filtered through Whatman No.42 filter paper and the filtrate was collected in the vials. The concentration of Fe, Mn, Zn, and Cu was estimated in Atomic absorption spectrometer (Tandon, 1993).

Calculation:

Weight of the soil used in the extraction : 10 g

Volume of DTPA extractant used : 20 mL

The Concentration of Fe or Zn or Mn or Cu

estimated in AAS : a ppm

Therefore the amount of available Micronutrients (ppm) 20 in the soil : $a \times ---- = y$

10

3.8. Statistical analysis

Throughout the investigation triplicates were maintained for each experiment. The data were expressed as the means of triplicates. All statistical analysis was performed by ANOVA procedures using SPSS software (Version 14). The significant differences among the means were compared by Tukey - HSD test and P < 0.05 was considered to be statistically significant.



4. EXPERIMENTAL RESULTS

4.1. Procurement of commercial available organic and biodynamic manure quality

All thirty two manures (BD500, BD500B, BD502, BD503, BD504, BD505, BD506, BD507, CPP, CPP-B, BD500-D, BD500-K, compost-K, compost-KG, compost-KK, CPP-K, compost-OYO, callies manure, coir pith compost, compost-B, cow urine, cow dung cake, FYM-P, goat manure, GP-FYM, jeewamirtham, panchakavya, compost— U S, compost— UM, vermicompost—M and vermicompost—R) were collected form Kurinji Organic Foods Pvt. Ltd., Genguvarpatti, Nadavan estate Kodiakanal, Dindugal district, Palani agriclinic, Hosur, Krishnagiri district, Badri, Palani, Dindugal district, Gudur, Andhra Pradesh and Ratnagiri, Maharashtra.

4.1.1. Physicochemical properties of commercial organic and biodynamic manures

Among the manures, CPP recorded a maximum pH 8.5, followed by goat manure, compost-K, BD500, compost-KG and BD500-D recorded pH (8.44, 8.43, 8.4, 8.34 and 8.04 respectively). The pH of panchakaviya (5.47), compost-UM (5.80) and compost-OYO (6.01) were recorded. Among the manures, GP-FYM and BD500-B had high level of electrical conductivity (5.60 dSm⁻¹) followed by BD505 (5.46 dS m⁻¹) and BD504 (5.38 dS m⁻¹) (**Table 2**). Electrical conductivity (1.14, 1.6 and 1.91 dS m⁻¹) was recorded in compost, compost-US and coir pith compost respectively. BD500-D contained a significantly high amount of organic carbon (43.50 %) on par with FYM-P (42.92 %), cow dung cake (41.76 %), cow urine (42.92 %) and vermicompost-R (40.97 %) The lowest amount of organic carbon was recorded in panchakavya (15.54 %) and BD505 (16.24 %).

Interestingly, the CPP-B had highest amount of nitrogen (2.85 %) significantly. The lowest amount of nitrogen (0.59 %) was recorded in panchakavya (**Table 2**). The highest amount of phosphorous (1.27, 1.19, 1.14 and 1.12 %) was significantly recorded in vermicompost-M, jeewamirtham, CPP-B and BD507. Similarly the significant high amount of potassium (1.20 and 1.04 %) was recorded in GP-FYM and goat manure, whereas the cow urine contained potassium (0.05 %) only in cow urine (**Table 2**). The highest amount of micronutrient such as magnesium (0.85 %), manganese (767 ppm), zinc (132 ppm) and copper (78 ppm) was recorded in BD500-K, compost-M, compost-KK respectively. whereas the amount of calcium (1.04 %), sodium (61 %) and iron (3219 ppm) were recorded in BD502 and compost-B (**Table 3**).

4.1.2. Quantity of Humic acid in commercial available organic and biodynamic manures

Among the thirty two manures, the biodynamic manures such as CPP- K (493.10 mg 100g⁻¹), compost-OYO (484 mg 100g⁻¹), BD505 (474.50 mg 100g⁻¹), compost-K (473.70 mg 100g⁻¹), BD500 (466.05 mg 100g⁻¹) and CPP-B (465.69 mg 100g⁻¹) recorded for highest amount of humic acid. Lowest amount of humic acid (280.66 281.93, 300.45 and 308.20 mg 100g⁻¹) was recorded in cow urine, compost-UM, BD500B and GP-FYM respectively (**Table 3**).

4.1.3. Activities of enzyme changes in commercial organic and biodynamic manures

Among the thirty two manures, the compost-K collected from Kurinji farms recorded for the highest activity of protease (493.10 µg tyrosine released g⁻¹ of manure

Humic acid	308.2±8.89	312.4±9.01	326.25±9.41	321.65±9.28	384.04±11.08	338,40±9.76	466.05±13.45	316 50+9 13	456 50+13 17	474 50±13 69	484.00±13.97	343.10±9.90	436.00±12.58	320.70±9.25	393.80±11.36	356.95±11.9	316.95±9.15	300.45±8.67	445.40±12.85	473.70±13.67	436.75±12.60	493.10±14.23	338 70+9 77	368.55±10.64	281.92±8.13	341.92 ± 9.86	374.84 ± 10.82	280.65 ± 8.10	465.68±13.44	391.99±11.31	402.25±11.61	365.64±10.55
Cu (ppm)	10±0.28	4±0.11	2±0.05	32±0.92	3±0.08	2±0.05	11 ± 0.31	4±0.11	17±0.49	9±0.25	66±1.90	43±1.24	41±1.18	4±0.11	38±1.09	4 ± 0.13	55±1.58	53±1.52	5±0.14	74±2.13	4±0.11	48±1.38	78±2.25	2 ± 0.05	41±1.18	11 ± 0.31	0.14 ± 0.005	39±1.12	25±0.72	21 ± 0.70	8±0.26	8±0.13
Zn (ppm)	29±0.83	14±0.40	36±1.03	34±0.98	39±1.12	77±2.22	88±2.54	75±2.16	114±3.29	96±2.77	67±1.93	82±2.36	85±2.45	66 ± 1.90	99±2.86	39±1.3	79±2.28	97±2.80	69 ± 1.99	80±2.30	78±2.25	73 ± 2.10	132 ± 3.81	57±1.64	32 ± 0.92	21 ± 0.60	7.6 ± 0.21	48 ± 1.38	25 ± 0.72	21 ± 0.70	45 ± 1.50	63±1.05
Mn (ppm)	597±17.23	193±5.57	265±7.64	767±22.14	707±20.40	237±6.84	451 ± 13.01	472±13.62	289±8.342	449±12.96	230±6.63	542±15.64	138±3.98	416±12.00	569±16.42	333±11.1	152±4.38	485±14.00	452±13.04	165±4.76	424±12.23	212±6.11	186 ± 5.36	413±11.92	203±5.86	232±6.69	13 ± 0.37	89±2.56	219 ± 6.32	351±11.7	206±6.86	482±8.03
Fe (ppm)	1853±53.49	1694±48.90	1786±51.55	2343±67.63	1766±50.98	2026±58.48	2987±86.22	2692±77.71	2577±74.39	2713±78.31	1848 ± 53.34	2431±70.17	2168±62.58	2111±60.93	3219±92.92	2026±67.53	2642±76.26	2956±85.33	2295±66.25	2162±62.41	2636±76.09	2426±70.03	2254 ± 65.06	2187±63.13	3892±112.3	3841 ± 110.88	51.2±1.47	2699±77.91	1660±47.92	1894 ± 63.13	2204±73.46	2271±37.85
Na (%)	0.21±0.006	0.12±0.006	0.17±0.006	0.26±0.006	0.18 ± 0.006	0.22 ± 0.006	0.21 ± 0.006	0.12 ± 0.003	0.11 ± 0.003	0.20 ± 0.006	0.09 ± 0.003	0.53 ± 0.017	0.12 ± 0.003	0.22 ± 0.006	0.61 ± 0.017	0.30 ± 0.009	0.09 ± 0.003	0.49 ± 0.011	0.22 ± 0.006	0.11 ± 0.003	0.19 ± 0.006	0.10 ± 0.003	0.11 ± 0.003	0.22 ± 0.006	0.19 ± 0.006	0.13 ± 0.006	0.09 ± 0.001	0.21 ± 0.006	0.57 ± 0.014	0.68 ± 0.023	0.61 ± 0.020	0.25±0.003
Mg. (%)	0.40±0.011	0.34±0.011	0.54±0.017	0.4/±0.011	0.33±0.008	0.45±0.011	0.79±0.023	0.69 ± 0.020	0.85 ± 0.026	0.58±0.017	0.31±0.011	0.79±0.023	0.28±0.005	0.80±0.020	0.83±0.023	0.73±0.023	0.26±0.005	0.81±0.023	0.80±0.023	0.34±0.011	0.6/±0.017	0.31±0.011	0.28±0.005	0.82±0.023	0.31±0.011	0.26±0.005	0.10±0.003	0.75±0.023	0.63±0.020	0.66±0.023	0.70±0.023	0.39±0.009
Sample No	GP-FYM	Callies Manure	Goat Manure	Compost	Cowdung Cake	BD500-D	BD500	BD504	BD500- K	BD505	Compost-OYO	CPP	Compost-KG	BD502	Compost-B	FYM-P	Coir pith compost	BD500B	BD200	Compost-K	BD503	CPP-K	Compost-KK	BD50/	Compost- UM	Compost-Us	Fanchakavya	Cow urine	CPP-B	Jeewamirtham	Vermicompost-M	v ennicompost-re

2h-1) followed by CPP-K (391.00 μg tyrosine released g-1 of manure 2h-1) and panchakavya (414.19 µg tyrosine released g⁻¹ of manure 2h⁻¹). The lowest activity of protease (128.40, 178.73 and 192.40 µg tyrosine released g⁻¹ of manure 2h⁻¹) was recorded in callies manure, GP-FYM from nandana farm, Gudur and BD502 respectively. The lowest cellulase activity (102.66 µg glucose released g⁻¹ of manure 24 h⁻¹) was recorded in BD507 (Table 4). The highest cellulase activity (299.20, 291.65, 287.55 287.23 and 283.60 μg glucose released g⁻¹ of manure 24 h⁻¹) was recorded in BD502, BD503, CPP-K, goat manure and BD500-D respectively. Among the manure, highest value of invertase (391.95 µg glucose released g⁻¹ of manure 24 h⁻¹) was recorded in compost-M followed by compost-B (357.75 µg glucose released g-1 of manure 24 h⁻¹) and BD506 (343.10 μg glucose released g⁻¹ of manure 24 h⁻¹). The lowest value of invertase (127.50 µg glucose released g⁻¹ of manure 24 h⁻¹) was recorded in callies manure. The highest activity of alkaline phosphatase (393.80 µg p-Nitrophenol released g-1 of manure h-1) was recorded in compost-M and the lowest (119.20, 126.05 and 135.70 µg p- Nitrophenol released g⁻¹ of manure h⁻¹) in GP-FYM, goat manure and BD500-K respectively (Table 4).

4.1.4. Microbial populations in commercial available organic and biodynamic manures

The more number of total bacteria was recorded in compost-K (30.38 x 10⁶ CFU g⁻¹) followed by CPP-K (26.12 x 10⁶ CFU g⁻¹), compost-KK (25.40 x 10⁶ CFU g⁻¹) and panchakavya (25.10 x 10⁶ CFU g⁻¹) (**Table 5**). The lowest number of total bacteria (12.02 and 11.06 x 10⁶ CFU g⁻¹) was recorded in organic manure of GP-FYM and

re	1.10	manife 24 h ⁻¹)	manure 24 h ⁻¹)	of manure h ⁻¹)
anure	manure 24)	160.85±4.64	138.50±4.00	119.2±11.36
anure	128.40±3.71	167.98±4.85	127.50±3.68	151.80±3.91
	264.10±7.62	287.23±8.29	170.70±4.93	126.04 ± 4.62
ure	308.80±8.91	217.10±6.27	391.95±11.32	256.55±9.90
	173.35±5.01	225.10±6.50	137.60±3.97	153.5±4.48
Cake	265.90±7.68	283.60±8.19	173.90±5.02	173.34±4.99
Q	372.5±10.75	248.45±7.17	318±9.18	233.40±9.28
	224.90±6.49	246.45±7.11	158.20±4.57	393.8±7.47
	262.55±7.58	277.15±8.00	168.50±4.86	135.69±7.19
K	278.60±8.04	238.40±6.88	172.60±4.98	160.20±4.53
	295.85±8.54	220.35±6.36	130.50±3.77	343.1±6.00
post-OYO	376.75±10.88	243.75±7.04	306.85±8.86	155.34 ± 8.67
	295.55±8.53	270.45±7.81	174.50±5.04	172.9±6.85
st-KG	192.40±5.55	299.20±8.64	272.90±7.88	321.65±10.11
	360,40±10.40	224.65±6.48	357.75±10.33	258.79±9.20
-B	157.45±5.25	180.40±6.01	237.35±7.91	249.35±10.34
	132.65±3.83	272.15±7.86	188.00±5.43	157.25±6.40
compost	336.10±9.70	239.05±6.90	248.35±7.17	207.99±7.17
~	268.80±7.76	241.30±6.97	343.10±9.90	300.45±9.90
	493.10±14.23	252.55±7.29	168.25 ± 4.86	237.35±4.85
y-K	223.80±6.46	291.65±8.42	310.15±8.95	350.35±8.95
	391.00±11.29	287.55±8.30	129.60±3.74	318.7±3.74
	306.05 ± 8.83	249.80±7.21	213.25±6.15	310.15±6.15
st-KK	228.95±6.61	240.34 ± 6.94	318.70±9.20	221.8±9.20
	300.69±8.68	102.66 ± 2.96	143.54 ± 4.14	282.69±8.57
1	336.82±9.72	197.99±5.72	266.15±7.68	297.18±9.54
	414.19±11.96	289.24±8.35	329.88±9.52	330.51±7.29
ya	337.22±9.73	141.51 ± 4.08	181.52±5.24	252.83±8.12
ine	356.54 ± 10.29	236.25±6.82	278.62±8.04	281.51±8.04
	296.21±8.55	147.85 ± 4.27	220.28±6.36	278.61±7.51
	377.16±10.89	222.52 ± 6.42	224.95±6.50	260.28±6.49
vermicompost-M 37	373.03±10.77	143.59±4.15	178.45±5.15	224.95±5.14

Fungi	4.38±0.127	4.98±0.144	5.19±0.150	5.9±0.170	6.03 ± 0.173	6.59 ± 0.191	6.21 ± 0.179	6.26 ± 0.179	6.86 ± 0.196	7.28±0.208	7.5±0.217	7.65±0.219	7.86±0.225	7.86±0.225	8.02±0.231	8.07±0.267	8.11 ± 0.237	8.23±0.237	8.44 ± 0.242	9.11 ± 0.266	9.30±0.268	9.68±0.277	11.10 ± 0.320	11.26 ± 0.323	7.70±0.222	7.25±0.208	9.06±0.263	6.34 ± 0.182	9.51 ± 0.274	6.51 ± 0.188	8.61 ± 0.248	7.25±0.208
Actinomycetes	12.49±0.36	8.99±0.26	8.50±0.25	16.52 ± 0.48	8.49±0.25	19.99±0.58	15.98±0.46	17.99±0.52	14.50±0.42	17.99±0.52	16.49±0.48	17.98±0.52	16.50±0.48	20.00±0.58	14.72±0.43	10.00±0.33	13.50±0.39	16.58±0.48	16.49±0.48	13.99 ± 0.40	17.00±0.49	18.50±0.53	16.49±0.48	23.50±0.68	14.64 ± 0.42	11.10 ± 0.32	18.83 ± 0.54	12.40 ± 0.36	14.05 ± 0.40	13.65±0.39	15.10 ± 0.44	11.23±0.32
Rhizobium	6.42±0.18	7.38±0.21	5.36±0.15	4.99±0.14	5.71±0.18	6.15 ± 0.17	5.76±0.01	8.44±0.24	7.26±0.20	7.86±0.22	9.59±0.27	3.48 ± 0.09	6.15 ± 0.17	8.07±0.23	7.74±0.22	5.94±0.20	6.42±0.18	4.14±0.12	11.10±0.32	9.96±0.28	11.26 ± 0.32	8.73±0.25	8.46±0.24	7.86±0.22	4.56±0.13	6.67 ± 0.19	10.14 ± 0.29	9.10 ± 0.26	8.61 ± 0.24	7.25±0.20	5.89 ± 0.17	7.70±0.22
Azospirillum CFU 10° g-1	1.07±0.03	1.00±0.02	1.05±0.03	1.80±0.05	0.91 ± 0.02	1.49 ± 0.04	1.36 ± 0.04	1.76±0.05	2.60±0.07	2.71 ± 0.08	1.36 ± 0.04	1.76 ± 0.05	2.18±0.06	1.43±0.04	1.55 ± 0.04	0.71 ± 0.02	1.07±0.02	1.32 ± 0.04	1.93 ± 0.05	1.10 ± 0.03	1.55 ± 0.04	2.96±0.08	1.46 ± 0.04	1.61 ± 0.04	1.05 ± 0.02	1.05 ± 0.02	2.32 ± 0.06	1.06 ± 0.02	1.94 ± 0.05	1.42±0.04	1.10 ± 0.03	0.98 ± 0.02
Azətobacter	1.56±0.046	1.36±0.04	1.55±0.04	3.12±0.09	2.08±0.05	3.08±0.08	2.40 ± 0.06	3.00±0.08	3.76 ± 0.10	4.10 ± 0.11	3.86 ± 0.10	2.36 ± 0.06	4.02±0.11	3.64 ± 0.10	2.24±0.06	2.90±0.09	3.96±0.11	3.74 ± 0.10	3.66±0.10	6.26±0.17	6.22±0.17	3.78 ± 0.10	4.70±0.13	4.02 ± 0.11	3.29±0.09	4.16 ± 0.12	4.14 ± 0.12	3.98 ± 0.12	5.09 ± 0.14	3.09 ± 0.08	4.28 ± 0.12	3.12 ± 0.09
Total Bacteria	12.02±0.34	13.42±0.38	15.80±0.45	22.42±0.64	16.38 ± 0.47	18.40 ± 0.53	17.62 ± 0.50	23.28±0.66	16.56±0.47	21.16 ± 0.61	22.44±0.64	17.06±0.49	21.54 ± 0.62	21.70±0.62	23.32±0.67	17.38±0.57	13.04±0.37	18.08±0.51	19.20±0.55	30.38±0.87	19.98±0.57	26.12 ± 0.75	25.40±0.73	22.90±0.66	12.72 ± 0.36	14.64 ± 0.42	25.10±0.72	18.83 ± 0.54	22.40 ± 0.64	11.05 ± 0.31	23.65 ± 0.68	14.99±0.43
Sample No	GP-FYM	Callies Manure	Goat Manüre	Compost	Cowdung Cake	BD500-D	BD500	BD504	BD500- K	BD505	Compost-OYO	CPP	Compost-KG	BD502	Compost-B	FYM -P	Coir pith compost	BD500B	BD506	Compost-K	BD503	CPP-K	Compost-KK	BD507	Compost- UM	Compost-US	Panchakavya	Cow urine	CPP-B	Jeewamirtham	Vermicompost-M	Vermicompost-R

jeewamirtham. Both compost-K and BD503 from Kurinji farms, Ganguvarpatti, recorded significantly highest number of Azotobacter (6.26 and 6.22 x 106 CFU g-1). The lowest number of Azotobacter (1.37, 1.55 and 1.56 x 10⁶ CFU g⁻¹) was recorded in three manures of callies manure, goat manure and GP-FYM. Among the three manures, CCP-K from Kurinji farms, Genguvarpatti, contained number of Azospirillum (2.96 x10⁶ CFU g⁻¹) followed by BD500-K from Kurinji farms, Genguvarpatti, (2.60 x 10⁶ CFU g⁻¹) and BD505 (2.71 x 10⁶ CFU g⁻¹). However organic manure of FYM-P from Poolambadi contained a few colonies of Azospirillum (0.71 x 10⁶ CFU g⁻¹). BD506 and BD503 contained a high number of Rhizobium sp. (11.10 and 11.26 x 10⁶ CFU g⁻¹) followed by panchakavya (10.14 x 10⁶ CFU g⁻¹), compost-K (9.96 x 10⁶ CFU g⁻¹) and compost-OYO (9.60 x 106 CFU g-1). Whereas CPP and BD500B contained low number of Rhizobium sp. (3.48 and 4.14 x 10^6 CFU g^{-1}). The BD 507, BD500-D and BD502 recorded highest significant number of actinomycetes (23.50, 20.00 and 20.00 x 10^6 CFU g⁻¹). The manures of callies, goat and cow dung cake had significantly low number of actinomycetes (9.0, 8.50 and 8.50 x 10⁶ CFU g⁻¹). Among the manures BD 507 and compost-KK from Kurinji farms, Ganguvarpatti, recorded significantly highest number of Fungi (11.26 and 11.10 x 10^6 CFU g⁻¹) followed by compost-K (9.12 x 10^6 CFU g⁻¹), BD 503 (9.30 x 10^6 CFU g^{-1}) and CPP-B (9.52 x 10^6 CFU g^{-1}). GP-FYM and callies manure recorded a lowest number of fungi (4.38 and 4.98 x 10⁶ CFU g⁻¹) (Table 5).

4.2. Impact of BD herbal preparation on compost properties

Comparative analysis of BDC (T_2) , BDCV – I (T_4) , BDCV – II (T_3) and Non – (T_1) were analysed (physico – chemical properties, microbial enumeration,

enzymes activities, biochemical properties and PGR) in different time interval [0 (D_1), 15 (D_2), 30 (D_3), 45 (D_4), 60 (D_5), 75 (D_6), 90 (D_7), 105 (D_8), 120 (D_9), 135 (D_{10}) and 150 (D_{11})] and the results were discussed below.

4.2.1. Changes in physico – chemical properties of biodynamic and non biodynamic compost

4.2.1.1. pH in compost

The residual pH of the different compost prepared at MCRC, Chennai and Vadakadambadi are illustrated in **Fig. 3.1b.** Among the compost treatments, BDCV-II recorded for neutral pH (7.93 and 7.91) followed by BDC (7.80), BDCV-I (7.77) and Non-BDC (7.74).

Among the two different compost (BDC and Non - BDC), high pH (7.93) was recorded in BDCV-II on 75th day and low pH (7.10) was recorded at 0th day in Non-BDC. However, the interaction effect between composts was found to be non significant.

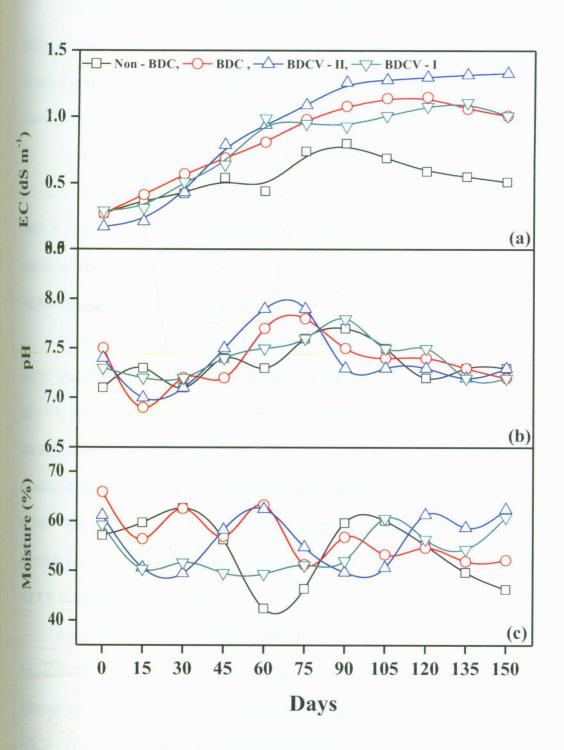
4.2.1.2. EC in compost

In all the treatments (BDC, BDCV - I, BDCV - II and Non - BDC), EC of the compost was recorded to be non significant at all the composting stages.

The EC values were found to be significant between the days and recorded the highest value (1.33 dS m⁻¹) at D_8 stage (105th day) and the lowest value (0.17 dS m⁻¹) at D_1 (0th day). The interaction effect of different treatments and various stages recorded the maximum value in T_3 at D_{11} stage (1.33 dS m⁻¹) and minimum in T_2 at D_1 (0.17 dS m⁻¹) (Fig. 3.1a).

Fig: 3.1 Changes in physico – chemical properties of biodynamic and non biodynamiccompost

a) Electrical conductivity (EC), b). PH, c). Moisture content



4.2.1.3. Moisture in compost

The moisture content was significantly influenced by all the treatments and the results are represented in **Fig. 3.1c**. Significantly higher moisture content (65.85 %) was recorded in BDC (T_2) and lower value (42.37 %) was recorded in Non-BDC (T_1) BDC recorded the second highest value (63.25 %), which was significantly on par with T_4 .

In case of different days, the highest value (58.20 %) was observed at D_4 (45 day) and the lowest value (49.65 %) was recorded at D_{10} (135 day). The interaction effect of treatments and various stages recorded maximum value in T_2 at D_1 (65.9 %) and minimum value (42.4 %) in T_1 at D_5 .

4.2.1.4. Organic Carbon in compost

The organic carbon content of the compost in all the treatments was significantly higher (**Fig 3.2a**). The organic carbon content was significantly higher (58.09 %) in BDCV-I, followed by BDCV-II T₃ (54.46 %), BDC T₂ (57.09 %) and Non-BDC T₁ (56.09 %) respectively. Organic carbon content was significantly altered over time and recorded higher value (58.01 %) at 0th day and the lowest value (34.08 %) was recorded at 150th Day. In the interaction effect of treatments with various stages, highest value (57.09 %) in T₂ at D₁ stage and the lowest value (33.05 %) were recorded in T₃ at D₁₁.

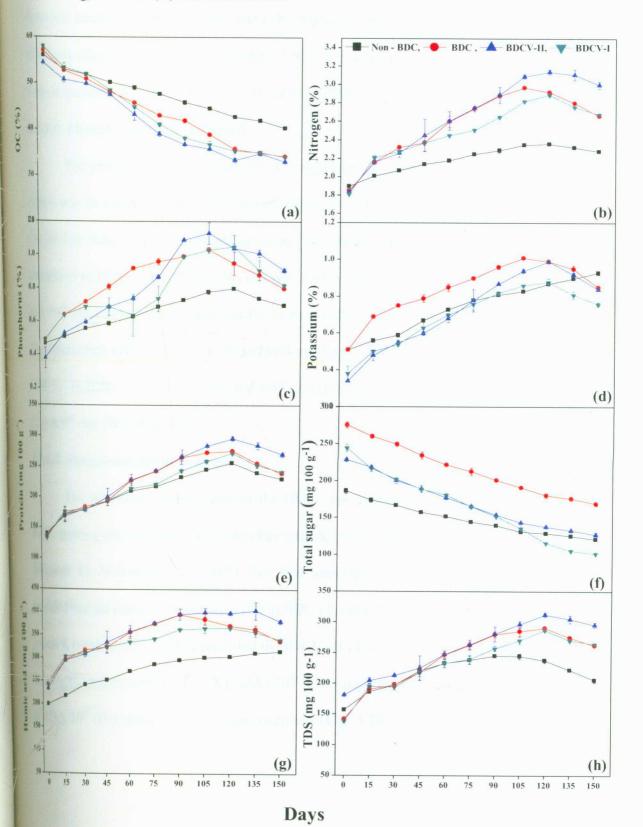
4.2.1.5. Nitrogen (N) in compost

A significant difference in nitrogen (N) of compost was recorded in BDC and Non-BDC. The nitrogen (N) was significantly higher (3.14 %) in BDCV-II (T₃), followed by (2.92 %) in BDC. The lowest nitrogen (N) (1.81 %) was recorded in BDCV-I (T₄). The nitrogen (N) was found to be highly significant in 105th (D₈), 120th

Fig:3.2.Changes in physico – chemical properties of biodynamic and non biodynamic compost

a) Organic carbon (OC), b) Nitrogen, c) Phosphorus, d) Potassium, e) Protein, f) Total sugar,

g) Humic acid, h) Total dissolved solid



 (D_9) and 135^{th} (D_{10}) days respectively and minimum at 0^{th} day (D_1) . Interaction effect between treatments and days recorded the highest value of 3.14 in BDCV-II (T_3) at 120^{th} day (D_9) , whereas the lowest value (1.81 %) in BDCV-I (T_4) at 0^{th} day (D_1) which was on par with BDCV-II (T_3) at 0^{th} day (D_1) and BDC (T_2) at 0^{th} day (D_1) (Fig. 3.2b).

4.2.1.6. Phosphorus (P) in compost

The phosphorus (P) in compost was significantly higher (1.09 %) in BDCV-II, which was on par with BDCV-I (T_4) (1.05 %) at 120^{th} (D_9) day. The Non-BDC recorded the lowest value (0.66 %) of phosphorus (P), which was significantly lesser, when compared to BDC. The phosphorus (P) was highly significant at 105^{th} (D_8), 120^{th} (D_9), and 90^{th} (D_7) days respectively and the lowest value (0.38 %) in BDCV-II at 0^{th} day. The interaction effect of treatments and various days recorded highest phosphorus (P) of 0.99 % in BDC (T_2), BDCV-I (T_4) at 90^{th} day (D_7) and lowest of 0.38 % in BDCV-II (T_9) at 0^{th} day (D_7) (Fig. 3.2c).

4.2.1.7. Potassium (K) in compost

The potassium (K) content in the BDC was significantly differing from Non-BDC. Among the composts, highest potassium (K) was recorded in BDC (T_2) (0.84 %), followed by Non-BDC (T_1) (0.74 %). The interaction between treatments and days recorded that the content of potassium (K) in BDC (T_2) at 105 (D_8) was highest (1.01 %) and lowest K content (0.34 %) was recorded in BDCV- II (T_3) at 0th (D_1). The potassium (K) was highly significant at 75th (D_6), and 120th (D_{10}), days respectively and the lowest at 0th (D_1), 30th (D_3) and 45th (D_4) days respectively (**Fig. 3.2d**).

4.2.1.8. Sugar content in compost

The sugar content of the compost (**Fig.3.2f**) was significantly influenced by different treatments. BDC (T_2) recorded significantly higher value of 228.0 mg 100 g⁻¹ followed by 189.1 and 164.6 mg 100 g⁻¹ in the BDCV-II (T_3) and BDCV-I (T_4) respectively. The lowest sugar value of 101.73 mg 100 g⁻¹ was recorded in BDCV-1 at 150th day (D_{11}). The highest sugar value (275.60 mg 100 g⁻¹) was recorded in the BDC (T_2) at 0th day (D_1). Among different treatments, the sugar content was progressively decreased and recorded the lowest value of 169.9 mg 100 g⁻¹ in the BDC (T_2) at 150th day (D_{11}).

4.2.1.9. Humic acid in compost

Humic acid content was significantly higher (228.0 mg 100 g⁻¹) in BDC (T_2) followed by BDCV-II (T_3) (189.1 mg 100 g⁻¹) and BDCV-I (T_4) (164.6 mg 100 g⁻¹). The Non-BDC (T_1) recorded the lowest humic acid content of 130.1 mg 100 g⁻¹. In case of various stages of composting, the maximum value of humic acid (404.21 mg 100 g⁻¹), was recorded in BDCV-II at 135th day (D_{10}) while 0th day (D_1) recorded the minimum value (199.78 mg 100 g⁻¹) in Non- BDC. The interactions among the treatment, the lowest humic acid of 316.07 mg 100 g⁻¹ was recorded in Non-BDC (T_1) at 150th day (D_{11}) and highest value (396.48, 398.70, 400.65 and 404.21 mg 100 g⁻¹) was recorded in BDCV-II (T_2) at 90th (D_7), 120th (D_9), 105th (D_8) and 135th (D_{10}) days respectively (Fig. 3.2g).

4.2.1.10. Total Dissolved Solid (TDS) in compost

From the result (**Fig. 3.2h**), it is evident that the total dissolved solid was significantly higher (256.8 mg 100 g⁻¹) in BDCV-II (T3), followed by BDC (T₂) of 240.7 mg 100 g⁻¹, BDCV-I (T₄) of 233.5 mg 100 g⁻¹. The lowest TDS value of 215.9 mg 100 g⁻¹ was recorded in Non - BDC. In case of different days of compost, the maximum value of 283.1 mg 100 g⁻¹ was recorded on 120th day (D₉), followed by the minimum value of 151.9 mg 100 g⁻¹ on 0th day (D₁). Interaction effects between treatments and days, recorded the highest TDS value of 312.4 mg 100 g⁻¹ in BDCV-II (T₃) at 120th day (D₉) and the lowest value of 138.8 mg 100 g⁻¹ in BDCV-I (T₄) at 0th day (D₁).

4.2.1.11. Protein content in compost

Protein content was significantly higher (233.1 mg 100 g⁻¹) in BDCV-II (T_3) (Fig. 3.2 e), followed by BDC (T_2) (224.45 mg 100 g⁻¹). The lowest value of 211.0 mg 100 g⁻¹ was recorded in Non - BDC (T_1).

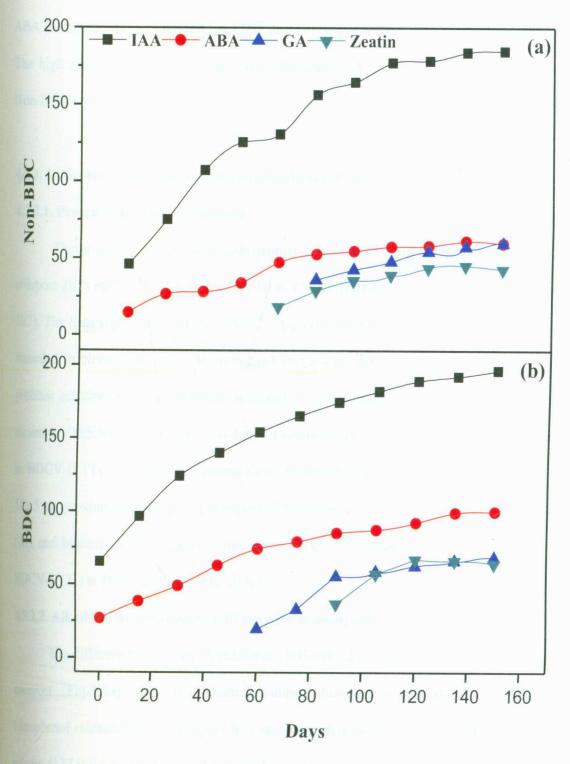
Among the various days of observation, the highest value (275.5 mg 100 g⁻¹) in BDC (T_2) at 120th day (D_9) and lowest value (136.4 mg 100 g⁻¹) in BDC (T_2) at 0th day (D_1) were recorded. Among the interaction between treatments and days, the higher protein (296.7 mg 100 g⁻¹) was recorded in BDCV-II (T_3) at 120th day (D_9) and lowest protein (133.4 mg 100 g⁻¹) in BDCV-I (T_4) at 0th day (D_1).

4.2.1.12. Plant growth regulators in compost

Among the composts, the high IAA (196.38 µg 100 g⁻¹) in BDC at 150th day compared to Non-BDC (184.81 µg 100 g⁻¹) (Fig. 3.5). The gibberllic acid production was recorded at 60th day of BDC and 75th day of Non-BDC. The high gibberillic acid

Fig: 3.5. Influence of BD preparation on plant growth regulator in biodynamic and non biodynamic compost

a). Non-BDC, b) BDC



(67.93 μ g 100 g⁻¹) in BDC at 150th day compared to Non-BDC. Significantly, the high ABA (99.63 μ g 100 g⁻¹) in BDC at 150th day compared to Non-BDC (59.74 μ g 100 g⁻¹). The high zeatin (66.86 μ g 100 g⁻¹) was recorded at 120th day of BDC com;pored to Non-BDC (43.63 μ g 100 g⁻¹)

4.2.2. Influence of enzyme activities in biodynamic and non biodynamic compost 4.2.2.1. Protease activity in compost

The protease activity was significantly higher (345.3 μg tyrosine released g⁻¹ of compost 2h⁻¹) in BDCV-II followed by BDC (333.6 μg tyrosine released g⁻¹ of compost 2h⁻¹). The lowest protease activity of 232.2 μg tyrosine released g⁻¹ of compost 2h⁻¹ was recorded in Non- BDC (T₁). With regard to various days of compost, the maximum protease activity (378.2 μg tyrosine released g⁻¹ of compost 2h⁻¹) at 105th day (D₈) and minimum (185.3 μg tyrosine released g⁻¹ of compost 2h⁻¹) at 0th day (D₁) were recorded in BDCV-II (T₃). Among the interactions treatment, the lowest protease activity of 181.5 μg tyrosine released g⁻¹ of compost 2h⁻¹ was recorded in Non-BDC (T₁) at 0th day (D₁) and highest (438.3 μg tyrosine released g⁻¹ of compost 2h⁻¹ was recorded in BDCV-II (T₃) at 105th day (D₈) (Fig. 3.3c).

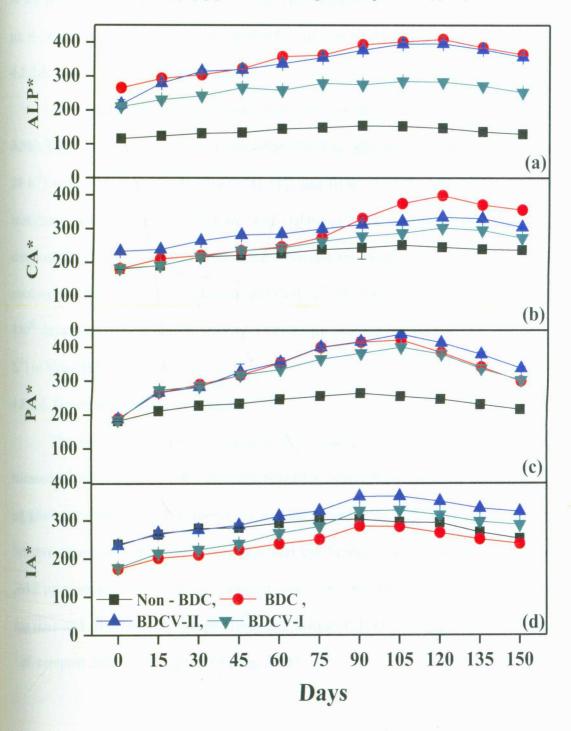
4.2.2.2. Alkaline Phosphatase (ALP) activity in compost

The different treatments significantly influenced on the alkaline phosphatase the compost (**Fig.3.3a**). The maximum alkaline phosphatase activity (350.2 µg p-Nitrophenol released g⁻¹ of compost h⁻¹) was recorded in BDC (T₂) and the minimum activity (137.9 µg p-Nitrophenol released g⁻¹ of compost h⁻¹) was recorded in Non –

Fig: 3.3 Influence of BD preparation on enzyme activities in biodynamic and non biodynamic compost

a) Alkaline phophatase activity (μg p-Nitrophenol released g ⁻¹ of compost h⁻¹) (ALP*),

- b) Cellulase activity (μg glucose released g⁻¹ of compost 24h⁻¹) (CA*),
- c) Protease (µg tyrosine released g⁻¹ of compost 2h⁻¹) (PA*),
- d) Invertase activity (μg glucose released g⁻¹ of compost 24h⁻¹)(IA*)



BDC (T_1). The interaction effect of different treatment and days recorded the highest activity (401.60 and 408.10 µg p- Nitrophenol released g^{-1} of compost h^{-1}) was recorded in the BDC (T_2) at 105^{th} (D_8) and 120^{th} day (D_9) respectively and lowest activity (115.17 µg p- Nitrophenol released g^{-1} of compost h^{-1}) in Non-BDC (T_1) at 0^{th} day (D_1).

4.2.2.3. Cellulase activity in compost

The different treatment significantly influenced on the cellulase activity (**Fig. 3.3b).** Highest cellulase activity (291.5 and 290.5 μ g glucose released g⁻¹ of compost 24 h⁻¹) were recorded in the BDCV-II (T₃) and BDC (T₂) of respectively. While the minimum activity was recorded in Non- BDC (179.83 μ g glucose released g⁻¹ of compost 24 h⁻¹). The interaction effect between the treatments and days recorded maximum activity (398.1 μ g glucose released g⁻¹ of compost 24 h⁻¹) in BDC (T₂) at 120th days (D₉) and minimum activity (179.83 μ g glucose released g⁻¹ of compost 24 h⁻¹) in Non-BDC (T₁) at 0th day (D₁).

4.2.2.4. Invertase activity in compost

The invertase activity of compost was significantly higher (311.8 μ g glucose released g⁻¹ of compost 24 h⁻¹) in BDC V-II (T₃). The lowest invertase activity of 237 μ g glucose released g⁻¹ of compost 24 h⁻¹ was recorded in BDC (T₂). The interaction between treatments and stages recorded that the higher invertase activity (362.5 and 363.2 μ g glucose released g⁻¹ of compost 24 h⁻¹) was recorded in BDCV-II (T₃) at 90th day (D₇) and 105th day D₈ respectively and lowest in T₂ (172.9 μ g glucose released g⁻¹ of compost 24 h⁻¹) at 0th day (D₁) (**Fig. 3.3d**).

4.2.3. Influence of BD preparation on microbial colonies in biodynamic and non biodynamic composting

4.2.3.1. Bacterial population

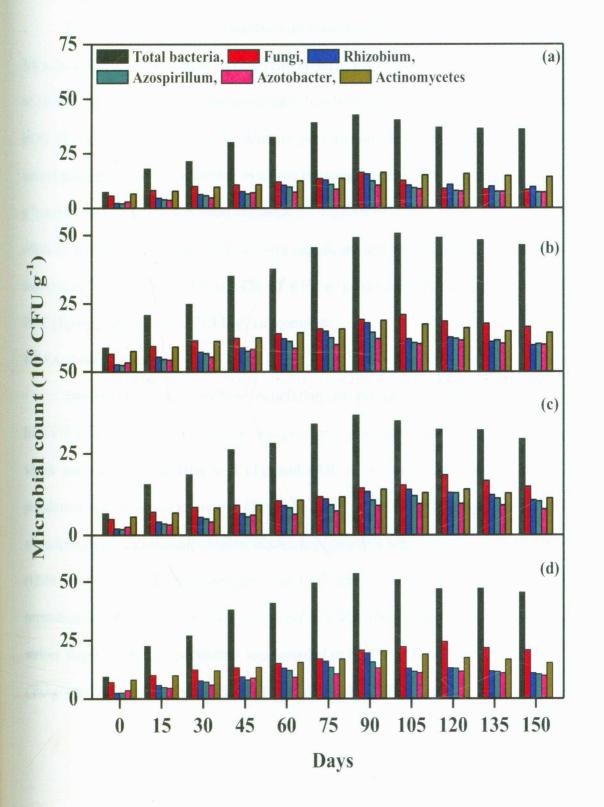
The different treatments not significantly influenced the total microbial population (**Fig. 3.4 a, b, c, d**) all the stages of observation. With respect to various days, significantly higher microbial population was recorded in all the treatments at D_5 (60 Day) (48.53x 10^6 CFU g^{-1}) and lower population was observed on D_1 stage (0^{th} Day) (19.2 x 10^6 CFU g^{-1}). The interaction effect of treatments and various days were significant and the maximum population was recorded in T_1 (non-biodynamic compost) at D_{11} stage (50.8x 10^6 CFU g^{-1}), which was on par with T_3 (BDCV-II) at D_5 (50.6x 10^6 CFU g^{-1}) and minimum value recorded in T_3 at D_6 Day (6.58x 10^6 CFU g^{-1}) of compost.

4.2.3.2. Fungal population

The results of fungal population are presented in (**Fig. 3.4 a, b, c, d**). Significantly higher fungal population was recorded in BDCV-I (T₄) (13.7 x 10⁶ CFU g⁻¹) and lower value was recorded in control (T1) (12.9 x 10⁶ CFU g⁻¹). BDCV-II (T₃) recorded the second highest population (13.6 x 10⁶ CFU g⁻¹), which was on par with BDCV-I (T₄). In case of different days, the highest value was observed at 150th (22.2 x 10⁶ CFU g⁻¹) and the lowest value was recorded at BDCV-II (8.07 x 10⁶ CFU g⁻¹). The interaction between treatments and days was found to be significant. Among the treatments, T₂ (BDC) at 150th recorded the highest compost fungal population (24.3 x 10⁶ CFU g⁻¹), while the lowest population was recorded in Non - BDC (T₁) at 0th day (5.58x 10⁶ CFU g⁻¹).

Fig: 3.4. Influence of BD preparation on microbial colonies in biodynamic and biodynamic composting

a). Non-BDC, b) BDC, c) BDCV-II, d) BDCV-I



4.2.3.3. Rhizobium

The different treatments significantly influenced the rhizobium population (**Fig. 3.4 a, b, c, d**)) at all the stages of observation. BDCV-I recorded the highest population of 10.04×10^6 CFU g⁻¹, while the minimum rhizobium population was recorded in Non-BDC (T_1) (9.68 x 10^6 CFU g⁻¹). With respect to various days, significantly higher microbial population was recorded in all the treatments at D_{10} (60 Day) (14.57x 10^6 CFU g⁻¹) and lower population was observed on D_1 (0^{th} Day) (5.19 x 10^6 CFU g⁻¹). The interaction effect of treatments and various days were significant and the maximum population was recorded in T_4 (BDCV- I) at D_{10} (19.47x 10^6 CFU g⁻¹) and minimum value recorded in Non-BDC (T_1) at 0^{th} day (2.19x 10^6 CFU g⁻¹) of compost.

4.2.3.4. Azosprillium:

The results on *Azosprillium* population are presented in (**Fig. 3.4 a, b, c, d**) BDCV-I (T_4) recorded the highest *Azosprillium* population of 8.97 x 10⁶ CFU g⁻¹, which was on par with BDCV-II (T_3) and BDC (T_2), while the minimum bacterial population was recorded in control (T_1) (8.56 x 10⁶ CFU g⁻¹). With respect to the effect of various days, significantly higher bacterial population was recorded in D_5 (60th day) (12.39 x 10⁶ CFU g⁻¹) which was par with 135th day (12.39 x 10⁶ CFU g⁻¹) and lower population was observed at D_1 (4.59x 10⁶ CFU g⁻¹). The interaction effects of treatments and various stages, maximum population was recorded in BDCV-I (T_4) at 135th day (15.62x 10⁶ CFU g⁻¹) and minimum in Non-BDC (T_1) at 0th day (2.05 x 10⁶ CFU g⁻¹).

4.2.3.5. Azotobacter:

The different treatments significantly influenced the *azotobacter* population (Fig. 3.4 a, b, c, d)) at all the stages of observation. BDCV-I recorded the highest population of 7.96 x 10⁶ CFU g⁻¹, which was on par with BDC (T₂), and BDC-II (T₃). The minimum *Azotobacter* population was recorded in Non-BDC (T₁) 7.56 x 10⁶ CFU g⁻¹). With respect to various days, significantly higher microbial population was recorded in all the treatments at D₁₀ (135 Day) (14.57x 10⁶ CFU g⁻¹) and lower population was observed on D₁ (0th Day) (5.19 x 10⁶ CFU g⁻¹). The interaction effect of treatments and various days were significant and the maximum population was recorded in T₄ (BDCV- I) at 120th day (19.47x 10⁶ CFU g⁻¹) and minimum value recorded in Non-BDC (T₁) at 0th Day (2.19x 10⁶ CFU g⁻¹) of compost.

4.2.3.6. Actinomycetes population

The results on actinomycetes population are presented in **Fig. 3.4 a, b, c, d.** In all the treatments the actinomycetes population was found to be non significant at all stages of observation regarding the various days highest and lowest values were recorded at 150^{th} (17.13 x 10^6 CFU g⁻¹) and 0^{th} (8.72 x 10^6 CFU g⁻¹), respectively. The interaction effect of different treatments with various days recorded maximum value in BDCV-I (T₄) at D₁₀ (135th day).

4.3. Evaluation of cow horn manure (BD500) maturation, impact of different dung and artificial containers

4.3.1. Impact of physicochemical properties of BD500

4.3.1.1. PH

The pH of cow horn manure (BD 500) with cowdung, buffalo and goat manure was neutral at 90th day. (Fig 4.1 a).

Among alternate containers (mud, plastic and glass) with different animal dung, pH scales of the manure were progressively changed into acid to neutral from 0th to 90th day. (Fig. 4.1.b, c, d, e)

4.3.1.2. EC

The Ec of cow horn manure (BD 500) with cowdung, buffalo and goat manure was observed has normal status (<= 1.00 dS m⁻¹). Similarly result was also observed in alternate containers (mud, plastic and glass) with different animal dung. (Fig.4.2 a,b,c,d,e)

4.3.1.3. Organic carbon

The organic carbon content of cow horn manure with different dung found to be not significant. Among different days of decomposition with cow dung, buffalo and goat dung in horn were maximum organic content 47.02, 48.44 and 48.18 respectively found in 0th day. (Fig. 4.3 b)

In case of alternate containers such as cow horn, mud, plastic and glass with cow, buffalo and goat dung manure shows increase organic content in 0th day of decomposition 51.11, 48.71 and 55.20 % was found in cow horn with cow, buffalo and

Fig: 4.1. Impact on pH of different dung and artificial containers

- a). Cowhorn with cowdung b). Cowhorn with cow, buffalo and goat dung
- c). Cowdung with horn, plastic and glass vessels
- d). Buffalodung with horn, plastic and glass vessels
- e). Goatdung with horn, plastic and glass vessels

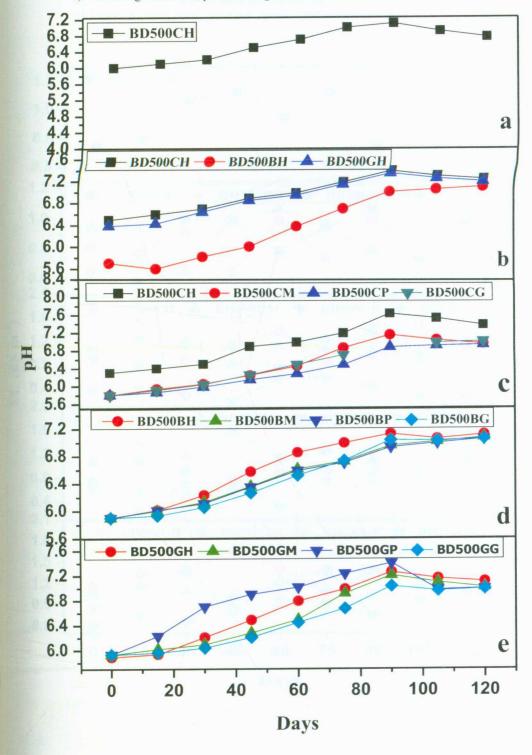
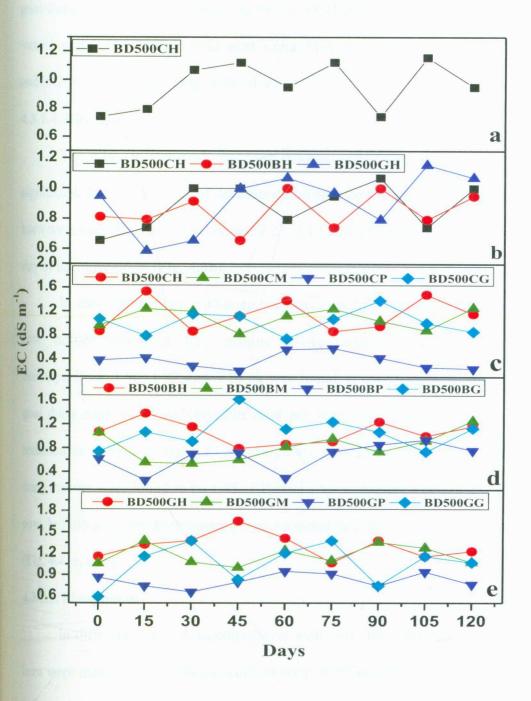


Fig: 4.2. Impact of EC on different dung and artificial containers

- a). Cowhorn with cowdung b). Cowhorn with cow, buffalo and goat dung
- c). Cowdung with horn, mud pot, plastic and glass vessels
- d). Buffalodung with horn, mud pot plastic and glass vessels
- e). Goatdung with horn, mud pot, plastic and glass vessels



goat dung manure respectively. For mud pot with cow, buffalo and goat dung were recorded as 51.11, 48.71 and 55.20%. In plastic containers with cow, buffalo and goat dung manure were found to be 51.11, 48.71 and 55.20 % respectively and glass vessel with cow, buffalo and goat dung manure were recorded as 51.11, 48.71 and 55.20% respectively. (Fig. 4.3 c, d, e).

4.3.1.4. Nitrogen

The nitrogen status of cow horn manure with different dung found to be not significant. In different days of decomposition with cow dung, buffalo and goat dung in horn were maximum nitrogen content 2.23, 2.12 and 3.07 % respectively found in 120th day of decomposition (**Fig. 4.4b**).

In case of alternate containers such as cow horn, mud, plastic and glass with cow, buffalo and goat dung manure shows increase nitrogen in 120th day of decomposition. 1.96, 1.97 and 3.10 % was found in cow horn with cow, buffalo and goat dung manure respectively. For mud pot with cow, buffalo and goat dung were recorded as 2.05, 2.38 and 2.83 %. In plastic containers with cow, buffalo and goat dung manure were found to be 1.85, 2.19 and 2.93 % respectively and glass vessel with cow, buffalo and goat dung manure were recorded as 2.10, 2.53 and 3.47 % respectively (Fig. 4.4. c, d, e).

4.3.1.5. Phosphorus

In different days of decomposition with cow dung, buffalo and goat dung in horn were maximum phosphorus content 0.64, 0.75 and 0.89 % respectively found in 90th day of decomposition. (Fig. 4.5b)

Fig: 4.3. Organic carbon content in BD 500 prepared with different dung and artificial containers

- a). Cowhorn with cowdung b). Cowdung with horn, mud pot, plastic and glass vessels
- c). Goatdung with horn, mud pot, plastic and glass vessels
- d). Buffalodung with horn, mud pot plastic and glass vessels
- e). Cowhorn with cow, buffalo and goat dung

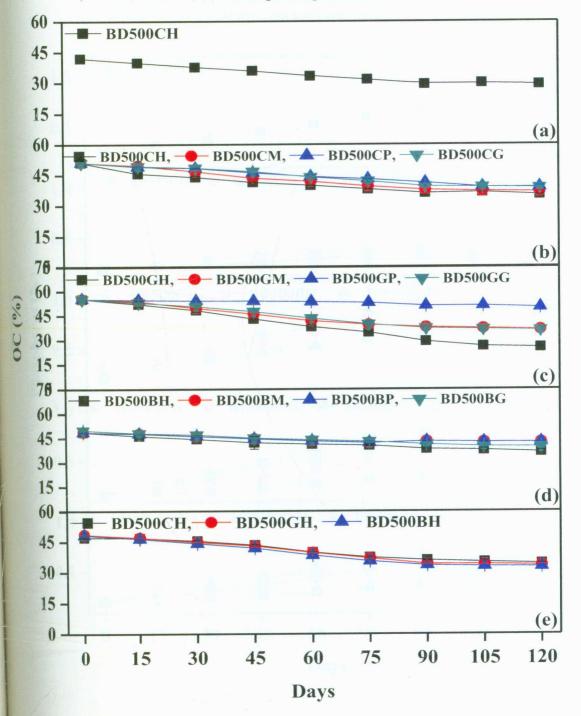


Fig:4.4. Nitrogen content in BD 500 prepared with different dung and artificial containers

- a). Cowhorn with cowdung b). Cowhorn with cow, buffalo and goat dung
- c). Cowdung with horn, mud pot, plastic and glass vessels
- d). Buffalodung with horn, mud pot plastic and glass vessels
- e). Goatdung with horn, mud pot, plastic and glass vessels

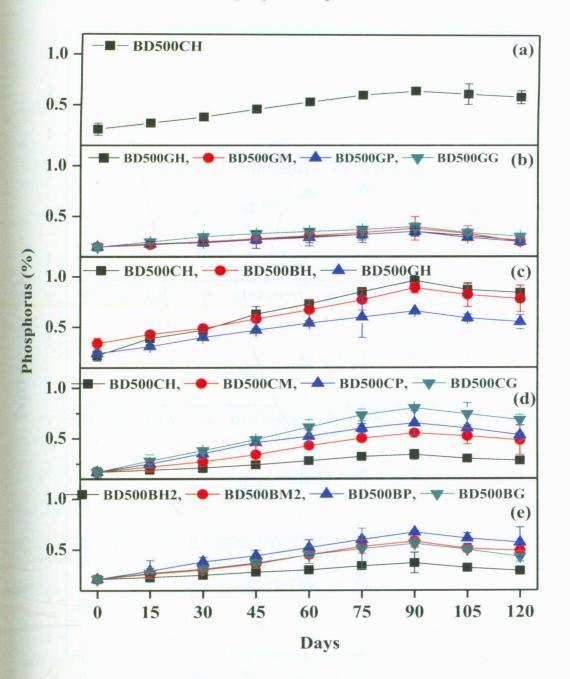
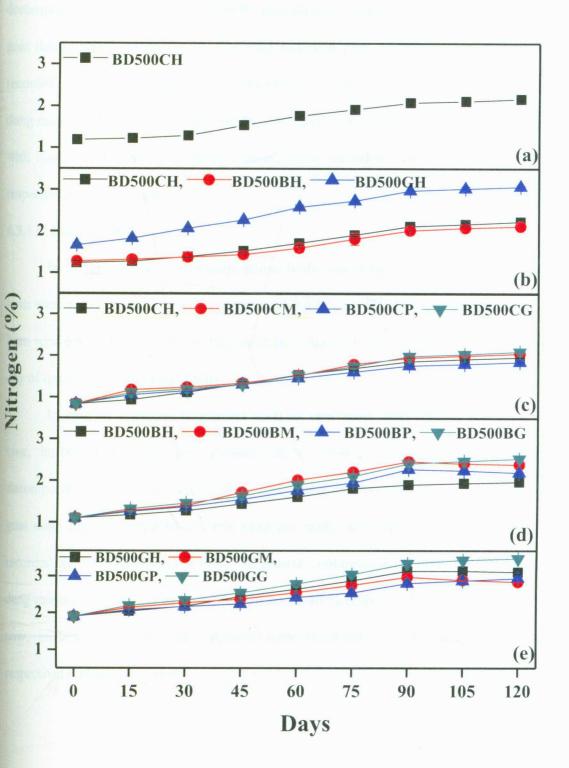


Fig: 4.5. Phosphorous content in BD 500 prepared with different dung and artificial containers

- a). Cowhorn with cowdung b). Goatdung with horn, mud pot, plastic and glass vessels
- c). Cowhorn with cow, buffalo and goat dung
- d). Cowdung with horn, mud pot, plastic and glass vessels
- e). Buffalodung with horn, mud pot plastic and glass vessels



In case of alternate containers such as cow horn, mud, plastic and glass with cow, buffalo and goat dung manure shows increase phosphorus in 90th day of decomposition. 0.34, 0.37 and 0.35 % was found in cow horn with cow, buffalo and goat dung manure respectively. For mud pot with cow, buffalo and goat dung were recorded as 0.55, 2.38 and 0.38 %. In plastic containers with cow, buffalo and goat dung manure were found to be 0.65 %, 0.67 and 0.35 % respectively and glass vessel with cow, buffalo and goat dung manure were recorded as 0.80, 0.56 and 0.40 % respectively (Fig. 4.5c, d, e).

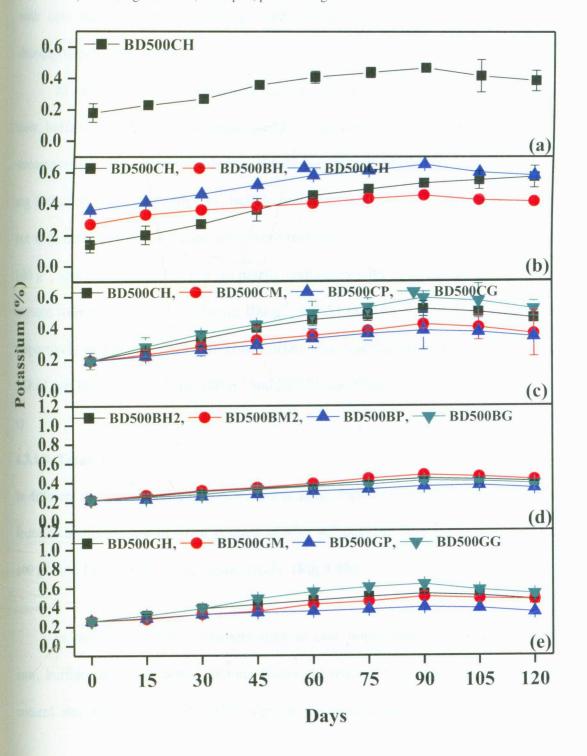
4.3.1.6. Potassium

In different days of decomposition with cow dung, buffalo and goat dung in horn were maximum potassium content was 0.47 % in 90th day for cowdung with cow horn where as buffalo and goat dung with horn shows 0.38 % and 0.60 % found in 90th day of decomposition. (Fig. 4.6.b).

In case of alternate containers such as cow horn, mud, plastic and glass with cow, buffalo and goat dung manure shows increase potassium in 90th day of decomposition. 0.52, 0.44 and 0.54 % was found in cow horn with cow, buffalo and goat dung manure respectively. For mud pot with cow, buffalo and goat dung were recorded as 0.42, 0.48, and 0.51%. In plastic containers with cow, buffalo and goat dung manure were found to be 0.38, 0.36 and 0.40 % respectively and glass vessel with cow, buffalo and goat dung manure were recorded as 0.59, 0.42 and 0.64 % respectively. (Fig. 4.6 c, d, e)

Fig: 4.6. Potassium content in BD 500 prepared with different dung and artificial containers

- a). Cowhorn with cowdung b). Cowhorn with cow, buffalo and goat dung
- c). Cowdung with horn, mud pot, plastic and glass vessels
- d). Buffalodung with horn, mud pot plastic and glass vessels
- e). Goatdung with horn, mud pot, plastic and glass vessels



4.3.1.7. Protein

In different days of decomposition with cow dung, buffalo and goat dung in cow horn were maximum protein content was 289.99 mg 100 g⁻¹ in 120th day for cowdung with cow horn, 242.08 mg 100 g⁻¹ and 255.11 mg 100 g⁻¹ found in 90th day of decomposition. (Fig. 4.7b)

In case of alternate containers such as cow horn, mud, plastic and glass with cow, buffalo and goat dung manure shows increase protein content up to 120th day of decomposition. The value recorded as 254.80 mg 100 g⁻¹, 249.03 mg 100 g⁻¹ and 256.35 mg 100 g⁻¹ in cow horn with cow, buffalo and goat dung manure respectively. For mud pot with cow, buffalo and goat dung were recorded as 266.33 mg 100 g⁻¹, 271.11 mg 100 g⁻¹, and 265.72 mg 100 g⁻¹. In plastic containers with cow, buffalo and goat dung manure were found to be 240.24 mg 100 g⁻¹, 261.51 mg 100 g⁻¹ and 252.37 mg 100 g⁻¹ respectively and glass vessel with cow, buffalo and goat dung manure were recorded as 273.61 mg 100 g⁻¹ 263.65 mg 100 g⁻¹ and 251.31 mg 100 g⁻¹ respectively. (**Fig.4.7 c, d, e**)

4.3.1.8. Total sugar

In different days of decomposition with cow dung, buffalo and goat dung in horn were found to be decreasing in sugar content at 0th - 90th day 149.78 mg 100 g⁻¹, 145.57 mg 100 g⁻¹ and 142.33 mg 100 g⁻¹ respectively. (**Fig.4.8b**)

In case of alternate containers such as cow horn, mud, plastic and glass with cow, buffalo and goat dung manure shows decreasing with decrease rate of sugar content was found from 0th to120th day of decomposition. In cow horn with cow,

Fig: 4.7. Protein content in BD 500 prepared with different dung and artificial containers

- a). Cowhorn with cowdung b). Cowhorn with cow, buffalo and goat dung
- c). Cowdung with horn, mud pot, plastic and glass vessels
- d). Buffalodung with horn, mud pot plastic and glass vessels
- e). Goatdung with horn, mud pot, plastic and glass vessels

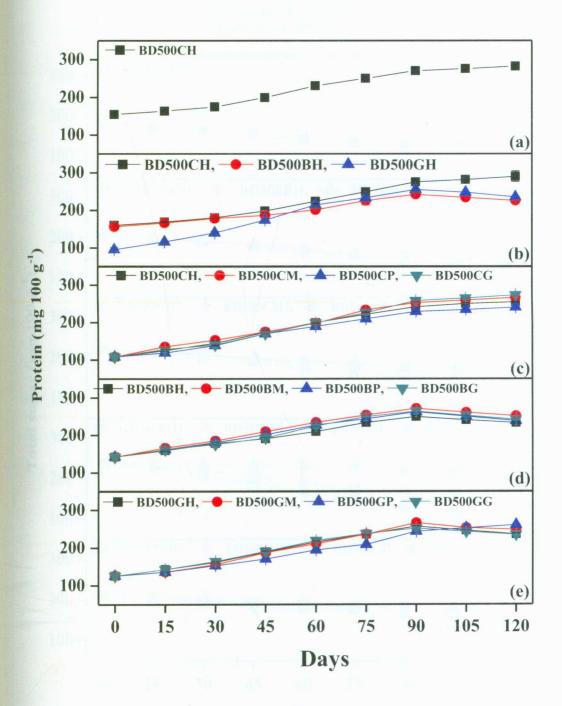
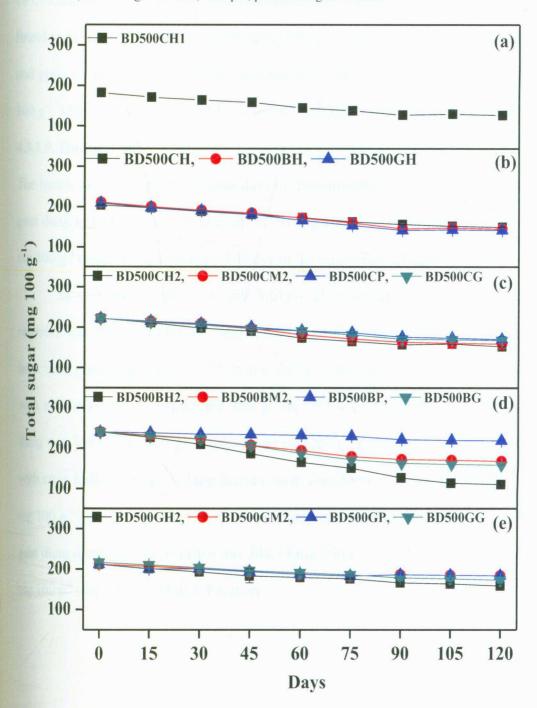


Fig: 4.8.Total sugar content in BD 500 prepared with different dung and artificial containers

- a). Cowhorn with cowdung b). Cowhorn with cow, buffalo and goat dung
- c). Cowdung with horn, mud pot, plastic and glass vessels
- d). Buffalodung with horn, mud pot plastic and glass vessels
- e). Goatdung with horn, mud pot, plastic and glass vessels



buffalo and goat dung manure of maximum sugar content were recorded as 153.10 mg 100 g⁻¹, 110.74 mg 100 g⁻¹ and 158.39 mg 100 g⁻¹ respectively. For mud pot with cow, buffalo and goat dung were recorded as 159.36 mg 100 g⁻¹, 168.93 mg 100 g⁻¹, and 183.95 mg 100 g⁻¹. In plastic containers with cow, buffalo and goat dung manure were found to be 170.77 mg 100 g⁻¹, 219.26 mg 100 g⁻¹ and 183.95 mg 100 g⁻¹ respectively and glass vessel with cow, buffalo and goat dung manure were recorded as 168.06 mg 100 g⁻¹ 158.91mg 100 g⁻¹ and 173.04 mg 100 g⁻¹ respectively. (**Fig.4.8 c, d, e**)

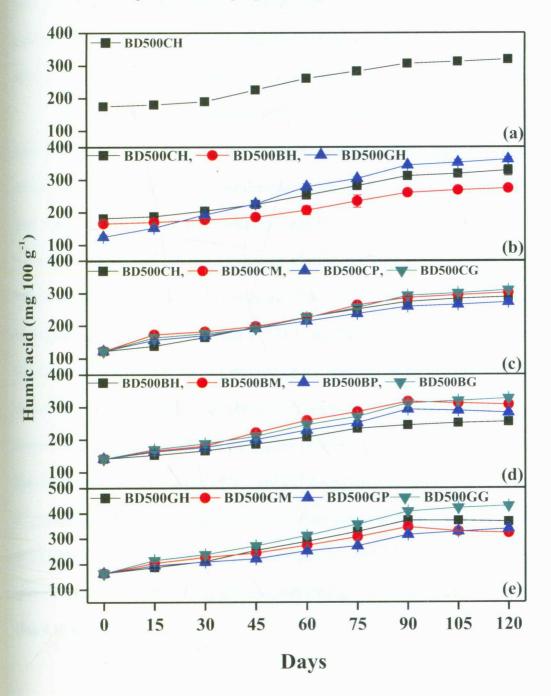
4.3.1.9. Humic acid

The humic acid content in different days of decomposition with cow dung, buffalo and goat dung in horn were maximum in 327.91 mg 100 g⁻¹, 273.31 mg 100 g⁻¹ and 362.00 mg 100 g⁻¹ respectively found in 120th day of decomposition.(**Fig.4.9b**)

In cow horn with cow and buffalo of maximum humic acid content was recorded has $288.12 \text{ mg } 100 \text{ g}^{-1}$ and $254.04 \text{ mg } 100 \text{ g}^{-1}$ in 120^{th} day , where as for cow horn with goat dung manure $372.25 \text{ mg } 100 \text{ g}^{-1}$ was found in 90^{h} day. For mud pot with cow recorded as $301.15 \text{ mg } 100 \text{ g}^{-1}$ in 120^{th} day, where as for buffalo and goat dung was $316.65 \text{ mg } 100 \text{ g}^{-1}$ and $345.44 \text{ mg } 100 \text{ g}^{-1}$ in 90^{th} day. In plastic containers with cow, buffalo and goat dung manure were found to be $271.66 \text{ mg } 100 \text{ g}^{-1}$, $282.34 \text{ mg } 100 \text{ g}^{-1}$ and $338.34 \text{ mg } 100 \text{ g}^{-1}$ respectively and glass vessel with cow, buffalo and goat dung manure were recorded has $308.39 \text{ mg } 100 \text{ g}^{-1}$, $326.28 \text{ mg } 100 \text{ g}^{-1}$ and $429.82 \text{ mg } 100 \text{ g}^{-1}$ respectively. (Fig.4.9 c, d, e)

Fig: 4.9. Humic acid content in BD 500 prepared with different dung and artificial containers

- a). Cowhorn with cowdung b). Cowhorn with cow, buffalo and goat dung
- c). Cowdung with horn, mud pot, plastic and glass vessels
- d). Buffalodung with horn, mud pot plastic and glass vessels
- e). Goatdung with horn, mud pot, plastic and glass vessels



4.3.2. Enzymes activities of BD 500

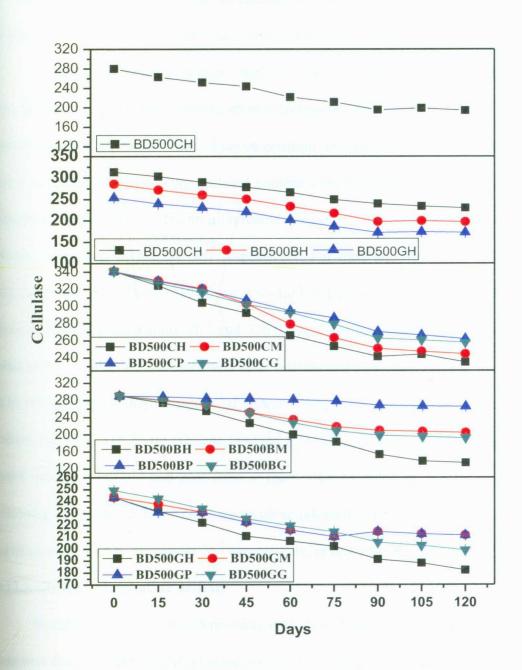
4.3.2.1. Cellulase activity

In different days of decomposition with cow dung, buffalo and goat dung in horn were found to be decreasing in cellulase activity, 229.66 µg glucose released g⁻¹ of manure 24 h⁻¹, 196.94 µg glucose released g⁻¹ of manure 24 h⁻¹ and 172.29 µg glucose released g⁻¹ of manure 24 h⁻¹ respectively upto 120th day of decomposition. (**Fig.4.10b**)

In case of alternate containers such as cow horn, mud, plastic and glass with cow, buffalo and goat dung manure shows decreasing with decrease rate of cellulase content was found upto 120th day of decomposition. In cow horn with cow, buffalo and goat dung manure of maximum cellulase content were recorded as 234.76 µg glucose released g-1 of manure 24 h-1, 134.06 µg glucose released g-1 of manure 24 h-1 and 182.14 μg glucose released g⁻¹ of manure 24 h⁻¹ respectively. For mud pot with cow, buffalo and goat dung were recorded as 244.35 µg glucose released g⁻¹ of manure 24 h⁻¹, 204.50 μg glucose released g⁻¹ of manure 24 h⁻¹, and 211.54 μg glucose released g⁻¹ of manure 24 h⁻¹ respectively. In plastic containers with cow, buffalo and goat dung manure were found to be 261.84 µg glucose released g⁻¹ of manure 24 h⁻¹, 265.42 µg glucose released g⁻¹ of manure 24 h⁻¹ and 211,54 µg glucose released g⁻¹ of manure 24 h-1 respectively and glass vessel with cow, buffalo and goat dung manure were recorded as 257.70 µg glucose released g⁻¹ of manure 24 h⁻¹, 192.37 µg glucose released g⁻¹ of manure 24 h⁻¹ and 199.00 µg glucose released g⁻¹ of manure 24 h⁻¹ respectively. (Fig.4.10 c, d, e)

Fig: 4.10. Cellulase activity in BD 500 prepared with different dung and artificial containers

- a) Cowhorn with cowdung b). Cowhorn with cow, buffalo and goat dung
- c). Cowdung with horn, mud pot, plastic and glass vessels
- d). Buffalodung with horn, mud pot plastic and glass vessels
- e). Goatdung with horn, mud pot, plastic and glass vessels



4.3.2.2. Protease activity

In different days of decomposition with cow dung, buffalo and goat dung in horn were found maximum protease activity 238.46 µg tyrosine released g⁻¹of manure 2h⁻¹, 229.15 µg tyrosine released g⁻¹of manure 2h⁻¹ and 332.33 µg tyrosine released g⁻¹of manure 2h⁻¹ respectively in 105th day of decomposition. (Fig 4.11b)

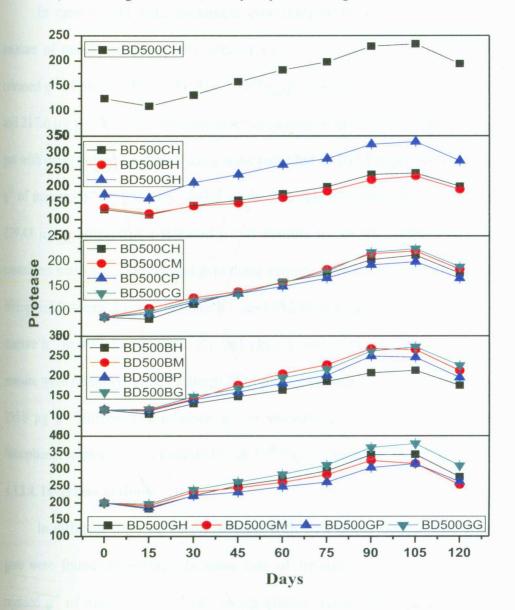
In case of alternate containers such as cow horn, mud, plastic and glass with cow, buffalo and goat dung manure shows decreasing with decrease rate of protease content was found upto 105th day of decomposition. In cow horn with cow, buffalo and goat dung manure of maximum protease content were recorded as 211.90 μg tyrosine released g⁻¹ of manure 2h⁻¹, 213.86 μg tyrosine released g⁻¹ of manure 2h⁻¹ and 344.68 μg tyrosine released g⁻¹ of manure 2h⁻¹ and 344.68 μg tyrosine released g⁻¹ of manure 2h⁻¹ pg tyrosine released g⁻¹ of manure 2h⁻¹, 268.11 μg tyrosine released g⁻¹ of manure 2h⁻¹ and 326.02 μg tyrosine released g⁻¹ of manure 2h⁻¹ respectively. In plastic containers with cow, buffalo and goat dung manure were found to be 198.58 μg tyrosine released g⁻¹ of manure 2h⁻¹, 249.93 μg tyrosine released g⁻¹ of manure 2h⁻¹ and 316.76 μg tyrosine released g⁻¹ of manure 2h⁻¹ respectively and glass vessel with cow, buffalo and goat dung manure were recorded as 224.75 μg tyrosine released g⁻¹ of manure 24h⁻¹, 272.61 μg tyrosine released g⁻¹ of manure 2h⁻¹ and 377.30 μg tyrosine released g⁻¹ of manure 2h⁻¹ respectively. (**Fig.4.11 c, d, e**)

4.3.2.3. Alkaline phophatase activity

In different days of decomposition with cow dung, buffalo and goat dung in horn were found maximum phosphatase activity 451.4 µg p- Nitrophenol released g⁻¹ of

Fig: 4.11. Protease activity in BD 500 prepared with different dung and artificial containers

- a) Cowhorn with cowdung b). Cowhorn with cow, buffalo and goat dung
- c). Cowdung with horn, mud pot, plastic and glass vessels
- d). Buffalodung with horn, mud pot plastic and glass vessels
- e). Goatdung with horn, mud pot, plastic and glass vessels



manure h⁻¹ in 75th day, 380.1 μg p- Nitrophenol released g⁻¹ of manure h⁻¹ and 347.0 μg p- Nitrophenol released g⁻¹ of manure h⁻¹ respectively in 105th day of decomposition. (Fig.4.12a)

In case of alternate containers cow horn with cow, buffalo and goat dung manure of maximum phosphate content were recorded as 167.5 μg p- Nitrophenol released g⁻¹ of manure h⁻¹ in75th day, 195.7 μg p- Nitrophenol released g⁻¹ of manure h⁻¹ and 217.6 μg p- Nitrophenol released g⁻¹ of manure h⁻¹ in 90th day respectively. For mud pot with cow, buffalo and goat dung were recorded as 263.8 μg p- Nitrophenol released g⁻¹ of manure h⁻¹ in 75th day, 307.5 μg p- Nitrophenol released g⁻¹ of manure h⁻¹ and 239.43 μg p- Nitrophenol released g⁻¹ of manure h⁻¹ in 90th respectively. In plastic containers with cow, buffalo and goat dung manure were found to be 317.4, 353.4 μg p-Nitrophenol released g⁻¹ of manure h⁻¹, and 213.86 6 μg p- Nitrophenol released g⁻¹ of manure h⁻¹ in 90th day respectively and glass vessel with cow, buffalo and goat dung manure were recorded as 384.1 μg p- Nitrophenol released g⁻¹ of manure h⁻¹ in 75th day, 295.9 μg p- Nitrophenol released g⁻¹ of manure h⁻¹ in 75th day respectively. (**Fig.4.12 c, d, e**)

4.3.2.4. Invertase activity

In different days of decomposition with cow dung, buffalo and goat dung in horn were found decreasing decrease rate of invertase activity 186.21 µg glucose released g⁻¹ of manure 24 h⁻¹, 167.40 µg glucose released g⁻¹ of manure 24 h⁻¹ and 155.88 µg glucose released g⁻¹ of manure 24 h⁻¹ respectively in 105th day of decomposition. (Fig.4.13b)

Fig: 4.12. Alkaline phophatase activity in BD 500 prepared with different dung and artificial containers

- a) Cowhorn with cowdung b). Cowhorn with cow, buffalo and goat dung
- c). Cowdung with horn, mud pot, plastic and glass vessels
- d). Buffalodung with horn, mud pot plastic and glass vessels
- e). Goatdung with horn, mud pot, plastic and glass vessels

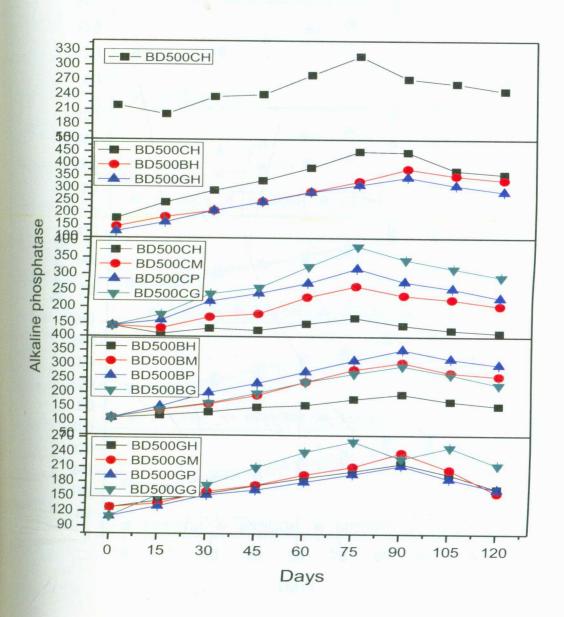
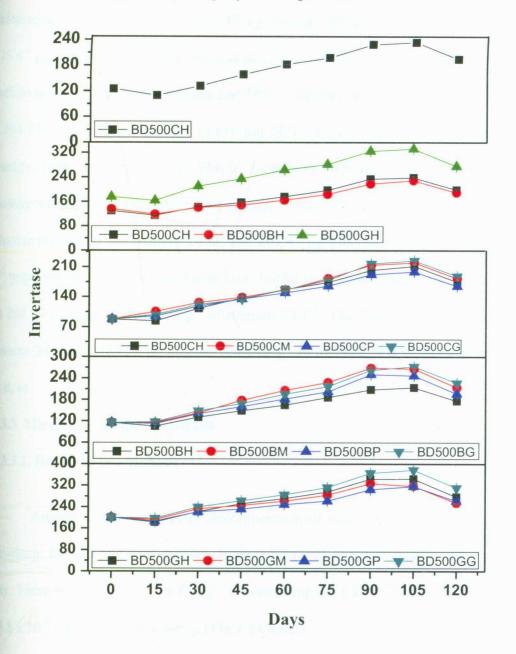


Fig: 4.13. Invertase activity in BD 500 prepared with different dung and artificial containers

- a) Cowhorn with cowdung b). Cowhorn with cow, buffalo and goat dung
- c). Cowdung with horn, mud pot, plastic and glass vessels
- d). Buffalodung with horn, mud pot plastic and glass vessels
- e). Goatdung with horn, mud pot, plastic and glass vessels



In case of alternate containers such as cow horn, mud, plastic and glass with cow, buffalo and goat dung manure shows decreasing with decrease rate of invertase content was found upto 120th day of decomposition. In cow horn with cow, buffalo and goat dung manure of maximum invertase content were recorded has 190.34 µg glucose released g⁻¹ of manure 24 h⁻¹, 127.35 µg glucose released g⁻¹ of manure 24 h⁻¹ and 173.47 µg glucose released g⁻¹ of manure 24 h⁻¹respectively. For mud pot with cow, buffalo and goat dung were recorded as 198.12 µg glucose released g⁻¹ of manure 24 h⁻ ¹, 194.27 μg glucose released g⁻¹ of manure 24 h⁻¹ and 201.47 μg glucose released g⁻¹ of manure 24 h⁻¹respectively. In plastic containers with cow, buffalo and goat dung manure were found to be 212.30 μg glucose released g⁻¹ of manure 24 h⁻¹, 252.15 μg glucose released g⁻¹ of manure 24 h⁻¹ and 201.47 µg glucose released g⁻¹ of manure 24 h⁻¹ respectively and glass vessel with cow, buffalo and goat dung manure were recorded as 208.94 µg glucose released g⁻¹ of manure 24 h⁻¹, 182.75 µg glucose released g⁻¹ of manure 24 h⁻¹and 189.52 µg glucose released g⁻¹ of manure 24 h⁻¹respectively. (Fig.4.13 c, d, e)

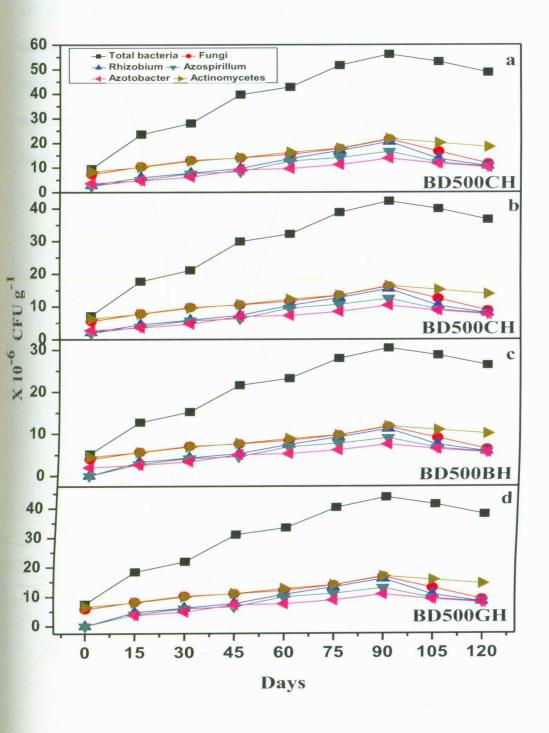
4.3.3. Microbial colonies of BD500

4.3.3.1. Bacterial population:

Among difference days of decomposition of cow horn manure (BD 500) with cowdung, buffalo and goat manure, highest bacterial population were observed in 90th day. There were 42.2 x 10⁶ CFU g⁻¹ in cow dung, 30.4 x 10⁶ CFU g⁻¹ in buffalo and 43.8 x 10⁶ CFU g⁻¹ in goat dung. (Fig.4.14 a,b,c)

Fig: 4.14. Microbial population in BD 500 prepared in cow horn with different dung

- a). Cow horn with cowdung b). Cow horm with cowdung
- c). Cow horm with buffalodung d). Cow horm withbuffalodung



In case of alternate containers such as mud, plastic and glass with cow, buffalo and goat dung manure shows highest bacterial population in 90th day of decomposition. (Fig. 4.15, 4.16, 4.17 (a,b,c)). The higher value of 48.4×10^6 CFU g⁻¹, 34.9×10^6 CFU g⁻¹ and 49.9×10^6 CFU g⁻¹ was found in mud pot with cow, buffalo and goat dung manure respectively. In plastic containers with cow, buffalo and goat dung manure were found to be 36.1×10^6 CFU g⁻¹, 26.0×10^6 CFU g⁻¹ and 37.1×10^6 CFU g⁻¹ respectively and glass vessel with cow, buffalo and goat dung manure were recorded has 40.9×10^6 CFU g⁻¹, 29.5×10^6 CFU g⁻¹ and 43.3×10^6 CFU g⁻¹ respectively.

Fig: 4.15. Microbial population in BD 500 prepared in cow dung with artificial containers

- a). Cowdung with cowhorn b). Cowdung with mudpot
- d). Cowdung with plastic containers e). Cowdung with glass vessels

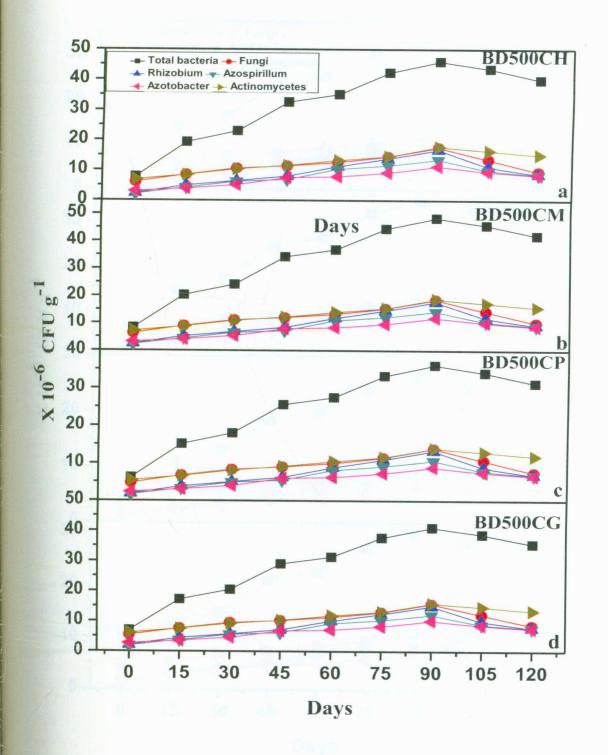


Fig: 4.16. Microbial population in BD 500 prepared in buffalo dung with artificial containers

- a). Buffalo dung with cowhorn). b). Buffalo dung with mudpot
- c). Buffalo dung with plastic containers d). Buffalo dung with glass vessels

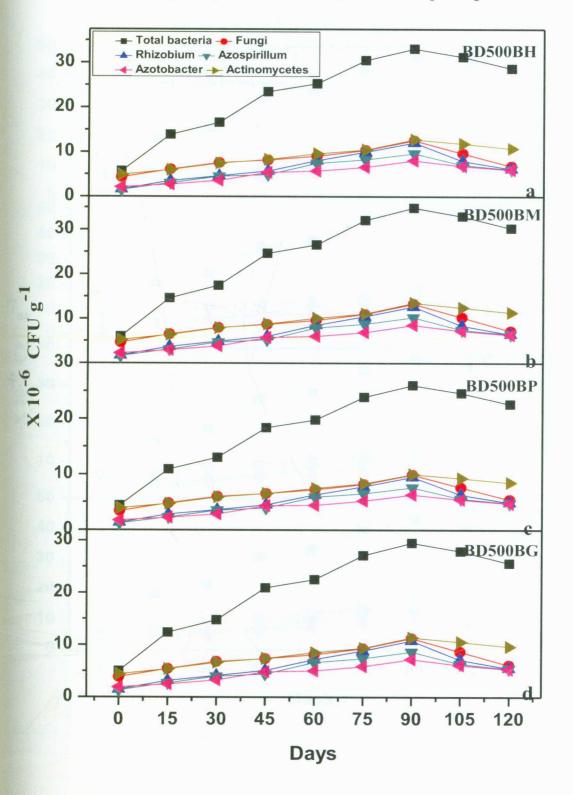
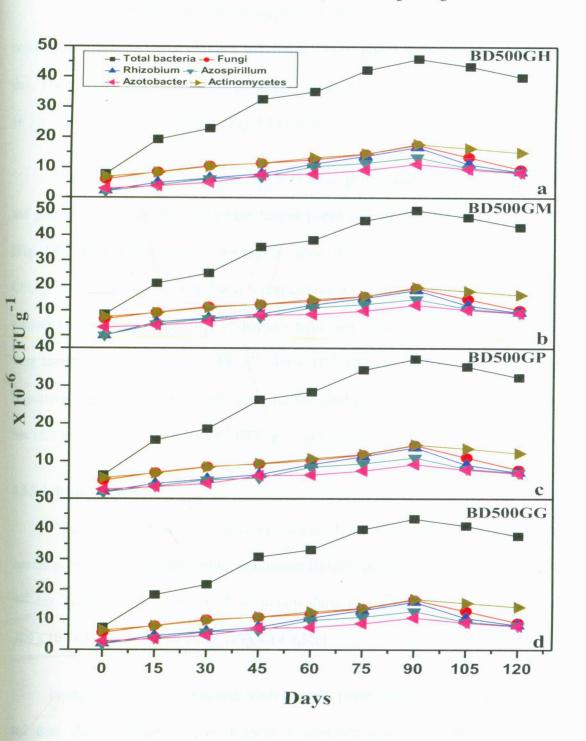


Fig: 4.17. Microbial population in BD 500 prepared in goat dung with artificial containers

- a). Goat dung with cowhorn). b). Goat dung with mudpot
- c). Goat dung with plastic containers d). Goat dung with glass vessels



4.3.3.2. Fungi population.

Among different days of decomposition of cow horn manure (BD 500) with cowdung, buffalo and goat manure, maximum fungal population were observed in 90^{th} day. There were 16.1 x 10 6 CFU g⁻¹ in cow dung, 11.6 x 10 6 CFU g⁻¹ in buffalo and 16.7 x 10 6 CFU g⁻¹ in goat dung. (Fig.4.14 a,b,c)

In case of alternate containers such as mud, plastic and glass with cow, buffalo and goat dung manure shows highest fungal population in 90th day of decomposition (Fig. 4.15, 4.16, 4.17 (a,b,c)). The higher value of 19.5 x 10⁶ CFU g⁻¹, 14.0 x 10⁶ CFU g⁻¹ and 20.2 x 10⁶ CFU g⁻¹ was found in mud pot with cow, buffalo and goat dung manure respectively. In plastic containers with cow, buffalo and goat dung manure were found to be 13.8 x 10⁶ CFU g⁻¹, 9.9 x 10⁶ CFU g⁻¹ and 14.2 x 10⁶ CFU g⁻¹ respectively and glass vessel with cow, buffalo and goat dung manure were recorded has 15.6 x 10⁶ CFU g⁻¹, 11.3 x 10⁶ CFU g⁻¹ and 16.5 x 10⁶ CFU g⁻¹ respectively.

4.3.3.3. Rhizobium

Among different days of decomposition of cow horn manure (BD 500) with cowdung, buffalo and goat manure, maximum Rhizobium population were observed in 90^{th} day. There are 15.4 x 10 6 CFU g⁻¹ in cow dung 11.1 x 10 6 CFU g⁻¹ in buffalo and 16.0×10^6 CFU g⁻¹ in goat dung. (Fig.4.14 a,b,c)

In case of alternate containers such as mud, plastic and glass with cow, buffalo and goat dung manure shows highest Rhizobium population in 90th day of

decomposition. The higher value of 18.6 x 10 6 CFU g⁻¹, 13.4 x 10 6 CFU g⁻¹ and 19.4 x 10 6 CFU g⁻¹ was found in mud pot with cow, buffalo and goat dung manure respectively. In plastic containers with cow, buffalo and goat dung manure were found to be 13.2 x 10 6 CFU g⁻¹, 9.5 x 10 6 CFU g⁻¹ and 13.6 x 10 6 CFU g⁻¹ respectively and glass vessel with cow, buffalo and goat dung manure were recorded has 14.9 x 10 6 CFU g⁻¹, 10.8 x 10 6 CFU g⁻¹ and 15.8 x 10 6 CFU g⁻¹ respectively. (**Fig. 4.15, 4.16, 4.17 (a,b,c)**).

4.3.3.4. Azospirillum population:

Among different days of decomposition of cow horn manure (BD 500) with cowdung, buffalo and goat manure, maximum Azospirillum population were observed in 90th day. There are 12.4 x 10 ⁶ CFU g⁻¹ in cow dung 8.9 x 10 ⁶ CFU g⁻¹ in buffalo and 12.9 x 10 ⁶ CFU g⁻¹ in goat dung. (**Fig.4.14 a,b,c**)

In case of alternate containers such as mud, plastic and glass with cow, buffalo and goat dung manure shows highest Azospirillum population in 90th day of decomposition. The higher value of 15.0 x 10 ⁶ CFU g⁻¹, 10.8 x 10 ⁶ CFU g⁻¹ and 15.6 x 10 ⁶ CFU g⁻¹ was found in mud pot with cow, buffalo and goat dung manure respectively. In plastic containers with cow, buffalo and goat dung manure were found to be 10.6 x 10 ⁶ CFU g⁻¹, 7.6 x 10 ⁶ CFU g⁻¹ and 10.9 x 10 ⁶ CFU g⁻¹ respectively and glass vessel with cow, buffalo and goat dung manure were recorded has 12.0 x 10 ⁶ CFU g⁻¹, 8.7 x 10 ⁶ CFU g⁻¹ and 9.3 x 10 6 CFU g⁻¹ respectively. (Fig. 4.15, 4.16, 4.17 (a,b,c)).

4.3.3.5. Azotobacter population:

Among different days of decomposition of cow horn manure (BD 500) with cowdung, buffalo and goat manure, maximum Azotobacter population were observed in 90^{th} day. There are 10.3×10^6 CFU g⁻¹ in cow dung 7.4×10^6 CFU g⁻¹ in buffalo and 10.7×10^6 CFU g⁻¹ in goat dung. (Fig.4.14 a,b,c)

In case of alternate containers such as mud, plastic and glass with cow, buffalo and goat dung manure shows highest Azotobacter population in 90th day of decomposition. (Fig. 4.15, 4.16, 4.17 (a,b,c)). The higher value of 12.5 x 10⁶ CFU g⁻¹, 9.0 x 10⁶ CFU g⁻¹ and 12.9 x 10⁶ CFU g⁻¹ was found in mud pot with cow, buffalo and goat dung manure respectively. In plastic containers with cow, buffalo and goat dung manure were found to be 8.8 x 10⁶ CFU g⁻¹, 6.3 x 10⁶ CFU g⁻¹ and 9.1 x 10⁶ CFU g⁻¹ respectively and glass vessel with cow, buffalo and goat dung manure were recorded has 10.0 x 10⁶ CFU g⁻¹, 7.2 x 10⁶ CFU g⁻¹ and 10.6 x 10⁶ CFU g⁻¹ respectively.

4.3.3.6. Actinomycetes population:

In different days of decomposition of cow horn manure (BD 500) with cowdung, buffalo and goat manure, maximum Actinomycetes population were observed in 90th day. There are 19.7 x 10⁶ CFU g⁻¹ in cow dung 14.2 x 10⁶ CFU g⁻¹ in buffalo and 20.5 x 10⁶ CFU g⁻¹ in goat dung. (**Fig.4.14 a,b,c**)

In case of alternate containers such as mud, plastic and glass with cow, buffalo and goat dung manure shows highest Actinomycetes population in 90^{th} day of decomposition. Fig. 4.15, 4.16, 4.17 (a,b,c)). The higher value of 12.5×10^6 CFU g⁻¹, 9.0×10^6 CFU g⁻¹ and 12.9×10^6 CFU g⁻¹ was found in mud pot with cow, buffalo and goat dung manure respectively. In plastic containers with cow, buffalo and goat dung manure were found to be 13.9×10^6 CFU g⁻¹, 10.0×10^6 CFU g⁻¹ and 14.3×10^6 CFU g⁻¹ respectively and glass vessel with cow, buffalo and goat dung manure were recorded has 15.8×10^6 CFU g⁻¹, 11.4×10^6 CFU g⁻¹ and 15.5×10^6 CFU g⁻¹ respectively.

4.3.2.5. Influence of cow horn manure BD500 on chromatogram image formation

The increasing concentrations (0.25 gram to 3 gram in 100 ml of NaOH 1%) of BD 500 increased the intensity colors, spikes and pattern in all 3 zones. The middle zone reflects for the presence of organic matter appears in saddle brown, ends in gray color (band width) with number of spikes (50). The outer zone has tan color which reflects the presence of humus (2 mg/ gram of manure) in the manure (plate 4). Various biodynamic manures were also processed for the development if chromatographic image (plate 4,6).

The various concentration of BD500 amended soil has resulted in 3 clear zones in the chromatograms. The inner zone of chromatographic images of BD has resulted in sandy brown and gray (plate 4, 6). The middle zone has prominent gray spikes in all the concentrations of chromatograms. In the outer zone of chromatograms wheat brown color was observed and a proportionate increase in intensity was recorded with concentration.

4.3.3.1. Effect of water extract of biodynamic manure on root growth of Spilianthus calva

The water biodynamic manures such BDC, Non-BDC, BD500CH, BD500CM, BD500CG and BD500CP were utilized for growing the shoot of *Spilianthus calva*. Among six different manures, highest root length (12.65, 11.63 and 11.25cm) was recorded in BDC, BD500CH and Non-BD500CM. The lowest root length (1.25 cm) was recorded in control followed by Non-BDC. The high number of root (13.5 Nos) was recorded in BDC followed by BD500CP (12.5 Nos). The low number of root (0.5 Nos) was recorded in control followed by Non-BDC (4.25 Nos) and BDCG (8.75). (Fig. 5.1)

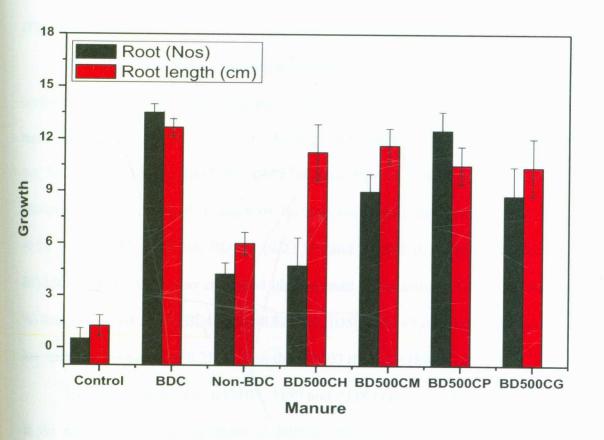
4.4. Effect of alternative herbs available in tropical region on production of biodynamic herbal preparation

4.4.1. Changes of physicochemical parameters of BD preparations (regular and alternatives)

The Eight different BD preparations namely: I) Yarrow (BD502) II) Chamomile (BD503), III) Stinging Nettle (BD504), V) Oak bark (BD505),V) *Aerva lanata* (BDA502), VI) *Tridax procumbens* (BDA503), VII) *Tragia involucrate* (BDA504) and VIII) *Casuarina sp.*(BDA505) were taken for physicochemical, biochemical and microbial properties analysis for the matured manure.

The Biodynamic preparation of BD502, BD 503, BD504, BD505, BDA502, BDA 503, BDA504 and BDA 505 recorded the pH values (7.25, 7.42, 7.21, 7.64, 7.37, 7.23, 7.52 and 7.38 respectively). The interaction effect of pH among the preparations was found to be non significant. Among them the BDA502 and BD505 recorded highly

Fig. 5.1. Effect of water extract of biodynamic manure on root growth of Spilianthus calva



significant level of Electrical Conductivity (EC) (0.65 dS m⁻¹) and (0.62 dS m⁻¹) followed by low significant in BD503 (0.39 dS m⁻¹) and BD505 (0.36 dS m⁻¹) (Fig.6.1b,c,).

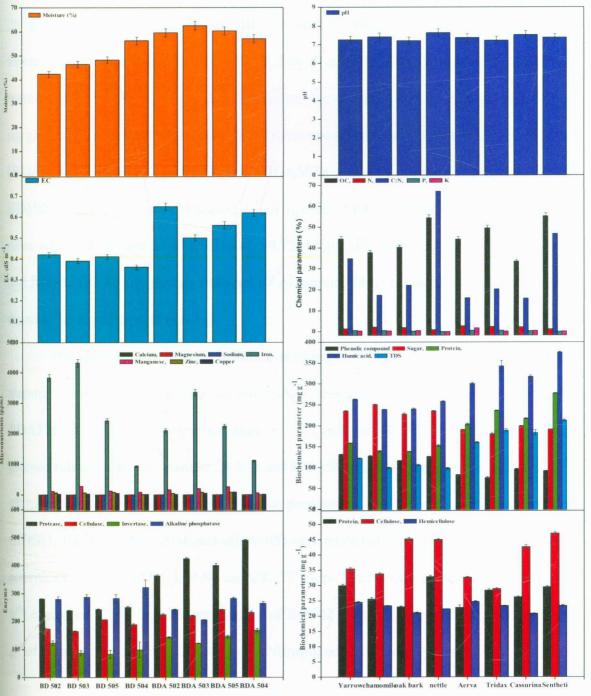
The BD alternative preparations of BDA502, BDA503, BDA504, BD505 contained significantly higher amount of nitrogen (2.74, 2.44, 2.12 and 1.18 %) than regular preparation of BD502 (1.27 %), BD503 (2.18 %), BD504 (1.82 %) and BD505 (0.81 %). Similarly BDA502, BDA503, BDA504 and BDA505contained high amount of phosphorous (0.63, 0.63, 0.50 and 0.04 %) than the regular BD preparation of BD502 (0.54 %), BD503 (0.40 %), BD504 (0.22 %) and BD505 (0.03 %). The BDA502, BDA504 and BDA505 also contained high amount of potassium (1.68, 0.50 and 0.14 %) than BD502 (0.27 %), BD504 (0.48 %) and BD505 (0.04 %). Whereas BDA503 had low amount of potassium (0.21 %) than the BD503 (0.25 %) (Fig.6.1d).

The low amount of iron (2103, 3359 and 2248 ppm) was recorded significantly in the alternative BD preparations of BD502, BDA503 and BDA504 compared to regular BD preparation of BD502 (3840 ppm), BD503(433 ppm), and BD504 (2432 ppm), Whereas high amount of iron (1111 ppm) recorded in BDA505 compared with regular preparation BD505 (932 ppm). Significantly high amount of Zinc (82 and 90 ppm) and copper (44 and 89 ppm) were recorded in BDA503 and BDA504, whereas BDA502 and BDA505 were recorded for the lowest value of zinc (48 and 3 ppm) and copper (9 and 7 ppm) compared to BD regular preparation of BD502 (17 ppm), BD503 (28 ppm), BD504 (50 ppm) and (8 ppm) (Fig.6.1e).

Fig: 6.1. Changes of physicochemical parameters of BD preparations (regular and alternatives)

- a). Moisture b). pH c).EC c).Chemical parameter(organic carbon, N, P, K)
- d). Micronutrient (Ca, Mg, Fe, Zn, Mn Na, Cu) e).Biochemical parameter(phenolic, sugar, acid, TDS) f).Enzymes(protease invertase, alk phosphates and cellulase)
- g).Biochemical parameter(cellulose and hemi cellulose)

humic



4.4.2. Changes of enzyme activities in BD preparation (Regular and Alternative)

The protease activities of alternative BD preparation (BDA504) found to be high (490.89 μg tyrosine released g⁻¹ of manure 2h⁻¹) followed BDA503 (425.37 μg tyrosine released g⁻¹ of manure 2h⁻¹) and BDA.505 (400.82 μg tyrosine released g⁻¹ of manure 2h⁻¹). The BD regular preparations (BD502, BD503, BD504 and BD505) recorded the lowest protease activities (281.36, 239.27, 244.49 and 251.37 μg tyrosine released g⁻¹ of manure 2h⁻¹). Similarly, the high invertase activities (168.57, 146.23, 143.88 and 122.85 μg glucose released g⁻¹ of manure 24 h⁻¹) was recorded in alternative BD preparations (BDA504, BDA505, BDA502 and BDA503) than BD regular preparation of BD502(123.47 μg glucose released g⁻¹ of manure 24 h⁻¹), BD503 (87.75 μg glucose released g⁻¹ of manure 24 h⁻¹), BD503 (83.86 μg glucose released g⁻¹ of manure 24 h⁻¹). The interaction effect was found to be significant (**Fig.6.1g**).

The significantly highest cellulase activities (242.66 μg glucose released g⁻¹ of manure 24 h⁻¹) was recorded in alternative preparation of BDA505 followed by BDA504 (232.61 μg glucose released g⁻¹ of manure 24 h⁻¹), BDA502 (224.64 μg glucose released g⁻¹ of manure 24 h⁻¹) and BDA503 (220.57 μg glucose released g⁻¹ of manure 24 h⁻¹) compared to BD regular preparation. The alternative BD preparation (BD503, BDA502, BDA504 and BDA505) was recorded lowest alkaline phosphatase activities (205.87, 242.64, 264.96 and 282.77 μg p- Nitrophenol released g⁻¹ of manure h⁻¹) compared to BD regular preparation of BD502 (281.11 μg p- Nitrophenol released g⁻¹ of manure h⁻¹), BD503 (288.43 μg p- Nitrophenol released g⁻¹ of manure h⁻¹), BD504

(322.09 μ g p- Nitrophenol released g⁻¹ of manure h⁻¹), BD505 (283.65 μ g p-Nitrophenol released g⁻¹ of manure h⁻¹) (**Fig.6.1g**).

4.4.3. Impact of biochemical properties in BD preparation (Regular and Alternative)

The alternative BD preparation of BDA503, BDA502, BDA504 and BDA505 recorded the low amount total sugar (181.08, 190.72, 191.56 and 199.19 mg 100 g⁻¹) and phenolic (76.35, 83.27, 92.43 and 96.48 mg 100 g⁻¹) compared to sugar (235.59, 250.86, 227.62 and 234.98 mg 100 g⁻¹) and phenolic compound (132.19, 128.08, 116.70 and 126.46 mg 100 g⁻¹) BD regular preparation of BD502, BD503, BD504 and BD505 (Fig.6.1f, h)..

The high amount of humic acid (375.26, 342.54, 317.79 and 300.72 mg 100 g⁻¹) and protein (277.96, 236.41, 217.44 and 204.09 mg 100 g⁻¹) were recorded in alternative BD preparation of BDA504, BDA503, BDA505 and BDA502 compared to humic acid (263.41, 238.83, 240.27 and 258.44 mg 100 g⁻¹) and protein (158.92, 139.49, 138.37 and 153.14 mg 100 g⁻¹) of BD regular preparation of BD502, BD503, BD504, BD505 (**Fig.6.1f**).

4.4.4. Impact of microbial populations in BD preparation (Regular and Alternative)

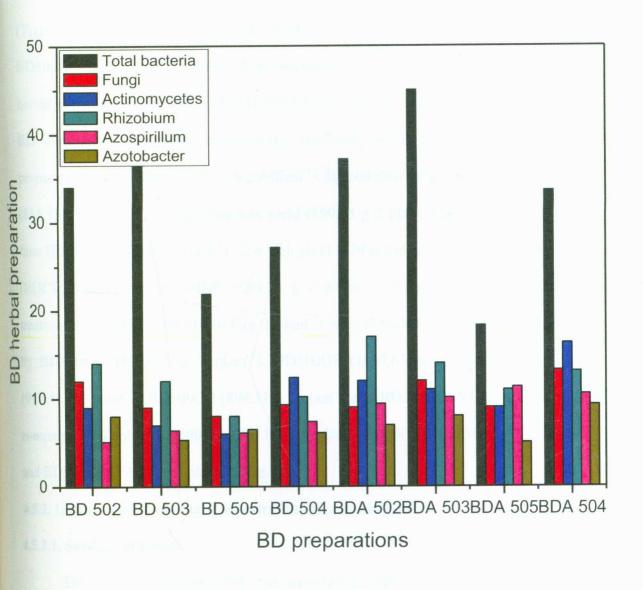
Investigation made on eight BD preparation (four regular and four alternative preparation) viz., BD502, BD503, BD504, BD505, BDA502, BD503, BD504 and BD505 prepared in Kurinji Organic Farms Pvt. Ltd, Genguvarpatti. Among the BD preparation, the alternative preparation of BDA503 recorded a maximum significant number of total bacteria (45 X 10⁶ CFU g⁻¹) than the BD503 (37 X 10⁶ CFU

g⁻¹), whereas the BDA505 recorded the low number of total bacteria (8.3). The alternative preparation (BDA504, BDA502, BDA503 and BDA505) recorded significantly high number of actinomycetes (9, 11, 12 and 16.3 X 10⁶ CFU g⁻¹) respectively) compared to BD regular preparation of BD502 (9 X 10⁶ CFU g⁻¹), BD503 (7 X 10⁶ CFU g⁻¹), BD504 (12.4 X 10⁶ CFU g⁻¹) and BD505 (6 X 10⁶ CFU g⁻¹). Similarly the *Rhizobium* like colonies were also recorded high number of population (17, 14, 13.1 and 11 X 10⁶ CFU g⁻¹) in alternative preparation of BDA502, BDA503, BDA504 and BDA505 respectively. The *Azospirillum* were also present similarly high number of population (11.3, 10.5, 10.1 and 9.4 X 10⁶ CFU g⁻¹) in alternative preparation of BDA505, BDA504, BDA503 and BDA502 compared to BD regular preparation of BD502 (5.1 X 10⁶ CFU g⁻¹), BD503 (6.4 X 10⁶ CFU g⁻¹), BD505 (6.3 X 10⁶ CFU g⁻¹) and BD504 (7.4 X 10⁶ CFU g⁻¹) (Fig.6.2)

4.5. Efficacy of different biodynamic manures on selected plants under laboratory and field trial

The polythene bag study with different treatment such as control (T₁), Chemical fertilizer (T₄), Non- BDC (T₂), biodynamic manures such as BDC (T₃), BD500CH (T₆) BD500CM (T₇), BD500CP (T₉) BD500CG (T₈), BD500BH (T₁₀), BD500BM (T₁₁), BD500BP (T₁₂), BD500BG (T₁₃), BD500GH (T₁₄), BD500GM (T₁₅), BD500GP (T₁₆), BD500GG (T₁₇), BDA502(T₁₈), BDA503(T₁₉), BDA504 (T₂₀), BDA505 (T₂₁), BD502 (T₂₂), BD503 (T₂₃), BD504 (T₂₄) and BD505 (T₂₅) was conducted for determining the biodynamic manures influence on tomato fruit and moringa leaves biomass yield were analyzed and the results were discussed below.

Fig: 6.2. Impact of microbial populations in BD preparation (Regular and Alternative)



4.5.1. Influence of biodynamic manures on tomato fruit yield

A maximum of fruit yield (1126.07 g 2 plant⁻¹) was recorded from BDA502 (T₁₈) followed by BD503 (1014 two plant⁻¹), BD50GM (1011.33 g 2 plant⁻¹) and BD500GG more than 1½ folds when compared to control. Compared between BD herbal preparations, the high yield (1126.07, 915.46 and 863.74 g 2 plant⁻¹) recorded in BD alternative preparation of BDA502, BDA504 and BDA505 than BD herbal preparation such as BD502 (844.28 g 2 plant⁻¹), BD504 (867.50 g 2 plant⁻¹) and BD505 (815.54 g 2 plant⁻¹), Whereas the low yield (890.75 g 2 plant⁻¹) recorded in BDA503 than BD503 (1014.09 g 2 plant⁻¹). The high yield (729 g 2 plant⁻¹) biodynamic compost (BDC) compared to Non-BDC (699.30 g 2 plant⁻¹). Among the BD500 manures treatments, the high yield (1011.33 g 2 plant⁻¹) were recorded in BD500GM followed by BD500GG (1008.02 g 2 plant⁻¹), BD500GP (1007.03 g 2 plant⁻¹), BD500BM (917.41 g 2 plant⁻¹), BD500CG (894.11 g 2 plant⁻¹) and BD500CH (892.57 g 2 plant⁻¹) compared to Control BD500GH (703.82 g 2 plant⁻¹), BD500CM (758.77 g 2 plant⁻¹) and BD500BP (799.32 g 2 plant⁻¹) (Fig.7.1).

4.5.2. Influence of biodynamic manures on Moringa olifera

4.5.2.1. Seed germination

The seed germination (100 %) recorded in BDC, BD500CM, BD500BM, BD500GM and BDA502 followed by BD500GP (91.67 %), BD500CH (91.67 %), BDA505 (91.67 %) and BD503 (83.33 %). The lowest seed germination (58.33 %) was recorded in control treatment followed by BD502 (66.67 %) (**Fig. 8.1**).

Fig: 7.1. Efficacy of different biodynamic manures on tomato fruit yield

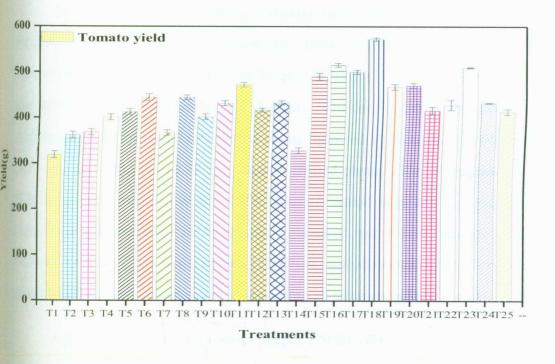
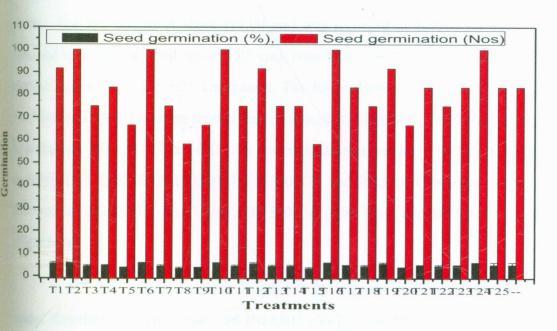


Fig: 8.1. Influence of biodynamic manures on seed germination of *Moringa olifera*



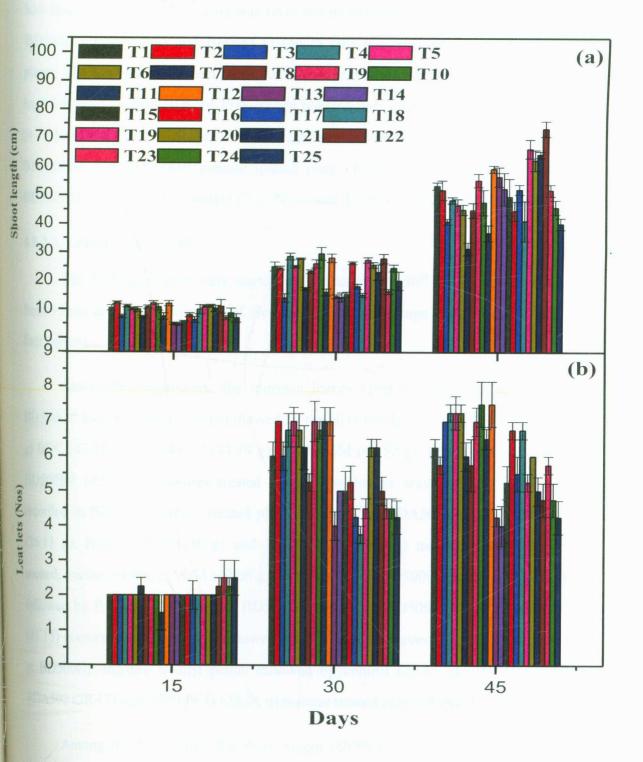
4.5.2.2. Biometric parameters

In 15th day of moringa plants, maximum shoot length (12.25 cm) was recorded in BD500CM manure treated experimental plants followed BD500GH (12.13 cm), BD500GP (12.00 cm), BD500CP (11.13 cm), BD502 (11.13 cm), BDA505 (11.00 cm) and BD504 (11.00 cm) manure treated plants. The low length of shoot (4.75 cm) was recorded in chemical fertilizers treated plants followed by Non-BDC (5.00 cm), control (5.75 cm) and BDA503 (6.38 cm) manure treated plants. The low number of leaf let (1.50 Nos) were recorded in BD500GG manure treated bags and high number leaf let (2.50 Nos) was recorded in biodynamic+chemical and BD505 manure treated bags followed by BDC (2.25 Nos), BD504 (2.25 Nos) and BD500BG (2.25 Nos). the number of leaf let (2.00 Nos) was recorded in remaining all treatments(Fig.8.2 a,b).

On 30th day, maximum shoot length (29.50 cm) was recorded in BD500GM manure treated experimental plants followed BD500CP (28.50 cm), BD500GP (28.00 cm), BD504 (27.75 cm), BD500BM (27.75 cm) and BDA505 (27.25 cm) manure treated plants. The low length of shoot (14.00 cm) was recorded in BD500CG treated plants followed by chemical fertilizers (14.25 cm), Non-BDC (14.50 cm), BDA504 (15.00 cm) manure treated control (15.25 cm) plants. The low number of leaf let (3.75 Nos) were recorded in BDA504 manure treated bags followed by Non-BDC (4.00 Nos) and manure treated plants BDA503 (4.25 Nos). The and high number leaf let (7.00 Nos) was recorded in BD500CM, BD500BH BD500GH, BD500GP and BD500GG manure treated plants followed by BD500GM (6.75 Nos), BD500BM (6.75 Nos) and BD500CP (6.75 Nos) (Fig.8.2a,b).

In 45th day, maximum shoot length (73.25 cm) was recorded in BD504 manure treated experimental plants followed BDA505 (66.25 cm), BD503 (64.25 cm), BD502 (62.25 cm), BD500GP (59.25 cm) and Non-BDC (56.50 cm) manure treated plants. The

Fig: 8.2. Influence of biodynamic manures on biometric of *Moringa olifera*a) Shoot length b) Leaf let



low length of shoot (31.25 cm) was recorded in BD500BG treated plants followed by BD500GG (36.75 cm) and BD500CG (40.75 cm) manure treated control (15.25 cm) plants. The low number of leaf let (4.00 Nos) were recorded in chemical fertilizer treated bags followed by Non-BDC (4.25 Nos) and biodynamic+ chemical (4.25 Nos), BDA504 (4.75 Nos) and BDC(4.75Nos). The and high number leaf let (7.50 Nos) was recorded in BD500GM and BD500GP manure treated plants followed by BD500CP (7.25 Nos), BD500BH (7.25 Nos), BD500BM (7.25 Nos), and BD500CG (7.00 Nos) (Fig.8.2a,b).

4.5.2.3. Leaf biomass yield

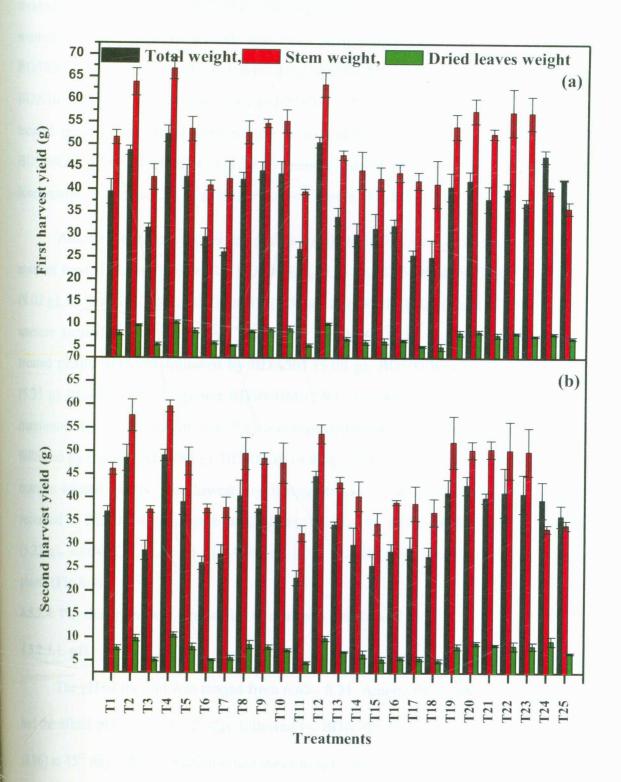
The Moringa leaves were started to first harvest at 50th day by cutting the plant from 15 cm above the ground level. Second harvest of moringa leaves after 50th days of first harvest.

Among the treatments, the moringa leaves yield (52.36 g) were recorded in BD500CP manure treated plants followed by the BD500 GP (50.55 g), BD500CM (48.63 g) BDC (47.51 g), BD500GH (44.19 g), BD500GM (43.52 g), BD500BH (42.80 g) and BD500BP (42.28 g) manures treated plants. The lowest leaves yield (24.99 g) was recorded in BDA504 manure treated plants followed by BDA503 (25.44 g), BD500BG (26.11 g), BD500GG (26.76 g) and BD500BM (29.46 g) manure treated plants. In second, maximum leaves yield (49.06 g) was recorded in BD500CP manure treated plants followed by BD500CM (48.49 g), BD500GP (44.58 g), BD5002 (42.69 g) and BD504 (41.15) manure treated plants. The lowest yield of second harvest (22.79 g) was recorded in BD500GP manure treated plants followed by control (25.45 g), BDA504 (27.39), BDA502 (28.47) and BD500CG (28.65 g) manure treated plants (Fig.8.3).

Among the treatments, the stem weight (66.86 g) were recorded in BD500CP manure treated plants followed by BD500GP (63.36 g), BD500CM (63.81 g), BD502 (57.54 g), BD504 (57.25 g), BD505 (57.07 g) and BD500GM (55.19 g) manure treated

Fig: 8.3. Efficacy of different biodynamic manures on *Moringa olifera* leaves yield

a). First harvest b). Second harvest



plants. The lowest stem weight (35.94 g) was recorded in Biodynamic+chemical manure treated plants followed by BD500GG (39.56 g), BDC (39.77 g) and BD500BM (40.90 g) manure treated plants. In second, maximum stem weight (59.57 g) was recorded in BD500CP manure treated plants followed by BD500CM (57.61 g), BD500GP (53.69 g), BDA505 (51.95 g), BD503 (50.45 g) and BD502 (50.27 g) and BD504 (50.23 g) manure treated plants. The lowest stem weight in second harvest (32.32 g) was recorded in BD500GG followed by BDC (33.39 g) manure treated plants, control (34.44 g) and biodynamic+chemical (34.17 g) manure treated plants (**Fig.8.3**).

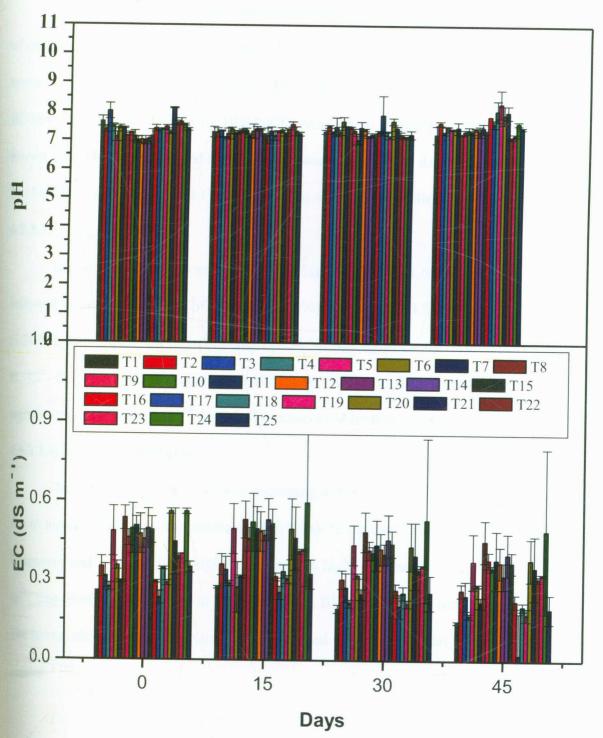
Among the treatments, the dry leaves weight (10.47 g) were recorded in BD500CP manure treated plants followed by BD500GP (10.11 g), BD500CM (9.73 g), BD500GM (9.02 g), BD500GH (8.84 g), BD500BH (8.56 g), BD500BP (8.46 g), and BD502 (8.40 g) manure treated plants. The lowest dry leaves weight (5.00 g) was recorded in manure treated plants BDA504 followed by BDA503 (5.09 g), BD500BG (5.22 g), BD500GG (5.35 g) BD500CG (5.55 g) and BD500BM (5.89 g) manure treated plants. In second, maximum dry leaves weight (10.57 g) was recorded in BD500CP manure treated plants followed by BD500CM (9.84 g), BD500GP (9.82 g), BD502 (8.84 g) and BD503 (8.47 g) manure treated plants. The lowest dry leaves weight of second harvest (4.61 g) was recorded in BD500GG manure treated plants followed by BDA504 (5.07 g), BD500BM (5.23 g), BD500CG (5.28 g), BDA503 (5.55 g) and BDA502 (5.63 g) manure treated plants (Fig.8.3).

4.5.2.4. Physico-chemical properties of moringa grown soil

4.5.2.3.1. pH

The pH of the soil was ranged from 6.65 - 8.31. Among the treatments, BDA503 had the alkali pH (8.31) at 45th day followed by BDA505 (8.12) at 0th day and BD502 (8.06) at 45th day. The interaction effect was found to be non significant (**Fig. 8.4a**).

Fig: 8.4. Effect of biodynamic manures on pH and EC content on *Moringa* olifera soil



4.5.2.3.2. EC

In all the treatments the EC of the soil was found to be non significant at all days of observation. Even through after the addition of manure the EC content of soil was come within normal range. The highest EC (0.60 dS m⁻¹) was recorded at 15th day in BD504 treated soil followed by BD500CG (0.54 dS m⁻¹) treated soil at 0th day. The lowest EC (0.02) was recorded in BD500GG treated soil at 45th day followed by control (0.15 dS m⁻¹) and chemical (0.17 dS m⁻¹) soil at 45th day (**Fig.8.4b**).

4.5.2.3.3. Organic Carbon

The organic carbon was higher (3.70 %) in BD500BM treated soil at 15th day followed BDA504 (3.53%), BD500CP (3.45 %), BD500BH (3.20 %) and BD500BM (3.20 %). The lowest soil organic carbon (0.54 %) was significantly recorded in control at 45th day followed by control (0.68 %) at 30th day. The organic carbon was significantly increasing at 15th day of all treatments of treated with manure (**Fig.8.6b**).

4.5.2.3.4. Available nitrogen

The soil available N level was increasing at the 15th day of moringa cultivation due to the effects of various treatments (**Fig.8.5a**). In case of interaction effect between treatments and duration were significant and the highest value (651.96 and 651.36 kg ha⁻¹) was recorded at 15th day in BD500BM and BDA504 manure treated soil sample. The lowest nitrogen (65.10 Kg ha⁻¹ at 45th day and 131.42 at 30th day) was recorded in control.

Fig:8.5. Effect of biodynamic manures on available nitrogen and phosphorus contents on *Moringa olifera* soil

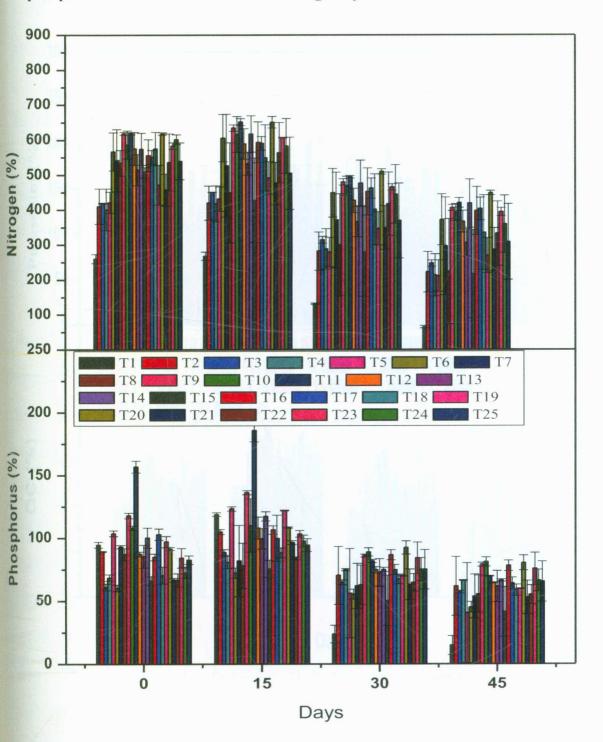
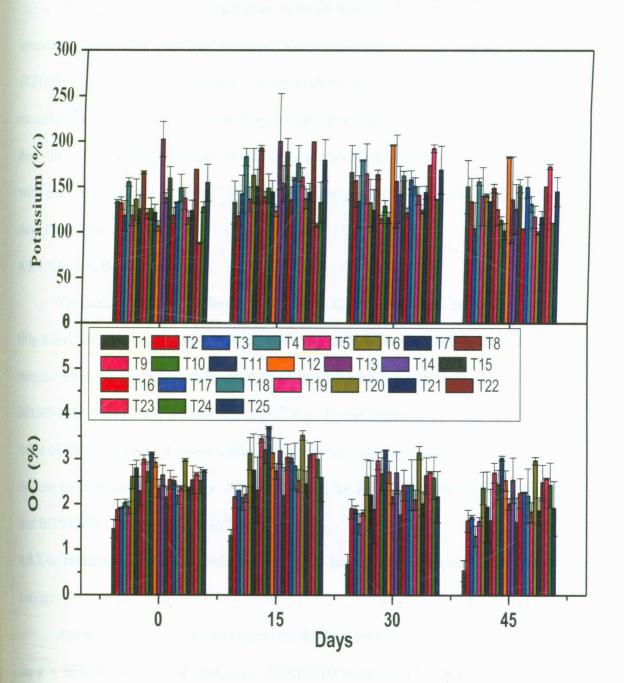


Fig:8.6. Effect of biodynamic manures on available potassium and organic carbon (OC) contents on *Moringa olifera* soil



4.5.2.3.5. Available phosphorus

The available P of soil higher (136.28 Kg ha⁻¹) was recorded in BD500CP manure treated soil followed by biodynamic+chemical (123.29 Kg ha⁻¹), BDA503 (121.93 Kg ha⁻¹) treated soil and control (118.84 Kg ha⁻¹) at 15th day. The control recorded the lowest soil available P value of 14.86 and 23.79 kg ha⁻¹ at 45th and 30th day. In case of different stages of duration, the soil available P was higher at 15th day with the value of 136.28 kg ha⁻¹ and lowest value (14.86 kg ha⁻¹) was recorded at 45th day (Fig.8.5b).

4.5.2.3.6. Available potassium

There is significant different in available K content of soil between treatments (Fig.8.6a). the highest available K (199.69 kg ha⁻¹) was recorded in the BD500BG manure treated soil followed by BD502 (199.46 kg ha⁻¹) treated soil at 15th day and BD503 (195.91 kg ha⁻¹) treated soil at 30th day. It was higher at 15th day (D₁) with the value of 199.69 kg ha⁻¹. Lowest value (87.73 kg ha⁻¹) was recorded at 0th day in BD503 manure treated soil followed by BDA504 (99.31 kg ha⁻¹), BD500BM (101.19 kg ha⁻¹) and BD500GP (103 kg ha⁻¹) at 45th day.

4.5.2.4. Influence of biodynamic manures on biochemical properties of Moringa leaves:

Among the twenty five treatment, the highest protein of moringa leaves (216.47 mg g⁻¹) in BDC treatment followed by BD500GH treatment (212.26 mg g⁻¹), BDA503 treatment (209.87 mg g⁻¹) and Biodynamic+chemical treatment (199.90 mg g⁻¹). The lowest protein of moringa leaves (111.69 mg g⁻¹) in control treatment followed by Non-

BDC treatment (120.64 mg g⁻¹), chemical fertilizer (144.71 mg g⁻¹) and BD500BM treatment (147.37 mg g⁻¹). The highest carbohydrate of moringa leaves (751.04 mg g⁻¹) in the Biodynamic+chemical treatment followed by BDC treatment (681.38 mg g⁻¹) BD500CH treatment (616.74mg g⁻¹) BD500BG treatment (616.33 mg g⁻¹) and BDA502 treatment (513.83 mg g⁻¹). The lowest carbohydrate of moringa leaves (202.40 mg g⁻¹) in the control treatment followed by chemical treatment (241.10 mg g⁻¹) and BD502 treatment (295.86 mg g⁻¹). Among twenty five treatment, the highest β-carotene of moringa leaves (0.51 %) in BD500CG treatment followed by BD500CH treatment (0.47 %), BD502 treatment (0.46 %), BD500GH treatment (0.46 %), BD500CM treatment (0.44 %), BDA505 treatment (0.41 %) and BD500BP (0.41 %). The lowest β-carotene of moringa leaves (0.22 %) in the control treatment followed by Non-BDC treatment (0.27 %), BDA502 treatment (0.30 %), BD503 treatment (0.30 %) and BD500GM treatment (0.30 %). Fig. 8.7, 8.8, 8.9)

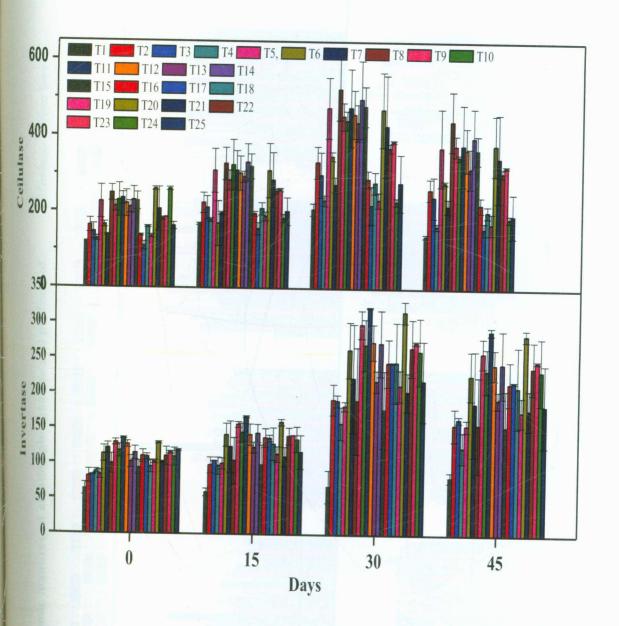
4.5.3. Influence of biodynamic manure (BD500) on ground nut and soil

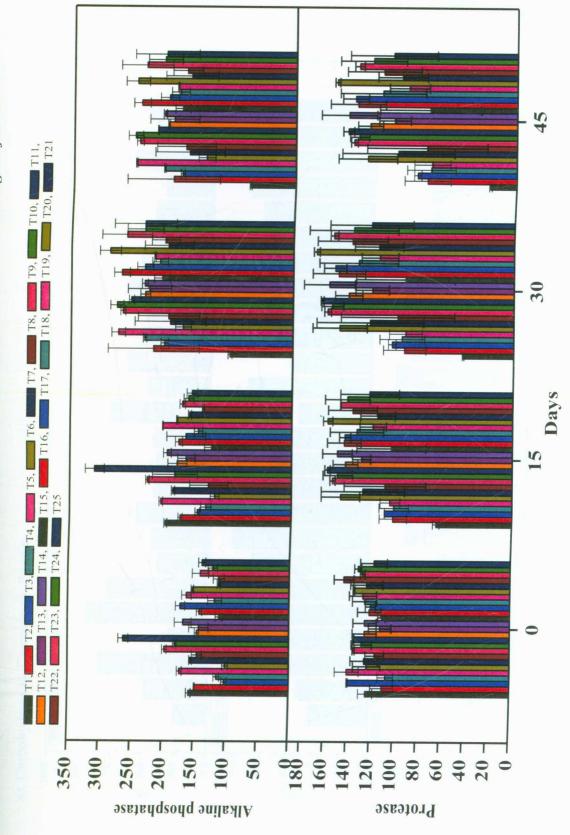
The field experiment with different treatment such as control (T_1) , Controll (T_2) organic, inorganic (Chemical) (T_3) , organic+chemical (T_4) vermiccompost (T_5) and biodynamic (BD500 (T_6)) was conducted for determining the biodynamic manures influence on ground nut yield and soil properties were analyzed and the results were discussed below.

4.5.3.1. Efficacy of biodynamic manure (BD500) on ground nut yield

The application of different organic manures influenced the pod, halum and dry matter yield (**Fig 9.5**). The highest pod yield of 3200 kg ha⁻¹, halum yield of 2968 kg

Fig: 8.7. Influence of biodynamic manures on cellulase and invertase of *Moringa olifera* leaves





B 9 T25 T9 T10 T11 T12 T13 T14 T15 T16 T17 T18 T19 T20 T21 T22 T23 T24 Days T8 a). Carbohydrate b). Carotene c). Protein Carbohydrate carotene "Protein 2 gm 0.1 248 = 800 0.0 0.5 0.4 0.3 009 200 200 160 120 -80 40 0 (%) ិខ្លួន ខ្លួក

ha⁻¹ and drymatter yield of 6168 kg ha⁻¹ were recorded in T4 (organic +chemical). The lowest pod yield of 2813 kg ha⁻¹, halum yield of 2488 kg ha⁻¹ and drymatter yield of 5300 kg ha⁻¹ were recorded in T1(control) (Fig. 9.4)

4.5.3.2. Effect of biodynamic manures on properties of groundnut cultivated soil 4.5.3.2.1. pH

The pH of the soil was slightly acidic ranged from 6.1 - 6.3. Among the treatments, slightly neutral pH of 6.3 was observed in T_2 (organic manure), which were on par with T_1 (Control). The slightly alkaline pH value of 6.1 was recorded in T_4 (Organic manure) T_5 (vermi compost) and T_6 (Biodynamic manure). In case of duration, slightly neutral pH of 6.4 was observed in D_6 (90 days). The interaction effect was found to be non significant (**Fig. 9.1a**).

4.5.3.2.2. EC

In all the treatments the EC of the soil was found to be non significant at all stages of observation (Fig. 9.1b). Even through after the addition of manure the EC content of soil was come with in normal range

4.5.3.2.3. Organic Carbon

The organic carbon content was higher (0.59 %) in T_4 (Organic and inorganic manure). It was on par with T_5 (vermi compost) and T_6 (Biodynamic manure). The lowest soil organic carbon content of 0.49 per cent (T_1) , which was on par with T_2 (organic) and T_3 (in organic) (Fig. 9.2d).

The interaction effects of treatments with various stages were found to be significant and recorded the highest value of 0.68 per cent in T_6 (biodynamic manure

Fig: 9.1. Effect of biodynamic manures on pH and EC of groundnut cultivated soil

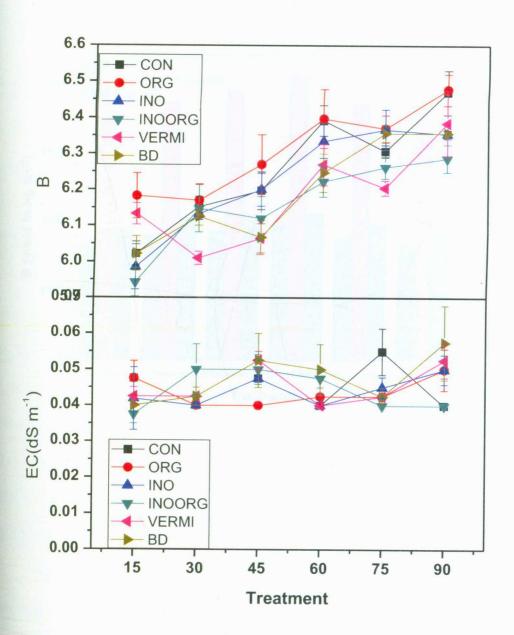
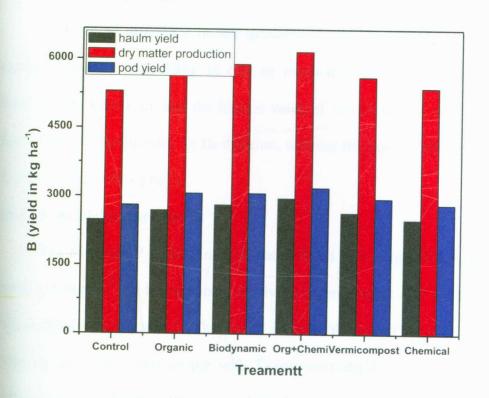


Fig 9.4. Efficacy of different biodynamic manures on yield of groundnut



application) at D_6 (90 DAYS) and the lowest value of 0.37 per cent was recorded in control (T_1) at D_5 (75 DAYS).

4.5.3.2.4. Available nitrogen

There is no difference in soil available N was observed due to the effects of various treatments (Fig. 9.2a). In case of interaction effect between treatments and duration were significant and the highest value of 125.15 kg ha⁻¹ was recorded in T_6 (application of biodynamic) at D_6 duration, whereas the lowest value was observed in T_6 at D_2 stage (80.08 kg ha⁻¹).

4.5.3.2.5. Available phosphorus

The available P content of soil status found to be high (Fig.9.2f) and it was higher (14.3kg ha⁻¹) in the treatment receiving vermicompost (T₅) which were on par with T₆ (biodynamic manure). The control recorded the lowest soil available P value of 10.04 kg ha⁻¹, which was on par with T₂ (organic) and T₃ (in organic). In case of different stages of duration, the soil available P was higher at 45 day (D₃) with the value of 13.1 kg ha⁻¹ and lowest value (10.3 kg ha⁻¹) was recorded at 30 day. The interaction effect was found to be non significant.

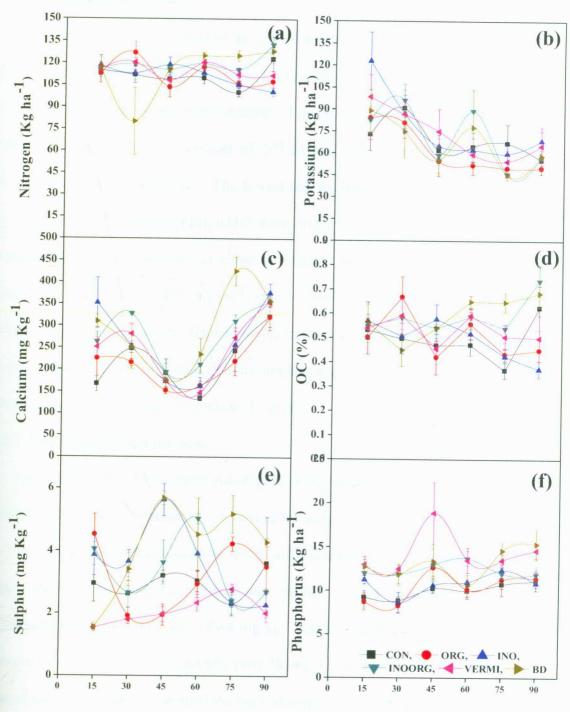
4.5.3.2.6. Available potassium

There no significant different in available K content of soil between treatments (Fig. 9.2b). But, in case of various stage of duration available K content of the soil status found to be significant. It was higher at 15 DAYS (D₁) with the value of 91.7 kg ha⁻¹. It was followed by D₂ (30 DAYS) (86.1 kg ha⁻¹) and lowest value was recorded in

Fig: 9.2. Effect of biodynamic manures on organic carbon and macronutrient of groundnut cultivated soil

a). Nitrogen b). Potassium c) Calcium d) Organic carbon(OC) e) Sulphur

f). Phosphorus



 D_5 (75 DAYS) (53.7 kg ha⁻¹), D_6 (90 DAYS) (59.1 kg ha⁻¹) and D_3 (45 DAYS) (61.7 kg ha⁻¹).

4.5.3.2.7. Exchangeable calcium

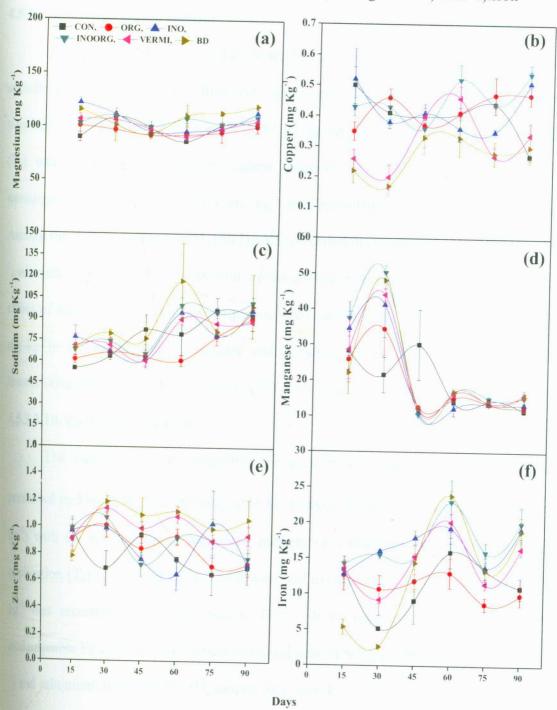
The exchangeable Ca content of the soil (Fig.9.2C) was significantly influenced by different treatments. Biodynamic manure (T₆) recorded significantly higher value of 292.6 mg kg⁻¹, followed by integrated nutrient (T₄) and inorganic fertilizer (T₃) which recorded the exchangeable Ca contents of 277.23 and 262.26 mg kg⁻¹ respectively, which were on par with each other. The lowest soil exchangeable Ca value of 216.18 mg kg⁻¹ was recorded in organic (T₂), which were on par with control (217.92 mg kg⁻¹). With regard to soil exchangeable Ca value at different stages, the highest value was recorded at 90 DAYS (D₆) (10.97 mg kg⁻¹) and the lowest value of 175.76 mg kg⁻¹ on 60 DAYS (D₄). The interaction effect between treatments with duration recorded the maximum value in T₆ (biodynamic manure application) at D₅ (425.83 mg kg⁻¹) and minimum value (134.81 mg kg⁻¹ in control (T₁ at D₄).

4.5.3.2.8. Exchangeable magnesium

The exchangeable Mg content was higher (108.29 mg kg⁻¹) (Fig. 9.3a) with the application of biodynamic manure (T₆) followed by inorganic fertilizer (T₃) (106.23 mg kg⁻¹). The organic manure recorded the lower soil exchangeable Mg content of 96.51 mg kg⁻¹. The exchangeable Mg content was slightly altered over time in all the treatments and the higher value of 108.68 mg kg⁻¹ was recorded at D₆ (90 DAYS) and the lowest value at the D₅ (75 DAYS) (101.78 mg kg⁻¹). The interaction effect of treatments and various stages recorded the highest magnesium content of 122.53 mg kg⁻¹

Fig: 9.3. Effect of biodynamic manures on magnesium, sodium, manganese, zinc and iron of groundnut cultivated soil

a). Magnesium b). Copper c). Sodium d). Manganease e). Zinc f). Iron



 1 in T_{7} (application of in organic) at D_{1} stage and lowest value of 86.49 mg kg $^{-1}$ in T1 at D_{4} stage.

4.5.3.2.9. Exchangeable sodium

From the results given in **Fig. 9.3c,** it is evident that the exchangeable Na was significantly influenced by different treatments. The exchangeable Na content was higher in biodynamic manure (T_6) (87.15 mg kg⁻¹), followed by integrated nutrient (T_4) which recorded the second highest value of 84.43 mg kg⁻¹. The lowest soil exchangeable Na value of 70.04 mg kg⁻¹ was recorded in inorganic fertilizer (T_2). Among the different duration, D_6 (90 DAYS) recorded the maximum value of 94 mg kg⁻¹, whereas D_1 (15 DAYS) recorded the minimum value of 66.95mg kg⁻¹. The interaction effect of treatments and various stages recorded the highest soil Na content of 117.06 mg kg⁻¹ in T_6 (application of organic and inorganic manures) at D_4 (60 DAYS) and lowest value of 55.13 mg kg⁻¹ in control (T_1) at D_1 stage.

4.5.3.2.10. Exchangeable iron

The data on soil exchangeable Fe at different stages of crop growth are presented in Fig. 9.3f. The exchangeable Fe content of the soil was higher (17.34 mg kg⁻¹) with the application of integrated nutrient (T₄) followed by inorganic fertilizer application (T₃) (16.50 mg kg⁻¹). The lowest soil exchangeable Fe content of 11.10 mg kg⁻¹ was recorded in organic manure (T₂). With regard to various stages, the exchangeable Fe content was found to be maximum at 60 days (D₄ stage) (17.34 mg kg⁻¹) and minimum at 30 DAYS (D₂ stage) (0.96 mg kg⁻¹). Among the interactions, the lewest exchangeable Fe of 2.63 mg kg⁻¹ was recorded in T₆ (biodynamic manure) at S₂

(30 DAYS). The treatment T_4 (application of organic and inorganic manures) at D_4 stage recorded highest exchangeable Fe value of 23.02 mg kg⁻¹.

4.5.3.2.11. Exchangeable manganese

There is no significant different in manganese content of soil between treatments. But, in case of various stage of duration Mn content of the soil status found to be significant. It was higher at 30 DAYS (D_2) with the value of 39.98 kg ha⁻¹. It was followed by D_1 (29.5 mg kg⁻¹) and lowest value was recorded in, D_4 (14.0 mg kg⁻¹), D_5 (14.4 mg kg⁻¹) D_6 (14.4 mg kg⁻¹) and D_3 (14.8 mg kg⁻¹). (**Fig.9.3d**)

The interaction effect of treatments and duration recorded the highest soil Mn content of 50.41 mg kg⁻¹in T_4 (application of organic and inorganic manures) at D_2 (30 DAYS) and lowest value of 10.65 mg kg⁻¹ in T_4 (application of organic and inorganic manures) at D_3 (45 DAYS).

4.5.3.2.12. Exchangeable zinc

The soil zinc content was significantly influenced by the different treatments and the results are presented in **Fig.9.3e**. Significantly higher zinc content was recorded with the application of biodynamic manure (T_6) (1.037mg kg⁻¹) and lower value was recorded in control (T_1) (0.782 mg kg⁻¹). Application of vermi compost (T_5) recorded the second highest value (0.990 mg g⁻¹), which was on par with biodynamic manure (T_6).

In case of different stages, the highest value was observed on 30 DAYS (1.012 mg kg⁻¹¹) and the lowest value on 90 DAYS (0.819 mg kg⁻¹).). Among the interactions, the lowest exchangeable Zn of 0.65 mg kg⁻¹was recorded in T_1 , T_3 at D_5

and D_4 stages respectively. The treatment T_6 (application of biodynamic manure) at D_4 stage recorded highest exchangeable Zn value of 1.12 mg kg⁻¹.

4.5.3.2.13. Exchangeable sulphur

The highest value of Sulphur in soil (3.68 mg kg⁻¹) was recorded in the treatment with application of inorganic manure (T_3), followed by inorganic + organic manures (T_4) (3.64 mg kg⁻¹), which were on par each other. The lowest value of 2.68 mg kg⁻¹ was recorded in organic (T_2) (**Fig9.2e**).

Regarding the various stages of observation, highest and lowest values were recorded at D_6 stage (4.41mg kg⁻¹) and D_5 stage (2.07 mg kg⁻¹), respectively. Among the interactions, the lowest exchangeable sulphur of 1.53 mg kg⁻¹was recorded in T_1 at D_5 . The treatment T_3 (application of inorganic manure) at D_3 stage recorded highest exchangeable sulphur value of 5.69 mg kg⁻¹.

DISCUSSION

5. Discussion

Organic agriculture is one among the broad spectrum of production methods which are socially and ecologically sustainable of the environment. Organic production system is a development vehicle for developing countries like India for achieving agro ecosystems (FAO, 2000; Ramesh et al., 2005). Different organic manures were analyzed in an apple pot trial and concluded that organic manure increased the levels of growth regulators in the soil and stimulated plant growth (Li-xiu et al., 1998). Recently, there has been increasing interest in biodynamic farming and gardening from 142 to 202 farms in France from 1989 and 1992 the (Bio-Dynamic Farming and Gardening Association in New Zealand, 1993). A mid-1980s survey in Europe recorded that 1,090 commercial biodynamic farms and gardens on 17,616 ha; 42 % of these were in Germany and 15 % in Holland (Koepf et al., 1989). Although biodynamic farming is practiced in cool and warm climates on all continents, the highest proportions of biodynamic farms are found in Western Europe, Australia, New Zealand and North America (Lampkin, 1990). On Australian bio-dynamic soils increases in organic matter from 0.9 % to 11.4 % have been registered in a few years in the top 4 inches and totally new organic matter levels were measured to a depth of 40 inches. The overall co₂ ratio in the soils increased from 10 to 586 tonnes per acre (Podolinsky, 1989).

Among the 32 different organic biodynamic manures, organic carbon (43.50 %), magnesium (0.85 %), manganese (767 ppm), Zinc (132 ppm) and copper (78 ppm) was recorded in BD500-D, BD500-K, compost-M, compost-KK respectively. The organic manures were shown both direct and indirect benefits to soil and plants, their

conservation and efficient use in agriculture assumed importance as essential components of nature friendly agriculture.

Several measurements suggested that BD composting is different from non - BD composting. Biodynamic-treated compost is consistently beneficial than non - BD compost. The level of nitrate increases in compost towards the time of full compost maturity (Poincelot, 1972).

In general, 13.6 kg of composting material can yield 5-7 kg of BD compost. This yield seems too small however it effects are notable. Yet, many bioactive compounds present in BD compost that protect the agriculturally important crops (Ries, 1984; Sasse, 1989). Yarrow, chamomile, stinging nettle and valerian containing bioactive compounds are the important ingredients in BD preparations (Hornok, 1992). Extracts of chamomile, for instance, have shown antibacterial and antifungal properties (Foster, 1990). The efficacy of BD and untreated composts on soil biological and fertility parameters and crop yield were made by Carpenter - Boggs, (1997) and Carpenter-Boggs *et al.* (2000a, 2000b).

Experiments were conducted to assess the utility and importance of cow horn in the preparation of cow horn manure (BD 500). It was observed that the preparation (cow dung) cow dung manure harvested after a period of 6 months from the cow horn was significantly better in terms of physical, chemical and biological properties when compared with the preparation (cow dung) harvested from bull horn, buffalo horn and mud pot. This correlates with the earlier works of Pfeiffer (1983) that BD 500 in cow horn had increased levels of micronutrients like calcium, copper, magnesium,

manganese thus stimulate soil micro-life with increase in micro flora and humusforming bacteria.

In the present study leaves mold sample contained a maximum amount of organic carbon of 28% which was less than 4% as recorded from the final compost samples collected at 60 cm depth after 8 weeks (Carpenter-Boggs et al., 2000). Cow Pat Pit remuni had shown a maximum amount of humic acid of 751 mg/g when compared to the rest of samples studied. Remuni C had shown a maximum number of total bacteria of 4.4 x 106 CFU/g. Overall the differences in microbial (bacteria and fungi) communities between organic and conventional systems are limited (Foissner, 1992; Girvan et al., 2003). However, there was an evidence of general trend towards elevated bacterial (Bossio et al., 1998; Scow et al., 1994) and fungal (Fraser et al., 1988; Shannon et al., 2002) abundance/activity in organic systems, Fraser et al. (1988) reported a microbial biomass of 10-26% in organic management. Auxins are a class of plant growth substance plays an essential role in coordination of many growth and behavioral processes in the plant life cycle. The behavior they played in plant growth was first revealed by a Dutch scientist named Fritz Went (1903-1990). Auxin induces new root formation by breaking root apical dominance induced by cytokinins. In horticulture, auxins, especially NAA and IBA, are commonly applied to stimulate root growth. However, high concentrations of auxin inhibit root elongation and instead enhance adventitious root formation. Removal of the root tip can lead to inhibition of secondary root formation. Among the biodynamic manures Remuni C contained a high

amount of auxin of 13.3 $\mu g/g$ which probably the first report on the Plant Growth Regulator in biodynamic manures.

The success of composting process depends on several basic conditions including: the moisture content (50-60%) (Jeris and Regan, 1973a; Jeris and Regan, 1973b), to reach a temperature of 50-60°C (Schultz, 1962, McGregor *et al.*, 1981), microbial characteristics such as microbial count and assessing the maturity or stability of compost (Hassen *et al.*, 2001; Tiquia *et al.*, 2002).

Compost application can also enhance several beneficial properties in amended soils, at physico-chemical and biological levels. While the change of physico-chemical properties (temperature, bulk density, N, pH, organic carbon etc.) during the composting process has been extensively studied (Harada and Inoko 1980; Garcia *et al.*, 1991), information on the biological properties and in particular, an enzymatic activity is rare, especially with regards to the assessment of compost maturity. In our study, there was an increasing trend during composting in temperature up to 4th day. On 4th day the subsurface, middle and bottom samples reached 60°C, 65°C and 70°C, respectively. Thereafter there was a decline in temperature on 60th day. On 90th day the subsurface, middle and bottom samples showed, 30°C, 32°C and 35°C were recorded. Martins and Dewes (1992) identified initial nitrogen content, temperature, high pH (>8) and turning as the main factors which affected gaseous emissions during composting of slurries.

The optimum water content for composting varies with the waste to be composted, but generally the mixture should be at 50-60% (Gajalakshmi and Abbasi,

2008). pH between 6.7 and 9.0 support good microbial activity during composting (de Bertoldi *et al.*, 1983; Miller, 1992). In our study the different organic and biodynamic manures samples exhibited the pH 7.0-8.3.

Among the twelve different types of organic and biodynamic manures viz., i) Cow Pat Pit (CPP), ii) vermicompost, iii) NADEP compost, iv) panchakavya, v) biodynamic compost (BD), vi) Cow horn manure (BD 500), biodynamic herbal preparations such as vii) BD 502, viii) BD 503, ix) BD 504, x) BD 505, xi) BD 506 and xii) BD 507 taken tin the present study, the CPP manure showed a maximum pH 8.6, whereas the compost prepared by Adas (2005) showed pH 8.1. The levels of nitrogen content in vermicompost and CPP were 2.13% and 2.09%, respectively. CPP manure contained a high level of auxin of 28.7 μ g/g. Further it showed CFU of bacteria 4.9 x 10^6 CFU/g when compared to 4.4 x 10^6 CFU/g of the manures prepared by Anith (2003). In addition CPP manure contained a high protein content of 4.96 μ g/g and subtilin of 0.967 mg/g.

The distance, pattern, colour and shape of the reaction area are significant for the interpretation of different substances present in the extract. The shape and colour chromatogram images revealed the rich amount of humus and available nutrients occur in the manure (Steiner, 1996). Steiner (1996) assessed the presence of NPK, organic carbon and humus through circular paper chromatogram image techniques. In the present study all the above 12 different samples were analysed by following circular paper chromatogram image techniques. The Cow Pat Pit (CPP) manure sample exhibited an inner zone of 1.8 cm with violet radiating spikes indicated the nature of

completely mineralized manure samples. The medium size of a middle zone of 1.8 cm (width) with brown coloured radiating spikes (39 nos) indicated the presence of high quantity of decomposed organic matter. The large size of outer zone (2.5 cm) with light brown colour indicated the presence of highly stable humus. The violet radiating spikes of inner zone, medium size of middle zone brown coloured spikes and brown colour radiating spikes considered the presence of good nutrients.

Humic acid is one of the major components of humic substances (Piccolo, 2002), which are dark brown and the major constituents of soil organic matter humus that contributes to soil chemical and physical quality and also the precursors of some fossil fuels. They also be found in peat, coal, many upland streams, dystrophic lakes and ocean water. Humic substances make up a large portion of the dark matter in humus and consist of heterogeneous mixtures of transformed biomolecules exhibiting a supramolecular structure (Fiorentino *et al.*, 2006). Humic substances arise by the microbial degradation of plant and animal tissues and ultimately the biomolecules such as lipids, proteins, carbohydrates and lignin dispersed in the environment after the death of living cells. Campitelli and Ceppi, (2008) observed that the contribution of humic acid (HA) from composted materials to soil cation exchange capacity and soil buffer capacity seems to be larger than that from the HA isolated from vermicomposting treatments.

Organic matter application of both from manure (Aoyama *et al.*, 1999; Gerzabeck *et al.*, 2001) and plant residues (Sainju *et al.*, 2003) also promotes aggregate formation. Sugahara and Inoko (1981) reported the humic acid properties of a city

refuse compost. Organic amendments such as green manures, stable manures and composts have long been recognized to facilitate biological control if applied before planting (Lumsden *et al.*, 1983). Organic manure was increased the availability of soil nutrients and stimulated plant growth Li-xiu *et al.* (1998).

Mixture of different organic manures contained maximum level of nutrients (Boeringa, 1980). Physicochemical properties of four different combinations of biodynamic manures prepared in the present study. The BDM I (CPP+BD 500) sample contained showed a high amount of nitrogen (1.83%), phosphorus of (3.94%) and potassium (3.58%) than other samples.

Biodynamic compost and organic compost

In the present investigation, evaluation of composts on their maturity was aimed to determine the completion of the composting process on the mineralization rate (Forster *et al.*, 1993; Grebus *et al.*, 1994). The biodynamic compost maturity was stabilize by day of 120-135 as the in heap method. The results of nitrogen, phosphorus content and potassium in compost at 120-135 were higher than other day of compost. These results correlated with finding of the Rynk, *et al.*, 1992, Michel *et al.*, 1996 and Tiquia and Tam, 2000 which depend on nature of organic material being composted (i.e. organic and nutrient contents, C/N ratio,self-heating capability), pile size, frequency of aeration, moisture content, and composting method.

Physicochemical properties of biodynamic compost and non has been significantly correlated with earlier studies of frequently measured parameters in composting, such as decreasing level organic carbon, narrower C: N ratio, increasing

nitrogen, phosphorus and potassium (Heinze and Breda, 1978; Ahrens, 1984; von Wistinghausen, 1984; von Wistinghausen, 1986; Finstein and Miller 1985; Tiquia et al., 2002; Benito et al., 2003; Goyal *et al.*, 2005; Kato *et al.*, 2005). Earlier studies of Iglesias-Jimenez and Perez-Garcia 1992; Boos et al., 1997; Carpenter-Boggs et al., 2000; Goyal *et al.*, 2005 revealed result of higher heating BD preparation treated compost than non treated compost (Fig.....).

Different between the Biodynamic (BDC, BDCV-II and BDCV-I) and control treatments of compost, most of parameters analyzed were significantly higher enzyme activities such as protease, cellulase, invertase and alkaline phophatase dehydrogenase activity was in the BD-treated compost (p < 0.05, Fig. 1). The greater enzyme activity showed in BD preparation treated compost and soil (Garcia et al., 1992; Carpenter-Boggs et al., 2000; Bachinger 1996; Fliessbach et al., 2007; Reeve, 1993; Reeve, 1993). Reeve et al., 2010 and Reeve et al., 2005were observed the statistically not significant in enzyme activities of BD treated compost, which is not correlated with our result.

BD500 manure

Most studies on organic amendments are conducted in complex systems involving interactions between plant, soil, and micro flora. These are difficult to assess whether a particular plant response to direct influence of organic amendment mediated by nutrients or growth regulatory compounds or is an indirect manifestation of changes in soil physical or biological conditions (Malik and Bradford, 2007). The water extract of biodynamic compost (BDC), Non-biodynamic compost and cow horn manure (BD500CH, BD500CM, BD500CP and BD500CG) were utilized as medium for growth

of *Spilianthus calva* shootcuttings. The result of this revealed high number and length of roots in water extract of BDC than control and Non-BDC.

This was in agreement with those of Chenu *et al.* (2000), Puget *et al.* (2000) and Tejada and Gonzalez (2003, 2004) who found that a good soil structure depended on the content and nature of the organic matter added. This organic matter promotes flocculation of clay minerals, which is an essential condition for the aggregation of soil particles. The increase in structural stability was especially evident in CGCC amended soils at the end of experimental period and this was probably due to different chemical composition of the wastes used. This difference is evident from the different concentrations of humic and fulvic acids.

Total C and OM are essential in soil restoration; concentrations of OM from 4 to 6% are considered necessary for a soil to maintain its productive functions, while concentrations over 10% can be beneficial for soil structural stability (Díaz-Fierros, 1999). The values of OM in the mixtures by the end of the experiment were slightly higher than those expected from the added amounts of GMV, probably as a consequence of additional enrichment in C due to the effect of plant remnants (Paradelo et al 2007).

Several authors reported that the supply of organic matter to soil through different amendments stimulated microbial populations (Weon et al., 1999; Lee et al., 2004 and Gomez et al., 2006). In agreement with our results, Fließbach and Mader (1997) found that tillage and fertilization had short-term effects after farmyard manure application. Soil biota exist in the labile fraction of soil organic matter involved in energy and

nutrient cycling; thus, microbial attributes may respond more quickly to changes in management practices or environmental conditions than physical and chemical properties (Doran *et al.*, 1996); however, repeated application would be recommended as a sustainable management practice to maintain the influence of organic amendment on microbial diversity (Gomez *et al.*, 2006).

Said-Pullicino et al. (2007a and 2007b) indicate that increase in soluble NH4-N results from ammonification of easily mineralizable organic N. As the composting process proceeds NH4-N concentration decreases as a result of immobilization, ammonification, and/or nitrification. After 200-250 days of composting, increased N03-N concentration has been reported (Cooperband et al., 2003; Said-Pullicino et al., 2007). The results of NH4-N and NO3-N content of the onion compost in 2006 indicate the nitrification of NH4-N. Sánchez-Monedero *et al.* (2001) demonstrated that the highest NO3-N concentration is produced at the end of maturation and can vary from 0.12% to 0.53%. Onion compost NO3-N concentration in 2006 was 0.02%, under the assumption that NO3-N was equal to TON-N (Sánchez -Monedero *et al.*, 1996, 1999).



6. SUMMARY

Biodynamic agriculture (BD) is a unique organic farming system that utilizes specific field sprays such as cow horn manure (BD500), horn silica (BD501) and fermented herbal preparations such as yarrow (BD502), chamomile (BD503), stinging nettle (BD504), oak bark (BD505), dandelion (BD506) and valerian (BD507) as compost additives. Biodynamic herbal preparations in consolation with cosmic forces enhance soil fertility and plant growth. Most of these herbs are naturally occurring or cultivated in temperate regions, harvested and utilized for the preparation of manures. A lot of sentiment about cow and availability of lactating cow horn in India is issues to produce large scale. Therefore the present investigation focused on identifying locally available herbs and alternative materials for the production of biodynamic herbal preparations, evaluate their physicochemical, biochemical and microbiological properties and study their efficacy on the growth of selected plants.

In order to study the physicochemical properties (pH, EC, moisture, organic carbon, (OC) nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu)), biochemical properties (protein, total sugar, humic acid, protease, cellulase, invertase and alkaline phsphatase) and microbiological studies (total bacteria, *Azospirillum, Azotobacter, Rhizobium* like colonies, *Actinimycetes* and fungi) of organic and biodynamic manures, different biodynamic manures such as biodynamic compost (BDC), cow pat pit (CPP), BD500, biodynamic herbal preparations (BD 502 - BD 507) and organic manures such as vermicompost, panchakavya, coir pith compost, and farmyard manure were

periodically obtained from Kurinji Organic Foods Pvt. Ltd., Genguvarpatti, Nadavan Estate Kodaikanal, Dindugal district, Palani agriclinic, Hosur, Krishnagiri District, Badri, Palani, Dindugal district, Nandana farms, Gudur, Andhra Pradesh and Ratnagiri, Maharashtra, India.

Among the thirty two organic and biodynamic manures, the goat manure and BD 507 contained high amount of nitrogen (2.34 %), potassium (1.20 %), and phosphorus (1.12 %). The lowest amount of nitrogen (0.91 %), phosphorus (0.15 %) and potassium (0.17 %) were recorded in immature compost and BD503. The lowest C: N ratio (10:1) was recorded in the goat manure and high C: N ratio (11:1-38:1) recorded in BD 500, BD 502-BD507, immature compost and BD compost.

Among the four enzymes tested in manures, BD compost obtained from Kurinji recorded for highest level of protease activity (493.10 μ g tyrosine released g⁻¹2h⁻¹) and total bacterial count (30.38 X10⁻⁶ CFU g⁻¹).

Enhancement of nutrients and maturation of compost were studied between normal compost heap and compost heap inoculated with biodynamic herbal preparations (BD502-BD507). The compost heap inoculated with BD herbal preparations reduced the time of compost maturity and enhanced the nutrients significantly.

The biodynamic compost such as BDC, BDCV-II and BDCV-I recorded for a significant reduction of C: N ratio (13:1, 14:1 and 12:1) on day 105 whereas the compost without BD herbal preparations recorded for high level of C: N ratio (19:1) on day 105.

The Non-biodynamic (Non-BDC) and biodynamic compost (BDC, BDCV-II and BDCV-I) was further analyzed for its extracellular enzymes such as cellulase, invertase, protease and alkaline phophatase. Significantly high cellulase activity (398.15, 334.12 and 302.71 µg glucose released from compost g⁻¹ 24h⁻¹) and protease (421.10, 438.30 and 400.0 µg tyrosine released from compost g⁻¹ 2h⁻¹) were recorded at 120th day and 105th day of BDC, BDCV-II and BDCV-I.

Decrease of total sugar (30.78, 34.44, 39.71 and 52.53 %), and increase of protein (45.44, 50.55, 54.28 and 51.08 %) and humic acid (33.88, 37.04, 41.65 and 33.17 %) were recorded on day 105 in biodynamic composts (Non-BDC, BDC, BDCV-II and BDCV-I). Significant amount of IAA (196.38 μg 100 g⁻¹) and ABA (99.63 μg 100 g⁻¹), GA (67.93 μg 100 g⁻¹) in 150th day and zeatin (66.86 μg 100 g⁻¹) in days 120th day were recorded in the BDC.

Cow horn manure (BD500) is considered as basal spray enhancing microbial activities by humus formation in biodynamic farms. Cow horn manure was prepared with lactating cow horn, filled with cow dung in the horn, buried under soil for 4 month and periodically evaluated its physicochemical, biochemical and microbiological properties. The cow horn manure contained highest amount of nitrogen (2.08, 2.12 and 2.17 %), recorded on 90th 105th and 120th day, whereas the phosphorus (0.64 %) and potassium (0.47 %) contents were high on 90th day.

Rudolf Steiner selected the lactating cow horn and cow dung for BD500 preparation whereas the availability of lactating cow horn is limited in India due to ethical and religious reasons. Hence artificial materials such as glass, mud pots and

plastic containers were used for the production of cow horn manure and periodically evaluated their physicochemical properties, biochemical properties and microbiological properties.

Among three artificial containers used for manure preparation, mud pot contained the cow dung recoded for low content of organic carbon (37.65 %), potassium (0.42 %), and high nitrogen (1.95 %) contents, average amount of phosphorus (0.66 %) and highest total bacterial count (48.4 X 10⁻⁶ CFU g⁻¹) and fungi (13.3 10⁻⁶ CFU g⁻¹) were recorded on 90th day. The mud pot manure revealed the physicochemical properties, biochemical properties and microbiological properties more or less similar that of cow horn manure whereas glass filled with cow dung recorded for the highest amount of phosphorus (0.80 %) and potassium (0.59 %).

Buffalo, goat and lactating cow dung were collected from Taramani, Chennai and used for the production of cow horn manure. The manure maturation was tested periodically by lifting the horn at every 15 days intervals and dungs were evaluated for their physicochemical properties, biochemical properties and microbiological properties.

Among the three dungs, cow horn containing the goat dung recorded for the highest amount of nitrogen (3.07 %) and humic acid (362 mg 100 g⁻¹) on 120th day, whereas the highest amount of phosphorus (0.89 %), potassium (0.65%), total bacterial count (43.8 X 10⁻⁶ CFU g⁻¹), fungi (16.7 X 10-6 CFU g⁻¹) and actinomycetes (16.9 X 10-6 CFU g⁻¹) recorded on 90th day. Average contents of carbon (34.2 %) and protein (255.11 mg 100 g⁻¹) were recorded on 90th day in cow horn goat dung manure.

The three dungs and four artificial containers were utilized individually for the production of manure. The mud pot with goat dung manure recorded highest potassium (0.86 %), IAA (309.8 μg/g), total bacteria count (49.9 X 10⁻⁶ CFU g⁻¹), fungi (19.1 X 10⁻⁶ CFU g⁻¹), *Rhizobium* like colonies (18.2 X 10⁻⁶ CFU g⁻¹) and *Actinomycetes* (19.9 X 10⁻⁶ CFU g⁻¹) whereas highest kinetin (76.0 and 75.3 μg 100 g⁻¹) was recorded in goat manure harvested from cow horn and mud pot container. The highest amount of humic acid (407 mg 100 g⁻¹) and nitrogen (3.34 %) were recorded in goat dung manure harvested from glass container on 90th day. The lowest carbon content (29.28 %) was recorded in goat dung manure harvested from cow horn. In case of non availability of lactating cow dung for preparation of BD500, the present investigation supported for the utilization of goat dung in cow horn.

Biodynamic preparations regulate the biological processes and strengthen the life forces on the farm. Five herbs such as yarrow (*Achillea millefolium*), chamomile (*Chamomilla officinalis*), stinging nettle (*Urtica dioca*), oak bark (*Quercus robur*), dandelion (*Taraxacum officinale*) and valerian (*Valeriana officinalis*) used for BD preparation (BD502 - BD507) are grown only in tropical regions and processed for the formulating the herbal preparations.

Identification of alternative herbs having similar active ingredients, functions and available in the tropical regions will have significant contribution in biodynamic agriculture in India. The locally available weeds having similar medicinal properties were identified as the alternative herbs such *Aerva lanata*, *Tridax procumbens*, *Tragia involucrate* and *Casuarina sp.* and utilized for preparation of BD alternative herbal preparation (BDA502-BDA505). The BD alternative herbal preparations were analyzed for their physicochemical properties, biochemical properties and microbiology

properties. Significant low C: N ratio (6.10) was recorded in BDA502 compared to BD502(34.82). High Amount of nitrogen (2.74 %), phosphorus (0.63 %) and potassium (1.68 %) were recorded in BDA 502 than BD 502.

Low amount of total sugar (199.19, 191.56 190.72 and 181.08 mg 100g⁻¹) in BDA505, BDA504, BDA502 and BDA503 were significantly recorded. High humic acid (300.72, 342.54, 317.79 and 375.26 mg 100 g⁻¹) and high protein (204.09, 236.41, 217.44 and 277.96 mg 100 g⁻¹) were recorded in BD Alternative herbal preparation (BDA 502, BDA 503, BDA505 and BDA504).

High IAA (115.78 μ g 100 g⁻¹) was recorded in BDA503 than BD503 (109.05 μ g 100 g⁻¹). The High zeatin (47.78 and 46.22 μ g 100 g⁻¹) was record in BDA505 and BD502 compared to BD505 (29.73 μ g 100 g⁻¹) and BD502 (42.27 μ g 100 g⁻¹).

The maximum extracellular enzyme activities such as cellulase (242.66 μg glucose released from manure g^{-1} 24h⁻¹) were recorded in BDA505 compared to BD505 206.99 μg glucose released from manure g^{-1} 24h⁻¹).

Effect of different manure (BDC, Non-BDC, BD500 manures (prepared with alternative source and vessels), BD preparations and chemical fertilizers) on growth of *Lycopersicon esculentus* were investigated. The highest fruit yield was recorded in BDA502 (572.17 g) and lowest yield (319.03g) in soil with Control treatment.

The BDC, Non-BDC, BD 500 manures (prepared with alternative source and vessels) and BD preparation were incorporated in soils and studied leaf biomass of Moringa (*Moringa oleifera*). Highest yield of dry leaves (10.47g/plant) of *M. oleifera* was recorded in soil amended with BD 500.

The effect of cow horn manure on soil properties and yield of ground nut was investigated. The field trials were conducted in 25 m² plot experiment at Extension Centre of Shri AMM Murugappa Chettiar Research Centre, Vadakadambadi.

The influence BD 500, FYM, chemical fertilizers soil treatments and untreated control studied on yield of ground nut (*Arachis hypogea L.*). The maximum pod yield and biomass (3200 and 6188 kg/ha) were recorded in soil amended with organic manure and BD 500.

The results revealed the mud pot was a alternative artificial contaner production of cow horn manure and local herbs for BD herbal preparation. The result of the *invitro* and field experiments indicates that there was improvement in soil fertility and growth of the crop using the manures. However, large scale production of manure and long term field experiments in different agro climatic zones are needed for confirmatory results.

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^{* -} Not Seen Original