SANJEEVAK AS A SOURCE OF NUTRIENTS AND PHYTOHORMONES FOR PRODUCTION AND PROPAGATION OF PLANTS

by
Richard Orendo Smith

Dissertation presented for the degree of Doctor of Philosophy in Agricultural Sciences at the University of Stellenbosch

Promoter: Dr. Andrei B. Rozanov
Faculty of Agrisciences
Department of Soil Science, SU
Co-Promoter: Prof. Tarak Kate
Sustainability Institute, SU

March 2012

DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Signature **Date**

SUMMARY

The use of cowdung as an organic fertilizer in Asian and African agriculture is an ancient practice. This explains its renewed interest, partly due to the financial inability of most farmers to purchase agrochemicals but also the ever increasing need to adopt greener technologies that do not adversely affect soil health, water quality, biodiversity and promote sustained or even increased food production. In this context, many innovative farmers have developed their own novel technologies based on the use of local resources. One such innovation is Sanjeevak (a mix of cow dung, cow urine, water and a handful of sugar); which showed very promising boosting effect on crop productivity. However, very little scientific work has so far been conducted to evaluate its effect as an organic product for soil amendments. The present study was subdivided into three main objectives. (i) To assess the fertilizing value, human health and ecological risk profiles of Sanjeevak; (ii) To screen Sanjeevak for phytohormones content using Salkowski colorimetric method and liquid chromatography – mass spectrometry (LC-MS) (iii) To evaluate Sanjeevak application at various rates on growth parameters and yield of various crops cultivated in glasshouse and field conditions.

Sanjeevak was assessed for its micro and macro nutrients contents. The analysis showed the presence of micronutrients such as Mg, Na, Ca and Zn at variable concentrations and phosphorus (P) (0.007%) and potassium (K) (0.063%). However, Sanjeevak content in total nitrogen (TN) (0.11%), and total organic carbon (TOC) (0.71%) was very low; suggesting that it may be a viable source of nutrients only if applied at higher and consistent rates or alternatively by improving its formulation.

Also, Sanjeevak was analysed for its microbiological characteristics and level of heavy metals content in comparison to the strictest legislations that regulate the use and application of wastewater sludge to agricultural land in South Africa. The findings showed that heavy metals, which averaged from 0.03±0.01 for Arsenic (As) to 4.74±0.92 mg/kg for Zinc (Zn) and feacal coliform was estimated at 1.2×10^2 CFU/g dry matter measured were considerably below the threshold (for Arsenic between 40 to 75 mg/kg dry weight; for Zinc between 2800 to 7500 mg/kg dry weight) and faecal coliform bacteria between 1000 to 1×10^7 CFU/g dry weight for application as a source of soil amendments.

Studies investigating the detection and concentration of phytohormones in Sanjeevak were carried out. In using the Salkowski colorimetric method to detect and quantify auxins from Sanjeevak and its composites (cow urine and dung), the results showed the presence of indole-3-acetic acid (IAA) at variable concentrations ranging from 20.38±2.1 ppm in cow urine, 20.1±6.6 ppm in cow dung, Sanjeevak 17.90±1.1 ppm to up to 138.31±12.6 ppm when LTRP was added to Sanjeevak bacterial cultures and by varying parameters such as incubaton time and temperature. Screening of the above mentioned samples for IAA using LC-MS analysis validated earlier findings. Further analysis of these results strongly emphasized the influence of bacteria in Sanjeevak in producing IAA.

Trials were carried out both in the glasshouse and the field. In the greenhouse, different Sanjeevak application rates consistently confirmed its root promoting effect on crops such as tomato, cucumber and grapevine and increased wheat yield independent of the nutrients it contains. Marginal increases were recorded between

treatments under field conditions; for example compost and compost + Sanjeevak 20.35 and 20.61 t/ha; and 2.46 and 2.60 t/ha compared to the control 11.67 t/ha and 1.29 t/ha respectively for tomato and maize. However, statistical analysis of the results obtained, revealed that there was no difference between treatments (control, compost, Sanjeevak and compost + Sanjeevak) for the same crop tested due to the high coefficient of variation of the data.

Therefore, the use of Sanjeevak as an organic source of soil amendments may be considered as a cheaper alternative to effective microorganisms (EM) technology made up of local and natural resources. As observed in the study, it may be best used in combination with a reliable source of plant nutrients.

OPSOMMING

Die gebruik van beesmis as 'n organiese kunsmis in Asië en Afrika is' n eeu-oue landbou praktyk. Dit verklaar die hernude belangstelling, deels vanweë die finansiële onvermoë van meeste boere om landbouchemikalieë aan te koop, maar ook as gevolg van die toenemende behoefte vir groener tegnologie wat nie nadelig is vir grond gesondheid, waterkwaliteit, biodiversiteit en wat volhoubaarheid of selfs verhoogde voedselproduksie bevorder. In hierdie konteks het baie vindingryke boere hul eie nuwe tegnologie, gebaseer op die gebruik van plaaslik verkrygde hulpbronne, ontwikkel. `n Voorbeeld hiervan is Sanjeevak ('n mengsel van beesmis, beesurine, water en die handvol melasse), wat belowende bevorderende effekte op gewas produktiwiteit en grond mikroflora getoon het. Tot dusver was daar egter baie min wetenskaplike werk gedoen om die effek daarvan as 'n organiese produkte vir grond wysigings te evalueer. Hierdie studie was verdeel in vier belangrike doelwitte. (i) Om die bemestingswaarde, menslike gesondheid en ekologiese risiko-profiele van Sanjeevak te evalueer; (ii) Om Sanjeevak vir fitohormone inhoud en vlakke met behulp van 'n kolorimetriese metode afgelei van dié van Salkowski en vloeistofchromatografie – massaspektrometrie (LC-MS) te besigtig; (iii) Glashuis en veld waarneming reaksies met betrekking tot groei parameters en opbrengs van verskeie gewasse na die toediening van Sanjeevak by verskillende tempos; (iv) Laastens, om die effek van die Sanjeevak voorbehandeling op saad ontkieming en voortplanting te toets in vergelyking met die metodes en tegnieke wat gereeld gebruik word.

Sanjeevak is geassesseer vir die mikro-en makro voedingstowwe inhoud. Die analise het die teenwoordigheid van mikrovoedingstowwe soos Mg, Na, Ca en Zn by wisselende konsentrasies, asook fosfor (P) (0.007%) en kalium (K) (0.063%), getoon. Sanjeevak inhoud van totale stikstof (TN) (0.11%), en die totale organiese koolstof (TOC) (0.71%) was egter baie laag, wat daarop dui dat dit slegs 'n lewensvatbare bron van voedingstowwe is indien dit by hoër en konsekwente tempos toegedien word of alternatiewelik wanneer formulering daarvan verbeter word. Sanjeevak was ook ontleed vir die mikrobiologiese eienskappe en die vlakke van swaar metale in vergelyking met die streng wetgewing wat die gebruik en toediening van afvalwater slyk op landbougrond in Suid-Afrika reguleer. Die bevindinge het getoon dat swaar metale en fekalieë kolivorm vlakke hier gemeet, aan die drumpel vereistes voldoen vir die toediening as ‘n grondverbeteringsmiddel.

Studies wat die opsporing en die konsentrasie van fitohormone in Sanjeevak ondersoek is uitgevoer. In die gebruik van die Salkowski kolorimetrise metode om die ouksiene op te spoor en te kwantifiseer uit Sanjeevak en sy mengsel (beesurine en mis), het die resultate die teenwoordigheid van indol-3-asynsuur (IAA) by wisselende konsentrasies wat wissel van 20 tot 140 ppm in beesurine, beesmis en Sanjeevak getoon. Evaluering van die bogenoemde monsters vir IAA met behulp van LC-MS-analise bevestig vroeër bevindings. Verdere ontleding van hierdie resultate beklemtoon sterk die invloed van Sanjeevak mikrobiota in fitohormone produksie.

Proewe is uitgevoer in die glashuis en die veld. In die glashuis eksperimente, is het die verskeie toedieningstempo van Sanjeevak herhalend die wortelbevorderende effekte bevestig op gewasse soos tamaties, komkommer en wingerdstok en dit het opbrengs

van koring verhoog, onafhanklik van die voedingstowwe wat dit bevat. Statistiese analise van die resultate verkry onder veldtoestande, het getoon dat daar geen verskil tussen die behandelings (kontrole, kompos, Sanjeevak en kompos + Sanjeevak) was nie, gegee dat dieselfde gewas getoets was.

Ten slotte, laboratorium-eksperimente op Sanjeevak as voor-behandeling om die beworteling te verbeter van die wingerdstok (Ramsey) onderstok steggies, het baie belowende resultate getoon in vergelyking met naftaleen asynsuur (NAA) voor-behandeling en die kontrole. Dit beklemtoon die feit dat Sanjeevak 'n alternatief kan wees en wat verdere studie verdien, hoofsaaklik as gevolg van sy lae-koste en omgewingsvriendelike prosedures.

Die gebruik van Sanjeevak as 'n organiese grondverbeteringsmiddel vir gewasproduksie en voortplanting kan beskou word as' n goedkoper alternatief tot effektiewe mikro-organisme (EM) tegnologie wat uit plaaslike en natuurlike hulpbronne saamgestel is. Soos waargeneem in die studie, kan dit die beste gebruik word in kombinasie met 'n betroubare bron van plantvoedingstowwe. Dus, moet die gebruik daarvan vir die produksie van gewasse en voortplanting aangemoedig word.

RESUMEE

L'utilisation des excréments de vaches comme engrais organique dans l'agriculture asiatique et africaine est une pratique très ancienne. Ceci explique son intérêt renouvelé, en partie due à aux restrictions monétaires de la plupart des agriculteurs d'acheter des produits agrochimiques, mais aussi la nécessité croissante d'adopter des technologies vertes qui ne nuissent pas à la qualité des sols, des eaux, la biodiversité et qui permettent d'améliorer la production agricole. C'est dans ce contexte que de nombreux paysans ont développé des techniques nouvelles dépendant des ressources naturelles et locales. L'utilisation de Sanjeevak (un mélange d'excréments de vaches, d'urine de vaches, l'eau et du sucre), a démontré sa capacité à accroître la productivité des plantes vivrières. Cependant, très peu de travaux scientifiques ont jusqu'à présent été menée pour étudier ce produit organique. La présente étude a été subdivisée en trois objectifs principaux. (i) Evaluation de la valeur fertilisante, et les profils de risques écologiques et sanitaires de Sanjeevak, (ii) Etudes de détection des phytohormones et leurs concentrations en utilisant une méthode colorimétrique adaptée de celle de Salkowski et la chromatographie liquide - spectrométrie de masse (LC-MS) (iii) Etude des effets de l'utilisation de Sanjeevak a différents taux d'applications sur la croissance et le développement des plants.

Sanjeevak a été évaluée pour son contenu en micro et macro nutriments. L'analyse a montré la présence d'oligo-éléments tels que Mg, Na, Ca et Zn à des concentrations variables. De plus, son contenu en éléments majors tels que le phosphore (P) (0.007%), le potassium (K) (0.063%), l'azote (N) (0.11%), et carbone (C) (0.71%) est très faible; suggérant qu'il pourrait être une source viable de nutriments que si elle est

appliquée à des taux plus élevés et répétés ou alternativement en améliorant sa formulation. En outre, Sanjeevak a été analysé pour ses caractéristiques microbiologiques et sa concentration en métaux lourds en comparaison a la législation qui réglemente l'utilisation et l'application de déchets liquide d'origines domestiques sur les terres agricoles en Afrique du Sud. Les résultats ont révélé que des métaux lourds et le niveau de coliformes fécaux mesuré était inférieur aux seuils d'application en tant que source d'amendements de sols agricoles.

Les études portant sur la détection et la concentration d'hormones végétales ont été effectuées. En utilisant la méthode colorimétrique de Salkowski pour détecter et quantifier les auxines de Sanjeevak, les urines et les excréments de vaches; les résultats ont révélé la présence d'acide indole-3-acétique (AIA) à des concentrations variables dans les urines, les excréments et Sanjeevak. Une autre analyse des échantillons mentionnés ci-dessus pour les AIA en utilisant LC-MS a validé les résultats obtenus au préalable. L'étude détaillée de ces résultats confirme l'influence des micro-organismes dans la production des hormones végétales.

Concernant les expériences sous serre, les différents taux d'application de Sanjeevak ont confirmé son effet stimulant à la croissance accélérée des racines des plantes telles que la tomate et les raisins et augmenté le rendement du blé indépendamment des nutriments qu'il contient. L'analyse statistique des résultats obtenus dans des conditions de terrain, a révélé qu'il n'y avait pas de différence entre les traitements (contrôle, compost, compost + Sanjeevak et Sanjeevak) pour la même plantes testées.

Par conséquent, l'utilisation de Sanjeevak comme un produit organique qui améliore la qualité des sols et le rendement des cultures vivrières; peu être considéré comme un

inoculum contenant des microorganismes constitué de ressources locales et naturelles. Comme l'a observé dans l'étude, il pourrait être mieux utilisé en combinaison avec une source fiable de nutriments végétaux.

DEDICATION

To my late father; SMITH VICTOR and sister; ENGANI AKPADJA DEDE JESSICA, both of whom I lost during the final stage of this research work. In the face of adversity, honouring your memories gave me the extra impetus that kept me going.

ACKNOWLEDGEMENTS

I am particularly grateful to my promoters, Dr. Andrei Rozanov and Prof. Tarak Kate for assiduously steering me clear off the many hurdles throughout this exciting and at times challenging endeavour in completing successfully this work. I wholeheartedly thank Dr. Andrei Rozanov for his sterling guidance throughout the duration of this research work, and his valuable comments on the manuscript. His easy-going and relaxed personality gave me the courage and confidence to approach him just at any available opportunity for discussions. I am indebted to Prof. Tarak Kate for his continued and inspiring encouragements and critical inputs on this research and the manuscript.

This dissertation draws from a wide variety of sources and expertises, but I have given recognisance to the work and contribution of those with whom I have been associated over the past four years. My thanks and most sincere appreciation is extended to all who have offered helping hands to support the completion of this work in various ways. They include lecturing, technical and supporting staff, from the Soil Science Department, Agronomy Department, Viticulture and Oenology Department, Plant Pathology Department, Horticulture Department, Animal Science Department Food Science Department, Welgevallen Research Farm; all from the University of Stellenbosch; the Sustainability Institute (SI) – colleagues from the soil science department (Stellenbosch university) – Special thanks are due to the Community Interaction Division of Stellenbosch university, the Association of African Universities (AAU) and the Gabonese Government who have given their financial assistance, enabling the fulfilment of this dissertation.

I acknowledge with great pleasure all the staff of the department of soil science who assisted me throughout the study period. My special thanks go to Nigel Robertson and Leonard Adams for their enthusiastic help in data collection and logistic matters. Equally I thank Ms. Annatjie French for kindly facilitating the procurement of products and equipments for my lab experiments and managing my research grant efficiently.

I express my profound appreciation for my flatmates (Victor Ngwang Kongor, Victor Misulu Mutamba, Carl Tshamala Mubenga, Xolani Madela) for their quiet inspiration and support; particularly the last two years of my PhD studies. They were undoubtedly one of my sources of strength and motivation and for instilling in me patience, endurance and humility.

I am immensely grateful to my family and friends for their patience and unwavering support.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

CHAPTER ONE

SANJJEVAK AS LIQUID ANAEROBICALLY COMPOSED ORGANIC GROWTH STIMULANT: THE ISSUES OF SUSTAINABLE CROP PRODUCTION MANAGEMENT AND BIOSAFETY (REVIEW)

1.1 Motivation

As stated by Groenewald (2009), according to the President of the International Fund for Agricultural Development (IFAD), small-scale agriculture is the largest private-sector activity in many African countries; not only does it feed families, but also provides employments, stimulates the growth of rural businesses and promotes broader development (Materechera 2010). To some extent; this is also true for many other developing nations outside the African continent such as India. However, agricultural production in much of the developing world is severely restricted by fragile ecosystems, a rapidly declining soil fertility and low use of external inputs such as agrochemicals and improved crop varieties (Smaling and Braun 1996). Thus, it is generally accepted that declining soil fertility leads to reduced yields which is a potential impediment to household food security and a symptom of poverty (Smaling 1993).

Accordingly, it is generally accepted that some of the direct causes of declining soil fertility include limited recycling of organic residues to the soil and a lack of adequate application of external sources of nutrients. Factors underpinning these direct causes include population pressure, poverty, high cost and/or limited access to agricultural inputs and credit, fragmented land holdings, insecure land tenure and farmers lack of information about viable alternative technologies (Dilallessa *2006*).

Currently, a strong emphasis is put on adopting conservation agricultural systems to reverse declining soil fertility and increase crops yield and per capita food production in many developing countries. This has led to an increased interest in biowaste materials as natural sources of soil fertility improvements due to their ability to improve plant growth, their ease of application and cost effective practice.

Consequently, where financial and logistic constraints on the availability of chemical fertilizers exist and naturally infertile soils are used for permanent production of crops; the use of biowaste materials (e.g., animal excreta and urine) for soil fertility improvement and crop productivity should assume particular importance (Mazzucato and Niemeijer, 2000; Bayu et al., 2004). As stated by Materechera (2010) animal manure is a vital resource not only for supplying plant nutrients but also for increasing soil organic matter content. It improves the pH of acid soils and calcareous soils, increases soil cation exchange capacity (CEC), water holding capacity of soils, improves soil aggregate stability, soil macro-structure and erosion resistance (Bayu et al., 2004). However, in taking into account the concentration at which animal manure is applied, then it is clear that the level of mineral nutrients added from animal farmyard would be in part responsible for any significant effect on plant growth as highlighted by Badaruddin et al. (1999). It is therefore assumed that plant regulating substances combined with mineral nutrients are responsible for yield increases (Badaruddin et al., 1999; Kate and Khadse 2002).

Cow dung application is an ancient practice used in Asian and African agriculture as a source of plant nutrients. The ever increasing need to use “green technologies” that

involve components which do not negatively affect soil health, water quality, biodiversity atmospheric and renewable energy sources require innovative and holistic approaches in farming (Kesavan, 2006; Swain et al., 2007). Other studies have successfully highlighted the activity of cow dung microbiota such as B. subtilis in producing growth regulator such as IAA which promoted yam sprouting (Swain et al., 2007), production of agriculturally important enzymes such as α-amylase (Swain et al., 2006) which have multiple beneficial impacts on crop growth and production and biocontrol activity against pathogens (Basak and Lee, 2000a, b; Swain and Ray, 2007). In terms of innovation; the fact that mixed crop-livestock farming systems are characteristics of small-scale agriculture in Asian and sub-Saharan Africa; if Sanjeevak is proven to be a safe and reliable source of beneficial bacteria for crop growth, then it can be used as a cheap and effective source of effective microorganisms (EM technology) to sustain or even improve agricultural productivity.

1.2 Introduction

The literature review will first emphasize on animal manure application and its effect on soil fertility properties and crop improvement. Then issues related to human health and the environment will be briefly discussed. Micro-organisms in the rhizosphere and/or in root-free environment and their ability to biosynthesize plant hormones will be briefly discussed. Also the detection and quantification of plant hormones in organic wastes derived-fertilizers will also be briefly discussed. Finally, the knowledge gap this study aims to bridge will be assessed.

1.3 Effects of manure application

The significance of manure in improving the physical, chemical and biological quality of agricultural soils is well documented (Sharpley and Smith, 1995; McGill et *al.,* 1986 and Meek et *al.,* 1982).

1.3.1 Chemical and physical properties

When added to the soil, manure can provide essential nutrients such as NPK and other micro-nutrients and improve soil nutrient levels. The composition of manures can vary depending mostly on the animals' diet, type of animals and the way manure is collected and strored and applied (Bayu et *al.,* 2004). According to De Ridder and Van Keulen (1990) and Eck and Stewart, (1995) manure can average approximately 2.0, 0.5 and 1.5 mg/kg (on dry weight basis) of N, P and K respectively and significant amounts of Ca, Mg, Na and many trace elements (Bayu et *al.,* 2004). Taking into account African conditions, where animal diets are often very poor; the composition of macronutrients is expected to be lower than the values mentioned earlier. However, not all the nutrients will be released from the manure in one season (Bayu et *al.,* 2004). In comparison to the commonly used chemical fertilizers, one of the benefits of applying manure is the provision of secondary nutrients (Lungu et al., 1993). The significance of manure as a source of micro-nutrients was demonstrated by Warman and Cooper (2000) who identified higher levels of B, Cu, Fe, Mn and Zn in manured than un-manured soils after a three years period of constant fresh and composted manure application at various rates.

There are several studies reported by researchers in which manure application significantly impacted on soil chemical properties (Hoffmann et *al.,* 2001; Powell, 1986;

Warman and Cooper, 2000; Lupwayi and Haque, 1999a; Kaihura et *al.,* 1999). For example, significant increases in total N and available P and K in soil following farmyard manure applications were reported in Ghana (Kwakye, 1988) and Nigeria (Agbenin and Goladi, 1997). Replenishment of soil nutrients and other soil fertility parameters, as a result of manure application is further highlighted by data from Niger (Bationo and Mokwunye, 1991).

Manure addition to the soil can change the pH of acid soils and decrease that of alkaline soils in general (Hoffmann et al., 2001; Lungu et *al.,* 1993; Wong et *al.,* 1998 as cited by Bayu et *al.,* 2004). Application of animal manure also provides other important elements like K, Mg and Ca, which can contribute to maintaining base saturation. The reaction of acid soils with composts and manures results in increased soil pH and decreased Al saturation (Hue, 1992). Ouédraogo et *al.* (2001) noted that 10 t/ha of compost application increased soil cation exchange capacity (CEC) from 4 to 6 mol kg^{-1}. Soil pH was also increased by compost applications.

The current emphasis on low-chemical-input, sustainable agricultural systems has led to renewed interests in adopting old farming practices such as crop rotation and use of manure as organic fertilizers (Belay et *al.,* 2001). These practices are important and have been shown to have beneficial effects on soil physical properties through improving the organic matter content of the soil (De Ridder and Van Keulen, 1990; Hoffmann et *al.,* 2001). For example, Mokwunye (1991) reported that application of manure in Nigeria once every three years at rates of 5 and 20 t/ha resulted in a two-fold increase in soil

organic matter content in the first three years of the investigation, as opposed to the unfertilized plot. Soil organic matter content in the manured plot was significantly superior to chemical fertilizers treated plots (See Bayu et *al.*, 2004). Similar effects of farmyard manure on the level of soil organic matter in African soils have been reported by researchers (Agbenin and Goladi, 1997 and Kaihura et *al.*, 1999).

1.3.2 Biological properties

Several studies have reported that organic farming leads to higher soil qualities with higher microbiological activities than conventional farming, due to flexible crop rotations and reduced application of agrochemicals (Marinari et *al.,* 2005). Drinkwater et *al.* (1995) reported higher pH, organic C and N, N mineralization potential and actinomycete abundance and diversity in organic fields than conventional ones. The microbial biomass plays a central role in the biochemical process and is especially important in determining the quality and health of the soil as stated by Kennedy and Papendick (1995). The current evidence points to the fact that the addition of organic residues can increase microbial pool size and activity (Palm et *al.,* 1997). Under temperate conditions, organic fertilizers cause a significant increase in soil microbial biomass than chemical fertilizers due mainly to an increase in organic C content (Bolton et *al.,* 1985). In a study conducted in South Africa, Belay et *al.* (2001) reported positive effects of farmyard manure on soil microbial properties (Table 2.1).

Table 1.1 Influence of residual manure on total and microbial C, N, and P, microbial number in 1998 and 1999 under long-term experiment established in 1939 in Pretoria, South Africa (Bayu et *al.,* 2004)

Treat.	Organic C	Total N	Bray-1 P	Biomass C	Biomass N	Biomass P	Bacteria ($\times10^5$)	Actinomycetes ($\times10^4$)	Fungi ($\times10^3$)
				mg/kg				g^{-1} dry soil	
Native	6300b	690b	27.8b	292a	13.5b	0.03a	6.9a	2.9b	39a
Control	5840b	591b	1.7c	140b	14.9ab	0.08a	7.0a	5.1a	8.0b
Manure	8890a	1051a	58.8a	236a	25.1a	0.09a	7.5a	4.1ab	5.8b

Note: Manure had been applied at a rate of 4.47 t/ha season until 1965 (two seasons per year) and at 8.94 t/ha season^{-1} from 1966 onwards until it was discontinued in 1989.

1.3.3 Increased crop production

The impact of manure application to increase production of different crops is well documented (Bayu et *al.,* 2004). Numerous studies in developing countries have reported considerable yield increases from manure application (Bekunda et *al.,* 1997; Powell, 1986). In an experiment conducted in Niger to study the effect of manure applied once every three years on the yield of pearl millet, Bationo and Mokwunye (1991) reported a doubling of millet yields a year after the first application of manure at the rate of 5 t/ha. When the manure was increased to 20 t/ha, there was no need for additional fertilizer.

In Kenya, Gibberd (1995) reported 58% and 75% yield increases in maize crops and intercrops, respectively, from manure application at the rates of 5 and 10 t/ha. In

Tanzania, Kaihura et *al.* (1999) reported maize grain yield increases of 1.732 kg/ha across eight distinct agricultural sites with manure applied at 20 t/ha. In Ghana, sorghum and millet grain yield increases were reported with the application of manure at 10 t/ha (Kwakye, 1988). In a CIMMYT coordinated study carried out in Sudan the application of farmyard manure at 10 t/ha produced the highest wheat yield response (14%), while approximately equivalent levels of NPK applied as chemical fertilizers had the lowest yield response (5.5%) (Badaruddin et *al.,* 1999). In India, Damodar Reddy et *al.* (2000) reported that manure application at the rate of 4, 8 and 16 t/ha per year increased soyabeans seed yield by 42%, 57% and 75% respectively and similarly wheat grain yield by 67%, 116% and 143% respectively over control. Ouédraogo et *al.* (2001) noted that compost application at rates of 10 t/ha and 5 t/ha tripled sorghum yield and increased it by 45% respectively compared to no-compost plots.

Vermicomposts produced commercially from cattle manure have been shown to promote the germination, growth, and yield of crop plants (Arancon et *al.,* 2008). Vermicomposts were applied to small replicated field plots planted with tomatoes (*Lycopersicon esculentum*) and bell peppers (*Capsicum anuum grossum*) at rates of 10 t/ha or 20 t/ha in 1999 and at rates of 5 t/ha or 10 t/ha in 2000. There were significant increases in shoot weights, leaf areas and total marketable fruit yields of pepper plants from plots treated with vermicomposts in comparison to those from plots treated with chemical fertilizers only (Arancon et *al.,* 2008). Some examples of Manure application rates and their impacts on crop yield and soil properties are summarized in table 1.2

Table 1.2 Summary: Examples of manure application and its impacts on crop yield and soil properties

Reference	Manure application	Region	Plant	Yield reached	Effects on soil properties
Bationo and Mokwunye 1991	5 t/ha applied once every three years	Niger	Pearl millet	doubling of millet yields a year after the first application	increased N and P content of the soil after two years of manuring
Gibberd 1995	5 and 10 t/ha	Kenya	Maize	58% and 75% increased yield grains in sole crops and intercrops	
Kaihura et al. 1999	20 t/ha	Tanzania	Maize	yield grain increases of 1732 kg/ha compared to unmanured plots	incrases of of soil N level by 0.03%, P by six fold and K by two fold
Badaruddin et al. 1999	10 t/ha	Soudan	wheat	increase wheat yield of about 14% under manure application compared to 5.5% under chemical fertilizer applied at equal N,P and K concentrations	
Powell 1986		Nigeria	Maize	grain yield increase of about 1 t/ha in manured plots over non-manured Plots	soil pH increase in manured plots (pH 5.8) as compared with non-manured plots (pH 5.1) three fold increase in N in manured plots
Mokwunye 1991	5 t/ha and 20 t/ha	Nigeria			two fold increase insoil organic matter levels
Goladi and Agbenin 1997 De Ridder and Van Keulen 1990		Nigeria Burkina Faso			increased CEC of the soil and sodium adsorption ratio
Warman and Cooper 2000	5 to 10 t/ha	Canada			higher levels of B, Cu, Fe Mn and Zn in manured compared to unmanured soils
Domador Reddy et al. 2000	4, 8 and 16 t/ha per year	India	Soybean and wheat	Yield seed increase of 42%, 57% and 75% respectively for soybean Yield grain increase of 67%, 116% and 146% respectively for wheat over the control	

1.4 Sanjeevak application in Agriculture

1.4.1 Procedure and application

As indicated by Kate and Khadse (2002), Sanjeevak (Table 1.3) is applied to crops during periods of vegetative growth and flowering initiation. It can be applied either to each single plant or to a larger area by pouring it into an irrigation channel. Every successive dose requires a gap of 8 to 10 days and adequate soil moisture is critical at the time of application. However, it has been observed that the basic formula of Sanjeevak as developed by Dabholkar has been frequently modified by some farmers based on their own field experience and as a result, different alternatives of the original formula are being applied (Pathe and Kate 2009).

Table 1.3 Standard requirements for Sanjeevak preparation: compounds required and their quantity.

Materials	**Quantity**
Cattle droppings (fresh)	10kg
Cattle urine	10 Litres
Molasses	100g
Water	180 Litres
Tank (size)	250 Litres

1.4.2 Effects on plant growth and development

The benefits of Sanjeevak application as reported by Kate and Khadse (2002) are mainly the followings:

1. It plays an important role as a growth promoter for various crop plants;
2. Stimulate a luxuriant vegetative growth;
3. Early fall of flowers and fruits is significantly limited.

1.4 Pathogens encountered in animal wastes

Manure and other wastes (such as respiratory secretions, urine and sloughed feathers, fur or skin) of various agricultural (livestock) animals often contain high concentrations (millions to billions per gram of wet weight faeces) of human pathogens (disease-infectious microorganisms). Per capita faecal production by agricultural animals such as cattle and swine far exceeds that of humans. Animal pathogens posing potential risks to human health include a variety of viruses (such as swine hepatitis E), bacteria (such as *Salomonella* species) and parasites (such as *Cryptosporidium parvum*), some of which are endemic to commercial livestock and difficult to eradicate from both animals and their production facilities (Sobsey et *al.,* 2001). Therefore, pathogens in animal manure and other wastes may cause potential risks to human and animal health both on and off animal agriculture production facilities if the wastes are not adequately treated and contained. The major viruses, bacterial and parasitic groups or species found in animal wastes are tabulated in Table 1.4

Table 1.4 some human pathogens potentially present in animal wastes (Source: Sobsey et *al.,* 2001)

Pathogen	
Viruses	Hepatitis E virus (swine), Reoviruses, Rotaviruses, Adenoviruses, Caliciviruses Infuenza viruses (Orthomyxoviruses)
Bacterium	Salmonella spp., Campylobacter spp., Escherichia coli, Aeromonas hydrophila, Yersinia enterocolitica, Vobrio ss., Leptospira spp.,
Parasites (Protozorans)	Crytosporidium parvum, Giardia lamblia and Balantidium coli

In the section that follows, a particular emphasis will be placed in describing coliform bacteria, including *Escherichia coli* found in animal wastes.

1.5.1 Escherichia coli

Although *Escherichia coli* may be found in faeces, water and soil, only a small proportion (<1%) are potentially harmful strains. Most strains of *E. coli* reside in the intestines of healthy animals and humans and are harmless, and in many cases beneficials (Dorn 1993; Kudva et *al.,* 1998 and Sahlström 2003). The harmful strains are called enterotoxogenic *Escherichia coli* (ETEC) and the most common of these is *Escherichia coli* O157:H7. Most *Escherichia coli* O157:H7 reported outbreaks are associated with the consumption of contaminated, undercooked, bovine food products (Boyce et *al.,* 1995; Griffin, 1995). Other sources of infection include contaminated, un-pasteurized apple cider, water (drinking and swimming), vegetables, mayonnaise, lamb, cured salami, and direct contact (animal to person

or/and person to person). Cattle are generally considered the major reservoir for this organism (Hu et *al.,* 1999). Studies have reported that once fruits and vegetables have been contaminated, it may be difficult to disinfect them (Maxy, 1982; Takeuchi et *al.,* 2000; Wachtel et *al.,* 2002a and Wachtel et *al.,* 2002b). Bacteria such as *E. coli* show preferential attachment to the interior of damaged fruits and vegetables than the surface (Takeuchi et *al.,* 2000) as the juice within the vegetables provides adequate growth medium (Maxy 1982).

E. coli O157:H7 successfully causes infections because of its low infectious dose (ID), which can be as few as ten cells (Rosen 2000). In cattle, *E. coli* O157:H7 occurs with an overall prevalence of 0.3 to 6.1% and the average length of time that faeces from an individual animal remain culture positive is 30 days (Kudva et *al.,* 1998). Wang et *al.* (1996) indicated that *E. coli* O157:H7 can survive and produce verotoxins for up to 10 weeks. In addition, *E. coli* O157:H7 is able to multiply in bovine faeces at temperature ranging from 22 and 37 °C. However, the occurrence of *E. coli* in the environment and its pathway from the environment to causing human health effects are not completely understood. The mechanism by which pathogenic *E. coli* causes disease varies between strains (Kaper et *al.,* 2004).

A number of factors contribute to the pathogenicity of this serotype; these factors include the production of Shiga toxin type 1 and/or 2, the *eae* genes, and a 60-Mda plasmid encoding adhesins and hemolysins (Kudva et al., 1998). *Escherichia coli*, depending on the infective strain, is an important foodborne pathogen that may cause different set of clinical manifestations in people, including severe bloody diarrhea and abdominal cramps, acute nonbloody diarrhea and thrombocytopenic purpura (syndrome similar to those of hemolytic uremic syndrome with central nervous system involvement). Young children, the elderly and people with immunocompromised systems are the most susceptive (Rosen 2000). The

infection can cause a serious complication called hemolytic uremic syndrome where the red blood cells are destroyed and the kidney fails to function. This occurs in about 2% to 7% of the cases. Death often occurs in patients with hemolytic uremic syndrome and thrombocytopenic purpura (Pell 1997).

Detection and enumeration of total coliform bacteria (including *E. coli*) in water is traditionally carried out by means of culturing methods (Lemarchanda et *al.,* 2005). Although these methods do not differentiate between pathogenic and non-pathogenic strains, they provide useful data that may be used as good indicators for quality control analysis of products such as sewage sludge and animal wastes applied in agricultural land, water-quality guidelines and institutional approaches to managing health-related water quality (Theron and Cloete 2004). For the purpose of this research study, the reference method used in South Africa for the detection of coliforms and *E. coli* in surface or waste water is an Miniaturized method (Most Probable Number) (SANS 9308-3).

1.5.2 Persistence of Escherichia coli in soil

The survival of microorganisms added to the soil is influenced by a number of factors (Santamaria and Toranzos 2003). Reductions in bacterial and viral population densities are observed under dry soil conditions. Hence, soil moisture favours the survival of viruses and bacteria (Gerba and Bitton 1984; Yeager and O'Brien 1979). Another important environmental factor affecting microbial movement is rainfall. It can result in pathogen spread by runoff from places where manure have been applied or by leaching through the soil profile. Bacterial and viral groundwater contamination increases during heavy rainfall. For example, the presence of coliforms was monitored for 9.4 m to 153.3 m wells. Coliforms were detected in both shallow and deep wells, with bacterial contamination coinciding with

the heaviest rainfalls (Gerba and Bitton 1984). Other factors such as water holding capacity, ultraviolet light from the sun, organic material and the hydrogen ion also contribute to the survival of microorganisms in soils (Rudolfs et *al.,* 1950; deRopp, 1999 and Chale-Matsau 2005).

One of the most important factors influencing the survival of bacteria in the soil is competition with existing soil microflora. In soils with low microbial activity, the newly added microorganisms may persist for much longer (Bitton 1994; Chale-Matsau 2005). Hence, the application of large quantities of manure to soil with low existing microbial activity will increase the ability of pathogens to persist in soils environment and increase the potential risk for transfer of pathogens to crops grown in soil (Chale-Matsau 2005). However, in biologically active soils, microorganism numbers are rapidly reduced due to competition (Penner, 1998; Chale-Matsau 2005). Faecal coliforms can survive for several years under optimum conditions (deRopp, 1999). In addition, Gagliardi and Karns (2000) reported that *E. coli* from manure applied to soil may survive, replicate and move vertically in the soil for more than two months.

1.5.3 Pathogens reductions by animal waste treatment processes

The reductions of some pathogens by some animal waste treatment processes have been researched and resolved in laboratory and pilot scale field studies. Thermophilic processes, such as pasteurization, thermophilic digestion and composting are capable of leading to significant (>4$\log_{10}$) pathogen inactivation. Hence, resulting treated residuals are likely to contain only low pathogen concentrations. However, most mesophilic biological treatment processes for animal manure are not likely to reduce pathogen levels by more than 1-2 log10

or 90-99% unless several treatment reactors or processes are used in series (MWPS, 2001). Estimated pathogen reductions in animal manures are summarized in Table 1.5

Table 1.5 Animal waste treatment processes and estimated pathogen reductions (Source: MWPS, 2001)

Treatment Process	Est. Pathogen Reduction (log10)	Comments
Physical		
Heat/Thermal Processes		
Mesophilic	Typically, 1-2	Depends on temperature, pathogen, contact time pH, etc
Thermophilic	Typically, >4	Depends on temperature, pathogen, contact time pH, etc
Freezing	Variable	Depends on pathogen, waste composition and conditions temperature, etc
Drying or dessication	Typically >4 at <1% moisture; Typically >4 at <5% moisture	Depends on pathogen, contact time, pH, etc
Gamma irradiation	Typically >3	Varies with pathogen, dose, waste, etc
Chemical		
High pH (>11)	Inactivation at high pH, e.g., alkaline/lime stabilization; >3-4	Varies with pathogen, contact ime, pH, etc
Low pH (<2 to <5)	Inactivation at low pH; acidification: typically, <2	Depends with pathogen, contact ime, pH, etc
Ammonia	Inactivation at higher pH where ammonia predominates	Varies with pathogen, contact ime, pH, other waste constituents
Biological Processes		
Aerobic mesophilic	Typically 1-2	Varies with pathogen, solids separation, contact time, reactor design, temp.
Aerobic, thermophilic (composting)	Typically >4	Depends on pathogen, solids separation, contact time, reactor design, mixing methods, temp.
Anaerobic, mesophilic	Typically 1-2	Depends on pathogen, solids separation, contact time, reactor design, temp.
Anaerobic, thermophilic	Typically >4	Depends on pathogen, solids separation, contact time, reactor design, temp.
Silage treatment, mesophilic	Variable	Depends on ensiling conditions and pathogen
Land application	Highly variable and largely unknown potentially high	Depends on site-specific factors: temperature, precipitation, vadose zone, loading sunlight, riparian buffers, etc

It is worth noting that pathogen reduction by animal waste treatment processes and management systems have been studied only for a few microbes, mainly indicator bacteria such as faecal coliforms. Therefore, removal and inactivation of the many different kinds of infective pathogen species in various waste treatment processes and management systems is uncertain and needs further analysis (MWPS, 2001).

1.5.4 Environmental and human health risks

Pathogens reduction by waste animal treatment processes and management systems has been studied only for a few microbes; for the most part indicator bacteria such as coliform bacteria (MWPS, 2001). Ideally, complete elimination of pathogens from animal excreta as manure and its subsequent use in soil fertilization for crop production is required. However, it has been indicated that if the numbers of pathogens in animal waste is decreased to an acceptable level, its use in agricultural land does not appear to result in unacceptable risk to human health (Apedaile, 2001; Tanner et *al.,* 2003). In addition, According to Vesilind (2003) coming into contact with small doses of pathogens is the sufficient challenge our bodies need to stay healthy as our enhanced health comes not from zero exposure, but from a sufficient exposure to pathogens. As argued by Chale-Matsau (2005), this could be different for situations where significant portions of the population are immuno-compromised patients (Dorrington et *al.,* 2002).

Bacteria pathogenic to humans have been shown to survive in animal slurries for a considerate amount of time (Jones, 1980; Larsen and Munch 1982; Kunte et *al.,* 1998; Pillai and Ricke 2002). Thus, the potential exists for the transmission of infectious diseases by a variety of routes, including bioaerosol transmission (Pillai et *al.,* 1996; Kudva et *al.,* 1998; Kunte et *al.,* 1998). Studies have revealed that the risk associated to adverse public health

effects from bioaerosols following land applied sludge is not alarming (Tanner et *al.,* 2003). However, it has been suggested that bacteria associated with manure have been responsible for both respiratory and intestinal diseases (Cole et *al.,* 1999). In addition, animal excreta as manure releases several gases; among them: ammonia, hydrogen sulphide, carbon dioxide and methane that pose health risks to both humans and animals. For example, higher concentrations of hydrogen sulphide may cause vomiting, nausea, diarrhea and acute asphyxiation (Cole et *al.,* 1999).

Methods are available to recover and detect some faecal indicator bacteria in animal manures and their treated residual solids and liquids. However, the methods for some indicators such as bacterial viruses (colipahges) and spores of *Clostridium perfingens* have not been properly verified and required further investigations. Equally, studies are needed to determine the true risk of enteric infections related to land application of animal manure. Among studies that necessitate further investigations, are:

(i) the survival of enteric microorganisms in different type of soils;

(ii) the ability of different type of soils to either protect or inactivate pathogenic microorganisms;

(iii) (iii) the ability of pathogens to invade and colonize vegetables that are eaten raw;

(iv) (iv) the development of methods for the detection and quantification of enteric pathogens in soils and

(v) Risk assessment (Santamaria and Toranzos 2003).

1.5 Regulatory framework for the use of animal waste in agricultural land

Most countries adopt a similar approach to protect the public from infection caused by pathogens originating from wastewater sludge. The use of wastewater sludge is regulated and these regulations stipulate how the sludge should be disinfected and/or how to minimize the chance of contamination through prescribed management practices (Chale-Matsau 2005).

In South Africa, humans and environmental risk assessment exposure to pathogens are regulated by various governmental departments; The Department of Water Affairs (DWA), Department of Environmental Affairs and Tourism (DEAT), Department of Health (DoH) and Department of Agriculture (DoA). The guidelines for wastewater sludge classification and application for agricultural use are summarized in a document titled "permissible utilisation of sludge" (WRC 1997; WRC 2002).

In the section that follows, biosynthesis of plant hormones and related plant growth regulating substances from plant and microorganisms will be discussed.

1.6 Plant hormones

Plant hormones are low-molecular weight, naturally occurring substances that operate at the micromolar (or even lower) concentrations to effectively co-ordinate all physiological and developmental processes during a plant's life cycle (Chiwocha et *al.,* 2003). These structurally diverse compounds have been considered to fall into five classes: ethylene and like molecules that promote ripening; abscisic acids (ABA) that are involved in dormancy and abscission; auxins that stimulate cell extension (typically in actively growing regions); gibberellins (GA) that are involved in shoot extension in light; and cytokinins (CK) that are

often found in roots and involved in cell division and interact with auxins to determine whether dividing cells become root or shoot cells. More recently, a variety of additional classes such as polyamines, jasmonates, salicylic acid, and brassinosteroids have been shown to act as growth regulators or signaling molecules (Davies 1995; Chiwocha et *al.,* 2003).

A diverse group of bacteria including species of Pseudomonas, *Azotobacter, Azospirillum, Bacillus, Klebsiella, Enterobacter, Xanthomonas and Serratia* have been reported to promote plant growth (Lucy at *al.,* 2004; Khalid et *al.,* 2004). Direct mechanisms of plant growth by free-living plant growth-promoting rhizobacteria (PGPR) include the provision of bioavailable phosphorus for plant uptake, nitrogen fixation for plant use, sequestration of iron for plants siderosphores, production of biological active substances like auxins, cytokinins and gibberellins, and lowering of plant ethylene levels (Glick, 1995; Glick et *al.,* 1999; Lucy et *al.,* 2004; Khalid et *al.,* 2004). There are a number of references in the literature that report that plant growth regulators, such as indole-acetic acids (auxins), gibberellins and cytokinins are produced by microorganisms, and there have been suggestions that the promotion of microbial activity in organic matter by earthworms would result in production of significant quantities of plant growth regulators (Tomati et *al.,* 1983, 1988, 1990; Tomati and Galli, 1995; Krishnamoorthy and Vajranabhiah, 1986; Edwards, 1998). Earthworm activity accelerates the humification of organic matter and their influence in increasing microbial populations enhances the presence of auxins and gibberellin-like substances as well as humic acids (Casenave de Sanfilippo et *al.,* 1990; Atiyeh et *al.,* 2002). In addition, humic acids have been shown to stimulate plant growth in auxin, gibberllin and cytokinin bioassays (Phuong and Tichy, 1976)

There have not been many reports of increased crop growth in soil amended with vermicomposts produced from cattle manure. Although, there are very few data in the

literature on possible mechanisms by which vermicomposts produce growth enhancement molecules. In recent times, (Atiyeh et *al.,* 2002; Arancon and Edwards, 2006b) lab experiments have demonstrated that organic matter degradation through the interactions between earthworms and micro-organisms, contain plant growth regulating materials, including plant growth hormones and humic acids, which are probably responsible for most of the increased germination, growth and yields of plants; independent to the nutrients they contain (Arancon et *al.,* 2008).

1.6.1 Biosynthesis of plant hormones

Anaerobic digestion in biogas plants (BGPs) is an alternative way to handle biowastes and generating energy in the form of methane (biogas), which include animal and human wastes (Sahlström, 2003). The main suppliers of biowastes are slaughterhouses, households, restaurants, food and beverage industries as well as sewage treatment plants (STPs) and animal farms. For example, it is reported that anaerobic methanogenic thermophilic digestion is a suitable process for the conversion of coffee residues to a digested product (slurry). The sieved solids of the slurry may then be used either as fertiliser or as growth medium in horticulture (Kostenberg and Marchaim 1993a, 1993b).

Biological tests with mung bean cuttings and *Grevillea* plantlets showed promotional effects on rooting of the slurry and its sieved fraction extract, washed with water (Kostenberg et al., 1995). Further analyses, using various extraction methods and TLC, HPLC and GC/MS to detect and quantify plant hormones, showed clearly the presence of IAA and IBA in free and bound forms in all the substrates (Kostenberg et *al.,* 1995). Most of the IAA identified as the free form had concentration ranging from 23.5 to 33.0 nmol g^{-1} which is almost ten times more than in the waste, and almost twice the total amount of IAA in coffee beans. It is

suggested that the high level of free IAA in the digested instant coffee waste are a result of catabolism of tryptophan by anaerobia bacteria (Kostenberg et *al.,* 1995).

1.6.2 Quantitative aspect of auxin effects

Auxins are involved in the regulation of cell division, cell growth, apical dominance, responses to directional stimuli and fruit setting (Davies, 1995). Auxins promote shoot growth, but a similar concentration inhibits root growth. Plant hormones are active at small concentrations, usually in the nanomolar range, although some responses begin at even lower concentrations (10 to 100 picomolars). Up to a certain concentration of the hormone, the response increases and then saturates. For some plant hormones, higher concentrations may lead to inhibited responses. For example, as stipulated by Sankhla and Daksha Sankhla (1972) auxins have been shown to be potent inhibitors of seed germination in several strains of lettuce. Alam and Khan (2002) showed that the foliar spray application of naphthalene acetic acid (NAA) at concentrations ranging from 5 to 25 mg/L significantly resulted in increased number of fruits and yield of tomato when compared to the control. Physiological experiments indicate that both exogenous and endogenous auxins are closely involved with several stages of carrot somatic embryogenesis (Michalczuk et *al.,* 1992). Experiments conducted on explants from different carrot roots revealed that in 14 days, bacterial media with indole-3-acetic acid (IAA) at 0.1 mg/L generally weighted twice as much as the basal control. Individual carrots behaved differently in their relative responsiveness to IAA. Responses to explants from individual carrots ranged from no increase in fresh weight to variable amounts of growth. Approximately, 20% of all carrots tested produced no response to IAA. Auxins play a key role in lateral root formation (Casson and Lindsey 2003). 1-naphthalene acetic acid (NAA), 2,3,5-triiodobenzoic acid (TIBA) and 6-benzylaminopurin

(6-BA) applied exogenously at 100 and 10 nmol/L to two maize inbred lines (478 and Wu312) induced the development of large numbers of lateral roots.

Based on the heterogeneous effects of auxins on growth and development of plants, it is evident that different quantities of auxin can produce different or even opposite effects (Leopold 1955). Thus, the growth can be promoted or inhibited by auxins. Each phenomenon in growth and development which is affected by auxins has its own concentration optimum, so that a given auxin concentration for growth of stem is higher than that for buds, which is in turn higher than that for roots (Table 1.6). Consequently, a given auxin application may promote and inhibit the growth of the other type of organs. The quantitative effects of auxins are further complicated by the interactions of auxins with other plant constituents. Thus, Skoog and Tsui (1948) found that the addition of auxins to tobacco stem sections inhibited bud formation, but in the presence of adenine; the auxin inhibition disappeared. Leopold and Guernsey (1953) demonstrated that auxins applied to pea seeds inhibited flowering, but that the additional application of carbohydrates reversed the inhibition. At variable concentrations, auxins effect at site-specific organ (roots, buds, stem) are affected in comparable way; their growth being inhibited by relatively high and promoted by relatively low auxin concentrations (Table 1.6).

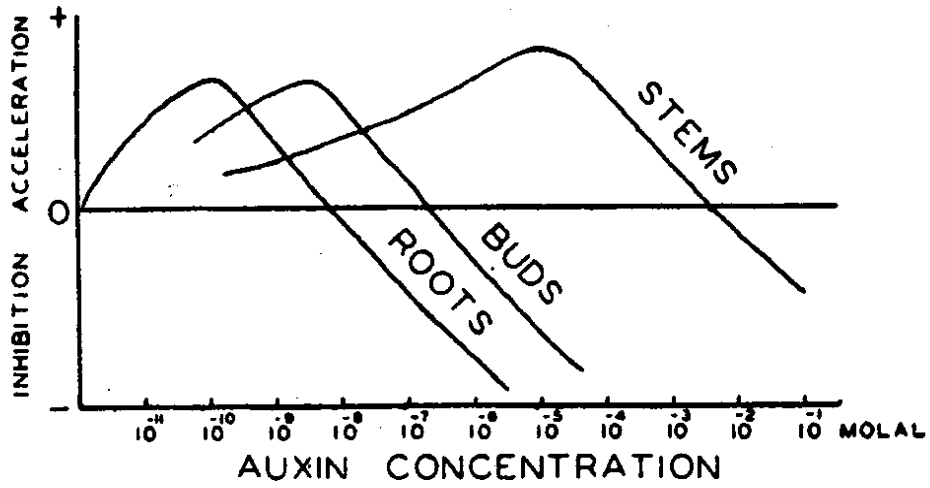


Figure 1.6 Inhibition and growth promotion of different organs as a function of auxin concentration (source: Thimann 1937)

1.7 Rationale of the study

Direct application of a fermented product (Sanjeevak) composed of cattle droppings and urine was reported to have shown promising results from field experiments carried out in India on a variety of crops in improving seedlings growth and yield (Kate and Khadse 2002). Thus, the primary motivation for this study is the following:

1. In addition to the obvious nutrient supply, plant hormones: auxins, cytokinins and gibberellins etc. may be present in Sanjeevak and produced by microorganisms. The latter may be having additional beneficial effect on seed germination, plant growth and development of seedlings from cuttings and possibly improvement in crop yield.

1.8 Objectives

The major objective of this study was to investigate the presence of phytohormones in Sanjeevak and their subsequent effects on growth parameters and yield. Specific objectives were the following:

1) Various samples were sreened for phytohormones (such as cow fodder, urine, cow droppings and Sanjeevak concentrate) using both a colorimetric method derived from that of Salkowski and LC-MS/MS;

2) We characterized Sanjeevak in terms of nutrient content, feacal coliform level and the risk associated to its use for agricultural production. In addition we studied Sanjeevak biogas production potential over time by focusing on its gases composition;

3) We studied the effect of Sanjeevak pre-treatment on seed germination and the rooting of grapevine rootstock cuttings;

4) We investigated the effects of Sanjeevak on crop growth, development and yield of selected crops cultivated in greenhouse conditions;

5) Finally, we studied the effect of Sanjeevak responses on crop yield in comparison to different combination of treatments (control, compost, and a combination of compost and Sanjeevak) under field trial conditions.

1.9 Hypotheses

1) From sanjeevak application on agricultural land, we expected phytohormones biosynthesis by microorganisms due to its reported increases in crop growth and yield compared to chemical fertilizer treatment;
2) The prospect for this section of the study was that the use of Sanjeevak as a source of enhanced soil properties for crop production could be promoted as an alternative for chemical fertilizer;

3) We expected the IAA produced by Sanjeevak microbiota to act as a catalyst for improved seed germination and stimulate the rooting of grapevine rootstock cuttings;
4) The hypothesis for objective 4 was that the study of the effect of Sanjeevak application as a source of soil amendment would lead to improved nutrional uptake that will subsequently result in sustained or possibly increased crop production under greenhouse conditions;
5) The inoculation of microbiota originating from Sanjeevak to agricultural land will result to improved plant growth and yield of a variety of crops under field trial conditions

1.10 Outline of the thesis

This dissertation aimed to assess Sanjeevak as a source of soil amendments and its impact on plant growth, development and ultimately yield.

Chapter 2, Sanjeevak and other samples (cow urine, cow dung and cow fodder) were screened for indole-3-acetic acid (IAA) content using a colorimetric method derived from that of Salkowski. The findings of these experiments revealed the presence of IAA. These results were further validated using LC-MS/MS analysis.

Chapter 3 provides a detailed assessment of Sanjeevak nutrient content, health and ecological risk profiles as well as its content and composition in biogas production. This chapter also briefly evaluates Sanjeevak as source of beneficial bacteria. The results indicated that in relation to the current legislation regulating the use of wastewater sludge for application on

agricultural land; none of the heavy metals and faecal coliform levels measured exceeded permissible threshold. However, the formulation process considered for this study proved to be not suitable for effective methane production. Also, the section highlighted the presence of micro-organisms known for producing phytohormones.

In chapter 4, the effect of Sanjeevak pre-treatment of carrot seeds and rootstock cuttings of grapevine as examples were studied. The results showed that grapevine rootstock cuttings under Sanjeevak treatment had improved rooting (percentage of root generated) relative to the control.

Chapter 5 and 6, describe the impact of Sanjeevak application in greenhouse and field experiment conditions as a source of soil amendments and its impact on plant growth, development and yield. The results highlighted Sanjeevak ability to increase plant development and yield of some crops.

Chapter 7 provides a general conclusion for this dissertation. An overview is provided of the most relevant findings from field and laboratory experiments. The outcomes of this dissertation may ultimately assist toward innovations in extension and advisory services for smallholder farmers with regard to agricultural practices that could sustain and eventually increase food production using natural resources.

1.11 References

Agbenin J.O and Goladin J.T (1997) Carbon, nitrogen and phosphorus dynamics under continuous cultivation as influenced by farmyard manure and inorganic fertilizers in the savanna of northern Nigeria. *Agric. Ecosyst. Environ*. 63: 17-24.

Altieri M.A. (1989) Agroecology: A new research and development paradigm for world agriculture. *Agriculture, Ecosystems & Environment* 27: 37-46

Apedaile E (2001) A perspective on biosolids management. The Canadian Journal of Infectious Diseases. 12(4) 202-204

Arancon N.G., Edwards C.A., Babenko A., Cannon J., Galvis P and Metzger J.D (2008) Influences of vermicomposts, produced by earthworms and microorganisms from cattle manure, food waste and paper waste, on the germination, growth and flowering of petunias in greenhouse. *Applied soil ecology* 39:91-99

Arancon N.Q and Edwards C.A (2006b). Effects of vermicomposts on plant growth. In: Proceedings of the Vermi-Technologies Symposium for Developing Countries, Department of Science and Technology—Philippine Council for Aquatic and Marine Research and Development, Los Banos, Philippines

Arthur, G.D., Jäger A.K and Van Staden J (2001) The release of cytokinin-like compounds from *Gingko biloba* leaf material during composting. Environmental and Experimental Botany 45: 55-61

Atiyeh R.M., Lee S., Edwards C.A., Arancon N.Q and Metzger J.D (2002) The influence of humic acids derived from earthworm-processed organic wastes on plant growth. *Bioresource Technology* 84: 7-14

Attia, K.K (1999) Yield and nutrient status in seeds of some faba bean varieties as affected by farmyard manure and different foliar regimes of micronutrients application. *Assiut J. Agric. Sci*. 35: 189-201.

Badaruddin M. Reynolds M.P and Ageed O.A.A (1999) Wheat management in warm environments: Effect of organic and inorganic fertilizers, irrigation frequency, and mulching. *Agron. J*. 91: 975-983

Basak A.B and Lee M.W (2000b) Efficacy of cowdung in controlling root rot and Fusarium wilt diseases of cucumber plants. http://plantpathsnu.ac.kr/ic2001/abstract.html. August 2005

Bationo A and Mokwunye A.U (1991) Role of manures and crop residues in alleviating soil fertility constraints to crop production: With special reference to the Sahelian and Sudanian zones of West Africa. *Fert. Res*. 29: 117-125.

Bayu W., Rethman N.F.G and Hammes P.S. (2004) The role of animal manure in sustainable soil fertility management in Sub-Saharan Africa. *Journal of Sustainable Agriculture,* Vol. 25 (2): 113-136

Bekunda M.A. Bationo A and Ssali H. (1997) Soil fertility management in Africa: A review of selected research trials. Pp. 63-79 In Buresh R.J, Sanchez P.A and Calhoun F. (eds) Replenishing soil fertility in Africa. SSSA Special publication No. 51 Madison, WI

Belay A, Claassens A.S, Wehner F.C and De Beer J.M (2001) Influence of residual manure on selected nutrient elements and microbial composition of soil under long-term crop rotation. *S. Afr. J. Plant Soil* 18: 1-6.

Beri V., Sidhu B.S., Gupta A.P., Tiwari R.C., Pareek R.P., Rupela O.P., Khera R and Ahluwalia JS (2003) Organic resources of a part of Indo-Gangetic plain and their utilization. PAU

Bitton G (1994) Wastewater microbiology. Wiley-Liss, New York. 478pp

Bolton H., Elliot L.F., Papendick R.I and Bezdicek D.F (1985) Soil microbial biomass and selected soil enzyme activities: Effect of fertilization and cropping practices. *Soil Biol. Biochem*. 17:297-302.

Boyce T.G., Swerdlow D.L and Griffin P.M (1995) Current concepts— *Escherichia coli* O157:H7 and the hemolytic-uremic syndrome. *N. Engl. J. Med*. 333: 364–368

Casenave de Sanfilippo, E., Arguello, J.A., Abdala, G., Orioli, G.A (1990) Content of Auxin-, inhibitor- and Gibberellin-like substances in humic acids. *Biologia Plantarum* 32, 346–351.

Chale-Matsau J.R.B (2005) Persistence of human pathogens in a crop grown in sewage sludge treated soil. University of Pretoria etd

Chiwocha D.S., Abrams S.R., Ambrose S.J., Cutler A.J., Loewen M., Ross A.R.S and Kermode A.R (2003) A method for profiling classes of plant hormones and their metabolites using liquid chromatography–electrospray ionization tandem mass spectrometry: an analysis of hormone regulation of thermodormancy of lettuce (*Lactuca sativa L.*) seeds, *Plant J.* 35: 405–417

Cole D.J., V.R. Hill, F.J Humenik, Sobsey M.D (1999) Health, safety and environmental concerns of farm animal waste. *Occupational Medicine* 14: 423-448

Damodar Reddy D., Subba Roa A and Rupa T.R (2000) Effects of continuous use of cattle manure and fertilizer phosphorus on crop yields and soil organic phosphorus in a Vertisol. *Bioresource Technology* 75:113-118

Davies P.J (1995) The plant hormones: their nature, occurrence, and functions. In plant Hormones: Physiology, Biochemistry and Molecular Biology, 2nd edn. (Davies PJ., ed.). Dordrecht, the Netherlands: Kluwer Academic Publishers, pp. 1-12

De Ridder N and Van Keulen H (1990) Some aspects of the role of organic matter in sustainable intensified arable farming systems in the west-African semi-arid-tropics (SAT). *Fert. Res*. 26:299-310.

DeRopp (1999) Worms and disease. In: Humanure Handbook. Jenkins, J (Ed.)Jenkins Publishing, PA. http://www.weblife.org/humanure/chapter7.html

Dilallessa T.D (2006) Effect of tillage system, residue management and nitrogen fertilization on maize production in western Ethopia. PhD Thesis

Dorn C.R (1993) Review of foodborne outbreakof Escherichia coli O157:H7 infection in the western United States. *J. Am. Vet. Med. Assoc.* 203 (11), 1583–1587.

Dorrington R., Bradshaw D and Budlender D (2002) HIV/AIDS profile in the provinces of South Africa. Indicators for 2002. Medical Research Council, South Africa 31pp

Eck, H.V. and Stewart, B.A. (1995) Manure. In: J.E. Rechcigl (ed.), Soil amendments and environmental quality, pp. 169-199. Lewis Publ., Boca Raton.

Edwards C.A (Ed.) (1998) Earthworm Ecology. CRC Press, Boca Raton, FL 389pp.

El-Attar H., E-Halfawi M and El-Haddad E (1982) Response of maize to farmyard manure and zinc. *FAO Soils bulletin* No.5 Rome, Italy

Francis CA, Flora CB and King LD (1990) Sustainable agriculture in temperate zones. John Wiley and Sons, Inc, New York.

Gagliardi J.V and Karns J.S (2000) Leaching of Escherichia coli O157:H7 in diverses soils under various agricultural management practices. *Applied and Environmental Microbiology* 66(3) 877-883

Gerba C.P and Bitton G (1984) Microbial polluants: their survival and transport pattern to groundwater. In: Bitton G, Gerba CP (eds) Groundwater pollution microbiology. Wiley, New York, pp 39-54

Gibberd V (1995) Yield responses of food crops to animal manure in semi-arid Kenya. *Trop. Sci.* 35:418-426

Glick B.R (1995) The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol.* 41: 109–117

Glick B.R., Patten C.L., Holguin G and Penrose D.M (1999) Biochemical and genetic mechanisms used by plant growth-promoting bacteria. Imperial College Press, London, UK.

Griffin P.M (1995) *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*, p. 739–761. *In* M. F. Blaser, P. D. Smith, J. I. Ravdin, H. B. Greenberg, and R. L. Guerrant (ed.), Infections of the gastrointestinal tract. Raven Press, Ltd., New York, N.Y.

Groenewald Y (2009) Malawi's fertile plan: smallscale farmers help the country to thrive. Mail Guard, 25(3), June 19-25, p 27. http://www.mg.co.za. Cited 28 July 2009

Hager A, Menzel H and Krauss A (1971) Versuche und hyopothese zur primarwirkung des auxins beim streckungswaschstum. *Planta* 100: 74-75

Haggag WM (2002) Sustainable agriculture management of plant diseases. J. Biol. Sci. 2(4): 280-284

Hameeda B, Rupela OP Gopal Reddy, Satyavani K (2006) Application of plant growth-promoting bacteria associated with composts and macro fauna for growth promotion of Pearl millet (*Pennisetum glaucum L.*) Biol Fertil Soils 43: 221-227

Hegazi A.M., El-Bagoury O.H., Mostafa M.T and El-Afandy K.T (1999) Yield and yield components of wheat as affected by organic manure and N-fertilizers under saline conditions at south Sinai. Desert Institute Bulletin 48 Egypt

Hewett, EW and Wareing, P.F (1973) Cytokinins in *Populus X robusta* Schneid: qualitative change during development. Physiol. Plant. 29: 386–389.

Hoffmann I., Gerling D., Kyiogwom U.B and Mane-Bielfeldt A (2001) Farmers' management strategies to maintain soil fertility in a remote area in northwest Nigeria *Agric. Ecosyst. Environ*. 86:263-275.

http://forestry.berkeley.edu/rangelandwq/pdfs/Atwillwssitn21.pdf

Hu Y., Zhang Q and Meitzler J.C (1999) Rapid and sensitive detection of Escherichia coli O157:H7 in bovine faeces by mulptiplex PCR. *Journal of applied microbiology* 87: 867-876

Hue N.V (1992) Correcting soil acidity of a highly weathered ultisol with chicken manure and sewage sludge. *Commun. Soil Sci. Plant Anal*. 23:241-264.

Ikombo, BM (1984) Effects of farmyard manure and fertilizers on maize in semi-arid areas of eastern Kenya. *East Afr. Aagric. Forestry J*. 44: 266-274

Jones P.W (1980) Health hazards associated with the handling of animal wastes. Vet. Rec. 106: 4-7

Kaihura F.B.S., Kullaya I.K., Kilasara M., Aune J.B., Singh B.R., Lal R and Arshad M.A (1999) Soil quality effects of accelerated erosion and management systems in three ecoregions of Tanzania. *Soil Till. Res*. 53:59-70.

Kaper J.B., Nataro J.P and Mobley H.L.T (2004) Pathogenic Escherichia coli. *Nature Reviews Microbiology*, 2: 123-140

Kate T and Khadse M. (2002) Extension of simple and low cost agricultural techniques for improving crop productivity of small & marginal farmers in Vidarbha region through grass root level NGOs. Unpublished report, Dharamitra Wardha

Kennedy A.C and R.I. Papendick R.I (1995) Microbial characteristics of soil quality. *J. Soil Water Cons*. 50:243-248.

Kesavan P.C (2006) From green revolution to evergreen revolution: pathways and terminologies. Curr. Sci. 90: 45-146

Khalid A, Arshad M and Zahir ZA (2004) Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. Journal of Applied Microbiology 96: 473-480

Kostenberg D and Marchaim U (1993a) Solid waste from the instant coffee industry as a substrate for anaerobic thermophilic digestion. *Water Sci. Technol* 27: 97 – 107

Kostenberg D and Marchaim U (1993b) Anaerobic digestion and horticultural value of solid waste from manufacture of instant coffee. *Environ Technol* 14: 973 – 980

Kostenberg D., Marchaim U., Watad A.A and Epstein E (1995) Biosynthesis of plant hormones during anaerobic digestion of instant coffee waste. *Plant Growth Regulation* 17: 127 – 132

Krishnamoorthy R.V and Vajranabhiah S.N (1986) Biological activity of earthworm casts: An assessment of plant growth promotor levels in casts. *Proc. Indian Acad. Sci*. (Anim. Sci.) 95: 341–351

Kudva I.T., Blanch, K and Hovde C.J (1998) Anaysis of Escherichia coli O157:H7 survival in ovine or bovine manure and manure slurry. *Appl. Environ. Microbiol.* 64: 3166–3174.

Kunte D.P., Yeole T.Y., Chiplonkar S.P and Ranade D.R (1998) Inactivation of Salmonella typhi by high levels of volatile fatty acids during anaerobic digestion. *J. Appl. Microbiol.* 84:138-142

Kwakye, P.K (1988) The influence of organic matter in combination with mineral fertilizers on crop yields and soil properties on a savanna soil in Ghana under continuous cropping. *International J. Trop. Agric*. 6:57-67.

Larsen H.E and Munch B (1982) Occurrence and survival of pathogenic bacteria in cattle and pig slurry. *In* communicable diseases resulting from storage, handling, transport and land spreading of manures. *Edited by* J.R Watton and E.G White. Commission of the European Communities. Brussels pp. 161-174

Lemarchanda K., Berthiaumea F., Maynarda C., Harelb J., Paymentc P., Bayardelled P., Massona L and Brousseau L (2005) Optimization of microbial DNA extraction and purification from raw wastewater samples for downstream pathogen detection by microarrays. *Journal of Microbiological Methods*, 63: 115-126

Lucy M. Reed E and Glick E. Bernard (2004) Applications of free living of plant growth promoting rhizobacteria. *Antonie van Leeuwenhoek* 86: 1-25

Lungu, O.I., Temba J, Chirwa B and Lungu C. (1993) Effects of lime and farmyard manure on soil acidity and maize growth on an acid Alfisol from Zambia. *Trop Agric. (Trinidad)* 70: 309-314.

Lupwayi N.Z and Haque I (1999a) Leucaena hedgerow intercropping and cattle manure application in the Ethiopian highlands. III. Nutrient balances. *Biol. Fert. Soils* 28:204-211.

Manici LM, Caputo F, Babibi V (2004) Effect of green manure on Pythium spp. population and microbial communities in intensive cropping systems. Plant Soil 263: 133–142

Marinari S., Mancinelli R., Campiglia E and Grego S (2005). Chemical and Biological indicators of soil quality in organic and conventional farming systems in Central Italy. *Ecological indicators* (Article in press)

Materechera, S.A (2010) Utilization and management practices of animal manure for replenishing soil fertility among small-scale crop farmers in semi-arid farming districts of the North West Province, South Africa. *Nutr Cycl Agroecosyst*

Maxy R.B (1982) Fate of microbial contaminants in lettuce juice. *Journal of Food Protection*. 45 (4) 335 - 339

Mazzucato V and Niemeijer D. (2000) The cultural economy of soil and water conservation: Market principles and social networks in Eastern Burkina Faso. Development and change Vol. 31 pp 831-855. Institute of social studies 2000. Published by Blackwell publishers, 108 Cowley Road, Oxford, OX4 1JF, UK.

McGill, W.B., Cannon, K.R., Robertson, T.A. & Cook, F.D. (1986) Dynamics of soil microbial biomass and water soluble organic carbon in Breton L. after 50 years of cropping to two rotations. *Can. J. Soil Sci*. 66: 1-19.

Meek, B., Graham, L. & Donovan, T. (1982) Long-term effects of manure on soil nitrogen, phosphorus, potassium, sodium, organic matter and water infiltration rate. *Soil Sci. Soc. Am. J*. 46:1014-1019.

Mishke, (1988) I.V., *Mikrobnye fitogormony v rastenievodstve* (Microbial Phytohormones in Crop Production), Riga: Zinatne

Mohamed E.I. and El-Aref K.A.O (1999) Farmyard manure as substitution of part or all chemical nitrogen fertilizer dose at planting for fertilizer maize (*Zea mays L.*) *Assiut J. Agric. Sci.* 30: 139-148

Mokwunye, A.U (1991) *Alleviating soil fertility constraints to increased crop production in West Africa*. Kluwer Academic Publ., Dordrecht, the Netherlands

Ouedraogo E. Mando A and Zombre N.P (2001) Use of compost to improve soil properties and crop productivity under low input agricultural system in West Africa. *Agriculture, Ecosystems and Environment* 84: 259-266

Palm C.A, Myers R.J.K and Nandwa S.M (1997) Combined use of organic and inorganic nutrient sources for soil fertility maintenance and replenishement. Pp. 193-217. In Buresh R.J, Sanchez P.A and Calhoun F (eds.) Replenishing soil fertility in Africa. SSSA Special publication N0. 51 Madison, WI

Pell, A.N (1997) Manure and microbes: Public and Animal health problem? Symposium: Manure Management. Journal of Diary Science Vol. 80 No. 10: 2673-2681

Penner K (1998) Microorganisms and foodborne illness. Food safety http://www.oznet.ksu.edu/library/fntr2/mf2269.pdf

Phuong H.K and Tichy V (1976) Activity of humus acids from peat as studied by means of some growth regulator bioassays. *Biologia Plantarum* 18, 195–199.

Pillai S.D and Ricke S.C (2002) Bioaerosols from municipal and animal wastes: background and contemporary issues. *Can. J. Microbiol*. 48: 681-696

Pillai S.D., Widmer K.W., Dowd S.E and Ricke S.C (1996) Occurrence of airborne bacterial pathogens and indicator organisms during land application of sewage sludge. *Appl. Environ. Microbiol*. 62: 296-299

Powell, JM (1986) Manure for cropping a case study from central Nigeria. *Expl. Agric*. 22:15-24.

Radke JK, Andrews RW, Janke RR, and Peters SE (1988) Low-input cropping systems and efficiency of water and nitrogen use. Pp. 193-218. In W.L. Hargrove(ed.) *Cropping strategies for efficient use of water and nitrogen*. ASA Special publication No 51. ASA, CSSA, SSSA, Madison, WI.

Rosen B.H (2000) Waterborne pathogens in agricultural watersheds. USDA – Natural Resources Conservation Service.

Rudolfs W., Falk L.L and Ragotzkie R.A (1950) Literature review on the occurrence and survival of enteric, pathogenic and relative organisms in soil, water, sewage, sludge and on vegetation. Sewage and Industrial wastes. 22: 1261-1281

Sahlström L. (2003) A review of survival of pathogenic bacteria in organic waste used in biogas plants. Biosource Technology 87: 161 – 166

Sanderson M.W., Gay J.M., Besser T.E., Hancock D.D., Fox L.K and Gay C.C (1995) Sensitivity of bacteriologic culture for detection of *Escherichia coli* O157:H7 in bovine feces. *J. Clin. Microbiol.* 33: 2616–2619.

Santamaria J and Toranzos G.A (2003) Enteric pathogens and soil: a short review. *Int Microbiol* 6:5-9

Sharpley, A.N. & Smith, S.J. (1995) Nitrogen and phosphorus forms in soils receiving manure. *Soil Sci*. 159:253-258.

Smaling E.M (1993) nutrient depletion in Sub Saharan Africa. In: van Reuler H, Prins WH (eds) The role of plant nutrients for sustainable food crop production in Sub-Saharan Africa. Dutch Association of fertilizer producers, The Netherlands.

Smaling E.M and Braun A.R (1996) Soil fertility research in Sub Saharan Africa: New dimensions, new challenges, Commum Soil Sci Plant Anal 29: 2571-2588

Sobsey M.D., Khatib L.A., Hill V.R., Alocilja E and Pillai S (2001) Pathogens in animal wastes and the impacts of waste management practices on their survival, transport and fate (White paper summaries) MidWest Plan Service (NWPS)

Srivastava LM (2002) General features of plant hormones, their analysis and quantitation. In Plant Growth and Development: Hormones and environment. In academic Press, pp 141-152

Swain M.R., Kar S, Padmaja G and Ray R.C (2006) Partial characterization and optimization of production extracellular α-amylase from Bacillus subtilis isolated from culturable cowdung microflora. Polish J. Microbiol. 55: 289-296

Swain M.R., Naskar S.K and Ray R.C (2007) Indole-3-acetic acid production and effect on sprouting of yam (Dioscorea rotundata L.) Minisetts by Bacillus subtilis Isolated from culturable cowdung microflora. Polish Journal of Microb

Takeuchi K and Franck J.F (2001) Penetration of *Escherichia Coli* O175: H7 into lettuce tissue as affected by Inoculum size and temperature and the effect of chlorine treatment cell viability. *Journal of Food Protection*. 63 (4) 434 – 440

Takeuchi K., Matute C.M., Hassan A.N and Frank J.F (2000) Comparison of the attachment of *Escherichia coli* O157:H7. *Listeria moccocytogenes, Salmonella typhimurium* and *Pseudomonas fluorescens* to lettuce leaf. *Journal of Food Protection*. 63(10) 1433-1437

Tanner B., Brooks J., Josephson K., Gerba C and Pepper I (2003) Evaluation of the potential for bioaerosols from land applied biosolids. Proceedings of the IWA Biosolids (2003) Conference. Wastewater sludge as a resource. 23-25 June 2003. Trondheim. Norway

Theron J and Cloete TE (2004) Emerging microbiological detection techniques. In: Microbial waterborne pathogens, Cloete, T.E. (ed), London, UK: IWA Publishing, pp 155-186

Tomati U and Galli E (1995) Earthworms, soil fertility and plant productivity. *Acta Zool. Fenn.* 196: 11–14.

Tomati U., Galli E., Grapppelli A and Dihena G (1990) Effect of earthworm casts on protein synthesis in radish (Raphanus sativum) and lettuce (Lactuca sativa) seedlings. *Biol. Fertil. Soil* 9:288–289.

Tomati U., Grappelli A and Galli E (1983) Fertility factors in earthworm humus. Proceedings International Symposium Agriculture Environment. Prospects in Earthworm Farming. *Publication Ministero della Ricerca Scientifica e Technologia*, Rome, pp. 49–56

Tomati U., Grappelli A and Galli E (1988). The hormonelike effect of earthworm casts on plant growth. *Biol. Fertil. Soils* 5: 288–294.

Tsavkelova E.A., Klimova S.Yu., Cherdyntseva T.A and Netrusov A.I (2006) Hormones and hormone-like Substances of microorganisms: A review *Applied Biochemistry and Microbiology* Vol. 42, No. 3 pp 229-235

U.S. Department of Agriculture Animal and Plant Health Inspection Service (1997) An update: *Escherichia coli* O157:H7 in humans and cattle, p. 1–28. *In* Report from Centers for Epidemiology and Animal Health. Centers for Epidemiology and Animal Health, Fort Collins, Colo.

Van Staden, J and Wareing, PF (1972) The effect of photoperiod on the levels of endogenous cytokinins in *Xanthium strumarium*. *Physiol. Plant.* 27: 331–337.

Vesilind P.A (2003) Pathogens in sludge: A case of sufficient challenge. Proceedings of the IWA Biosolids 2003 Conference, wastewater sludge as a resource. 23-25 June 2003. Trondheim. Norway

Wachtel M.R., Whitehead L.C and Mandrell R.E (2002a) Association of *Escherichia coli* O157:H7 with preharvest leaf lettuce upon contaminated of irrigated water. Journal of Food Protection 65 (1) 18-25

Wachtel M.R., Whitehead L.C and Mandrell R.E (2002b) Prevalence of *Escherichia coli* associated with cabbage crop inadvertently irrigated with partially treated sewage wastewater. Journal of Food Protection 65 (3) 471-475

Wang G., Zhao T and Doyle M.P (1996) Fate of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces. *Appl. Environ. Microbiol.* 62: 2567–2570

Warman P.R and Cooper J.M (2000) Fertilization of a mixed forage crop with fresh and composted chicken manure and NPK fertilizer: Effects on soil and tissue Ca, Mg, S, B, Cu, Mn and Zn. *Can. J. Soil Sci*. 80:345-352.

Wells J.G., Shipman L.D., Greene K.D., Sowers E.G., Green J.H., Cameron D.N., Downes F.P., Martin M.L., Griffin P.M., Ostroff S.M., Potter M.E., Tauxe R.V and Wachsmuth I.K (1991) Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. *J. Clin. Microbiol.* 29: 985–989.

Wong M.T.F., Nortcliff S and Swift R.S (1998) Method of determining the acid ameliorating capacity of plant residue compost, urban waste compost, farmyard manure, and peat applied to tropical soils. *Commun. Soil Sci Plant Anal*. 29:2927-2937.

WRC (1997) Permissible utilization and disposal of sewage sludge. *Water Research Commission.* TT 85/97

WRC (2002) Addendum to the guideline on permissible utilization and disposal of sewage sludge. Edition 1 *Water Research Commission*. T154/0

CHAPTER TWO

Screening of Indole-3-acetic acid (IAA) from Sanjeevak microbiota using Salkowski reagent and Liquid Chromatography-Mass Spectrometry (LC-MS)

2.1 Introduction

Plant hormones are widely used in the agricultural, horticultural, and biotechnological industries; primarily to alter plant growth and development. It is well known that hormones regulate a range of cellular and physiological processes which include cell division, cell enlargement and differentiation, flowering, fruit ripening, movement (tropisms), seed dormancy and germination, senescence, leaf abscission and stomatal conductance (Arshad and Frankenberger 1991; Lucy et al., 2004; Tsavkelova et al., 2005; Lwin et al., 2008).

Phytohormones are synthesized by both plants and microorganisms (Arshad and Frankenberger 1991; Lwin et al., 2008). The ability to synthesize phytohormones is extensively distributed among soil and plant associated bacteria. It is estimated that close to 80% of bacteria isolated from plant rhizosphere are able to produce indole-3-acetic acid (IAA). Diverse soil organisms including soil bacteria, fungi and algae are also capable of producing physiologically active quantities of auxins. However, microorganisms isolated from the rhizosphere of various crops are more likely to release auxins than those from root-free environment (Sarwar et al., 1992). Narayanaswami and Veeraju (1969) found a 3-fold higher IAA content in the rhizosphere compared to non-rhizosphere environments. Also, Rossi et *al.* (1984) found that auxin-like compounds were greater in the rhizosphere soil under maize cultivation compared with non-rhizosphere soil, especially during seedling emergence.

IAA has been identified from a variety of samples, such as human urine where it is believed to be synthesized by intestinal bacteria from tryptophan (TRP) in the diet (Went and Thimann, 1937); digested instant coffee waste as a result of catabolism of TRP by anaerobic bacteria (Kostenberg et al., 1995), anaerobically digested cow manure was reported to display a growth promotion effect on herb plants (Marchaim, 1983; Raviv et al., 1993) and auxin groups incorporated in humic acids extracted from cattle manure vermicompost (Canellas et al., 2000).

Previous studied have identified plant hormones such IAA in cow urine (Weissbach et al., 1958; Allison et al., 1974), and cow dung (Swaminathan, 2005; Sreenivasa et al., 2009). However, this is the first quantitative study aimed at investigating IAA content through the feeding chain of animals such as dairy cattle and the influence bacteria in sanjeevak do have on IAA production when environmental parameters such as incubation time, concentration of LTRP and temperature are altered.

The objectives of this study were (i) to detect and quantify indole-3-acetic acid (IAA) through the feeding chain: Animal fodder => cow dung => cow urine, => Sanjeevak concentrate; using Salkowski colorimetric method and liquid chromatographic-mass spectrometry (LC-MS). (ii) to investigate the effect of temperature, incubation time and TRP (biocatalyst) addition to Sanjeevak bacterial cultures on IAA synthesis. (iii) to monitor the overall influence of Sanjeevak maturation on IAA content.

2.2 Materials and Methods

2.2.1 Biosynthesis of auxins

Materials

Cow dung, fodder, urine were collected from the University of Stellenbosch experimental farm. Sanjeevak (a mix of cow dung, cow urine, water and a handful of sugar) was made and stored in a capped container at room temperature and allow to mature under anaerobic conditions.

Methods

Sample preparation: Cow dung samples were oven dried at 65°C to a constant weight. The urine used in this study was provided by dairy cows from the dairy farm facility of the experimental farm of the University of Stellenbosch. The collection was done manually at every milking session using a 15 L plastic container that was placed underneath at every opportunity a cow urinated. Part of the urine was immediately used to make Sanjeevak; the remaining part was stored in tightly capped plastic containers (100 ml plastic bottles) in the refrigerator until ready for use. Cow fodders were randomly collected from the university experimental farm. Both oven dried cow dung and cow fodder were ground to powder for use in laboratory analyses.

Method of assay: The L-Tryptophan (L-TRP) derived auxin production assay as follows: 1.5g (powdered samples) / 2 ml (liquid samples) were placed in 50 ml Erlenmeyer flask and treated with 6 ml of phosphate buffer (0.2 M, pH 7.0) and 4 ml of the L-TRP solution. The phosphate buffer (0.2 M, pH 7.0) was made using (0.2 M) of each Na_2HPO_4 and NaH_2PO_4

prepared separately as stock solutions and appropriate volumes were mixed to obtain the buffer of a desired pH. The flasks were covered with parafilm and incubated in darkness at variable temperatures (ranging from room temperature to 40°C) for 12 hrs to 48 hrs on a shaker (~150 rev/min) depending of the experiment requirements. After incubation, the flask contents were treated with 2 ml of Trichloroacetic acid (5 g/100 ml H_2O) to terminate the reaction and 1 ml of calcium chloride (0.5 M) to facilitate filtration. The sample solution was subsequently filtered through Whatman filter paper No. 2. Investigations were also undertaken to test samples for their auxin content in the absence of L-TRP.

Colorimetric determination of auxins: 3 ml of sample filtrate was added to a test tube and treated with 2 ml of the Salkowski reagent (colour developing agent). The Salkowski reagent was prepared using 2 ml of 0.5 M $FeCl_3$ mixed with 98 ml of 35% Perchloric acid (Gordon and Webber, 1951). The mixture was allowed to stand for 30 min for colour development. The intensity of the colour developed was measured at 535 nm by using spectrophotometer. This wavelength was chosen since it is commonly used in auxin colorimetric assays derived from Salkowski (Sarwar et al., 1992).

The colorimetric method also forms chromophoric complexes with other auxin compounds (such as indoleacetamide and indolepyruvic acid) synthesized by bacteria in addition to Indole-3-acetic acid (IAA); as a result the unit IAA equivalents was chosen to express auxin production (Sarwar et al., 1992; Glickmann and Dessaux 1995).

Standard IAA solutions: A dilution series with concentrations of IAA (0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm) were prepared (Fig. 2.1), treated with Salkowski reagent and the developed colour were measured at 535 nm by using the spectrophotometer (Fig. 2.2). IAA concentration in the samples analysed using Salkowski reagent was determined using the

following equation: X = Y÷ (0.0183); with Y the colour developed measured at 535 nm by using spectrophotometer.

Figure 2.1 Samples of the attained solutions of indole-acetic acid (1) compared with standards (II)

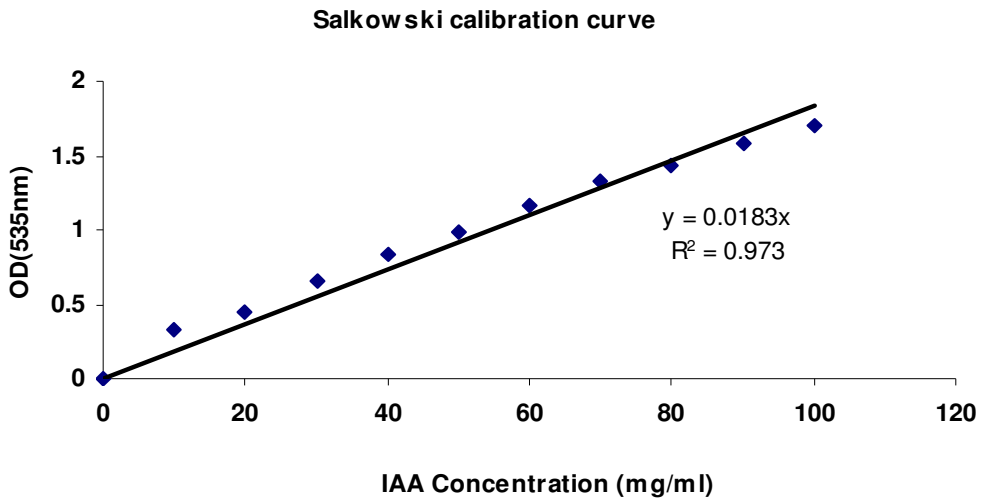


Figure 2.2 OD_{535} values were measured as a function of IAA concentration

Liquid Chromatography and Mass Spectrophotometry (LC-MS)

LC-MS/MS analysis was performed with a Waters Acquity UPLC system coupled to a Waters Xevo TQ MS. Compounds were separated on a Waters Acquity BEH phenyl column (100 x 2.1 mm, 1.7 µm) at 50 °C using a 0.01% acetic acid / acetonitrile gradient: Starting with 85% 0.01% acetic acid and 15% acetonitrile for 0.1 min at a flow rate of 0.3 ml/min. The acetonitrile was increased linearly to 70% over 2.9 min at a flow rate of 0.3 ml/min. The acetonitrile was then increased to 95% over 0.1 min at a flow rate of 0.5 ml/min and maintained for 0.4 min. The column was re-equilibrated for 1.5 min. The injection volume was 5 µl.

IAA was ionized using electrospray ionisation in positive mode (ESI+) and the multiple reaction monitoring (MRM) transition of m/z = 176.2 > 130 (cone voltage = 15 V; collision energy = 10 eV) was acquired. The source capillary was at 3.7 kV. The source and

desolvation temperatures were 150 ºC and 450 ºC, respectively. The desolvation and cone gas flows were 600 and 60 L/h, respectively.

2.3 Results and Discussion

2.3.1 Auxin biosynthesis

The evaluation of cow urine, cow dung, cattle fodder and Sanjeevak showed that all the samples investigated contained auxins (Table 2.1). The presence of IAA in cow urine and Sanjeevak was further confirmed by LC-MS analytic method (MRM chromatogram is presented in Appendix 2).

Further analyses were done on Sanjeevak using LC-MS analytic method to identify gibberelic acid (GA_3) and cytokinins (zeatin and kinetin) formations; however they were found undetectable at concentration ranging from 0.001 to 10 ppm. Of significant interests are the levels of auxins concentration in cow urine, cow dung and cow fodder samples; both supplemented with or without L-TRP (Table 2.1). Cow urine, cow dung and cow fodder samples preparations incubated at room temperature were relatively higher in IAA equivalents than Sanjeevak in the absence or presence of L-TRP. However, IAA equivalents in response to L-TRP addition were doubled in cow dung. This; we think, may be caused by TRP conversion to IAA catalysed by bacteria in cow dung. A marginal increase in auxins content was observed for cow urine in response to its incubation with L-TRP. This may be attributed either to the saturation of the reaction mixture with the substrate or the low content of specific bacteria to convert L-TRP to IAA.

As discussed earlier, Went and Thimann (1937) first isolated and identified IAA from human urine, and they argued that it was probably synthesized by intestinal bacteria from tryptophan in the diet. Also, IAA was found to be biosynthesized from tryptophan by fungi (Gruen,

1959) and bacteria such as *Azospirillum spp.* (Tien et al., 1979; El-Khawas and Adachi, 1999; Abbas Akbari et al., 2007), *Pseudomonas, Azotobacter* and *Agrobacerium* (Costacurta and Vanderleyden 1995; Lwin et al., 2008). Therefore, the detection of IAA in cow urine (MRM chromatogram is presented in Appendix 2) is most probably the product of tryptophan metabolism by the microbial population in the rumen (Weissbach et al., 1958; Allison et al., 1974).

Table 2.1 Effect of L-tryptophan on auxins production by cow urine, manure and fodder and Sanjeevak (mean ± SE)

Treatments	IAA equivalents (ppm)
Cow fodder*	34.37 ± 11.05
Cow urine*	20.38 ± 2.06
Cow dung*	20.06 ± 6.65
Cow urine + L-TRP (10 µM)*	23.79 ± 3.28
Cow dung + L-TRP (10 µM)*	52.66 ± 3.00
Sanjeevak†	17.90 ± 1.10
Sanjeevak + L-TRP (10 µM)†	64.29 ± 9.77
Sanjeevak‡	16.87 ± 1.43
Sanjeevak + LTRP (10 µM)‡	138.31 ± 12.64

* Samples that were incubated in darkness at room temperature (RT) for 12 hrs on a shaker

† Samples that were incubated in darkness at 30°C for 48 hrs on a shaker

‡ Samples that were incubated in darkness at 40°C for 48 hrs on a shaker

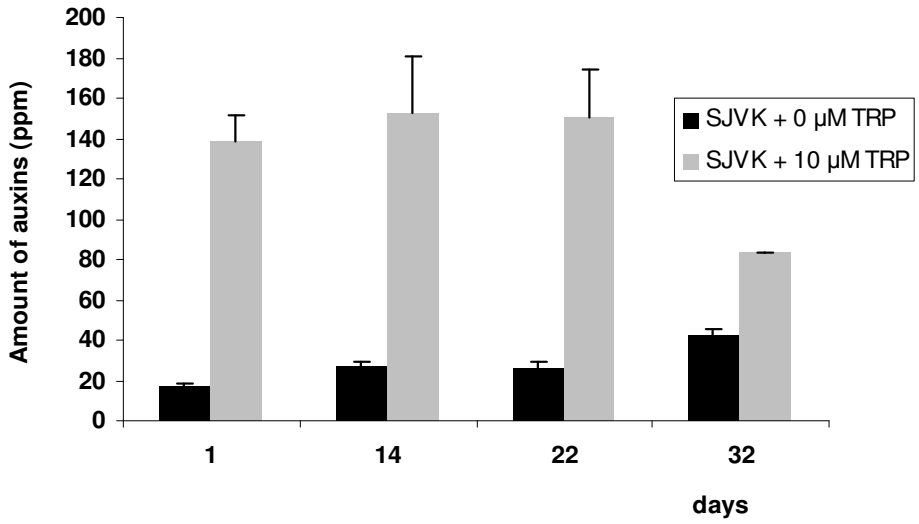


Figure 2.4 Effect of L-tryptophan on IAA production by Sanjeevak microbiota (mean ± SE)

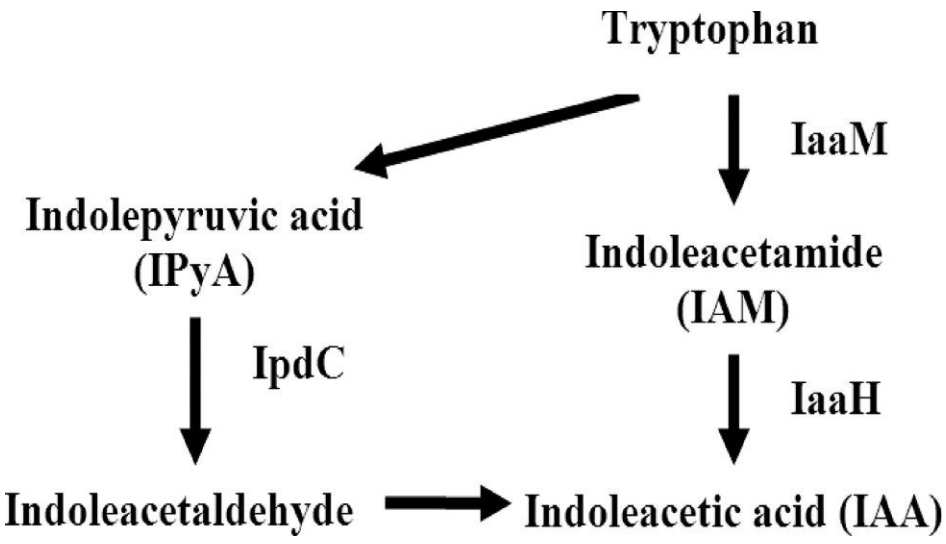


Figure 2.5 Tryptophan-dependent pathways of bacterial indole-3-acetic acid (IAA) biosynthesis (Spaepen et al., 2007)

L-TRP is a recognized biocatalyst for IAA synthesis because addition of this amino acid to cultures of bacteria that are known to synthesise IAA stimulates an increase in IAA production (Figure 2.5) (Oberhänsli et al., 1991; Costacurta and Vanderleyden 1995; Spaepen et al., 2007).

Figure 2.4 shows auxins production by bacteria in Sanjeevak supplemented with both no tryptophan and tryptophan at 10 µM. The production of auxins gradually increased from 1 to 14 days. Between day 14 and 22, auxins content did not vary; however auxins biosynthesis markedly declined by almost 50% from 22 to 32 days in Sanjeevak supplemented with tryptophan. In contrast, from 22 to 32 days auxins biosynthesis significantly increased to 41.93 from 26.13 ppm in Sanjeevak not supplemented with TRP. Overall, auxins response to Sanjeevak supplemented with TRP (10 µM) was up to 8-fold greater (from 16.87 to 138.31 ppm) compared to the control (Figure 2.4).

These findings rather suggest a physiological than nutritional effect. In addition, Incubation at 40°C promoted auxins biosynthesis in Sanjeevak by 2.2-fold compared to incubation at 30°C (Table 2.3). This reflects the fact that mesophilic Sanjeevak bacterial cultures are active in this biochemical reaction. Furthermore, earlier studies have demonstrated that increased auxins biosynthesis was closely related to the growth of bacteria producing IAA in tryptophan-supplemented medium (Swain et *al.,* 2007). Also, Unyayar et *al.,* (2000) argued that during the stationery phase some IAA producing bacteria might be able to get maximum tryptophan from the dead bacterial mass which could in turn result in more IAA production. It is well documented that a single bacterial strain may often use more than one biosynthesis pathways for IAA production (Pattern and Glick, 1996; Swain et *al.,* 2007). For example, as previously stated by Swain et *al* (2007) in *Enterobacter cloacae*, IAA was produced via

indole-3-pyruvic acid (tryptophan => indole-3-pyruvic acid => indoleacetaldehyde => indole-3-acetic acid) (Koga et *al.,* 1991); in *Pseudomonas syringae*, IAA biosynthesis happens predominantly via indole-3-acetamide (tryptophan => indole-3-acetamide => indole-3-acetic acid) (Hutcheson and Kosuge, 1985; Kosuge and Sanger, 1987) whilst in *Pseudomonas fluorescens*, IAA is synthesized from tryptophan via the following pathway (tryptophan => indole-3-acetaldehyde => indole-3-acetic acid) (Oberhansli et al., 1991). Swain et *al* (2007) further emphasized the fact that IAA production was also found to take place via tryptamine in *Agrobacterium tumefaciens* and via indole-3-acetonitrile in *Alcaligene faecalis* and *A. tumefaciens* (Costacurta and Vanderleyden, 1995; Kobayashi et al., 1995). The use of many biosynthesis pathways for IAA production requires additional energy, substrate and enzymes to metabolize tryptophan to indole-3-acetic acid. Also, it is possible that part of the auxin produced may be degraded by other microorganisms (Arshad and Frankenberger 1991). Indeed, IAA is a biodegradable molecule (Levau and Gerards 2007). As argued by Leveau and Gerards (2007) IAA catabolism has previously been demonstrated in soils under conditions that promote bacteria growth (Proctor 1958; Riviere et al., 1966; Chandramohan and Mahadevan 1968; Martens and Frankenberger 1993). Leveau and Lindow (2005) reported on the quantitative correlation between increase in bacterial biomass and IAA degradation. Therefore, under certain conditions bacteria may use IAA as an ideal source of food. IAA carries the two most abundant elements of bacterial cell (C and N) in a single molecule, with a C/N ratio of 8.6. Hypothetically, if half of the carbon is used for the generation of energy, the remaining C/N ratio is 4.3; which is close to C/N of 3.6 for a typical bacterium (Neidhardt et al., 1990; Leveau and Lindow 2005). Hence, these may possibly explain the decreased IAA biosynthesis by almost 50% between 22 to 32 days in Sanjeevak supplemented with tryptophan.

The presence of naturally beneficial microorganisms mainly, yeast, fungi and bacteria such as *actinomycetes*, *azotobacter*, free living N_2 fixers and phosphate Solubilizing microorganisms (PSM) have all been previously identified in cow dung (Swaminathan, 2005; Sreenivasa et al., 2009) and also Sanjeevak (Pathe and Kate 2009). Indole-3-acetic acid and other growth regulators have been widely reported in the literature to be synthesized by microorganisms in the rhizosphere or in root-free environments (Costacurta and Vanderleyden 1995). For example, *Bacillus subtilis* isolated from cultivable bacteria in cowdung (Swain et al., 2007), bacteria isolates from Beejamrutha (a mix of cow dung, cow urine, water, lime and soil) (Sreenivasa et al., 2009) were shown to produce phytohormones. Therefore, our results strongly suggest that phenotypic character of bacteria in Sanjeevak appears to have more of an influence on IAA production. Such results confirm those from other studies (Swain et *al.*, 2007; Sreenivasa et *al.,* 2009).

2.4 Conclusion

To the best of my knowledge, this is the first report on the production of indole-3-acetic acid (IAA) in Sanjeevak. The findings this research investigation revealed the consistent presence of IAA from cow feeds, cow urine and dung to Sanjeevak concentrate although at variable concentrations. In addition, the influence of TRP (a known biocatalyst of IAA in bacteria) added to Sanjeevak bacterial cultures were highlighted. The results of this study showed that environmental factors such as temperature and incubation time also increased substantially IAA production in Sanjeevak. However, the influence of the maturation process did not effectively translate to increased IAA production; emphasizing the fact that brewing Sanjeevak is not a necessary step to increased IAA synthesis from bacteria. In contrast, lengthy period of maturation leads to decreased IAA content due IAA catabolism. This research provides clear evidence that IAA was produced by Sanjeevak bacterial cultures.

2.5 References

Abbas Akbari Gh., Arab S.M., Alikhani H.A., Allahadadi I and Arzanesh M.H (2007) Isolation and selection of indigenous Azospirillum spp. And the IAA of superior strains effects on wheat roots. World journal of agricultural sciences 3 (4) 523-529

Alley J.C (1961) Factors affecting the rooting of grape cuttings II. Growth regulators. 11th Annual meeting of the American Society of Enologists, California June 23-24

Allison J.M., Robinson I.M., Baetz A.L (1974) Tryptophan biosynthesis from indole-3-acetic acid by anaerobic bacteria from the rumen. Journal of Bacteriology 117 (1) 175-180

Aracon N.Q., Edwards C.A., Bierman p., Metzger J.D and Lucht C (2005) Effects of vermicomposts produced from cattle manure, food waste and paper waste on the growth and yield of peppers in the field. Pedobiologia 49: 297-306

Arshad M and Frankenberger jr W.T (1993) Microbial production of plant growth regulators. In meeting, Jr., F.B. (Ed.) Soil microbial ecology: Applications in agricultural and environmental management. Marcell Dekker, New York, pp. 307-347

Canellas L.P., Olivares F.L., Okorokova A.L and Facanha A.R (2000) Humic acids isolated from earthworm compast enhance root elongation, lateral root emergence and plasma H+ ATPase activity in maize roots. Plant Physiol. 130: 1951-1957

Chandramohan D and Mahadevan A (1968) Indole acetic acid metabolism in soils. Curr Sci 37: 112–113.

Costacurta A. and Vanderleyden J (1995) Synthesis of phytohormones by plant associated bacteria. Crit. Rev. Microbiol. 21: 1-18

El-Jhawas H and Adachi K (1999) Indefication and quantification of auxins in culture media of Azospirillum and Klebsiella and their effect on rice roots. Biol. Fertil. Soils, 28: 377-381

Gordon S.A and Weber R.P (1951) Colorimetric estimation of indole acetic acid. Plant Physiol. 26: 192-195

Gruen H.E (1959) Auxins and plant. Annu. Rev. Plant Physiol 10: 405-440

Hutcheson S and Kosuge T (1985) Regulation of 3-indoleacetic acid production in Pseudomonas syringae pv. Savastanoi (purification and properties of trptophan 2-monooxygenase) J. Biol. Chem. 260: 6281-6287

Johan H.J. Leveau J.H.J and Gerards S (2008) Discoveryofa bacterial gene cluster for catabolismof the plant hormone indole 3-acetic acid. FEMS Microbiol Ecol 65: 238–250

K. Mya Lwin., M. Myint Han., M. Myint and Khaing Oo Z. (2008) Screening of indole-3-acetic acid (IAA) producing plant growth promoting rhizobacteria (Pseudomonas sp and Azobacter sp.) and study on the IAA production of the best IAA producer strain. GMSARN International conference on sustainable development: Issues and prospects for the GMS 12-14 Nov. 2008

Kate T and Khadse M. (2002) Extension of simple and low cost agricultural techniques for improving crop productivity of small & marginal farmers in Vidarbha region through grass root level NGOs. Unpublished report, Dharamitra Wardha

Kate T and Pathe S. (2009) Scientific validation of the nutrient management practices evolved by some innovative farmers. Unpublished report, Dharamitra Wardha

Kobayashi M., Suzuki T., Fujita T., Masuda M and Shimizu S (1995) Occurrence of enzymes involved in biosynthesis of indole-3-acetic from indole-3-acetinitrile in plant assocuated bacteria, Agrbacterium and rhizobium. Proc. Natl. Acad. Sci. USA. 92: 714-718

Kostenberg D., Marchaim U., Watad A.A and Epstein E (1995) Biosynthesis of plant hormones during anaerobic digestion of instant coffee waste. *Plant Growth Regulation* 17: 127 – 132

Kosuge T and Sanger M (1987) Indole acetic acid, its synthesis and regulation: basis for tumorigen city in plant disease. Recent. Adv. Phytochem 20: 147-161

Leveau J.H.J and Lindow S.E (2005) Utilization of the Plant Hormone Indole-3-Acetic Acid for Growth by *Pseudomonas putida* Strain 1290. Applied and Environmental Microbiology vol 71 No. 5 p. 2365–2371

Lucy M, Reed E and Glick B.R (2004) Application of free living plant growth-promoting rhizobacteria. Antomie van Leewenhoek 86: 1-25

Marchaim U (1983) Anaerobic digestion of agricultural wastes: the economic lie in the effluent uses. Inc., Watertown, MA. Pp. 342-355

Martens DA and Frankenberger WT (1993) Stability of microbialproduced auxins derived from L-tryptophan added to soil. Soil Sci 155: 263–271.

Milton J.A, Robinson I.M and Baetz A.L (1974) Tryptophan biosynthesis from indole-3-acetic acid by anaerobic bacteria from the rumen. Journal of Bacteriology 117 (1) 175-180

Narayanaswami R and Veerraju V (1969) IAA synthesis in paddy soil as influence by ammonium sulfate fertilization. Curr. Sci. 38: 517-518

Neidhardt, F.C., Ingraham J. L and Schaechter M (1990) Physiology of the bacterial cell: a molecular approach. Sinauer Associates, Inc., Sunderland, Mass.

Oberhansli T., Defago G and Hass D (1991) Indole-3-acetic acid (IAA) synthesis in the biocontrol starin CHAO of Pseudomonas fluoresces: role of tryptophan side chain oxidase. J. Gen. Microbiol 137: 2273-2279

Pattern C.L and Glick B.P (1995) Bacterial biosynthesis of indole-3-acetic acid. Can. J. Microbiol 42: 207-220

Proctor M.H (1958) Bacterial dissimilation of indoleacetic acid: a new route of breakdown of the indole nucleus. Nature 181:1345.

Raviv M., Chen Y., Geler Z., Medina S., Putievsky E and Inbar Y (1983) Slurry production by methanogenic fermentation of cow manure as a growth medium for some horticultural crops. Acta Hortic 150: 563-573

Riviere, J., and Berthier B (1964) Action des microorganismes de la rhizosphe re sur la croissance du ble´. III. Isolement et identification des bacteries degradant l'acide indole-3-acetique. Ann. Inst. Pasteur 3:250–256.

Rossi W, Grappelli A and Pietrosanti W (1984) Pytohormones in soil microorganisms. Soil Sci. 48: 135-139

Sarwar M., Arshad M., Martens A.D and Frankenberger jr W.T. (1992) Tryptophan-dependent biosynthesis of auxins in soil. Plant and Soil 147: 207-215

Sreenivasa M.N., Bagaraj Naik and Bhat S.N (2009) Beejamrutha: A source for beneficial bacteria. Karnakata J. Agric. Sci. 22 (5) 1038-1040

Swain M.R., Naskar S.K and Ray R.C (2007) Indole-3-acetic acid production and effect on sprouting of yam (Dioscorea rotundata L.) Minisetts by Bacillus subtilis Isolated from culturable cowdung microflora. Polish Journal of Microbiology 57 (2) 103-110

Swaminathan C (2005) Food production through vrkshayurvedic way. Technologies for natural farmng. Agriculture College & Research Institute, Madurai, Tamilnadu, India pp: 18-22

Tien T.M., Gaskins M.H and Hubbell D.H (1979) Plant substances produced by Azospirillum brasilence and their effect on the growth of pearl millet (Pennisetum americanum L.) Appl. Environ. Microbiol. 37: 1016-1024

Unyayar S., Unyayar A and Unal E (2000) Production of auxin and abscisic acid by Phanerochaete chrysosporium ME446 immobilized on polyurethane foam. Turk J. Biol 24: 769-774

Went F.W and Thimann K.V (1937) Phytohormones. Macmillan Co., New York, N.Y.

Weissbach H, King W, Sjoerdsma A and Udenfriend S (1959) Formation of indole-3-acetic acid and trptamine in animals: A method for estimation of indole-3-actic acid in tissues. J. Biological Chemistry 234: 81-85

CHAPTER THREE

Sanjeevak: An assessment of its nutrient content, health, ecological risk profiles and its content and composition in biogas production

3.1 Introduction

The search to find viable and cost-effective alternatives to improve soil fertility and properties for sustainable crop production has resulted in the recycling of biowastes including human and animal wastes. The fertilizing value of organic wastes such as urine, human and animal excreta and their use as soil amendments has recently been the subject of greater attention among researchers (Morgan 2003, Prajapati and Gajurel 2003, Jonsson et *al.,* 2004; Mnkeni et *al.,* 2005; Guzha et *al.,* 2005 and Mnkeni et *al.,* 2008). Trials conducted in Zimbabwe and South Africa showed significant increased yields of different vegetables and maize, grown in sandy soils because of using human urine as a fertilizer (Morgan 2003; Guzha et *al.,* 2005 and Mnkeni et *al.,* 2008).

The agricultural use of human and animal wastes is seen as a viable cost effective management option; both for the agricultural and wastewater industry. Organic wastes can assist in increasing the organic content of soil. For example, South Africa cultivated soils are low in organic matter due to its rapid decomposition caused by climatic conditions. This has contributed to a widespread deterioration of soil physical properties. The improvement of the physical properties of soils (water holding capacity, permeability etc.) as a result of an increase in organic carbon plays an important role in promoting the agricultural application of wastewater sludge in South Africa. Subsistence and small-scale farmers can particularly benefit from the agricultural use of organic wastes, since the farmer will benefit financially

due to savings on commercial fertilizers. In addition to animal waste materials containing valuable nutrients; they are also a source of beneficial bacteria. Brea and Brown (1974) reported an increased growth of several plants upon inoculation with *Azotobacter paspali*. This increased yield was attributed to the bacterial production of plant hormones (Arshad and Frenkenberger 1991). Indole-3-acetic acid (IAA) is an important product of tryptophan metabolism by the microbial population of the rumen (Allison et al., 1974). Equally, IAA is also synthesized by bacteria from human feces (Weissbach et al., 1959; Chung et al., 1975; Kögl et al., 1933). Previously, the production of plant hormones has been widely reported for *Azotobacter* (Ahmad et al., 2005) and *Azospirillum* (Zakharova et al., 2000; Ona et al., 2003).

However, the presence of a wide variety of potentially harmful chemicals such as heavy metals in wastewater (although in trace amounts) with the potential of uptake by plants and animals, together with the potential of animal pathogens in the forms of bacteria, viruses and other pathogenic agents means that human and animal wastes need to be assessed and treated if necessary in order to meet international requirements prior to their use for agriculture production (Snyman and Herselman 2006).

There are numerous studies focusing on the biosafety use of wastewater sludge for food production (Chale-Matsau 2005 and reference therein). However, not nearly enough is done when it comes to providing proper guidelines in terms of the biosafety of animal waste materials use in agricultural land. The objectives of this study were (i) to characterise Sanjeevak in terms of its nutrient and heavy metals contents over time (ii) to evaluate Sanjeevak as a potential source of hazard in terms of its coliform bacteria load (iii) to assess

Sanjeevak biogas production; specially methane content. The findings of this study will help formulate approaches for Sanjeevak use in agriculture.

3.2 Materials and methods

Waste materials

Freshly deposited cattle droppings and urine were regularly collected from Welgevallen dairy farm at the University of Stellenbosch experimental Farm, Republic of South Africa.

Sanjeevak preparation and sampling

Cattle faeces and urine were mixed with water in the following proportions (1:1:18), with a handful of sugar; then fermented. The fermentation of Sanjeevak (T0) was carried out in 25 L plastic bucket under an aerobic system, kept at room temperature for a period of ± 45 days, and replicated four times. The mixes were sampled 1, 3, 5, 7, 10, 14, 18, 21, 25, 29, 37 and 45 days after initiation

Figure 3.1 Sanjeevak preparation

Methods

Macro and micronutrients and heavy metals

Sanjeevak samples were oven-dried by heating at 65°C for 3 days to a constant weight to determine dry mass content. The pH was recorded directly from Sanjeevak suspension. Extractable mineral N concentrations (NH_4-H and NO_3-H) were determined colorimetrically in 0.5 M K_2SO_4 extracts in the ratio of 1:10 (v:v) Sanjeevak to extractant, using a modified indophenol blue technique (Sims et al., 1995). Soluble P was assessed using NH_4-HCl reagent (Olsen and Sommers, 1982). Colour in the sample filtrates was developed with stannous chloride and ammonium paramolybdate and hypochlorite reagents. Absorbance was measured using a Bio-Tek EL211sx automated microplate reader. A more complete nutrient analysis was made for other Sanjeevak samples after nitric acid/Perchloric acid digestion (Singer and Hanson, 1969). The extracts were analyzed for P, K, Ca, Mg, B, Mn, As, Cd, Cr,

Cu, Pb, Hg, Ni and Zn using atomic absorption spectroscopy (AAS). Total organic C and N were measured in Sanjeevak by dry combustion using a carlo-Erba apparatus.

Detection and enumeration of Escherichia coli (E. coli) and coliform bacteria

Analysis was performed according to South African National Standard for the detection and enumeration of *E. coli* and coliform bacteria in surface or waste water using a miniaturized method (Most Probable Number) (SANS 9308-3:2004).

Gas production and composition

Reactor design

A laboratory-scale anaerobic reactor was set up and used. The reactor had an operational volume of 50 ml (total height of 70 mm and internal diameter of 200 mm). The test was carried out as triplicate batch experiments. 20 ml of Sanjeevak was transferred in each reactors using a pipette; no inoculum was added to the reactors. The reactor was subsequently kept at room temperature and allowed to stabilize to for ± 48 hours in order to allow the bacterial community to acclimatise and start the digesting process of waste materials and release biogas.

Gas chromatography

Gas samples (± 0.2 ml) were taken from the headspace of the reactors through the septum using a syringe with pressure lock. The syringe was redrawn and the sample was injected directly into the gas chromatograph (GC) (Varian 3300). The GC was equipped with a thermal conductivity detector and a 2.0 m x 3.0 mm i.d column filled with Hayesep Q

(Supelco, Bellefonte, PA), 80/100 mesh. Helium was the carrier gas and parameters for the flow rate were set at 30 ml.min^{-1} and the oven temperature was adjusted to 55°C. The GC was set up to only detect cabon dioxide (CO_2), methane (CH_4) and nitrogen (N_2). The gas composition was determined as described by Sigge and Britz (2007).

3.3 Results and Discussion

In figure 2.2, changes of pH value through the maturation process of Sanjeevak are shown. pH values in Sanjeevak decreased steadily after 18 days. However, between day 25 and day 45, there was no significant difference in pH values. Gas composition remained on average relatively constant (7% $N_{2;}$ represented by peak 2) and (93% CO_2 represented by peak 1) (GC chromatogram is presented in Appendix 1). Georgacakis et *al.* (1982) and Patni and Jui (1985) reported the pH of manure slurries is determined by the strength of the HCO_3^- /CO_3^{2-} and NH_4^+/NH_3 buffer systems and the concentration of volatile fatty acids (VFA). Georgacakis et *al.* (1982) further explained that as the concentration of VFA increased, the importance of the HCO_3^- /CO_3^{2-} buffer system decreased and the pH of the slurry may be determined by VFA and ammonia concentrations. As stated by Strauch (1987) decreased pH values during cattle dung digestion may be caused by microbial activity stimulated by the natural bacterial flora producing VFA. In addition, gas composition produced by the anaerobic digestion of Sanjeevak was analysed using the gas chromatograph (GC). The findings revealed that most the biogas generated was carbon dioxide (CO_2) and nitrogen (N_2). This result underlines the fact that, only the acidogenic phase of the anaerobic digestion of Sanjeevak driven by acidogenic bacteria occurred; leading to the production of VFA + CO_2 + N_2 + biomass (Phase I). However, the methanogenic phase of the anaerobic degradation of Sanjeevak did not occur (Phase II), mostly due to the lack of adequate active inoculum (methanogenic bacteria) added or present in the mixture to drive the conversion of VFA +

CO_2 + H_2 to methane (CH_4). This has contributed to the observed decreased of pH values from 8.54 at initiation to 7.1 after 45 days of Sanjeevak anaerobic digestion. These results reflect the fact that the reduction of alkalinity is most likely influenced by VFA, total ammonia (NH_4^+ + NH_3) and CO_2 release.

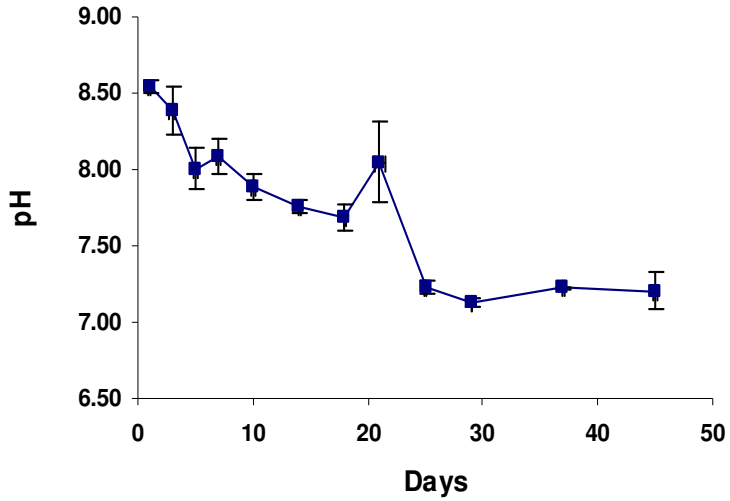


Figure 3.2 Changes in pH value of Sanjeevak maturation process (mean ± SE).

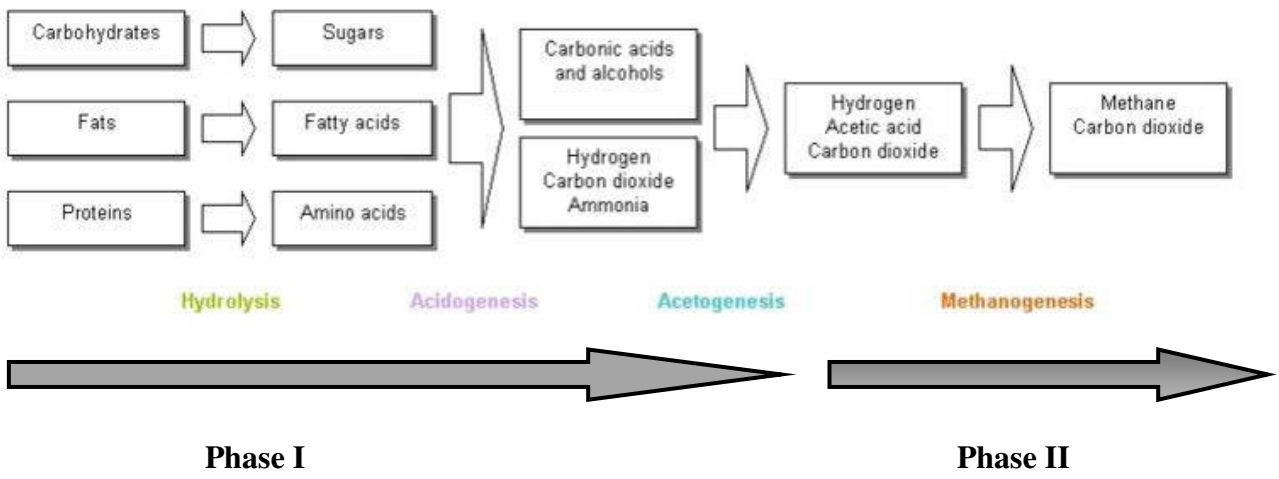


Figure 3.3 Biogas production process

In the findings of our experiments, there were small variations in N and K concentrations over time (Figure 3.4A, C) but not of great significance. However, steady increase of extractable P over the length of the maturation period is probably related to the gradual decomposition of organic matter in Sanjeevak. Based on past findings, the major proportion of the nutrients excreted is found in the urine (Jönsson et *al.,* 2004). The nutrients in urine are in ionic form and their availability has been found to compare very well with chemical fertilizers. Hence, the plant availability of urine N is the same as that of chemical urea or ammonium fertilizer; close to 90-100% of urine N is found as urea and ammonium and has been verified in fertilizing experiments (Kirchman and Pettersson, 1995; Richert Stintzing et *al.,* 2001; Morgan 2003; Guzha et *al.,* 2005 and Mnkeni et *al.,* 2008). P in the urine is entirely (95 – 100%) inorganic and is released in the form of phosphate ions (Lentner et *al.,* 1981). These ions are directly plant-available; as a result their availability has been found to be similar to that of chemical phosphate (Kirchman and Pettersson, 1995). K is excreted as ions, which are directly plant-available.

Animal excreta contain both water-soluble nutrients and nutrients that are combined in large particles not soluble in water (Jönsson et *al.,* 2004). However, the plant availability of nutrients in faecal materials is lower and slower compared to that of the urine nutrients. This is due to the fact that the main proportion of the P and a large proportion of the N originate from undigested materials and these substances need to be degraded in the soil mainly through microbial activity to become available to plants. As a result, this may explain the low degree of variation of total N and extractable P and K in Sanjeevak.

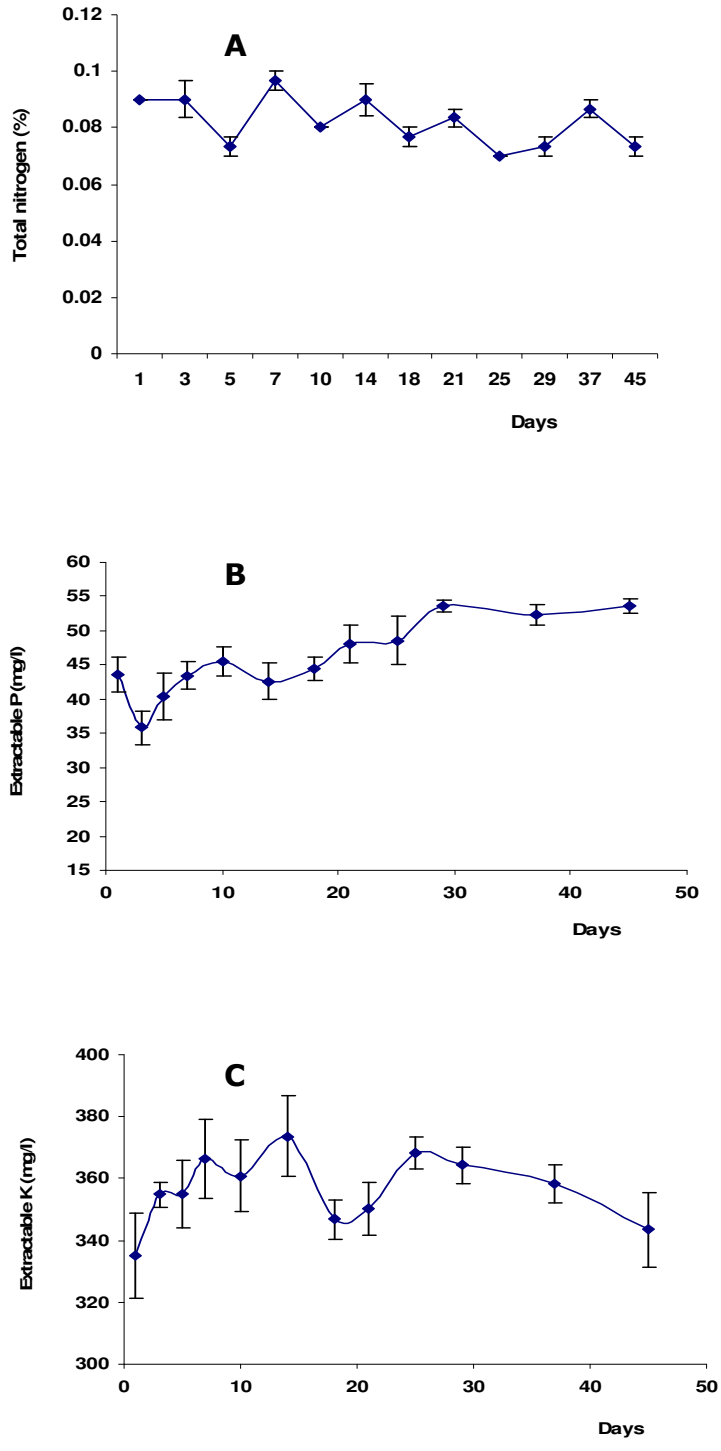


Figure 3.4 Total N (A), extractable P (B) and extractable K (C) contents in Sanjeevak (mean ± SE)

The analysis of heavy metal compositions and concentrations was conducted to evaluate the potential environmental and human health risks associated with animal organic wastes applications to agricultural land. As previously reported by Jönsson et *al.* (2004), the levels of heavy metals are generally very low in excreta, depending on the amounts present in consumed products. Equally, heavy metal contents in urine tend to be low depending on consumed food, probably because they are filtered through the kidneys when they enter the human body. (Jönsson et *al.,* 1997; Vinnerås, 2002; Palmquist et *al.,* 2004).

Table 3.1 various formulations of organic growth promoters (Kate and Pathe 2009)

Ingredients	*Sanjeevak (T0)*	*Sanjeevak* 1 (T1)	*Sanjeevak* 2 (T2)		*Sanjeevak* 3 (T3)	*Sanjeevak* 4 (T4)	*Sanjeevak* 5(T5)	*Panchagavya* (T6)	*Amritpani* (T7)
			(a) (T2a)	(b) (T2b)					
Cow dung (kg)	10	10	10	60	10	15	10	20	10
Cow urine (L)	10	10	5	5	5	15	10	20	-
Jaggery (mg)	100	250	250	250	1	500	1	-	-
Flour of pulses (kg)		-	-	-	1	-	2	-	-
Ant hill soil (kg)		-	-	-	-	-	1	-	-
Cow ghee (mg)		-	-	-	-	-	-	250	250
Cow milk (ml)		-	-	-	-	-	-	250	-
Cow curd (mg)		-	-	-	-	-	-	250	-
Honey (mg)		-	-	-	-	-	-	-	500
Water (L)	180	10	10	10	10	15	10	10	200

Table 3.2 Total concentrations (mg kg^{-1} dry weight) of heavy metals in Sanjeevak, compared to different classes of pollutant in wastewater sludges (mean ± SE)

	Pollutant class			
mg/kg	**a**	**b**	**c**	**Sanjeevak**
Arsenic (As)	<40	40-75	>75	n.d
Cadmium (Cd)	<40	40-85	>85	n.d
Chromium (Cr)	<1200	1200-3000	>3000	0.03 ± 0.01
Copper (Cu)	<1500	1500-4300	>4300	0.86 ± 0.18
Lead (Pb)	<300	300-840	>840	0.03 ± 0.01
Mercury (Hg)	<15	15-55	>55	1.76 ± 0.09
Nickel (Ni)	<420	420	>420	0.14 ± 0.05
Zinc (Zn)	<2800	2800-7500	>7500	4.74 ± 0.92

n.d.: Not detected

Additionally, microbiological analyses were carried out to identify and quantify faecal coliform load in Sanjeevak for health and safety considerations and to compare their levels with the guidelines for the permissible utilization of sludge in agriculture applications based on the South African legislation.

Table 3.3 Total feacal coliform count in Sanjeevak (cfu g^{-1} weight dry matter), compared to microbiological classes in wastewater sludge

	Microbiological Class			
	A	**B**	**C**	**Sanjeevak**
Feacal coliform (CFU/g dry)	1000-10000	1×10^{6} - 1×10^{7}	$>1\times10^{7}$	1.2×10^{2}
Helminth ova (viable ova/g dry)	0.25-1	1-4	>4	N/A

N/A: Not Applicable

Table 3.4 Summary: Permissible utilization of sludge in agricultural applications (Snyman and Herselman 2006)

South Arica Sludge Classification		Is agricultural use an option?	Any additional restrictions and requirements	Notes
Microbiological class	**A**	Yes (i)	No	Could potentially be used as sealable product
	B	Qualified yes (ii)	Yes	General restrictions/ requirements apply.
	C	Maybe (iii)	Yes	General restrictions/ requirements apply.
Pollutant class	**a**	Yes (i)	No	Could potentially be used as sealable product
	b	Qualified yes (ii)	Yes	General restrictions/ requirements apply.
	c	No (v)	Not applicable	May not be used in agricultural practices

The analysis of our results showed that the level of heavy metals (Table 3.2) and feacal coliforms measured in Sanjeevak (Table 3.3) did fall under pollutant class (a) and microbiological class (A) respectively. Therefore, Sanjeevak does not pose any potential health and environmental risks if used for cropland amendments (Table 3.4) (Snyman and Herselman 2006).

Table 3.5 Micro organisms' analysis of OGPs (Kate and Pathe 2009)

Treatment	Total bacterial count/ml	Total fungal count/ml	Actinomycetes count/ml	Azotobacter count/ml	*Pseudomonas fluorescens* count / ml	PSM count / ml
T1	3.0×10^7	1.03×10^6	2.00×10^6	1.21×10^6	9.8×10^2	1.13×10^4
T2(a)	1.26×10^8	1.46×10^6	2.85×10^6	1.32×10^6	1.11×10^2	1.71×10^4
T2(b)	1.21×10^8	1.11×10^6	2.18×10^6	1.16×10^6	2.11×10^2	1.11×10^4
T3	2.01×10^7	1.51×10^6	1.70×10^6	1.11×10^6	8.6×10^2	2.15×10^4
T4	1.22×10^8	2.02×10^6	2.54×10^6	1.28×10^6	7.7×10^2	1.12×10^4
T5	1.45×10^8	2.12×10^6	2.44×10^6	2.15×10^6	1.00×10^2	2.01×10^5
T6	1.51×10^8	2.50×10^7	2.13×10^6	1.31×10^6	9.6×10^2	2.08×10^5
T7	1.03×10^6	1.12×10^5	< 30 colonies	< 30 colonies	< 30 colonies	< 30 colonies

The microbiological analysis of the different formulations of organic growth promoters (OGPs) highlighted the presence of fungi, *Actinomycetes, Azotobacter, Pseudomonas fluorescens* and phosphate Solubilizing microorganisms (PSM) (Table 3.5). These micro-organisms have previously been studied for their impact in improving plant growth and development (Lucy et al. 2004). *Azotobacter spp* and *Pseudomonas spp* were isolated from different rhizospheres of plants and found to be physiologically capable of producing IAA at concentration ranging from 0.1 to 80 ppm (Lwin et al. 2008). Alam et al (2001) reported increased total dry matter, grain yield and nitrogen accumulation by 6 to 24% over two years of field study. Swain et al. (2007) reported that when yam minisetts were dipped in *B. subtilis* suspension an increase sprouts number, roots and shoots length, root and shoot fresh weights and root: shoot ratio in comparison to the control (minesetts not treated with *B. subtilis*) could be observed. Similarly, fresh cow dung slurry treatment on yam minisetts confirmed results obtained with *B. subtilis* inoculation. Previously, *Bacillus subtilis* strains were found to produce IAA in nutrient broth. Further, inoculation with *Bacillus sp.* in field study environments increased yield of 15.3 to 33% and 0 to 114% respectively for sorghum and wheat (Broadbent et al. 1977; Kloepper et al. 1977) (for more details, see Table 3.6).

Table 3.6 Summary – examples of free-living bacteria inoculum tested on various crop

Bacteria	Crop plant	Conditions	Plant responses	Reference
Bacillus sp.	Sorghum	Field	Increased yield of 15.3 to 33%	Broadbent et al. 1977
Bacillus sp.	Wheat	Field	Changes in yield of 0 to 114%	Kloepper et al. 1977
Bacillus subtilis B2	Onion	Growth chamber	Significant increases in shoot dry weight (12-94%); only two out of 24 test sites produced negative results	Reddy and Rahe 1989
Pseudomonas fluorescens 63-49, 15, 13, R1GC4	Cucumber	Field	Strain 63-49 significantly increases fruit number by 12% and fruit weight by 18% Strains 13, 15, R1GC4 slightly increase yields	McCullaugh et al. 1996
Pseudomonas spp.	Lettuce, Cucumber, Tomato and Canola	Hydroponic growth chamber	Increases of root and shoot weights for all plants tested Most significant positive growth responses in lettuce, tomato and cucumber	Van Peer and Schippers 1998
Pseudomonas spp	Potato	Field	Changes in yield of -14 to 33%	Kloepper et al. 1988

2.4 Conclusion

With regard to the strictest current legislations that regulate the use of wastewater sludge for agricultural purposes, the assessment of Sanjeevak revealed that none of heavy metals and faecal coliform bacteria levels measured exceeded permissible limits for application to agricultural land. Sanjeevak could potentially be used as a sealable product. The analysis of Sanjeevak as a viable source of nutrient showed that macro and micro nutrients were present although in small concentrations. In addition, fairly stable composition and concentration of nutrients from the different sanjeevak formulation was observed, irrespective of the duration of the maturation of Sanjeevak. In terms of biogas production, the current formulation of Sanjeevak proved to be not suitable for methane production.

3.5 References

Allison J.M., Robinson I.M., Baetz A.L (1974) Tryptophan biosynthesis from indole-3-acetic acid by anaerobic bacteria from the rumen. Journal of Bacteriology 117 (1) 175-180

Alonso E.., Callejon, M., Jimenez, J.C and Ternero M (2000) Determination of heavy metals in sewage sludge by microwave acid digestion and inductively coupled plasma atomic emission spectrometry. Toxicol. Environ. Chem. 75, 207–214

Broadbent P., Baker K.F., Franks N and Holland J (1977) Effect of *Bacillus spp*. On increased growth of seedlings in steamed and in non-treated soil. Pytopathol. 67: 1027-1034

Chale-Matsau J.R.B (2005) Persistence of human pathogens in a crop grown in sewage sludge treated soil. University of Pretoria etd

Guzha E, Nhapi I and Rockstrom J (2005) An assessment of the effect of human faeces and urine on maize production and water productivity. Physics and Chemistry of the Earth 30: 840-845

Jönsson H., Richert Stintzing A., Vinneras B and Salomon E (2004) Guidelines on the use of urine and faeces in crop production. EcoSanRes Publications Series. Report 2004-2. Stockholm, Sweden. Available from www.ecosanres.org

Jönsson, H., Stenström T.A., Svensson J and Sundin A (1997) 'Source separated urine Nutrient and heavy metal content, water saving and faecal contamination'. *Water Science and Technology* 35(9):145-152.

Kate T and Khadse M. (2002) Extension of simple and low cost agricultural techniques for improving crop productivity of small & marginal farmers in Vidarbha region through grass root level NGOs. Unpublished report, Dharamitra Wardha

Kate T and Pathe S. (2009) Scientific validation of the nutrient management practices evolved by some innovative farmers. Unpublished report, Dharamitra Wardha

Kirchmann, H. and Pettersson S (1995) Human urine – chemical composition and fertilizer efficiency. *Fertilizer Research* 40:149-154.

Klopper J.W., Schoth M.N and Miller T.D (1980) Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathol 70: 1078-1082

Lentner C., Lentner C and Wink A (1981) *Units of Measurement, Body Fluids, Composition of the Body, Nutrition. Geigy Scientific tables*. Ciba-Geigy, Basel, Switzerland

McCulagh M., Utkhede R., Menzies J.G., Punja Z.K and Paulits T.C (1996) Evaluation of plant growth promoting rhizobacteria for biological control of of Phythium root rot of cucumbers grown in rockwool and effects on yield. Europ. J. Plant Pathol. 102: 747-755

Mnkeni P.N.S, Austin A and Kutu F.R (2005) Preliminary studies on the evaluation of human urine as a source of nutrients for vegetables in the Eastern Cape Province, South Africa. In: Ecological Sanitation: a Sustainable Integrated Solution. Proc. of the Third International Ecological Sanitation Conference, Durban, South Africa, 23-26 May 2005, pp. 418-426. http://conference2005.ecosan.org/papers/mnkeni_et_al.pdf

Mnkeni P.N.S, Kutu F.R and Muchaonyerwa P (2008) Evaluation of the human urine as a source of nutrients for selected vegetables and maize under tunnel house conditions in the Eastern Cape, South Africa. Waste Management & Research 26: 132-139

Morgan P. (2003) Experiments using urine and humus derived from ecological toilets as a source of nutrients for growing crops. Paper presented a Third World Water Forum, 16-23 March 2003. Available at http://aquamor.tripod.com/KYOTO.htlm

Palmquist H and Jönsson H (2004) Urine, faeces, greywater, greywater and biodegradable solid waste as potential fertilizers. In: *Ecosan – closing the loop*. Proceedings of the 2nd International Symposium on Ecological Sanitation, Incorporating the 1st IWA Specialist Group Conference on Sustainable Sanitation, 7th-11th April, Lübeck, Germany, pp. 587-594

Prajapati K.M and Gajurel D.R (2003) Nutrient uptake by different vegetable plants from source separated human urine. In EcoSan – closing the loop. Proc. of the second International symposium on ecological sanitation, incorporating the First IWA Specialist Group Conference on Sustainable Sanitation, 7-11 April 2003, Lubeck, Germany, pp. 631-634

Reddy M.S and Rahe J.E (1989) Growth effects associated with seed bacterization not correlated with population of Bacillus subtilis inoculant in onion seedling rhizospheres. Soil Biol. Biochem. 21: 373-378

Richert Stintzing, A., Rodhe L and Åkerhielm H (2001) *Human urine as fertilizer – plant nutrients, application technique and environmental effects* (In Swedish, English summary). JTI- Rapport Lantbruk & Industri 278

Sigge, G. & Britz, T. (2007). UASB treatment of a highly alkaline fruit-cannery lye-peeling wastewater. *Water S. A.*, 33, 275-278

Snyman HG and Herselman JE (2006) Guidelines for the utilization and disposal of wastewater sludge. Vol 2, Requirements for the agricultural use of wastewater sludge. *Water Research Commission* (WRC) Report No TT 262/06. Pretoria

Van Peer R. and Schippers B (1988) Plant growth responses to bacterization with selected *Pseudomonas spp*. Strains and rhizosphere microbial development in hydroponic cultures. Can. J. Microbiol. 35: 456-463

Vinnerås, B (2002) *Possibilities for sustainable nutrient recycling by faecal separation combined with urine diversion.* Agraria 353, Acta Universitatis Agriculturae Sueciae, Swedish University of Agricultural Sciences. Uppsala, Sweden

CHAPTER FOUR

Sanjeevak pre-treatment to improve carrot seeds germination and rooting of grapevine (*Vitis viniferas L.*) rootstock cuttings

4.1 Introduction

Pre-germination seed treatment is a well established technique that is being used to increase the rate and/or uniformity of emergence of seeds leading to fully mature plants (Taylor et al., 1998). Pre-sowing seed treatments are used to suppress germination inhibitors; soften seed coats and drive physiological processes as reported by Habib (2010). Many methods exist such as soaking the seeds into water (Jun and Ling 2004) or solutions with known matric potentials (Kaffka et al., 1997), chilling and stratification (Schutz Rave 1999; Jun and Ling 2004), temperature (Demel 1996; 1998; Demel and Mulualem, 1996), physical scarification (abrasion removal of seed coat layers) chemical scarification (with HCl, H_2SO_4 and HNO_3), growth regulators (IAA, NAA, IBA, GA etc.) and sound stimulation pre-treatment significantly affect seed germination (Duan et al., 2004; Khazaei, 2001; Bockarie and Duryea, 1993; Durrant and Mash 1991) and have been recommended for improved germination in dormant seeds (Habib, 2010).

Equally, improving the rooting of cuttings of many plant species that are difficult to root is critical in plant propagation (Köse 2007). Treatments are often applied on cuttings to increase the rooting percentage, including plant growth regulators, especially auxins,

carbohydrates, other chemical substances, and plant-growth promoting rhizobacteria (Alley 1961; Hartmann et al. 1990, Smart et al. 2002, Kose et al. 2003). Plant growth regulators have contributed to improving the rooting of succulent, soft wood and hardwood planting materials (Alley 1961). Through the use of various concentrations of indolebutyric acid (IBA) and naphthalene acetic acid (NAA), Rodriguez (1957) reported an improved rooting of rootstock grapevine cuttings which would normally root with difficulty. Further, Kracke and Cristoferi (1983) reported that IBA and NAA treatments increased the rooting percentage from 50% (control) to 62% for IBA and 66% for NAA of hardwood cuttings of grapevine rootstocks "140 Ruggeri" (hard to root). However, although these techniques and chemicals have been shown to be effective in improving seed germination and root formation; they remain out of the reach for many small and subsistence farmers due to financial and logistic constraints.

To the best of our knowledge, reearch studies that looked at the impact of pre-sowing treatments of plant seeds or cuttings (for example grapevine cuttings) aimed at improving their germination and rootings using natural resources (such as Sanjeevak) rather that synthetic chemicals are lacking. The undertstanding of such impacts; would be useful in evaluating the consequences on crop productivity; especially for rural farmers in developing countries. The first objective of this study was to investigate the influence of Sanjeevak as a source of bacteria producing IAA on seed germination using carrot as an example. The second objective of this study was carried out to test the effect of Sanjeevak concentrates vs. naphtaleneacetic acid (NAA) on the rooting of grapevine

(Ramsey) rootstock cuttings as an example, in search for alternative, low-cost and environmentally friendly procedures.

4.2 Materials and methods

4.2.1 The effect of Inorganic fertilizer solution vs. Sanjeevak on seed germination of carrots

Plant materials

This experiment was carried out at the Soil Science department of Stellenbosch University. Carrot seeds were purchased from *AgriMark* in Stellenbosch.

Seed germination

They were subjected to five treatments which consisted of soaking in distilled water, 18 ppm and 9 ppm Sanjeevak containing IAA and 0.55g N and 0.22g N of fertilizing solution respectively for 24 hours to hasten germination and arranged based on completely randomized design (CRD), replicated four times (Note: Sanjeevak was prepared as in chapter three). Percentage and rate of germination were investigated over time. 20 seeds per treatment were placed in 9 cm diameter petri dishes on Whitman filter paper moistened with distilled water and kept at room temperature. Germination counts were made daily for 10 days. Seeds were recorded as germinated when the tip of the radicle had grown free of the seed coat. Percentage of germination (PG) and T10 (time necessary in days to reach 10% of final germination percentage) were determined for all tests.

4.2.2 The effect of NAA vs. Sanjeevak concentrates on the rooting of cuttings from grapevine (Ramsey) rootstocks

Plant material

The experiment was conducted at the soil science department, university of Stellenbosch (South Africa). Actively growing young shoots of Ramsey rootstock cuttings were collected in June from a mature plant in the vineyard of Voor-GroenberG Nurseries (Wellington, South Africa), which had been pruned in February. The collected shoots were stripped off all leaves, excised into 50 cm in length. Cuttings of ~ 1 cm in diameter with at least one bud and similar length were prepared into bundle of 100 and stored at 4 °C until used for the experiment. Ramsey is a variety of grapevine which would root only with difficulty or not at all using conventional method (Alley 1961).

Rooting

For in vitro rooting, cuttings of 50 cm in length in bundles of 10 cuttings replicated three times for each treatment were used to measure rooting rate; total root biomass produced, and shoot emergence. The treatments consisted of dipping the base of the cuttings to a depth of 2-3 cm in for approximately 30 seconds in 250, 500, 1000 ppm 1-Naphthaleneacetic acid (NAA) respectively. Equally, cuttings were also dipped in three different concentrations of Sanjeevak containing variable concentrations of IAA calculated from the results presented in Chapter 2 .The control consisted of water only. All cuttings were inserted into Erlenmeyer flask in bundles of 10 containing tap water at room temperature. Water was replaced from the Erlenmeyer flask in 5 to 7 day intervals

Observations were recorded in 2 to 3 day intervsls for 44 days. The experiment was conducted in factorial design

Measurement and statistical analysis

For carrot seeds, percentage of germination (PG) and T10 were determined for all tests . All experiments were with 4 replicates and 20 seeds per petri-dish and the experiments were repated twice. For grapevine cuttings, rooting (%) and root biomass (mg) were determined 44 days after initiation. All experiments were with 3 replicates and 10 cuttings per replicate and the experiments were repated just once.

All data were checked for normality and homogeneity of variance with the Shapiro-Wilks W test and Levene's test respectively. When data were not normally distributed even after transformations, non parametric statistics were used. The results of all carrot and rootstock cuttings of grapevine parameters where data were taken; were analysed using the measures analysis of variance (ANOVA). Fischer's least significant difference (LSD) post-hoc test was used to determine the significance of any differences between speficic groups in parametric cases. All statistics were analysed with STATISTICA 10 software.

4.3 Results and discussion

Seed germination

Percentage of germination (PG) and T10 of germination are given in Tables 4.1 and 4.2. Comparison of mean showed that after 10 days of germination, all the treatments had statistically similar rates of germination (P>0.05), distilled water (61.25%), 4.4g N/L (63.75%), 2.2gN/L (72.5%), Sanjeevak containing 9.0 ppm of IAA (66.25%) and Sanjeevak containing 18 ppm of IAA (60%). However, carrot seeds pre-treated with Sanjeevak containing 18 ppm of IAA had the lower germination rate.whilst the highest level of germination was obtained for seeds pre-treated with 2.2gN/L of fertilizer solution. Although we were expecting to obtain difference between treatments in comparison the the control, the lack of statistical difference for carrot seeds germination between treatments can be attributed to the fact that the treatments applied were not sufficient to either break dormancy or leachout chemical inhibitors from seed coat to get earlier or more uniform germination. As a result, the influence of chemicals (Sanjeevak concentrates and fertilizer solutions) seeds treatments in comparison to distilled water was statiscally similar (Table 4.1). These findings are in accordance with that obtained by Pan and Basu (1985).

Table 4.1 Effect of seed soaking under different treatments on carrot seeds germination (PG and T_{10})

Treatments	Concentration	PG (%)	T_{10}
Distilled water	0	61.25	61.25
Sanjeevak	18 ppm (IAA)	60.00	60
	9.0 ppm (IAA)	66.25	66.25
Fertilizer solution	4.4g N/L	63.75	63.75
	2.2g N/L	72.50	72.5
LSD (P = 0.05)	-	0.50	0.61

T_{10} = Time necessary (in days) to reach 10% of final germination percentage

Table 4.2 Effect of time and treatments on carrot seeds germination (%)

Days	Distilled water	F.S (N) 4.4g/L	F.S (N) 2.2g/L	Sanjeevak 18 ppm (IAA)	Sanjeevak 9 ppm (IAA)
0	0	0	0	0	0
5	30	26.25	23.75	16.25	30
6	46.25	47.5	46.25	37.5	47.5
7	57.5	57.5	62.5	48.75	60
8	60	60	68.75	56.25	65
10	61.25	63.75	72.5	60	66.25

Kate and Pathe (2009) reported the effect of vermiwash on seed germination from a variety of plants. Vermiwash is a mixed culture, containing soil bacteria combined with earthworms (Zambare et al., 2008; NIIR Board, 2008). In this study, the effect of vermiwash on seed germination was compared to that of plant hormones at various concentrations (Table 4.3). Vermiwash effect on seed germination and root growth was at its optimum at concentrations between 5 to 10%, for Indole-3-acetic acid mostly at 300ppm, Indole-3-butyric acid (IBA) at concentrations ranging between 50 to 200ppm;

NAA between 10 and 100ppm and gibberlic acid (GA) between 50 and 200ppm; depending on the crop tested.

Table 4.3 Optimum concentration of vermiwash and plant hormones on seed germination and root growth of various crop plants (Kate and Pathe 2009)

	Tested concentrations	Sorghum	Soyabean	Pigeon Pea	Wheat	Bengal gram
IAA (ppm)	0, 10, 25, 50, 100, 200, 500, 750, 1000	300	300	300	200	300
IBA (ppm)	0, 10, 25, 50, 100, 200, 500, 750, 1000	200	100	100	100	50
NAA (ppm)	0, 10, 25, 50, 100, 200, 500, 750, 1000	50	10	10	100	100
GA (ppm)	0, 10, 25, 50, 100, 200, 500, 750, 1000	200	50	50	100	50
Vermiwash (%)	0, 1, 2.5, 5, 7.5, 10	5	7.5	10	5	7.5

In Table 4.4, the effect of vermiwash on root growth was compared to that of GA. Taking the control as the benchmark, root length increases of 125.12% and 139.42% are achieved with sorghum treated with 200 ppm GA and 5% vermiwash respectively. Equally, root length increases for soyabeans of about 150.4% and 77.23% are attained using 50 ppm GA and 7.5% vermiwash respectively.

Table 4.4 Effect of vermiwash versus GA on crop root growth (Kate and Pathe 2009)

	Control	GA			Vermiwash		
	RL	Conc. (ppm)	RL	*Increased growth	Conc. (%)	RL	*Increased growth
Sorghum	3.5	200	7.88	125.14	5	8.38	139.42
Soyabeans	1.23	50	3.08	150.40	7.5	2.18	77.23
Pigeon Pea	2.01	50	2.72	35.32	10	2.86	42.28
Wheat	3.26	100	3.08	4.60	5	3.52	7.97
Bengal gram	2.20	50	3.92	78.18	7.5	4.50	104.54

**Increased growth refers to the percentage of root growth increase using the control as a baseline*

R.L: Root Length

In Figure 4.1, root length for soyabeans treated with 7.5% vermiwash was found to compare quite well with GA treatment at 50 ppm. The same results were observed for Pigeon pea and Bengal gram. These findings may be attributed to vermiwash contain in proteases, α-amylase (Swain et al., 2007), phosphatases, but also microorganisms such as *Azotobacter sp., Agrobacterium sp, Rhizobium sp* and phosphate solubilizing bacteria and the effects of their properties in altering plant root growth and development as reported previously in other studies (Lucy et al., 2004; Zambare et al., 2008).

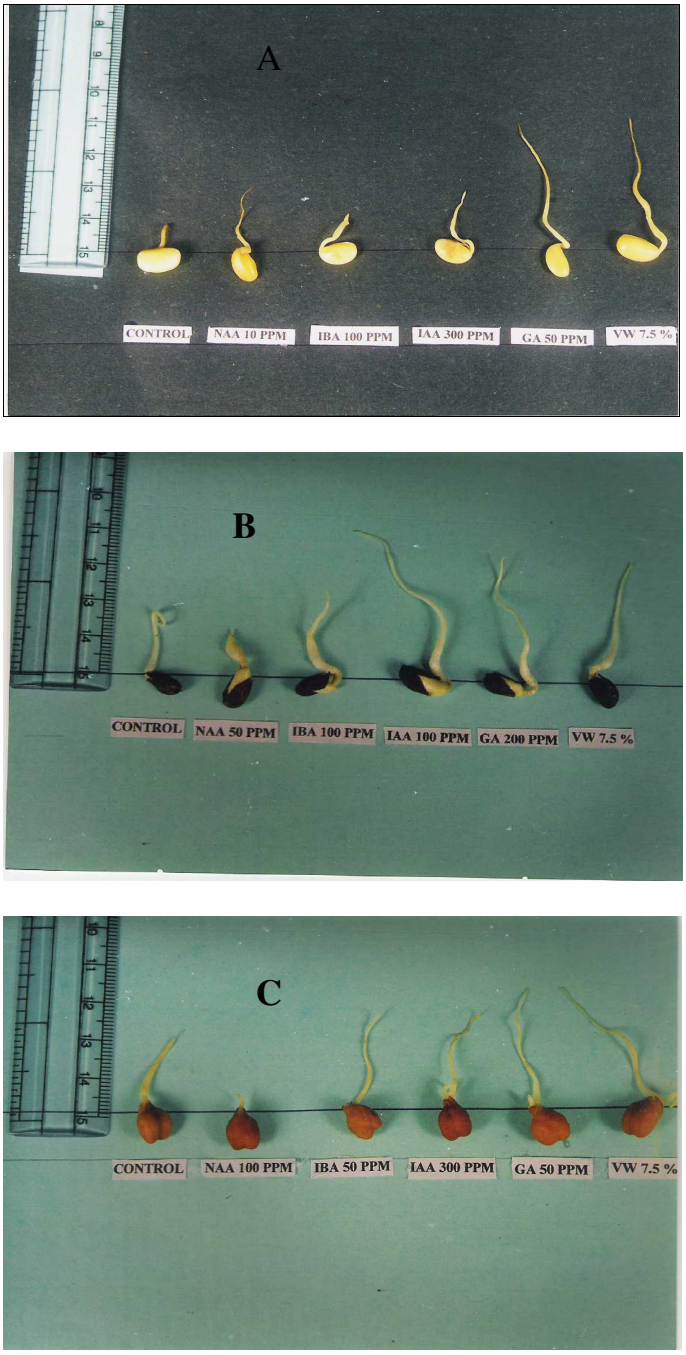


Figure 4.1 Seed germination experiments for Soyabeans (A), Pigeon pea (B) and Gram (**C**): Effect of vermiwash vs. plant hormones (Kate and Pathe 2009)

Rooting

The root developed in the control and Sanjeevak were somewhat thin and long; whilst root regenerated from cuttings treated with NAA were fleshy and to some extent adventitious. The number of cuttings that rooted in Sanjeevak concentrate (9 ppm of IAA equivalents) was the highest (Table 4.5 and Figure 4.3)). The treatments with the least rooting effects were the control (Figure 4.2) and Sanjeevak concentrate (4.5 ppm of IAA equivalents) with 30% and 26.7%; and weight of roots 0.6 mg/cutting and 0.7 mg/cutting respectively. However, the treatments with the most significant responses to rooting were Sanjeevak concentrate (9 ppm of IAA equivalents) and 250 ppm NAA with 86.7% and 73.3% respectively. Of the various treatments, cuttings tested with 1000 ppm NAA, on average produced significantly higher root biomass per cutting (7.8 mg) (Table 4.4).

The results as shown in Table 4.5 indicate that root cuttings treated with NAA yielded the most root biomass compared to the control and Sanjeevak treated cuttings; and in some instance also improve the rooting rate. However, Sanjeevak concentrates improved significantly the percentage of cuttings that form root and root biomass per cutting by up to 49% compared to the control. Overall, statistical analysis of the results showed that root cuttings treated with 250 ppm, 1000 ppm NAA and Sanjeevak concentrate (18 ppm of IAA equivalents) had comparable rooting effect as highlighted in Table 4.5

Figure 4.2 Rooting experiments of grapevine rootstock cuttings under laboratory conditions: (control)

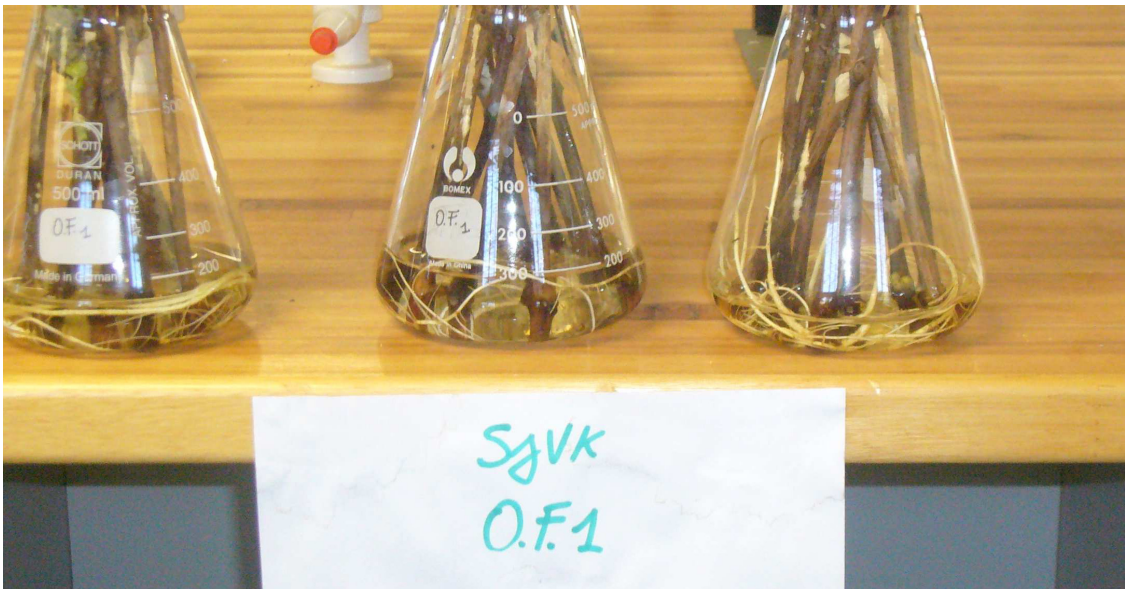


Figure 4.3 Rooting experiments of grapevine rootstock cuttings under laboratory conditions pre-treated with Sanjeevak containing 9 ppm of IAA equivalents

Table 4.5 Effects of NAA vs. Sanjeevak on rooting rate and biomass produced per cutting of grape rootstock cuttings.

Treatment	Concentration	Rooting (%)	Root biomass per cutting (mg)
Control	0	30a	0.6a
NAA	250 ppm	73.3c	3.8b
	500 ppm	43.3d	2.73a
	1000 ppm	66.7c	7.8c
Sanjeevak	18.0 ppm	60c	1.46ab
	9.0 ppm	86.7b	2.46a
	4.5 ppm	26.7a	0.7a

Means within each column followed by the same letter are not significantly at p<0.05 according to the least significant differences (LSD) test

In the present study, grapevine cuttings pre-treated with Sanjeevak markedly increased the number of cuttings that forms root (Table 4.5). It is clear that Sanjeevak as a source of natural substances has root promoting effects on grapevine cuttings. Moreover, a positive correlation between the ease of rooting and auxin levels has previously been reported with two distinct cultivars of grapevine rootstock (Kracke et al., 1981). In addition, applied auxins on a variety of plant species generally translated to positive rooting (Audus, 1959).

4.4 Conclusion

This study emphasized the fact that Sanjeevak pre-treatments effectively improved the rooting of grapevine cuttings of Ramsey (hard to root) compared to NAA treatment and the control, but not carrot seeds germination. However, further experimental works in the laboratory and in the field under natural conditions are required, using a larger sample size to practically ascertain whether Sanjeevak treatment can be an efficiency and low-cost technique to improve both seed germination and root formation of grapevine rootstock cuttings that have difficulties rooting for the wine industry.

4.5 References

Alley J.C (1961) Factors affecting the rooting of grape cuttings II. Growth regulators. 11th Annual meeting of the American Society of Enologists, California June 23-24

Austin R.B., Longden, P.C. and Hutchinson J. (1969) Some effect of "hardening" carrot seeds. Ann. Bot., 33: 883—895

Demel T (1996). The effect of different pre-sowing seed treatments, temperature and light on the germination of five *Senna* species from Ethiopia. New For. 11: 155-171

Demel T (1998). Germination of *Acacia origena, A. pillispina* and *Pterolobium stellatum* in response to pre-sowing seed treatments, temperature and light. J. Arid Environ. 38: 551-560

Demel T, Mulualem T (1996). The effect of different pre-sowing seed treatments, temperature and light on the germination of *Tamarinusindica L.* a multipurpose tree. J. Trop. For. 12: 73-79

Duan CB, Wang W, Liu J, Chen J, Lian J, Zhao H (2004). Effect of chemical and physical factors to improve the germination rate of *Echinacea angustifolia* seeds. Colloids and Surfaces B: 37: 101-105

Durrant MJ, Mash SJ (1991). Sugar beet seed step treatments to improve germination under cold, wet conditions. Plant Growth Regul. 10(1): 45-55.

Hegarty, T.W (1970) The possibility of increasing field establishment by seed hardening. Hortic. Res., 10: 59—64

Kaffka SR, Brittan K, Bab T, Canevari M, Ehler L, Peterson G (1997). Sugar beet stand stablishment. Sugar beet Research. Univ. Calif., Davis

Kate T and Khadse M. (2002) Extension of simple and low cost agricultural techniques for improving crop productivity of small & marginal farmers in Vidarbha region through grass root level NGOs. Unpublished report, Dharamitra Wardha

Kate T and Pathe S. (2009) Scientific validation of the nutrient management practices evolved by some innovative farmers. Unpublished report, Dharamitra Wardha

Khazaei H (2001). Improvement of sugarbeet (*Beta vulgaris*) seed germination with water treatment. J. Agric. Sci. Technol. 15 (1): 115-119

Kracke H and Cristoferi G (1983) Effect of IBA and NAA treatments on the endogenous hormomes in grapevine rootstock hardwood cuttings. Acta Horticulturae 137: 95-102

Lucy M, Reed E and Glick B.R (2004) Application of free living plant growth-promoting rhizobacteria. Antomie van Leewenhoek 86: 1-25

Nadjafi F, Banayan M, Tabrizi L, Rastgoo M (2006) Seed germination and dormancy breaking techniques for *Ferula gummosa* and *Teucrium polium.* J. Arid Environ. 64: 542-547.

NIIR Board. 2008. The complete technology book on vermiculture and vermicompost. National Institute of Industrial Research, New Delhi, India. p. 1.

Pan D and Basu R.N (1985) Mid-Storage and pre-sowing seed treatments for Lettuce and Carrot *Scientia Horticulturae,* 25: 11—19

Schutz W, Rave G (1999). The effect of cold stratification and light on seed germination of temperature sedges (*Carex*) from various habitats and implications for regenerative strategies. Plant Ecol. 144: 215-230

Swain M.R., Naskar S.K and Ray R.C (2007) Indole-3-acetic acid production and effect on sprouting of yam (Dioscorea rotundata L.) Minisetts by Bacillus subtilis Isolated from culturable cowdung microflora. Polish Journal of Microbiology 57 (2) 103-110

Taylor AG, Allen PS, Bennet MA, Brandford KJ, Burris JS, Misra MK (1998). Seed enhancements. Seed Sci. Res. 8: 245-256

Zambare V.P, Padul M.V., Yadav A.A and Shete T.B (2008) Vermiwash: Biochemical and microbiological approach as ecofriendly soil conditioner. ARPN Journal of of Agricultural and Biological Science 3 (4): 1-5

CHAPTER FIVE

Effects of Sanjeevak on the growth and yield of cucumbers, wheat, baby marrows and tomatoes under greenhouse conditions

5.1 Introduction

In recent times, there has been a growing awareness of possible adverse environmental and economic impacts regarding the excessive use of agrochemicals for food production. This has stimulated interest in the utilization of organic amendments such as composts, vermicomposts or organic wastes as sources of soil amendments and environmental conservation for small-holder farming and urban agricultural systems (Aracon et *al.,* 2003, 2007, 2008). As argued by Guzha et *al.* (2005), to ensure that sustainable food security is attained through increased food production, it is essential that cheap and readily available sources of nutrients and soil ameliorants be considered. It is for this reason that Sanjeevak as a source of soil amendments is being studied for its observed effects on improving plant growth and yield (Kate and Khadse, 2002; Pathe and Kate, 2009; Manjengwa 2011). Sanjeevak is a fermented product composed of cattle faeces and urine; it has shown significant promises in field studies in India to improve seedlings development and the yield of various crops (Kate and Khadse, 2002; Kate and Pathe, 2009).

There have been numerous available studies in the literature that investigated the use of animal manure as source plant nutrients and organic materials versus chemical fertilizers

for food production (Bayu et al., 2004 and reference therein). To date, however; there have been very few attempts to design experiments that investigate both the use of plant nutrients and plant regulating substances (such as plant hormones, enzymes and other substances) within animal waste materials and their combined benefial use on crop production. The research reported herein was conducted to evaluate the potential of utilizing a mix of cattle wastes (Sanjeevak), as sources of phytohormones as observed in previous work (Kate and Khadse, 2002; Swaminathan, 2005; Swain et al., 2007; Sreenivasa et al., 2009; Pathe and Kate 2009) for the increased plant growth and development independent of the nutrients it contains for the biomass production and yield parameters of various crop plants in sandy soils.

5.2 Materials and methods

5.2.1 Materials

The urine used as well as the dund in this study was obtained from dairy cattle at the University of Stellenbosch experimental farm (Welgevallen), Republic of South Africa. The collection of ± 5-L urine was done over a week period at every required opportunity during the period of the study; using a 10-L containers. The urine was collected from cows whenever they were urinating by manually and carefully placing the container underneath their genitals during milking sessions once in the morning and the afternoon daily. Cow dung was freshly collected from the kraal (the enclosure in which they are kept during the night). Cattle dung and urine were mixed with water in the following proportions (1:1:18), then fermented with Jaggery (molasses). The fermentation of

Sanjeevak was carried out in 25 l plastic bucket covered with lids under an aerobic system and kept in the greenhouse throughout the duration of the pot experiment (Read chapter 3 for additional details information about Sanjeevak preparation)

For the first set of pot experiments, we used sandy soil materials from building construction obtained from a building warehouse shop in Stellenbosch primarily because of its reduced content in organic matter and nutrients. The soil contained 0.04% N, 60.63 mg/kg P and available 139.70 mg/kg K respectively. It also showed a pH (in water) of 8.37 and electrical conductivity of 0.089 mS/cm. For the second set of pot experiments conducted in tunnel house, we used filtered silica sand (Fe, 0_2, 80% $Si0_2$) purchased from *AgriMark* Stellenbosch to minimize the adverse affects the soil pH on crop growth and development experience in the first pot trials. The soil had a pH (in water) of 6.4 and electrical conductivity of 0.015 mS/cm.

Sanjeevak nutrient compositions and concentrations

Table 5.1 Selected initial Sanjeevak characteristics applied

pH (H2O)	7.00
Dry mass (%)	21.27
EC (mS m-1)	526.00
TOC (%)	0.71
TN (%)	0.11
$NH4^+$ (mg l^{-1})	351.75
$NO3^-$ (mg l^{-1})	5.36
P (mg l^{-1})	68.79
Mg (mg l^{-1})	69.18
K (mg l^{-1})	632.77
S (mg l^{-1})	33.30
Ca (mg l^{-1})	142.00
Mn (mg l^{-1})	0.72
B (mg l^{-1})	0.47
Na (mg l^{-1})	206.67
As (mg l^{-1})	0.10
Cd (mg l^{-1})	0.00
Cu (mg l^{-1})	0.07
Cr (mg l^{-1})	0.01
Hg (mg l^{-1})	0.44
Pb (mg l^{-1})	0.01
Ni (mg l^{-1})	0.03
Zn (mg l^{-1})	0.32

The bulk Sanjeevak contained 0.1% N, 0.007% P and 0.063% K and had a pH of 7.00 (pH value depends on the maturation stage) and electrical conductivity (EC) of 0.053 mS/cm.

5.2.2 The fertilizer value of Sanjeevak on cucumber and wheat plants

For pot experiments conducted in the greenhouse; using sandy soil materials; Sanjeevak had 0.44% N, 0.028% P and 0.25% K; which translated to 4.4g N, 0.28g P and 2.52g K per 1000 ml. Mineral fertilizer of the same NPK concentration as to that of Sanjeevak was formulated as a source of inorganic fertilizer and translated into 12.56g NH_4NO_3,

1.2g KH_2PO_4, and 4.56g KCl per pot experiment. The treatments were 0, 0.55, 1.1 and 2.75g N per pot from both sources and were arranged in a randomized complete block design (RCB) and replicated six times and in greenhouse under controlled conditions (temp 25-28°C) to ensure statistical validity. The major factors investigated were nutrients and plant growth regulating materials.

Experiments with cucumber (*Cucumis sativus*) were started on January 12, 2010 and for wheat (*Triticum sp)* on December 24, 2009. The fertilization of the pot experiments consisted of 5 rounds of fertilization at a rate of 125 mL of both Sanjeevak and inorganic chemical solutions containing equal concentration of NPK, and then uniformly poured onto potted soil.

Five cucumber and wheat seeds were sown into each pot respectively. Only cucumber pots were thinned to one seedling per pot 11 days after germination. The treatments were initially irrigated daily with 50 mL/pot tap water using an automated irrigation system until plants were fully grown at which time irrigation rate was increased due to increased evapo-transpiration water losses. Also, regular weeding of each pot was carried out until harvest, which was 14 and 12 weeks after planting (WAP) for wheat and cucumber respectively. The trials were terminated on the 07 April 2010 at which time fresh biomass and height for both crops were recorded. All harvested plant samples were oven dried at 65°C to a constant weight to determine dry matter yield and for use in laboratory analyses.

5.2.3 Analytical methods

Fifty-two days after sowing, all plant materials from each treatment (Control/Sanjeevak/Inorganic fertilizer) were harvested for plant height, shoot and root fresh and dry weight measurements. Dry shoot were then ground in a ball mill and analyzed for total P and K concentrations as described by Okalebo et *al.* (2002). Soil from each pot was removed at the end of the trial, air-dried, ground (< 2 mm). The content of total N in each plant and soil sample was determined using a Carlo Erba NA 1500 C/N analyzer. Soil pH and electrical conductivity (EC) were determined in soil water ratio of 1:2.5.

The pH was recorded directly from suspension of Sanjeevak. Sanjeevak samples were oven dried by heating at 65°C for 3 days to a constant weight to determine dry mass content. Extractable mineral N concentrations (NH_4-H and NO_3-H) were determined colorimetrically in 0.5 M K_2SO_4 extracts in the ratio of 1:10 (v:v) Sanjeevak to extractant, using a modified indophenol blue technique (Sims et *al.,* 1995). Soluble P was assessed using NH_4-HCl reagent (Olsen and Sommers, 1982). Colour in the sample filtrates was developed with stannous chloride and ammonium paramolybdate and hypochlorite reagents. Absorbance was measured using a Bio-Tek EL211sx automated microplate reader. A more complete nutrient analysis was made for other Sanjeevak samples after nitric acid/Perchloric acid digestion (Singer and Hanson, 1969). The extracts were analyzed for P, K, Ca, Mg, B, Mn, As, Cd, Cr, Cu, Pb, Hg, Ni and Zn using inductively coupled plasma atomic emission spectroscopy (ICP-AES). Total organic C and N were measured in Sanjeevak by dry combustion using a carlo-Erba apparatus.

5.2.4 The effect of Sanjeevak as a source of plant regulating substances on baby marrow and tomato production

For pot experiments carried out in a tunnel house;.the treatments were 0 and 500 ml per 5-kg soil pot for baby marrow (*Cucurbita pepo*) and 0, 1.4 L and 2.4 L per 5-kg soil pot for tomato (*Lycospersicon esculentum*). Thus, the experiment had a total of 2 and 3 treatments per test respectively for baby marrow and tomato crops, which were replicated twelve times and arranged in a randomized complete block (RCB) design.in the tunnel house. Sanjeevak was applied by uniformly pouring it onto the potted soil at different time interval in dose of 500 ml or exceeding it. All treatments were irrigated daily with 600 ml a of standardized fertilizer solution (E.C = 1.1) until harvest, which was 11 weeks after planting (WAP) for baby marrow and 17 WAP for tomato. The trials were terminated on 18 January 2011 for baby marrow and 02 March 2011 for tomato at which time fresh biomass for both crops and fruit yields were harvested and recorded.

Statistical analyis

All data were checked for normality and homogeneity of variance with the Shapiro-Wilks W test and Levene's test respectively. When data were not normally distributed even after transformations, non parametric statistics were used. The results of all carrot and rootstock cuttings of grapevine parameters where data were taken; were analysed using the measures analysis of variance (ANOVA). The Bonnferoni test was used to compare means of growth data (fresh and dried root, shoot and total biomass), EC, and pH for each treatment. All statistics were analysed with STATISTICA 10 software.

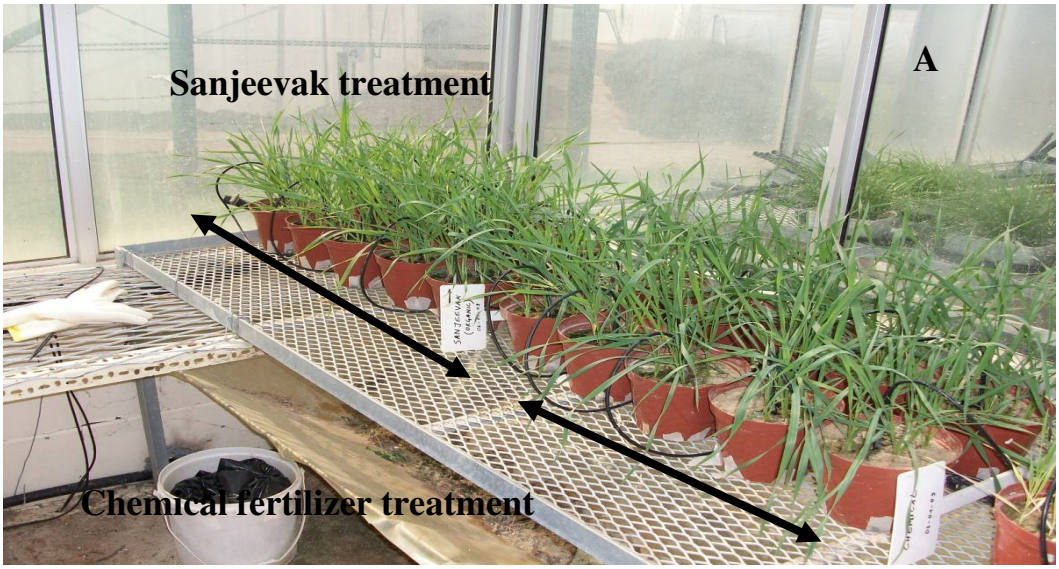


Figure 5.1 Greenhouse experiments of wheat (A) cultivation under Sanjeevak and chemical fertilizer treatments four (4) weeks after sowing.

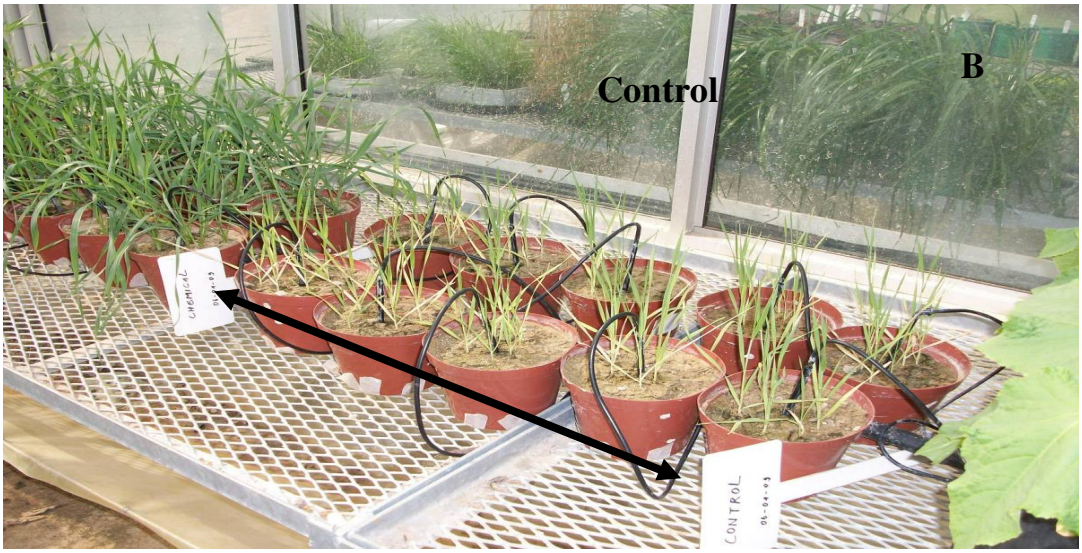


Figure 5.2 Greenhouse experiments of wheat (B) cultivation under control conditions, four (4) weeks after sowing.

Figure 5.3 Greenhouse experiments of cucumber (**C**) cultivation under Sanjeevak, treatments four (4) weeks after sowing.

Figure 5.3 Greenhouse experiments of cucumber (D) cultivation under chemical fertilizer treatments four (4) weeks after sowing.

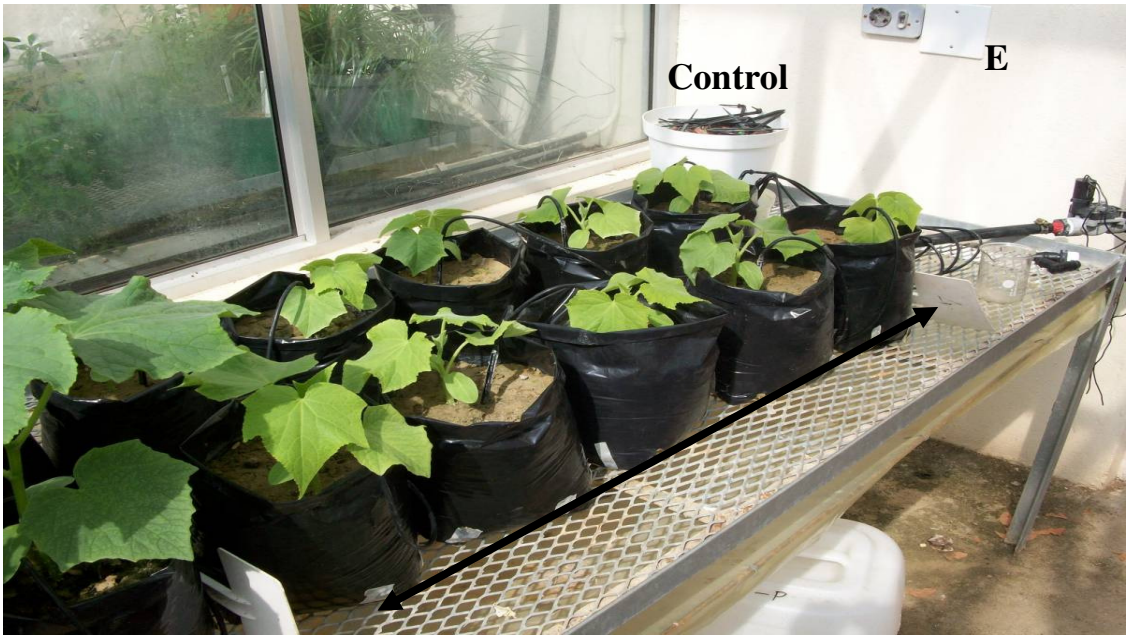


Figure 5.4 Greenhouse experiments of cucumber (**E**) under control conditions, four (4) weeks after sowing.

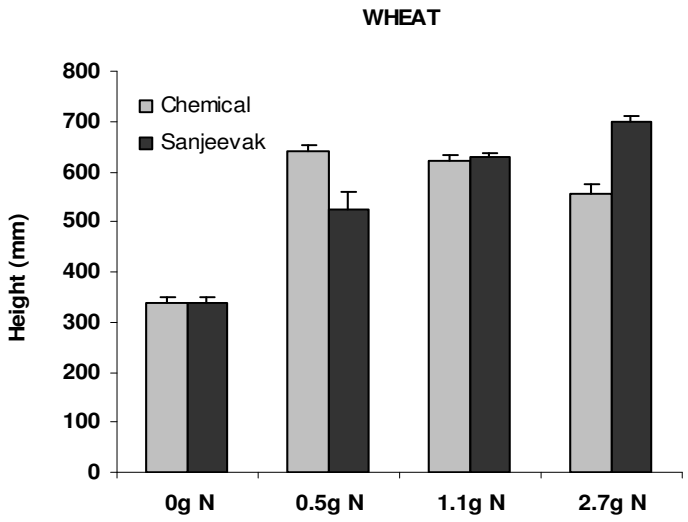
5.3 Results and Discussion

5.3.1 The effects of Sanjeevak and inorganic fertilizer application on cucumber growth and biomass yields

Application of similar concentrations of Total NPK as follows: 1.1g N, 0.07g P and 0.63g K per pot from both Sanjeevak and inorganic fertilizer treatments, resulted in cucumber total biomass production increased relative to the control. Cucumber biomass yields obtained 12 WAP were generally higher for both sources; with consistently greater yields from inorganic fertilizer treatments. Equally, the heights of cucumber plants grown in inorganic fertilizers were greater and significantly different from those of plants grown in Sanjeevak and the control (Figure 5.5). Cucumber root and shoot weights were greatest in inorganic fertilizer treatments (Table 5.2). These results seem to suggest that higher cucumber biomass and growth rate (height) in chemically fertilized treatments compared to Sanjeevak are due to higher nutrients uptake. The increased growth and biomass following Sanjeevak and chemical fertilizer (NPK) is related to higher NPK uptake to the growing cucumber plants as illustrated by higher shoot and root biomass relative to the control. These findings were in agreement with that of Etesami et al. (2008) who reported that one of the most critical channel used by bacteria to affect the growth and development of plants is by producing IAA. This plant hormone (IAA) alters the root system development; resulting in increased nutritional uptake and eventually increased plant growth as reported in this study.

5.3.2 The effects of Sanjeevak and inorganic fertilizer application on wheat growth, biomass and grain yields

The growth parameter (heights) of wheat plants fertilized with Sanjeevak and inorganic fertilizer were not significantly different but increased relative to the control (Figure 5.5). Shoot, root, total fresh and dried biomass increased with increased Sanjeevak and inorganic fertilizer rates; specially applied at rates of 0.55g and 1.1g N per pot, with consistently greater yields from inorganic fertilizers. However, total biomass (fresh and dried) and wheat grain yields obtained 14 WAP were generally highest at 2.75g N pot^{-1} for both sources; with significantly greater yields from Sanjeevak (Tables 5.3 and 5.4). Wheat biomass and yield production increased with Sanjeevak and inorganic fertilizer application relative to the control and peaked at 1.1g and 2.75g N pot for inorganic fertilizer and Sanjeevak respectively (Table 5.3).



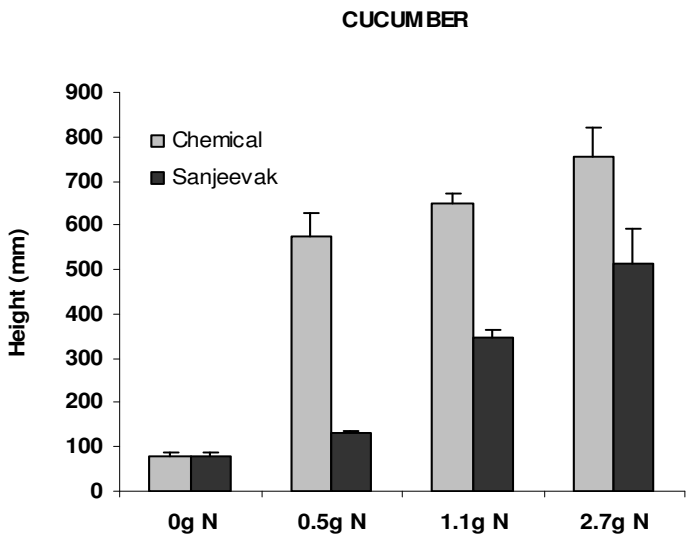


Figure 5.5 Effect of Sanjeevak vs. inorganic fertilizer on wheat and cucumber heights (mean ± SE).

Table 5.2 Mean values of selected growth parameters of cucumber as affected by similar Sanjeevak and chemical fertilizer NPK application rate

		Fresh biomass (g pot^{-1})		Dried biomass (g pot^{-1})		Total biomass (g pot^{-1})	
N sources	Rates (g N per 12 kg soil pot)	Shoot	Root	Shoot	Root	Fresh	Dried
Control	0	2.5a	0.95a	0.32a	0.13a	3.45a	0.45a
Chemical	0.55	51.82b	9.35b	7.93bc	0.83bc	61.17de	8.76cd
	1.1	93.93c	18.15b	10.03be	1.22b	112.08g	11.25c
	2.75	134.57c	32.4c	19.6e	2.12e	166.97dg	21.72g
Sanjeevak	0.55	8.03e	1.52a	1.57d	0.28ad	9.55f	1.85be
	1.1	26.17d	6.42ab	4.75c	0.48cd	32.59de	5.23d
Sanjeevak	2.75	48.98db	11.28ab	8.62bcd	0.88abc	60.26ef	9.5cdef

Means within each column followed by the same letter are not significantly at p<0.05 according to the Bonferroni test

Table 5.3 Mean values of selected growth parameters of wheat as affected by similar Sanjeevak and chemical fertilizer NPK application rate

		Fresh biomass (g pot^{-1})		Dried biomass (g pot^{-1})		Total biomass (g pot^{-1})	
N sources	Rates (g N per 12 kg soil pot)	Shoot	Root	Shoot	Root	Fresh	Dried
Control	0	0.70a	0.33ab	0.30a	0.20a	1.03a	0.50a
Chemical	0.55	6.18c	20.02abc	3.00b	4.40c	26.20c	7.40de
	1.1	19.58e	30.63abc	7.30c	9.30ac	50.21c	16.60bde
	2.75	24.62e	10.8c	9.2cd	2.30bc	35.42c	11.50ce
Sanjeevak	0.55	5.2cd	8.48abc	2.2ab	2.80ac	13.68bc	5.00bcd
	1.1	11.3cd	10.48bc	4.8bd	2.60c	21.78c	7.40d
Sanjeevak	2.75	27.87bde	33.20abc	10.1abc	10.80ac	61.07abc	20.90bcd

Means within each column followed by the same letter are not significantly at p<0.05 according to the Bonferroni test

At 2.75g N pot^{-1}, wheat grain yields under Sanjeevak treatment was almost double that of inorganic fertilizers; suggesting that the higher yields obtained with Sanjeevak may be attributed to better crop nutrition due to increased root length and weight (Tables 5.3 and 5.4). In addition to N, P and K; Sanjeevak contains some amounts of Na and Ca and lower concentration of S and Mg in plant available forms (Table 5.1). Therefore, these results may reflect the interactive and synergic impact of nutrients and IAA within Sanjeevak as previously reported in Chapter 4.

Table 5.4 Yield parameters of wheat as affected by varying Sanjeevak and inorganic fertilizer N application rates.

N sources	Rates (g N per 6-kg soil pot)	Fresh yield weight (g)	Dry yield weight (g)	Fresh yield weight (g)	Dry yield weight (g)
Control	0	7.10	6.60	1.20	1.10
Chemical	0.55	36.80	30.00	6.10	5.00
	1.1	72.50	52.00	12.10	8.70
	2.75	64.10	39.80	10.70	6.60
Sanjeevak	0.55	26.20	21.20	4.40	3.50
	1.1	56.30	37.90	9.40	6.30
	2.75	117.30	74.60	19.4	12.40

5.3.3 Effect of Sanjeevak vs. inorganic fertilizer applications on soil pH and electrical conductivity (EC) in wheat and cucumber pots

Cucumber (salt sensitive) and wheat (salt tolerant) were affected differently by Sanjeevak and inorganic fertilizer applications. Soil residual pH both for cucumber and wheat crop plants did not vary significantly with either Sanjeevak or inorganic fertilizer rates. However, EC increased in soil planted with cucumber fertilized with inorganic fertilizer relative to the control (Table 5.5). In both treatments, although the same amount of salts was added to the soil, the EC was much higher in soil planted with cucumber. This may underline the fact that wheat; as a salt tolerant crop plant, takes up more salts such as chloride and bicarbonate and cations such as K from inorganic fertilizing source and the soil, resulting in the lower EC in the residual soil. However the magnitude of the effect of increased soil residual EC in pots planted with cucumber was of such a level that it possibly may not have affected the growth of the plants (Table 5.2). This was in agreement with Mnkeni et al (2005) who reported a much lower EC for beetroot (tolerant

to salinity) compared to carrot (sensitive to salinity) under the same amount of salts applied to the soil.

Table 5.5 Effect of Sanjeevak and inorganic fertilizer applications on soil residual pH and electrical conductivity (mS cm^{-1})

N sources	Rates (g N per 6-kg soil pot)	Wheat pH	Wheat EC	Cucumber pH	Cucumber EC
Control	0	8.40	0.09	8.70	0.12a
Chemical	0.55	8.80	0.11	8.60	0.14ab
	1.1	8.60	0.13	8.40	0.37cd
	2.75	8.70	0.15	8.30	0.55bd
Sanjeevak	0.55	8.50	0.10	8.50	0.13a
	1.1	8.60	0.12	8.60	0.11ac
	2.75	8.40	0.11	8.60	0.15ac

Means within each column followed by the same letter are not significantly at p<0.05 according to the Bonferroni test

5.3.4 The effects of Sanjeevak as an auxin-like substance on baby marrow and tomato production

The data presented (Table 5.6) display considerably accelerated rates of tomato growth, particularly in terms of root biomass compared to the control in response to different application rates of Sanjeevak that we tested in the tunnel house experiments independent of nutrient supply. The statistical analysis of variance of the treatment effect was analysed using the Bonferroni test and it showed that tomato fruit yield (number of fruits per plant) had no significant difference between treatments. However, in terms of fruit yield (weight), the study showed that tomato fruit yield from the control was significantly larger than Sanjeevak treatments at $P \leq 0.05$. This may be attributed to the difference

between the fresh weight of marketable and non-marketable size fruits per plant (data not shown).

Measurement of baby marrow yield parameters (shoot, root and fruit yields) obtained 11 WAP from both the control and Sanjeevak treated plots revealed no significant difference between treatments (Table 5.7). The lack of increased root weight in Sanjeevak treated plots may be related to its low application rate (0.5 L) compared to tomato (up to 2.4L) per pot.

Table 5.6 Mean values of selected growth parameters of tomato as affected by different Sanjeevak treatments vs. control

Treatment	Concentration (g N per 6-kg soil pot)	Dry shoot weight (g)	Fresh root weight (g)	Fresh fruits per plant	Fresh fruits per plant (g)
Control	0	67.64	53.50a	10.54a	439.00c
Sanjeevak	1	98.64	111.30b	9.18a	279.50d
	2	73.04	119.90b	8.59a	291.80d

Means within each column followed by the same letter are not significantly at p<0.05 according to the Bonferroni test

Table 5.7 Mean values of selected growth parameters of baby marrow as affected by Sanjeevak treatment vs. control

Treatment	Fresh biomass		Total biomass per plant	Yield (g pot^{-1}) per plant (g)
	Shoot (g pot^{-1})	Root (g pot^{-1})		
Control	274	18	292	273
Sanjeevak	253	21	274	256

5.4 Conclusion

The first part of the study revealed that the use of Sanjeevak may be potentially as effective as inorganic fertilizer as a source of soil nutrients for crop production if used at proper agronomic rates. Furthermore, the findings obtained in the second part of the study seemed to indicate that Sanjeevak applied at higher rates can stimulate positively root growth and development. However, the effects of consistent and higher application doses of Sanjeevak on yields should be further and thoroughly investigated.

5.5 References

Albuzio A., Concheri G., Nardi S and Dell'Agnola, G (1994) Effect of humic fractions of different molecular size on the development of oat seedlings grown in varied nutritional conditions. In: Senesi N., Miano, T.M. (Eds.), Humic Substances in the Global Environment and Implications on Human Health. *Elsevier Science B.V.,* pp. 199–204

Arancon N.G., Edwards C.A., Babenko A., Cannon J., Galvis P and Metzger J.D (2008) Influences of vermicomposts, produced by earthworms and microorganisms from cattle manure, food waste and paper waste, on the germination, growth and flowering of petunias in greenhouse. *Applied soil ecology* 39:91-99

Bayu W., Rethman N.F.G and Hammes P.S. (2004) The role of animal manure in sustainable soil fertility management in Sub-Saharan Africa. *Journal of Sustainable Agriculture,* Vol. 25 (2): 113-136

Cacco G and Dell'Agnola G (1984) Plant growth regulator activity of soluble humic complexes. *Can. J. Soil Sci.* 64, 225–228

Guzha E, Nhapi I and Rockstrom J (2005) An assessment of the effect of human faeces and urine on maize production and water productivity. Physics and Chemistry of the Earth 30: 840-845

Hayes M.H.B and Wilson W.S (1997) Humic substances, peats and sludges. Health and environmental aspects. *Roy. Soc. Chem.* 172, 496

Kate T and Khadse M. (2002) Extension of simple and low cost agricultural techniques for improving crop productivity of small & marginal farmers in Vidarbha region through grass root level NGOs. Unpublished report, Dharamitra Wardha

Kate T and Pathe S. (2009) Scientific validation of the nutrient management practices evolved by some innovative farmers. Unpublished report, Dharamitra Wardha

Lee Y.S and Bartlett R.J (1976) Stimulation of plant growth by humic substances. *J. Am. Soc. Soil Sci.* 40, 876–879

Manjengwa M. G (2011) Animal traction and small scale farming: A Stellenbosch case study. MPhil Thesis, Stellenbosch University

Mnkeni P.N.S, Kutu F.R and Muchaonyerwa P (2008) Evaluation of the human urine as a source of nutrients for selected vegetables and maize under tunnel house conditions in the Eastern Cape, South Africa. Waste Management & Research 26: 132-139

Muscolo A., Bovalo F., Gionfriddo F and Nardi S (1999) Earthworm humic matter produces auxin-like effects on Daucus carota cell growth and nitrate metabolism. *Soil Biol. Biochem.* 31, 1303–1311

Muscolo A., Felici M., Concheri G and Nardi S (1993) Effect of earthworm humic substances on esterase and peroxidase activity during growth of leaf explants of Nicotiana plumbaginifolia. *Biol. Fertil. Soils* 15, 127–131

Muscolo A., Panuccio M.R., Abenavoli M.R., Concheri G and Nardi S (1996) Effect of molecular complexity and acidity of earthworm faeces humic fractions on glutamate dehydrogenase, glutamine synthetase, and phosphenolpyruvate carboxylase in Daucus carota a II cell. *Biol. Fertil. Soils* 22, 83–88

Nardi, S., Arnoldi G and Dell'Agnola G (1988) Release of hormonelike activities from Alloborophora rosea and Alloborophora caliginosa feces. *J. Soil Sci.* 68, 563–657

Sreenivasa M.N., Bagaraj Naik and Bhat S.N (2009) Beejamrutha: A source for beneficial bacteria. Karnakata J. Agric. Sci. 22 (5) 1038-1040

Swain M.R., Naskar S.K and Ray R.C (2007) Indole-3-acetic acid production and effect on sprouting of yam (Dioscorea rotundata L.) Minisetts by Bacillus subtilis Isolated from culturable cowdung microflora. Polish Journal of Microbiology 57 (2) 103-110

Swaminathan C (2005) Food production through vrkshayurvedic way. Technologies for natural farmng. Agriculture College & Research Institute, Madurai, Tamilnadu, India pp: 18-22

Valdrighi M.M., Pera A., Agnolucci M., Frassinetti S., Lunardi D and Vallini G (1996) Effects of compost-derived humic acids on vegetable biomass production and microbial growth within a plant (Cichorium intybus)-soil system: a comparative study. *Agric. Ecosyst. Environ.* 58, 133–144

CHAPTER SIX

Pilot Study: An assessment of the effect of liquid manure (Sanjeevak) on selected vegetables and maize yields in the Western Cape, South Africa

6.1 Introduction

Taking into account the continuing poor productivity of smallholder farming systems in sub-Saharan Africa (SSA) and the alarming reports of soils degradation due to nutrient mining and soil erosion (Giller et *al.,* 2009), the use of eco-friendly technologies to improve soil properties for sustainable crop production appears to offer great potential to address these problems.

According to Carter et *al.* (1992), the use of animal manure to improve soil fertility and crop production assumes particular importance where financial and logistic constraints on the availability of inorganic fertilizer exists, and where naturally infertile soils are used for permanent production of crops (Bayu et *al.,* 2004). As stated by Materechera (2009), the declining fertility of African soils because of soil nutrient mining is a major cause of decreased crop yield and per capita food production on the continent and on the medium to long-term, a key source of land degradation and environmental damage (Henao and Baanate 2006; Stoorvogel and Smaling 1990). Although the use of mineral fertilizer is commonly applied to overcome nutrient depletion in soils, its use by the majority of small-scale farmers in sub-Saharan Africa is limited due to various constraints

(Materechera 2009). To ensure sustainable food security is attained via increased food production, it is essential that cheap and readily available sources of soil amendments are considered. Some authors argue that the logical path of disposing of human and animal wastes is in agriculture as this makes use of valuable nutrients, whilst at the same time avoiding environmental pollution associated with waste disposal into water bodies (Guhza et *al.,* 2005). Hence, liquid manure such as Sanjeevak is being utilized for agricultural production (Kate and Khadse 2002).

There is a large body of knowledge available in the literature that has documented the use of animal manure as source plant nutrients or organic matter versus chemical fertilizers for food production under field conditions (Bayu et al., 2004 and reference therein). However; few of these studies have attempted to investigate both the use of plant nutrients and plant regulating substances (such as plant hormones, enzymes and other substances) within animal waste materials and their combined benefial use on crop production.The study reported herein was carried out to investigate the potential for utilizing a mixture of animal wastes (Sanjeevak) as a source of soil amendments (plant nutrients and plant regulating substances) for maize, tomato, and lettuce and carrot production among small holders with the view to consider the advantages to be gained for its correct use.

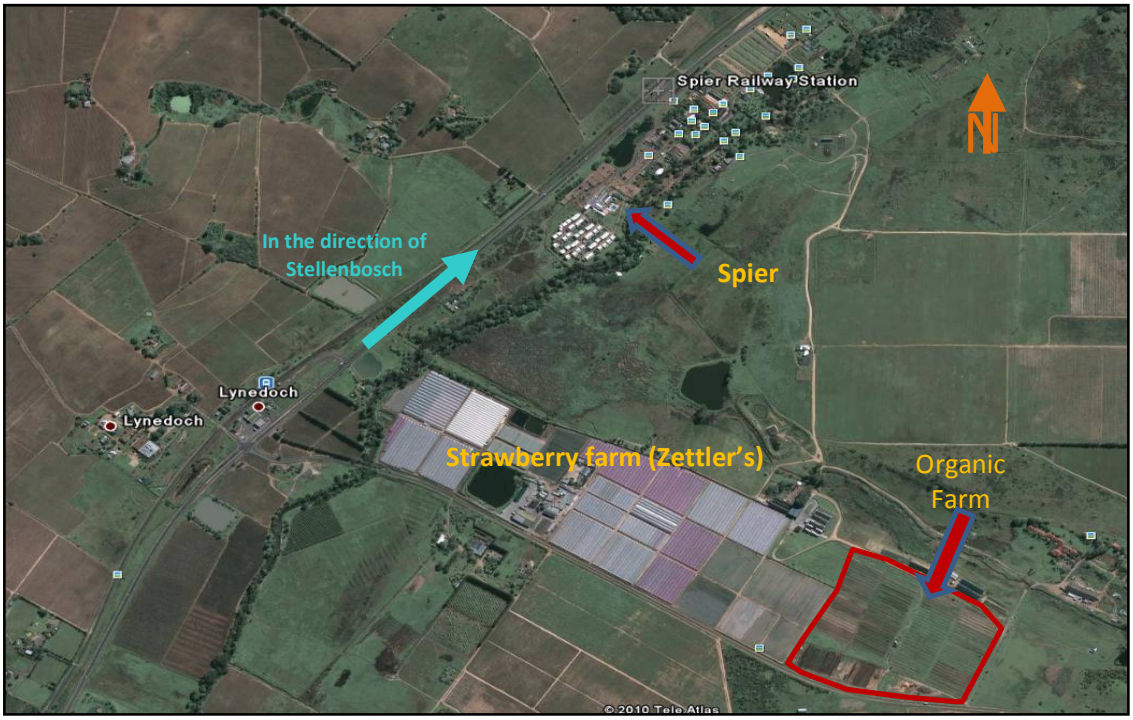
6.2 Methodology

6.2.1 Study area

The study was conducted in the Lynedoch region, (Western Cape province – South Africa) in Spier wine estate lands (Figure 6.1). The farm; strictly organic, is located approximately 4 km southeast of the Spier railway station (33°59'15.92''S and 18°47'20.20''E). The organic farm was initially managed under conventional farming practices and cultivated with tobacco until early 1980s. For approximately nineteen years, the ten hectare farmland lay fallow and in 1999 under *Go-organic at Spier*[*] production resumed, this time under organic production management practices. The local climate is Mediterranean in character with an annual winter rainfall between 600 and 800 mm. The average summer temperature is 29° C and average winter temperature is 19° C (Moloto, 2009).

Figure: 6.1 Organic farm's location (study area)

[*] Go Organic at Spier is a joint venture with seven emerging farmers; together owning 27.5% of the business. 100 hectares of the land Spier used to lease from the local municipality is used by the company and it is funded by the government's Land Reform Credit Facility. The farm is now one of South Africa's largest commercial organic farms, fully certified by Ecocert and supply fresh vegetables to leading supermarkets in the Western Cape and overseas.



6.2.2 Experimental setup

The study was designed as a two factor experimental design consisting of 9 m by 7 m randomized blocks with three replications to ensure statistical validity. The major factors investigated were plant growth regulating substances. The field experiments (Figure 6.3 and 6.4) consisted of four levels of treatments consisting of the following:

i. Plot 4: control plot where maize, carrot, tomato and lettuce were planted and allowed to grow without any external inputs applied.

ii. Plot 1: compost treatment was applied at the rate of 40t/ha. The compost (Table 6.2) contained 0.35 mg/kg N, 0.07 mg/kg P, 0.16 mg/kg K and total organic carbon (TC) 5.72 mg/kg.

iii. Plot 2: both compost and Sanjeevak were applied at the rate of 40t/ha and 2963 L/ha respectively. The compost contained 0.35 mg/kg N, 0.07 mg/kg P, 0.16 mg/kg K and total organic carbon (TC) 5.72 mg/kg and Sanjeevak (Table 6.1) contained 0.1% N, 63.9 mg/l P and 1741 mg/l K and plant growth regulators.

iv. Plot 3: Sanjeevak was applied at the rate of 2963 L/ha as a liquid formulation of biological inoculants.

The plots were randomly arranged in the sense that no particular treatment had a fixed position in the field. Measurement of the harvest was done by weighting the maize cobs, tomato fruits, carrot fruits and lettuce biomass and the data expressed as yield per hectare (t/ha).

Figure 6.2 view of lay out for the field experiments.

6.2.3 Analytical methods

The pH (in water) and EC were recorded directly from Sanjeevak and compost suspension. A more complete nutrient analysis was made for other Sanjeevak and compost samples after nitric acid/Perchloric acid digestion (Singer and Hanson, 1969). The extracts were analyzed for P, K, Ca, Mg, B, Mn, Na using atomic absorption spectroscopy (AAS). Total organic C and N were measured by dry combustion using a carlo-Erba apparatus.

Table 6.1 Selected chemical properties of Sanjeevak used

TC	TN	EC	P	Mg	K	Ca	Mn	Na
%	%	mS/cm			mg/l			
0.7	0.1	0.053	68.8	69.2	632.7	142	0.7	206.7

Table 6.2 Selected chemical properties of compost applied

pH	Mn	Na	P	Mg	K	Ca	TC	TN
%					(%)			
7.7	26.95	108	0.07	0.06	0.16	0.74	5.72	0.35

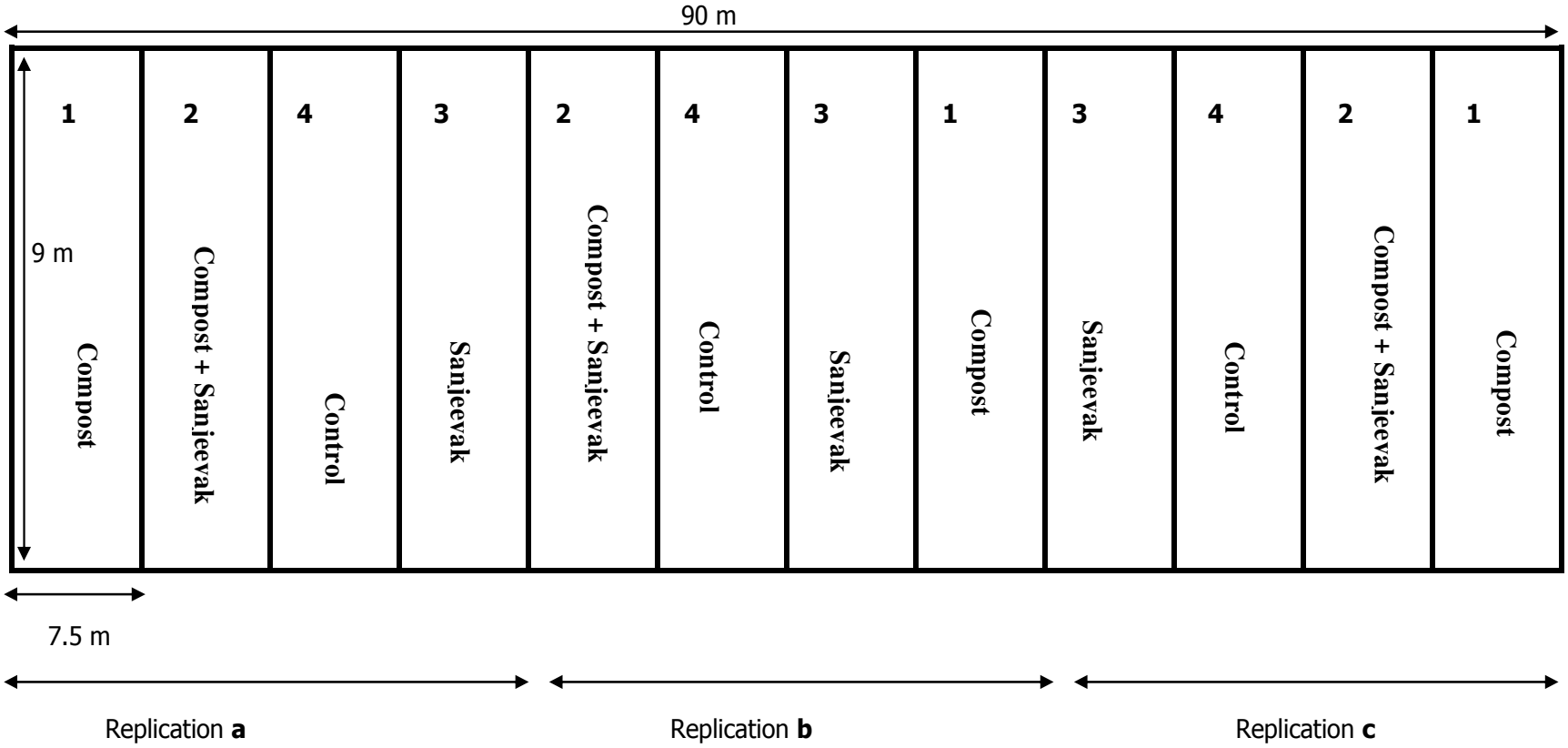


Figure 6.3 Layout design of the experimental field trials used for growing maize, lettuce, carrot and tomato

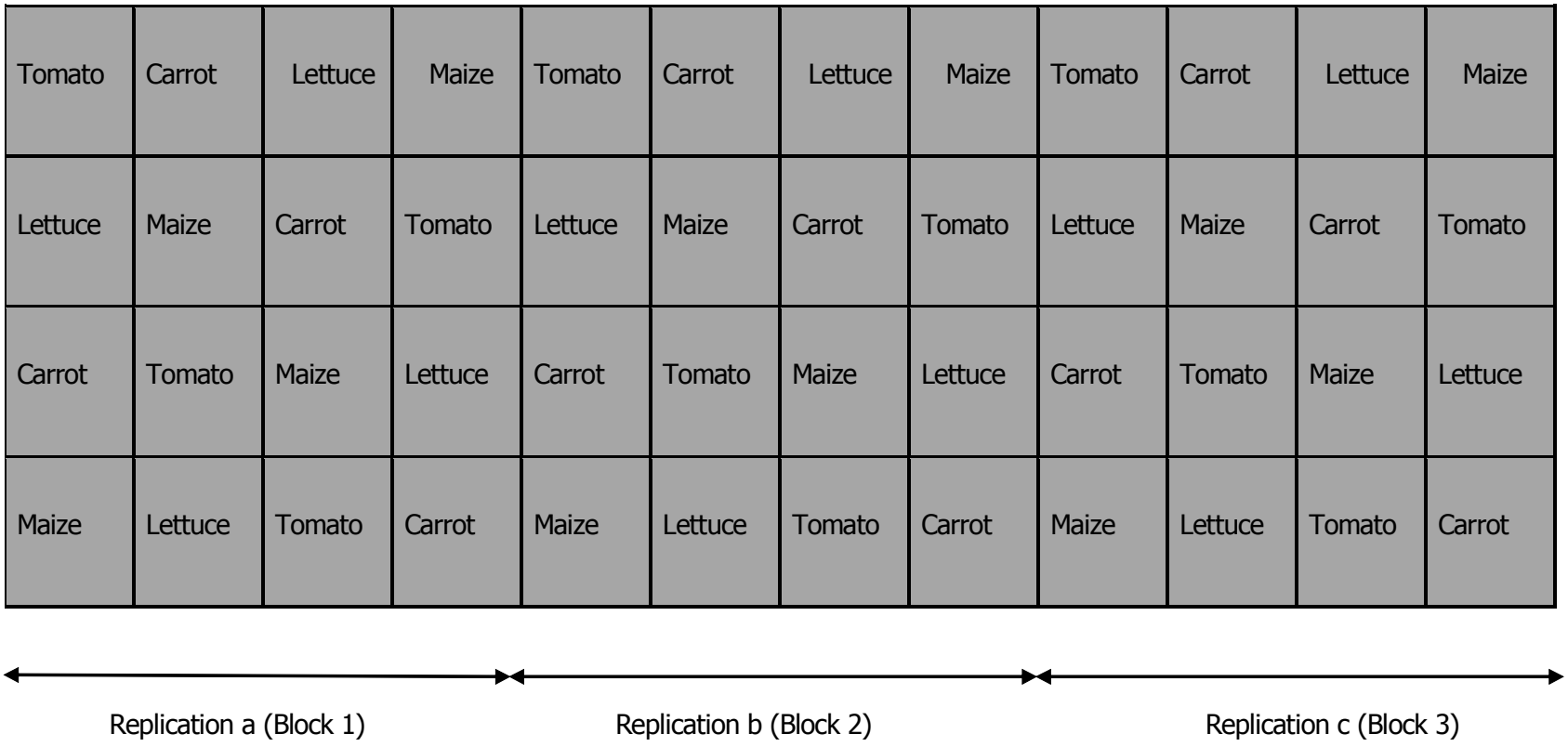


Figure 6.4 Layout design for the field trial experiments (planting outline)

Statistical analysis

All data were checked for normality and homogeneity of variance with the Shapiro-Wilks W test and Levene's test respectively. Yield data for carrot, maize, tomato and lettuce yields were analysed using the measures analysis of variance (ANOVA). Fischer's least significant difference (LSD) post-hoc test was used to determine the significance of any differences between speficic treatments in parametric cases. All statistics were analysed with STATISTICA 10 software.

6.3 Results and discussion

Maize, tomato, carrot and lettuce from different 9 m × 7 m plots were harvested and weighted, with the data expressed as yield per hectare (t/ha)(Figure 6.5). The results showed that the compost + Sanjeevak and compost only treatments were the best; especially for tomato and maize. In most cases, both Sanjeevak treatment and the control had similar yields recorded for the various crops tested, but were lower than compost and compost + Sanjeevak.

Measurement of harvest of both tomato and maize yields from different treatment indicated that a combination of compost + Sanjeevak may provide a farmer of a good return of his capital investment if Sanjeevak was applied consistently and at higher rates and obtained at little to no cost. Compost treatments were applied at the rate of 40t/ha. The compost contained 0.35 mg/kg N, 0.07 mg/kg P, 0.16 mg/kg K and total organic carbon (TC) 5.72 mg/kg; which translated to 156kg N/ha, 39kg P/ha and 78kg K/ha.

However, Sanjeevak treatments were applied at the rate of 20L/plot (9 m × 7 m). Sanjeevak contained 0.1% N, 68.8 mg/l P and 632.7 mg/l K; which translated to 3.17kg N/ha, 0.22kg P/ha and 2kg K/ha.

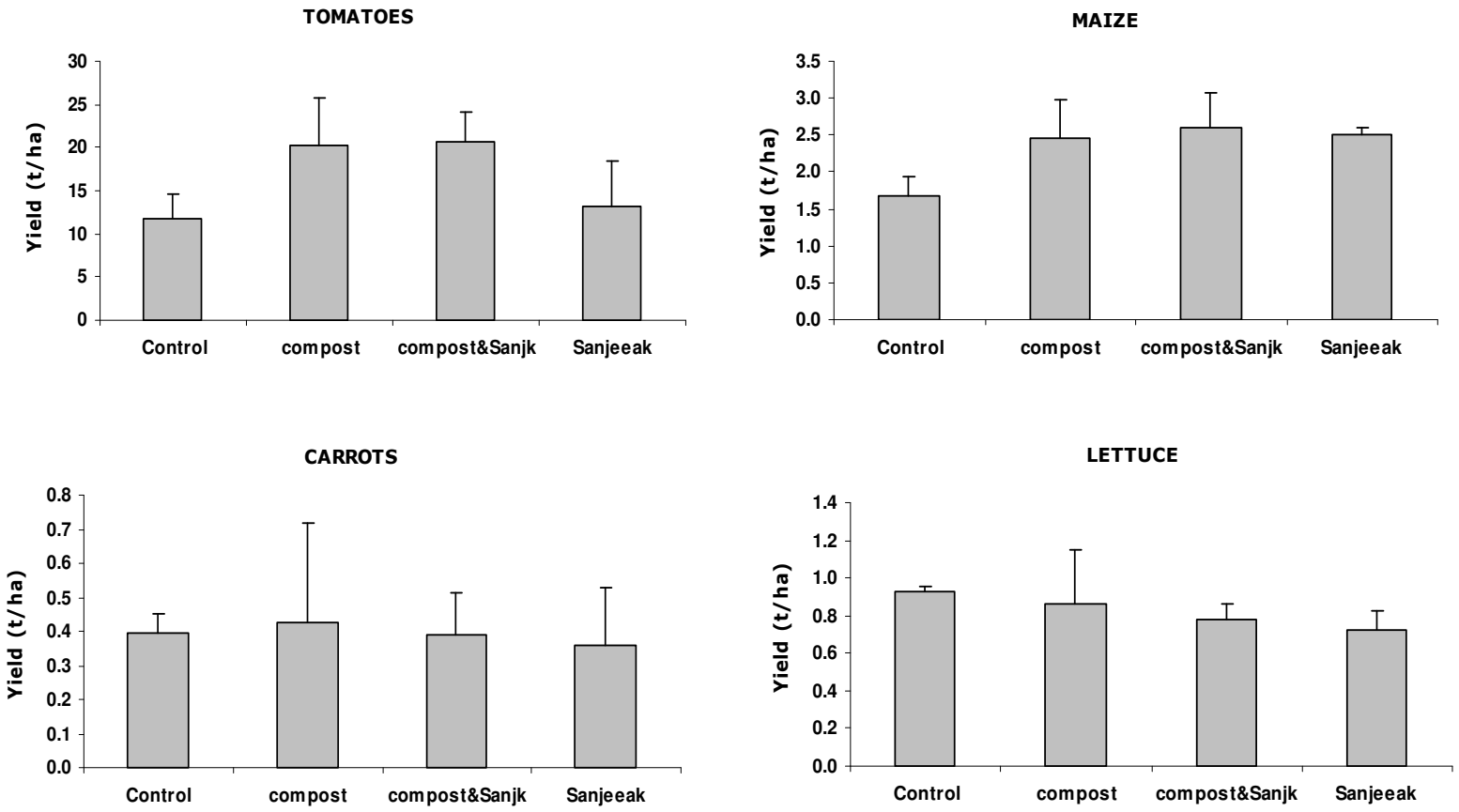


Figure 6.5 Average yields/ha for Tomatoes, Carrots, Maize and Lettuce harvested from the four treatments (mean ± SE)

Research results indicate that organic fertilizers (eg., compost, animal manure, vermicompost) are viable biological sources with positive environmental and ecological benefits (Araji et *al.,* 2001). Nitrogen, phosphorous and potassium are the elements that are most frequently required in relatively large amounts. Soil fertility analysis was conducted before the start of the field trials (Table 6.3) and some weeks after harvest (Table 6.4).

Table 6.3 Properties of the soil used before the start of the field trials (mean ± SE)

Treatment	pH	C (%)	NH4-N	NHO3-N	P	K
				mg/kg		
Control	5.90± 0.20	0.79± 0.53	8.72± 0.23	13.10± 5.95	273.33±172.81	96.00± 40.95
Compost+Sanjeevak	5.96 ± 0.12	1.10 ± 0.42	9.20 ± 1.52	15.87± 6.10	344.00± 49.43	93.33± 45.36
Compost	5.96± 0.12	0.77 ± 0.05	7.33 ± 2.05	14.57± 4.18	313.00± 17.35	71.66± 32.08
Sanjeevak	5.93± 0.06	0.82± 0.19	7.18 ± 1.01	15.87± 5.43	295.33± 42.22	76.33± 35.28

Table 6.4 Properties of the soil used after crop harvests (mean ± SE)

Treatment	pH	C (%)	NH4-N	NHO3-N	P	K
				mg/kg		
Control	5.93± 0.15	1.39± 0.30	5.80± 0.16	11.74± 4.18	275.74±62.08	54.94± 44.14
Compost+Sanjeevak	5.80 ± 0.10	1.60 ± 0.50	6.36 ± 0.30	18.38± 6.00	329.73± 74.52	77.41± 34.94
Compost	5.96± 0.21	1.36 ± 0.13	6.19 ± 0.43	13.22± 1.51	319.50± 16.46	62.72± 15.94
Sanjeevak	5.70± 0.00	1.33± 0.27	6.55 ± 0.41	12.73± 3.09	331.42± 36.37	59.86± 29.90

The results showed that pH values, phosphorus and nitrogen concentrations were not markedly affected by compost and/or Sanjeevak applications to soils for the duration of the experiment. However, total organic carbon (TOC) content increased in all the plots (control 75.95%, compost + Sanjeevak 45.5%, compost 76.62% and Sanjeevak 64.2%). This may be attributed to crop root exudates (C source) released in the rhizosphere. Potassium (K) decreased (control 42.7%, compost + Sanjeevak 17%, compost 12.5% and

Sanjeevak 21.6%) in soils was observed after harvest. This reflects the fact that it is its accumulation in plant tissues that is the principal source for its decline in soils.

In terms of its nutrient content (NPK), the average quantity of Sanjeevak applied to crops in the study was insufficient to achieve an economic yield. However, based on the soil macro-nutrient levels before the start of the trials (Table 6.3) and after harvest (Table 6.4), we strongly believe the yield levels achieved especially for plots under Sanjeevak treatment and the control are mostly attributable to past conventional farming practices of the land under commercial tobacco cultivation (Moloto, 2009). On the other hand, taking the control as the benchmark, yield increases of about 76.65%, 74.37% and 13.27% are achieved using compost + Sanjeevak, compost and Sanjeevak respectively for tomato. Equally, relative production increases for maize of about 54.5%, 49.24% and 46.9% were reached using compost + Sanjeevak, Sanjeevak and compost only respectively. Statistical analysis of the data, revealed that at $P<0.05$ there was no difference between treatments for the same crop tested. The lack of position correlation between treatments (including the control) can be attributed to high coefficients of variation of yield data within and between treatments (Appendixes 11 to 14).

Nevertheless, in comparing compost and compost + Sanjeevak treatments; yield increases from 20.35 to 20.61 t/ha for tomato and 2.46 to 2.60 t/ha for maize were recorded; which is equivalent to increases in yield of 265 Kg and 126 kg per hectare for tomato and maize respectively due to Sanjeevak application. Also, yield increases from 11. 67 to 13.21 t/ha for tomato and 1.67 to 2.51 t/ha for maize respectively for the control and Sanjeevak treatments were observed. These equal to increases in yield of 1547 kg and 831 kg per

hectare due to Sanjeevak application. Since compost and compost + Sanjeevak treatments received all the needed nutrients at almost equal concentration, and the control and Sanjeevak treatments were not directly treated with nutrients input. As a result, the contribution of Sanjeevak microorganisms' properties on the increased crop yield of tomato and maize in compost + Sanjeevak compared to compost only and Sanjeevak compared to the control can be ascribed as an influence. Etesami et al. (2008) reported that one of the most critical channel used by bacteria to affect the growth and development of plants is by producing IAA. This plant hormone (IAA) alters the root system development; resulting in increased nutritional uptake and eventually increased plant growth. This may be in accordance with the fact that population and diversity of microorganisms that produce plant hormones and enzymes may be responsible for the marginal reported increases in yield in this study as discussed by other authors (Swaminathan, 2005; Swain et al., 2007; Sreenivasa et al., 2009; Pathe and Kate 2009). As a result, Sanjeevak may be best applied as a source of effective microorganisms in combination with a reliable source of nutrients.

Table 6.5 Soil amendments application (Kate and Phate 2009)

	Soybean	Pigeon pea	Wheat	Bengal gram
Chemical fertilizer (kg/ha)	30:75:30	25:50:00	120:60:60	25:40:00
Sanjeevak (L/ha)	3×60150	5×60150	3×60150	3×60150

Measurement of crop grain yields (Table 6.6) cultivated using different formulations of organic growth promoters (refers to Table 3.1 in Chapter 3), compared to the control and

chemical fertilizer application (Table 6.5) showed that Sanjeevak applied consistently and at much higher rates can lead to comparable or higher yields than chemical fertilizer treated crops (Pathe and Kate 2009).

Table 6.6 Average grain yields (t/ha) from the different formulations of organic growth promoters (Kate and Phate 2009)

	Wheat	Bengal gram	Soyabeans	Pigeon pea
Control	0.127	0.432	0.694	1.299
Chemical	0.763	0.334	0.890	1.383
T1	0.638	0.577	0.972	1.532
T2	0.621	0.759	1.005	1.615
T3	0.606	0.736	0.832	1.814
T4	0.857	0.704	0.857	1.698
T5	0.863	0.653	0.873	1.930
T6	0.852	0.637	1.206	1.609
T7	0.364	0.793	1.120	2.236

For example, wheat grain yield under chemical fertilizer treatment was 0.763 t/ha; which is 6 times higher than wheat grain yield for the control treatment. For Sanjeevak, wheat grain yield ranged between 0.638 to 0.863 t/ha; these equal up to 7 times higher yield than the control and an increase of about 13% compared to chemical fertilizer. Also, taking pigeon pea as an example, grain yield obtained for the control treatment was 1.3 t/ha compared to 1.4 t/ha for chemical fertilizer treatment. For Sanjeevak treatments, pigeon pea grain yield ranged between 1.5 to 1.9 t/ha, which is a yield improvement of 0.1 to 0.5 t/ha or 7 to 35% compared to both the control and the chemical fertilizer

treatment. In general, yield from crops treated with Sanjeevak can be comparable or significantly improved to chemically fertilized plots; depending on the level of application of treatment materials.

As previously indicated, (Chapter 3) Sanjeevak concentration in macronutrient averages 0.11% N, 0.007% P and 0.063% K; which translates to 1.1g N, 0.07g P and 0.63g K per L. Taking these levels of concentrations as baseline and considering Sanjeevak formulation (T0) it is possible to provide agronomic requirements for the use of Sanjeevak as a source of nutrients; with the added advantage that Sanjeevak contains bacteria that are capable of producing plant hormones, compared to chemical fertilizers for a variety of crop plants (Table 6.7).

Table 6.7 Agronomic requirements for Sanjeevak (T0) application based on N and P content vs. chemical fertilizer of various crops per hectare (FSSA – Fertilizer handbook, 1989)

	Tomatoes	Wheat	Lettuce	Carrots
Chemical fertilizer (kg/ha)	120:80:00	120:60:00	100:80:00	70:80:00
Manure (feces + urine)	feces: 6t urine: 6t	feces: 6t urine: 6t	feces: 5t urine: 5t	feces: 3.5t urine: 3.5t

6.4 Conclusion

Sanjeevak can only be used effectively as a reliable source of nutrients and phytohormones to improve crop yields if applied consistently and at agronomic rates

because of its relative low content of macro nutrients (N, P and K) per litre. Alternatively, its formulation can be modified, such as reducing its dilution coefficient (by reducing the quantity of water added in the mix), and/or increasing the proportion of dung and urine mixed), etc. to obtain greater concentration of nutrients per litre prior to its application. However, the study indicated that a higher yield may be achieved if the farmer uses both compost and Sanjeevak. Taking all the above mentioned recommendations into consideration regarding the improvements of Sanjeevak preparation, further research field trials are required; by considering diverse ranges of application rates of Sanjeevak and assessing its response to plant growth parameters and yield potential for a variety of crops; season after season.

6.5 References

Araji A.A., Abdo Z.O and Joyce P (2001) Efficient use of animal manure on cropland – economic analysis. *Biosource Technology* 79: 179-191

Bayu W., Rethman N.F.G and Hammes P.S. (2004) The role of animal manure in sustainable soil fertility management in Sub-Saharan Africa. *Journal of Sustainable Agriculture,* Vol. 25 (2): 113-136

Carter D.C., Harris J.B. Yongquist and Persaud N. (1992) Soil properties, crop water use and cereal yield in Botswana after additions of mulch and manure. *Field Crops Res*. 30: 97-107

Giller Ken E., Witter E., Corbells M and Tittonell P (2009) Conservation agriculture and smallholder farming in Africa: The heretic's view. *Field Crops Res.* 114: 23-34

Guzha E, Nhapi I and Rockstrom J (2005) An assessment of the effect of human faeces and urine on maize production and water productivity. *Physics and Chemistry of the Earth* 30: 840-845

Henao J and Baanate C (2006) Agricultural production and soil nutrient mining in Africa: Implications for resource conservation and policy development. IFDC Technical Bulletin, Muscle Shoals, Alabama, USA

Kate T and Khadse M. (2002) Extension of simple and low cost agricultural techniques for improving crop productivity of small & marginal farmers in Vibdarbha region through grass root level NGOs. Unpublished report, Dharamitra Wardha

Kate T and Pathe S. (2009) Scientific validation of the nutrient management practices evolved by some innovative farmers. Unpublished report, Dharamitra Wardha

Materechera S.A (2010) Utilization and management practices of animal manure for replenishing soil fertility among small-scale crop farmers in semi-arid farming districts of the North West Province, South Africa. *Nutr Cycl Agroecosyst*

Moloto K.P (2009) The potential of sustainable agricultural practices to enhance soil carbon sequestration and improve soil quality. Msc thesis

Powers W.L., Wallingford G.W and Murphy L.S (1975) Research status on effects of land application of animal manure. EPS-660/2-75-010. US Government Printing Office, Washington DC 20402. Stock No. 055-001-01206

Singer M.J and Hanson L (1969) Lead accumulation in soils near highways in the Twin Cities metro area. Soil Science Society of America proceedings 33: 152-153

Stoorvogel J.J and Smaling EMA (1990) Assessment of soil nutrient depletion in sub-Saharan Africa: 1983-2000, report 28, vol 1-4. The winad Staring Centre, Wageningen, Netherlands

Sreenivasa M.N., Bagaraj Naik and Bhat S.N (2009) Beejamrutha: A source for beneficial bacteria. Karnakata J. Agric. Sci. 22 (5) 1038-1040

Swain M.R., Naskar S.K and Ray R.C (2007) Indole-3-acetic acid production and effect on sprouting of yam (Dioscorea rotundata L.) Minisetts by Bacillus subtilis Isolated from culturable cowdung microflora. Polish Journal of Microbiology 57 (2) 103-110

Swaminathan C (2005) Food production through vrkshayurvedic way. Technologies for natural farmng. Agriculture College & Research Institute, Madurai, Tamilnadu, India pp: 18-22

CHAPTER SEVEN

7.1 GENERAL CONCLUSION

The use of agrochemicals is generally applied to overcome nutrient depletion in soils. However, its use by the majority of small-scale farmers in the developing world such as Sub Saharan Africa (SSA) and India is severely restricted by logistic constraints and financial inability of most farmers to pay for them. As a result, the declining fertility of soils in those regions because of nutrient mining is a major cause of concern for decreased food production but also as a key source of land degradation and environmental damage. However, mixed crop-livestock farming systems are characteristics of small-scale agriculture in developing countries; therefore, animal waste materials (cow dung and urine) can be an essential resource of cheap effective microorganisms (EM) but also for supplying nutrients, improve soil properties such as organic matter content, soil pH, CEC, water holding capacity and microbial abundance and diversity; all essential for effective soil fertility improvements.

Studies investigating the detection and quantity of phytohormones in Sanjeevak were carried out (Chapter 2). In using both the Salkowski colorimetric method and LC-MS analytic method to detect and quantify auxins from Sanjeevak and its composites (cow urine and droppings), the results revealed the presence of IAA equivalents in cow urine, manure and Sanjeevak. Cow urine and dung contents of auxins in comparison to Sanjeevak, strongly indicated the influence of Sanjeevak microbiota in producing phytohormones. However, the study also highlighted the fact that fermenting Sanjeevak

had no significant impact on IAA production level. Rather, what the study revealed was that incubating Sanjeevak by altering various external environmental parameters (such as temperature, time of incubation, amount of tryptophan applied etc.) proved to be more effective in increasing the amount of IAA produced. Overall, the present investigation demonstrated the fact the Sanjeevak preparation may be applied just after its preparation without requiring further maturation process to stimulate its ability to produce IAA.

Sanjeevak was assessed for its micro and macro nutrients contents. The analysis showed the presence of micronutrients such as Mg, Na, Ca, Zn at variable concentrations and phosphorus (P) (0.007%) and potassium (K) (0.063%). However, Sanjeevak content in total nitrogen (TN) (0.11%), and total organic carbon (TOC) (0.71%) was very low; suggesting that it may only be considered a viable source of nutrients only if applied at higher rates and consistently (Chapter 3) or by modifying its formulation in such a way that its content in nutrients will be enhanced. Sanjeevak was also analysed for its coliform bacterial load and level of heavy metals contents in relation to the strictest legislation that regulates the use and application of wastewater sludge to agricultural lands in South Africa. The findings revealed that heavy metals and feacal coliform bacteria levels measured did not exceed permissible limits for application as a source of soil properties improvements for crop production (Chapter 3).Analysis to determine the potential for biogas production from Sanjeevak was also conducted. However, the results showed that if biogas production is considered, then different processes need to be explored. For example, the use of an inoculum (methanogenic bacteria) to act as a catalyst for methane production from Sanjeevak, as observed in many studies.

An investigation was conducted to study the effect of Sanjeevak versus inorganic fertilizers application at equal NPK rates on growth and yield parameters of various crops. The results showed that shoot, root, total fresh and dried biomass increased with increased Sanjeevak and inorganic fertilizer rates; specially applied at lower rates (0.55 to 1.1g N pot^{-1}) with consistently greater yields from inorganic fertilizers. However, total biomass (fresh and dried) and wheat grain yield were generally highest at 2.75g N pot^{-1} for both sources; with significantly greater yields from Sanjeevak (Chapter 5); highlighting the effectiveness of higher and consistent application doses of Sanjeevak; not only as a source of organic fertilizers but most probably as an Inoculum containing bacteria producing plant growth regulators. Further analysis of Sanjeevak as a mixture containing plant growth substances, indicated consistently its root promoting effect on crops such as tomato, wheat, cucumber and grapevine (Chapter 4 and 5). This is consistent with other studies on animal waste materials pertaining to the role of microorganisms in producing phytohormones (Swain et *al.,* 2007; Sreenivasa et *al.,* 2009).

Finally, Chapter 6 emphasized the fact; Sanjeevak applied at agronomic rates may be comparable or possibly more effective than equivalent chemical fertilizer application in terms of crop yield production level. In addition, agronomic requirements for Sanjeevak application based on N and P concentration was provided as a benchmark for future use for some crops.

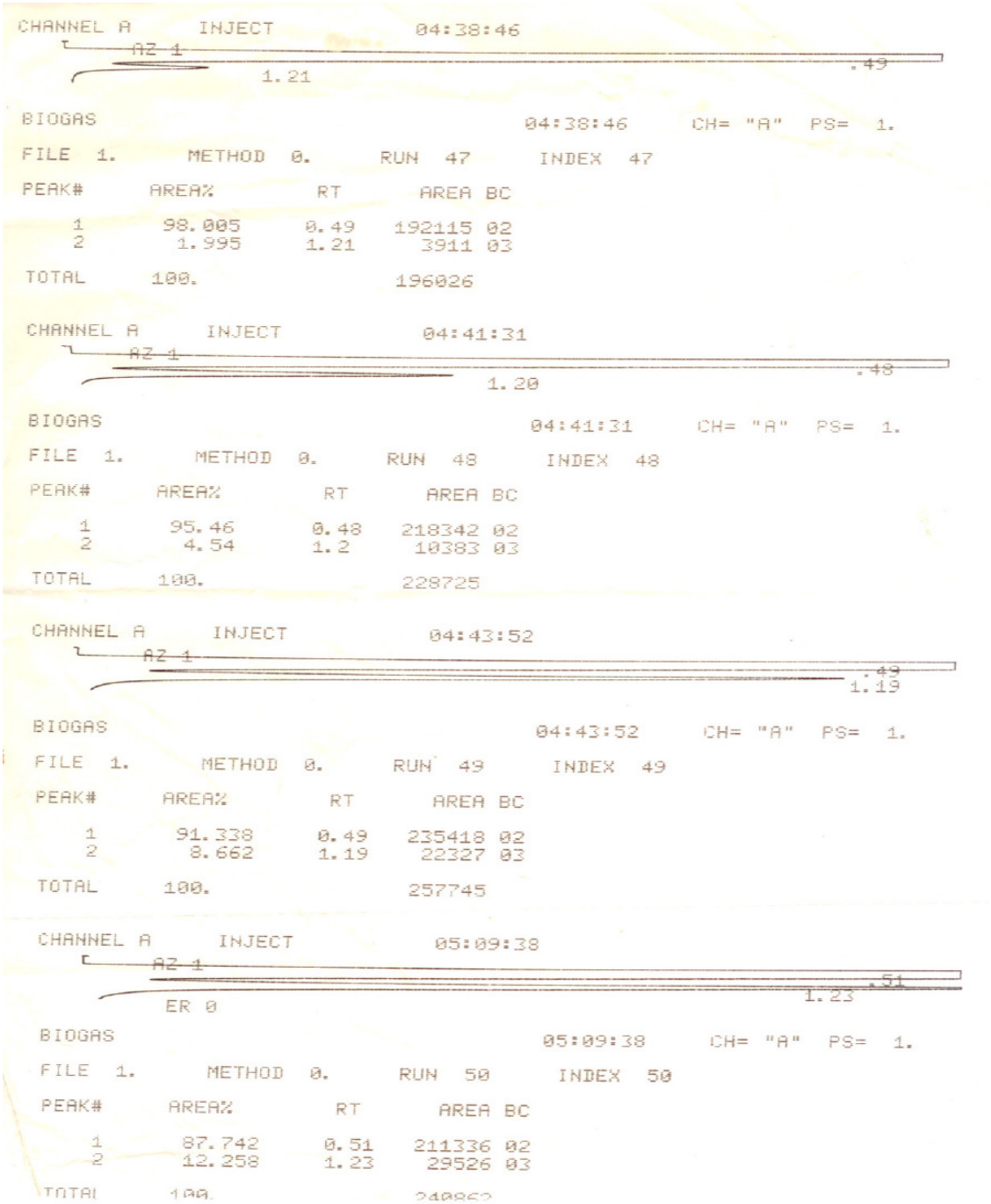
In terms of the primary hypothesis that motivated this research, the study successfully confirmed that Sanjeevak as a source of soil amendments contains all the essential plant nutrients although in varied and small quantities; but most importantly was a source of phytohormones such as IAA. Indeed, a set of experiments emphasized the fact that the identification and measurement of IAA in Sanjeevak may be ascribed to Sanjeevak microbiota activities and properties. In terms of testing the objectives to be attained from this thesis, the study successfully demonstrated the fact that Sanjeevak application as a source soil amendments led to increased growth and development of some of the crop plants investigated. However in the context of this study, the field and greenhouse experiments showed the limiting impact that external environmental parameters may have on data validation of laboratory findings. Nevertheless, in terms of innovation and improvement of our current knowledge of traditional technologies developed by smallholder farmers; the findings of this thesis provide a good scientific understanding of the utilisation of organic waste materials in agriculture for food production.

On the whole, the study will be first and foremost useful to farmers and policymakers who have to decide on the need to promote the use by smallholder farmers of organic waste materials from animal origin on their agricultural land as a source of soil properties improvements. However, aspects aimed at improving the formulation and efficiency of Sanjeevak that need to be investigated, include the following:

- Sanjeevak microbiota should be further investigated for its physiological ability to synthesize plant hormones and also by studying precursor (biocatalyst)-inoculum interactions. For example, Methionine (L-MET) for ethylene production, Adenine (ADE) and isopentyl alcohol (IA) for cytokinins.
- The use of cheap alternative biocatalysts for plant hormones biosynthesis from bacteria in Sanjeevak. For example, replacing purified tryptophan with soyabeans which is rich in tryptophan (0.59g TRP/ 100g of soyabeans).
- Long term experimentations with frequent and increased application of Sanjeevak, possibly using various formulations should be supported and will ultimately further help our understanding of its influence on growth and yield parameters of crop plants.

APPENDICES

Appendix 1: Gas chromatography (GC) analysis showing Sanjeevak gas composition (carbon dioxide, methane and nitrogen) in percentage terms.



CHANNEL A INJECT 04:38:46
AZ 1
1.21 .49

BIOGAS 04:38:46 CH= "A" PS= 1.
FILE 1. METHOD 0. RUN 47 INDEX 47

PEAK#	AREA%	RT	AREA BC
1	98.005	0.49	192115 02
2	1.995	1.21	3911 03
TOTAL	100.		196026

CHANNEL A INJECT 04:41:31
AZ 1
1.20 .48

BIOGAS 04:41:31 CH= "A" PS= 1.
FILE 1. METHOD 0. RUN 48 INDEX 48

PEAK#	AREA%	RT	AREA BC
1	95.46	0.48	218342 02
2	4.54	1.2	10383 03
TOTAL	100.		228725

CHANNEL A INJECT 04:43:52
AZ 1
.49 1.19

BIOGAS 04:43:52 CH= "A" PS= 1.
FILE 1. METHOD 0. RUN 49 INDEX 49

PEAK#	AREA%	RT	AREA BC
1	91.338	0.49	235418 02
2	8.662	1.19	22327 03
TOTAL	100.		257745

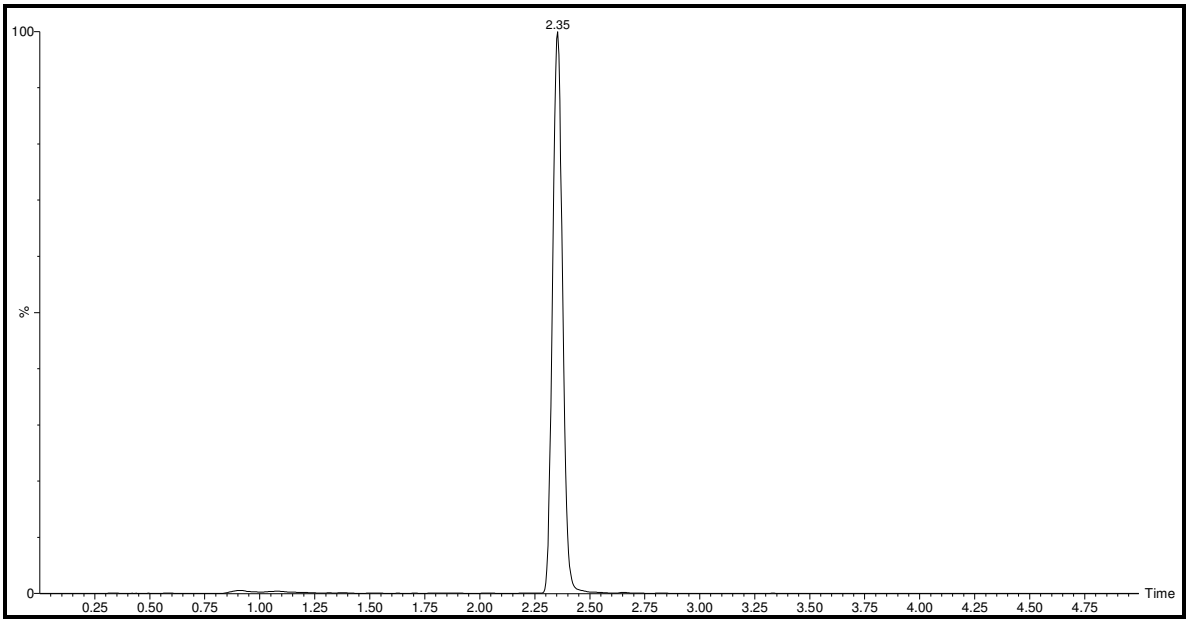
CHANNEL A INJECT 05:09:38
AZ 1
.51 1.23
ER 0

BIOGAS 05:09:38 CH= "A" PS= 1.
FILE 1. METHOD 0. RUN 50 INDEX 50

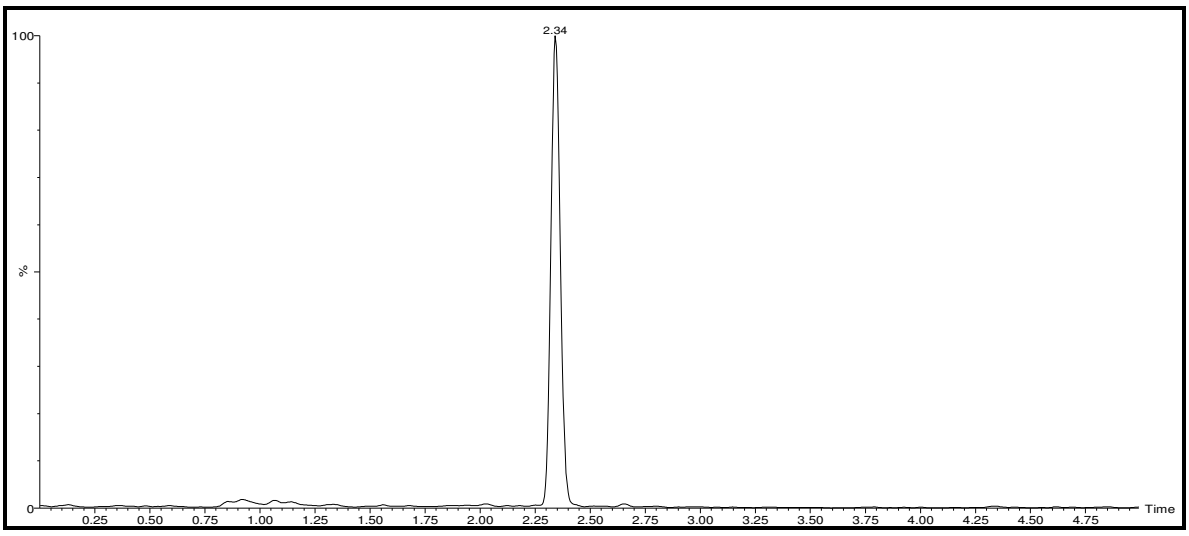
PEAK#	AREA%	RT	AREA BC
1	87.742	0.51	211336 02
2	12.258	1.23	29526 03
TOTAL	100.		240862

Appendix 2: IAA MRM chromatograms of cow urine and Sanjeevak filtrates obtained by LC-MS analysis – Chapter 2.

IAA MRM chromatogram: Sanjeevak filtrate sample



IAA MRM chromatogram: Urine sample



Appendix 3: Analysis of variance table showing the effect of Sanjeevak vs. NAA pre-treatments at various concentrations on rooting and produced root biomass grapevine (Ramsey) rootstock cuttings (*Vitis viniferas L)*

	Variables	SS	DF	MS	F	P
Biomass	Treatment	35.2837	1	35.2837	21.04822	0.000303
	Concentration	40.1913	3	13.3971	7.99229	0.001763
	Treatment*Concentration	48.6046	3	16.2015	9.66534	0.000705
Rooting	Treatment	37.50	1	37.50	0.1324	0.720767
	Concentration	5379.17	3	1793.06	6.3284	0.004918
	Treatment*concentration	5445.83	3	1815.28	6.4069	0.004672

Appendix 4: Analysis of variance table showing the individual and interactive the effect of Sanjeevak vs. inorganic fertilizer on soil residual pH and EC under cucumber cultivation (*Cucumis sativus*)

	Variables	SS	DF	MS	F	P
pH (in H2O)	Treatment	0.005	1	0.005	0.04	0.840837
	Concentration	0.926	3	0.309	2.52	0.071541
	Treatment*Concentration	0.920	3	0.307	2.51	0.072813
pH (in KCl)	Treatment	0.145	1	0.145	3.32	0.076056
	Concentration	0.350	3	0.117	2.67	0.060637
	Treatment*concentration	0.408	3	0.136	3.11	0.037113
EC	Treatment	330290	1	330290	36.1166	0.000000
	Concentration	419855	3	139952	15.3034	0.000001
	Treatment*concentration	343587	3	114529	12.5235	0.000006

Appendix 5: Analysis of variance table showing the individual and interactive effect of Sanjeevak vs. inorganic fertilizer on soil residual pH and EC under wheat cultivation (*Triticum sp)*

	Variables	SS	DF	MS	F	P
pH (in H2O)	Treatment	0.240	1	0.240	1.13	0.294203
	Concentration	1.218	3	0.406	1.91	0.143755
	Treatment*Concentration	0.658	3	0.219	1.03	0.288926
pH (in KCl)	Treatment	0.258	1	0.258	1.79	0.188837
	Concentration	0.618	3	0.206	1.43	0.249456
	Treatment*concentration	0.220	3	0.0.073	0.51	0.679921
EC	Treatment	1348.7	1	1348.7	0.6380	0.429268
	Concentration	11755.3	3	3918.4	1.8536	0.153479
	Treatment*concentration	2395.1	3	798.4	0.37777	0.769596

Appendix 6: Analysis of variance table showing the individual and interactive effect of Sanjeevak vs. inorganic fertilizer on wheat (*Triticum sp)* height, shoot (fresh and dried), root (fresh and dried) and total biomass (fresh and dried)

	Variables	SS	DF	MS	F	P
Height	Treatment	4.502	1	4.502	0.7618	0.387976
	Concentration	603.654	3	201.218	34.0497	0.000000
	Treatment*Concentration	18.682	3	6.227	1.0538	0.379445
Shoot (Fresh)	Treatment	27.150	1	27.150	0.7654	0.386861
	Concentration	4585.846	3	1528.615	43.0948	0.000000
	Treatment*concentration	213.279	3	71.093	2.0043	0.128813
Shoot (dried)	Treatment	4.502	1	4.502	0.7618	0.387976
	Concentration	603.654	3	201.218	34.0497	0.000000
	Treatment*concentration	18.682	3	6.227	1.0538	0.379445
Root (Fresh)	Treatment	64.64	1	64.635	0.2485	0.620866
	Concentration	3522.31	3	1174.105	4.51396	0.008085
	Treatment*concentration	3057.77	3	1019.255	3.91863	0.015236
Root (dried)	Treatment	0.060	1	0.0602	0.00135	0.970873
	Concentration	304.992	3	101.6641	2.27961	0.094087
	Treatment*concentration	359.782	3	119.9274	2.68912	0.059160
Biomass (F)	Treatment	157.57	1	175.57	0.38569	0.538095
	Concentration	15052.31	3	5017.44	11.02252	0.000021
	Treatment*concentration	4693.56	3	1564.52	3.43701	0.025731
Biomass (D)	Treatment	37.453	1	37.453	0.50811	0.480097
	Concentration	1062.174	3	354.058	4.80331	0.005976
	Treatment*concentration	1276.975	3	425.658	5.77467	0.002227

Appendix 7: Analysis of variance table showing the individual and interactive effect of Sanjeevak vs. inorganic fertilizer on cucumber (*Cucumis sativus*) height, shoot (fresh and dried), root (fresh and dried) and total biomass (fresh and dried)

	Variables	SS	DF	MS	F	P
Height	Treatment	740033	1	740033	74.2729	0.000000
	Concentration	2033392	3	677797	68.0266	0.000000
	Treatment*Concentration	312079	3	104026	10.4405	0.000033
Shoot (Fresh)	Treatment	29146.2	1	29146.2	158.4916	0.000000
	Concentration	53320.7	3	17773.6	96.6495	0.000000
	Treatment*concentration	12355.3	3	4118.4	22.3952	0.000000
Shoot (dried)	Treatment	384.201	1	384.201	57.1048	0.00000
	Concentration	1198.772	3	399.591	59.3922	0.00000
	Treatment*concentration	183.044	3	61.015	9.0688	0.000010
Root (Fresh)	Treatment	1212.264	1	1212.264	45.6472	0.000000
	Concentration	2827.000	3	942.333	35.4831	0.000000
	Treatment*concentration	661.274	3	220.425	8.3000	0.000216
Root (dried)	Treatment	4.64663	1	4.64663	54.5620	0.000000
	Concentration	11.28591	3	3.76197	44.1741	0.000000
	Treatment*concentration	2.24336	3	0.74779	8.7807	0.000142
Biomass (F)	Treatment	39768.0	1	39768.0	140.1603	0.000000
	Concentration	74647.0	3	24882.3	87.6965	0.000000
	Treatment*concentration	16877.9	3	5626.0	19.8284	0.000000
Biomass (D)	Treatment	461.280	1	461.280	60.0078	0.000000
	Concentration	1414.962	3	471.654	61.3573	0.000000
	Treatment*concentration	213.295	3	71.098	9.2492	0.000009

Appendix 8: Levene's test of homogeneity of variances - analysis of variance table showing the effect of treatments (on maize, carrot, lettuce and tomato yields.

Variables	MS Effect	MS Error	F	P
Maize	0.194527	0.0888995	2.185831	0.167509
Carrot	0.055384	0.011516	4.809482	0.033648
Lettuce	0.066807	0.009245	7.226483	0.011501
Tomato	7.193749	11.911717	0.603645	0.630779

Appendix 9 Results for selected soil properties analysis of the soil used per plot before the start of the field trials

		pH	C	NH4-H	NO3-H	P	K
			%	mg/kg			
Block 1	1a	5.9	0.83	9.61	18.77	324	105
	2a	5.9	1.5	8.27	22.02	398	128
	3a	6	0.88	8.19	18.25	325	117
	4a	5.9	1.31	8.46	18.69	430	124
Block 2	1b	6.1	0.73	6.74	14.52	293	69
	2b	5.9	1.15	8.38	15.79	333	110
	3b	5.9	0.96	6.18	19.71	314	58
	4b	5.7	0.26	8.84	6.85	88	115
Block 3	1c	5.9	0.77	5.65	10.42	322	41
	2c	6.1	0.66	10.96	9.82	301	42
	3c	5.9	0.6	7.18	9.66	247	54
	4c	6.1	0.82	8.87	13.76	302	49

Appendix 10 Results for selected soil properties analyis of the soil used per plot after harvest of the field trials

		pH	C	NH4-H	NO3-H	P	K
			%	mg/kg			
Block 1	1a	5.8	1.46	6.67	14.37	329.2	77.33
	2a	5.8	2.15	6.4	22.42	405.3	115.02
	3a	5.7	1.63	6.88	15.81	372.3	92.7
	4a	5.7	1.74	5.92	16.4	347.26	105.77
Block 2	1b	5.9	1.21	6.1	13.79	328.8	45.72
	2b	5.7	1.5	6.64	21.23	327.6	71.25
	3b	5.7	1.1	6.68	12.74	319.33	52.69
	4b	5.4	1.25	5.86	10.53	235.72	32.79
Block 3	1c	6.2	1.41	5.82	11.51	300.5	65.13
	2c	5.9	1.17	6.05	11.49	256.3	45.97
	3c	5.7	1.28	6.09	9.64	302.64	34.2
	4c	5.5	1.2	5.62	8.31	244.26	26.27

Appendix 11 Yield data from fiel experiment for tomato fruits (t/ha)

	Control	Compost	Compost + Sanjeevak	Sanjeevak
Replication	t/ha			
1	9.83	29.08	27.19	20.57
2	17.28	10.46	19.38	16.09
3	7.88	21.49	15.26	2.98
Average	11.67	20.34	20.61	13.21
Stdev	4.96	9.36	6.06	9.14
SE	2.86	5.40	3.50	5.28

Appendix 12 Yield data from field experiments for maize cobs (t/ha)

	Control	Compost	Compost + Sanjeevak	Sanjeevak
Replication	t/ha			
1	1.29	3.47	2.73	2.35
2	2.16	1.71	1.68	2.68
3	1.56	2.21	3.35	2.47
Average	1.67	2.46	2.59	2.50
Stdev	0.44	0.91	0.85	0.17
SE	0.26	0.52	0.49	0.10

Appendix 13 Yield data from field experiments for carrot fruits (t/ha)

	Control	Compost	Compost + Sanjeevak	Sanjeevak
Replication	t/ha			
1	0.46	1.02	0.63	0.69
2	0.44	0.13	0.25	0.16
3	0.28	0.13	0.28	0.22
Average	0.39	0.42	0.39	0.36
Stdev	0.10	0.51	0.21	0.30
SE	0.06	0.30	0.12	0.17

Appendix 14 Yield data from field experiments for lettuce (t/ha)

	Control	Compost	Compost + Sanjeevak	Sanjeevak
Replication	t/ha			
1	0.91	1.43	0.82	0.87
2	0.87	0.59	0.89	0.77
3	0.99	0.55	0.63	0.54
Average	0.92	0.86	0.78	0.73
Stdev	0.06	0.50	0.14	0.17
SE	0.04	0.29	0.08	0.10

Stdev: Standard deviation; SE: Standard Error