SEED TREATMENT OF MAIZE, SORGHUM AND SUNFLOWER WITH EFFECTIVE MICRO-ORGANISMS

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Declaration of independent work

I, the undersigned, herby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature: …….....

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Date: ……..

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Dedication

I dedicate this work to my grandfathers

N.C.P. Oosthuizen;

M.T.J. van Tonder.

The secret of success is constancy to purpose. – Disraeli

Abstract

A series of incubation studies and greenhouse experiments were conducted to evaluate the use of EM seed treatments, at different application levels, handling techniques and soil conditions on germination and seedling vigour of selected cultivars of maize, sorghum and sunflower.

Two incubation studies were conducted to evaluate the germination and seedling vigour of maize, sorghum and sunflower seeds treated with M-EM from three different suppliers, multiplied at two different ratios (1% and 3%) and diluted at three different levels (0.01%, 0.1% and 1.0%) compared to a control treated with pure water. Results revealed no significant differences under optimum germination conditions, while seedlings under cold stress indicated that M-EM treatments positively affected germination and seedling vigour compared to the control treatments.

Two incubation studies were also conducted to evaluate the germination and seedling vigour of maize, sorghum and sunflower seeds treated with M-EM from three different suppliers, multiplied at two different ratios (1% and 3%) and exposed to the influences of irradiation and temperature fluctuation. From the results became clear that the correct storage and handling is essential in optimizing the effect of M-EM on seeds. Even though M-EM was exposed to irradiation and temperature fluctuation, M-EM still had positive effects on germination and seedling vigour.

Pot experiments were conducted to determine the effect of EM as seed treatment, at different dilutions, on germination, seedling vigour and dry mass of maize, sorghum and sunflower at different planted depths. Germination were not affected by the M-EM treatment, while shoot length results indicated that seed treated with M-EM could have significant effect on seedling survival. A greater effect was visible on the shoot length of shallow planted seeds, than on deeper planted seeds. From the results no single company, ratio or dilution could be prescribed as paramount.

To further investigate the effect of M-EM subjected to the influences of irradiation and temperature fluctuation; maize, sorghum and sunflower seeds were treated with M-EM from three different suppliers, multiplied at two different

ratios (1% and 3%) and exposed to the influences of irradiation and temperature fluctuation and planted in soil. M-EM treatments only benefited the germination of deeper planted sorghum seeds compared to the control treatments. The shoot lengths of deeper planted maize and sunflower seed were positively increased by the M-EM treatments while also resulting in significant results for the overall shoot length of sorghum.

The third pot study was conducted to determine the influence of EM as a seed treatment on maize, sorghum and sunflower planted in three different soils, namely: sterilized soil, soil treated with M-EM and *Fusarium* containing soil. Germination and seedling vigour results of the sterilized and M-EM treated soil revealed to be superior to that of the *Fusarium* containing soil. From the results was concluded that M-EM treatments will probably improve early seedling growth of maize, sorghum and sunflower compared to untreated seed and that M-EM seed treatment and a pre-plant EM soil treatment might assist seeds in unfavourable germination and growth conditions.

List of Content

Language and style in this dissertation are in accordance with the requirements of the *South African Journal of Plant and Soil.* This dissertation represents a compilation of manuscripts where each chapter is an individual entity and some redundancy between chapters has, therefore, been unavoidable.

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List of Abbreviations

EM	-	Effective micro-organisms
S-EM	-	Stock Effective micro-organisms
M-EM	-	Multiplied Effective micro-organisms
A	-	Multiplied ratio A
B	-	Multiplied ratio B
R-S	-	Rise to set
24H	-	24 Hours
nm	-	Nanometer
pH	-	Acidity
ha	-	Hectare
^{o}C	-	Degree Celsius
N	-	Nitrogen
P	-	Phosphorus
K	-	Potassium
ℓ	-	Liter
ml	-	Milliliter
mm	-	Millimeter
cm	-	Centimeter
g	-	Gram
kg	-	Kilogram

Chapter 1
General Introduction

1.1 Motivation and problem identification

Soil microbiologists and microbial ecologists divided soil micro-organisms into two groups namely the beneficial and the harmful micro-organisms. This division is based on their effect on soil quality, plant health, plant growth and yield (Higa & Parr, 1994). A more specific classification for the beneficial micro-organisms was given by Professor T. Higa. The author refers to the organisms as “Effective micro-organisms” also known as “EM” (Higa & Parr, 1994). Effective micro-organisms are microbial inoculants which shift the microbiological equilibrium towards a better quality soil, enhanced crop production and protection, preserving natural resources and creating a more sustainable agriculture and environment (Higa & Parr, 1994).

EM is created through an organic process of fermentation and is not chemically synthesized or genetically engineered. EM contains over 80 selected types of micro-organisms (Woodward, 2003; Singh, 2007) including populations of lactic acid bacteria, photosynthetic bacteria, actinomycetes, fermenting fungi, yeasts and other types of organisms (Higa & Parr, 1994). This indicates that EM can be seen as a natural technology which does not have any known unfavourable effects on plants, animals, humans, or the ecosystem after more than a decade of application (Higa & Wood, 1998).

It has been scientifically documented that EM produces organic acids, plant hormones, vitamins, and antibiotics through fermentation reactions (Higa, 1996). These products are necessary to benefit growing plants and the soil by:

1. Protecting plants from soil-borne pathogens, insects, and diseases;
2. Stimulating plant growth, thereby increasing the yield and quality of crops;
3. Solubilisation of nutrients from materials of limited solubility, e.g. rock phosphate (Higa, 1996);
4. Complexion of heavy metals to restrict their uptake by plants;

5. Providing simple organic molecules for direct uptake by plants, e.g. amino acids;
6. Improving the chemical and physical properties of soils (Higa, 1996).

Although high-quality seed is expensive, the value of using high-quality seed continues to increase because of changing production systems (Lipps *et al*., 1998) and the establishment of new diseases, thus ensuring that expensive seeds have the best chance of survival to produce high yields is necessary. One of the benefits of EM is that EM ensures better germination by effectively suppressing certain disease organisms (Siqueira *et al.,* 1993). While also stimulating plant growth which increase vigour (Primavesi, 1997), leading to higher crop yields. Seed treatment with EM may therefore be used to enhance seedling emergence and crop performance during the growing season as rapid seedling emergence and an even stand are vital in maximizing the yield of all crops (Lipps *et al*., 1998).

According to Higa and Parr (1994), EM is able to increase the beneficial properties of the best soil and crop production practices. EM is not a replacement for the production practices, but can be seen as an extra dimension for optimizing practices such as conservation tillage, crop rotation, using organic amendments, crop residue recycling and pest bio-control. The ability of EM to suppress diseases (Primavesi, 1997) and to stimulate plant growth (Higa,1996) lead to the assumption that the introduction of EM into the production of economically important summer crops such as maize, sorghum and sunflower in the Free State province of South Africa, might play an important part in securing crop production.

The use of pesticides and fertilisers by farmers has increased the productivity of agricultural systems, though these methods often result in environmental deterioration and unsustainable systems (Condor Golec *et al.,* 2007). EM is known to actively increase the biodiversity of the micro-flora, which in turn increases the yield of a crop (Condor Golec *et al*., 2007). Thus the use of EM should be able to correct the damage in the microbial ecology of soil and may reduce the need for fertilizer. An additional benefit, according to Siqueira *et al.* (1993), is that microbial inoculants such as EM may increase the rate of germination so that weak seeds can survive and produce normal plantlets.

Commercially available EM can be bought in the form of Stock EM (S-EM) and this form of EM can be stored for up to six months (EMROSA, 2006; A. Rosenberg, personal communication, March 2009). The S-EM can be propagated for economical reasons into Multi-EM, by mixing with molasses and water which acts as food for the micro-organisms (Boermetem, 2008; EMROSA, 2008; A. Rosenberg, personal communication, March 2009). The M-EM, has the same strength as the original EM and can be diluted for application, just as with the original EM (D. Anthony, personal communication, March 2009). EM is multiplied in ratios of 30 to 100 times.

However, the availability of scientific research and publicised literature on the influence of EM as a seed treatment on the germination and vigour of different cultivars of maize, sorghum and sunflower is limited. There is no clear indication by EM producers at what ratio EM should be multiplied and at what dilution multiplied EM should be used as a seed treatment. The present work is an attempt to evaluate the effect of different handling techniques on EM and its effect on plant germination and vigour subjected to different environmental conditions.

1.2 Objectives

The overall objective of this study is to evaluate the use of EM seed treatments, at different application rates, handling techniques and soil conditions on germination and vigour of selected cultivars of maize, sorghum and sunflower.

1.2.1 Main objectives:

- To determine and compare the effect of EM from selected suppliers as seed treatment, at different application rates (two multiplied ratios nl. M-EM (A) at a ratio of 1% S-EM, 7% molasses and 92% water, M-EM (B) at a ratio of 3% S-EM, 5% molasses and 92% water, at three dilutions nl. 0.01%, 0.1% and 1%.), on the germination and seedling vigour of selected cultivars of maize, sorghum and sunflower under favourable as well as unfavourable germination conditions in incubation studies;

- To determine and compare the effect of EM from selected suppliers as seed treatment, subjected to irradiation and temperature fluctuation, on the seedling vigour of selected cultivars of maize, sorghum and sunflower under favourable as well as unfavourable germination conditions in incubation studies;
- To determine and compare the effect of EM from selected suppliers as seed treatment, at different application rates (two multiplied ratios nl. M-EM (A) at a ratio of 1% S-EM, 7% molasses and 92% water, M-EM (B) at a ratio of 3% S-EM, 5% molasses and 92% water, at three dilutions nl. 0.01%, 0.1% and 1%.), on germination and seedling vigour of selected cultivars of maize, sorghum and sunflower in pot experiments;
- To determine and compare the effect of EM from selected suppliers as seed treatment, subjected to irradiation and temperature fluctuation, on germination and seedling vigour of selected cultivars of maize, sorghum and sunflower in pot experiments;
- To determine and compare the effect of EM from selected suppliers as seed treatment, on germination and seedling vigour of selected cultivars of maize, sorghum and sunflower planted in sterilized, EM treated and *Fusarium* containing soil, in pot experiments.

1.3 Hypotheses

- EM as seed treatment at different application rates does not have the same effect on germination and seedling vigour;
- Irradiation and temperature fluctuation play an important roll in the effectiveness of EM as a seed treatment;
- Seed treatment with EM promotes faster and more uniform germination as well as improved seedling vigour.

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Chapter 2
Literature study

2.1 Introduction

The increase in world population has lead to the intensification of agricultural systems (Condor Golec *et al*., 2007). In South Africa the population has grown with 23% from 1996 (STATS, 2001) to 2010 (STATS, 2010) and South African farmers had to tern to the intensive use of chemicals in terms of fertilizer, pesticide, herbicide and fungicide in an attempt to secure production for the increase in demand. Due to the use of pesticides the productivity of agricultural systems has increased but environmental deterioration and unsustainable systems are the consequences of these ways of management (Condor Golec *et al*., 2007).

Due to use of unsustainable production systems used in South Africa, the land size available for the production of maize and sorghum, respectively, decreased by 28% and 60% between 1996 and 2010 (Grain SA, 2011). Nevertheless, with the help of research the average yield per hectare in South Africa has increased across all grain crops. The yield of maize had increased by 58% from the 1995/96 to 2010/11 seasons, while sorghum and sunflower yields increased by 16% and 5%, respectively (Grain SA, 2011). Improved production techniques in agriculture, due to applied research, led to the increased yields and a decrease in inputs.

The use of the environmentally friendly effective micro organisms (EM) as an input is highly recommended in the production of agriculture crops. This is due to the beneficial effect thereof on soil as claimed by supplier companies (Condor Golec *et al*., 2007). These beneficial effects include amongst others, the fixation of atmospheric nitrogen (Higa & Parr, 1994; EMROSA, 2008; Anthony, 2009, personal communication), the decomposition of organic wastes and residues (Higa & Parr, 1994; Anon, 1995; Waltz *et al*., 2001; Anthony, 2009, personal communication) and the suppression of soil-borne pathogens (Higa & Parr, 1994; Anon, 1995; Higa, 1996; EMROSA, 2008). With EM supplier companies excessively elaborating on successful trail results, the importance of

thorough scientific research about the effect of EM on crop production is necessary before the use of EM in crops could be justified.

2.2 Chemical usage in modern agriculture

The excessive use of fertiliser, pesticides and fungicides in modern agriculture, has left the present farmer with an increase in input costs and a decrease in productive land. According to Woodward (2003) the worldwide intensive agricultural usage of high quantities of chemicals leads to 7.5% of arable land being abandoned every 10 years, because of the destruction of soil enriching micro-organisms.

2.2.1 Chemical fertilizers

Mineral fertilizers are important in modern agriculture and is the quickest way of supplying nutrients to the plant and activating various plant enzymes (Tisdale *et al*., 1990 as cited by Shah *et al*., 2001). However, their high cost and short supply at the time of need prevent farmers from using recommended doses at all times (Shah *et al*., 2001). Shaffer (2001) reported on a test which was done on twenty-nine USA chemical fertilizers which were tested for twenty-two toxic heavy metals (Table 2.1). The results indicated that all twenty-nine fertilizers contained the twenty-two toxic heavy metals (Shaffer, 2001). The build-up of these toxic heavy metals in the soil can lead to the increase in soil acidity (Panchaban, 1991; Shaffer, 2001), the degrading of soil structure (Panchaban, 1991) and the pollution of surface and ground water through runoff and leaching (Shaffer, 2001). This necessitates the exploration of alternative potential sources of plant nutrients with the minimum use of mineral fertilizers.

Table 2.1 Twenty two toxic heavy metals which were found in twenty nine fertilizers (Shaffer, 2001).

Metal Tested	Number of Fertilizers Containing the Metal
Aluminium (Al)	29
Antimony (Sb)	29
Arsenic (As)	29
Barium (Ba)	29
Beryllium (Be)	29
Boron (B)	29
Cadmium (Cd)	29
Chromium (Cr)	29
Cobalt (Co)	29
Copper (Cu)	29
Iron (Fe)	29
Lead (Pb)	29
Manganese (Mn)	29
Mercury (Hg)	29
Molybdenum (Mo)	29
Nickel (Ni)	29
Selenium (Se)	29
Silver (Ag)	29
Thallium (Tl)	29
Vanadium (V)	29
Uranium (U)	29
Zinc (Zn)	29

2.2.2 Chemical pesticides and fungicides

The worldwide usage of pesticides has reached 2.6 million tons yearly of which 85% is for agriculture (Woodward, 2003). The data in Table 2.2 is from the National Centre for Food and Agricultural Policy in Washington D.C., which shows the pesticide use of the USA in 1997. Interestingly the two most used ingredients, namely oil at 46.3 million kg per year and sulphur at 78 million pounds per year, are approved for use in organic crop production (Avery, 2006).

Table 2.2 Ten Most Used Pesticides in the United States of America in 1997 (Gianessi & Marcelli, 2000).

Rank Active Ingredient	Millions kg/Year
1 Oil (I)	46.3
2 Sulphur (F)	35.5
3 Atrazine (H)	34
4 Metolachlor (H)	30.4
5 Metam Sodium (O)	27.2
6 Sulphuric Acid (O)	21.8
7 2,4-D (H)	18.6
8 1,3-D (O)	15.9
9 Glyphosate (H)	15.9
10 Methyl Bromide (O)	15
F = Fungicide, H = Herbicide, I = Insecticide, O = Other	

Some of the currently understood consequences for soil ecology, biodiversity, groundwater and health from the use of pesticides and fungicides are:

- Loss of micro-organisms: leading to the increase of agricultural pests and decrease in soil nutritive qualities. The obliteration of beneficial predators removes the homeostatic mechanisms for keeping dangerous agricultural pests in check, leading to an increase and not a decrease in the use of pesticides (Woodward, 2003). In the last 50 years the USA farmers have, with intensive pesticide use, doubled the loss of crops to pests;
- Climate change – global warming: agricultural practices cause severe decrease in the oxidation rates of atmospheric methane, more so in arable soils compared to forest soils (Woodward, 2003);
- Increased cost, diminished returns: the increase in cost of energy requirements of intensive agriculture, whether fuel for machinery or fertilizer for plants and even pesticides, increases year after year. There are also losses due to proliferation of pests, and effect on agricultural soils from pesticide pollution (Woodward, 2003);
- Loss of nutrients and antioxidants: antioxidants such as flavonoids, inositol, ubiquinone, saponin, low molecular polysaccharides, polyhenols and chelates of minerals are powerful and are found in Effective micro-organisms. These anti-oxidant substances are proven to provide humans with disease suppressions (Woodward, 2003).

This clearly necessitates the exploration of alternative potential sources of pest and fungi control with the minimum use of harmful chemicals. The negative effect that modern agricultural practices has on the environment through the use of chemicals either in the form of fertilizer or in the form of pesticides and fungicides necessitates the exploration for alternative and regenerative crop production systems such as organic farming and the use of Effective micro-organisms.

2.3 Beneficial and harmful micro-organisms

Soil micro-organisms are divided into two groups, namely the beneficial and the harmful micro-organisms, based on their effect on soil and plants (Higa & Parr, 1994). Beneficial micro-organisms are those that can fix atmospheric nitrogen, decompose organic wastes and produce bioactive compounds that stimulate plant growth such as vitamins, hormones and enzymes. Harmful micro-organisms are those which are able to induce plant diseases, and stimulate soil-borne pathogens that negatively affect plant growth and health (Higa & Parr, 1994).

2.3.1 Functions of beneficial micro-organisms (EM)

Some general functions of beneficial soil micro-organisms as they influence soil quality, crop production, and plant health, are indicated below:

- Fixation of atmospheric nitrogen (Higa & Parr, 1994; EMROSA, 2008; Anthony, 2009, personal communication);
- Decomposition of organic wastes and residues (Higa & Parr, 1994; Anon, 1995; Waltz *et al*., 2001; Anthony, 2009, personal communication);
- Suppression of soil-borne pathogens (Higa & Parr, 1994; Anon, 1995; Higa, 1996; EMROSA, 2008);
- Recycling and increased availability of plant nutrients (Higa & Parr, 1994; Anon, 1995; Waltz *et al*., 2001);
- Degradation of toxicants, including pesticides (Higa & Parr, 1994);
- Production of antibiotics and other bio-active compounds (Higa & Parr, 1994; Konoplya & Higa, 2001);
- Production of simple organic molecules for plant uptake (Higa & Parr, 1994; Anon, 1995; Higa, 1996; Waltz *et al*., 2001);

- Complexion of heavy metals to limit plant uptake (Higa & Parr, 1994; Higa, 1996);
- Solubilisation of insoluble nutrient sources (Higa & Parr, 1994; Higa, 1996);
- Production of polysaccharides to improve soil aggregation (Higa & Parr, 1994; Anon, 1995; Higa, 1996);
- Increase in plant germination, flowering, fruiting and ripening (Anon, 1995; Higa, 1996; Konoplya & Higa, 2001; EMROSA, 2008; Anthony, 2009, personal communication);
- Enhance the photosynthetic capacity of crops (Anon, 1995; Higa, 1996; Konoplya & Higa, 2001).

2.3.2 Functions of harmful micro-organisms according to Higa and Parr (1994)

The influence of harmful soil micro-organisms as they influence soil quality, crop production, and plant health, includes:

- Induction of plant diseases;
- Stimulation of soil-borne pathogens;
- Immobilization of plant nutrients;
- Inhibition of seed germination;
- Inhibition of plant growth and development;
- Production of phytotoxic substances.

2.4 The main components of EM

EM is a fluid of effective micro-organisms, not containing any mineral or having any nutritional value (Higa & Wood, 1998), nor a fertilizer. EM is however, used in creating beneficial conditions in soil. There are five main types of bacteria used to set up EM into a solution (Condor Golec *et al*., 2007). This solution is inoculated into soil for its beneficial qualities on the soil itself and on production, each with its own specific benefits (Anon, 1995).

2.4.1 Photosynthetic bacteria

These are independent, self supporting micro-organisms (Condor Golec *et al*., 2007). The main species include *Rhodopseudomonas palustrus* and *Rhodobacter spaeroides* (Diver, 2001 as cited by

Szymanski & Patterson, 2003). These bacteria produce amino acids, nucleic acids, bio-active substances and sugars (Anon, 1995), substances from the secretions of roots, and organic matter by using sunlight (Condor Golec *et al*., 2007) and the warmth of soil as sources of energy (Anthony, 2009, personal communication). Energy from infrared bands of solar radiation from 700 nm to 1200 nm cannot be used by the plants (Condor Golec *et al*., 2007), however photosynthetic bacteria can use these solar radiations to produce organic matter, and thus the effectiveness of the plant is increased (Anon, 1995; Anthony, 2009, personal communication). The metabolites are absorbed by the plants directly and act as substrates for bacteria raising the biodiversity of the micro-flora. The addition of photosynthetic bacteria into soil enhances other effective micro-organisms (Anon, 1995; Condor Golec *et al*., 2007; Anthony, 2009, personal communication).

2.4.2 Lactic acid bacteria

Lactic acid bacteria produce lactic acid from sugars (Anon, 1995; Condor Golec *et al*., 2007; Anthony, 2009, personal communication). The main bacteria species included in lactic acid bacteria is *Lactobacillus plantarum, L.casei* and *Streptoccus lactis* (Diver, 2001 as cited by Szymanski & Patterson, 2003). Food such as yoghurt from milk, pickles from cucumbers and sauerkraut from cabbage (Tortora *et al*., 1995) are made by using lactic acid bacteria. Lactic acid is nevertheless a strong sterilizer (Anon, 1995) and is suppressive against harmful micro-organisms (Anthony, 2009, personal communication) and the decomposition of organic matter is rapidly increased (Condor Golec *et al*., 2007). Lactic acid bacteria furthermore enhance the breakdown of organic matter such as lignin and cellulose, and ferment those materials which normally take a long time. Lactic acid bacteria possess the ability to suppress *Fusarium* proliferation which is a harmful micro-organism causing disease problems in continuous cropping. *Fusarium* promotes the enhancement of harmful nematodes (Anon, 1995; Condor Golec *et al*., 2007; Anthony, 2009, personal communication). As lactic acid bacteria restrains the propagation and function of *Fusarium*, the occurrence of harmful nematodes will disappear steadily (Condor Golec *et al*., 2007).

2.4.3 Yeasts

Yeasts are non-filamentous, unicellular fungi that are typically spherical or oval (Tortora *et al*., 1995). The main species include *Saccharomyces cerevisiae* and *Candida utilis* (Diver, 2001 as cited by Szymanski & Patterson, 2003). Yeasts synthesizes antimicrobial and valuable substances for plant growth with amino acids and sugars, which is concealed by photosynthetic bacteria, organic matter and plant roots (Anon, 1995). Bio-active substances produced by yeasts like hormones and enzymes, promote vigorous cell and root split. Their discharges are valuable substances for effective micro-organisms such as lactic acid bacteria and actinomycetes (Condor Golec *et al*., 2007; Anthony, 2009, personal communication). Yeasts have been found in comparable numbers in soils of Antarctica, in grasslands, in cultivated fields and forests and they are sometimes particularly numerous on the roots of certain plants (Alexander, 1977).

2.4.4 Actinomycetes

Actinomycetes are a broad group of bacteria that form thread-like filaments in the soil, producing antimicrobial substances from amino acids concealed by photosynthetic bacteria and organic matter (Alexander, 1977). The main species include *Streptomyces albus* and *S. griseus* (Diver, 2001 as cited by Szymanski & Patterson, 2003). These antimicrobial substances restrain harmful fungi and bacteria and are able to co-exist with photosynthetic bacteria and therefore both species improve the quality of the soil ecosystem by raising the antimicrobial activity of soil (Condor Golec *et al*., 2007). These organisms are found in very large numbers in soil and produce a gaseous substance called *geosmin*, which gives the soil its characteristic musty odour (Tortora *et al*., 1995). They are particularly responsive to pH changes with populations being maximum at pH values above 6.0 and almost absent at pH 5.0 (Waltz *et al*., 2001).

2.4.5 Fermenting Fungi

Fermenting fungi such as *Aspergillus oryzae, Mucor hiemalis* (Diver, 2001 as cited by Szymanski & Patterson, 2003) and *Penicillium* (Condor Golec *et al*., 2007) decompose organic matter swiftly to produce alcohol, esters and antimicrobial substances. They suppress foul odours and avoid

infestation of damaging insects and harmful maggots (Anon, 1995; Condor Golec *et al*., 2007; Anthony, 2009 personal communication).

2.5 The effect of EM on soil organic amendments

According to Panchaban (1991) infertile and inefficient soils can be a result of infertile parent material such as sandstone, extreme soil erosion and nutrient run-off, intensive tillage, cropping cycles and inadequate use of chemical fertilizer. The best method to improve the productivity of these extremely marginal and infertile soils is through frequent addition of organic amendments and residues (Hornick & Parr, 1987) supplemented with sensible amounts of chemical fertilizers (Panchaban, 1991).

Micro-organisms decompose and ferment raw organic material into humus, containing nutrients and hormones which help the plant grow (Khaliq *et al.*, 2006). Micro-organisms are also responsible for providing these hormones, nutrients and minerals in a transferable form to the plants via the root ecology (Higa & Parr, 1994; Anon, 1995; Higa, 1996; Woodward, 2003). While organic amendments assist in improving soil physical properties (Khaliq *et al.*, 2006) and the withholding of plant nutrients in the soil-root zone (Hornick & Parr, 1987) where they can be used effectively by plants (Panchaban, 1991).

It is of utmost importance to regularly add organic materials such as animal manures and crop residues for the maintenance of fertility and productivity in agricultural soils (Hornick & Parr, 1987). These plant and animal remnants decompose in the soil into humus which discharge compounds of nitrogen, phosphate and potassium which then could be absorbed by plants (Hewitt & Brazier, 1986). Organic fertilisers can be applied at high rates because they contain little or no soluble salt and without risk of damaging crop roots, which may occur with the use of heavy doses of inorganic fertilisers (Hewitt & Brazier, 1986).

Organic amendments can be in the form of any plant based material, from animal manures to crop residues, with the main sources of agricultural organic amendments listed below:

- Ploughed-in plant remains, stubble and straw;
- Ploughed-in pasture;
- Green manure crops, mainly legumes;
- Farmyard manure;
- Industrial organic waste.

Higa and Wididana (1991) conducted a study on the effect of EM on soil and organic amendments. The authors found that cultivation depth and porosity was significantly higher in soil treated with EM and dry grass than in soil treated only with dry grass. There was however, no significant difference in bulk density throughout the study. Aggregation was higher for all EM treatments than with the control and EM decreased with application of chemical fertilizer (Higa & Wididana, 1991). Little difference was seen in the effect of EM treatment on soil pH and on nutrients such as nitrate, ammonium, and potassium. The effect of EM on soil physical properties suggests that EM can induce plant roots to penetrate soil more effectively. Soil treated with EM became more friable and porous, less compact, and promoted deeper cultivation. Micro-organisms, particularly fungi, can bind soil particles into more stable aggregates (Higa & Wididana, 1991).

A study by Condor Golec *et al*. (2007) supports the findings of Higa & Wididana (1991), as the results indicated that there were no differences in the physical properties such as bulk density and compaction in a citrus soil analysis after the use of EM, although the humus in the soil had increased. This is an indication of the fermentation ability of EM.

2.6 The influence of EM seed treatment

According to Tamilnadu (2009) seeds may be treated with a variety of substances ranging from conditioned water to chemicals in an effort to increase crop yield. The listed objectives play a roll in the choice to treat seed;

- Preventing the spread of diseases;
- Protecting seed against seed rot and seedling blight;

- Improving seed germination;
- Controlling soil insects.

2.6.1 The effect of EM seed treatment on germination and vigour

A study by Siqueira *et al.* (1993) evaluated the influence of EM and Vairo (a bio-fertilizer) on seed germination and vigour. The trials were conducted on cucumber, carrot, beet, tomato, pepper, corn, pea, burdock and beans. Siqueira *et al.* (1993) found significant differences among treatments in the germination percentage of pea, beet, pepper, tomato, cucumber, corn, carrot, beans and burdock. The EM treatment had the greatest number of germinated seeds. The seedling root lengths for cucumber, beet, pea, pepper and carrot were significantly greater than the control. The root lengths of tomato seedlings which were treated with EM were comparable to those with the bio fertilizer (Vairo) and control treatments (Siqueira *et al.,* 1993). The roots of the cucumber plantlets were comparable to the bio fertilizer treatment. Total weight of pea, beet, carrot, bean, burdock and corn were significantly higher with EM treatment than the control. However, pepper, cucumber and tomato were not different from the control. Siqueira *et al.* (1993) also found that the weights for pea, corn and beet with EM were greater than with the bio fertilizer treatment. For most of the crops tested by Siqueira *et al.* (1993) the root length and total seedling weight were greatest for the EM treatments compared to the control and to the bio fertilizer treatment at a 5% level of probability.

Tokeshi and Changas (1997) treated seed of Cleopatra tangerine with EM to determine the effect of EM on germination and vigour. The following conclusions were drafted after completion of the study:

- EM presents a similar effect as gibberelic acid, improving the emergence and vigour of the seed;
- The seedling survival ability increased with EM seed treatment;
- EM treated seedlings were superior to that of the control.

2.6.2 The effect of EM seed treatment on soil chemical and microbial content

Lim *et al*. (1997) conducted a study on the use of EM on rice and maize. This was done to determine the influence of the treatment of seed, the seedling nursery bed, the field, and a combination of all with EM. Results indicated that the amount of beneficial micro-organisms, the availability of nutrients and the content of organic matter increased where EM was applied to soil and enhanced the neutralization of soil (Lim *et al*., 1997). The incidence of aerobic bacteria, anaerobic bacteria, nitrogen-fixing bacteria and Actinomycetes increased 10.5, 17.8, 49.6 and 1.7 fold respectively compared to the control (Lim *et al*., 1997). EM also increased the content of soluble nutrients. Soil content of soluble nitrogen, phosphorous and potassium improved with 4.4, 3.6 and 2.8 mg $100g^{-1}$ soil respectively. Soil treated with EM had a pH of 0.1 higher than that of the control. The increase of soluble N, P and K content may possibly be attributed to the activity of nitrogen fixers and organic acids produced by different organisms of EM (Lim *et al*., 1997).

2.6.3 The effect of EM seed treatment on diseases

A study was conducted by Primavesi (1997) on the seed treatment with EM and micronutrients for controlling rice and maize diseases. The study was conducted to determine whether seed treatment with EM and micronutrients could reduce the incidence of disease and parasite injury and prevent nutrient imbalances after germination. Results revealed that EM treatments were effective in suppressing *Spodoptera* and *Elasmopalpus* and resulted in a maize yield of 30% higher than the other treatments. These results are an indication of both the parasite-suppressive ability of EM, and its beneficial effects on plant growth and yield (Primavesi, 1997).

2.6.4 Influence of EM on maize growth, yield and quality

Studies conducted by researchers on the effect of EM on maize mostly resulted in a positive effect on yield and other production parameters. These studies include research by Panchaban in 1991, Shah *et al*. in 2001 and by Lim *et al*. in 1997.

Panchaban (1991) conducted a study to compare the result of EM with other conventional types of fertilizer (chemical and organic) and lime on the growth and yield of maize under field conditions. The results of the field study indicated that EM treatment improved the decomposition of bagasse (an organic fertilizer) and released obtainable plant nutrients at a rate that could maintain the growth and yield of maize (Panchaban, 1991).

Results from a study by Shah *et al*. (2001) on the effect of different fertilizers and EM on growth, yield and quality of maize revealed that the highest grain yield of 4.72 t ha^{-1} was obtained with the application of 150 kg N + 75 kg P_2O_5 + 30 ℓ EM ha^{-1}. The increase in yield was attributed to increased leaf area, improved number of grains per cob (572.40) and higher weight per 1000 grains (234.30 g). The protein content (10.03%) was however, higher with the application of 75 kg N + 37.5 kg P_2O_5 + 60 ℓ EM ha^{-1}. A conclusion was drawn which indicated that fertilizer in combination with EM could have a highly significant effect on grain yield (Shah *et al*., 2001).

The results which were found by Lim *et al*. (1997) on the use of EM on rice and maize also indicated positive results in yield as was found by Panchaban (1991) and Shah *et al*. (2001). Lim *et al*. (1997) found that the use of EM increased the yield of rice and maize over each control as follows; 7.2% and 7.4% where only seeds were treated; 7.1% and 7.4% in seedling nursery bed treatment; 4.2% and 13% in field treatment; 9.5% and 14.9% in combination treatment, 9.0% and 30% on continuous application of EM. The growth of crop plants was improved as a whole, therefore resulting in yield increase (Lim *et al*., 1997).

2.7 Continuous application of EM

The continuous treatment of the same soil with EM over a long period increases the density of EM in the soil which in tern leads to improved effects on soil health and production. Lim *et al*. (1997) found in their study that the rice yield after three years of continuous EM application was higher than that of the first year. From the study Lim *et al*. (1997) concluded that EM should be applied several times in order to increase the concentration of EM in soil. This could be achieved by a combination of various treatments such as seed treatment, nursery seedbed treatment and main field application. Such combined treatments are able to

intensify the density of EM in every soil and plant based cultural operation. Daly (2004) reported that after four years of EM use, soil structure had improved, yield improved and stabilized, weed management improved and quality of produce improved.

2.8 Theories on the effectiveness of EM

Various research results contradict each other, leading to theories being formed as reasons for the contradictions. According to Condor Golec *et al.* (2007) there are factors in the ecosystem of the soil which reduce the effectiveness of EM. To achieve the favourable effect of EM in the soil is complicated, since the number of micro-organisms added by the EM solution is insignificant compared to the sum of micro-organisms in the soil (about 10^9) and therefore no effects are expected (Condor Golec *et al*., 2007). There is a complex competitive and symbiotic relation between micro-organisms in the soil. Adding EM disrupts the relation and this leads to the destruction of the EM and a quick restoration of the initial equilibrium in the soil (Condor Golec *et al*., 2007).

The ecosystem of the soil is difficult to change. The probability exists that in other areas where EM has been tested by diverse departments of different universities in tropical countries, EM had positive effects because of the reduced amount of micro fauna in the soil (Condor Golec *et al*., 2007). In contrast, research done at Wageningen University, The Netherlands, the main conclusion was that EM is ineffective (Condor Golec *et al*., 2007). This conclusion is supported by results of Ncube (2008) who evaluated the effect of EM on soil properties and yield of vegetables. Ncube (2008) concluded that EM has inconsistent results, and no significant effect on production. EM even had a depressive effect on fruit yield in some cases (Ncube, 2008).

2.9 Applying EM in practice

EM can be bought from commercial companies in the form of Stock EM (S-EM) and this form of EM can be stored for up to six months in the right conditions (EMROSA, 2006; A. Rosenberg, personal communication, March 2009). When a producer plans to use the EM, EM can be multiplied into Multi-EM (M-EM). When EM is multiplied the EM is not diluted, EM multiplies in ratios of 30 to 100 times.

M-EM can safely be used for one month (EMROSA, 2006) if conditions are favourable (A. Rosenberg, personal communication, March 2009).

2.9.1 Stock EM

S-EM has a sweet-sour smell and taste, and has a pH of below 3.7 (Anon, 1995; EMROSA, 2008; D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009). EM needs to be stored in an airtight plastic container. Because EM consists of live organisms, EM produces gas, which needs to be released occasionally (A. Rosenberg, personal communication, March 2009). For smaller quantities this can be done by opening the container as needed. With large quantities, the most convenient would be to glue a tube into a hole in the lid and place the other end into a bottle of water, so gas can escape and no oxygen can flow back (D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009).

The best temperature for storage is between 15^{o}C and 20^{o}C, with a little fluctuation of less than 10^{o}C in 24 hours (EMROSA, 2008; A. Rosenberg, personal communication, March 2009). EM needs to be stored away from direct sunlight, preferably in a storeroom (EMROSA, 2008; D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009). Ideally blankets or bubble sheets should be used as insulator at the top and the sides of the containers (EMROSA, 2008). The container should be placed directly onto the ground in summer for coolness and in winter the insulation should be placed under the base of the container as well (D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009).

2.9.2 Multiplied EM

S-EM is multiplied to save money (D. Anthony, personal communication, March 2009). The micro-organisms are triggered into a growing phase and multiply in an anaerobic condition. EM keeps on multiplying until the microbial units and components are the same as that of the S-EM.

To multiply EM into M-EM the following ingredients are required (Figure 2.1):

1. 1-3% good quality S-EM (Boermetem, 2008; EMROSA, 2008; A. Rosenberg, personal communication, March 2009).
2. 5-7% pure liquid cane molasses (Boermetem, 2008; EMROSA, 2008; A. Rosenberg, personal communication, March 2009).
3. 92% water (EMROSA, 2008; A. Rosenberg, personal communication, March 2009).

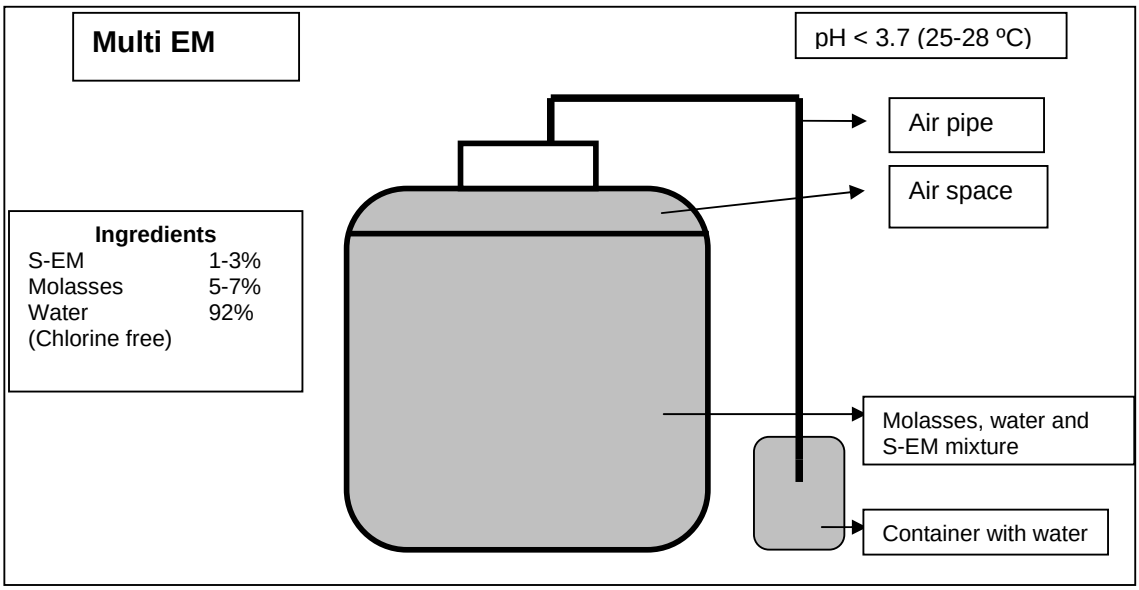

Figure 2.1 Ingredients and process of making M-EM from S-EM using water and molasses with a breather pipe system (A. Rosenberg, personal communication, March 2009).

Borehole water suitable for drinking (A. Rosenberg, personal communication, March 2009) or municipal water can be used. If municipal water is used, the containers must be left open in the sun for up to a day to get rid of the chlorine, before being used (EMROSA, 2008).

The procedure for preparing M-EM comprises the mixing of the molasses with warm water in order to dissolve the molasses completely, mixing the S-EM into the molasses-water, filling the container with good quality (ideally warm) water, leaving a gap at the top for air and mixing the ingredients well (A. Rosenberg, personal communication, March 2009). The container should be sealed airtight and gas should be release often by opening the container or by

using a breather pipe system (D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009).

Good quality M-EM

When the M-EM is ready, the M-EM will smell and taste as the S-EM did (A. Rosenberg, personal communication, March 2009). The M-EM will be ready in three to 14 days, depending on the temperature. For a favourable environment the pH needs to be below 3.7 (Boermetem, 2008; A. Rosenberg, personal communication, March 2009). M-EM should be used within 30 days of reaching the desired pH level (Boermetem, 2008; EMROSA, 2008; D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009).

2.9.3 Application

EM can be applied as a seed treatment, pre-planting treatment, again at planting and then every three to four weeks during crop growth (D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009). EM can also be applied to crop residues after harvest and just before incorporating residues into the soil (D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009).

2.9.3.1 Seed treatment

According to D. Anthony (personal communication, March 2009) seeds should be soaked for five to ten minutes in a one to one thousand dilution. A. Rosenberg (personal communication, March 2009) states that small seeds should be soaked for up to 30 minutes, and large seeds such as maize for up to 8 hours. Seeds should be dried under shade to avoid sticking together (D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009). This process promotes faster and even germination as well as healthy growth of plants (A. Rosenberg, personal communication, March 2009). Seed treatment with EM is not advocated for plants of the legume family (A. Rosenberg, personal communication, March 2009).

2.9.3.2 Pre-planting

Apply EM to the soil between two to three weeks prior to planting (D. Anthony, personal communication, March 2009). Spray 30 ℓ to 50 ℓ ha^{-1} EM in a dilution of 1:100 and cultivate weeds that emerge after 10 - 14 days (D. Anthony, personal communication, March 2009).

2.9.3.3 Planting.

Apply 30 ℓ to 50 ℓ ha^{-1} EM with Fertilizer (D. Anthony, personal communication, March 2009).

2.9.3.4 Plant treatment

Dilute M-EM 1:500 to 1:1000 (D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009) and spray the dilution onto the plants every three to four weeks during the growth period (D. Anthony, personal communication, March 2009).

2.9.3.5 After harvest

Apply an M-EM dilution of 1:200 during or after harvest on the crop rests. This will help in the breakdown of the rests into organic material (D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009).

2.9.3.6 Soil treatment

M-EM at 1 ℓ ha^{-1} or S-EM 1 ℓ ha^{-1} should be diluted with water at a ratio of 1:100 to 1:500 (D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009). This diluted EM should be sprayed on the soil before cultivation. EM should not be sprayed onto the produced product as the EM might have a negative impact on the quality of the product (D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009).

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Chapter 3

The influence of Effective micro-organisms at different dilutions, on the germination and seedling vigour of maize, sorghum and sunflower

Abstract

Two incubation experiments were conducted to determine and compare the effect of Multiplied effective micro-organisms (M-EM) from three selected suppliers multiplied at two ratios and diluted at three levels on, i) germination and ii) seedling vigour of maize, sorghum and sunflower under favourable conditions and after exposure to cold stress.

Two cultivars of maize, sorghum and sunflower were used in both experiments where Effective micro-organisms (EM) was multiplied at two ratios namely: 1% and 3%. Seeds were treated for seven hours with diluted M-EM at three levels namely: 0.01%, 0.1% and 1%. In the first experiment treated seeds were germinated in favourable conditions (25^{o}C) while, in the second experiment treated seeds were first exposed to cold stress (10^{o}C) for seven days prior to favourable conditions (25^{o}C).

Germination percentages under favourable conditions were not significantly increased by M-EM treatments compared to the control treatments. Under cold stress conditions germination and seedling vigour of M-EM treated seeds were in some cases significantly improved compared to the control. M-EM of all three suppliers at both multiplied ratios and at all three dilutions, lead to positive as well as negative results. M-EM as a seed treatment may therefore have an improved positive effect on germination and seedling vigour during stressed cropping seasons.

Keywords: Cold test, favourable germination conditions, seed treatment, Multi-EM.

3.1 Introduction

Effective micro-organisms (EM) consist of a wide variety of effective, beneficial and non-pathogenic micro-organisms. EM is produced through a natural process and is therefore not chemically synthesized or genetically engineered (EMROJP,

2010). EM is distributed in a liquid form, whiles not a fertilizer and therefore does not have a mineral or nutritional value (Higa & Wood, 1998). EM can be propagated by mixing with molasses and water which acts as food for the micro-organisms (Boermetem, 2008; EMROSA, 2008; A. Rosenberg, personal communication, March 2009). This is known as Multi-EM (M-EM) (EMROSA, 2008). EM is multiplied for economical reasons (D. Anthony, personal communication, March 2009) and after propagation into M-EM, M-EM has the same strength as the original EM and can be diluted for application, just as with the original EM (D. Anthony, personal communication, March 2009).

Seed can be inoculated with EM which gives the seed an added advantage. EM inoculation promotes faster and more uniform germination as well as healthy growth of plants (A. Rosenberg, personal communication, March 2009). According to D. Anthony (personal communication, March 2009) and A. Rosenberg (personal communication, March 2009), seeds should be soaked in a one to one thousand dilution. However, D. Anthony (personal communication, March 2009) suggests soaking seeds for five to ten minutes, while A. Rosenberg (personal communication, March 2009) suggests that small seeds should be soaked for up to 30 minutes, and large seeds such as maize for up to 8 hours. After soaking, seeds should be dried in the shade to avoid them from sticking together (D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009).

The effects of EM seed treatment on plant growth were studied by Siqueira *et al.* (1993), Primavesi (1997) and Tokeshi and Changas (1997). All three studies indicated that seedlings of seed treated with EM were superior to that of the respective controls. Siqueira *et al.* (1993) found that soaking seeds for 10 minutes in undiluted EM significantly increased germination, root length and total seedling weight. This was confirmed by Tokeshi and Changas (1997) who also found that EM improves the emergence and vigour of seeds, which increases the seedling's ability to survive. Primavesi (1997) concluded that not only did EM have beneficial effects on plant growth and yield but also has the ability to suppress parasites.

Because of the beneficial effects achieved through the treatment of other crop seeds with EM, EM seems of value to evaluate what the effect of EM seed treatments will be on selected summer grain crops. Therefore, the objective of

this study was to determine the effect of EM seed treatment, at different dilutions, on the germination and seedling vigour of maize, sorghum and sunflower. This was evaluated in incubation studies under favourable germination conditions for i) good quality seed and ii) good quality seed, subjected to cold stress.

3.2 Material and methods

3.2.1 Location and experimental layout

Two independent experiments were conducted in a laboratory of the School for Agricultural and Environmental Sciences of the Central University of Technology, Free State. Maize of the cultivars, PAN 6236 (cultivar 1) and PAN 6053 (cultivar 2), sorghum of the cultivars, PAN 8247 (cultivar 3) and PAN 8816 (cultivar 4), and sunflower of the cultivars, PAN 7351 (cultivar 5) and PAN 7033 (cultivar 6), were used in both experiments. Seeds were surface sterilized in a 3.5% sodium hypochlorite solution for 10 minutes and subsequently triple rinsed in pure water. A total of 800 and 2400 seeds were used per cultivar for the two experiments respectively. Each experiment was replicated four times.

3.2.1.1 The maize, sorghum and sunflower cultivars

- PAN 6236 (cultivar 1) is an ultra early yellow maize, which achieves excellent results under irrigation as well as high potential dry land conditions. The cultivar does exceptionally well in the Orange River area and other warm irrigation regions (PANNAR, 2011).
- PAN 6053 (cultivar 2) is medium maturing white maize cultivar, with excellent yield potential and proven reliability under low rainfall conditions, producing yields at low plant populations (PANNAR, 2011).
- PAN 8247 (cultivar 3) is a sorghum with good yield potential and has a very uniform plant type (PANNAR, 2011).
- PAN 8816 (cultivar 4) is a popular sorghum and recommended for the main planting in all sorghum production areas. The cultivar has an excellent yield potential and stability (PANNAR, 2011).
- PAN 7351 (cultivar 5) is a sunflower with a wide area adaptability, a high yield potential and a good stability, with outstanding performance in commercial plantings (PANNAR, 2011).

- PAN 7033 (cultivar 6) is a top performer sunflower in cultivar trails over the past three years and is recommended for the main bulk planting in all production regions (PANNAR, 2011).

3.2.2 M-EM dilutions

Generally fallible Stock-EM (S-EM) was bought from three different commercial companies. Due to a secrecy agreement names will be withheld and in this document the products will be revered to as S1, S2 and S3. Multi-EM (M-EM) was produced of each of the three suppliers S-EM at the following ratios:

- M-EM (A) at a ratio of 1% S-EM, 7% molasses and 92% water.
- M-EM (B) at a ratio of 3% S-EM, 5% molasses and 92% water.

After the M-EM stood for 14 days to multiply, each of the three M-EM (A) and three M-EM (B) was diluted with water at three levels. The first was at 0.01%, the second at 0.1% (which is also the standard dilution in practice) and the third was a dilution of 1%. A control that consisted of soaking seeds in purified water was prepared for comparison. Ten seeds of each cultivar were soaked for seven hours in the three dilutions of M-EM (A) and M-EM (B) of each supplier EM, in a dark environment. After soaking, the seeds were left to dry in the laboratory.

To simplify statistical analysis and interpretation of results, EM suppliers, multiplied ratios, dilutions and the control treatments were pooled into 20 treatment combinations (Table 3.1), which will be referred to as treatments throughout the rest of this chapter. Treatment abbreviations are coded and are not an indication of the supplier company.

Table 3.1 Treatment combinations 1 to 20 with regard to treatment abbreviation, EM supplier company, multiplied ratio and dilution.

Treatment number	Treatment abbreviation	Supplier company	Multiplied ratio	Dilution
1	S1 A 0.01%	1	A	0.01%
2	S1 A 0.1%	1	A	0.1%
3	S1 A 1%	1	A	1%
4	S1 B 0.01%	1	B	0.01%
5	S1 B 0.1%	1	B	0.1%
6	S1 B 1%	1	B	1%
7	S2 A 0.01%	2	A	0.01%
8	S2 A 0.1%	2	A	0.1%
9	S2 A 1%	2	A	1%
10	S2 B 0.01%	2	B	0.01%
11	S2 B 0.1%	2	B	0.1%
12	S2 B 1%	2	B	1%
13	S3 A 0.01%	3	A	0.01%
14	S3 A 0.1%	3	A	0.1%
15	S3 A 1%	3	A	1%
16	S3 B 0.01%	3	B	0.01%
17	S3 B 0.1%	3	B	0.1%
18	S3 B 1%	3	B	1%
19	Control	Control	N/A	N/A
20	Control	Control	N/A	N/A

3.2.3 Experiment 1: Germination under favourable conditions

Dried seeds were placed in 90 mm-diameter Petri dishes on filter paper which were moistened with 10 ml pure water and covered with a second filter paper. The Petri dishes were sealed in plastic Ziploc bags to prevent moisture loss. The seeds were placed in a temperature controlled cabinet at 25^{o}C and the experiment was terminated after seven days (Table 3.2).

Table 3.2 Favourable conditions – EM seed treatment variables per crop. Stock EM from three different suppliers were multiplied at two ratios (1% and 3%) and diluted at three dilutions (0.01%, 0.1% and 1%). Each replication had two control treatments which consisted of untreated seed.

	Maize	Sorghum	Sunflower
Cultivars	2	2	2
Number of seeds per Petri dish	10	10	10
Replications	4	4	4
EM suppliers	3	3	3
Multiplied ratios	2	2	2
Dilutions	3	3	3
Control	2	2	2
Total number of seeds	1600	1600	1600

3.2.4 Experiment 2: Germination and seedling vigour of seed subjected to the cold test

The cold test was executed as described in the ISTA Handbook of Vigour Test Methods by Hampton and TeKrony (1995):

1. On the day before planting, pure water was cooled overnight to 10^{o}C.
2. A double layer of paper towels (230mm×280mm) were saturated with approximately 35 ml of the cooled water. The dried seeds in each treatment were placed on the double layer of saturated paper towels in two rows of five seeds each, 6 cm and 12 cm from the top edge of the towels. A single saturated paper towel was placed over the two lower towels covering the seed.
3. The three towels were then rolled up. Care was taken to ensure that the towels did not warm up above 10^{o}C during and after preparation.
4. The rolled towels were placed upright in a plastic container before they were transferred to the cold (10^{o}C) chamber. Each rolled towel was placed in a plastic bag, to keep upright and separated. The plastic bags were sealed off to prevent loss of moisture and cross contamination.
5. The containers were kept in the cold chamber at 10^{o}C in darkness for seven days.
6. After the cold treatment the containers were moved to the germination chamber at 25^{o}C also in darkness

Table 3.3 stipulates the quantity of seeds that was used for each grain crop and the combination of variables in the experiment.

Table 3.3 Cold test – M-EM seed treatment variables per crop. Stock EM from three different suppliers were multiplied at two ratios (1% and 3%) and diluted at three dilutions (0.01%, 0.1% and 1%). Each replication had two control treatments which consisted of untreated seed.

	Maize	**Sorghum**	**Sunflower**
Cultivars	2	2	2
Number of seeds	10	10	10
Replications	12	12	12
EM suppliers	3	3	3
Dilutions	3	3	3
Multiplied ratios	2	2	2
Control	2	2	2
Total number of seeds	4800	4800	4800

3.2.5 Measurements

In experiment 1, germination was scored at a radical protrusion of 3 mm. Petri dishes were inspected in 24 hour intervals for seven days after planting. For experiment 2, germination was scored at a radical protrusion of 3 mm and seedling lengths were measured at 48, 96 and 168 hour intervals after transfer to the germination chamber to determine vigour.

3.2.6 Statistical analysis

A factorial analysis of variance (ANOVA) was performed on the germination and seedling vigour with cultivars, suppliers, EM ratios, EM dilutions and time as factors. P-values were used to compare means at a 5% probability level, using STATISTICA version 8.0 (Statsoft Inc., 2004).

3.3 Results and discussion

3.3.1 Experiment 1: Germination under favourable conditions

The general trend for all crops germinated at highly favourable conditions was that germination started slowly within the first 24 hours, followed by a rapid increase to the 48 hour measurement, while most of the seeds were germinated after 72 hours (Figures 3.1a - f). The only significant interaction between cultivar, time and treatment was for maize (Table 3.4).

Table 3.4 Analysis of variance (ANOVA) of germination of maize, sorghum and sunflower, germinated under favourable temperature and moisture conditions, as affected by cultivar, time and treatment.

	p-values		
Effect	**Maize**	**Sorghum**	**Sunflower**
Cultivar	0.0018	0.0000	N/S
Time	0.0000	0.0000	0.0000
Treatment	0.0000	0.0000	0.0000
Cultivar*Time	0.0000	0.0016	0.0000
Cultivar*Treatment	0.0439	0.0030	0.0133
Time*Treatment	0.0026	N/S	0.0383
Cultivar*Time*Treatment	0.0104	N/S	N/S

Significant differences over time were, however, mainly caused by differences in germination percentage between days (Figure 3.1.1 - 3.1.3). This was also true for the first degree interaction between time and treatment for all crops. Since the effect of time on germination is to be expected, the discussion will rather be focussed on the first degree interactions between cultivar and time as well as cultivar and treatment for all crops.

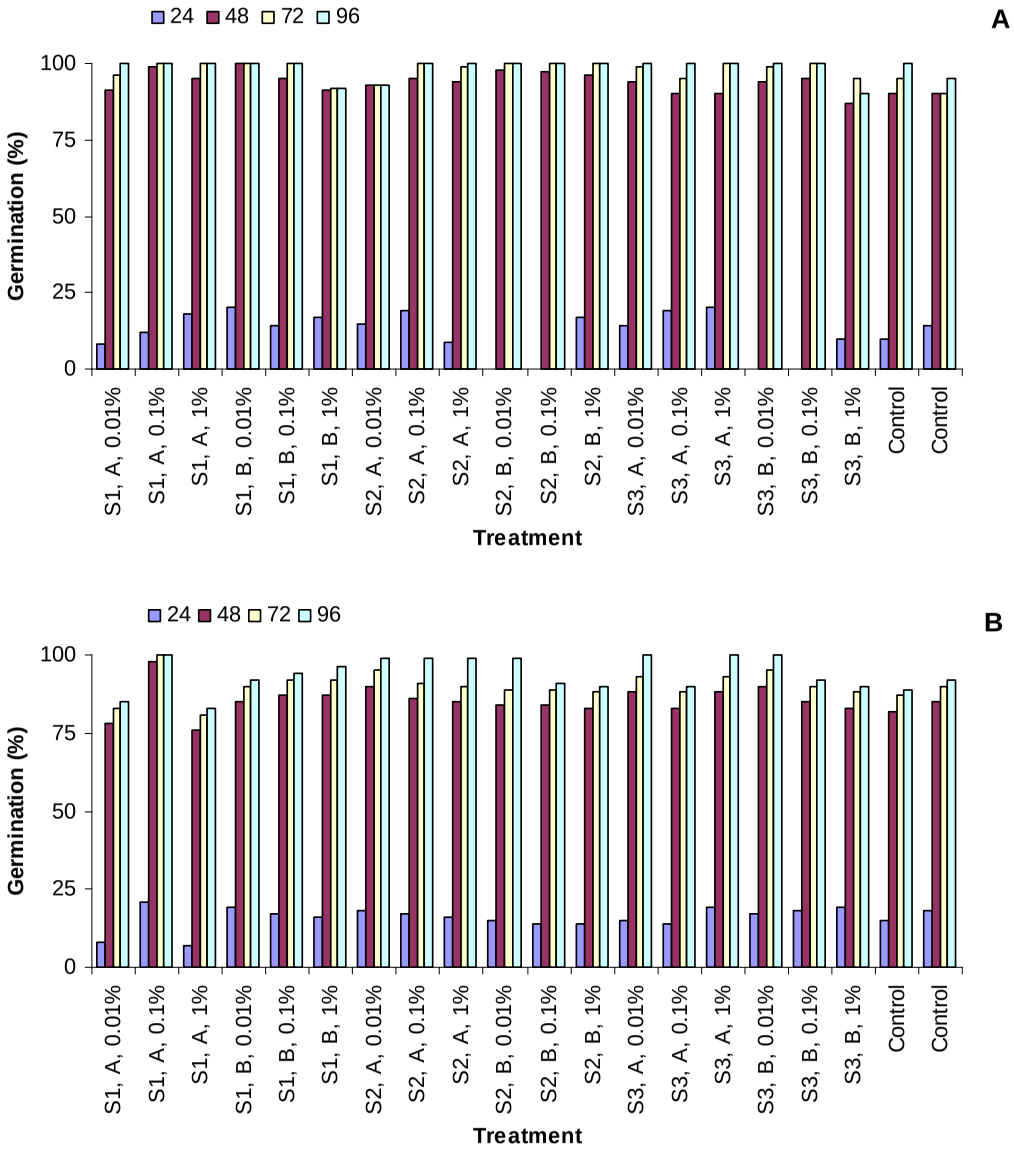

Figure 3.1.1 Germination of maize: a) cultivar 1 and b) cultivar 2, under favourable conditions in a temperature controlled chamber as observed over time intervals of 24 hours.

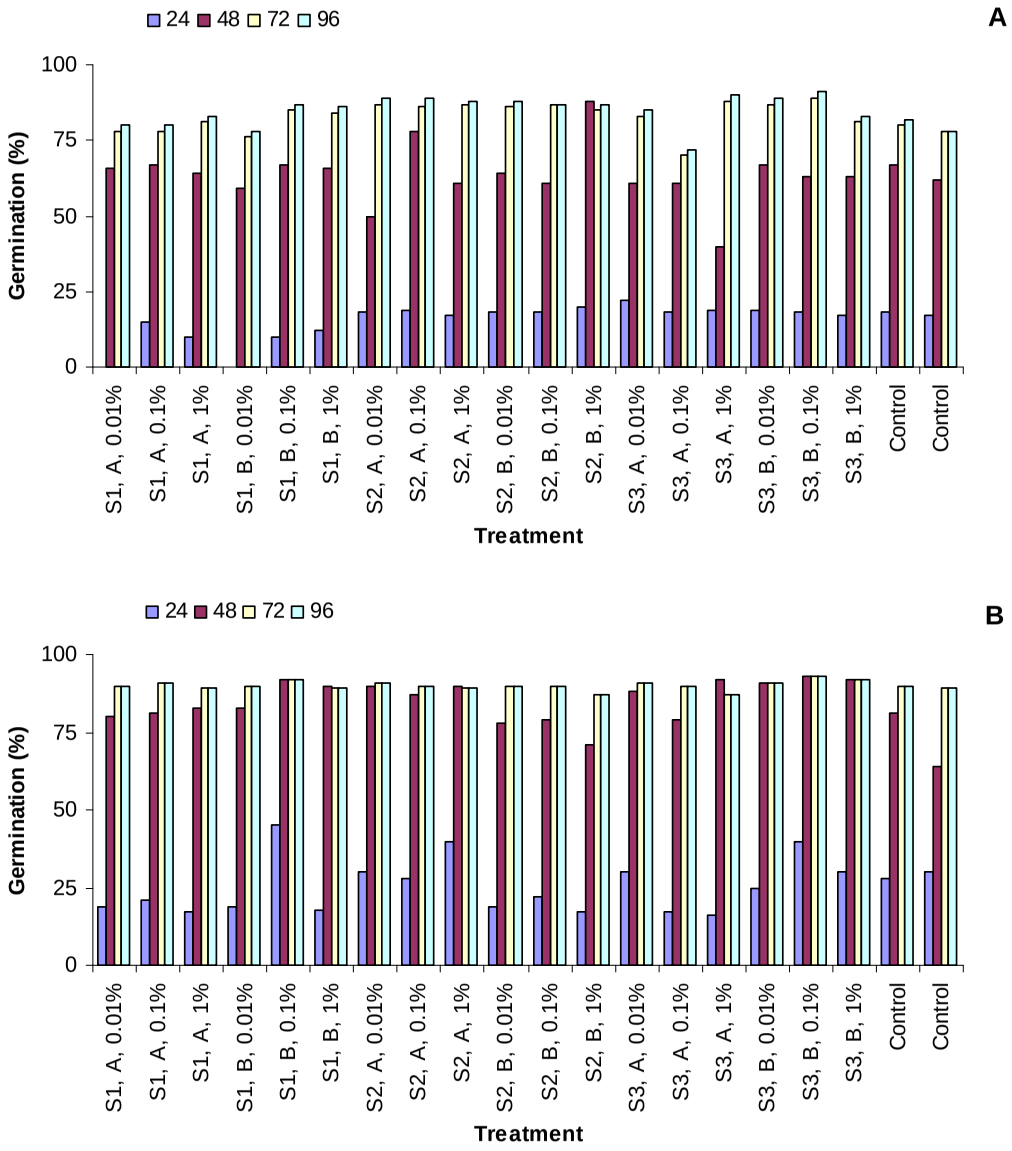

Figure 3.1.2 Germination of sorghum: a) cultivar 3 and b) cultivar 4, under favourable conditions in a temperature controlled chamber as observed over time intervals of 24 hours.

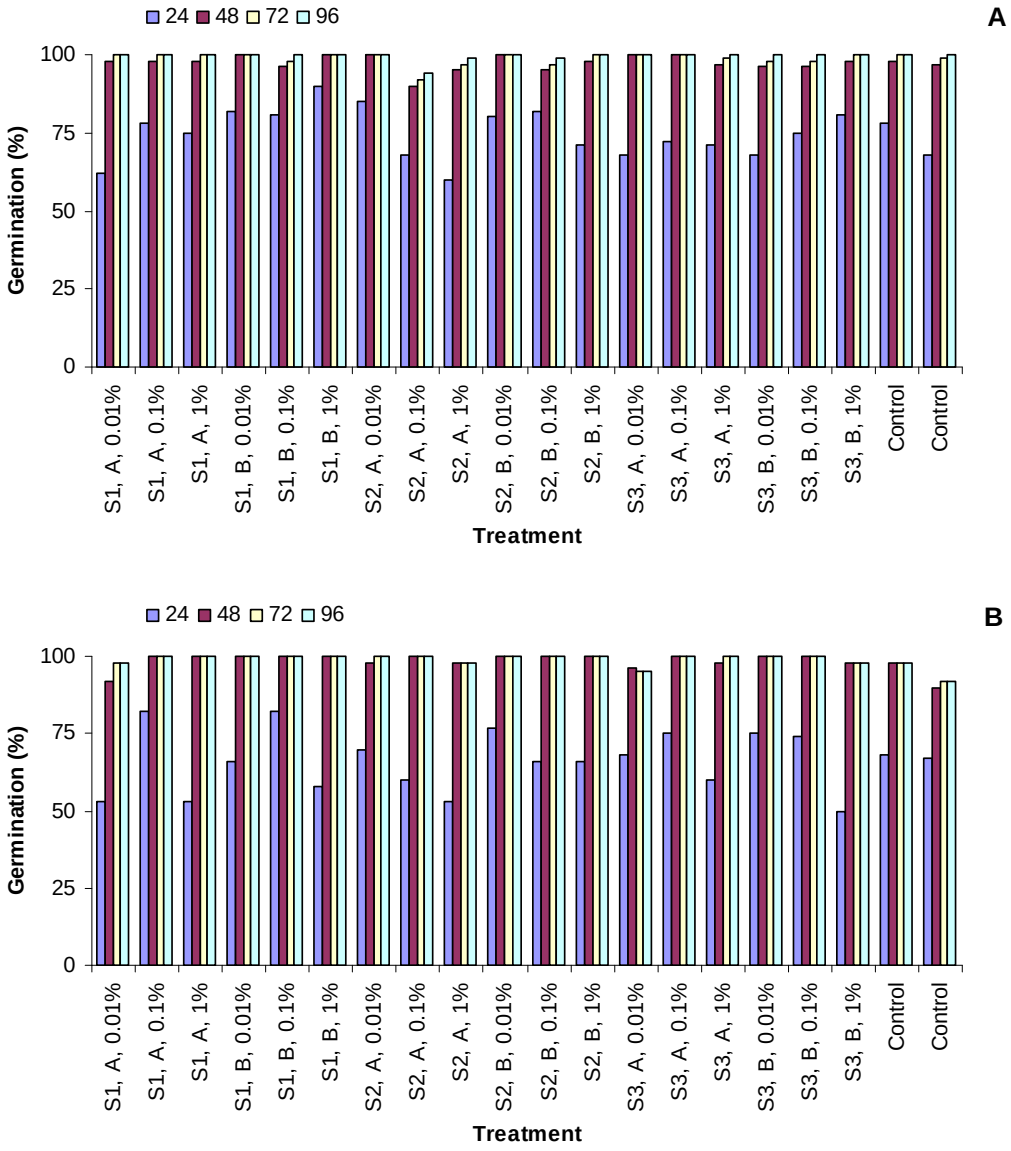

Figure 3.1.3 Germination of sunflower: a) cultivar 5 and b) cultivar 6, under favourable conditions in a temperature controlled chamber as observed over time intervals of 24 hours.

The interaction between cultivar and treatment for all crops resulted in no trend with regard to EM ratios or concentrations of the various suppliers when compared to the control treatments (Figure 3.2a - c).

Maize cultivar 1 had no treatment which improved germination significantly but to the contrary had three treatments which led to a significant decline in germination namely: S2 A 1%, S3 A 1%, and S3 B 0.1% (Figure 3.2a). S1 A 0.1% had a significant increase in germination compared to the control treatments for maize cultivar 2. Sorghum cultivars 3 and 4 had significant

increased germination over that of the control treatments when treated with S2 B 0.1% and S1 B 0.1% respectively (Figure 3.2b).

The germination of sunflower cultivar 5 treatment with S1 B 1% was significantly increased over that of its control treatments (Figure 3.2c). For cultivar 6 there was no treatment which increased or decreased germination with a significant margin in comparison with the control treatments. With all six cultivars seeds being of high quality and germinated under optimal conditions created in the germination chamber, a low number of significant differences were expected.

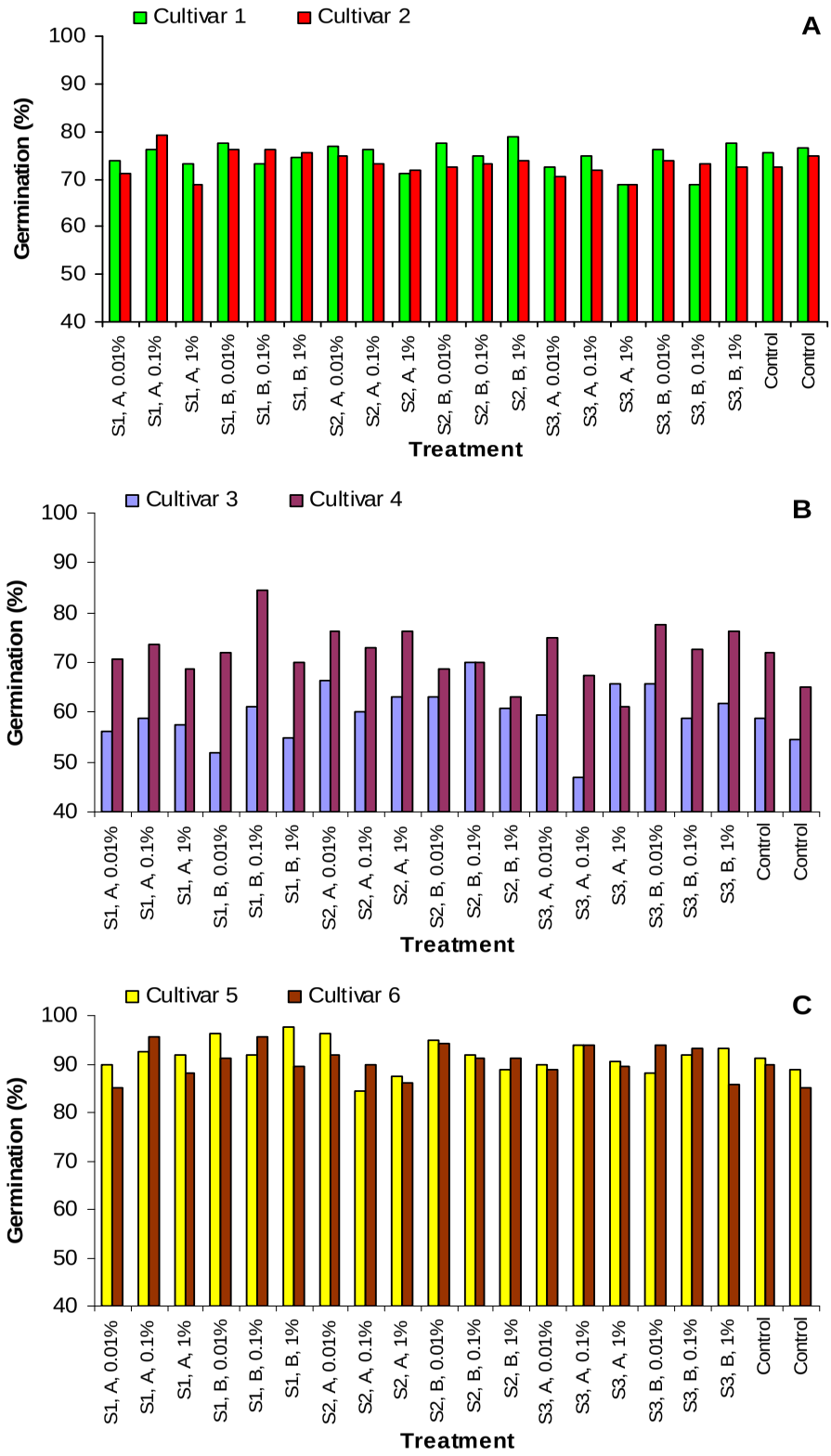

Figure 3.2 Interaction between cultivar and treatment (consisting of a supplier, multiplied ratio and dilutions) for the germination of a) maize, b) sorghum, and c) sunflower under favourable conditions in a temperature controlled chamber.

From Figure 3.3a - c, may be concluded that the interaction between time and cultivar was mainly due to the rapid acceleration of germination over the first 72 hours. Sunflower (Figure 3.3c) germinated faster than both maize (Figure 3.3a) and sorghum (Figure 3.3b) in the first 24 hours, with sunflower reaching maximum germination at the 48 hour mark.

For maize and sorghum some germination still occurred after 48 hours, while the curves flattened at the 72 hour mark with minimal germination there after. All six cultivars were found to be highly vigorous in the optimal germination conditions created in the germination chamber.

In the case of maize and sunflower (Figure 3.3a & c) there was a significant difference in germination between cultivars at the 24 and 48 hour interval, respectively. The difference in germination between cultivars was 7.85% for maize after 48 hours, and 9.25% for sunflower after 24 hours. These differences were, however, nullified by the next time interval. For both crops, these differences seem to be of no importance due to the germination differences being reduced to an insignificant difference at the next measurement interval.

Sorghum (Figure 3.3b) had a significant difference in germination between cultivars from the 24 hour to the 96 hour interval. Cultivar 4 outperformed cultivar 3 by 13.62%, 17.75%, 8.37%, and 8% respectively at the 24, 48, 72 and 96 hour intervals. Cultivar 4 therefore germinated faster than cultivar 3 and its exposure to seedling diseases and unfavourable climatic conditions was shorter during early seedling growth.

Even though no significantly positive results were found from the M-EM seed treatment germinated under favourable germination conditions, field conditions are not always favourable and thus experiments were replicated under cold stress conditions.

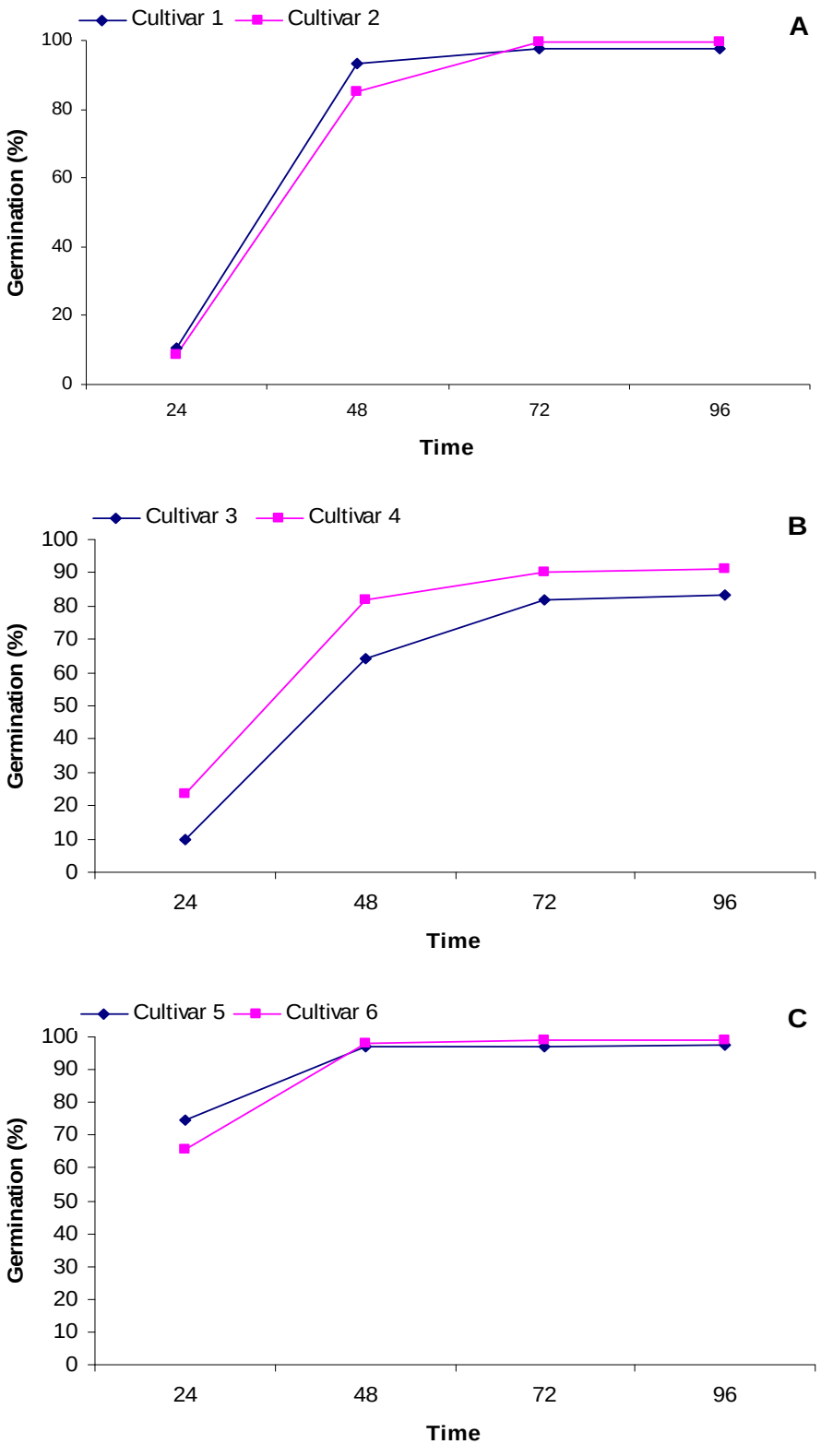

Figure 3.3 Germination of a) maize, b) sorghum, and c) sunflower under favourable conditions in a temperature controlled chamber as observed over time intervals of 24 hours for two cultivars.

3.3.2 Experiment 2: Germination and seedling vigour of seed subjected to the cold test

3.3.2.1 Germination rate after the cold test

The large trend in non-significance of results for this experiment may be because seeds were only inspected for germination on day two after the cold chamber, and therefore most of the seeds had already passed the rapid initial germination period (Table 3.5).

Table 3.5 Analysis of variance (ANOVA) of germination of maize, sorghum and sunflower, germinated under cold stress conditions, as affected by cultivar, time and treatment.

	p-values		
Effect	**Maize**	**Sorghum**	**Sunflower**
Cultivar	0.0000	0.0000	N/S
Time	0.0000	0.0000	N/S
Treatment	N/S	N/S	N/S
Cultivar*Time	N/S	N/S	N/S
Cultivar*Treatment	0.0000	N/S	N/S
Days*Treatment	N/S	N/S	N/S
Cultivar*Time*Treatment	N/S	N/S	N/S

The significant interaction between cultivar and treatment for maize was the result of four treatments of cultivar 1 that germinated significantly better than the two control treatments (Figure 3.4). These include seeds treated with S1 B 0.1%, S2 A 0.1%, S2 A 1% and S3 A 0.01%. There was however, no significant difference between any of the treatments and the control treatments for cultivar 2, while the control treatments even outperformed many of the treatments. The reason for cultivar 2 outperforming cultivar 1 may be ascribed to the difference in cultivar characteristics in terms of stress tolerance. Characteristics of cultivar 2 are that cultivar 2 has excellent stress tolerance and is highly adaptable (Pannar, 2011).

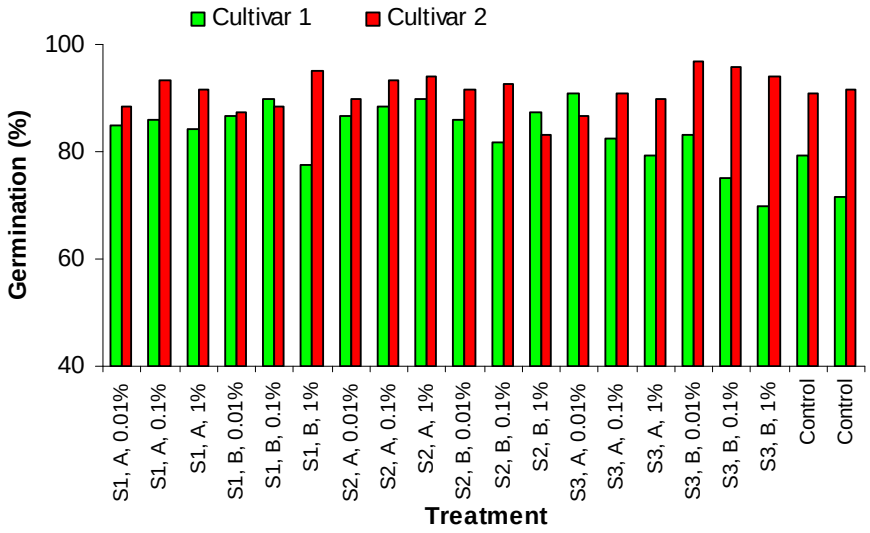

Figure 3.4 Interaction between cultivar and treatment for the germination of maize after seeds were subjected to cold stress.

Significant differences occurred between cultivars over time for the germination of sorghum seeds after exposure to cold stress. From Figure 3.5 is clear that cultivar 4 outperformed cultivar 3 under cold stress conditions. The reason for cultivar 4 outperforming cultivar 3 may be ascribed to the difference in cultivar characteristics, where cultivar 4 is described as an excellent cultivar that be planted in all sorghum production areas (Pannar, 2011).

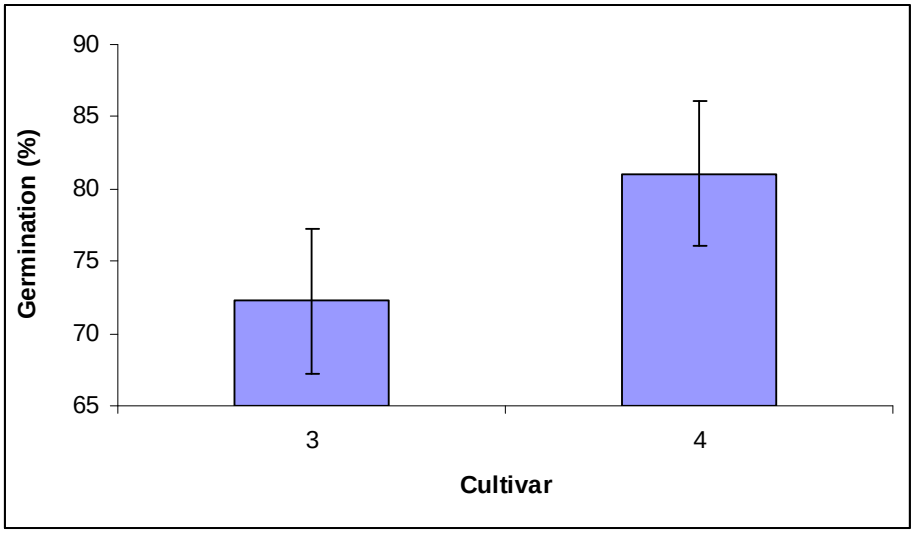

Figure 3.5 Variation in germination of two sorghum cultivars exposed to a cold treatment. Vertical bars denote 0.95 confidence intervals.

Sorghum germination started slow after the seed was removed from the cold stress unit and placed in optimum germination conditions (Figure 3.6). Most seeds germinated within 48 hours, while there was a significant increase of 12.87% in germination from the 48 to 96 hour interval. There was however no significant increases in germination between the 96 hour and the 168 hour intervals. This indicated that germination peaked at 96 hours after the cold treatment and stabilised thereafter, compared to germination under favourable conditions where germination peaked at 48 hours. The delay in germination would lead to seeds being exposed longer to germination impeding factors such as seed rot and insect damage.

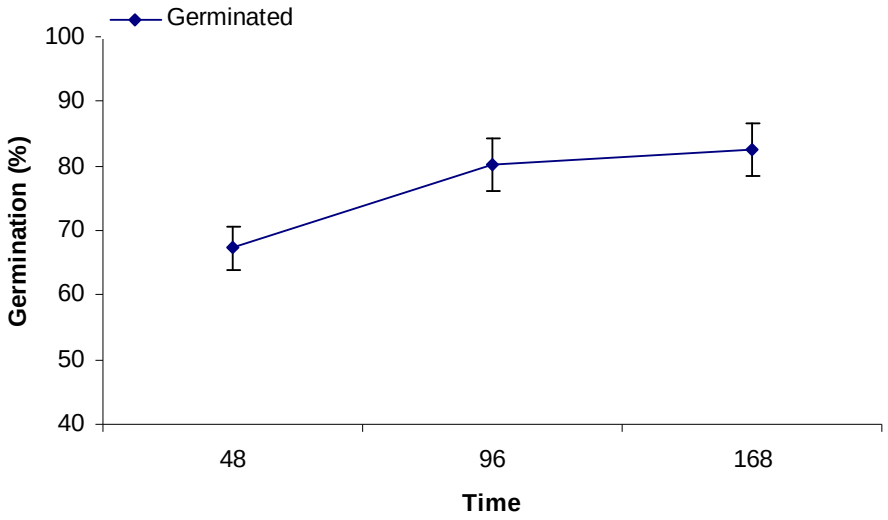

Figure 3.6 Germination of sorghum exposed to a cold treatment as observed over time intervals of 48, 96 and 168 hours after the cold chamber.

3.3.2.2 Seedling vigour after the cold test

The analysis of variance of seedling vigour as influenced by cultivar, time and treatment is summarised in Table 3.5 for maize, sorghum and sunflower. Sorghum had a significant third degree interaction between cultivar, time and treatment (Table 3.5). The discussion for maize will focus on the second degree interactions between cultivar and treatment and between cultivar and time, while the discussion for sunflower will focus on the second degree interactions between cultivar and treatment and time and treatment.

Table 3.6 Analysis of variance (ANOVA) of plant length of maize, sorghum and sunflower, exposed to cold stress conditions, as affected by cultivar, time and treatment.

	p-values		
Effect	**Maize**	**Sorghum**	**Sunflower**
Cultivar	0.0000	0.0000	0.0000
Time	0.0000	0.0000	0.0000
Treatment	0.0060	0.0076	0.0006
Cultivar*Time	0.0000	0.0480	N/S
Cultivar*Treatment	0.0171	0.0004	0.0000
Time*Treatment	N/S	N/S	0.0027
Cultivar*Time*Treatment	N/S	0.0453	N/S

There was a significant interaction between cultivar, time and treatment for sorghum (Figure 3.7a - c), with significant increases in plant length only developing at the 96 hour interval. The plant length of cultivar 3 was significantly increased over the control by treatment with S1 A 1%, S2 A 1%; S3 A 0.1%, S3 A 0.01% and S3 B 0.01%. However, these were nullified after 168 hours. Cultivar 4 had three treatments which showed a significant increase in plant length over the control at the 168 hour measurement interval, namely treatment with S1 A 0.01%, S1 B 0.01% and S1 B 0.1%. It is interesting to note that the treatments detrimentally affected plant length of cultivar 3.

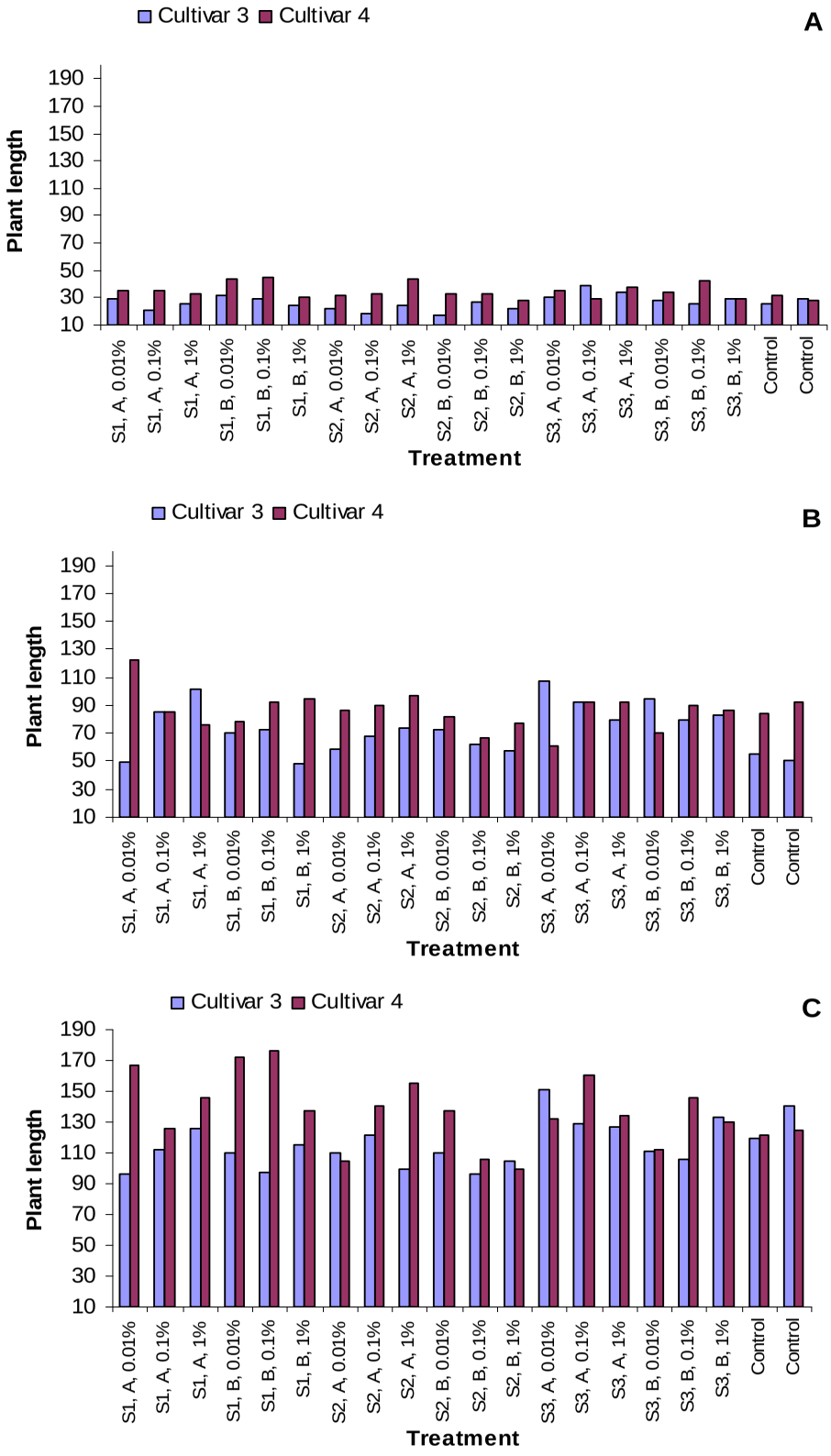

Figure 3.7 Plant length of sorghum a) 48 hours, b) 96 hours, and c) 168 hours, under stress conditions in a cold test observed with regard to EM ratios and concentrations.

The interaction between cultivar and time had a significant effect on the plant length of maize. Cultivar 2 showed significantly higher seedling vigour compared to cultivar 1 from the 96 hour interval onward (Figure 3.8). The average total plant length of cultivar 2 exceeded that of cultivar 1 by 19.7 mm at the 96 hour interval and 48.4 mm at the 168 hour interval. These results can be linked to that of the cold stress germination (Figure 3.4) where cultivar 2 outperformed cultivar 1.

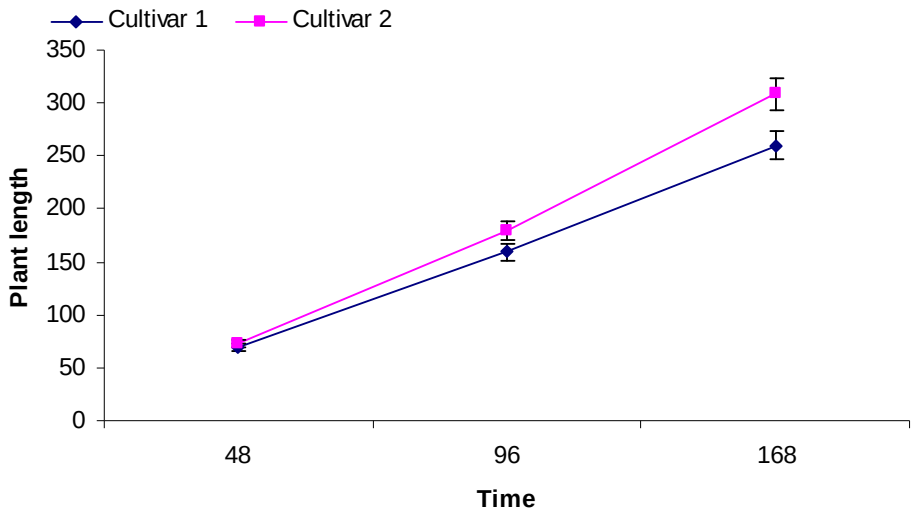

Figure 3.8 Plant lengths of two maize cultivars, after cold stress conditions observed over 48 hour intervals.

For maize, the plant lengths of cultivar 1 (Figure 3.9a) treated with S1 A 0.1%, S2 A 0.1%, S3 A 0.01% and S1 B 0.01% was significantly increased compared to the control treatments.

There were no treatments which resulted in significantly greater plant lengths for maize cultivar 2 over the control. S2 B 1% led to a significant lack in vigour for cultivar 2 in respect to the control treatments. As with germination difference between cultivar 1 and cultivar 2, the results indicate that M-EM can improve seedling vigour for cultivars which is less tolerant to stress conditions.

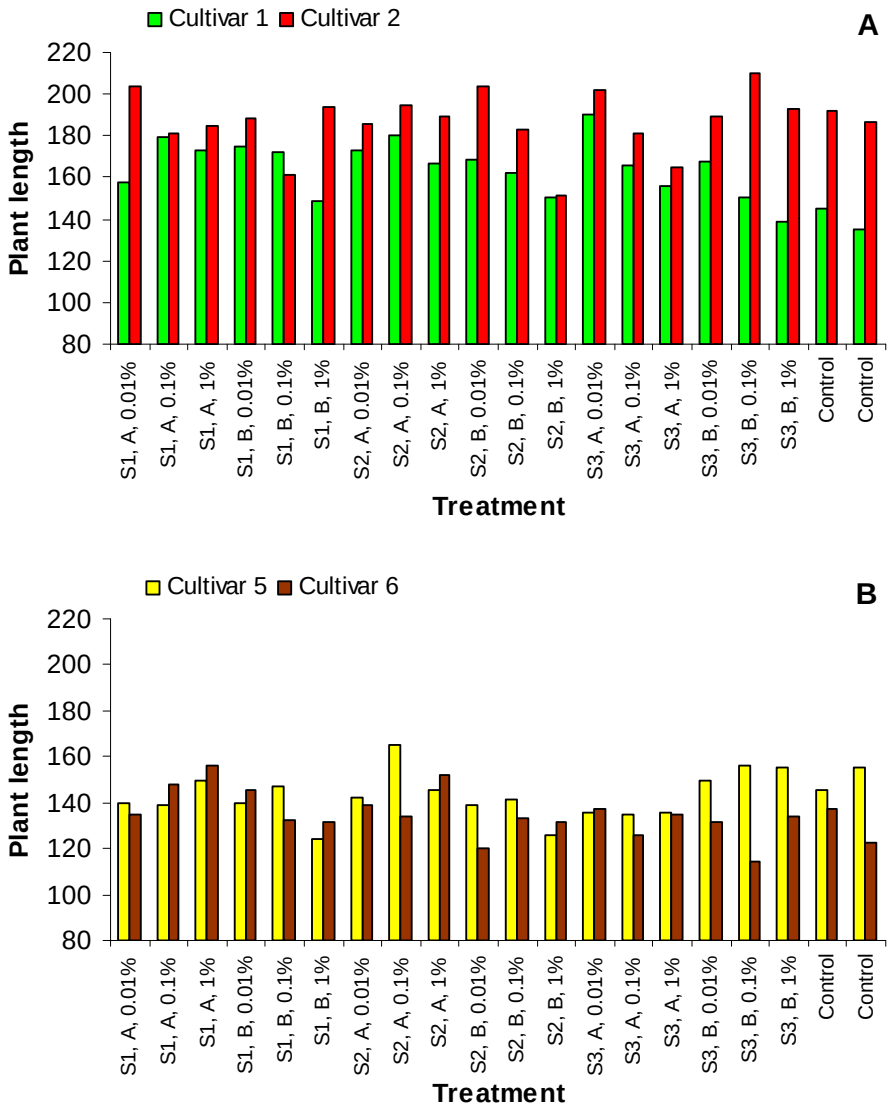

Figure 3.9 Plant lengths of maize a) and sunflower b) under stress conditions in a cold test observed with regard to EM ratios and concentrations.

For sunflower, both S1 B 1% and S2 B 1% significantly reduced the vigour of cultivar 5 compared to the control (Figure 3.9b) while S2 A 0.1% increased plant vigour of cultivar 5. For cultivar 6 S1 A 1% and S2 A 1% increased plant length over that of the control treatments. From this is possible to say that a B-ratio of S1 and S2 at a concentration of 1% significantly reduced the vigour of cultivar 5 and that the A-ratio of S1 and S2 at a concentration of 1% increased the vigour of cultivar 6.

3.4 Conclusion

Seed inoculated with M-EM is said to have a faster and more uniform germination as well as having improved plant growth. However, there is no clear indication by EM producers at what ratio EM should be multiplied or at what dilution multiplied EM should be used as a seed treatment. The purpose of this study was therefore to ascertain the effectiveness of M-EM at different dilutions, on the germination and seedling vigour of maize, sorghum and sunflower. The results clearly suggest that M-EM as a seed treatment was ineffective on germination under optimum conditions, but rather had notable positive influence on germination and plant vigour after cold stress conditions. S1, S2 and S3 at both multiplied ratios, and at all three dilutions, lead to positive as well as negative results with no significant differences between them. Based on the results of the study, M-EM may have a beneficial effect on germination and seedling vigour of some maize, sorghum and sunflower cultivars. However, remaining unclear is which product is superior and at what dilution and ratios the different product should be used. Also notable that M-EM improved germination and seedling vigour of cultivars less tolerant to stress conditions. As can be seen in the case of sorghum, where cultivar 4 outperformed cultivar 3 in all the experiments, but results of M-EM treated seeds of cultivar 3 were more improved than that of cultivar 4 compared to its respective control treatments. Thus, further research is needed before a product and dilution of M-EM as a seed treatment can be recommended for maize, sorghum and sunflower.

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Chapter 4

The influence of Effective micro-organisms exposed to irradiation and temperature fluctuation on germination and seedling vigour of maize, sorghum and sunflower

Abstract

Two incubation experiments were conducted to determine and compare the effect of Multiplied Effective micro-organisms (M-EM) from three selected suppliers multiplied at two ratios and exposed to irradiation and temperature fluctuation on, i) the germination and ii) seedling vigour of maize, sorghum and sunflower under favourable conditions and after exposure to cold stress in incubation studies.

Stock-EM (S-EM) from three suppliers was multiplied to produce Multi-EM (M-EM) at two different ratios each, namely: 1% and 3%. M-EM was subjected to the influence of irradiation and temperature fluctuation by different exposure rates to direct sunlight and uncontrolled temperature fluctuation in an open field. The M-EM were divided into three groups from which the first group was left in an open field from sunrise to sunset, the second group was left for 24 hours in the same field, and the third group was left in a dark room with minimum temperature fluctuation for 30 days. Seeds were then treated with these M-EM which had been influenced by temperature fluctuation and irradiation. In the first experiment of this study, seeds were treated with the diluted M-EM and germinated in optimum germination conditions ($25^{o}C$) to investigate the influence on germination. In the second part of this study M-EM treatments were repeated but the seeds were subjected to the cold test ($10^{o}C$).

Results indicated that the handling and storage techniques played a very important role in the effect of M-EM on germination and vigour of the crops which were used for these experiments. The 30 day stored M-EM treatments, under favourable germination conditions were the only treatments which had a significant positive effect on germination. For germination under cold stress no treatment had any significant effect on any of the crops. The plant vigour of cultivar 2 was significantly affected by most of the M-EM treatments compared to the control treatments. Therefore, cultivar characteristics might have an effect on

the effectiveness of M-EM treatment. A conclusion can be drown that the handling of S-EM, M-EM and diluted M-EM in the manner as described by the supplier is highly important.

Keywords: Stock-EM, Multi-EM, cold test, favourable germination conditions.

4.1 Introduction

Effective micro-organisms (EM) contain over 80 selected types of micro-organisms (Woodward, 2003; Singh, 2007). These include populations of lactic acid bacteria, photosynthetic bacteria, actinomycetes, fermenting fungi and yeasts (Higa & Parr, 1994). EM are microbial inoculants which shift the microbiological balance towards a better quality soil, enhancing crop production and protection. EM helps to preserve natural resources and create a more sustainable agricultural environment (Higa & Parr, 1994). Micro-organisms are also successfully applied to promote seed germination and plant growth of numerous crop species (Sangakkara and Attanayake, 1993).

EM, being a mixture of living micro-organisms, needs to be kept away from direct sunlight. EM should preferably be kept in a storeroom with little temperature fluctuation (EMROSA, 2008; D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009). In addition, EM should not be stored in a refrigerator (D. Anthony, personal communication, March 2009) since significant temperature fluctuations influence micro-organism survival (Szymanski and Patterson, 2003). According to Alexander (1977), each micro-organism has its own optimum temperature range for growth, outside of which development is brought to a standstill.

Exposure to irradiation and temperature fluctuation may have a great effect on the efficiency of EM to promote germination and seedling vigour. Crop producers must thus ensure that they adhere to the recommendations of the EM manufacturers for storage and handling, to avoid killing these micro-organisms. The best storage and handling practices include: storage between 15^{o}C and 20^{o}C with fluctuation of less than 10^{o}C in 24 hours (EMROSA, 2008; A. Rosenberg, personal communication, March 2009), storage of EM out of direct sunlight and using EM in adequate time. Stock-EM should not be stored for more than six months while Multiplied-EM should not be stored for longer than one month (EMROSA, 2006). If EM is stored longer than the prescribed period, the activity of the micro-organisms start to decrease (EMROSA, 2006).

Negligent storage and in-field handling of EM by crop producers may lead to their exposure to irradiation, temperature fluctuation and the prolonged storage of Multiplied-EM. Therefore, the objectives of this study were to determine the influence of EM that was exposed to irradiation and temperature fluctuation as well as prolonged storage of M-EM on i) germination and ii) seedling vigour of maize, sorghum and sunflower.

4.2 Material and methods

4.2.1 Location and experimental layout

Two independent experiments were conducted in the Laboratory of the School for Agricultural and Environmental Sciences of the Central University of Technology, Free State. Maize of the cultivars, PAN 6236B (cultivar 1) and PAN 6053 (cultivar 2), sorghum of the cultivars, PAN 8247 (cultivar 3) and PAN 8816 (cultivar 4), and sunflower of the cultivars, PAN 7351 (cultivar 5) and PAN 7033 (cultivar 6), were used in both experiments. Seeds were surface sterilized in a 3.5% sodium hypochlorite solution for 10 minutes and subsequently triple rinsed in pure water. A total of 800 and 2400 seeds were used per cultivar for the germination and seedling vigour experiment, respectively. Each experiment was replicated four times.

4.2.1.1 The maize, sorghum and sunflower cultivars

- PAN 6236 (cultivar 1) is an ultra early yellow maize, which achieves excellent results under irrigation as well as high potential dry land conditions. The cultivar does exceptionally well in the Orange River area and other warm irrigation regions (PANNAR, 2011).
- PAN 6053 (cultivar 2) is medium maturing white maize cultivar, with excellent yield potential and proven reliability under low rainfall conditions, producing yields at low plant populations (PANNAR, 2011).
- PAN 8247 (cultivar 3) is a sorghum with good yield potential and has a very uniform plant type (PANNAR, 2011).
- PAN 8816 (cultivar 4) is a popular sorghum and recommended for the main planting in all sorghum production areas. The cultivar has an excellent yield potential and stability (PANNAR, 2011).

- PAN 7351 (cultivar 5) is a sunflower with a wide area adaptability, a high yield potential and a good stability, with outstanding performance in commercial plantings (PANNAR, 2011).
- PAN 7033 (cultivar 6) is a top performer sunflower in cultivar trails over the past three years and is recommended for the main bulk planting in all production regions (PANNAR, 2011).

4.2.2 Treatment of M-EM

Generally fallible Stock-EM (S-EM) was acquired from three different commercial companies. Due to a secrecy agreement names will be withheld and in this document the products will be revered to as S1, S2 and S3. The S-EM was multiplied into Multi-EM (M-EM) at the following ratios:

- M-EM (A) at a ratio of 1% S-EM, 7% molasses and 92% water.
- M-EM (B) at a ratio of 3% S-EM, 5% molasses and 92% water.

M-EM (A) and (B) of each of the three companies were allowed to stand for 14 days to multiply in optimum prescribed conditions. Each of the three M-EM (A) and (B) was further divided into three bottles with a capacity of 2 ℓ each. The three bottles of both M-EM ratios were exposed to different environmental conditions, namely, 1) the first bottle was placed in an open field from sunrise to sunset, 2) the second bottle was placed in an open field for 24 hours, and 3) the last bottle was stored in a room with little temperature fluctuation and out of direct sunlight for 30 days. The M-EM bottles were left in a field just outside Bloemfontein during November and December of 2009. The average minimum and maximum temperature for that time was 14^{o}C and 30^{o}C (Weather and Climate, 2011). The room that was used for storage was a laboratory with an air cooling system, which was used to regulate temperature between 15^{o}C and 20^{o}C.

4.2.3 Treatment of seeds

Seeds were soaked for seven hours in a 0.1% dilution of the two M-EM ratios, which had different amounts of exposure to irradiation and temperature fluctuation. A control that consisted of soaking seeds in purified water was prepared for comparison. After soaking, the seeds were left to dry in the laboratory.

To simplify statistical analysis and interpretation of results, EM suppliers, multiplied ratios, exposure rates and the control treatments were pooled into 20 treatment combinations (Table 4.1), which will be referred to as treatments throughout the rest of this chapter. Treatment abbreviations are coded and are not an indication of the supplier company.

Table 4.1 Treatment combinations 1 to 20 with regards to treatment abbreviation, EM supplier company, multiplied ratios and exposure rate.

Treatment number	Treatment abbreviation	Supplier company	Multiplied ratio	Exposed rates
1	S1 A R-S	1	A	Rise - Set
2	S1 A 24H	1	A	24 Hours
3	S1 A 30	1	A	30 Days
4	S1 B R-S	1	B	Rise - Set
5	S1 B 24H	1	B	24 Hours
6	S1 B 30	1	B	30 Days
7	S2 A R-S	2	A	Rise - Set
8	S2 A 24H	2	A	24 Hours
9	S2 A 30	2	A	30 Days
10	S2 B R-S	2	B	Rise - Set
11	S2 B 24H	2	B	24 Hours
12	S2 B 30	2	B	30 Days
13	S3 A R-S	3	A	Rise - Set
14	S3 A 24H	3	A	24 Hours
15	S3 A 30	3	A	30 Days
16	S3 B R-S	3	B	Rise - Set
17	S3 B 24H	3	B	24 Hours
18	S3 B 30	3	B	30 Days
19	Control	Control	N/A	N/A
20	Control	Control	N/A	N/A

4.2.4 Experiment 1: Germination under favourable conditions

Dried seed were placed in 90 mm-diameter Petri dishes between two filter papers moistened with 10 ml purified water. The Petri dishes were placed in a temperature controlled cabinet at 25^{o}C and in darkness. The dishes were sealed in plastic Ziploc bags to prevent moisture loss. The Petri dishes were inspected in 24 hour intervals and the experiment was terminated after seven days. Table 4.2 indicates the quantity of seed that was used for each grain crop and the combination of variables in the experiment.

Table 4.2 Favourable conditions – Maize, sorghum and sunflower were used in this study with the listed variables effecting study layout and results of the germination under favourable conditions experiment.

	Maize	**Sorghum**	**Sunflower**
Cultivars	2	2	2
Number of seeds	10	10	10
Replications	4	4	4
EM suppliers	3	3	3
Multiplied ratios	2	2	2
Exposure rates	3	3	3
Control	2	2	2
Total number of seeds	1600	1600	1600

4.2.5 Experiment 2: Germination and seedling vigour subjected to the cold test

The cold test was executed as described in the ISTA Handbook of Vigour Test Methods by Hampton and TeKrony (1995):

1. On the day before planting, purified water was cooled overnight to 10^{o}C.
2. A double layer of paper towels (230mm×280mm) were saturated with approximately 35 ml of the cooled purified water. The seeds in each treatment were placed on the double layer of saturated paper towels in two rows of five seeds each, 6 cm and 12 cm from the top edge of the towels. A single saturated paper towel was placed over the two lower towels covering the seed.
3. The three towels were then rolled up. Care was taken to ensure that the towels did not warm up above 10^{o}C during and after preparation.
4. The rolled towels were placed upright in a plastic bucket when they were transferred to the cold (10^{o}C) chamber. Each rolled towel was placed in a plastic bag, to keep the rolles upright and separated. The plastic bags were sealed of to prevent loss of moisture and cross contamination.
5. The containers were kept in the cold chamber at 10^{o}C in darkness for seven days.
6. After the cold treatment the containers were moved to the germination chamber at 25^{o}C also in darkness.

Table 4.3 indicates the quantity of seeds that was used for each grain crop and the combination of variables in the experiment.

Table 4.3 Cold test – Maize, sorghum and sunflower were used in this study with the listed variables effecting study layout and results of the cold test.

	Maize	**Sorghum**	**Sunflower**
Cultivars	2	2	2
Number of seeds	10	10	10
Replications	12	12	12
EM suppliers	3	3	3
Exposure rates	3	3	3
Multiplied ratios	2	2	2
Control	2	2	2
Total number of seeds	4800	4800	4800

4.2.6 Measurements

In experiment 1, germination was scored at a radical protrusion of 3 mm. Petri dishes were inspected in 24 hour intervals for seven days after planting. For experiment 2, germination was scored at a radical protrusion of 3 mm and seedling lengths were measured at 48, 96 and 168 hour intervals after transfer to the germination chamber to determine vigour.

4.2.7 Statistical analysis

A factorial analysis of variance (ANOVA) was performed on the germination and seedling vigour with cultivars, suppliers, EM ratios, exposure rates and time as factors. P-values were used to compare means at a 5% probability level, using STATISTICA version 8.0 (Statsoft Inc., 2004).

4.3 Results and discussion

4.3.1 Experiment 1: Germination under favourable conditions

There was a significant second degree interaction for sorghum between cultivar, time and treatment (Table 4.4). Maize had significant first degree interactions for cultivar and time, cultivar and treatment and for time and treatment. Significant interactions for sunflower occurred between cultivar and treatment and time and treatment.

Table 4.4 Analysis of variance (ANOVA) of germination of maize, sorghum and sunflower, germinated under favourable temperature and moisture conditions as affected by cultivar, time and treatment.

	p-values		
Effect	**Maize**	**Sorghum**	**Sunflower**
Cultivar	0.0001	0.0000	N/S
Time	0.0000	0.0000	0.0000
Treatment	0.0000	0.0000	0.0000
Cultivar * Time	0.0000	N/S	N/S
Cultivar * Treatment	0.0000	0.0000	0.0441
Time * Treatment	0.0000	0.0000	0.0000
Cultivar * Time * Treatment	N/S	0.0020	N/S

Germination of sorghum indicated a clear trend with regard to handling technique and multiplied ratio compared to the control treatments (Figures 4.1a - d). All M-EM treatments that were stored for 30 days resulted in significantly improved germination of both cultivars, compared to all other EM treatments and the untreated control treatments in the first 48 hours (Figure 4.1a & b). The control and some of the other treatments caught up after 96 hours (Figure 4.1d).

At the 96 hour interval, germination of S3 B R-S treated seed of cultivar 3 was significantly lower compared to both control treatments, while other treatments such as S1 B 24H and S3 A 24H also germinated poorer than the control. Using M-EM stored for 30 days at the correct conditions resulted in faster germination in the first 72 hours. This may prove beneficial since seeds and seedlings will be susceptible to unfavourable conditions as well as diseases for a shorter period compared to untreated seeds.

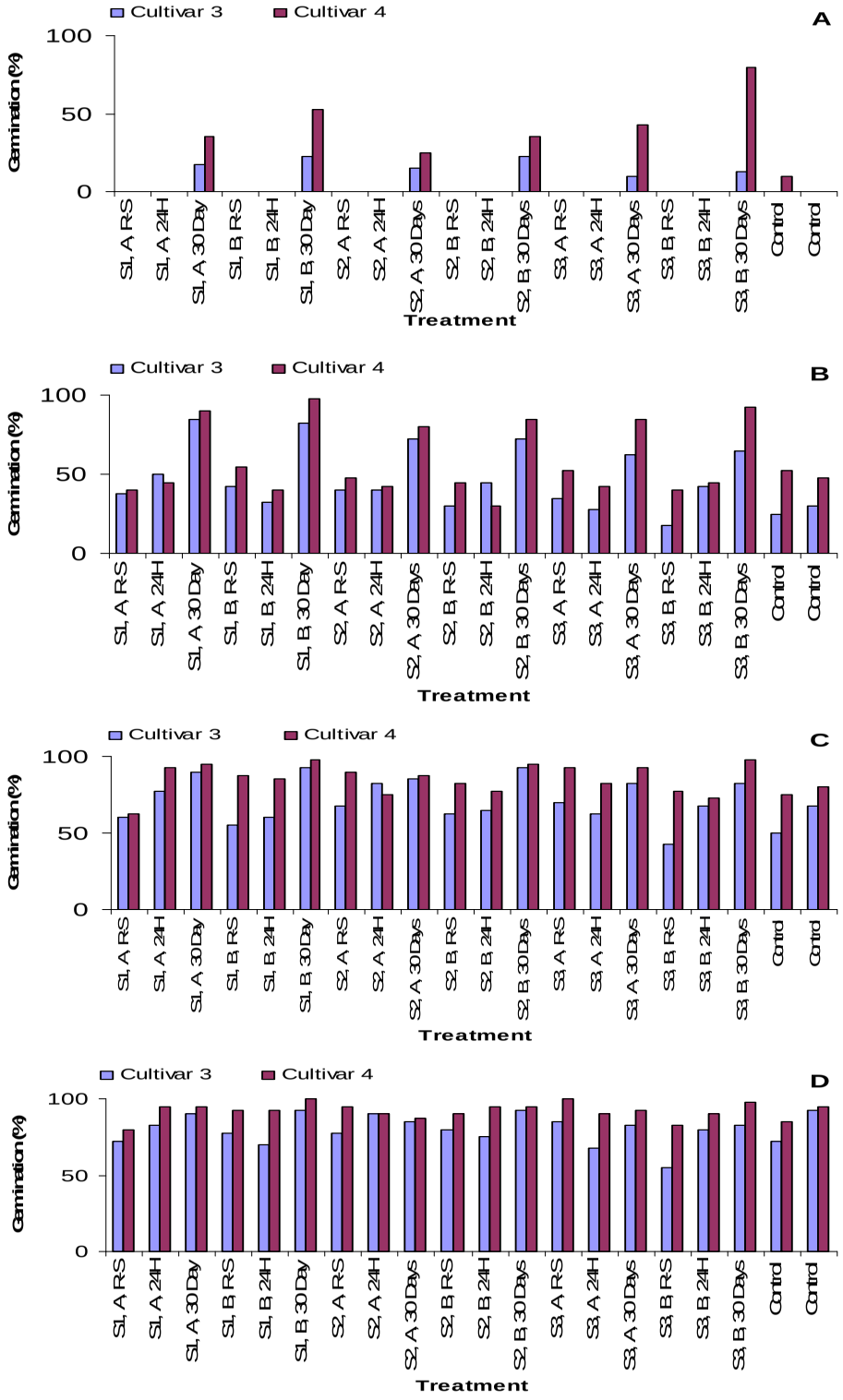

Figure 4.1 Germination of two sorghum cultivars at a) 24, b) 48, c) 72 and d) 96 hours after planted in Petri dishes under favourable conditions, as observed with regard to cultivar and treatments (consisting of a supplier, multiplied ratio and exposure rates).

As for sorghum, what was obvious from Figure 4.2a and b was that the M-EM 30 treatments improved early germination compared to all other treatments for both maize and sunflower. All M-EM 30 treatments of all EM suppliers at both multiplied ratios out-performed the control treatments significantly after 24 and 48 hours for sunflower and maize, respectively. After 72 hours all differences was at a non significant level, excluding the germination of maize treated with S3 B R-S which were significantly lower compared to the control treatments, as was in the case of sorghum treated with S3 B R-S. As with sorghum, maize and sunflower seeds treated with EM, showed improved germination in the first 48 hours, giving seedlings a head start which may prove beneficial especially under unfavourable conditions.

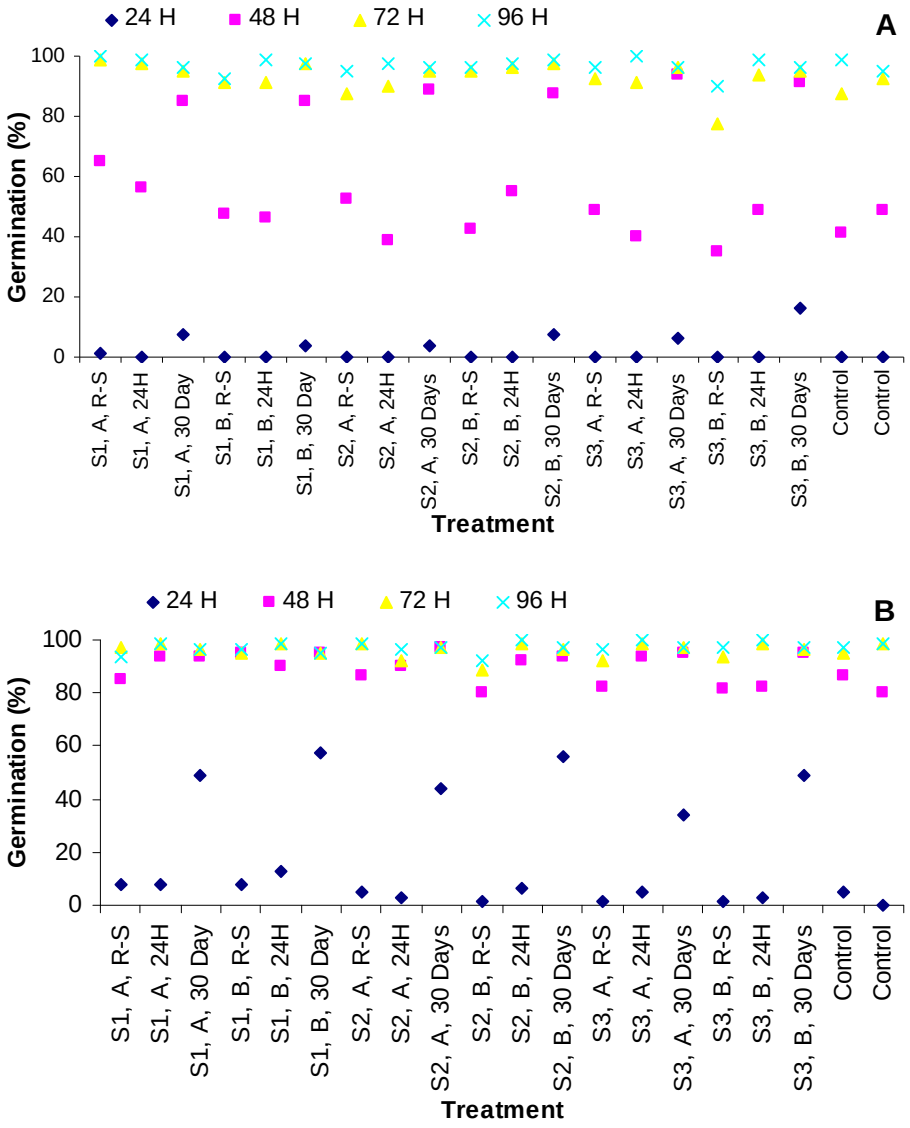

Figure 4.2 Germination of a) maize and b) sunflower under favourable conditions in a temperature controlled chamber observed with regard to M-EM ratios and handling techniques compared to the control treatments over four time intervals.

The interaction between cultivar and treatment for maize and sunflower (Figure 4.3a & b) indicated a significant difference in germination between handling techniques. The M-EM 30 treatment at both ratios increased germination with a significant margin above that of all control treatments for both maize cultivars. S1 A R-S, S1 A 24H and S2 A R-S treatments, also increased germination with a significant level over that of the control treatments for cultivar 1. This may prove that M-EM may be beneficial to germination of maize seeds even when not treated correctly.

Both sunflower cultivars (cultivar 5 and 6) had a significant increase in germination when treated with M-EM 30, compared to the control treatments. While some R-S and 24H treatments for cultivar 6 and some R-S treatments for cultivar 5, reduced germination compared to their respected control treatments. For both maize and sunflower, treatment with 30 day M-EM results in improved early germination which may result in stronger seedlings.

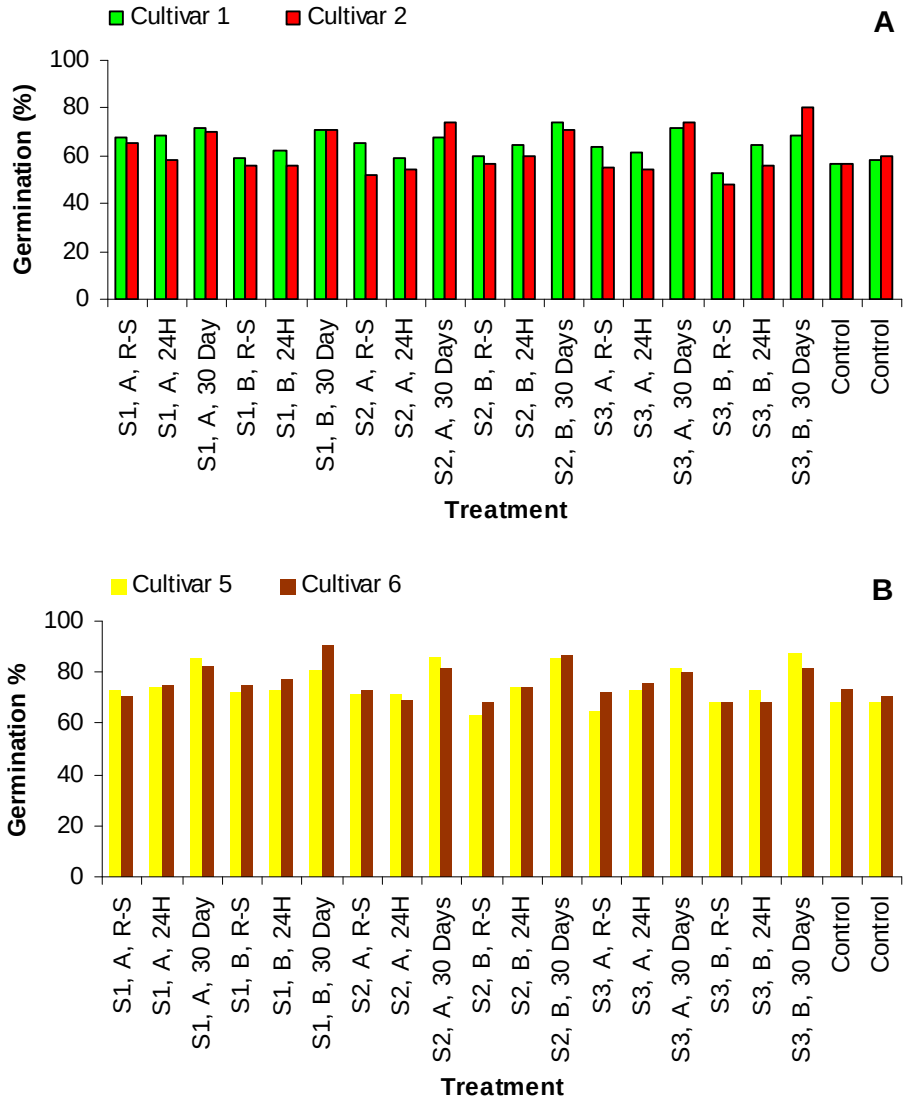

Figure 4.3 Germination of two a) maize and b) sunflower cultivars under favourable conditions in a temperature controlled chamber observed with regard to M-EM ratios and handling techniques.

As visible in Figure 4.4 was expected that germination percentage of maize would increase with time up to a point from where no further germination takes

place. The difference in initial germination speed (after 48 hours) was nullified over time and therefore seems to be of non importance. A similar difference in germination between cultivar 1 and 2 at the 48 hour interval was observed in chapter three, where germination was also reduced to a non significant level at the 72 hour interval.

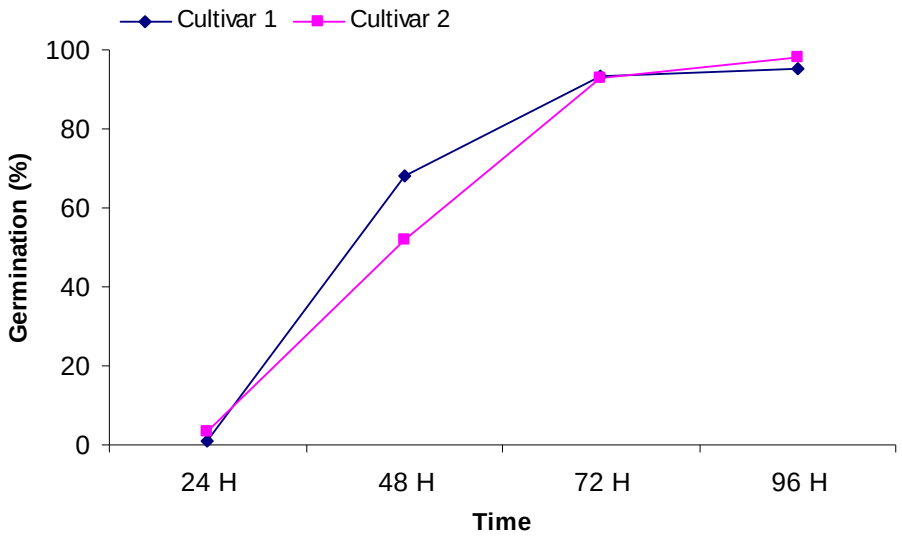

Figure 4.4 Germination of two maize cultivars under favourable conditions in a temperature controlled chamber as observed over time intervals of 24 hours observed with regard to cultivars.

4.3.2 Experiment 2: Germination and seedling vigour after exposure to the cold test

4.3.2.1 Germination rate after the cold test

There was a significant third degree interaction between cultivar, time and treatment for sunflower (Table 4.5). Maize had a significant second degree interaction between cultivar and time. Because an increase in germination over time was expected, discussion of germination results for sorghum will only focus on the first degree interaction for cultivar.

Table 4.5 Analysis of variance (ANOVA) of germination of maize, sorghum and sunflower, germinated under cold stress conditions as affected by cultivar, time and treatment.

	p-values		
Effect	**Maize**	**Sorghum**	**Sunflower**
Cultivar	0.0000	0.0000	0.0492
Time	0.0000	0.0000	N/S
Treatment	N/S	N/S	0.0170
Cultivar * Time	0.0319	N/S	N/S
Cultivar * Treatment	N/S	N/S	N/S
Time * Treatment	N/S	N/S	N/S
Cultivar * Time * Treatment	N/S	N/S	0.0125

The third degree interaction for the two sunflower cultivars did not produce any treatments which germinated significantly better than both control treatments in each respective time interval. This may be the result of the delay in the determination of germination, since germination was only measured 48 hours after the seedlings was moved from the cold chamber (10^{o}C) to the germination chamber (25^{o}C). Germination within all three measurement intervals (48, 96 and 168 hours) for all treatments was between 80% and 100% (data not shown).

Germination results of maize indicated a difference in tolerance to cold stress between cultivars (Figure 4.5). Cultivar 2 had a germination advantage of 10.88%, 14.75% and 16.25% over cultivar 1 at the 48, 96 and 168 hour intervals, respectively. This is confirmed by the fact that cultivar 1 is an excellent performer in warm irrigated regions and that cultivar 2 is described as having excellent stress tolerance.

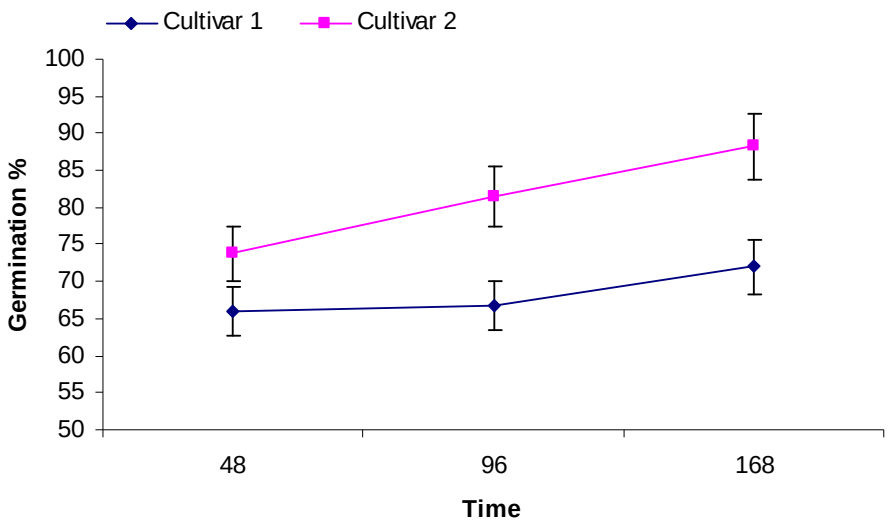

Figure 4.5 Germination of two maize cultivars, under cold stress germination conditions observed over 48, 96 and 168 hour intervals.

There was a significant difference in germination between the two sorghum cultivars (Figure 4.6). Cultivar 4 outperformed cultivar 3 with a significant margin as a result of differences in cultivar characteristics with regard to stress tolerance.

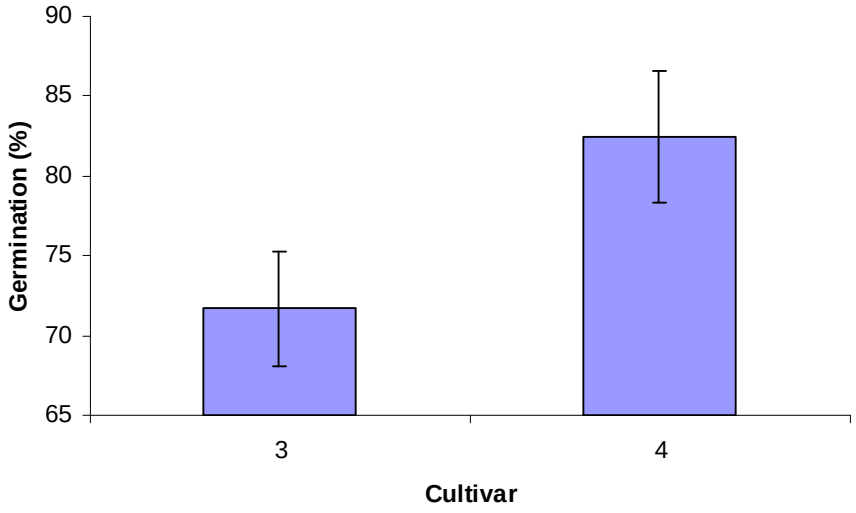

Figure 4.6 Germination of two sorghum cultivars under cold stress germination conditions, observing difference in germination averages between the two cultivars. Vertical bars denote 0.95 confidence intervals.

4.3.2.2 Seedling vigour after the cold test

The plant length measurements for maize and sunflower after exposure to cold stress conditions, resulted in a significant third degree interaction between cultivar, time and treatment (Table 4.6). Sorghum had a second degree interaction at a significant level between cultivar and treatment.

Table 4.6 Analysis of variance (ANOVA) of the plant lengths of maize, sorghum and sunflower, exposed to cold stress, as affected by cultivar, time and treatment.

	p-values		
Effect	**Maize**	**Sorghum**	**Sunflower**
Cultivar	0.0055	0.0000	N/S
Time	0.0000	0.0000	0.0000
Treatment	0.0000	N/S	0.0001
Cultivar * Time	N/S	N/S	0.0000
Cultivar * Treatment	0.0000	0.0111	0.0476
Time * Treatment	0.0095	N/S	0.0104
Cultivar * Time * Treatment	0.0000	N/S	0.0054

Plant lengths of maize and sunflower at the 48 hour measurement did not reveal significant differences between treatments and the control treatments (data not shown). However at the 96 hour measurement interval there were treatments which significantly affected plant lengths positively and negatively compared to the control treatments (Figure 4.7a & b).

Cultivar 2 was the only cultivar that was significantly positively affected by the M-EM treatment. Only the three treatments of S3 B which did not increase plant length. The plant length of cultivar 1 were negatively affected by six treatments including S1 A R-S, S1 A 30, S1 B R-S, S2 A 24H, S3 A 24H and S3 A 30 treatments.

Cultivar 5 had four treatments which affected plant length negatively namely: S2 A R-S, S2 A 24H, S3 B R-S, S3 B 24H treatments in addition to the three treatments of cultivar 6, S1 B 30, S2 A 24H and S3 A R-S. Even though M-EM 30 had a positive effect on germination, M-EM 30 did not have an effect on seedling vigour, except for on cultivar 2, with its excellent stress tolerance characteristic (Pannar, 2011). Therefore the effect on cultivar 2 could rather be accredited to its characteristics in combination with M-EM treatment.

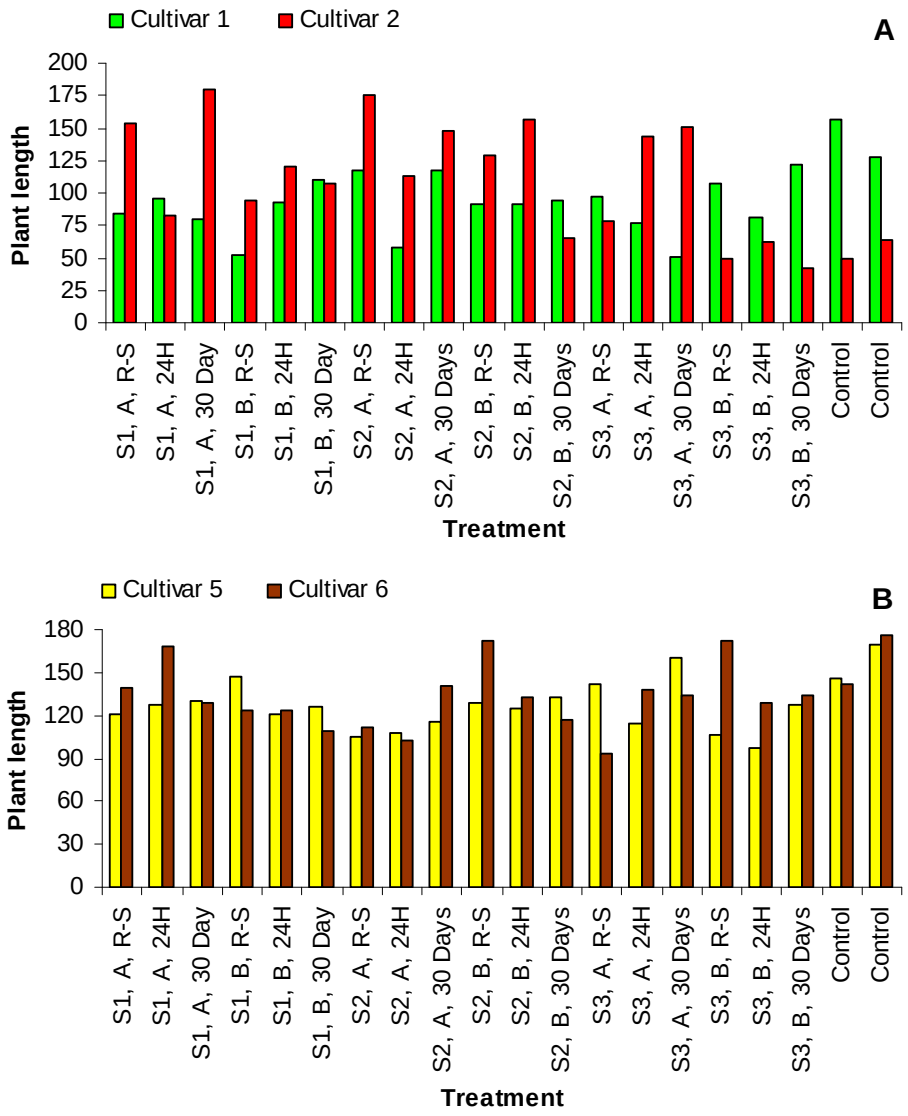

Figure 4.7 Plant lengths of a) two maize and b) two sunflower cultivars at 96 hours intervals, under stress conditions in a cold test, observed with regard to treatments and compared to the control treatments.

Plant length measurements of the two sorghum cultivars (Figure 4.8) revealed that for cultivar 3 only S1 B R-S treatment affected plant length by significantly increasing plant length over both control treatments. Cultivar 4 had no treatments which had a significant effect on plant length.

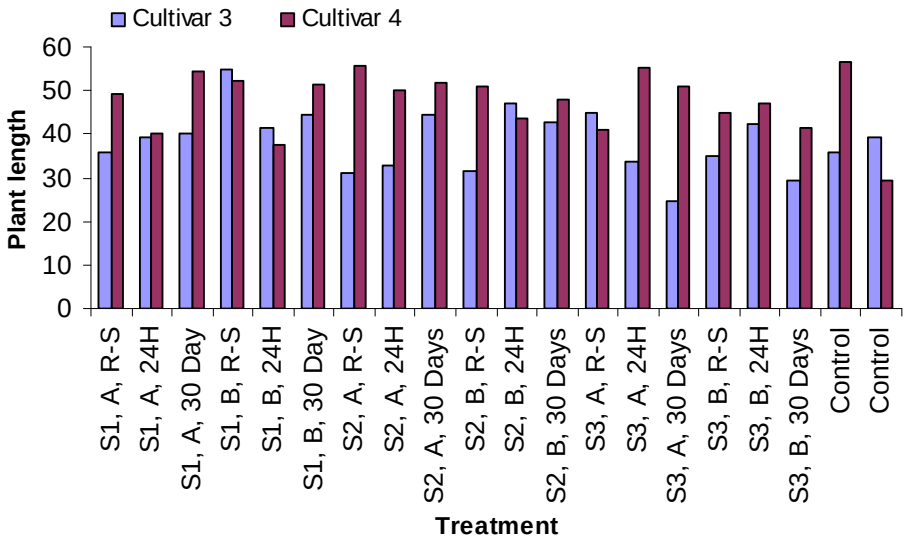

Figure 4.8 Plant lengths of two Sorghum cultivars under stress conditions, in a cold test observed in regards to treatments and compared to the control treatments.

4.4 Conclusion

Significant temperature fluctuations influence micro-organism survival thus, M-EM being a mixture of living micro-organisms needs to be stored in a storeroom with little temperature fluctuation and away from direct sunlight. However, there is no clear indication what effect M-EM as seed treatment, exposed to irradiation and temperature fluctuation will have on the germination and seedling vigour of maize, sorghum and sunflower. The purpose of the present study was therefore to ascertain the effectiveness of M-EM at different exposure rates to irradiation and temperature fluctuation, on the germination and seedling vigour of maize, sorghum and sunflower. The results indicated that under favourable germination conditions the only positive effect of M-EM seed treatment were with the 30 day stored M-EM. This indicated that with the correct handling of M-EM germination can be improved, while irradiation and temperature fluctuation nullifies the effect of M-EM on germination. Therefore producers have to consider the correct handling of EM as important in order to get positive germination results. Under the cold stress conditions the plant lengths of cultivars 2 and 3 were positively affected by only a few M-EM treatments. M-EM application therefore seems to be beneficial to seedling growth only for maize and sorghum cultivars which are more susceptible to stress. The study therefore highlighted the importance of

handling S-EM, M-EM and diluted M-EM in the manner as described by the supplier at all times.

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Chapter 5

The influence of Effective micro-organisms at different dilutions, on the germination and seedling vigour of maize, sorghum and sunflower in soil

Abstract

Pot experiments were conducted to determine the effect of effective micro-organisms (EM) as seed treatment, at different dilutions, on germination, seedling vigour and dry mass of maize, sorghum and sunflower at different planted depths.

Maize was planted at depths of 50 mm and 100 mm, sorghum at 30 mm and 60 mm, and sunflower at 25 mm and 50 mm. The Stock-EM (S-EM) from three suppliers was multiplied at two different ratios each, namely: 1% and 3%. In this study these two different ratios Multi-EM were diluted at three levels namely: 0.01%, 0.1% and 1%. Seeds were treated with the diluted M-EM and planted in untreated soil to investigate the influence on germination, vigour and dry mass.

The results indicate that the treatment of seed with M-EM did not have a prominent effect on germination. Shoot length results, however, indicated that seed treated with M-EM might have a significant effect on seedlings survival, which might lead to an increase in yield. A greater effect was visible on the shoot length of shallow planted seeds, than on deeper planted seeds. Shoot length results were more affected by M-EM treatment than the results of germination or dry mass. The only significantly affected dry mass was that of sorghum, which were increased by six M-EM treatments from different companies, at different ratios and at different dilutions. No real trend could be found between supplier companies and multiplied ratios, seeing that all three companies and both ratios lead to positive and negative results. The findings also indicate that under these experimental conditions the most prominent treatment were with S3 A 0.1% leading to the most significant affect across the three crops for germination, shoot length and dry mass.

Keywords: Multi-EM, seed treatment, planting depth, dry mass.

5.1 Introduction

Effective micro-organisms (EM) contains over 80 selected types of micro-organisms (Woodward, 2003; Singh, 2007). These include populations of lactic acid bacteria, photosynthetic bacteria, yeasts and fermenting fungi (Higa & Parr, 1994; Diver, 2001 as cited by Szymanski & Patterson, 2003). EM cultures do not contain any genetically modified micro-organisms and is made up of mixed cultures of microbial species which are found in natural environments, throughout the world (Anon, 1995). These natural occurring micro-organisms are known to increase the bio-diversity of the micro flora which in return increases the yield of crops (Condor Golec *et al*., 2007). Photosynthetic bacteria are the main component of EM (Anon, 1995), working synergistically with other micro-organisms to provide the nutritional requirements to plants and also reducing manifestation of diseases (Condor Golec *et al*., 2007).

EM has a broad variety of applications and has no adverse effects on plants, animals or humans. In terms of the production of crops, EM can be applied as a seed treatment, as pre-planting treatment, as planting treatment, every three to four weeks during crop growth and can usefully be applied to crop residues after harvest and just before incorporating residues into the soil (D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009). Crop seeds inoculated with EM increase the microbial diversity of the soil (Lindros, 2010) and increase seed viability (EdenBound, 2010).

No clear indication is given by supplier companies, as a direction of use, for the application of EM as a seed treatment. Efficient Microbes (2010) recommends that seeds should be soaked in a one to ten thousand dilution. In contrast, D. Anthony (personal communication, March 2009) and EdenBound (2010) recommend that seeds should be soaked in a one to one thousand dilution for 5 to 10 minutes. A. Rosenberg (personal communication, March 2009) recommends that small seeds should be soaked for up to 30 minutes in a one to one thousand dilution, and large seeds, such as maize, for up to 8 hours. EM soaked seeds should further be dried in the shade before planting to avoid them from sticking together (D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009; EdenBound, 2010).

Recommendations by suppliers therefore vary greatly and there exists a lack of knowledge from research results in terms of the treatment dilutions of seeds with EM. Therefore, the objective of this study was to determine the effect of EM as seed treatment, at different dilutions, on germination, seedling vigour and dry weight of maize, sorghum and sunflower in pot experiments.

5.2 Material and methods

5.2.1 Location and experimental layout

The experiment was conducted in a greenhouse of the School for Agricultural and Environmental Sciences of the Central University of Technology, Free State. Plastic growing bags, with a 100 mm diameter and 1 ℓ capacity, were used as pots. The bags had holes at the bottom and a sandy soil was used as growth medium. The bags were maintained in a naturally ventilated greenhouse without temperature control. Seeds were hand planted and the soil was compacted by applying minimal hand pressure on top of the soil. The soil in the bags was moistened with pure water as needed. Maize of the cultivars, PAN 6236 (cultivar 1) and PAN 6053 (cultivar 2), sorghum of the cultivars, PAN 8247 (cultivar 3) and PAN 8816 (cultivar 4), and sunflower of the cultivars, PAN 7351 (cultivar 5) and PAN 7033 (cultivar 6), were used in the three experiments. More than 1700 seeds were used per cultivar and the experiment was replicated four times.

5.2.1.1 The maize, sorghum and sunflower cultivars

- PAN 6236 (cultivar 1) is an ultra early yellow maize, which achieves excellent results under irrigation as well as high potential dry land conditions. The cultivar does exceptionally well in the Orange River area and other warm irrigation regions (PANNAR, 2011).
- PAN 6053 (cultivar 2) is medium maturing white maize cultivar, with excellent yield potential and proven reliability under low rainfall conditions, producing yields at low plant populations (PANNAR, 2011).
- PAN 8247 (cultivar 3) is a sorghum with good yield potential and has a very uniform plant type (PANNAR, 2011).

- PAN 8816 (cultivar 4) is a popular sorghum and recommended for the main planting in all sorghum production areas. The cultivar has an excellent yield potential and stability (PANNAR, 2011).
- PAN 7351 (cultivar 5) is a sunflower with a wide area adaptability, a high yield potential and a good stability, with outstanding performance in commercial plantings (PANNAR, 2011).
- PAN 7033 (cultivar 6) is a top performer sunflower in cultivar trails over the past three years and is recommended for the main bulk planting in all production regions (PANNAR, 2011).

Different planting depths were used in the pot experiments and are listed in Table 5.1.

Table 5.1 Maize, sorghum and sunflower were planted at two depths in untreated sandy soil.

Crop		Depth 1	Depth 2
Maize	-	50 mm	100 mm
Sorghum	-	30 mm	60 mm
Sunflower	-	25 mm	50 mm

5.2.2 M-EM treatments

Generally fallible Stock-EM (S-EM) was obtained from three different commercial companies and due to a secrecy agreement names will be withheld and in this document the products will be revered to as S1, S2 and S3. Multi-EM (M-EM) was produced from S-EM of each of the three suppliers at the following ratios:

- M-EM (A) at a ratio of 1% S-EM, 7% molasses and 92% water.
- M-EM (B) at a ratio of 3% S-EM, 5% molasses and 92% water.

After the M-EM stood for 14 days to multiply each of the M-EM's were diluted with water at three levels namely: 0.01%, 0.1% (which is also the standard dilution in practice) and 1%. A control that consisted of soaking seeds in purified water was prepared for comparison. Seeds of each cultivar were soaked for seven hours in the three different dilutions M-EM (A) and M-EM (B) in a dark environment. M-EM seed treatment variables per crop for the experiment are summarised in Table 5.2. After soaking, seeds were left to dry in the laboratory.

Dried seeds were planted at 10 seeds per 100 mm-diameter bag filled with untreated soil.

Table 5.2 M-EM seed treatment variables per crop. Stock EM from three different suppliers were multiplied at two ratios (1% and 3%) and diluted at three levels (0.01%, 0.1% and 1%), all of the seeds were planted in untreated soil.

	Maize	**Sorghum**	**Sunflower**
Cultivars	2	2	2
Number of seeds	10	10	10
Planting depth	2	2	2
Replications	4	4	4
EM suppliers	3	3	3
Multiplied ratios	2	2	2
Application rates	3	3	3
Number of control seeds	320	320	320
Total number of seeds	3200	3200	3200

To simplify statistical analysis and interpretation of results, EM suppliers, multiplied ratios, dilutions and the control treatments were pooled into 20 treatment combinations (Table 5.3), which will be referred to as treatments throughout the rest of this chapter. Treatment abbreviations are coded and are not an indication of the supplier company.

Table 5.3 Treatment combinations 1 to 20 with regard to treatment abbreviation, EM supplier company, multiplied ratio and dilution.

Treatment number	Treatment abbreviation	Supplier company	Multiplied ratio	Dilution
1	S1 A 0.01%	1	A	0.01%
2	S1 A 0.1%	1	A	0.10%
3	S1 A 1%	1	A	1%
4	S1 B 0.01%	1	B	0.01%
5	S1 B 0.1%	1	B	0.10%
6	S1 B 1%	1	B	1%
7	S2 A 0.01%	2	A	0.01%
8	S2 A 0.1%	2	A	0.10%
9	S2 A 1%	2	A	1%
10	S2 B 0.01%	2	B	0.01%
11	S2 B 0.1%	2	B	0.10%
12	S2 B 1%	2	B	1%
13	S3 A 0.01%	3	A	0.01%
14	S3 A 0.1%	3	A	0.10%
15	S3 A 1%	3	A	1%
16	S3 B 0.01%	3	B	0.01%
17	S3 B 0.1%	3	B	0.10%
18	S3 B 1%	3	B	1%
19	Control	Control	N/A	N/A
20	Control	Control	N/A	N/A

5.2.3 Measurements

Seedlings were scored as germinated at a shoot emersion length of 3 mm above the soil level on day seven and day 14 after planting. Vigour was also determined at both days seven and 14 by measuring shoot length above soil. On measurement day 14 the seedlings were cut-off at soil level and dried.

5.2.4 Statistical analysis

A factorial analysis of variance (ANOVA) was performed on the germination, seedling vigour and dry mass with cultivars, suppliers, M-EM ratios, M-EM dilutions and planting depth as factors on both day seven and day 14. P-values were used to compare means at a 5% probability level, using STATISTICA version 8.0 (Statsoft Inc., 2004).

5.3 Results and discussion

The discussion will focus on the effect of the treatments on cultivars at different depths. Where the significant difference of day seven maintained itself into day 14, both results will be discussed. Where results became significant or insignificant between day seven and day 14, only the significant results of day 14 will be discussed. This is because the significant effect of day seven could not be maintained.

5.3.1 Germination rate

There was no visible trend, caused by the treatments. Maize at measurement day 14 (Table 5.4) had a significant second degree interaction between cultivar, depth and treatment. Sorghum on day 14 only had significant main effects for cultivar, depth and treatment and sunflower had a significant first degree interaction between cultivar and treatments.

Table 5.4 Analysis of variance (ANOVA) of the germination of maize, sorghum and sunflower, grown in pots, as affected by cultivar, depth and treatments on day seven and day 14.

	p-values					
Effect	**Maize 7**	**Maize 14**	**Sorghum 7**	**Sorghum 14**	**Sunflower 7**	**Sunflower 14**
Cultivar	N/S	0.0047	0.0000	0.0000	0.0356	N/S
Depth	N/S	0.0112	0.0000	0.0000	0.0000	0.0000
Treatment	0.0258	N/S	0.0093	0.0266	N/S	N/S
Cultivar * Depth	0.0000	0.0000	0.0018	N/S	N/S	N/S
Cultivar * Treatment	N/S	N/S	N/S	N/S	0.0071	0.0108
Depth * Treatment	N/S	N/S	N/S	N/S	N/S	N/S
Cultivar * Depth * Treatment	N/S	0.0465	N/S	N/S	N/S	N/S

At measurement day 14 (Figure 5.1), the only significant effect on the germination of maize was caused by S1 B 0.01%, which significantly decreased the germination rate of cultivar 2 at depth 2 compared to the control treatments.

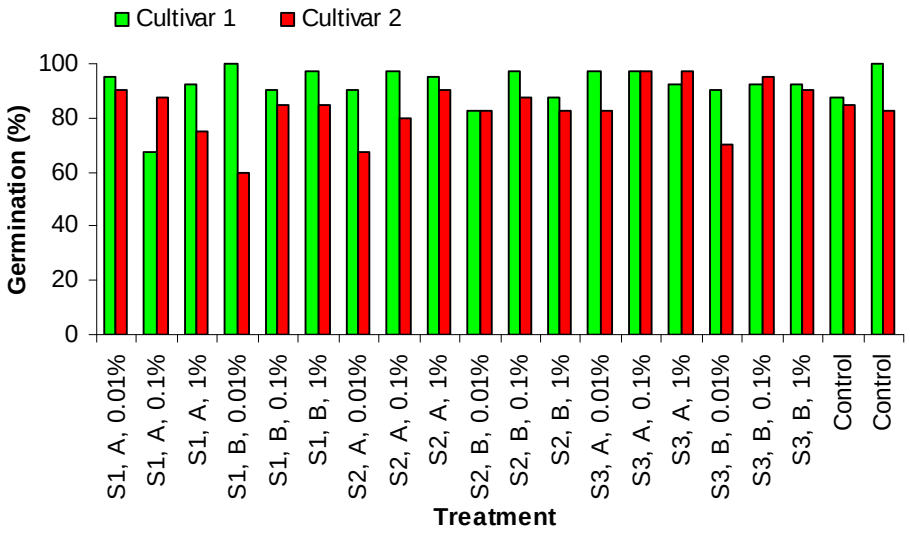

Figure 5.1 Germination of two maize cultivars on day 14 at a depth of 100 mm, observed with regard to cultivar and treatment (consisting of a supplier, multiplied ratio and dilutions) planted in untreated soil.

From Figure 5.2 for sorghum, clearly indicate that cultivar 4 outperformed cultivar 3 in terms of germination. The results in the germination studies under cold stress germination in chapter four and chapter five also had the same result.

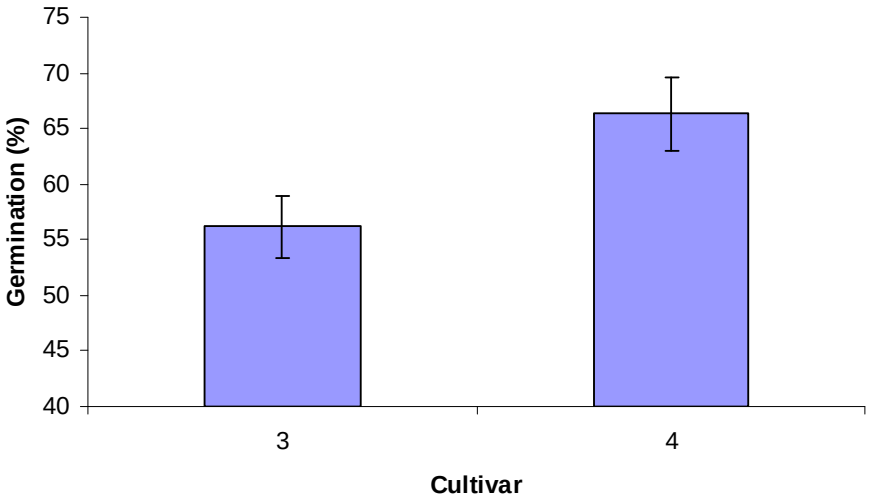

Figure 5.2 Germination of two sorghum cultivars on day 14, observed for two different cultivars.

The average germination of sorghum (Figure 5.3) compared in terms of treatments revealed three treatments which increased germination significantly

compared to the control treatments. These treatments were all diluted at the prescribed dilution of 0.1% which includes S1 A 0.1%, S2 B 0.1% and S3 A 0.1%.

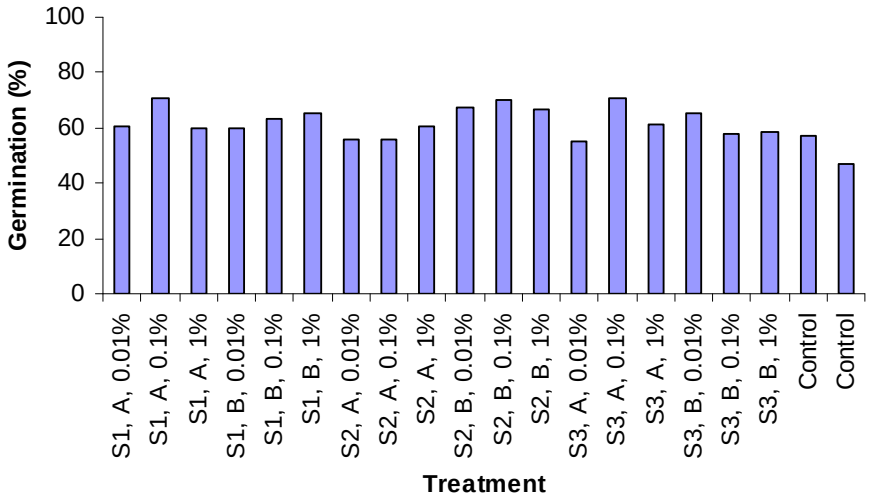

Figure 5.3 Average germination of sorghum on day 14, observed with regard to M-EM ratios and concentrations compared to the control treatments.

The germination of the two sunflower cultivars at measurement day seven did not reveal any treatments which had a significant effect compared to the control treatments. However, at the 14 day measurement interval (Figure 5.4) the only significant interaction was caused by S1 A 1% treatment, which significantly decreased germination of cultivar 6.

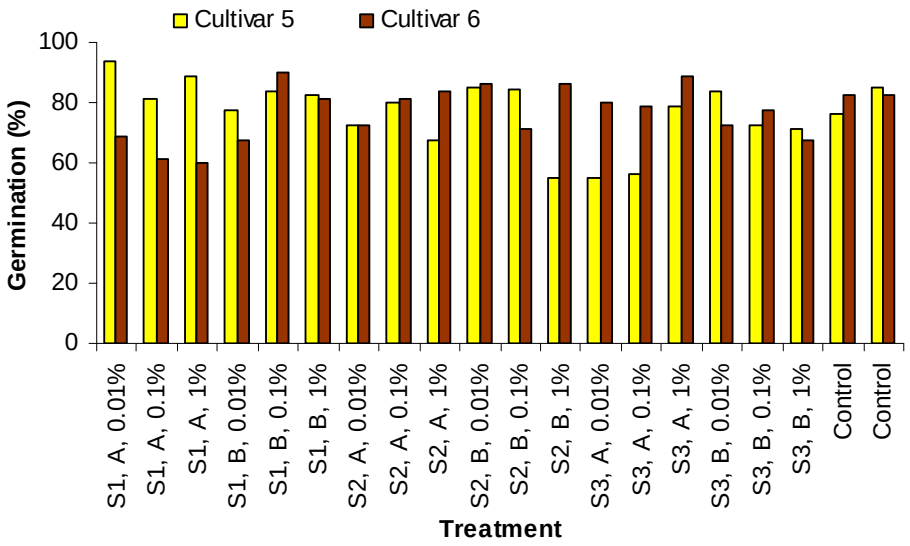

Figure 5.4 Germination of two sunflower cultivars on day 14, observed with regard to M-EM ratios and concentrations compared to the control treatments.

5.3.2 Shoot lengths

All shoot lengths (Table 5.5) of maize, sorghum and sunflower on day seven and day 14, had significant third degree interactions with regard to cultivar, depth and treatment. Significant differences between shoot lengths in terms of planting depths were expected, since each cultivar was planted at its minimum and maximum prescribed planting depths.

Table 5.5 Analysis of variance (ANOVA) of shoot length of maize, sorghum and sunflower, grown in pots, as affected by cultivar, depth and treatments on day seven and day 14.

	p-values					
Effect	**Maize 7**	**Maize 14**	**Sorghum 7**	**Sorghum 14**	**Sunflower 7**	**Sunflower 14**
Cultivar	0.0000	0.0000	0.0001	0.0000	N/S	0.0000
Depth	0.0000	N/S	0.0000	0.0000	0.0000	0.0000
Treatment	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Cultivar * Depth	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Cultivar * Treatment	0.0000	0.0000	0.0000	N/S	0.0000	0.0000
Depth * Treatment	0.0000	0.0017	0.0000	N/S	0.0000	0.0000
Cultivar * Depth * Treatment	0.0000	0.0001	0.0000	0.0425	0.0000	0.0000

For maize, there were some treatments which significantly increased shoot length over that of the control treatments on day seven (data not shown). However, as time passed to day 14 (Figure 5.5), only a few treatments could persist in their improved performance.

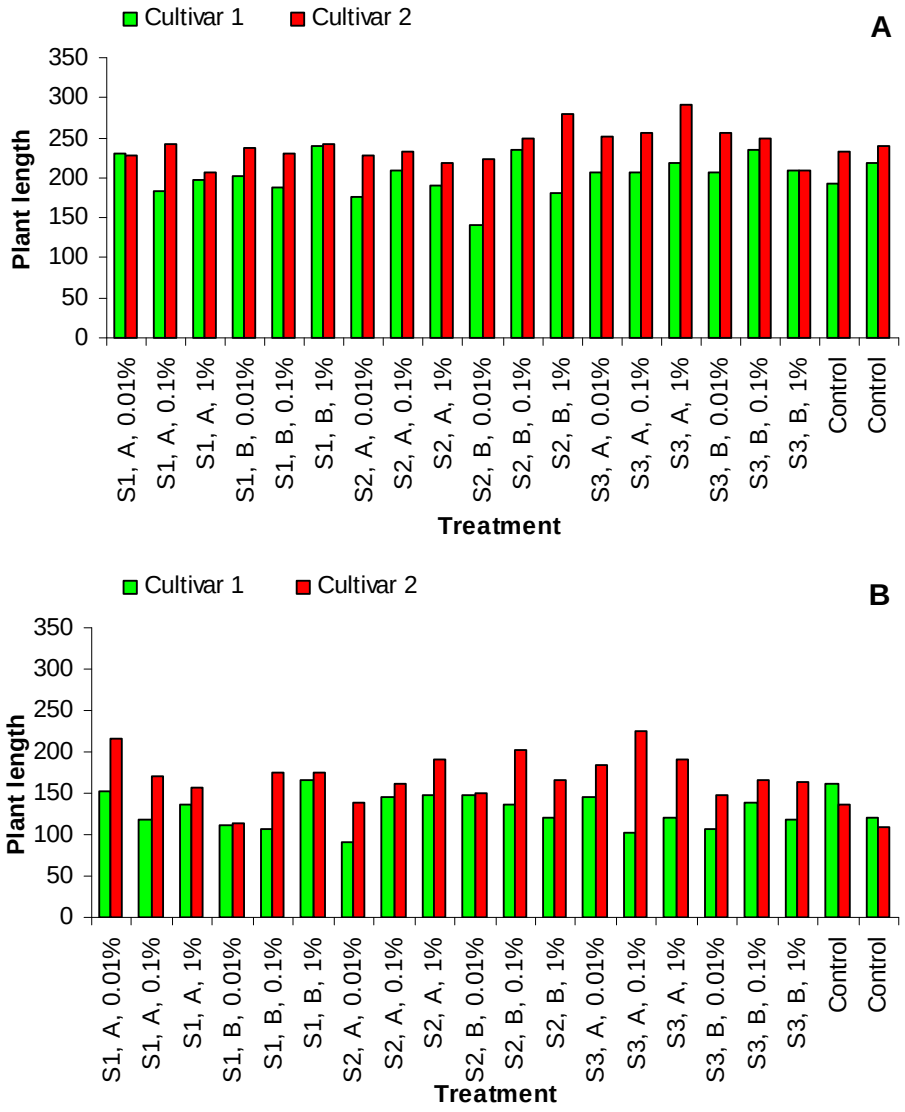

Figure 5.5 Shoot length of two maize cultivars on day 14 at two depths, a) 50 mm, and b) 100 mm, observed with regard to M-EM ratios and concentrations compared to the control treatments.

At the 50 mm depth, the only significant difference between treatments and controls was S2 B 0.01% which significantly reduced shoot length of cultivar 1 on measurement day 14 (Figure 5.5a). For cultivar 2, S2 B 1% significantly

increased shoot length over that of both control treatments on measurement days seven (data not shown) and 14 while S3 A 1% increased shoot length significantly on day 14.

At the 100 mm depth, only cultivar 2 had treatments which increased shoot length significantly on day 14 namely: S1 B 0.1%, S1 B 1%, S2 A 1%, S2 B 0.1%, S3 A 0.01%, S3 A 0.1%, S1 A 0.01% and S3 A 1% (Figure 5.5b). The two latter treatments outperformed the control treatments on both measurement days.

Sorghum planted 30 mm deep revealed that cultivar 3 had 12 treatments which significantly improved shoot length over the control treatments on measurement day seven (data not shown) and 14 (Figure 5.6). They are listed as follow: S1 A 0.01%, S1 A 0.1%, S1 B 0.01%, S1 B 0.1%, S1 B 1%, S2 B 0.01%, S2 B 0.1%, S2 B 1%, S3 A 0.1%, S3 A 1%, S3 B 0.01% and S3 B 0.1%. Cultivar 4 had no treatments on measurement day 14 which increased shoot length significantly.

At a 60 mm planting depth no treatments significantly affected shoot length compared to the control treatments for both cultivars 3 and 4 on measurement day 14 (data not shown).

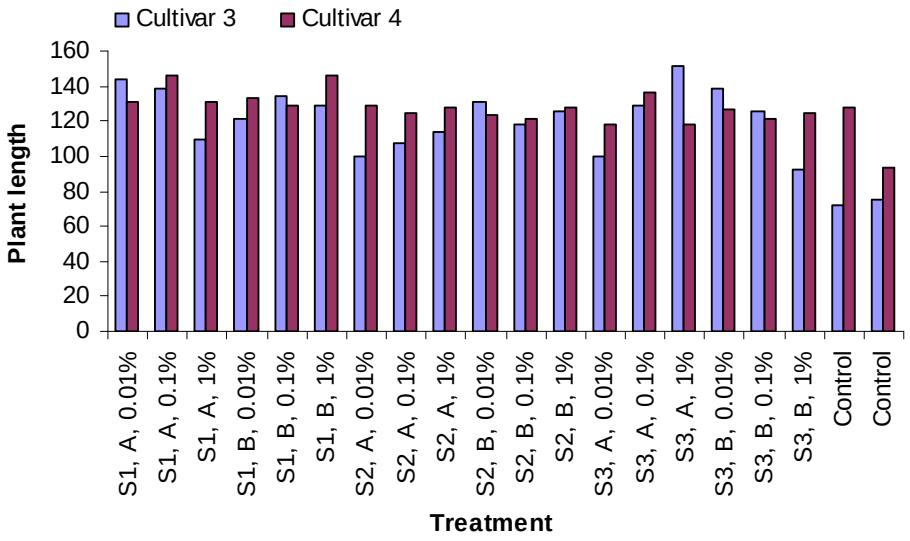

Figure 5.6 Shoot length of two sorghum cultivars on day 14 at 30 mm, observed with regard to M-EM ratios (1% & 3%) and concentrations (0.01%, 0.1% & 1%) compared to the control treatments.

For sunflower, cultivar 5 had four treatments which increased shoot length with a significant margin over that of the control treatments at a planting depth of 25 mm at both day seven (data not shown) and 14 namely: S1 A 1%, S1 B 0.01%, S1 B 1% and S1 B 0.1% (Figure 5.7a). Cultivar 6 had three treatments which increased shoot length on day seven (data not shown) and day 14 when planted 25 mm deep, namely: S1 B 0.1%, S2 A 0.01% and S2 A 0.1%, while treatments S1 B 1%, S2 B 0.01% and S3 A 1% only increased shoot length on day 14.

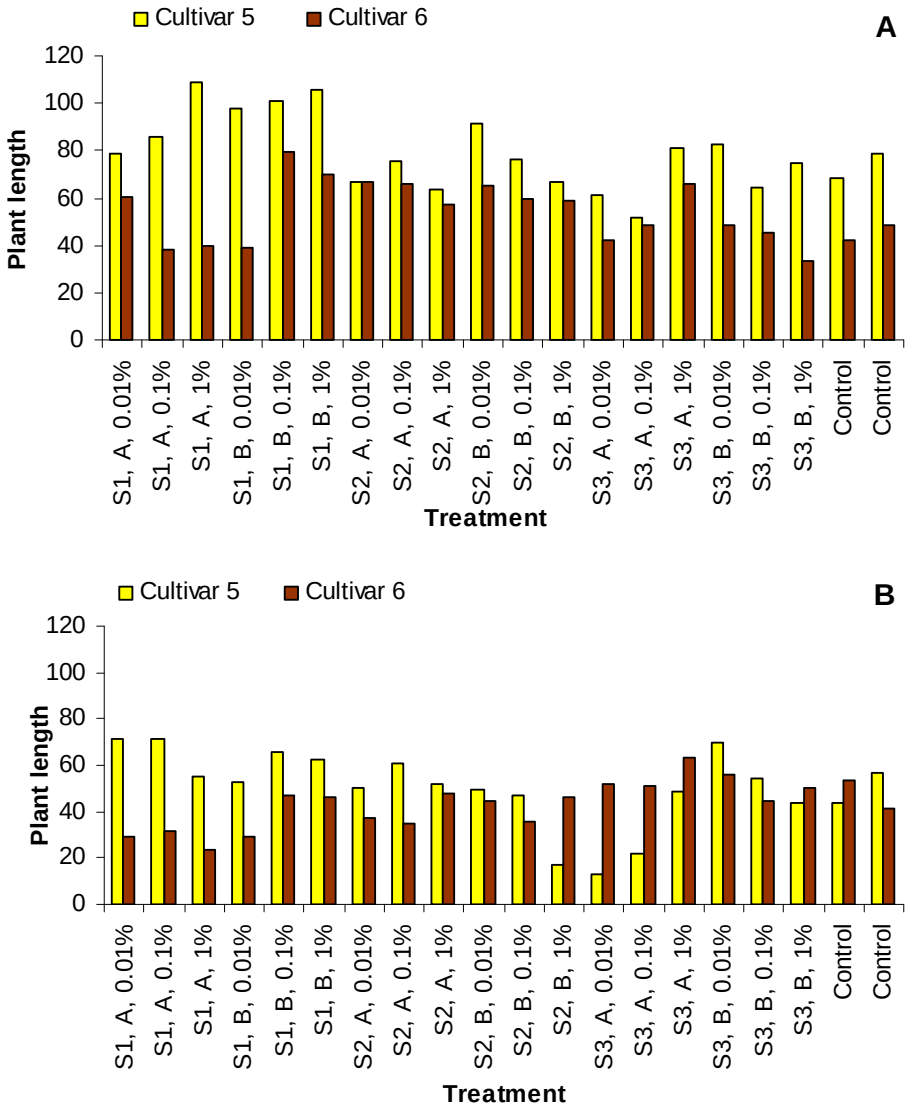

Figure 5.7 Shoot length of two sunflower cultivars on day 14 at two depths, a) 25 mm, and b) 50 mm, observed with regard to M-EM ratios and concentrations compared to the control treatments.

At a planting depth of 50 mm, both sunflower cultivars experienced significant decreased shoot lengths compared to control treatments on day 14 (Figure 5.7b). Cultivar 5 had three treatments which negatively effected shoot length, namely; S2 B 1%, S3 A 0.01% and S3 A 0.1%, and cultivar 6 reacted negatively to the S1 A 1% treatment. Visible from these results that M-EM treatment had more significant effects on shoot length of sorghum and sunflower at the shallow planted depth and that the extra strain placed on the seedlings by the deeper planting depth enhanced the negative effect of M-EM. However, the results of maize were contradictory, with M-EM treated seedlings of cultivar 2 revealing significant increase in shoot length compared to the control treatments at the deeper planting depth of 100 mm.

5.3.3 Dry mass

Maize had a significant first degree interaction between cultivar and depth, while sorghum had significant main effects with treatments, depth and cultivars (Table 5.8). Sunflower had significant main affects from treatments and depths.

Table 5.6 Analysis of variance (ANOVA) of the dry mass of maize, sorghum and sunflower, grown in pots, as affected by cultivar, depth and treatment on day 14.

	p-values		
Effect	**Maize**	**Sorghum**	**Sunflower**
Cultivar	0.0036	0.0000	N/S
Depth	N/S	0.0000	0.0004
Treatment	N/S	0.0428	0.0471
Cultivar * Depth	0.0000	N/S	N/S
Cultivar * Treatment	N/S	N/S	N/S
Depth * Treatment	N/S	N/S	N/S
Cultivar * Depth * Treatment	N/S	N/S	N/S

Maize cultivar 2 had a significantly heavier dry mass than cultivar 1 at both 50 mm and 100 mm planting depths (Figure 5.8). The difference between cultivar 2 and cultivar 1 was 0.0882g at 50 mm, while this difference increased with 0.0035g at 100 mm. The difference was expected since cultivar 1 was also outperformed by cultivar 2 in terms of germination and shoot lengths. The reason for cultivar 2 outperforming cultivar 1 may be ascribed to the difference in

cultivar characteristics in terms of adaptability and stress tolerance. Characteristics of cultivar 2 are that cultivar 2 is highly adaptable and has excellent stress tolerance (Pannar, 2011).

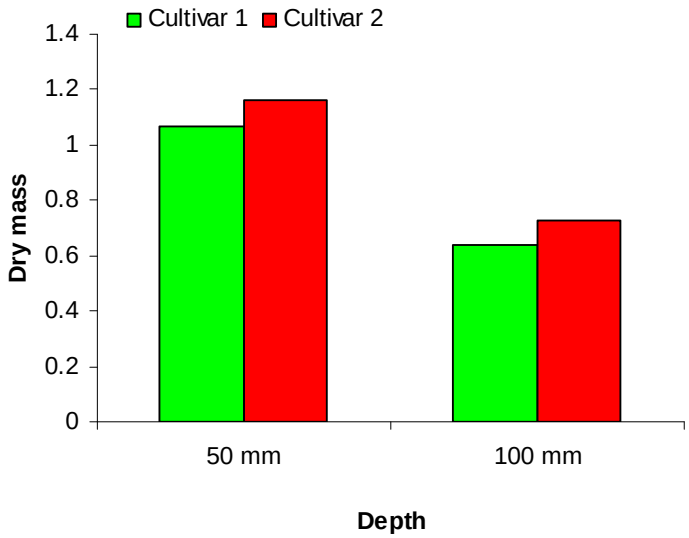

Figure 5.8 Dry mass of two maize cultivars on day 14, planted at two depths, namely; 50 mm and 100 mm.

Figure 5.9 clearly indicates that the dry mass of sorghum cultivar 4 was significantly more than that of cultivar 3. The average dry mass of cultivar 3 is 0.1055g and that of cultivar 4 is 0.1645g. The germination advantage that cultivar 4 had over cultivar 3 and the shoot length difference visible in Figure 5.2, may be part of the reason for the difference in dry mass.

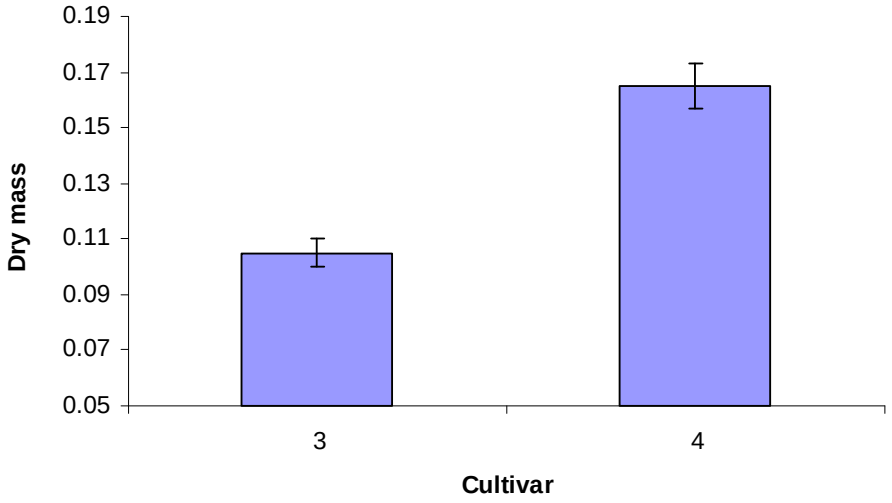

Figure 5.9 Dry mass of two sorghum cultivars on day 14.

Dry mass of M-EM treated seedlings were higher than the control seedlings for all M-EM treatments (Figure 5.10). The average dry mass of sorghum was, however, significantly increased over both control treatments by six treatments. These treatments were as follows: S1 A 0.1%, S1 A 1%, S1 B 1%, S2 B 0.01%, S2 B 0.1% and S3 A 0.1%.

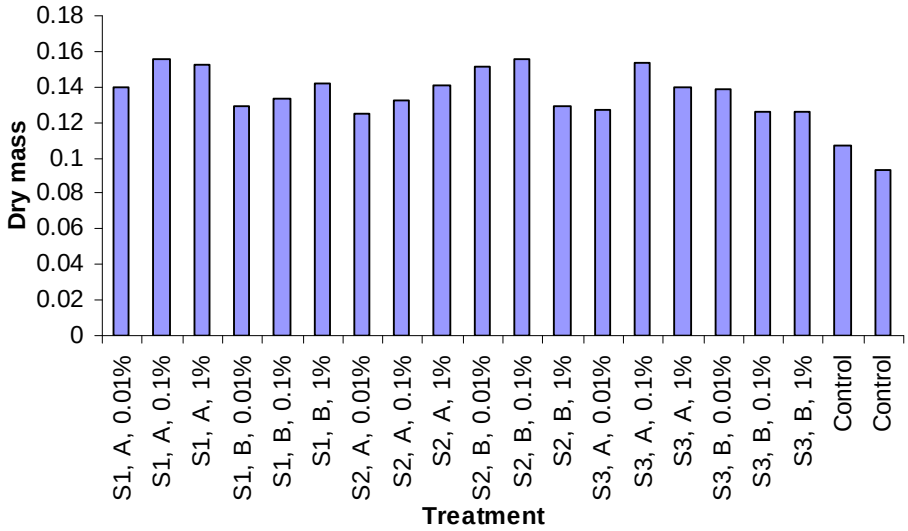

Figure 5.10 Dry mass of sorghum on day 14, observed with regard to M-EM ratios and concentrations compared to the control treatments.

The dry mass of sunflower was affected at a significant level by only two treatments namely: S1 B 0.01% and S2 A 0.01% (Figure 5.11). Both these two treatments had significantly lower dry mass compared to the control treatments.

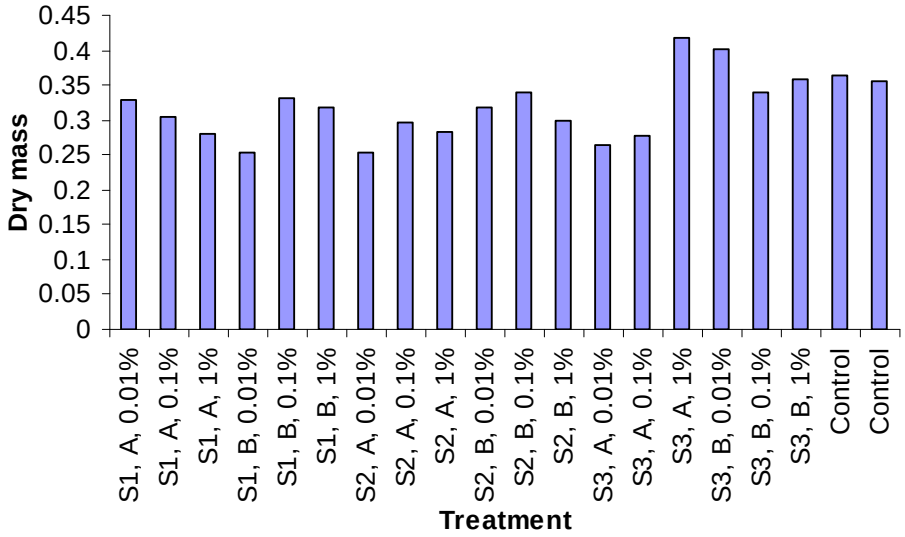

Figure 5.11 The dry mass of sunflower on day 14, observed with regard to M-EM ratios and concentrations compared to the control treatments.

5.4 Conclusion

M-EM has a broad variety of applications and can be applied as a seed treatment or as a soil or plant treatment at almost any time during the season to improve crop production. However, there seems to be a lack in research in terms of the effectiveness of M-EM as a seed treatment at different dilutions. The purpose of the present study was therefore to ascertain the effectiveness of using M-EM at different dilutions as a seed treatment on the germination, seedling vigour and dry mass production of maize, sorghum and sunflower. The results indicate that under these experimental conditions the treatment of seed with M-EM did not have a prominent effect on germination. Several M-EM treatments did however increase shoot length of all three crops significantly while others had detrimental effects. Dry mass of sorghum was increased by M-EM treatment even though not significant in all cases, with no effect on maize and sunflowers dry mass. The most prominent dilutions were at the 0.1% and 1% levels, with neither multiplied ratio nor supplier company having any real effect on outcomes. S3 A 0.1% and S2 B 0.1% had the most positive effect across the three crops for germination,

shoot length and dry mass. From the results can be concluded that M-EM might affect shoot length and dry mass in early seedling growth. M-EM treated seedlings will thus, faster overcome germination and growth straining factors such as climate and insects, than untreated seedlings. Depth experiments were executed to place more strain on seedlings to promote the effects of the treatments, however, no notable effect was found. Further research may be required to determine the effect of M-EM at these dilutions on the germination, vigour and dry mass of maize, sorghum and sunflower under more stressed environmental conditions.

5.5 References

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Chapter 6

The influence of Effective micro-organisms exposed to irradiation and temperature fluctuation on germination and seedling vigour of maize, sorghum and sunflower, in soil

Abstract

Pot experiments were conducted to determine the influence of effective micro-organisms (EM) as a seed treatment on maize, sorghum and sunflower at different planting depths. In this study M-EM (multiplied at 1% and 3% ratios) were subjected to the influence of irradiation and temperature fluctuation.

The M-EM were divided into three groups from which the first group was left in an open field from sunrise to sunset, the second group was left for 24 hours in the same field, and the third group was left in a room for 30 days. Seeds were then treated with these M-EM which had been influenced by temperature fluctuation and irradiation and planted at different depths in untreated soil to investigate the influence on germination and seedling vigour.

M-EM treatments did not have an significant effect on the germination results of maize or sunflower. Germination of deeper planted sorghum seeds was significantly improved by S1 B 30 compared to control treatments. Positive effects from M-EM treatment were visible on the shoot length results of deeper planted maize and sunflower and an overall shoot length of sorghum. Results of the shoot length experiments might be an indication that M-EM, even if stored in undesired conditions, might have a positive effect on germination and seedling vigour. In conclusion, the exposure of M-EM to irradiation, temperature fluctuations or even prolonged storage might compromise the effectiveness of on crop production, even though M-EM still might have a positive effect on crops.

Keywords: Stock-EM, Multi-EM, seed treatment, shoot length.

6.1 Introduction

During the past decades, a microbial inoculation referred to as Effective Micro-organisms (EM) has been used with considerable success in nature farming and organic farming systems in Japan and throughout the Asia-Pacific region (Iwaishi, 2000). EM is a mixed culture of naturally-occurring, beneficial micro-organisms, predominately lactic acid bacteria, photosynthetic bacteria, yeast, actinomycetes and fungi (Higa & Parr, 1994; Diver, 2001 as cited by Szymanski & Patterson, 2003; Singh, 2007) that has reportedly enhanced soil quality and biodiversity and increased the growth, yield and quality of crops (Higa & Parr, 1994; Condor Golec *et al*., 2007).

The correct storage conditions play a vital role in the survival of EM. EM bought in the form of Stock EM (S-EM) can be stored for up to six months under the right conditions (Anon, 1995; EMROSA, 2006; A. Rosenberg, personal communication, March 2009). Stock EM (S-EM) can be multiplied into Multi-EM (M-EM) to save on costs, however, in this multiplied state EM can only be safely used for one month if storage conditions were favourable (EMROSA, 2006; A. Rosenberg, personal communication, March 2009). While diluted M-EM should be used within three days (Anon, 1995).

The correct storage conditions depend on temperature control and protection from sunlight. According to Szymanski and Patterson (2003), environmental temperature plays a major role in micro-organism survival, with significant temperature fluctuations reducing their ability to survive. This is due to the fact that EM consists of live organisms for which the best temperature for storage ranges between 15^{o}C and 20^{o}C. Fluctuation of temperature should be less than 10^{o}C in 24 hours (EMROSA, 2008; A. Rosenberg, personal communication, March 2009). A good practise is to insulate the EM and M-EM containers with blankets or bubble sheets to protect the organisms from temperature fluctuations (A. Rosenberg, personal communication, March 2009). An additional important storage factor playing a roll in micro-organism survival is the need to store the EM away from direct sunlight (EMROSA, 2008; D. Anthony, personal communication, March 2009; A. Rosenberg, personal communication, March 2009).

With temperature and sunlight influencing EM survival, knowing what effect in-field handling by the farmer will have on the ability of EM to improve

germination, seedling vigour and plant growth is essential. Therefore, the objectives of this study was to determine the effect of Effective Micro-organisms (EM) seed treatment, subjected to irradiation and temperature fluctuation, on the germination and seedling vigour of maize, sorghum and sunflower in pot experiments.

6.2 Material and methods

6.2.1 Location and experimental layout

The experiment was conducted at a greenhouse of the School for Agricultural and Environmental Sciences of the Central University of Technology, Free State. Plastic growing bags, with a 100 mm diameter and 1 ℓ capacity, were used as pots. The bags had drainage holes in the bottom (to prevent over-watering) and a sandy soil was used as growth medium. The bags were maintained in a naturally ventilated greenhouse without temperature control. Seed were hand planted and the soil was compacted by applying minimal hand pressure on top of the soil. The soil in the bags was moistened with pure water as needed. Maize of the cultivars, PAN 6236 (cultivar 1) and PAN 6053 (cultivar 2), sorghum of the cultivars, PAN 8247 (cultivar 3) and PAN 8816 (cultivar 4), and sunflower of the cultivars, PAN 7351 (cultivar 5) and PAN 7033 (cultivar 6), were used in the three experiments. More than 1700 seeds were used per cultivar and the experiment was replicated four times.

6.2.1.1 The maize, sorghum and sunflower cultivars

- PAN 6236 (cultivar 1) is an ultra early yellow maize, which achieves excellent results under irrigation as well as high potential dry land conditions. The cultivar does exceptionally well in the Orange River area and other warm irrigation regions (PANNAR, 2011).
- PAN 6053 (cultivar 2) is medium maturing white maize cultivar, with excellent yield potential and proven reliability under low rainfall conditions, producing yields at low plant populations (PANNAR, 2011).
- PAN 8247 (cultivar 3) is a sorghum with good yield potential and has a very uniform plant type (PANNAR, 2011).

- PAN 8816 (cultivar 4) is a popular sorghum and recommended for the main planting in all sorghum production areas. The cultivar has an excellent yield potential and stability (PANNAR, 2011).
- PAN 7351 (cultivar 5) is a sunflower with a wide area adaptability, a high yield potential and a good stability, with outstanding performance in commercial plantings (PANNAR, 2011).
- PAN 7033 (cultivar 6) is a top performer sunflower in cultivar trails over the past three years and is recommended for the main bulk planting in all production regions (PANNAR, 2011).

Different planting depths were used in the pot experiments and are listed in Table 6.1.

Table 6.1 Maize, sorghum and sunflower were planted at two depths in untreated sandy soil.

Crop		Depth 1	Depth 2
Maize	-	50 mm	100 mm
Sorghum	-	30 mm	60 mm
Sunflower	-	25 mm	50 mm

6.2.2 M-EM treatments

Generally fallible Stock-EM (S-EM) from three different commercial companies was used to produce Multi-EM (M-EM). Due to a secrecy agreement names will be withheld and in this document the products will be revered to as S1, S2 and S3. M-EM was propagated at the following ratios:

- M-EM (A) at a ratio of 1% S-EM, 7% molasses and 92% water.
- M-EM (B) at a ratio of 3% S-EM, 5% molasses and 92% water.

M-EM (A) and (B) of each of the three companies were allowed to stand for 14 days to multiply in optimum prescribed conditions. Each of the three M-EM (A) and (B) was further divided into three bottles with a capacity of 2 ℓ each. The three bottles of both M-EM ratios were exposed to different environmental conditions, namely: 1) the first bottle was placed in an open field from sunrise to sunset, 2) the second bottle was placed in an open field for 24 hours, and 3) the last bottle was stored in a room with little temperature fluctuation and out of direct sunlight for 30 days. The M-EM bottles were left in a field just outside Bloemfontein during November and December of 2009. The average minimum

and maximum temperature for that time was 14^{o}C and 30^{o}C (Weather and Climate, 2011). The room that was used for storage was a laboratory with an air cooling system, which were used to regulate temperature between 15^{o}C and 20^{o}C.

6.2.3 Treatment of seeds

Seeds were soaked for seven hours in a 0.1% dilution of the two M-EM ratios, which had different amounts of exposure to irradiation and temperature fluctuation. A control that consisted of soaking seeds in purified water was prepared for comparison. After soaking, the seeds were left to dry in the laboratory. Dried seeds were planted 10 seeds per 100 mm-diameter bag. The bags were filled with untreated soil and placed in the greenhouse.

Table 6.2 Maize, sorghum and sunflower were used in this study with the listed variables effecting study layout and results of the experiment. In each replication there were 320 control seeds and all of the seeds were planted in untreated soil.

	Maize	**Sorghum**	**Sunflower**
Cultivars	2	2	2
Number of seeds	10	10	10
Planting depth	2	2	2
Replications	4	4	4
EM suppliers	3	3	3
Multiplied ratios	2	2	2
Exposure rates	3	3	3
Number of control seeds	320	320	320
Total number of seeds	3200	3200	3200

To simplify statistical analysis and interpretation of results, EM suppliers, multiplied ratios, exposure rates and the control treatments were pooled into 20 treatment combinations (Table 6.3), which will be referred to as treatments throughout the rest of this chapter. Treatment abbreviations are coded and are not an indication of the supplier company.

Table 6.3 Treatment combinations 1 to 20 in terms of EM supplier company, multiplied ratios, exposure rates and control treatments with which seed were treated for seven hours before planting.

Treatment number	Treatment abbreviation	Supplier company	Multiplied ratio	Exposed rates
1	S1 A R-S	1	A	Rise - Set
2	S1 A 24H	1	A	24 Hours
3	S1 A 30	1	A	30 Days
4	S1 B R-S	1	B	Rise - Set
5	S1 B 24H	1	B	24 Hours
6	S1 B 30	1	B	30 Days
7	S2 A R-S	2	A	Rise - Set
8	S2 A 24H	2	A	24 Hours
9	S2 A 30	2	A	30 Days
10	S2 B R-S	2	B	Rise - Set
11	S2 B 24H	2	B	24 Hours
12	S2 B 30	2	B	30 Days
13	S3 A R-S	3	A	Rise - Set
14	S3 A 24H	3	A	24 Hours
15	S3 A 30	3	A	30 Days
16	S3 B R-S	3	B	Rise - Set
17	S3 B 24H	3	B	24 Hours
18	S3 B 30	3	B	30 Days
19	Control	Control	N/A	N/A
20	Control	Control	N/A	N/A

6.2.4 Measurements

Seedlings were scored as germinated at a shoot emersions length of 3 mm above the soil level on day seven and day 14 after planting. Vigour was determined by measuring shoot length on day seven and day 14 after planting.

6.2.5 Statistical analysis

A factorial analysis of variance (ANOVA) was performed on the germination and seedling vigour with cultivars, suppliers, M-EM ratios, exposure rates and planting depth as factors on both day seven and day 14. P-values were used to compare means at a 5% probability level, using STATISTICA version 8.0 (Statsoft Inc., 2004).

6.3 Results and discussion

The discussion will focus on the effect of the treatments on cultivars at different depths. Where the significant difference of day seven maintained itself into day

14, both results will be discussed. Where results became significant or insignificant between day seven and day 14, only the significant results of day 14 will be discussed. This is because the significant effect of day seven could not persist.

6.3.1 Germination rate

The objective of planting seeds at different depths was to stress seeds which might result in the difference between treatments becoming more visible. Thus, the difference in germination and seedling vigour in terms of different depths was expected (Table 6.4). Sorghum had a significant first degree interaction between cultivar and treatment and between depth and treatment, while sunflower had a significant first degree interaction between cultivar and depth.

Table 6.4 Analysis of variance (ANOVA) of the germination of maize, sorghum and sunflower, grown in pots, as affected by cultivar, depth and treatment on day seven and day 14.

	p-values					
Effect	**Maize 7**	**Maize 14**	**Sorghum 7**	**Sorghum 14**	**Sunflower 7**	**Sunflower 14**
Cultivar	N/S	0.0192	0.0063	0.0014	0.0003	N/S
Depth	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000
Treatment	N/S	N/S	0.0381	0.0152	N/S	N/S
Cultivar * Depth	0.0016	N/S	0.0000	N/S	N/S	0.0017
Cultivar * Treatment	N/S	N/S	N/S	0.0315	0.0116	N/S
Depth * Treatment	N/S	N/S	0.0353	0.0141	N/S	N/S
Cultivar * Depth * Treatment	N/S	N/S	N/S	N/S	N/S	N/S

There was a significant difference between the germination of cultivar 1 and cultivar 2 (Figure 6.1). Cultivar 2 had a 7.6% higher germination rate than cultivar 1. Cultivar 2 also outperformed cultivar 1 under cold stress germination in chapter 5. This is an indication that cultivar 2 germinates better than cultivar 1 and can be ascribed to an array of cultivar characteristics which includes stress tolerance and adaptability.

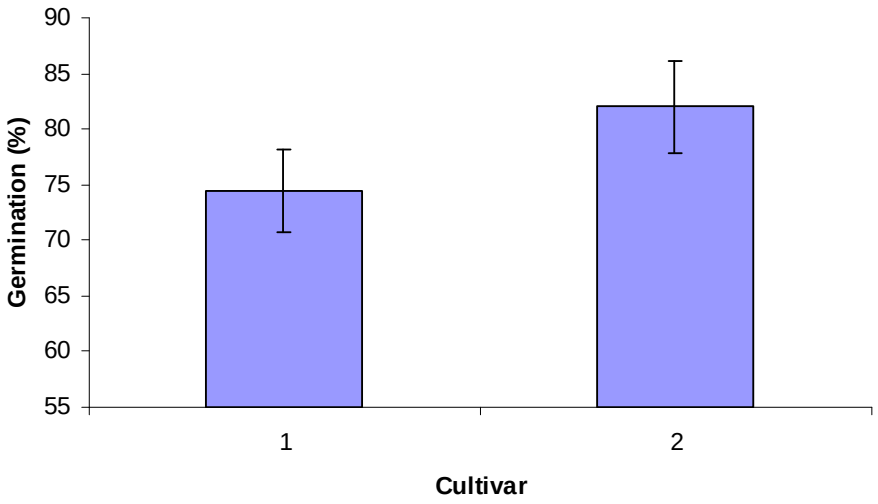

Figure 6.1 Germination of two maize cultivars on day 14, with regard to difference in cultivars germination.

The germination differences of sorghum cultivars on day 14 only revealed that S1 A 24H significantly reduced germination of cultivar 3 compared to its two control treatments (Figure 6.2). There were no treatments which increased germination for cultivar 3 as well as for cultivar 4 at a significant level.

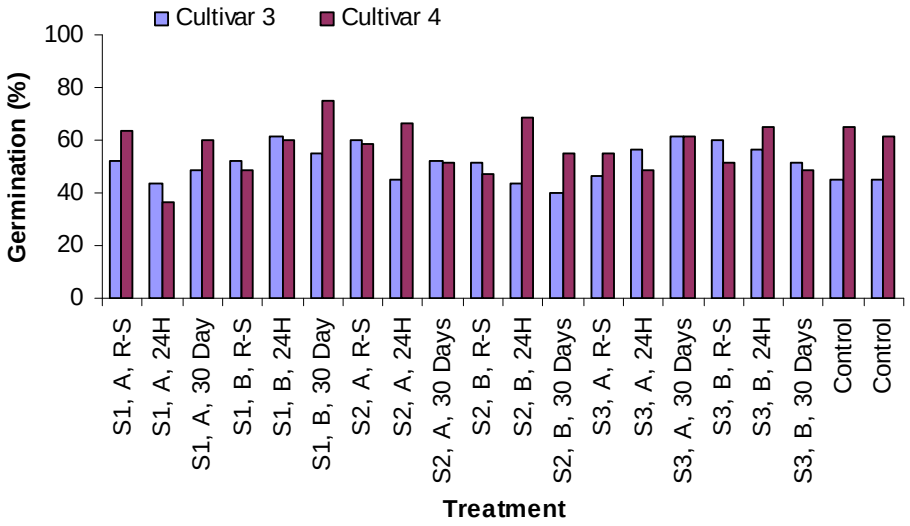

Figure 6.2 Germination of two sorghum cultivars on day 14, observed in regard to M-EM ratios and concentrations compared to the control treatments.

For sorghum, seeds germinated better at 30 mm planting depth than at 60 mm, as expected (Figure 6.3.). M-EM treated seed did not improve germination at the

shallow depth of 30 mm. However, when planted deeper, germination was more variable with some M-EM treatments improving the germination of sorghum although not significantly and although no trend with regard to treatment was observed. Only S1 B 30 improved germination significantly compared to control treatments with 60 mm planting depths.

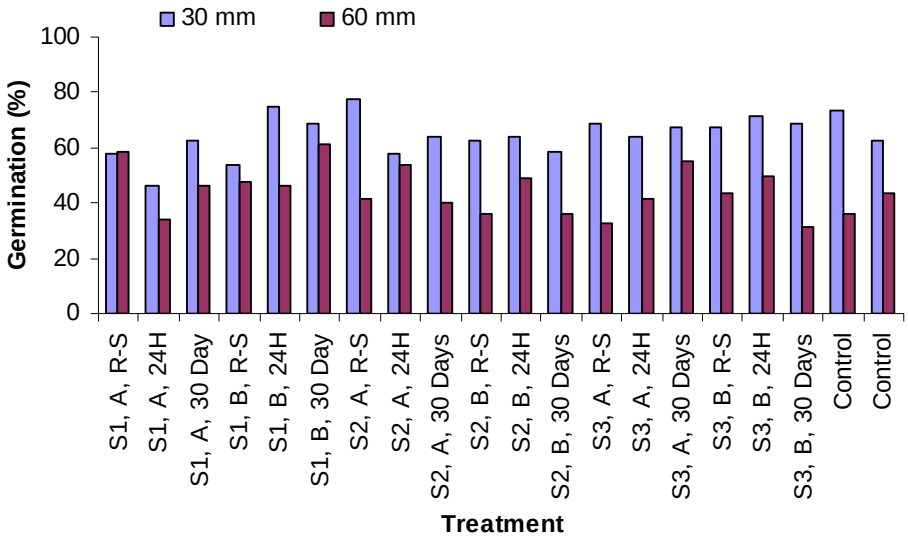

Figure 6.3 Germination of sorghum planted at two depths on day 14, observed with regard to M-EM ratios and exposure rates.

The germination of sunflower at day 14 revealed that cultivar 6 significantly outperformed cultivar 5 at 25 mm planting depth (Figure 6.4). Cultivar 6 had an average germination of 93.87% compared to cultivar 5 at 84.62%. The difference at 50 mm was however not significant.

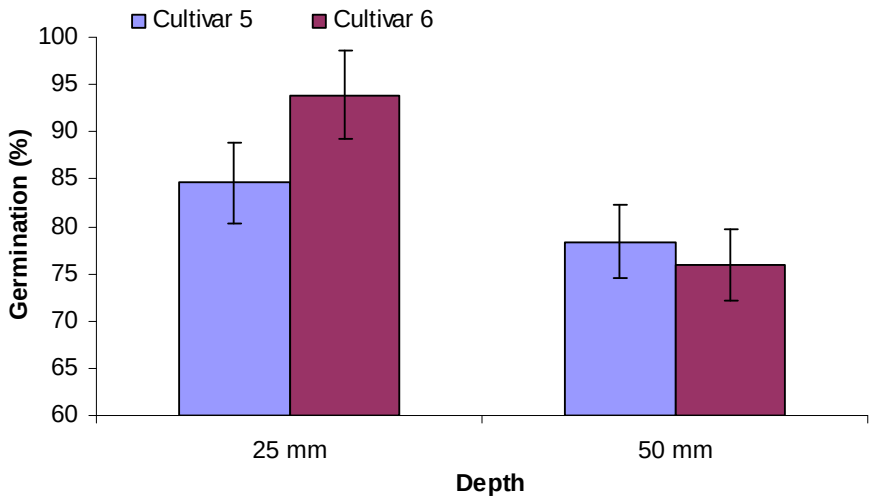

Figure 6.4 Germination of two sunflower cultivars on day 14 with regard to two different planting depths, 25 mm and 50 mm.

6.3.2 Shoot lengths

Maize and sunflower both had significant second degree interactions between cultivar, depth and treatment at both day seven and 14 (Table 6.5). Sorghum on day seven also had a significant second degree interaction but at day 14 only had significant first degree interactions between cultivar and depth, cultivar and treatment and between depth and treatment.

Table 6.5 Analysis of variance (ANOVA) of shoot length of maize, sorghum and sunflower, grown in pots, as affected by cultivar, depth and exposure rate on day seven and day 14.

	p-values					
Effect	**Maize 7**	**Maize 14**	**Sorghum 7**	**Sorghum 14**	**Sunflower 7**	**Sunflower 14**
Cultivar	N/S	0.0000	0.0245	0.0000	N/S	0.0000
Depth	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Treatment	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000
Cultivar * Depth	0.0000	N/S	0.0000	0.0016	0.0041	0.0055
Cultivar * Treatment	0.0000	0.0000	0.0000	0.0014	0.0000	0.0000
Depth * Treatment	0.0000	0.0000	0.0000	0.0344	0.0000	0.0000
Cultivar * Depth * Treatment	0.0000	0.0000	0.0323	N/S	0.0000	0.0000

Maize shoot lengths at 50 mm were significantly affected by M-EM treatments (Figure 6.5a). Cultivar 1 had six treatments which had a significant positive effect on day seven as well as day 14 compared to both control treatments namely:

S2 A 24 H, S2 A 30, S2 B R-S, S2 B 30, S3 A R-S and S3 B 30. Treatment with S3 B R-S only produced a significant positive effect on day 14. Cultivar 2 had three treatments which had a significant negative effect on shoot length namely: S1 A R-S, S1 B 24H and S2 A 30.

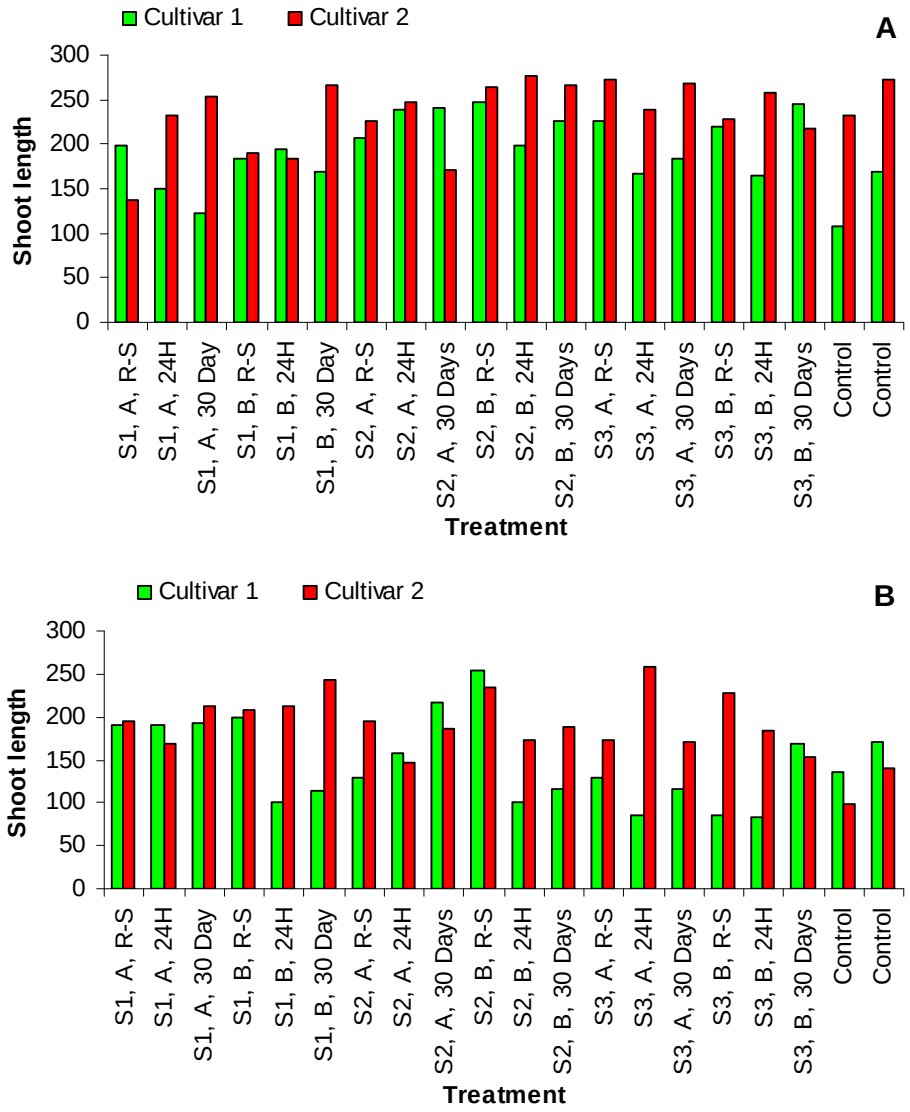

Figure 6.5 Shoot length of two maize cultivars on day 14 at two depths, a) 50 mm, and b) 100 mm, observed with regard to M-EM ratios and exposure rates.

At the 100 mm planting depth, cultivar 1 at day seven and day 14 had three treatments which had a significantly negative effect on shoot length, namely: S3 A 24H, S3 B R-S and S3 B 24H (Figure 6.5b). At day 14 only S2 B R-S had a significant positive effect on cultivar 1 compared to the control treatments.

Cultivar 2 at 100 mm had 11 treatments which had a significant positive effect on shoot length on day 14, namely: S1 A R-S, S1 A 30, S1 B R-S, S1 B 24H, S1 B 30, S2 A R-S, S2 A 30, S2 B R-S, S2 B 30, S3 B R-S and S3 A 24H. The latter also outperformed both control treatments at day 7. Most M-EM treatments did better than the control, at the deeper planting depth, which is also the more stressed planting depth, especially in terms of cultivar 2. Cultivar 2 is also known for its adaptability and stress tolerance ability.

For sorghum, the significant interaction between cultivar and depth at day 14 indicated that cultivar 3 had a significantly shorter average shoot length than that of cultivar 4 (Figure 6.6). The sorghum shoot length figures of chapters four, five and six also indicated that cultivar 4 had an overall longer shoot length than cultivar 3. Cultivar 4 is known as the best sorghum hybrid in the Pannar package, with an average height between 112 cm and 117 cm (Pannar, 2011), while little is known of cultivar 3 since cultivar 3 has been removed from the seed range of the company.

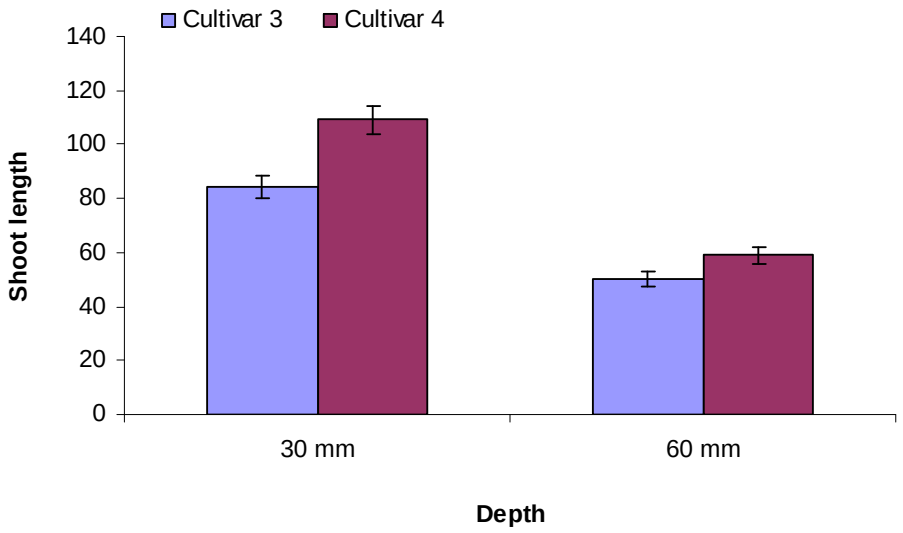

Figure 6.6 Average shoot lengths of two sorghum cultivars on day 14 for two different planting depths, namely: 30 mm and 60 mm.

In Figure 6.7 the average shoot length per cultivar per treatment was determined for the two combined depths. S3 B R-S significantly increased plant length of cultivar 3 and S1 B 30 increased shoot length for cultivar 4, both compared to the control treatments across the two depths. Treatments with S1 A R-S, S1 A 30,

S1 B 24H, S1 B 30, S2 A R-S, S2 A 24H and S3 A 30 had increased shoot lengths compared to both control treatments although not significantly so. This might be an indication that M-EM can have a positive influence on shoot length despite handling M-EM in an undesirable manner.

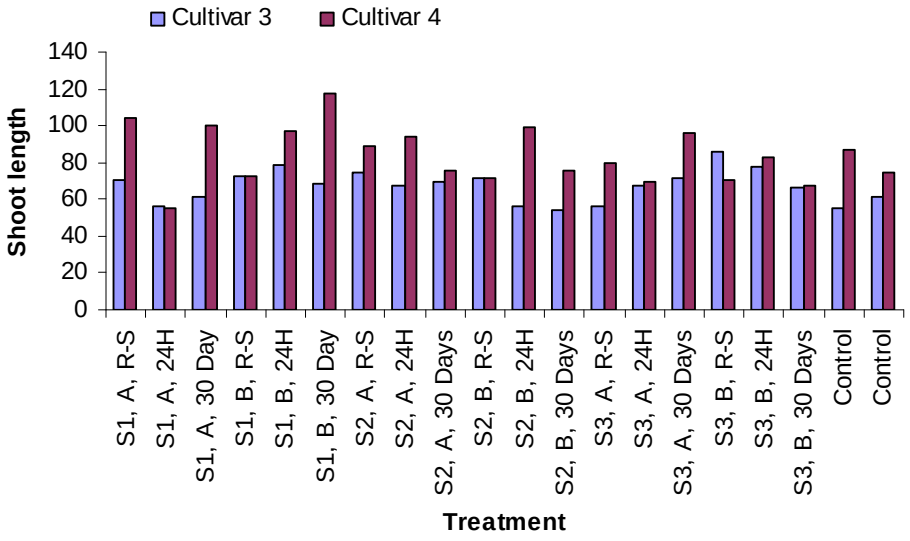

Figure 6.7 Shoot length of two sorghum cultivars on day 14, with regard to M-EM ratios and exposure rates.

The shoot length of sorghum planted 30 mm deep did not deliver any treatments which increased shoot length with a significant margin (Figure 6.8). However at 60 mm, S1 A R-S increased shoot length with a significant margin over that of the control treatments. The difference between 60 mm and 30 mm planting depth varied significantly, this is caused by the extra time needed by the seedlings to emerge above soil level. Under field conditions the deeper planted seeds would also be exposed to a lower soil temperature than shallow planted seeds, which would also reduce germination rate.

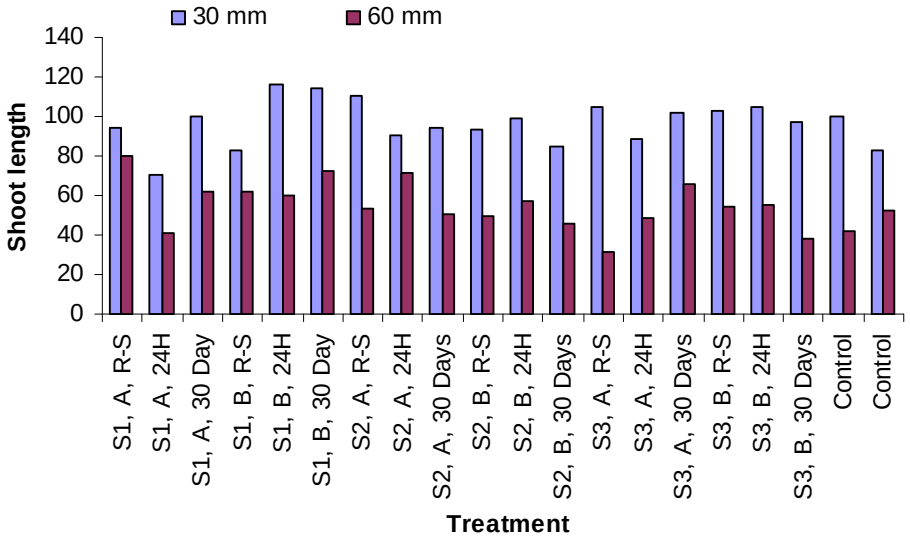

Figure 6.8 Average shoot length of sorghum planted at two depths on day 14 with regard to M-EM ratios and exposure rates.

Sunflower cultivar 5, planted 25 mm deep, had 10 treatments which resulted in significantly shorter shoot lengths compared to the two control treatments on both day seven and day 14 (Figure 6.9a). The treatments were as follows: S1 A R-S, S2 A R-S, S2 A 24H, S2 A 30, S2 B R-S, S2 B 24H, S2 B 30, S3 A R-S, S3 B R-S and S3 B 30. Cultivar 6 did not have any treatments which had a significant effect on shoot length.

At 50 mm, cultivar 5 had three treatments which significantly increased shoot length on day seven and day 14, namely: S1 A R-S, S1 A 24H and S1 A 30 while S1 B R-S only had significant results on day 14 (Figure 6.9b). Cultivar 6 had two treatments which significantly increased shoot length, namely: S1 A R-S and S2 A 24H. Again, the beneficial results of M-EM were observed only through the application of some form of stress, e.g. increased planting depth in the case of this experiment.

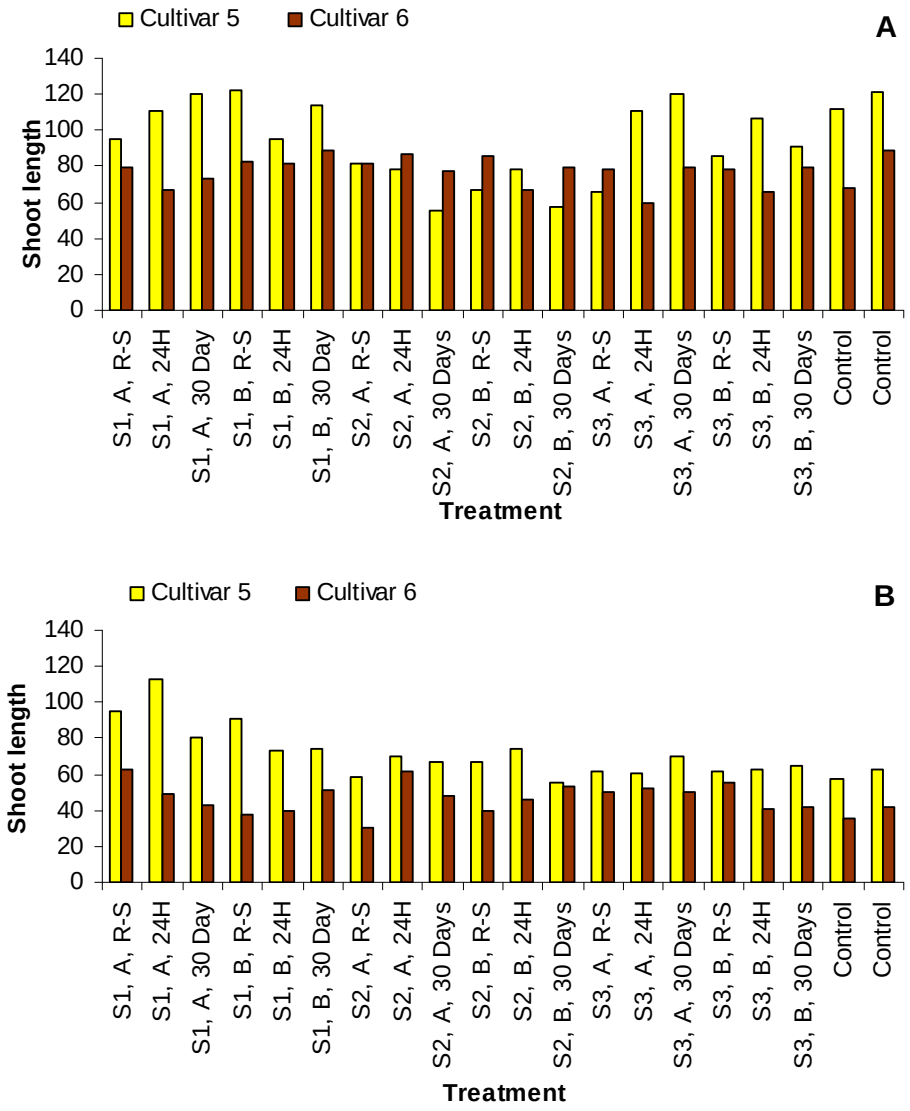

Figure 6.9 Shoot length of two sunflower cultivars on day 14 at two depths, a) 25 mm, and b) 50 mm, with regard to M-EM ratios and exposure rates.

6.4 Conclusion

The correct storage conditions play a vital role in the survival of EM. Maintaining proper conditions depend on temperature control and the protection from sunlight. There has been very little research reported on what effect M-EM exposed to irradiation and temperature fluctuation will have on plant production. The purpose of the present study was therefore to determine the effect of EM seed treatment, subjected to irradiation and temperature fluctuation, on the germination and seedling vigour of maize, sorghum and sunflower in pot experiments. The findings of the experiment revealed that M-EM exposed to

irradiation, temperature fluctuation and prolonged storage did not only effect germination and vigour positively but, also had and equal amount of significant negative effects on results compared to the control treatments. Results indicated as was expected, that seeds germinated faster at the shallow planting depth compared to the deeper depth. M-EM treatments did not have significant effects on the germination of maize or sunflower. A positive effect was however, detectable on the germination of deeper planted sorghum seeds, with no visible trend with regard to treatments. Only S1 B 30 improved germination at a significant level compared to control treatments. Shoot length results indicated that M-EM had more positive effects at the deeper planting depth for maize and sunflower and an overall positive effect was detectable on the shoot length of sorghum. Result of the shoot length experiments might be an indication that M-EM, even if stored in undesired conditions, might have a positive effect on germination and seedling vigour. As can be concluded from the experiments the exposure of M-EM to irradiation, temperature fluctuations or even prolonged storage might compromise the effectiveness of M-EM on crop production, even though M-EM still might have a positive effect on crops. Further research is needed, however, to determine the effect of M-EM exposed to irradiation and temperature fluctuation on the yield of maize, sorghum and sunflower, exposed to longer stressed conditions.

6.5 References

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Chapter 7

The influence of Effective micro-organism on germination and seedling vigour of maize, sorghum and sunflower exposed to sterilized, EM treated and *Fusarium* containing soils

Abstract

Pot experiments were conducted to determine the influence of effective micro-organisms (EM) as a seed treatment on different grain crops planted at different depths, under different soil microbial conditions.

In this study seeds of maize, sorghum and sunflower were treated with M-EM (multiplied at 1 % and 3% ratios) and planted at two depths in differently treated soil. The first part of the soil was sterilized, the second was treated with M-EM and the third part was inoculated with *Fusarium*.

The germination of maize and sorghum was not significantly improved by any of the M-EM treatment at any depth and in any soil treatment. Germination of sunflower was however, greatly influenced by M-EM treatments especially in M-EM treated soil. Although germination percentage of sunflower was sometimes slightly lower in M-EM treated soil, compared to sterilized soil, the M-EM treatments generally increased germination compared to the control. Germination in sterile soil revealed to be superior regardless of treatment. M-EM treatments mostly increased seedling vigour of maize, sorghum and sunflower although not always significantly above that of the control treatments. This result was emphasized by seeds planted at the maximum recommended depth. This indicated that M-EM treatments will probably improve early seedling growth of maize, sorghum and sunflower compared to untreated seed. M-EM seed treatment and a pre-plant M-EM soil treatment might assist seeds in unfavourable germination and growth conditions. M-EM treatment did however, rarely have an improved effect on seedling vigour in *Fusarium* treated soil.

Keywords: Multi-EM, shoot length, seed treatment, soil treatment.

7.1 Introduction

Effective micro-organisms (EM) consists of a wide variety of effective, beneficial (Higa & Parr, 1994) and non-pathogenic micro-organisms (Higa & Wood, 1998), produced through a natural process (EMROJP, 2010). EM does not contain chemicals and could thus not be described as a pesticide (Anon, 1995). In Agriculture EM has been used to improve the soil and generate quality, healthy crops at a greater yield with a decrease in pests and diseases without the use of agricultural chemicals (Higa & Wood, 1998).

All fungi are heterotrophic and most are saprophytic, but some invade plant roots and are pathogens such as *Fusarium* (Wild, 1993). Plant fungal pathogens are responsible for billions of dollars of crop damage worldwide in all countries where agriculture is practiced (Maier *et al*., 2000). Almost all commercial crops are subjected to plant fungal attacks, which can result in diseases of seeds, roots, stems, leaves, fruit or grain kernels (Maier *et al*., 2000). Almost all plant pathogenic fungi spend some of their time on the host plant and the remainder of their lives in soil or in plant debris within the soil (Maier *et al*., 2000). Thus, the survival and effect of the pathogen are controlled mainly by soil environmental factors including biotic (microbial) and abiotic factors such as temperature and moisture (Maier *et al*., 2000).

Fusarium thrives inside the plant and fills up the plants intercellular spaces (Owens, 2010). This actually helps the plant survive some problems encountered during the growing season, however when the seed develops, infected with *Fusarium* which in turn produce toxins (Owens, 2010). *Fusarium* is associated with seeds of many members of the Poaceae, including maize and wheat (Galli *et al*., 2005) The application of EM in crop production is said to control root rot, nematodes, *Fusarium* and other diseases as well as harmful gasses in soil (EMROSA, 2006).

EM has a variety of applications including pre-planting soil treatment and seed treatment to name only two. In the case of pre-planting treatment, EM should be applied to the soil two to three weeks before planting (D. Anthony, personal communication, March 2009; EdenBound, 2010) at a rate of 30 ℓ to 50 ℓ ha^{-1} in a dilution of 1:100 (D. Anthony, personal communication, March 2009). EM as a seed treatment can be used to promote faster and even germination as well as healthy growth of plants (A. Rosenberg, personal communication, March

2009). According to A. Rosenberg (personal communication, March 2009) seeds should be soaked in 0.1% EM, small seeds for up to 30 minutes, and large seeds such as maize for up to 8 hours.

The objectives of this study was to determine the effect of effective Micro-organisms (EM) as seed treatment on the germination and seedling vigour of maize, sorghum and sunflower planted in sterilized soil, in soil treated with EM and soil containing *Fusarium,* in pot experiments.

7.2 Material and methods

7.2.1 Location and experimental layout

The experiment was conducted at a greenhouse of the School for Agricultural and Environmental Sciences of the Central University of Technology, Free State. Plastic growing bags, with a 100 mm diameter and 1 ℓ capacity, were used as pots. The bags had holes in the bottom to prevent over watering and a sandy soil was used as the growth medium. Seed were hand planted and the soil was compacted by applying minimal hand pressure on top of the soil. The bags were maintained in a naturally ventilated greenhouse without temperature control and the bags were moistened with pure water as needed. Maize of the cultivars, PAN 6236 (cultivar 1) and PAN 6053 (cultivar 2), sorghum of the cultivars, PAN 8247 (cultivar 3) and PAN 8816 (cultivar 4), and sunflower of the cultivars, PAN 7351 (cultivar 5) and PAN 7033 (cultivar 6), were used in the three experiments. Approximately 1600 seeds were used per cultivar and the experiment was replicated four times.

7.2.1.1 The maize, sorghum and sunflower cultivars

- PAN 6236 (cultivar 1) is an ultra early yellow maize, which achieves excellent results under irrigation as well as high potential dry land conditions. The cultivar does exceptionally well in the Orange River area and other warm irrigation regions (PANNAR, 2011).
- PAN 6053 (cultivar 2) is medium maturing white maize cultivar, with excellent yield potential and proven reliability under low rainfall conditions, producing yields at low plant populations (PANNAR, 2011).

- PAN 8247 (cultivar 3) is a sorghum with good yield potential and has a very uniform plant type (PANNAR, 2011).
- PAN 8816 (cultivar 4) is a popular sorghum and recommended for the main planting in all sorghum production areas. The cultivar has an excellent yield potential and stability (PANNAR, 2011).
- PAN 7351 (cultivar 5) is a sunflower with a wide area adaptability, a high yield potential and a good stability, with outstanding performance in commercial plantings (PANNAR, 2011).
- PAN 7033 (cultivar 6) is a top performer sunflower in cultivar trails over the past three years and is recommended for the main bulk planting in all production regions (PANNAR, 2011).

Two different planting depths for each crop, as indicated in Table 7.1.

Table 7.1 Maize, sorghum and sunflower were planted at two depths in three pre-treated sandy soils.

Crop		**Depth 1**	**Depth 2**
Maize	-	50 mm	100 mm
Sorghum	-	30 mm	60 mm
Sunflower	-	25 mm	50 mm

EM variables which where used in the study can be noted in Table 7.2.

Table 7.2 M-EM treated seed variables per crop planted in pre-treated soil. Stock EM from three different suppliers was multiplied at two ratios (1% and 3%) and a dilution of 0.1%. Soil was divided into three pre-treated groups.

	Maize	Sorghum	Sunflower
Cultivars	2	2	2
Number of seeds	10	10	10
Planting depth	2	2	2
Replications	4	4	4
EM suppliers	3	3	3
Multiplied ratios	2	2	2
Number of control seeds	160	160	160
Soil treatments	3	3	3
Total number of seeds	3360	3360	3360

7.2.2 M-EM treatment

Generally fallible Stock-EM (S-EM) was bought from three different commercial companies. Due to a secrecy agreement names will be withheld and in this document the products will be revered to as S1, S2 and S3. Multi-EM (M-EM) was produced of each of the three suppliers EM at the following ratios:

- M-EM (A) at a ratio of 1% S-EM, 7% molasses and 92% water.
- M-EM (B) at a ratio of 3% S-EM, 5% molasses and 92% water.

Seeds were soaked for seven hours in a 0.1% dilution of the M-EM, which were given 14 days to multiply. A control that consisted of soaking seeds in purified water was prepared for comparison. After soaking, the seeds were left to dry in the laboratory. Dried seeds were planted 10 seeds per 100 mm-diameter bag.

7.2.3 Soil treatment

The bags were filled with soil from the same source but which had different treatments, namely: sterilized soil, M-EM treated soil and soil containing *Fusarium.*

7.2.3.1 Sterilized soil

The soil in this section of the experiment was sterilized with the Microwave Oven Method as described by Pottorff (2009). One kg of moistened soil was placed in a polypropylene bag in the centre of a microwave oven with the top of the bag open. Treatment of the soil took 2.5 minutes at full power of about 650 watts (Pottorff, 2009).

7.2.3.2 EM treatment of soil

The soil of the M-EM treated section was sprayed with M-EM, 21 days prior to planting at a rate of 30 $\ell\ ha^{-1}$ in a 1:100 water dilution.

7.2.3.3 *Fusarium* treated soil

This experiment was planted in soil containing *Fusarium. Fusarium* was cultured at the Agricultural Research Council (ARC) in Potchefstroom. After culturing, the *Fusarium* was inoculated into the soil at the ARC (O. Rhode, personal communication, November 2009).

To simplify statistical analysis and interpretation of results, EM suppliers, multiplied ratios and the control treatments were pooled into seven treatment combinations (Table 7.3), which will be referred to as treatments throughout the rest of this chapter. Treatment abbreviations are coded and are not an indication of the supplier company.

Table 7.3 Treatment 1 to 7 with regard to treatment abbreviation, EM supplier company and multiplied ratios.

Treatment number	Treatment abbreviation	Supplier company	Multiplied ratio
1	S1, A	1	A
2	S1, B	1	B
3	S2, A	2	A
4	S2, B	2	B
5	S3, A	3	A
6	S3, B	3	B
7	Control	Control	N/A

7.2.4 Measurements

Seedlings were scored as germinated at shoot length emersions of 3 mm above the soil level at day seven and day 14 after planting. Vigour was determined by measuring seedling length at day seven and day 14 after planting.

7.2.5 Statistical analysis

A factorial analysis of variance (ANOVA) was performed on the germination and seedling vigour with cultivars, suppliers, M-EM ratios, soil and planting depth as factors on both day seven and day 14. P-values were used to compare means at a 5% probability level, using STATISTICA version 8.0 (Statsoft Inc., 2004).

7.3 Results and discussion

The discussion will not focus on the results of day seven but, if the significant difference of day seven maintained itself onto day 14 the results will be discussed. If the significance changed from day seven to day 14, only significant interactions of day 14 will be discussed, since the significant difference of day seven could not persist.

7.3.1 Germination rate

The objective of planting seeds at different depths was to stress seeds which might result in the difference between treatments becoming more visible. Thus, the difference in germination and seedling vigour in terms of the first degree interaction between depths was expected. There was no visible trend that could be detected, caused by the treatments. Maize at measurement day 14 had a significant second degree interaction between cultivar, depth and soil (Table 7.4). Sorghum on day 14 only had main effects for soils and depths. Sunflower had significant second degree interactions between cultivar, depth and soil, between cultivar, treatment and soil and between depth, treatment and soil.

Table 7.4 Analysis of variance (ANOVA) of the germination of maize, sorghum and sunflower, grown in pre-treated soils, as affected by cultivar, depth, soil and treatment on day seven and day 14.

	p-values					
Effect	**Maize 7**	**Maize 14**	**Sorghum 7**	**Sorghum 14**	**Sunflower 7**	**Sunflower 14**
Cultivar	N/S	N/S	0.0000	N/S	0.0169	N/S
Depth	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Treatment	N/S	N/S	0.0348	N/S	N/S	0.0067
Soil	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Cultivar * Depth	N/S	N/S	N/S	N/S	0.0132	N/S
Cultivar * Treatment	0.0016	N/S	N/S	N/S	N/S	0.0003
Depth * Treatment	0.0156	0.0160	N/S	N/S	0.0085	0.0092
Cultivar * Soil	0.0002	0.0000	0.0004	N/S	0.0000	0.0000
Depth * Soil	0.0009	0.0000	0.0058	N/S	0.0000	0.0000
Treatment * Soil	0.0244	N/S	N/S	N/S	N/S	0.0008
Cultivar * Depth * Treatment	0.0363	N/S	0.0277	N/S	N/S	N/S
Cultivar * Depth * Soil	0.0000	0.0000	0.0000	N/S	0.0261	0.0276
Cultivar * Treatment * Soil	0.0000	N/S	N/S	N/S	N/S	0.0002
Depth * Treatment * Soil	0.0050	N/S	0.0016	N/S	N/S	0.0015
Cultivar * Depth * Treatment * Soil	0.0130	N/S	N/S	N/S	N/S	N/S

In both sterile and M-EM treated soil, maize cultivar 1 did not have a significant difference in terms of germination between 50 mm and 100 mm planting depth (Figure 7.1a & b). Cultivar 2 however, had significantly more germinated seeds at 50 mm than at 100 mm. This might be due to the extra stress caused by the difference in depth.

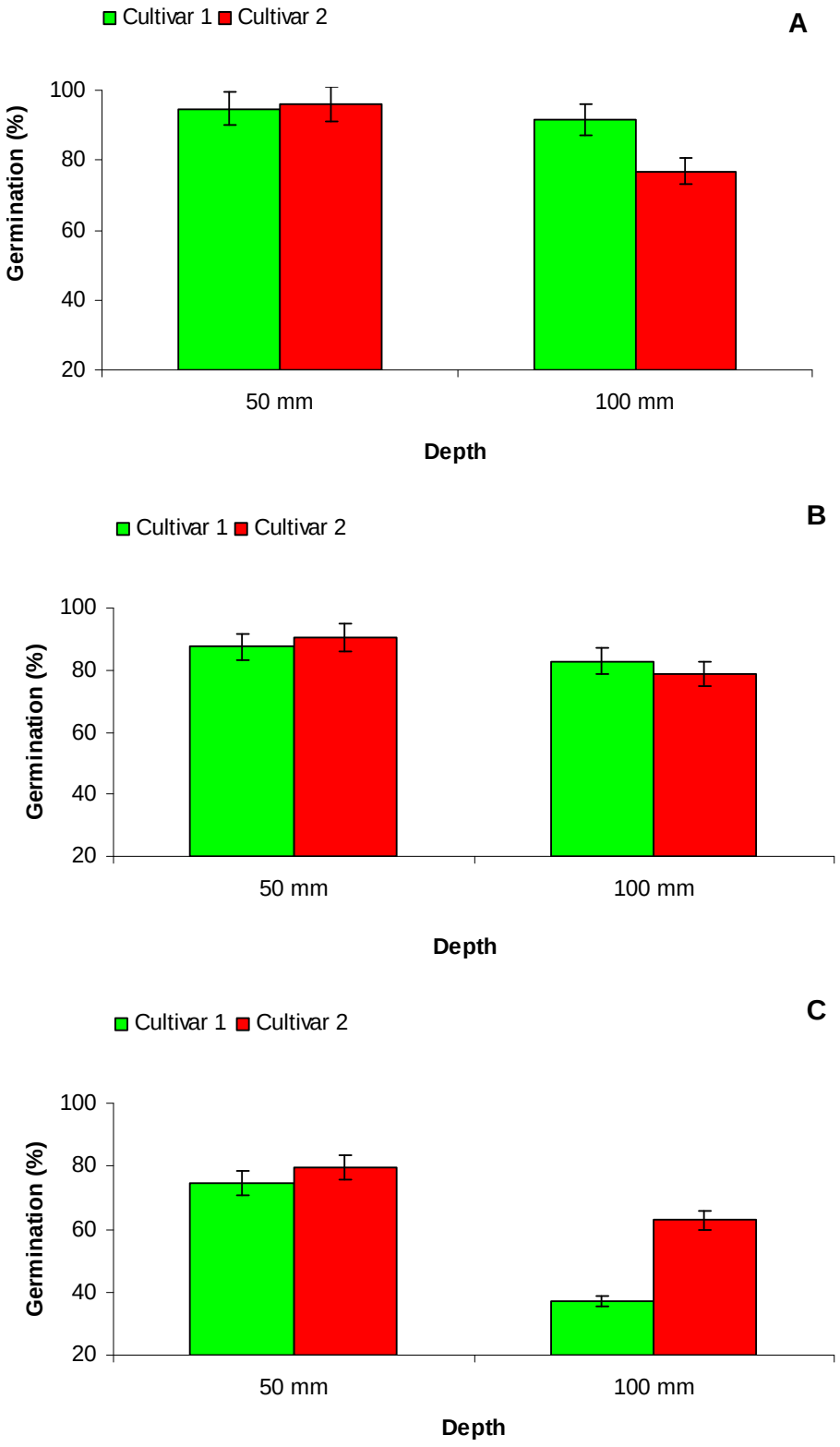


Figure 7.1 Germination of two maize cultivars on day 14, with regard to different planting depths and soil treatments where a) sterile soil, b) M-EM treated soil and c) soil containing *Fusarium*.

In *Fusarium* containing soil (Figure 7.1c), both cultivars had significantly better germination at 50 mm than at 100 mm. The difference between 50 mm and 100 mm was 37.5% and 16.79%, respectively for cultivar 1 and cultivar 2. Compared to the other soils, the *Fusarium* treated soil had a very low germination percentage at 100 mm depth.

No significant improvement on the germination of maize on day 14 was indicated at both planting depth 50 mm and 100 mm by M-EM treatment (Figure 7.2). Treatment with S1 A and S3 B did however, improve germination at both depths compared to the control treatments.

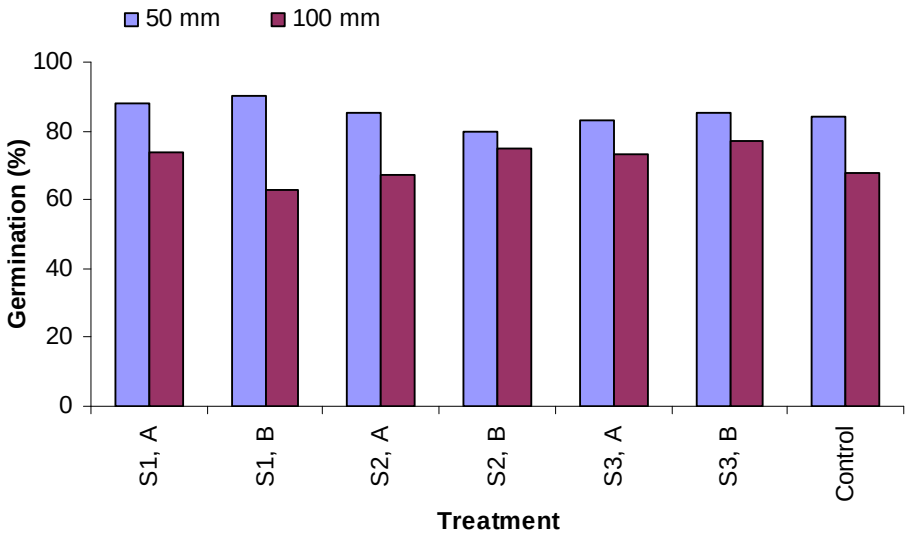


Figure 7.2 Germination of maize on day 14, planted at two different depths, namely: 50 mm and 100 mm.

The germination of sorghum differed significantly between soil treatments. From Figure 7.3 is clear that sorghum planted in soil 1 germinated significantly better than in soil 2 or soil 3. The germination in soil 2 was also significantly better than that in soil 3. This indicated that soil treated once-off with M-EM could not improve germination percentage over untreated sterilised soil. Soil 3 contains *Fusarium* which is known for the death of affected plants, resulting in thin stands or numerous skips in rows (Wrather, 2009) or in this case a lack of emerged seedlings.

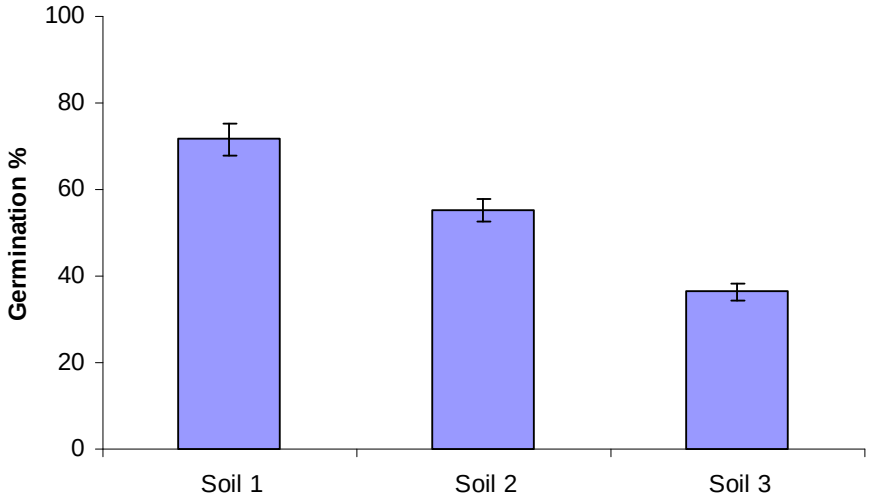


Figure 7.3 Average germination of sorghum on day 14, with regard to three differently treated soils namely: 1) sterile soil, 2) M-EM treated soil and 3) soil containing *Fusarium*.

The germination of sunflower (Figure 7.4) revealed that the germination in soils 1 and 2 were significantly higher compared to soil 3 at both depths. The results clearly indicate that the negative effect of soil 3 was more at the deeper planted seeds (50 mm). Thus, the additional stress caused by the depth, caused seedlings being longer exposed to unfavourable conditions leading to slower emergence. According to Afonin *et al.* (2008) a low sunflower stand, premature drying and a reduced yield of between 10% and 50% could be caused by *Fusarium*.

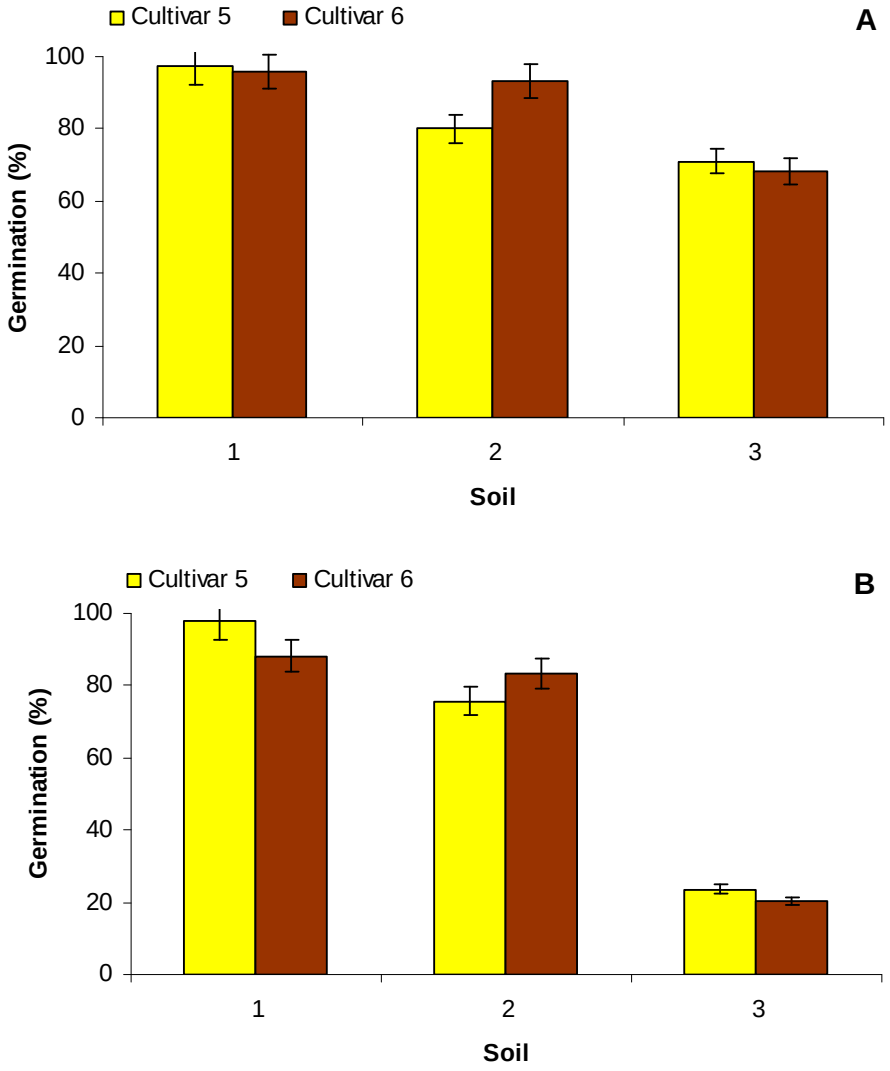


Figure 7.4 Germination of two sunflower cultivars on day 14, observed with regard to three differently treated soils and at a) 25 mm and b) 50 mm depth.

Sunflower in sterile soil had an overall good germination level with no treatment having any significant influence compared to the control treatment (Figure 7.5a). Germination of cultivar 5 planted in M-EM treated soil were significantly improved by S1 A and S3 A (Figure 7.5b). All M-EM treatments improved germination of cultivar 6 compared to the control, although only S1 A and S3 A were significantly so.

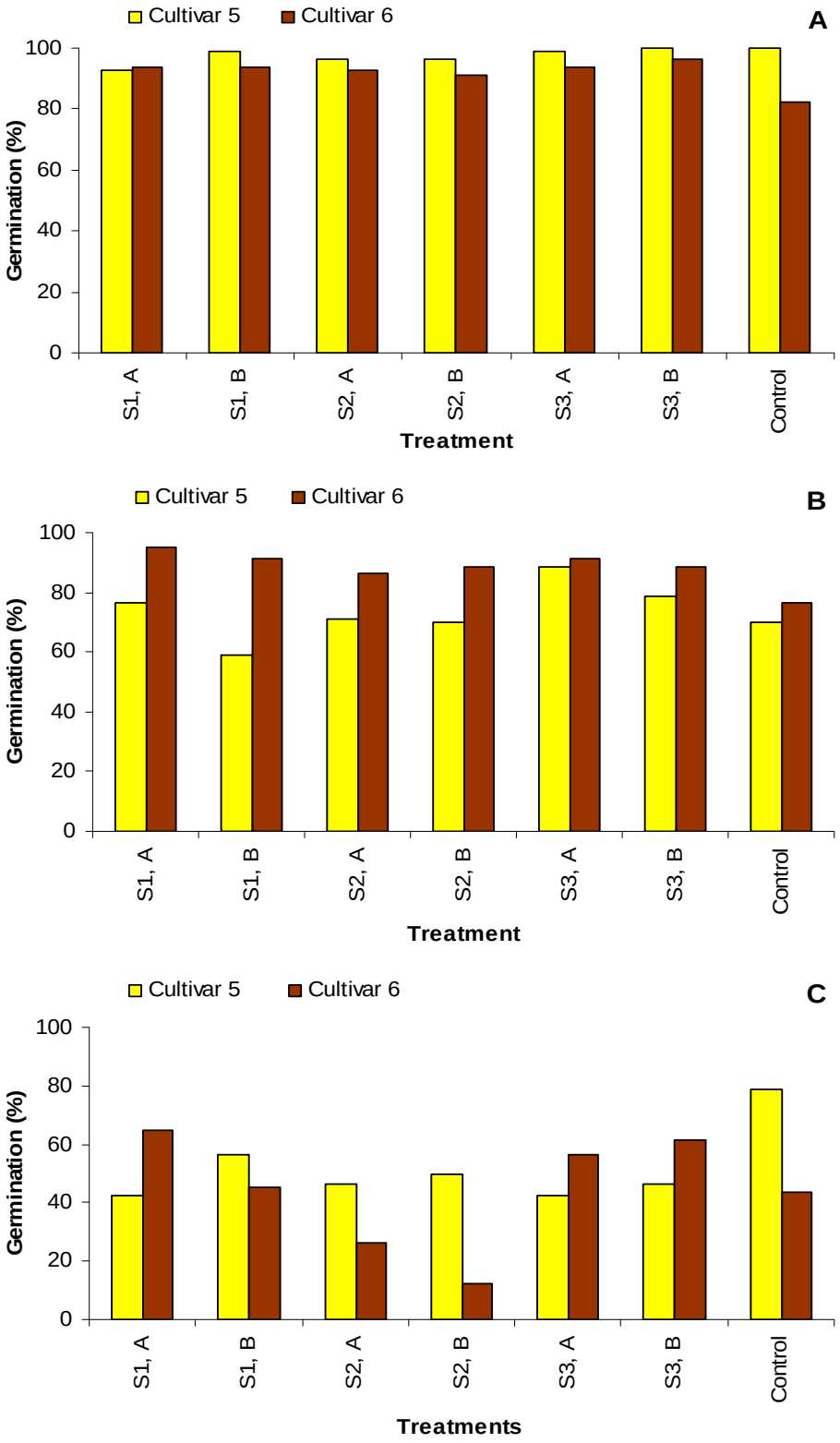


Figure 7.5 Germination of two sunflower cultivars on day 14 planted in three pre-treated soils, a) sterile, b) M-EM treated and c) *Fusarium* containing, with regard to M-EM ratios.

The germination of sunflower in soil which contains *Fusarium* had some M-EM treatments which had significantly positive as well as negative effects compared to the control treatments (Figure 7.5c). Germination on day 14 was negatively affected by al M-EM treatments compared to the control treatment for cultivar 5. Two M-EM treatments significantly increased germination of cultivar 6 namely: S1 A and S3 B. Cultivar 6 treated with S2 A and S2 B showed a significant reduction in germination.

Sunflower in sterile soil revealed no significant differences between the treatments of 25 mm and 50 mm (Figure 7.6a). In M-EM treated soil at a depth of 25 mm two treatments, S1 B and S2 A significantly reduced germination. At 50 mm all six M-EM treatments significantly increased germination (Figure 7.6b). This indicates that M-EM treatment improved germination under stressed conditions. In the soil containing *Fusarium*, the germination at a depth of 25 mm was significantly reduced by S2 B. The germination at 50 mm revealed five M-M-EM treatments which also significantly reduced germination, namely: S1 A, S1 B, S2 A, S2 B and S3 A.

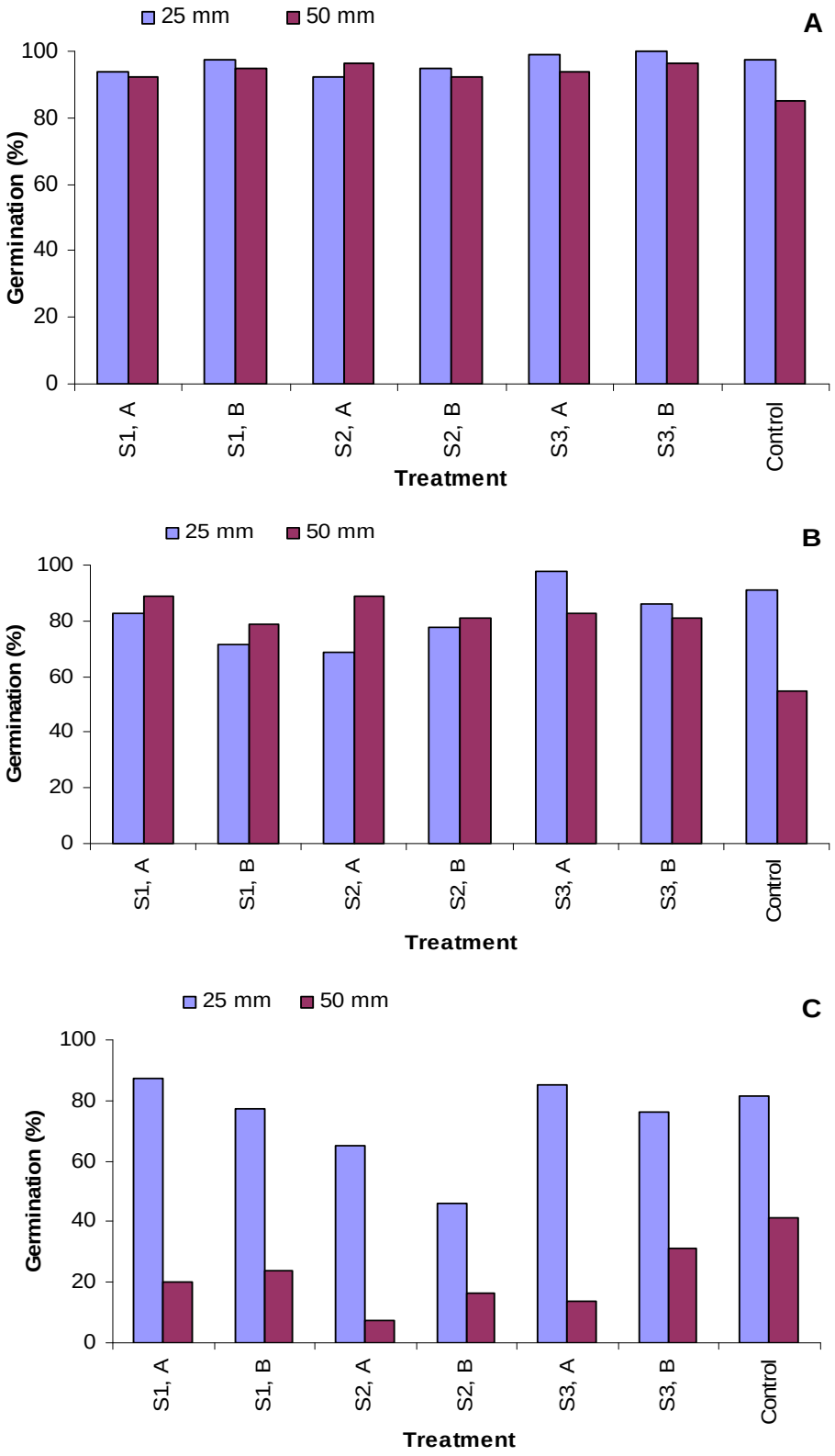


Figure 7.6 Germination of sunflower planted at two different depths on day 14 at three soil treatments, a) sterile, b) M-EM treated and c) Fusarium containing soil, with regard to M-EM ratios.

7.3.2 Shoot length

Shoot length of maize and sunflower on day seven and day 14 had significant third degree interactions with regard to cultivar, depth, treatment and soil (Table 7.5). Sorghum on day 14 had second degree interactions between cultivar, depth and treatment, between cultivar, depth and soil and between depth, treatment and soil.

Table 7.5 Analysis of variance (ANOVA) of shoot length of maize, sorghum and sunflower, grown in pots, as affected by cultivar, depth, soil and treatment on day seven and day 14.

	p-values					
Effect	**Maize 7**	**Maize 14**	**Sorghum 7**	**Sorghum 14**	**Sunflower 7**	**Sunflower 14**
Cultivar	0.0053	0.0000	0.0000	0.0000	0.0000	0.0000
Depth	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Treatment	0.0423	0.0387	0.0000	0.0002	0.0000	0.0006
Soil	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Cultivar * Depth	0.0077	0.0060	N/S	N/S	N/S	0.0048
Cultivar * Treatment	0.0000	N/S	0.0004	0.0045	0.0000	0.0000
Depth * Treatment	0.0001	0.0000	0.0000	0.0004	0.0161	0.0000
Cultivar * Soil	0.0000	0.0000	N/S	0.0278	0.0000	0.0000
Depth * Soil	0.0000	0.0000	0.0000	N/S	0.0000	0.0000
Treatment * Soil	0.0000	0.0000	0.0039	N/S	0.0000	0.0000
Cultivar * Depth * Treatment	0.0000	0.0013	0.0001	0.0404	0.0000	0.0164
Cultivar * Depth * Soil	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Cultivar * Treatment * Soil	0.0000	0.0000	0.0680	N/S	0.0000	0.0000
Depth * Treatment * Soil	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
Cultivar * Depth * Treatment * Soil	0.0000	0.0000	0.0104	N/S	0.0000	0.0000

Shoot lengths of maize cultivar 1 planted in sterile soil (Figure 7.7a) revealed that at a planting depth of 50 mm, four M-EM treatments significantly reduced shoot length compared to the control treatment. S1 A and S2 A only reduced shoot lengths on day 14 while S2 B and S3 A reduced shoot length significantly on days seven and 14.

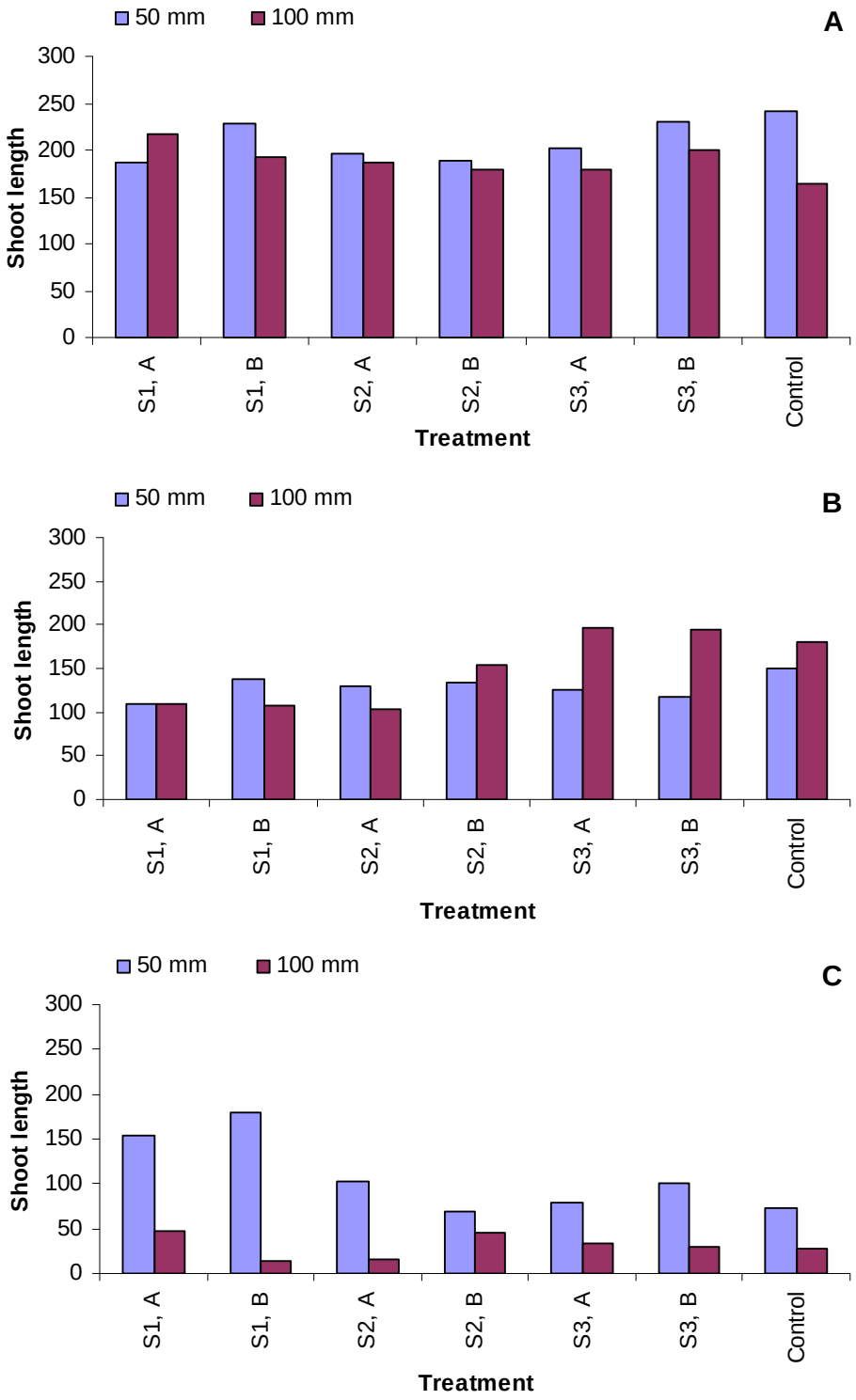


Figure 7.7 Shoot length of maize cultivar 1 on day 14 planted at two depths in differently treated soil, a) sterile, b) M-EM treated and c) *Fusarium* containing soil, with regard to M-EM ratios.

Planting depth of 100 mm had two treatments which significantly increased shoot length on both day seven and day 14, namely: S1 A and S3 B. All M-EM increased shoot length compared to the control at 100 mm depth, even though not all at a significant level. Shoot length of maize cultivar 1 planted in soil treated with M-EM (Figure 7.7b) were significantly negatively affected by S1 A and S3 B at 50 mm and by S1 A, S1 B and S2 A at 100 mm. The shoot length at 50 mm, in soil containing *Fusarium* (Figure 7.7c) was significantly positively affected by S1 A and S1 B compared to the control treatment. There were however, no significant treatment results for 100 mm.

Shoot lengths of maize cultivar 2 planted in sterile soil (Figure 7.8a) at planting depth 100 mm were significantly increased by treatment with S1 A, S1 B, S2 B and S3 on day seven as well as day 14. Shoot length of maize planted in M-EM treated soil (Figure 7.8b), at depth 50 mm were significantly reduced by S1 A, S1 B, S2 B and S3 A on day seven as well as day 14 compared to the control treatment. At 100 mm planted depth, all six M-EM treatments increased the shoot length with a significant margin on day 14 while S2 A and S2 B also increased shoot length on day 7. For both these soils, maize seedling vigour was increased above that of the control by M-EM seed treatment when seeds were planted at the maximum recommended depth. In *Fusarium* containing soil (Figure 7.8c) and a planting depth of 100 mm only S3 B treatment did not significantly reduce shoot lengths compared to the control treatment on day seven and 14.

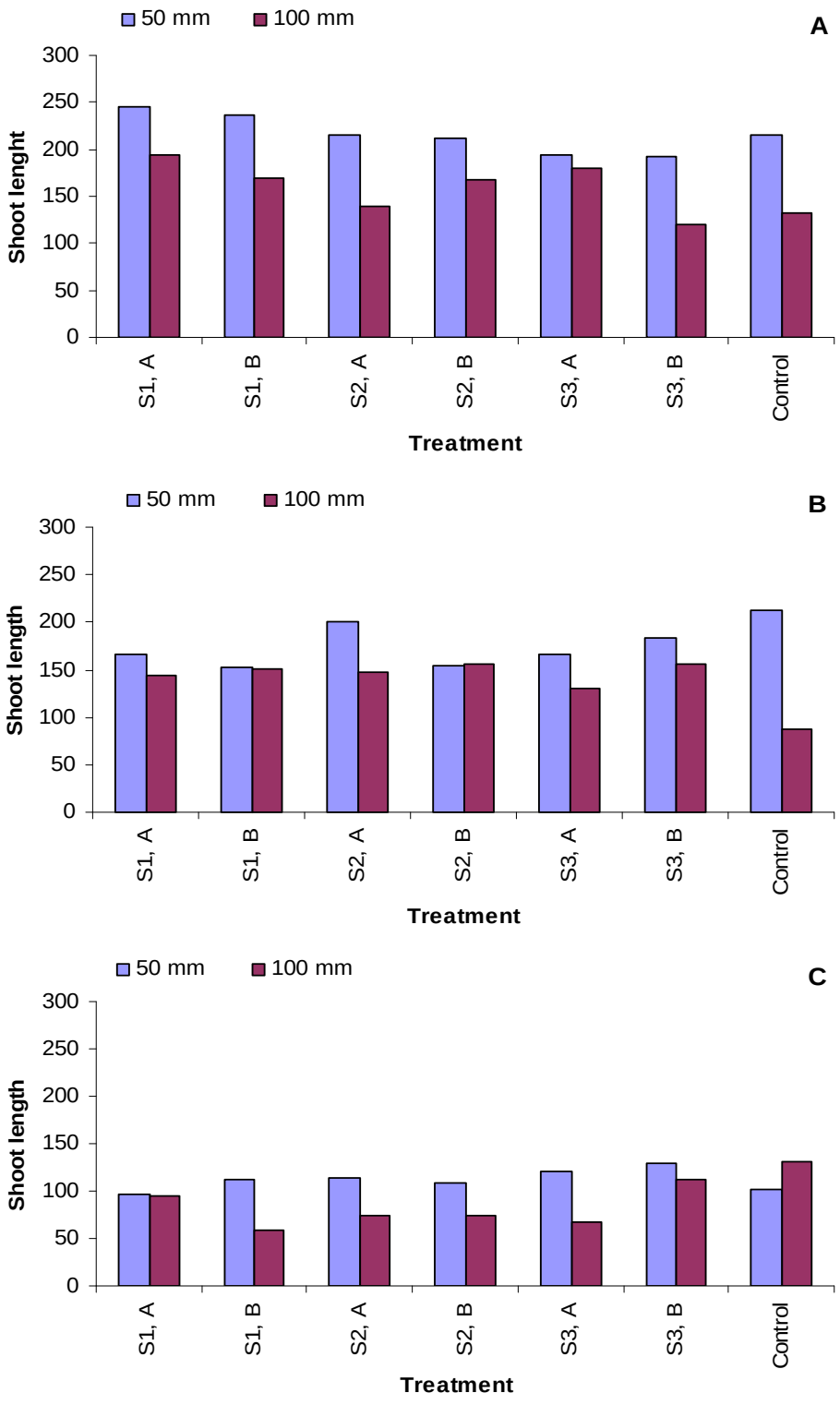


Figure 7.8 Shoot length of maize cultivar 2 on day 14 planted at two depths in differently treated soil, a) sterile, b) M-EM treated and c) *Fusarium* containing soil, with regard to M-EM ratios.

Sorghum cultivar 3 planted at 30 mm indicated that five out of the six M-EM treatments significantly increased shoot length, compared to the control treatment (Figure 7.9a). These treatments are S1 A, S1 B, S2 A, S2 B and S3 A. For cultivar 4, S2 A, S2 B and S3 A had longer shoots than the control treatment although only S2 B significantly increased shoot length at 30 mm. At 60 mm shoot length was increased by S1 A and S3 A for cultivar 3, although not significantly, while significantly increased by treatment with S1 B for cultivar 4, compared to the control treatments (Figure 7.9b).

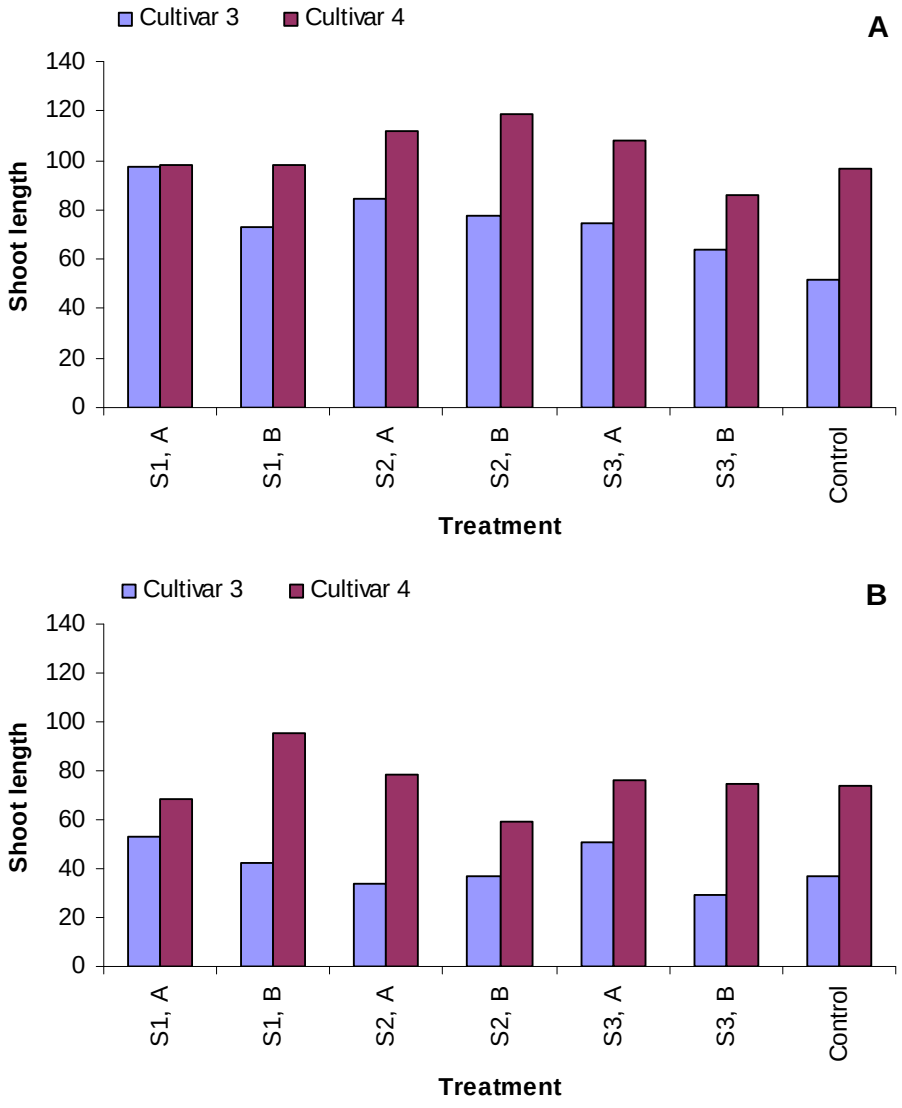


Figure 7.9 Shoot length of two sorghum cultivars on day 14 planted at two depths, a) 30 mm and b) 60 mm, with regard to M-EM ratios and concentrations.

From Figure 7.10 a clear indication was seen that soil 3 (containing *Fusarium*) had the most depressing effect on shoot length of sorghum at both planting depths. Sorghum seedlings in both soil 1 and soil 2 had significantly longer shoot lengths than those in soil 3.

The shoot length of soil 1 also did significantly better than soil 2, except in the case of cultivar 3 at planting depth of 60 mm.

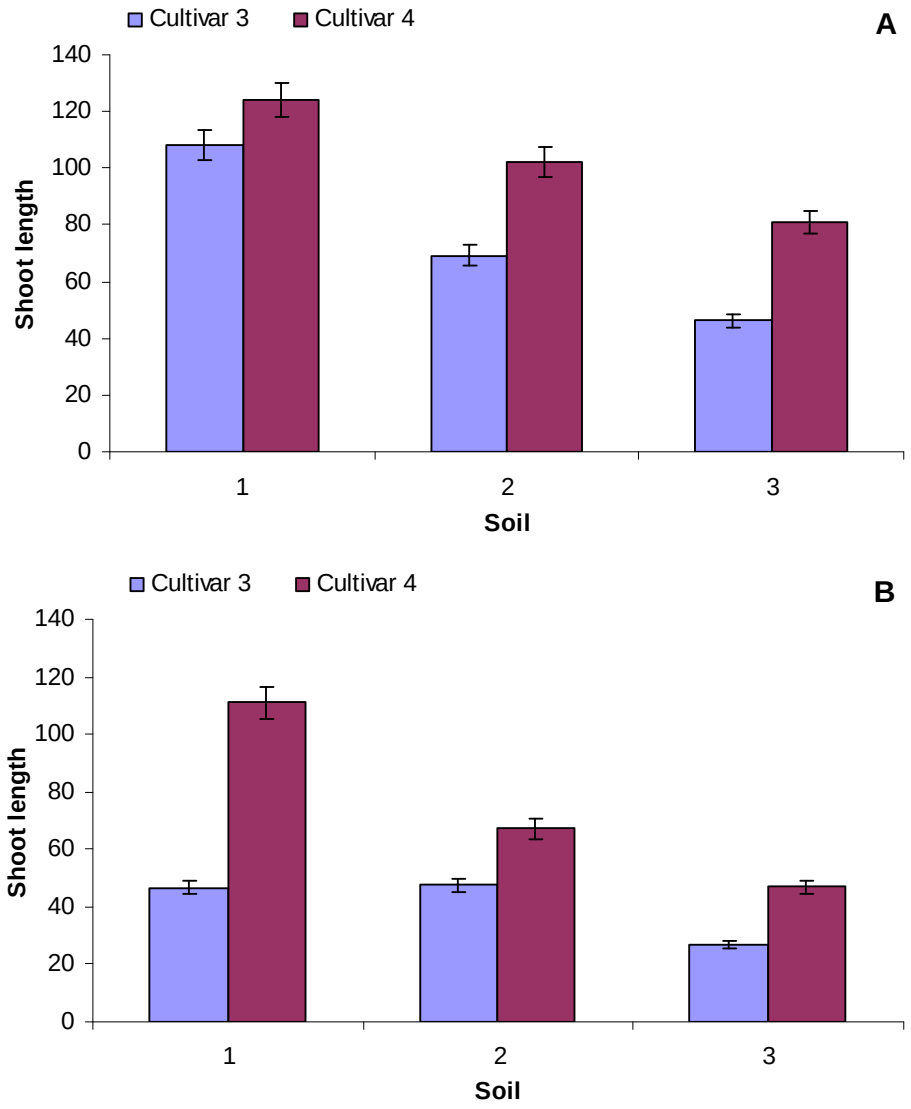


Figure 7.10 Germination of two sorghum cultivars on day 14, with regard to three differently treated soils and at a) 30 mm and b) 60 mm.

In Figure 7.11a - c, the response of sorghum seedling growth to different planting depths and soils are shown. In sterile soil, sorghum planted at a depth of 30 mm had five M-EM treatments significantly increasing the shoot length compared to the control treatment. These treatments were S1 A, S1 B, S2 A, S2 B and S3 A. At depth 60 mm, all M-EM treatments resulted in shorter shoots compared to the control, while S1 A and S2 B significantly reduced shoot lengths.

Both S1 B and S3 A significantly increased shoot length at 60 mm depth in M-EM treated soil, while S1 A also resulted in longer seedlings. In the *Fusarium* treated soil all M-EM treatments with the exception of S3 B increased shoot length at 60 mm, although only S1 A was significantly longer.

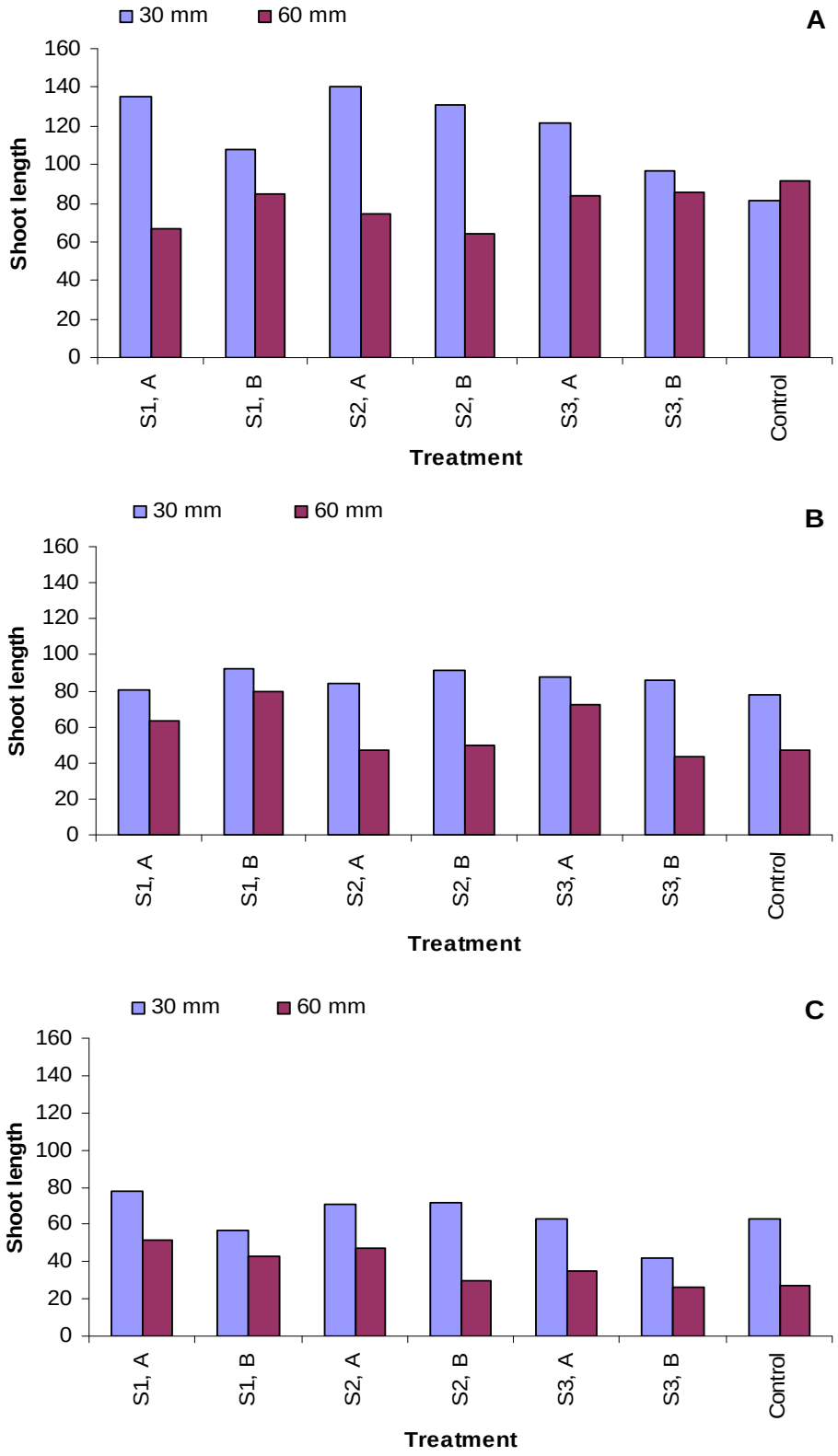
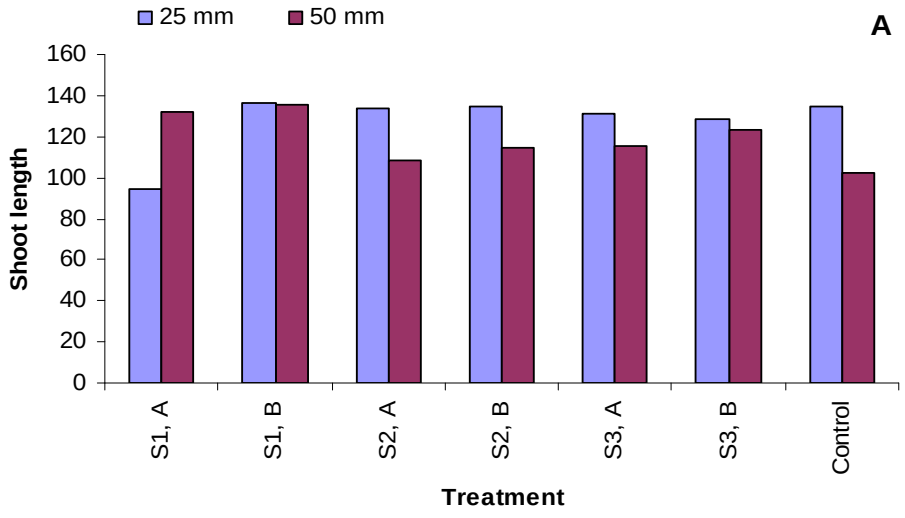


Figure 7.11 Shoot length of sorghum on day 14 planted at two depths in differently treated soil, a) sterile, b) M-EM treated and c) *Fusarium* containing soil, with regard to M-EM ratios.

The shoot length of sunflower cultivar 5 planted in sterile soil at 25 mm was reduced significantly by S1 A at both day seven and day 14 compared to the control treatment (Figure 7.12a). At 50 mm, all M-EM treatments improved shoot length compared to the control, although only S1 A, S3 A and S3 B did so significantly on days seven and 14 and S1 B only significantly improved on day 14.

In M-EM treated soil S1 A, S1 B, S2 A and S2 B significantly reduced shoot length on day 14 at 25 mm planting depth (Figure 7.12b). All of the named treatments accept S1 A also had a significantly reduced shoot length on day 7. At 50 mm planting depth, S1 A, S2 A, S2 B, S3 A and S3 B significantly improved shoot length of cultivar 5, compared to the control treatment.

In the *Fusarium* containing soil, all M-EM treatments resulted in shorter seedlings compared to the control at 25 mm (Figure 7.12c). Four M-EM treatments significantly reduced shoot length on both day seven and day 14, namely: S1 A, S1 B, S2 A and S3 B. At 50 mm, five of the six M-EM treatments significantly reduced shoot length of cultivar 5 compared to the control treatment. These include S1 A, S2 A, S2 B, S3 A and S3 B. Although not significant, S1 B also reduced shoot length.



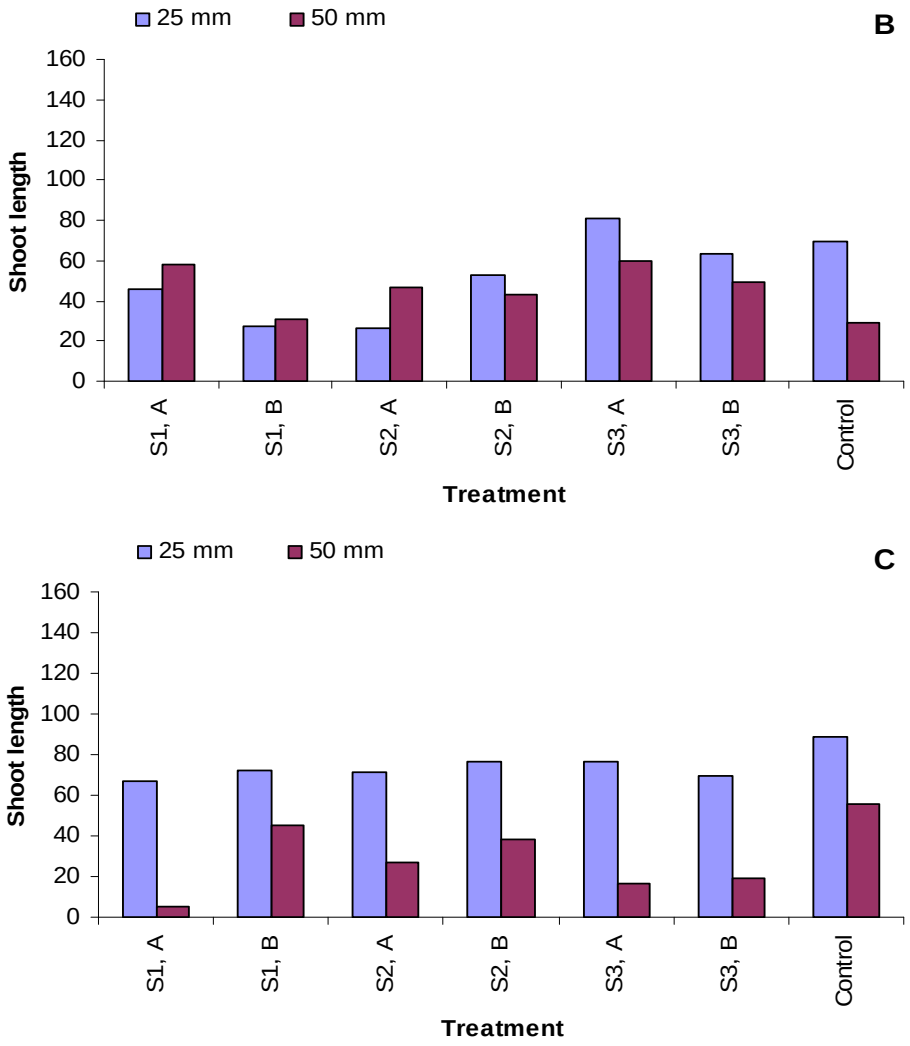


Figure 7.12 Shoot length of sunflower cultivar 5 on day 14 planted at two depths in three differently treated soil namely: a) sterile, b) M-EM treated and c) *Fusarium* containing soil, with regard to M-EM ratios.

Results for cultivar 6 in sterile soil indicated that at 25 mm, only S3 A significantly improved shoot length on day seven and day 14 (Figure 7.13a). At 50 mm, all M-EM treatments resulted in increased shoot lengths. Of these, only S2 A increased shoot length significantly on both day seven and 14, while S1 A, S2 B, S3 A and S3 B only significantly increased shoot lengths on day 14.

All M-EM treatments of sunflower cultivar 6 planted 50 mm deep in M-EM treated soil increased shoot length significantly (Figure 7.13b). S1 A, S1 B, S2 A, and S3 A significantly improved shoot length on day seven and day 14, while S2 B and S3 B only significantly improved shoot lengths on day 14.

All M-EM treatments, except S2 A increased shoot length over the control for cultivar 6 planted 25 mm deep in *Fusarium* containing soil (Figure 7.13c). Of these S1 A and S3 A significantly improved shoot length on day seven as well as day 14 while S2 B increased shoot length on day 14. Treatment with S1 A, S1 B, S3 A and S3 B increased shoot length compared to the control at 50 mm planting depth. S1 A and S3 B increased shoot length significantly on day 14 at 50 mm.

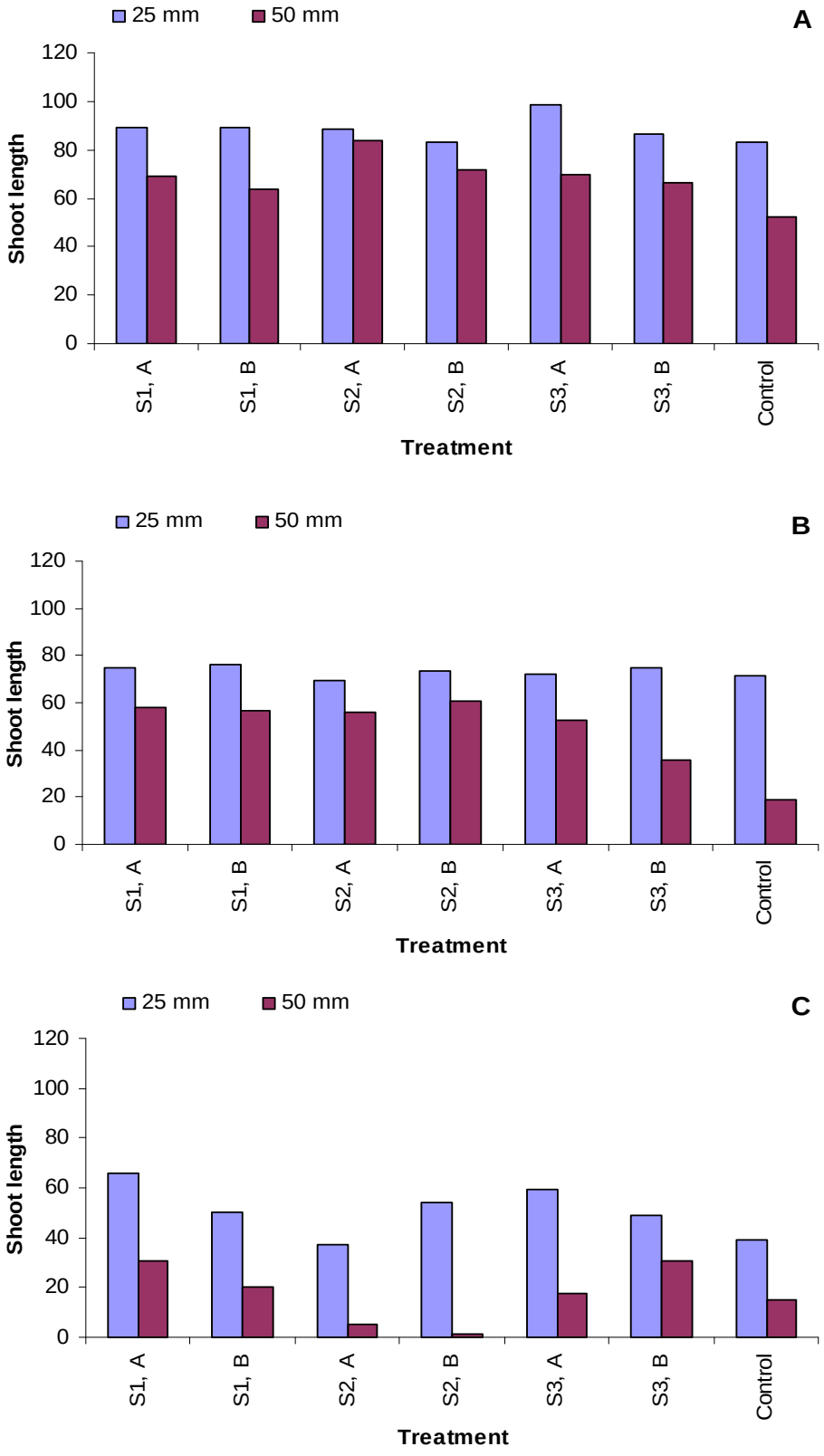


Figure 7.13 Shoot length of sunflower cultivar 6 on day 14 planted at two depths in differently treated soil, namely: a) sterile, b) M-EM treated and c) *Fusarium* containing soil, with regard to EM ratios.

7.4 Conclusion

The application of EM in crop production is said to control root rot, nematodes, *Fusarium* and other diseases as well as harmful gasses in soil. M-EM has however, a variety of applications including pre-planting soil treatment, seed treatment and plant rest treatment. Nevertheless, there has been very little research reported on the effect of M-EM in different soil microbial conditions. Therefore the purpose of the present study was to determine the effect of effective micro-organisms (EM) as seed treatment on the germination and seedling vigour of maize, sorghum and sunflower planted in sterilized soil, in soil treated with M-EM and soil containing *Fusarium,* in pot experiments. For germination, no M-EM treatment significantly improved the germination of maize or sorghum at any depth and in any soil treatment. Germination of sunflower was however, greatly influenced by M-EM treatments, especially in M-EM treated soil. In sterile soil germination of sunflower proved to be superior regardless of treatment. This is to be expected, since there is nothing in the soil to prevent good germination of untreated seeds. Although germination percentage of sunflower was sometimes slightly lower in M-EM treated soil, compared to sterilized soil, the M-EM treatments generally increased germination compared to the control. S1 A and S3 A significantly increased germination of sunflower in M-EM treated soil. At a planting depth of 50 mm, all M-EM treatments significantly increased germination.

Generally, seedling vigour was not improved by M-EM treatment in sterilised soil, irrespective of planting depth. As with germination, this was expected due to the lack of unfavourable soil conditions. Since soils are seldom sterilised in practice, these results do not supply an indication of the efficiency of M-EM as seed treatment. Although not always significant, maize, sorghum and sunflower seedling vigour was mostly increased above that of the control treatments by M-EM treatments. This result was accentuated when seeds were planted at the maximum recommended depth. This indicated that M-EM treatments will probably improve early seedling growth of maize, sorghum and sunflower compared to untreated seed. Especially when seeds are planted deeply and with the aid of a M-EM pre-plant treatment. M-EM treatment rarely had an improved effect on seedling vigour in *Fusarium* treated soil. In fact,

untreated seedlings often showed significantly better vigour compared to M-EM treated seedlings. Large differences in germination and seedling vigour between sterilised and M-EM treated soil, and *Fusarium* treated soil, indicated a good *Fusarium* infestation in soil 3. The results from this study therefore indicate that M-EM seed treatment had no advantage over untreated seed when planted in *Fusarium* containing soil.

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Chapter 8

General conclusion

It has been scientifically documented that effective micro-organisms (EM) protects plants from soil-borne pathogens, insects and diseases. Furthermore, EM is known to stimulate plant growth thereby increasing the yield and quality of crops. However, the availability of scientific research and published literature on the influence of M-EM as a seed treatment on the germination and seedling vigour of different cultivars of maize, sorghum and sunflower is limited. There is also no clear indication by EM producers at what ratio EM should be multiplied and at what dilution multiplied EM should be used as a seed treatment. The effect that EM seed treatment will have on maize, sorghum and sunflower is also unknown.

The present work therefore, was an attempt to evaluate the use of EM seed treatments, at different application rates, handling techniques and soil conditions on germination and seedling vigour of maize, sorghum and sunflower. Incubation studies were conducted in Chapter three, where EM was multiplied at two ratios, namely: A (1% Stock EM (S-EM), 7% molasses and 92% water) and B (3% S-EM, 5% molasses and 92% water) and thereafter diluted at three levels (0.01%, 0.1% and 1.0%). The objective was to determine the effectiveness of M-EM as seed treatment at different dilutions on the germination and seedling vigour of maize, sorghum and sunflower under 1) optimum germination conditions and 2) cold stress. From the results obtained in the first experiment revealed that M-EM seed treatment were ineffective in significantly increasing the amount of seeds germinated per 24 hour cycle, compared to the control treatments. However, in the second experiment rather notable difference on germination and seedling vigour was visible after the seeds were exposed to cold stress. Differences were observed between all supplier companies, at both multiplied ratios A and B, and at all three dilutions. The results were, however, contrasting with no single treatment responding only significantly positive or significantly negative. Notable was that M-EM seed treatment had a greater effect on cultivars less tolerant to stress conditions. However, remained unclear which product is superior and at what dilution and ratios the different products should be applied.

Therefore, the study was repeated in Chapter five to determine the effect of M-EM seed treatment on the germination, seedling vigour and dry mass of maize, sorghum and sunflower in pot experiments using untreated soil. The same multiplied ratios and dilutions used in Chapter three were used in this experiment. The results supported those in Chapter 3. Revealing that there were no real differences between either multiplied ratios, or supplier companies. The results indicate that the treatment of seed with M-EM did not have a significant effect on germination or on dry mass of maize, sorghum and sunflower under the experimental conditions. Some Multi-EM (M-EM) treatments did increase the dry mass of sorghum however, at a non-significant level. The shoot length results revealed that M-EM significantly increased shoot lengths of all three crops, compared to the control treatments. The most significant effects were at the 0.1% and 1% dilutions, although neither multiplied ratio A or B, nor supplier company had any real effect on shoot length. From the results a conclusion was drawn that the treatment of seeds with M-EM might increase seedlings' survivability, by improving seedling vigour (increased shoot length) and in some instances dry mass of young seedlings.

The importance of handling S-EM, M-EM and diluted M-EM in the manner as described by the supplier at all times was highlighted by the study whereby M-EM was exposed to different rates of irradiation and temperature fluctuation. The purpose of the incubation study in Chapter four was to establish the effectiveness of M-EM exposed to irradiation and temperature fluctuation on the germination and seedling vigour of maize, sorghum and sunflower. EM was multiplied into M-EM at two ratios, namely: A (1% S-EM, 7% molasses and 92% water) and B (3% S-EM, 5% molasses and 92% water). The M-EM was then exposed to irradiation and temperature fluctuation at three different rates namely; Rise–Set, 24 hours and 30 days. Treatments Rise–Set and 24 hours was left in a open field from sun rise to sun set and for 24 hours respectively, while the 30 day treatment was stored under optimal conditions in a store room and out of direct sunlight. The results indicated that if seeds were treated with the exposed M-EM and germinated under favourable germination conditions (25^{o}C), only the 30 day stored M-EM could generate a positive effect. This indicates that irradiation and temperature fluctuation has a negative effect on the effectiveness of M-EM seed treatment on germination. When the experiment was repeated under cold stress

(10^{o}C) conditions, only the plant lengths of maize PAN 6053 and sorghum PAN 8247 were positively affected by a few M-EM treatments. However, results were contrasting between treatments and therefore no dilution or multiplied ratio could be singled out.

To further test the effect of irradiation and temperature fluctuation on M-EM and its effect on maize, sorghum and sunflower, seeds were treated with M-EM, exposed to the same level of irradiation and temperature fluctuation, and planted in pots which were filled with untreated soil. Results in Chapter six supported the findings of that of Chapter four. M-EM exposed to irradiation and temperature fluctuation could not persistently produce the same results while, results contrasted each other by not only effecting germination and vigour significantly positively but, also significant negatively compared to the control treatments. The germination of deeper planted sorghum seeds, were positively effected by M-EM treatment, with no visible trend with regard to treatments. Only treatment with S1 B 30 lead to a significant improvement in germination compared to control treatments. While an overall positive effect was observed in the shoot length results of sorghum, M-EM had a more notable effect on the deeper planted maize and sunflower. A conclusion was drown that exposure to irradiation and temperature fluctuation has a bigger negative effect on M-EM than prolonged storage and that M-EM stored under unfavourable conditions might still have a positive effect on seedling survival.

Finally a study (Chapter seven) was conducted to determine the effect of M-EM seed treatment on the germination and seedling vigour of maize, sorghum and sunflower planted in different soil conditions, namely: sterilized soil, soil treated with M-EM and soil containing *Fusarium.* The results in terms of germination suggest that M-EM treatment was unable to significantly improve the germination of neither maize nor sorghum in any soil treatment. Sunflower germination was especially influenced by M-EM treatments, in M-EM treated soil. Germination in sterile soil proved to be better irrespective of treatment. M-EM treatments had an overall positive effect on the germination of sunflower planted in sterile or EM treated soil. S1 A and S3 A stood out by significantly increasing the germination of sunflower in M-EM treated soil. Seedling vigour of seeds treated with M-EM had an advantage over that of the control seeds in sterilised soil, even though the advantage was at a non-significant level in most cases.

Results indicated that M-EM seed treatment might increase the ability of a seedling to survive, especially under stressed conditions. Soil treated with M-EM before plant, might have an added benefit on the effect of M-EM treated seed. With the control treatments outperforming M-EM treated seedlings in *Fusarium* containing soil, *Fusarium* proved to be detrimental to the effectiveness of M-EM as a seed treatment in this study.

From the added strain caused by the deeper planting depth results indicated that M-EM seed treatment might be able to aid seeds in germination and also aid seedlings to overcome vigour depressing factors.

It can be concluded that neither supplier company, nor multiplied ratio, revealed significantly important differences in these experiments and that a dilution between 0.1% and 1% might be superior to a dilution of 0.01%. Although some positive results were obtained from the exposure to irradiation and temperature fluctuation, the manner in which EM, M-EM or even diluted M-EM is used and stored might play a crucial role in the effectiveness of EM. EM should at all times be handled as described by the supplier. From this study a conclusion was drown that the effect of using M-EM might increase with an increase in application rate.

Although the two dilutions of 0.1% and 1% preformed better than the control treatments, further research is needed before the use of EM as a seed treatment at a specific dilution could be recommended for use in maize, sorghum and sunflower production. Further research is also needed on the effect of the continuous use of M-EM on different soil types.

The results obtained from the use of EM are not easily nor instantly measurable, but rather a long term project with long term results. However, studies as this one, are necessary and needed to provide scientific information on this complex application uses of EM.