



International Journal of
**Agricultural
Research**

ISSN 1816-4897



Academic
Journals Inc.

www.academicjournals.com

Effect of Effective Microorganisms (EM) Seed Treatment and Types of Potting Mix on the Emergence and Growth of Coffee (*Coffea arabica* L.) Seedlings

¹Ali Mohammed, ²Wosene Gebreselassie and ²Tigist Nardos

¹Department of Post-Harvest Management, Jimma University, College of Agriculture and Veterinary Medicine, P.O. Box 307, Jimma, Ethiopia

²Department of Horticulture and Plant Science, Jimma University, College of Agriculture and Veterinary Medicine, P.O. Box 307, Jimma, Ethiopia

Corresponding Author: Tigist Nardos, Department of Horticulture and Plant Science, Jimma University, College of Agriculture and Veterinary Medicine, P.O. Box 307, Jimma, Ethiopia

ABSTRACT

Coffee (*Coffea arabica* L.) is primarily propagated by seedlings and produced directly from seeds. Effective Microorganisms (EM) has been used for the purpose of improving the germination of different crops. This study was initiated to assess the effect of EM on emergence and subsequent growth of coffee seedlings in different potting mix as a media for growth. The study was conducted in Jimma, Ethiopia from November 2011 until June 2012. The experimental units were laid out in a 5×3 factorial arrangement with Randomized Complete Block Design with three replications. The EM seed treatment had five levels of soaking h (3.5, 4.5, 5.5, 6.5 h and soaking in pure water for 72 h) while the potting mixes had three proportions of Forest Soil (FS) and EM Compost (EMC): (100% FS, 75% FS: 25% EMC and 50% FS: 50% EMC). The highest (76.47%) emergence was recorded from forest soil and EM compost mixture of 75:25 and seeds soaked in pure water for 72 h. Forest soil combined with a 4.5 h soaking of coffee seeds in EM solution resulted in the largest seedling height, number of primary branches and total dry matter. According to this study, EM solution may not be preferred than soaking in water as far as hastening of emergency is concerned. However, the use of forest soil as a pot media and soaking coffee seeds in EM solution for 4.5 h results in a relatively vigor coffee seedlings for transplanting.

Key words: Coffee, Effective Microorganisms (EM), EM-compost, pre-sowing treatment

INTRODUCTION

The family Rubiaceae comprises about 5000 species of mainly trees and shrubs within about 400 genera. The two most important genera are *Coffea* which provides beverages and *Cinchona* which provides medicinal products. The genus *Coffea* has more than 70 species, but only two species, *Coffea arabica* L. and *Coffea canephora* Pierre, contributing 70 and 30% of world's coffee, respectively (Vieira *et al.*, 2006).

To date, regardless of the considerable effort being made to arrive at effective vegetative and micro-vegetative propagation techniques, coffee is primarily propagated by seedlings and produced directly from seeds. However, coffee has a slow and non uniform germination in terms of time which makes difficult to obtain uniform and vigor seedlings at the time of transplanting. In addition coffee loses its viability quickly (Amaral *et al.*, 2006). Emergence of *C. arabica* seedlings from the soil starts 50-60 days after sowing in the warmer periods of the year (Maestri and

Vieira, 1961) and can take up to 90 days when temperatures are lower (Went, 1957; Wrigley, 1988). This is because of the endogenous ABA-like substances and exogenous ABA that cause inhibition of germination by preventing growth of the embryo in the seed (Valio, 1976). In addition the presence of the parchment (endocarp) severely inhibits the germination of coffee seeds (Valio, 1980). As a result, obtaining high quality seedlings at the appropriate time of planting in many coffee production regions in Ethiopia and around the world is difficult.

In response to the difficulty in coffee germination several efforts have been made to improve the germination capacity of coffee seeds. Different chemicals like hydrochloric acid, sulphuric acid, boric acid, pantothenic acid, ascorbic acid, nicotinic acid, riboflavin, inositol, thiourea, indoleacetic acid, indolepropionic acid, naphthaleneacetic acid, GA₃, copper sulphate, manganese chloride, zinc sulphate and pyridoxine were all tested to improve the germination capacity of coffee seeds (Gopal and Ramaiah, 1971).

Successful efforts were reported in enhancing seed germination of *Arabica* coffee through constant temperatures (Wellman, 1961; Went, 1957; Riley, 1981; Valio, 1976), pre-soak (Huxley, 1967), warm stratification (Riley, 1981), dark germination (Valio, 1976), removal of endocarp (Gopal and Ramaiah, 1972; Van der Vossen, 1979), removal of endocarp and pre-soak in Kinetin (Valio, 1976), Thiamine Folic acid and Ferrous sulphate (Gopal and Ramaiah, 1971). Most of the works done were, however, for laboratory consumption and in practice there is no effective way of germination enhancement technique with the exception of the recent report by Gebreselassie *et al.* (2010) which reported the result of parchment removal and subsequent soaking in pure water for 72 h as an effective method of enhancing *Arabica* coffee seed germination. However, 72 h seed soaking seems still longer and calls for other methods of soaking to promote effective germination of coffee seeds.

Effective Microorganisms (EM) are microbial inoculants comprised mainly of lactic acid bacteria, photosynthetic bacteria, yeasts and actinomycetes that are commonly found in soil. The diverse applications of EM include improving soil conditions for better plant growth, treating waste water, controlling pests and diseases, improving animal growth, enhancing compost production and extending the shelf life of harvested crops (Zimmermann and Kamukuenjandje, 2008). EM has been used for the purpose of improving the germination of different crops (Siqueira *et al.*, 1993). Siqueira *et al.* (1993) studied the effect of seed treatment by EM and biofertilizer observed a substantial increase in the percentage of germination and vigor for many vegetable seeds including tomato, pepper, carrot, beetroot, peas, cucumber and corn. According to Harakawa and Higa (1989), Sangakkara and Attanayake (1993), Siqueira *et al.* (1993), Tokeshi and Chagas (2010) and Daly and Stewart (1999), Effective Microorganisms (EM) have hormonal effects similar to the gibberelic acid. In an attempt to improve the germination and emergence speed of citrus seedlings, Tokeshi and Chagas (2010) tested EM treatment (0.1% v/v) for 30 min on Cleopatra citrus seeds and discovered that EM seed treatment increased seed germination and the percentage of vigor increase by 810, 944, 646 and 552% at 20, 21, 22 and 23 days, respectively.

Soaking seeds in EM diluted 100 times speeded up germination and increased the proportion of seeds which germinated in most of the species tested, in comparison to the control seeds soaked in water. However, these increases were usually not sufficient and even in a few species germination was actually seen decreased. Rosenberg and Linders (2004) warn that legume seeds should not be treated by EM. Similarly, Mutumbulwa (2006) reported that *Albizia anthelmintica* seeds soaked in water germinated better (55%) than seeds soaked in 1% EM (15% only). The author also found that EM soaking resulted in faster germination for *Kigelia africana*, with all the

EM-soaked seeds germinating after 13 days, while all those soaked in water germinated after 25 days. Zimmermann and Kamukuenjandje (2008) found that EM soaking slightly reduced the number of days taken to the germination of parsley and chives, but slightly lowered the germination rate and subsequent growth of celery. An independent trial with seed of devil's claw, *Harpagophytum procumbens*, showed that EM soaking increased germination from 5.5-12%.

In a study conducted in Bangladesh by Khan *et al.* (2006) tested a microbial inoculant as Effective Microorganisms (EM) applied to *Cassia fistula* to study its influence on growth and development of seedlings. EM solutions at different concentrations (0.1, 0.5, 1, 2, 5 and 10%) were used as a treatment and applied in polybags one week before and after sowing seeds. The result showed that germination and subsequent physical growth parameters of seedlings were found significantly higher in the treated plants than the controls where maximum growth was recorded from at 2% EM solution.

To date, there is no documented scientific work on the effect of EM seed treatment and EM fermented compost on the germination capacity of coffee seeds. Furthermore, the current use of forest soils (the recommended best potting media for coffee by Institute of Agricultural Research (1996), does not take into account the ever-dwindling forest of Ethiopia. Therefore, there is a substantial and urgent need to formulate a potting media that takes into account environmental sustainability of coffee seedling production. Hence, this study was initiated to assess: (1) The effect of EM on emergence and subsequent growth of and coffee seedlings and (2) The interaction effect between EM seed treatment and the type of potting mix on the germination and subsequent growth of coffee seedlings.

MATERIALS AND METHODS

Study site: The study was conducted at Jimma University College of Agriculture and Veterinary Medicine, coffee nursery site from November 2011 until June 2012. Jimma is located at approximate geographic coordinates of latitude 07°42'N and longitude of 36°50'E at an altitude of 1710 m above sea level. It receives an annual average rainfall of 1600 mm and has mean minimum and maximum temperatures of 11.4 and 26.8°C, respectively (Amina *et al.*, 2012). The major soil types that represent the study area are black to red soils; chromic nitosol; combiosl bottom land. The soil is clay loam in texture (Daba *et al.*, 2012).

Experimental design and treatments: The experiment consisted of two factors namely EM Seed treatment and potting medium each having five and three levels, respectively. The experimental units were laid out in a 5×3 factorial arrangement with Randomized Complete Block Design (RCBD) with three replications. The EM seed treatment had five levels of different soaking h (3.5, 4.5, 5.5, 6.5 h and control) while the potting mixes had three different proportions of Forest Soil (FS) and EM compost (EMC): (100% FS, 75% FS: 25% EMC and 50% FS: 50% EMC).

Forest soil preparation: Forest soil was collected from the middle of the forest at Eladale farm of the College. It was air dried, manually crushed and passed through 2 mm sieve to remove clods, plant roots and other foreign materials (Yakob *et al.*, 1998). The sieved soil was filled to black polythene bag of 12 cm wide and 25 cm length as per the treatments in proportion to the EM or soil.

Compost preparation: The EM compost was prepared in a pit from coffee husk, biomass of parthenium and chicken feces with additional 200 mL EM solution which was sprinkled on the

compost material at the first pit filling and first turning. Based on our preliminary study, in this way the compost matured within 40 days.

EM treatment of seeds: The seeds for the experiment were soaked in EM stalk water (0.1%) for 3.5, 4.5, 5.5 and 6.5 h. The control seeds were soaked in pure water for 72 h. Then the seeds were drained off and sown immediately.

Potting mixes and arrangement: The forest soil as well as the EM compost material was sieved before pot filling. Then the EM compost was mixed as per the treatment combinations with forest soil. A single experimental unit (treatment) consisted of 16 pots and they were arranged in square fashion (4×4) on nursery bed with 60 cm spacing between experimental units and 1.5 m between replications.

Seed preparation and sowing: Red ripe cherries from coffee cultivar 74-40, which is resistant to Coffee Berry Disease (CBD), were picked and added to water to skim off the floaters that were suspected of poor seed germination and seedling growth. The cherries were then hand pulped and washed off their mucilage with repeated change of water. Then, the seeds were uniformly treated with wood ash to avoid sticking together, cracking of the parchment and to keep away flies during drying under shade. Seeds were then dried to a moisture content of 18-20% and stored under dry and cool condition until sowing time.

Four seeds were sown per polythene bag. Thinning to one seedling in a bag was done 90 days after sowing when all normal viable seedlings were expected to emerge. Every routine nursery activity was practiced uniformly to all experimental units as per the recommendation of the Jimma Agricultural Research Center (Institute of Agricultural Research, 1996).

Data collection: Emergence count was done from each experiment 90 days after sowing when all potential seeds were believed to emerge. Then, the attributes of non-destructive parameters were measured by taking two seedlings from the inner most four of each experimental unit: Plant height (cm), stem diameter (cm) and number of primary branches. Plant height was measured from the base to the tip of the seedling using a ruler. Stem diameter was measured at the base near the medium surface using a caliper. Primary branches were also counted.

Each seedling sampled for measurement of non-destructive parameters was brought to the laboratory for destructive parameters. The polythene bag containing the roots of the seedlings was immersed in a bucket filled with water and roots were allowed to separate carefully from the soil still being in water. The roots were subsequently washed with clean water, dried with water adsorbent cloth and fresh weight was measured. Seedlings were then cut with a scissor at collar point to separate the shoot from the root and fresh weight of each was weighed using sensitive balance. Finally, the shoot and root parts were oven dried at 100°C until a constant weight as described by Adjet-Twum and Solomon (1982) and dry matter measurement was made using sensitive balance.

Statistical analysis: The data were subject to analysis of variance (ANOVA) for the design. Mean separation was also done for treatment of significant difference GenStat, using 12.1 version (GenStat, 2007).

RESULTS AND DISCUSSION

The results showed that there was a statistically significant difference ($p < 0.05$) between treatments for percent of emergence 90 days after sowing. The highest (76.47%) emergence was recorded from forest soil and EM compost mixture of 75:25 and seeds soaked in pure water for 72 h while the lowest emergence (23.53%) was obtained from pots filled with forest soil alone and seeds soaked for 3.5 h in an EM solution (Table 1).

There was a statistically significant difference ($p < 0.05$) between treatments in plant height. Forest soil combined with a 4.5 h soaking of coffee seeds in EM solution resulted in the largest seedling height (0.75 m) in contrast to the smallest height (0.4 m) which was recorded from 50:50 soil mix and seeds soaked in water (Table 2). A statistical significant difference ($p < 0.05$) was also observed in number of braches among the treatments where forest soil combined with a 4.5 h soaking of coffee seeds in EM solution showed the highest number of primary branches (12.17). The smallest number of branches was obtained from 50:50 soil mix and seeds soaked in water (Table 2). Total dry matter also brought a statistically significant difference ($p < 0.05$) among the treatments. Maximum of the dry matter (54.6 g) was obtained from seedlings raised in sole forest soil and seeds soaked for 4.5 h in EM solution. A soil media of 50:50 and soaking in pure water for 72 h resulted in the lowest dry matter content (19.8 g) of seedlings before transplanting (Table 2). There was however, no significant difference observed in girth among the treatments.

The better emergence was resulted from seeds soaked in pure water for 72 h and sown in a pot filled with forest soil and EM compost mixture of 75:25.

A positive effect of EM solution in hastening of the seedlings was expected because in many reports (Siqueira *et al.*, 1993; Zimmermann and Kamukuenjandje, 2008) it was mentioned that treating different seeds with EM solution hastened emergency in many crop seeds. However, seeds soaked in pure water for 72 h had better performance in emergency compared to the EM-treated

Table 1: Emergence of coffee seeds as affected by different media mix of forest soil with EM compost and soaking h in EM solution

Treatments		
Media mix*	Soaking h	Emergence 90% days after sowing
100:0	3.5	23.53 ^a
	4.5	54.90 ^{ab}
	5.5	41.18 ^{ab}
	6.5	33.33 ^a
	Control	52.94 ^{ab}
50:50	3.5	25.49 ^a
	4.5	29.41 ^a
	5.5	33.33 ^a
	6.5	25.49 ^a
	Control	60.78 ^{ab}
75:25	3.5	23.53 ^a
	4.5	49.02 ^{ab}
	5.5	37.25 ^{ab}
	6.5	47.06 ^{ab}
	Control	76.47 ^b
L.S.D		39.80
CV (%)		58.2

*100:0: Forest soil only, 50:50: 50% forest soil+50% EM compost, 75:25: 75% forest soil+25% EM compost

Table 2: Some agronomic parameters of coffee seeds as affected by different media mix of forest soil with EM compost and soaking h in EM solution

Treatments		Parameters			
Media mix*	Soaking h	Height (m)	Girth (cm)	No. of primary branch	Total dry weight (g)
100:0	3.5	0.53 ^{abc}	0.850	8.00 ^{ab}	24.3 ^{ab}
	4.5	0.75 ^d	1.133	9.83 ^{ab}	54.6 ^c
	5.5	0.53 ^{abc}	0.900	12.17 ^b	31.4 ^{ab}
	6.5	0.56 ^{abcd}	0.833	10.33 ^{ab}	30.9 ^{ab}
	Control	0.45 ^{ab}	0.883	7.83 ^{ab}	24.5 ^{ab}
50:50	3.5	0.47 ^{abc}	1.033	10.33 ^{ab}	38.2 ^{abc}
	4.5	0.64 ^{bcd}	0.917	7.83 ^{ab}	33.9 ^{ab}
	5.5	0.54 ^{abc}	0.717	7.33 ^a	22.9 ^{ab}
	6.5	0.49 ^{abc}	0.850	8.17 ^{ab}	24.3 ^{ab}
	Control	0.40 ^a	0.769	6.62 ^a	19.8 ^a
75:25	3.5	0.63 ^{bcd}	0.817	8.33 ^{ab}	29.7 ^{ab}
	4.5	0.67 ^{cd}	0.983	10.50 ^{ab}	38.9 ^{abc}
	5.5	0.62 ^{bcd}	0.900	8.83 ^{ab}	34.7 ^{ab}
	6.5	0.63 ^{bcd}	0.933	10.67 ^{ab}	37.9 ^{abc}
	Control	0.57 ^{abcd}	0.917	7.83 ^{ab}	28.3 ^{ab}
L.S.D		0.21	NS	4.53	17.52
CV (%)		22.3	22.8	30.1	33.1

*100:0: Forest soil only, 50:50: 50% forest soil+50% EM compost, 75:25: 75% forest soil+25% EM compost

seeds. This suggests that EM-solution was not a better option than pure water in pre-sowing treatment of coffee seeds to enhance emergency once EM compost is incorporated in a media. It was reported by Gebreselassie *et al.* (2010) that coffee seeds soaked in pure water for 72 h were better in emergency and subsequent growth than non-treated and less time treated seeds.

As far as performance in agronomic traits of seedlings before transplanting to the actual field is concerned, the better performance in the measured parameters by seedlings rose on forest soil and seeds treated for 4.5 h by EM solution were better and consistent in plant height, number of primary branches and total dry matter of the seedlings. As far as the effect of forest soil is concerned, it might be because the forest soil had ideal chemical fertility for the growth of seedlings with high organic matter and major plant nutrients. As it was mentioned in materials and methods, the soil was harvested from under trees. In a different study, it was suggested by Kufa (2011) that might be due to the high litter fall mainly from indigenous upper canopy trees.

CONCLUSION

From this study, it can be concluded that soaking coffee seeds in EM solution may not be preferred than soaking in pure water as far as hastening of emergency is concerned. However, better and consistent performance in agronomic parameters (seedling height, number of primary branch and total dry matter content) were evidenced in EM-treated seeds after emergence. Therefore, the use of forest soil as a pot media and soaking coffee seeds in EM solution for 4.5 h can result in a relatively vigor coffee seedlings for transplanting.

ACKNOWLEDGMENTS

We are thankful to Jimma University College of Agriculture and Veterinary Medicine for making money available for the study. We also thank Mr. Daniel Damtew for his help in collecting data and field supervision.

REFERENCES

- Adjet-Twum, D. and L. Solomon, 1982. Some Research Methods in Agronomy and Crop Physiology. Institute of Agricultural Research, Addis Ababa, Ethiopia.
- Amaral, J.A.T., A.B. Rena and J.F.T. Amaral, 2006. Crescimento vegetativo sazonal do cafeeiro e sua relacao com fotoperiodo, frutificacao, resistencia estomatica e fotossintese [Seasonal vegetative growth of the coffee plant and its relationship with the photoperiod, fructification, stomatic resistance and photosynthesis]. Pesq. Agropec. Bras. Brasilia, 41: 377-384.
- Amina, J.G., D. Belew and A. Mohammed, 2012. Yield and quality of indeterminate tomato (*Lycopersicon esculentum* Mill.) varieties with staking methods in jimma. Singapore J. Sci. Res., 2: 33-46.
- Daba, M., D. Belew, W. Gebreselassie and W. Sori, 2012. Growth and yield performance of anchote [*Coccinia abyssinica* (Lam.) Cogn.] in response to contrasting environment. Asian J. Plant Sci., 11: 172-181.
- Daly, M.J. and D.P.C. Stewart, 1999. Influence of Effective Microorganisms (EM) on vegetable production and carbon mineralization-A preliminary investigation. J. Sustainable Agric., 14: 2-3.
- Gebreselassie, W., A. Mohammed and A. Netsere, 2010. Pre-sowing treatment of coffee (*Coffea arabica* L.) seeds to enhance emergence and subsequent growth of seedlings. Res. J. Seed Sci., 3: 218-226.
- GenStat, 2007. GenStat. 12th Edn., VSN International Ltd., Hemel Hempstead, UK.
- Gopal, N.H. and P.K. Ramaiah, 1971. Studies on hastening of germination of *Arabica* coffee seeds and further growth of seedlings. Ind. Coffee, 35: 459-462.
- Gopal, N.H. and P.K. Ramaiah, 1972. Studies on the physiology of germination of coffee seed. I. Observations on sprouting. J. Coffee Res., 2: 14-19.
- Harakawa, T. and T. Higa, 1989. Effective microorganisms in farming: Weeding effect of EM 4 in paddy fields. Proceedings of the 1st International Conference on Nature Farming, October, 17-21, 1989, Thailand, USDA/USAID.
- Huxley, P.A., 1967. The effects of artificial shading on some growth characteristics of arabica and robusta coffee seedlings. I. The effects of shading on dry weight, leaf area and derived growth data. J. Applied Ecol., 4: 291-308.
- Institute of Agricultural Research, 1996. Recommended Production Technologies for Coffee and Associated Crops. Jimma Agricultural Research Center, Jimma, Ethiopia pp: 17.
- Khan, B.M., M.K. Hossain and M.A.U. Mridha, 2006. Growth performance of *Cassia fistula* L. Seedlings as Affected by formulated microbial inoculants. Bangladesh J. Bot., 35: 181-184.
- Kufa, T., 2011. Chemical properties of wild coffee forest soils in Ethiopia and management implications. Agric. Sci., 2: 443-450.
- Maestri, M. and C. Vieira, 1961. A note on the reduction of the percentage of coffee seed (*Coffea arabica* L. var. bourbon) by the action of gibberellic acid. Rev. Ceres, 11: 247-249.
- Mutumbulwa, A., 2006. Effect of EM on germination of various tree species at Okahandja. In-service Training Project, Polytechnic of Namibia, Windhoek, Namibia.
- Riley, J.M., 1981. Growing rare fruit from seed. CRFG Yearbook, 13: 1-47.
- Rosenberg, A. and T. Linders, 2004. Organic Agriculture: A Handbook. Lindros, South Africa, ISBN-13: 9780620309523, Pages: 223.
- Sangakkara, U.R. and A.M.U. Attanayake, 1993. Effect of EM on germination and seedling growth of rice. Proceedings of the 3rd International Conference on Nature Farming, October, 5-7, 1993, Santa Barbara, Ca., pp: 223-227.

- Siqueira, M.F.B., C.P. Sudre, L.H. Almeida, A.P.R. Pegorer and F. Akiba, 1993. Influence of effective microorganisms on seed germination and plantlet vigor of selected crops. Proceedings of 3rd International Conference on Kyusei Nature Farming, October 5-7, 1993, Santa Barbara, Ca., pp: 244-245.
- Tokeshi, H. and P.R.R. Chagas, 2010. Hormonal effect of EM on citrus germination. EM Research Organization, 16 May, 2010. <http://emrojapan.com/emdb/content/106.html>
- Valio, I.F.M., 1976. Germination of coffee seeds (*Coffea arabica* L. cv. mundonovo). J. Exp. Botany, 27: 983-991.
- Valio, I.F.M., 1980. Inhibition of germination of coffee seeds (*Coffea arabica* L. cv. Mundo Novo) by the endocarp. J. Seed Technol., 5: 32-39.
- Van der Vossen, H.A.M., 1979. Methods of preserving the viability of coffee seed in storage. Seed Sci. Technol., 7: 65-74.
- Vieira, L.G.E., A.C. Andrade, C.A. Colombo, A.H.A. Moraes and A. Metha *et al.*, 2006. Brazilian coffee genome project: An EST-based genomic resource. Br. J. Plant Physiol., 18: 95-108.
- Wellman, F.L., 1961. Coffee, Botany, Cultivation and Utilization. Leonard Hill Books, London.
- Went, F.W., 1957. The Experimental Control of Plant Growth. Chronica Botanica Co., Waltham, Mass.
- Wrigley, G., 1988. Coffee. Tropical Agriculture Series. Longman Scientific and Technology, New York, ISBN-13: 978-0470208656, pp:639.
- Yakob, E., K. Taye and Y. Alemseged, 1998. Varietal and age impact on Arabica coffee leaf growth parameters at three locations. Proceedings of the 3rd Conference of Agronomy and Crop Physiology Society of Ethiopia, May 29-30, Addis Ababa, Ethiopia, pp: 38-51.
- Zimmermann, I. and R.T. Kamukuenjandje, 2008. Overview of a variety of trials on agricultural applications of Effective Microorganisms (EM). Agricola, 8: 1-24.