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Alternative methods of biological control in maintaining the viability of stored coffee seeds

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The objective of this work was to evaluate the efficiency of different alternative methods of biological control in maintaining the viability of coffee seeds. For the control of fungus contamination in seeds, dehydrated and powdered medicinal plants in doses of 200 g kg^{-1} of seed, were tested with chemical fungicides mancozebe (Dithane® NT 4 g kg-1 of seed), potassium sorbate (300 g L^{-1}) and sodium benzoate (300 g L^{-1}) and three biological products, Trichodermil® SP (1 g kg^{-1} of seed), Trichodel® (50 g kg^{-1} of seed) and Trichoplus® (50 g kg^{-1} of seed). Samples of 200 g of seeds were conditioned in three different packages: polypropylene flasks, kraft paper bags and polyethylene + nylon bags. After, they were stored in cold chamber at 16 ± 3°C of temperature, for a period up to 15 months with evaluations for each three months. Seeds conditioned in kraft paper bags presented percentage of germination higher than those conditioned in polypropylene flasks or polyethylene + nylon bags. In seeds treated with rosmarin, garlic and Trichoplus®, there was a reduction in the total microbial, and the best germinated on 12 and 15 months of storage.

Key words: *Coffea arabica,* package, quality, storage.

INTRODUCTION

Obtaining coffee seedlings with high quality standard and on time for cultivation is hampered by the non-conservation of the germinative power of seeds by periods higher than six months after harvest (Sguarezi et al., 2001). During the storage, the viability of seeds is influenced by factors like species, cultivar, physiological quality, moisture content, relative air humidity, temperature, storage period, packaging type and the action of fungus and insects (Carvalho and Nakagawa, 2000).

Given the high incidence of microorganisms in important crops, the search for new antimicrobial agents extracted from plants, has been an alternative for the control of these pathogenic microorganisms, which is an alternative to the synthetic products. Beyond this, before now, there is no fungicide registered in the Agriculture

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Ministry Pecuary and Supplying (MAPA) to control microorganisms in coffee seeds during storage (MAPA, 2009).

Alternative methods that control proliferation of microorganisms in coffee seeds, without causing significant damages to the environment, have been intensely studied and the use of medicinal plants like alternative to the use of synthetic products is very promising (Souza et al., 2007).

Approaches developed with crude extracts and essentials oils of medicinal plants have demonstrated the potential of natural products on phytopathogens (Moreira et al., 2004). The aim of this work was to evaluate the effect of medicinal plants, biological products and chemical alternative compounds on *Coffea arabica* seeds stored within different packages, up to15 months in cold chamber.

MATERIALS AND METHODS

The assays were developed in the following laboratories: Laboratory of Seeds for Research, Crop Science Department, Laboratory of Seed Pathology and Post-harvest of the Phytopathology Department, both at Federal University of Viçosa and Laboratory of Microbiological Analyzes of Food and Water of EPAMIG in Viçosa- MG, Brazil. The coffee seeds (*C. arabica* L.), cultivar Catuaí Vermelho IAC 44 were obtained from the experimental Farm Vale do Piranga, Oratórios- MG Brazil, in 2012 and 2013. The fruits were manually selected in the cherry stage. Then, the fruits were peeled and desmucilled by natural fermentation per 12 h. After that, fruits were dried until the moisture content of 42% was achieved.

For the control of fungus contamination of seeds, the following dehydrated and powered medicinal plants were tested: rosemary (*Rosmarinus officinalis* L.), basil (*Ocimum americanum* L.), garlic (*Allium sativum* L.), cinnamon (*Cinnamomum zeylanicum* L.), horsetail (*Equisetum arvense* L.), clove (*Caryophyllus* L. *aromaticus*), fennel (*Pimpinella anisum* L.), ginger (*Zingiber officinalis* W.) and basil (*Ocimum basillium* L.). The material was acquired from Flowers and herbal Pharmacist Ltda, Piracicaba-SP, and applied in doses of 200 g kg^{-1} of seed. The chemical fungicide Mancozebe (Dithane® NT, Dow AgroSciences Industrial Ltda, São Paulo/SP - Brasil) 4 g kg^{-1} of seed, potassium sorbate (300 g L^{-1}) and sodium benzoate (300 g L^{-1}) were also tested, and the seeds were immersed for one minute in each solution. At the end, three biological products were tested, Trichodermil® SP (*Trichoderma harzianum*, ESALQ 1306, Koppert Brazil, Piracicaba/SP – Brazil) 1 g kg^{-1} of seeds, Trichodel® (*Trichoderma* spp., ECCB Caxias do Sul/RS - Brazil) 50 g kg^{-1} of seeds and Trichoplus® (*Trichoderma* spp. and *T. harzianum* JCO Indústria e Comércio de Fertilizantes LTDA, Barreira/BA - Brazil) 50 g kg^{-1} of seeds.

Samples of 200 g of seeds were stored in three different packages: polypropylene flasks, kraft paper bags and polyethylene nylon bags. After, they were aconditioned in cold chamber at 16 ± 3°C and moisture content of 60 ± 3%, for a period up to 15 months with the following analyses in each three months.

Germination test

Germination test was composed of four replications of 50 seeds without endocarps (parchments), totaling 200 seeds per treatment. It was assayed in germitest paper moistened with 2.5 times the weight of the paper at 30°C in germinator type B.O.D, with evaluations in each 30 days until the end of the experiment. The final count of germination test was realized in the 30th day after the initiation of test, according to the recommendation of Rules for Seed Analysis (Brasil, 2009).

Determination of moisture content

The method used involved oven drying at 105 ± 3°C for 24 h (Brasil, 2009). The samples were weighed with analytical scale of 0.001g of precision, with four replications of 50 g of seeds each.

Evaluation of the length of primary root

Seven days after the initiation of germination test, the seeds were directed with the embryo down. On the 30th day, the measurements were taken using a graduated ruler, with distance between the final part of primary root to the collar region. The average length (cm) of roots was obtained by the division of summation of the measurements recorded by the number of roots.

Count of filamentous fungus and yeasts

According to the methodology of MAPA (Brasil, 2003), the authors used a minimum of two decimal dilutions and one duplicate for each dilution. The incubation of plates was done at 25 ± 1°C, for five to seven days at B.O.D.

Evaluation of the efficiency of products

The measurable response (dependent variable) was the count of colony forming units by amount of seeds (CFU g^{-1} of seeds), expressed in terms of decimal reduction (g): $Y = \log N_0/N$, where: Y= number of decimal reductions achieved by treating, N_0= initial number (CFU g^{-1} of seeds) and N = number of survivors (CFU g^{-1} of seeds). The results were expressed in colony forming units (CFU g^{-1} of seeds).

Identification of filamentous fungus

The morphology of vegetative and reproductive structures was observed on stereomicroscope and on light microscope. With the aid of dichotomous keys was realized the fungus identification at gender level and in some cases, after the determination of the gender, the collected material was compared with the descriptions of fungus published for the determination of species.

The experimental design used was completely randomized with four replications. The treatments were established in factorial scheme 16 × 3 × b5 (types of fungicides × types of packages × periods of evaluation). The average test used for the treatments was Dunnet unilateral at 5%, and interest is not in the differences between the treatments, but when there are treatments which are superior to the control and the maconzebe (reference treatment). To evaluate the effect of packages and the times, Tukey test at 5% was used. And for the microbiological analyses, the descriptive statistical was used.

RESULTS AND DISCUSSION

During the storage significant reduction was observed in the moisture content of the seeds conditioned in kraft paper, with moisture content of 14.3 ± 1.9%, while seeds

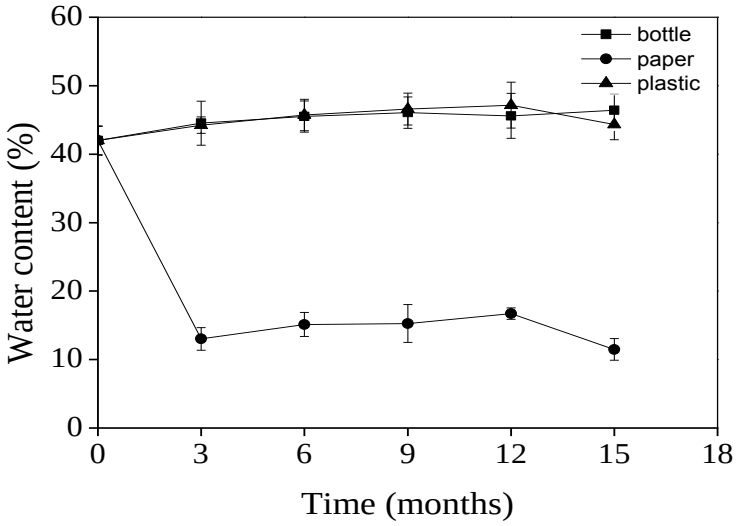

Figure 1. Moisture content of coffee seeds aconditioned in different packages and stored in cold chamber for 15 months.

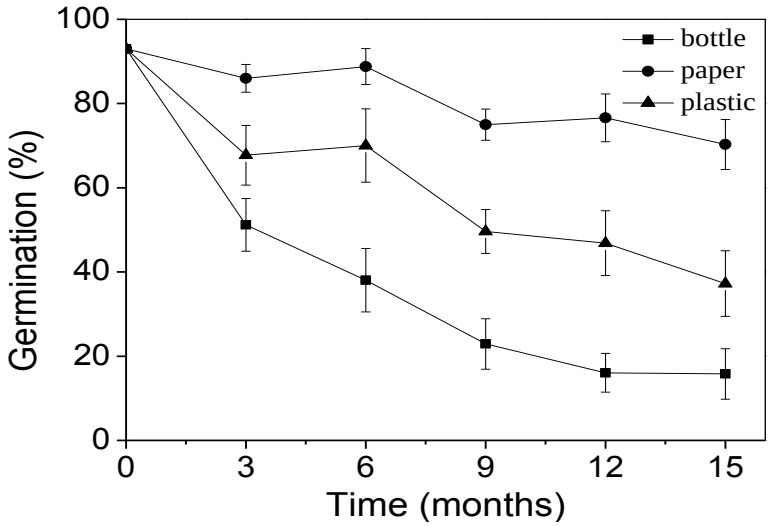

Figure 2. Germination of coffee seeds conditioned in different packages during 15 months (±standard error, n =16).

conditioned in polypropylene flasks or in polyethylene nylon bags presented low reduction in the water content during the storage (45.6 ± 4.1% and 45.6 ± 6.1%, respectively) (Figure 1). The variation in the water content of seeds was more intense in the package of paper making this kind of package to offers little resistance to the water vapor exchanges between the seeds and the environment. Similar results were found by Silva et al. (2010) and Alves and Lin (2003) with cotton seed.

Independently of the treatment, the paper package presented higher efficiency in the viability conservation of seeds, with germination above 70% at 15 months of storage to fungus control, possibly by the lower moisture content which does not favor the proliferation of microorganisms capable to impair the germination. The high water content of stored seeds in others packages (flasks and plastic), may have negatively affected the germination of seeds to keep the metabolism of seeds more active, providing better conditions for the degradation of reserves and the microorganisms proliferation.

The type of package also afected the seeds from sixteen different treatments in relation to the viability conservation ($p < 0.05$). Seeds conditioned in kraft paper presented percentage of germination significantly higher than those conditioned in flasks or in polyethylene nylon bags (Figure 2). The higher germination in seeds stored in this package may be related to the gas exchange

Table 1. Germination percentage (%) of coffee seeds (*Coffea arabica*) treated with biological products, chemical products and medicinal plants aconditioned in polypropylene flasks, kraft paper bags and polyethylene+nylon bags.

Treatment	Polypropylene flasks					Kraft paper					Polyethylene + nylon bags				
	Months of storage					Months of storage					Months of storage				
	3	6	9	12	15	3	6	9	12	15	3	6	9	12	15
Control	85	81	46	--	--	84	84	57	51	31	81	80	54	51	--
Rosemary	92	97^{C}	89^{CM}	91^{CM}	86^{CM}	86	92	90^{C}	89^{C}	91^{CM}	94^{C}	95^{C}	93^{C}	91^{C}	87^{C}
Hoary Basil (*Ocimum americanum* L.)	18	--	--	--	--	88	91	77^{C}	76^{C}	80^{C}	83	81	59	83^{C}	23^{C}
Garlic	94	90	87^{CM}	78^{CM}	79^{CM}	96^{C}	91	70^{C}	87^{C}	81^{CM}	90	88	78^{C}	93^{C}	86^{C}
Benzoate	--	--	--	--	--	87	85	59	75^{C}	64^{C}	35	--	--	--	--
Cinnamon	75	--	--	--	--	67	84	68	73^{C}	72^{C}	81	93^{C}	90^{C}	93^{C}	83^{C}
Horsetail	30	--	--	--	--	84	92	83^{C}	74^{C}	63^{C}	75	62	--	--	--
Clove	48	--	--	--	--	85	91	69^{C}	73^{C}	69^{C}	37	43	16	--	--
Fennel	--	--	--	--	--	80	88	69^{C}	68^{C}	70^{C}	73	43	--	--	--
Ginger	--	--	--	--	--	77	89	76^{C}	78^{C}	72^{C}	80	81	--	--	--
Basil *(Ocimum basillium*)	19	6	--	--	--	84	81	68	86^{C}	77^{C}	13	24	22	--	--
Sorbate	--	--	--	--	--	93	91	78^{C}	54	--	--	--	--	--	--
Trichodel®	93	65	--	--	--	94	95^{C}	94^{C}	83^{C}	85^{CM}	88	94^{C}	76^{C}	90^{C}	89^{C}
Trichodermil®	89	79	--	--	--	94	86	73^{C}	86^{C}	76^{C}	94^{C}	86	80^{C}	83^{C}	93^{CM}
Trichoplus®	88	94^{C}	93^{CM}	88^{CM}	88^{CM}	90	96^{C}	87^{C}	91^{C}	92^{CM}	92^{C}	94^{C}	89^{C}	85^{C}	56^{C}
Mancozebe	91	92^{C}	52	--	--	--	88	86^{C}	84^{C}	72^{C}	92^{C}	95^{C}	88^{C}	84^{C}	81^{C}

*C = significant (P < 0.05) in relation to the control and M = significant (P < 0.05) in relation to the Mancozebe by the unilateral test Dunnet; -- no germination.

capacity allowing the moisture equilibrium of the intern and the extern environment, favoring the reduction of seeds moisture (Figure 1). Like mentioned before, the reduction of moisture content could de disadvantage of the pathogenic microorganisms proliferation on seeds, allowing their higher germination as well as contributing to the cellular metabolism reduction of these seeds, what directly affecting the content of reserves used in the initial germination.

In relation to the microbiological control, high initial contamination of filamentous fungus and yeasts in coffee seeds (7.8×10^5 CFU g^{-1} seeds) was observed. The filamentous fungus predominant in coffee seeds were: *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp. and *Fusarium* sp. Seeds conditioned in flasks and treated with garlic, rosemary and Trichoplus® provided germination above 70% during all the period of evaluation with significant reduction in the total microbial, and the highest germinated at 12 and 15 months of stored (Tables 1 and 2). At nine months of storage, they were significantly higher than those treated with Mancozeb and the control, remaining like this until the end of the experiment (Table 1).

In paper kraft package, the higher reduction of fungal population was observed in seeds treated with garlic and rosemary. In the other treatments, including the control, there was increase of fungal population and reduction in the germinative power of seeds (Table 3). In plastic bags packages, better results in the reduction of microbiological population was also observed in treatments with garlic and rosemary. In other treatments, including the control, there was increase in the fungal population during the storage (Table 4).

The use of vegetative extracts, mainly garlic and rosemary, were efficient in the control of

Table 2. Decimal reductions (Y) of coffee seeds (*Coffea arabica*) treated with biological products, chemical products, and medicinal plants aconditioned in polypropylene flasks.

Treatment	Months of storage				
	3	6	9	12	15
	Y	Y	Y	Y	Y
Control	-0.81	-1.56	-1.56	-2.04	--
Rosemary	0.46	0.48	0.78	1.02	2.59
Hoary Basil (*Ocimum americanum* L.)	-0.89	-2.15	--	--	--
Garlic	0.39	0.57	1.41	1.46	1.44
Benzoate	1.85	--*	--	--	--
Cinnamon	1.32	-1.31	--	--	--
Horsetail	-1.11	0.11	--	--	--
Clove	2.89	3.85	--	--	--
Fennel	-2.06	-2.63	--	--	--
Ginger	-1.39	-2.43	--	--	--
Basil (*Ocimum basillium*)	-2.11	-1.89	-1.91	--	--
Sorbate	2.89	--	--	--	--
Trichodel®	3.90	-0.77	-3.22	--	--
Trichodermil®	2.89	4.94	4.94	--	--
Trichoplus®®	0.46	0.36	0.85	0.24	0.75
Mancozebe	1.34	2.41	2.61	2.11	--

*--Germination equal to zero in period of anterior evaluation, however without microbiological evaluation.

Table 3. Decimal reductions (Y) of coffee seeds (*Coffea arabica*) treated with biological products, chemical products and medicinal plants dehydrated, powdered and aconditioned in kraft paper bags.

Treatment	Months of storage				
	3	6	9	12	15
	Y	Y	Y	Y	Y
Control	0.48	0.18	0.06	-0.01	0.10
Rosemary	0.34	0.44	0.99	0.59	1.30
Hoary Basil (*Ocimum americanum* L.)	-0.97	0.59	-0.45	0.20	-0.01
Garlic	0.55	0.37	1.57	1.53	1.72
Benzoate	1.31	2.16	2.35	2.41	1.51
Cinnamon	2.99	1.69	-0.36	1.29	2.49
Horsetail	0.41	-0.78	0.05	-0.19	-0.39
Clove	2.89	4.94	2.92	4.59	4.94
Fennel	-1.15	-0.52	-1.22	-1.69	-1.87
Ginger	-0.22	-0.36	-0.22	-0.52	0.55
Basil (*Ocimum basillium*)	-1.15	-0.54	-0.22	-0.49	0.05
Sorbate	2.89	3.90	3.59	3.43	--
Trichodel®	3.90	0.19	-2.71	-0.11	--
Trichodermil®	2.89	4.94	4.94	4.94	4.94
Trichoplus®®	0.29	0.14	0.55	0.10	0.14
Mancozebe	1.17	0.95	2.14	2.02	1.49

-- Germination equal to zero, so there was no possible measure for the decimal reduction.

Table 4. Decimal reductions (Y) of coffee seeds (Coffea arabica) treated with biological products, chemical products, and medicinal plants dehydrated and powdered aconditioned in polyethylene+ nylon bags.

Treatment	Months of storage				
	3	6	9	12	15
	Y	Y	Y	Y	Y
Control	-0.56	0.48	0.81	1.31	-0.90
Rosemary	0.55	0.49	1.19	0.85	2.69
Hoary Basil (*Ocimum americanum* L.)	1.81	-1.22	-1.01	0.29	-0.41
Garlic	0.51	0.20	1.41	1.49	1.18
Benzoate	1.99	0.53	0.69	1.49	--
Cinnamon	0.11	0.29	0.61	0.46	1.14
Horsetail	-1.15	1.11	-1.90	-2.28	--
Clove	2.89	2.51	3.16	2.36	--
Fennel	-1.41	-2.01	-1.47	--	--
Ginger	-1.25	-1.57	-1.96	-3.83	--
Basil*(Ocimum basillium)*	-1.11	-1.28	-0.47	0.99	--
Sorbate	2.89	3.90	4.94	--	--
Trichodel®	3.90	-0.19	-2.65	-1.96	--
Trichodermil®	2.89	4.94	4.94	4.94	4.94
Trichoplus®®	0.75	0.59	0.89	0.55	0.85
Mancozebe	2.26	1.95	2.85	2.09	1.12

*-- Germination equal to zero, so there was no possible measure for the decimal reduction.

microorganisms by having substances like terpenoids, essentials oils, alkaloids (Barrera-Necha et al., 2008), which can act in the microorganisms control. Silva et al., (2012) verified that the aqueous extract of garlic promoted a relative inhibition of the development *in vitro* phytopathogenic mycelium. In the present study, garlic presented excellent fungus control, which can be related to their chemical composition.

Several studies show that allicin, S-allyl cysteine, Allyl cysteine Mercapto, diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide are volatile compounds presented in garlic and which presents antioxidant activity (Cecilia and Olubunmi, 2014; Dias et al., 2011; Queiroz et al., 2006). These antioxidant compounds may have influenced the maintenance of seeds quality, not just controlling the proliferation of microorganisms, but also contributing to the preservation of germination of seeds by acting on reactive oxygen species which generate oxidation chain reactions and affecting the reserve compounds, membranes, genetic material between others (Cecilia and Olubunmi, 2014; Perelló et al., 2013; Baraka et al., 2011; Santos et al., 2010)

In relation to rosemary product, the α-pineno, 1,8-cineol and the camphor, major constituents of the essential oil (Ribeiro et al., 2012), are the compounds that may have assisted in maintaining the viability of the seeds. According to the same authors, these compounds present inhibitory effects on fungus and bacteria growth, which can explain the efficiency of these treatments in the maintenance of viability of coffee seeds in the present study. Brand et al. (2010) verified that higher reduction occurs in growth of fungus and increase in the viability of coffee seeds, using autoclaved aqueous extract of rosemary in doses of 2.5%. Similar results were observed by Souza et al. (2010) using rosemary extract in different concentrations in seeds of *Inga edulis*. This fungicide effect of extract, has effect on many microorganisms (bacteria and fungus) which can affect several forms of the physiological quality and, in some cases, completely inhibit the germination of seeds (Lopes et al., 2011; Neto et al., 2012.).

Leite et al. (2012) showed that the use of vegetal extract did not affect the seeds metabolism of *Mimosa caesalpiniaefolia* Benth and even favored the control of pathogens, increasing the viability of seeds. The same behavior related to the speed of germination was also reported by Xavier et al. (2012), in seeds of cowpea-beans (*Vigna unguiculata* L.). Between the biological products, Trichodermil® and Trichoplus® were more effective in the reduction of fungus population in all the packages. This efficiency can be attributed to several mechanisms of action used by fungus, like the production of metabolites and enzymes with antifungal properties, the hyperparasitic and the competition for nutrients (Harman et al., 2004), and avirulent symbionts associated with plants (Carvalho et al., 2011). It is also suggested that they have effect like growth vegetal stimulators and also the production of auxin analogs

(Harman et al., 2004; Vinale et al., 2008).

Conclusion

The paper packaging is best suited for the preservation of coffee seeds, independent of the microorganism control treatment used. Garlic, rosemary and organic products are effective in controlling microorganisms and maintenance of coffee seed viability stored for 15 months with higher germination required for minimum of 70%.

Conflict of interest

The author has not declared any conflict of interest.

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