INTERACTION EFFECTS OF EFFECTIVE MICRO-ORGANISMS AND PROLONGED STORAGE ON GERMINATION AND SEEDLING VIGOUR OF MAIZE, SORGHUM AND SUNFLOWER

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Abstract

A study involving two incubation experiments and a germination experiment in sandy soil was conducted to determine the influence of Multiplied Effective Micro-organisms (M-EM) that were exposed to different levels of irradiation and temperature fluctuation as well as prolonged storage, on the germination and seedling vigour of maize, sorghum and sunflower. Irrespective of poor handling and/or prolonged storage of M-EM, seed treatment with M-EM improved germination under optimal conditions for all crops compared to the control. Increased planting depths and cold stress were used to create conditions where seed treatment with M-EM may improve germination and seedling growth. Seed treatment with M-EM significantly improved germination and seedling vigour of the stress-tolerant maize cultivar following cold stress. Similar improved seedling vigour results were observed for the stress-tolerant sunflower cultivar at increased planting depth. It was concluded that the beneficial influence of M-EM as a seed treatment was remarkable, even after exposure of M-EM to unfavourable environmental conditions and prolonged storage before application. Further research under field conditions and in different cultivation systems are required before the large scale application of M-EM as seed treatment can be recommended.

Keywords: cold stress, irradiation, multi-EM, planting depth, temperature

1. INTRODUCTION

Effective micro-organisms (EM) consist of over 80 selected types of micro-organisms (Singh, 2007), which include populations of lactic acid and photosynthetic bacteria, actinomycetes, fermenting fungi and yeasts (Lee et al., 2008; Javaid, 2010). According to Higa (1991), EM can improve crop growth through the secretion of growth regulator-like substances. These include organic acids, enzymes, antioxidants, and metallic chelates secreted mainly by the lactic acid bacteria (Higa & Chinen, 1998 cited by Szymanski & Patterson, 2003). In addition, EM and especially the photosynthetic bacteria can produce plant hormones such as gibberellins, through the fermentation of organic material (Higa, 1991; Javaid, 2010). These bioactive gibberellins control plant growth and development aspects such as seed germination, stem elongation, leaf expansion, and flower and seed development (Yamaguchi, 2008).

According to Golec, González and Lokare (2007), yeasts can also produce hormones and enzymes, which promote active cell and root division. Some authors observed beneficial effects of multiplied-EM (M-EM) on germination and seedling vigour for a variety of crops (Sangakkara & Attanayake, 1993; Van Tonder, 2011). According to Sangakkara and Attanayake (1993), germination and seedling growth of rice were improved through the application of M-EM. Previous research on M-EM as seed treatment for maize and grain sorghum also reported that M-EM improved germination and seedling vigour significantly under adverse environmental conditions (Van Tonder, 2011).

The aforementioned beneficial effects of EM are possible on condition that the micro-organisms survive throughout the pre- to post-treatment process. As a mixture of living micro-organisms, EM needs to be protected from direct sunlight and EM products must be stored in a room with adiabatic temperature (Gourlay, 2006). According to Alexander (1977), each microorganism has its own optimum temperature range for growth, outside of which development is halted, while significant temperature fluctuations can be detrimental to micro-organism survival (Szymanski & Patterson, 2003). Therefore, to ensure efficiency of EM as a growth stimulant, crop producers must take care not to expose their EM solutions to irradiation and temperature fluctuation. The best storage and handling practices include storage between 20°C and 30°C with fluctuation of less than 10°C in 24 hours, storage of EM out of direct sunlight and using EM in adequate time (Gourlay, 2006). An ideal practice is to insulate the EM and M-EM containers with blankets or bubble sheets to protect the organisms from temperature fluctuations (A. Rosenberg, 2009, Personal communication). Furthermore, Gourlay (2006) suggested that stock-EM should not be stored for more than six months, while M-EM should not be stored for a period exceeding one month.

Unwittingly, producers do not always realise that storage and handling of EM during planting may result in the destruction of the micro-organisms, and as a result they refrain from using EM again, because they did not achieve the anticipated results (D. Anthony, 2009, Personal communication). Since crop producers sometimes do not have another option but to store the M-EM in the field during planting, or they are just ignorant to the impact of their action, research was conducted to determine whether these factors really have a significant impact on the efficiency of M-EM. Therefore, the objectives of this study were to determine the influence of M-EM that was exposed to irradiation and temperature fluctuation as well as prolonged storage of M-EM on the germination and seedling vigour of maize, sorghum and sunflower.

2. MATERIAL AND METHODS

Location and experimental layout

Three independent experiments were conducted in the Laboratory and Greenhouse complex 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 (Pannar Seed (Pty.) Limited). Seed surfaces were sterilised in a 3.5% sodium hypochlorite solution for 10 minutes. Thereafter, the seed was triple rinsed in pure water. A total of 800, 2400 and 1600 seeds were used respectively per cultivar for the germination and seedling vigour experiments. Each experiment was replicated four times.

Cultivar Characteristics

Cultivar 1 (PAN 6236) is an ultra-early yellow maize that achieves excellent results under irrigation, and high potential under dry land conditions. Cultivar 2 (PAN 6053) is a medium maturing white maize cultivar, with excellent yield potential and proven reliability under low rainfall conditions, producing yields at low plant populations. Cultivars 3 (PAN 8247) and 4 (PAN 8816) are commonly used sorghum cultivars with good yield potential and a very uniform plant type/stability. Cultivars 5 (PAN 7351) and 6 (PAN 7033) are commonly used sunflower cultivars with a wide area adaptability, a high yield potential and a good stability.

M-EM dilutions and seed treatment

Multi-EM (M-EM) was produced from a commercial Stock EM (S-EM) at the following ratios:

- M-EM A: 1% S-EM, 7% molasses and 92% water
- M-EM B: 3% S-EM, 5% molasses and 92% water

M-EM was allowed to multiply for 14 days. Thereafter, the M-EM A and M-EM B were 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. The first bottle was placed in an open field from sunrise to sunset, whilst the second bottle was placed in an open field for 24 hours, and 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 of Bloemfontein, South Africa, during November and December 2009. The average minimum and maximum temperatures are summarised in Table 1 (University of the Free State Weather Station).

The third bottle of each of the M-EM treatments was stored in a laboratory with an air cooling system, which was used to regulate the temperature between 15 and 20°C.

Table 1 Weekly average minimum, maximum and change in temperature (°C) for 24-hour periods and daylight periods (sunrise to sunset) from 1 November to 31 December 2009.

	24 Hours			Daylight		
	Min.	**Max.**	**Change**	**Min.**	**Max.**	**Change**
Week 1	12.7	24.7	12.0	14.4	24.7	10.3
Week 2	11.7	25.3	13.6	14.6	24.8	10.3
Week 3	9.9	21.0	11.1	11.4	21.0	9.5
Week 4	15.0	29.2	14.1	17.4	29.2	11.8
Week 5	15.8	31.3	15.4	18.5	31.3	12.8
Week 6	16.3	29.4	13.1	17.7	29.4	11.7
Week 7	15.0	30.9	15.9	17.6	30.9	13.3
Week 8	18.3	32.7	14.4	19.7	32.7	13.0
Average	14.3	28.1	13.7	16.4	28.0	11.6

Seeds were soaked for seven hours in a 0.1% dilution (the standard dilution in practice) 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. Multiplied ratios, exposure rates and the control treatments were pooled into seven treatment combinations (Table 2), which was referred to as treatments.

Table 2 Treatment combinations 1 to 7 with regard to treatment abbreviation, multiplied ratio and exposure to irradiation and temperature fluctuation as well as prolonged storage.

Treatment number	Treatment abbreviation	Multiplied ratio	Exposure
1	A S-S	A	Sunrise-Sunset
2	A 24H	A	24 Hours
3	A S	A	Storeroom
4	B S-S	B	Sunrise-Sunset
5	B 24H	B	24 Hours
6	B S	B	Storeroom
7	Control	-	-

Experiment 1: Germination under favourable conditions

Ten dried seeds of each cultivar and treatment combination were placed in 90 mm diameter Petri dishes, and each treatment combination was replicated four times.

Seeds were placed on filter paper moistened with 10 ml sterilised 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°C. The experiment was terminated after seven days, and Petri dishes were inspected every 24 hours.

Experiment 2: Germination and seedling vigour after cold stress

The cold test was executed as described in the ISTA Handbook of Vigour Test Methods by Hampton and TeKrony (1995). On the day before planting, pure water was cooled overnight to 10°C. 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. The three towels were rolled and care was taken to ensure that the towels did not warm up above 10°C during and after preparation. The rolled towels were placed upright in a plastic container before they were transferred to the cold (10°C) chamber. Each rolled towel was placed in a plastic bag, to keep them upright and separated, but also to prevent loss of moisture and cross contamination. The containers were kept in a cold, dark chamber at 10°C for seven days.

After the cold treatment the containers were moved to the germination chamber at 25°C, also in darkness. This procedure was followed for all crops, cultivars and treatment combinations. Each experiment was performed in triplicate.

Experiment 3: Germination, seedling vigour and seedling growth in sandy soil

Plastic growing bags, with a 100 mm diameter, 1 ℓ capacity and drainage holes in the bottom, were used. Sandy soil was used as growth medium. The bags were maintained in a naturally ventilated greenhouse without temperature control. Seed was hand planted and the soil compacted by applying minimal hand pressure on top of the soil, and the soil in the bags was moistened with pure water as needed.

Different planting depths were used to create favourable conditions in which seed treatment with M-EM may improve germination and establish strong seedlings early after planting. Seeding depths were 50 mm (optimal) and 100 mm for maize; 30 mm (optimal) and 60 mm for sorghum; and 25 mm (optimal) and 50 mm for sunflower. Dried seeds were planted at 10 seeds per 100 mm diameter bag.

Measurements

In both experiments, a seed was recorded as germinated when the radical protruded by 3 mm or more. The Petri dishes were inspected in 24 hour intervals for seven days after planting. For experiment 2, plant lengths were measured at 48, 96 and 168 hour intervals following their transfer to the germination chamber to determine vigour. For experiment 3, germination was scored as soon as a shoot emerged above the soil level. Seedling vigour was determined by measuring shoot length on day 14 after planting. Seedling growth was determined as the dry mass of seedlings after day 14.

Statistical analysis

A factorial analysis of variance (ANOVA) was performed on the germination and seedling vigour with time factor, cultivar and treatment as factors. P-values were used to compare means at 0.95 confidence levels, using STATISTICA version 8.0 (STATISTICA, 2004).

3. RESULTS AND DISCUSSION

Experiment 1: Germination under favourable conditions

As expected, the time factor played a significant role in the statistical data as germination increased rapidly initially (Table 3). In combination with cultivar, a significant difference in germination percentage occurred between both maize cultivars on day two (68% for cultivar 1 vs. 52% for cultivar 2), although this effect was nullified after three days (data not shown). The significant difference in germination between the two cultivars of sorghum was profound for the total experimental period. Similar to other trials by Van Tonder (2012) on sorghum, cultivar 4 outperformed cultivar 3.

Table 3 Significant levels of main effects namely time factor (T), cultivar (C) and treatment (ST), as well as interactions for maize, sorghum and sunflower germinated under favourable conditions.

	p-values		
Effect	**Maize**	**Sorghum**	**Sunflower**
T	0.000	0.000	0.000
C	0.002	0.000	0.373
ST	0.014	0.021	0.028
T x C	0.000	ns	ns
T x ST	ns	ns	ns
C x ST	ns	ns	ns
T x C x ST	ns	ns	ns

(ns = not significant at $p<0.05$)

For both maize and sorghum, the germination of seed treated with M-EM, regardless of the duration of exposure to unfavourable environmental conditions, was significantly higher than the control (Figure 1 and 2).

This was also true for sunflower, although the B 24H and B S treatment did not significantly improve germination compared to the control (Figure 3).

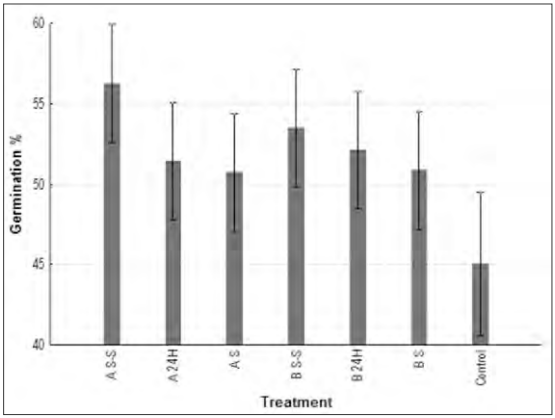

Figure 1: Germination percentage of maize seed treated with M-EM that were exposed to poor handling techniques and prolonged storage, and germinated under favourable conditions in a temperature controlled chamber. Vertical bars denote 0.95 confidence intervals.

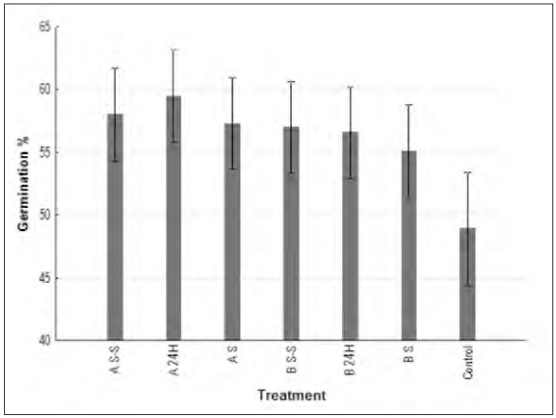

Figure 2: Germination percentage of sorghum seed treated with M-EM that were exposed to poor handling techniques and prolonged storage, and germinated under favourable conditions in a temperature controlled chamber. Vertical bars denote 0.95 confidence intervals.

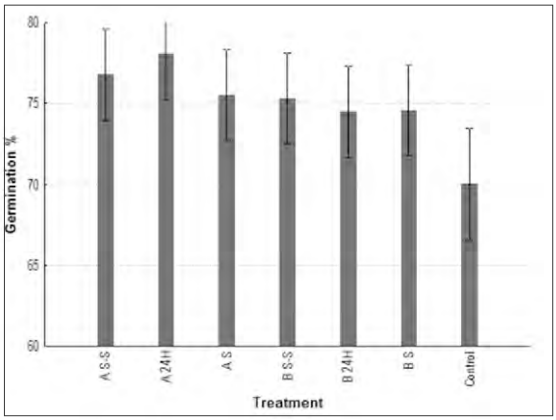

Figure 3: Germination percentage of sunflower seed treated with M-EM that were exposed to poor handling techniques and prolonged storage, and germinated under favourable conditions in a temperature controlled chamber. Vertical bars denote 0.95 confidence intervals.

Experiment 2: Germination and seedling vigour following cold stress

Over time, germination and seedling vigour of maize were highly significant for the two cultivars and seven treatments. (Table 4). In contrast, only the cultivars of sorghum differed significantly from each other with regard to germination and seedling vigour. Although sunflower did not have a significant effect on germination, the seedling vigour was significantly affected in both cultivars over time and treatments over time (Table 4).

Table 4: Interaction effects of time (T), cultivar (C) and seed treatment (ST) on germination and seedling vigour of maize, sorghum and sunflower following exposure of seed to cold stress.

	Germination			Seedling vigour		
Effect	**Maize**	**Sorghum**	**Sunflower**	**Maize**	**Sorghum**	**Sunflower**
T	0.009	0.000	ns	0.000	0.000	0.000
C	0.000	0.000	ns	0.056	0.000	ns
ST	ns	ns	ns	0.005	ns	0.000
T x C	ns	ns	ns	ns	ns	0.000
T x ST	ns	ns	ns	0.029	ns	0.004
C x ST	ns	ns	ns	0.000	ns	ns
T x C x ST	0.012	ns	ns	0.000	ns	ns

(ns = not significant at $p<0.05$)

Germination rate following cold stress

Most of the M-EM treated seed of maize cultivar 2 resulted in improved germination on day 4 in comparison with the control, although not significant (Figure 4). For maize cultivar 1, day 4, the control as well as B S-S and B S resulted in significantly higher germination than B 24H. In an earlier experiment by Van Tonder et al. (2012), the germination of cultivar 1 (a cultivar less tolerant to stress conditions) was significantly improved over the control with the application of M-EM, especially under unfavourable environmental conditions. The results speculatively indicate that the handling and storage treatments in this experiment significantly reduced the beneficial effects of M-EM. However, no definite trend could be observed with regard to the handling and storage techniques of any of the cultivars studied.

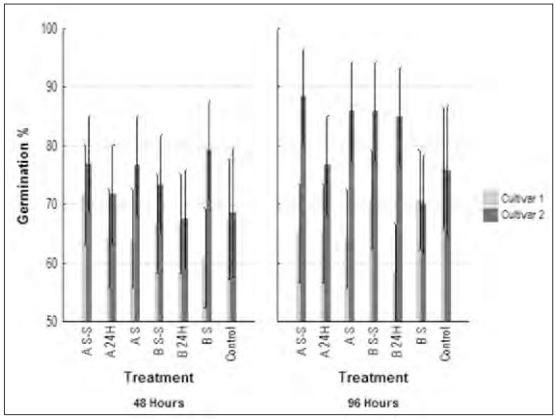

Figure 4: Germination percentage of maize seed that was subjected to cold stress and thereafter treated with M-EM that were exposed to poor handling techniques and prolonged storage. Vertical bars denote 0.95 confidence intervals.

Seedling vigour following cold stress

All seedlings of maize cultivar 1 were significantly smaller than the control on day 4 (Figure 5). In contrast, seedlings of maize cultivar 2 were significantly bigger than the control, except for those of seed treated with BS. This confirmed the results of the germination test and may indicate that, while the beneficial effects of EM was nullified by poor handling and storage techniques, it was conceived to have a negative effect on seedling vigour for cultivars less tolerant to stress conditions (such as cultivar 1). However, cultivars more tolerant to stress (such as cultivar 2) may still be able to utilize the leftover of M-EM following exposure of the micro-organisms to unfavourable experimental conditions.

As for maize cultivar 1, the seedling vigour of both sunflower cultivars was significantly reduced after four days by the application of M-EM exposed to poor handling and storage, compared to the control (Figure 6). Since there was no significant difference between seedlings of both sunflower cultivars after four days (127.5 mm and 134.3 mm), these results were purely ascribed to the influence of M-EM treatment.

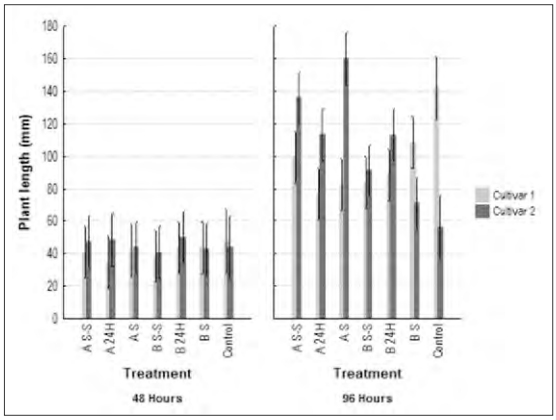

Figure 5: Plant lengths of maize seed that was subjected to cold stress and thereafter treated with M-EM that were exposed to poor handling techniques and prolonged storage. Vertical bars denote 0.95 confidence intervals.

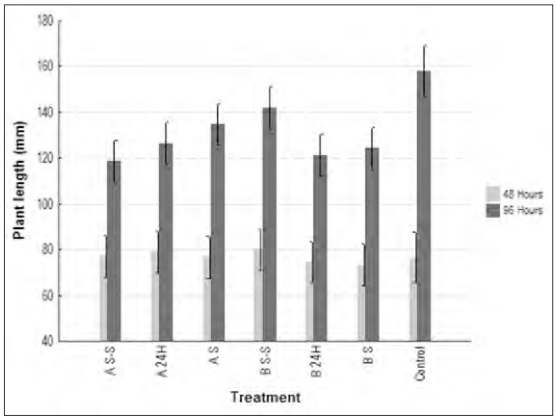

Figure 6: Plant lengths of sunflower seed that was subjected to cold stress and thereafter treated with M-EM that were exposed to poor handling techniques and prolonged storage. Vertical bars denote 0.95 confidence intervals.

Experiment 3: Germination, seedling vigour and seedling growth in sandy soil

The treatment of maize and sorghum seed with M-EM did not significantly affect germination and seedling dry mass, while it did play a significant role in seedling vigour (Table 5).

Table 5: Interaction effects of cultivar (C), depth (D) and seed treatment (ST) on germination; plant height and seedling dry mass of maize, sorghum and sunflower.

	Germination			Seedling dry mass		Seedling height		
Effect	**Maize**	**Sorghum**	**Sunflower**	**Maize**	**Sorghum**	**Maize**	**Sorghum**	**Sunflower**
C	ns	0.011	0.001	0.000	0.000	0.000	0.000	0.000
D	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ST	ns	ns	0.050	ns	ns	0.000	0.042	ns
C x D	ns	0.000	ns	0.039	0.000	ns	0.003	0.027
C x ST	ns	ns	ns	ns	ns	0.003	0.011	0.001
D x ST	ns	ns	ns	ns	ns	0.001	ns	0.000
C x D x ST	ns	ns	ns	ns	ns	0.000	ns	0.007

(ns = not significant at $p<0.05$)

As expected, depth had a significant influence on germination and seedling dry mass. Germination rates for maize and sunflower planted at the optimal seeding depth of 50 mm and 25 mm respectively, were correspondingly 28.1% and 23.8% higher compared to seeds that were planted deeper (100 mm and 50 mm respectively). Germination of sorghum was also significantly reduced by a mean value of 40% when seeds were planted deeper (60 mm), while cultivar 3 performed significantly better (13%) than cultivar 4 at 30 mm, as opposed to previous results. In contrast, seedlings of cultivars 3 and 4 did not vary in dry mass for the deeper seeding depth, while cultivar 4 outperformed cultivar 3 by a significant margin at optimal seeding depth of 30 mm. The same trend was observed for maize seedling dry mass, with cultivar 2 significantly outperforming cultivar 1 at an optimal seeding depth of 50 mm. The reasoning behind using different planting depths was to create conditions where seed treatment with M-EM may improve germination and establish strong seedlings early after planting. However, the only treatment effect with regard to germination was observed for sunflower (Figure 7), where all treatments of M-EM A marginally germinated better than the control. Significant effects were only observed for A 24H with regard to all M-EM B treatments, while A S-S and A S were significant compared to B 24H.

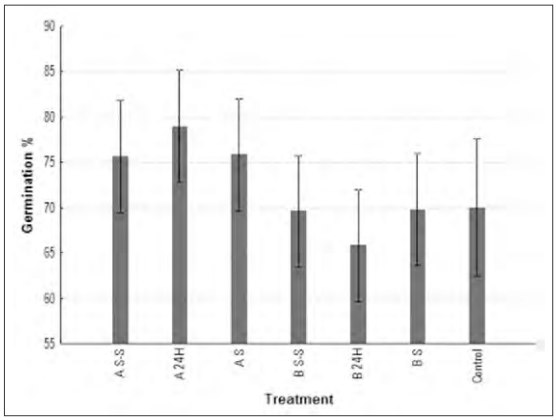

Figure 7: Germination of sunflower seed treated with M-EM that were exposed to poor handling techniques and prolonged storage, and germinated in sandy soil. Vertical bars denote 0.95 confidence intervals.

Mixed results were observed with regard to seedling vigour and seed treatment with M-EM. Seedlings of maize cultivar 1 seed planted at optimal depth (50 mm) and treated with M-EM, even though they were exposed to various levels of unfavourable environmental conditions or prolonged storage, were significantly more vigorous compared to the control (Figure 8). This significant effect was also the case for seedlings of maize cultivar 2, but at a deeper seeding depth. Since maize cultivar 2 is more tolerant to stress, it performed better at deeper seeding depths than cultivar 1, with the exception of the control, which may be ascribed to the influence of M-EM that presumably improved seedling vigour of cultivar 2.

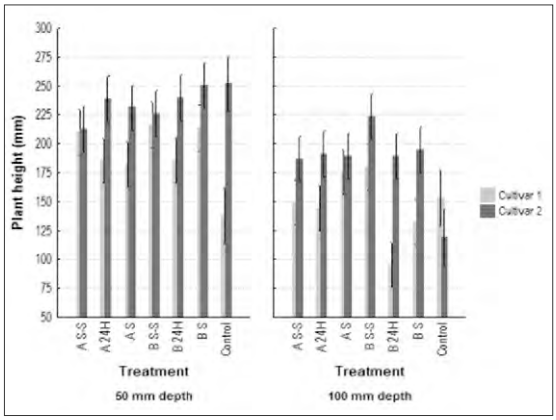

Figure 8: Plant heights of two cultivars of maize in pot experiments as a result of seed treated with M-EM that were exposed to poor handling techniques and prolonged storage. Vertical bars denote 0.95 confidence intervals.

There was no trend for seedling height of both sorghum cultivars with regard to treatment, with no single treatment performing significantly better or worse than the controls. Treatment of seed from sunflower cultivar 5 with M-EM exposed to various levels of unfavourable conditions or prolonged storage, significantly reduced plant height of all treatments compared to the control at the optimal seeding depth of 25 mm (Figure 9). However, the opposite result was observed for seeds of cultivar 5 which were placed 50 mm deep. This indicated that M-EM had a beneficial effect on seedling vigour even after it was exposed to unfavourable environmental conditions or prolonged storage. There was no significant difference between seedlings of cultivar 6 in all treatments, although seeds planted deeper into the soil resulted in significantly smaller seedlings than the control seeds.

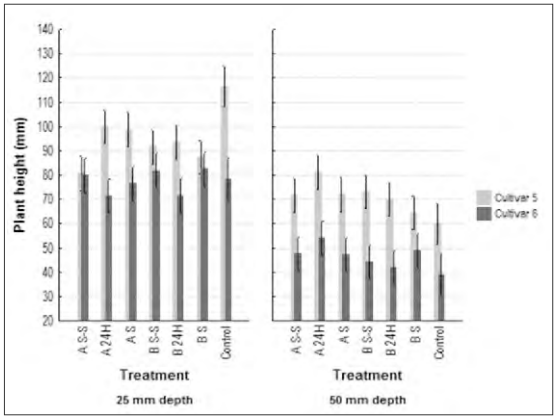

Figure 9: Seedling vigour of two cultivars of sunflower in pot experiments as a result of seed treated with M-EM that were exposed to poor handling techniques and prolonged storage. Vertical bars denote 0.95 confidence intervals.

4. CONCLUSIONS

It is generally conceivable that temperature fluctuations influence micro-organism survival, and therefore M-EM needs to be stored in a storeroom with minimal temperature fluctuation and away from direct sunlight. The purpose of this study was therefore to determine the influence of M-EM that was exposed to irradiation and temperature fluctuation as well as prolonged storage of M-EM on the germination and seedling vigour of maize, sorghum and sunflower.

For all crops M-EM seed treatment significantly improved germination under optimal conditions compared to the control, irrespective of the level of exposure of M-EM to unfavourable environmental conditions and prolonged storage. However, after treated seeds of all crops were exposed to cold stress, germination was significantly reduced by all treatments, while there was no trend indicating which handling and storage techniques gave the best results.

Similar results were observed for seedling vigour of treated maize seed (cultivar 1) subjected to cold stress. Results indicated that poor handling and storage techniques of M-EM may even have a negative effect on seedling vigour for maize cultivars less tolerant to stress conditions (e.g. cultivar 1), as well as for both sunflower cultivars. In contrast, seedling vigour of maize cultivars more tolerant to stress (e.g. cultivar 2) may be improved by treating seed with M-EM after exposure to unfavourable conditions or prolonged storage, compared to the control. However, this will depend on the amount of micro-organisms that survived the unfavourable handling techniques and prolonged storage, which is in turn dependent on the harshness of environmental conditions to which M-EM were exposed. For this study, the average change in minimum and maximum temperature for November to December was not extreme, e.g. 11.6°C for the Sunrise to Sunset treatment and 13.7°C for the 24 Hour treatment.

The third experiment used planting depth to create conditions where seed treatment with M-EM may improve germination and seedling vigour in the early stages after planting. The only treatment effect with regard to germination was observed for sunflower, where all treatments of M-EM A marginally germinated better than the control. From the same results, M-EM A was found to be significantly superior to M-EM B. In contrast to seedling vigour results for maize cultivar 1 after exposure to cold stress, seedlings of cultivar 1 planted 50 mm deep and treated with M-EM that were exposed to unfavourable environmental conditions or prolonged storage, were significantly more vigorous than the control. A similar result was observed for maize seedlings of cultivar 2 planted 100 mm deep, and this correlated with previous results of vigour after cold stress. Similar to previous results for seedling vigour after cold stress, seedlings of sunflower cultivar 5 from all treatments and planted 25 mm deep were significantly smaller than the control. However, with deeper placement of seed, cultivar 5 had significantly bigger plants for all M-EM treatments than the control, even though M-EM were exposed to temperature fluctuations and prolonged storage.

It can be concluded that the beneficial influence of M-EM as a seed treatment is remarkable, even if M-EM were exposed to moderate irradiation, temperature fluctuation and prolonged storage before application. However, in combination with cold stress, the same treatment may have a negative effect on germination, while cultivars less tolerant to stress can also be less vigorous. Nevertheless, this effect may be nullified by the use of cultivars more tolerant to stress as well as the prevention of exposure of M-EM to harsh environmental conditions.

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