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International Journal of Current Research
Vol. 7, Issue, 11, pp.23091-23098, November, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

COFFEE SEEDS CONSERVATION IN NATURAL ENVIRONMENT WITH ALTERNATIVE FUNGI CONTROL

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ARTICLE INFO

Article History:
Received 14th August, 2015
Received in revised form
25th September, 2015
Accepted 17th October, 2015
Published online 30th November, 2015

Key words:

Coffeaarábica,
Viability,
Storage,
Quality.

ABSTRACT

Coffe seeds are classified like intermediate, presenting difficulties in production of viable and vigor seeds for a long period of time. The objective of this work was to evaluate the viability of coffee seeds using different microbial control agents storage in natural environment. The fruits were selected in cherry phase, peeled and desmucilated in natural fermentation for 12 hours, with initial water content of 42%. For the control of fungus contamination in seeds, were tested dehydrated and powdered medicinal plants in doses of 200 g kg-1 of seed, chemical fungicides mancozeb (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 200g of seeds were conditioned in three different packages: polypropylene flasks, kraft paper bags and polyethylene nylon bags for a period of 15 months with evaluations each three months. Seeds conditioned in kraft paper bags presented germination average, higher than those conditioned in polypropylene flasks or polyethylene+nylon bags, however the polyethylene nylon bags were most efficient in keep the longevity of seeds. In this study, medicinal plants that allowed better control of fungi population during the storage were, rosemary, garlic and clove in all packaging.

Citation: Marcelo de Freitas Ribeiro, Eduardo Fontes Araújo, Genaina Aparecida de Souza, Raquel Maria de Oliveira Pires, Roberto Fontes Araújo, Paola Andrea Hormaza Martinez and Marcelo Resende de Freitas Ribeiro, 2015. "Coffee seeds conservation in natural environment with alternative fungi control", *International Journal of Current Research*, 7, (11), 23091-23098.

INTRODUCTION

The low longevity of coffee seeds has been attributed to the sensibility to dissection. Physical, physiological and biochemical alterations that occurs during the storage, affects the quality of these seeds and consequently the production of seedlings with high pattern of quality in time for implantation of crop (Sguarezi *et al*., 2001). During the storage, the viability of seeds is influenced by many factors, like specie, cultivar, physiological quality and the water content of seeds, the moisture content, the temperature during the storage, the type of package, the period of storage and the action of fungis and insects (Carvalho and Nakagawa, 2000).

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Small producers do not have appropriate locals to the storage and keep seeds in environmental conditions. The lack of temperature and humidity control during the storage is the main responsible by the loss of seeds vigor. Between the causes of this loss, the insect attack and pathogenic microorganisms. In this moment is common the practice of the producers to use the chemical products for the control, affecting both the human health and the environment. Thus, alternative methods which control the proliferation of microorganisms in coffee seeds, without causing significant damages to the environment and that keeps the viability of seeds, are becoming a challenge. The use of vegetal extracts are efficient in the control of microorganisms by having terpenoids, essential oils and alkaloids (Barrera-Nechа*et al*., 2008), lecithin, polypeptides and phenolic and polyphenols substances, like; simple phenolic, phenolic acids and quinone (Stern *et al*., 1996), beyond flavones and flavonols.

In this way, the objective of this work was to evaluate the effect of ground medicinal plants, biological products and alternative chemical compounds in Arabica coffee seeds, stored in natural environment in three different packages.

MATERIALS AND METHODS

The work was developed in the Laboratory of Seeds Research in the department of Crop Science, in the Seeds Pathology and Postharvest Laboratory of Plant Pathology Department at Universidade Federal de Viçosa and in the Microbiological Analysis of food and water Laboratory, at the Empresa de PesquisaAgropecuária de Minas Gerais (EPAMIG), regional unity EPAMIG Zona da Mata, Viçosa-MG. Coffee seeds (*Coffeaarabica*L.), of cultivar Red catuai IAC44, were acquired in field of seeds production registered on MAPA, from experimental farm of Vale do Piranga, Oratórios-MG.
The fruits were manually selected in the cherry stage. At the same day were desmucilated and hulled by natural fermentation during 12 hours. The drying was realized under sun, how is commonly used by seeds producers, until achieve the water content of 42%.

For the fungus contamination control of seeds, were tested the following dehydrated and ground medicinal plants: rosemary (*Rosmarinus officinalis* L.), basil (*Ocimumamericanum* L.), garlic (*Allium sativum*L.), cinnamon (*Cinnamomumzeylanicum* L.), horsetail (*Equisetum arvense*L.), clove (*Caryophyllusaromaticus* L.), fennel (*Pimpinellaanisum* L.), ginger (*Zingiber officinalis* W.) andbasil(*Ocimumbasillium* L.). The material was acquired from the Flores e ErvasFarmacêutico CompanyLtda, Piracicaba-SP, being applied in dose of 200 g $kg^{-1}$of seed. The chemical fungicides mancozeb (Dithane® NT 4 g $kg^{-1}$of seed), potassium sorbate (300 g L^{-1}) e sodium benzoate (300 g L^{-1}) were also tested, being the seeds immersed for one minute in solution. . At the end, was tested three biological products, Trichodermil® SP (1 g kg^{-1} of seeds), Trichodel® (50 g kg^{-1} of seeds) and Trichoplus®® (50 g kg^{-1} of seeds).

Samples of 200g of seeds were conditioned in three different packages: polypropylene flasks, kraft paper bags and polyethylene nylon bags sealed with sealing. After, were stored in natural environment where the minimum relative humidity ranged between 30 and 60% and the maximum remained next to 100%. The minimum values of temperature reached 5 ° C and maximum above 30°C for a period of until 15 months with evaluations in each three months. The following tests realized were:

Germination test

The germination test was composed of four replications of 50 seeds without parchments, totalizing 200 seeds per treatment. Was realized in germitest paper moistened with 2,5 times the weight of the paper in temperature of 30 ºC 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 installation of teste, according the recommended by Rules for Seed Analysis (Brasil, 2009).

Determination of water content

Realized the standard test in stove at 105 ± 3°C during 24 hours (Brasil, 2009) with the aid of a digital balance of 0,001g of precision, with four replications of 50 grams of seeds each.

Evaluation of the length of primary root

The seven days after the installation of germination test, the seeds were directed with the embryo to down and in the 30°C day was realized the measurement, with the aid of graduated ruler, of the distance between the final part of primary root until 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 with the methodology of MAPA (Brasil, 2003). Were used at minimum two decimal dilutions and one duplicate for each dilution. The incubation of plates were done at 25 ± 1°C, by 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: was observed the morphology of vegetative and reproductive structures 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 16x3x5 (types of fungicides x types of packages x periods of evaluation). The average test used for the treatments was Dunnet unilateral at 5%, cause de interest is not on the differences between the treatments, but if there are treatments which are superior to the control and to the maconzebe (reference treatment). To evaluate de effect of packages and the times, was used Tukey test 5%. And for the microbiological analyses was used the descriptive statistical.

RESULTS

Germination and vigor of seeds treated with different medicinal plants

During the evaluated period, there was an increase in the water content when seeds were stored in flasks and in plastic bags, this, related to the initial water content of 42%. On the other hand, was observed in seeds stored in kraft paper, reduction in the water content to values below than 20%.

The relative humidity and the temperature varied during the experimental period between 30% and 60% and the maximum remained next to 100%. The environmental temperature achieved minimum values of 5° C and maximum above 30 °C. Seeds conditioned in flasks, treated with rosemary, garlic, trichodel, trichodermil and mancozeb promoted percentage of germination higher than the control at three months of storage, not differing significantly to mancozeb (treatment – comparison) (Table 1). However, in general, the kraft paper package was more efficient in keeping the seeds germination during the six months of storage. An interesting result was obtained when seeds were stored in nylon package with polyethylene and treated with mancozeb, cause they kept viable for 15 months with germination higher than 70%.

At six months of storage, the percentage of germination of seeds treated with rosemary, garlic and trichoplus were higher than those of control and the treatment with mancozeb (Table 1). From nine months of storage, no seeds were observed, independently of the treatments and the packages used; kraft paper and polypropylene flasks. For seeds conditioned in nylon bags with polypropylene, only seeds treated with mancozeb presented germination above 80% during the period of storage and higher to the control, from sixth months.

After the verification of germination of seeds stored, the evaluation of root length was realized. In this way, was verified that practically the same treatments which presented results significantly higher than control in relation to germination kept the same behavior in relation to the root length, indicating higher vigor in relation to the other treatments and control (Table 2).

Table 1. Average of germination percentage (%) of coffee seeds treated with biological products, chemical compounds and dehydrated and ground medicinal plants conditioned in polypropylene flasks, kraft paper bags and polyethylene+nylon bags, stored in natural environment between 3 and 15 months

Treatments	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
Rosemary	86^{C}	93^{CM}	--	--	--	88	88^{C}	--	--	--	93	90^{C}	--	--	--
Basil	--	--	--	--	--	76	61	--	--	--	45	34	--	--	--
Garlic	92^{C}	86^{CM}	--	--	--	93	83^{C}	--	--	--	93	89^{C}	--	--	--
Benzoate	--	--	--	--	--	47	63	--	--	--	7	--	--	--	--
Cinnamon	--	--	--	--	--	86	71	--	--	--	88	73	--	--	--
Horsetail	--	--	--	--	--	70	70	--	--	--	39	9	--	--	--
Clove	--	--	--	--	--	53	23	--	--	--	43	--	--	--	--
Fennel	--	--	--	--	--	89	70	--	--	--	--	--	--	--	--
Ginger	--	--	--	--	--	72	62	--	--	--	--	--	--	--	--
Basil	--	--	--	--	--	80	62	--	--	--	--	--	--	--	--
Sorbate	--	--	--	--	--	63	77^{C}	--	--	--	--	--	--	--	--
Trichodel®	85^{C}	7	--	--	--	92	77^{C}	--	--	--	90	92^{C}	--	--	--
Trichodermil®	86^{C}	2	--	--	--	92	54	--	--	--	94	97^{C}	12^{C}	--	--
Trichoplus®	90^{C}	93^{CM}	--	--	--	91	79^{C}	--	--	--	88	79^{C}	--	--	--
Mancozeb	93^{C}	42^{C}	--	--	--	92	82^{C}	--	--	--	92	94^{C}	88^{C}	86^{C}	84^{C}
Control	71	11	--	--	--	83	64	--	--	--	91	64	--	--	--

*C = significant (P < 0,05) in relation to the control and M = significant (P < 0,05) in relation to the mancozeb and – the germination was zero

Table 2. Average of root length (cm) of coffee seedlings treated with biological products, chemical compounds and dehydrated and ground medicinal plants conditioned in polypropylene flasks, kraft paper bags and polyethylene+nylon bags, stored in natural environment between 3 and 15 months

Treatments	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
Rosemary	$2{,}85^{C}$	4,38	--	--	--	2,61	3,61	--	--	--	2,51	4,34	--	--	--
Basil	--	--	--	--	--	2,49	$5{,}15^{CM}$	--	--	--	1,58	3,9	--	--	--
Garlic	$3{,}70^{CM}$	4,3	--	--	--	$3{,}37^{CM}$	3,4	--	--	--	$3{,}75^{C}$	3,8	--	--	--
Benzoate	--	--	--	--	--	1,89	$4{,}73^{C}$	--	--	--	1,27	0	--	--	--
Cinnamon	--	--	--	--	--	2,79	$4{,}62^{C}$	--	--	--	$3{,}19^{C}$	$4{,}85^{C}$	--	--	--
Horsetail	--	--	--	--	--	2,18	$4{,}35^{C}$	--	--	--	$2{,}62^{C}$	3,72	--	--	--
Clove	--	--	--	--	--	2,06	$4{,}73^{C}$	--	--	--	$2{,}59^{C}$	--	--	--	--
Fennel	--	--	--	--	--	$3{,}14^{C}$	$4{,}19^{C}$	--	--	--	--	--	--	--	--
Ginger	--	--	--	--	--	2,15	$5{,}04^{C}$	--	--	--	--	--	--	--	--
Basil	--	--	--	--	--	2,33	3,37	--	--	--	--	--	--	--	--
Sorbate	--	--	--	--	--	1,65	2,98	--	--	--	--	--	--	--	--
Trichodel®	$3{,}31^{CM}$	1,96	--	--	--	2,51	3,71	--	--	--	$2{,}89^{C}$	4,2	--	--	--
Trichodermil®	$2{,}90^{C}$	2,3	--	--	--	2,94	$4{,}09^{C}$	--	--	--	$3{,}87^{CM}$	4	$1{,}50^{C}$	--	--
Trichoplus®	2,52	4,22	--	--	--	$3{,}38^{CM}$	$5{,}00^{C}$	--	--	--	$2{,}78^{C}$	4,45	--	--	--
Mancozebe	$2{,}75^{C}$	3,69	--	--	--	2,81	$4{,}39^{C}$	--	--	--	$3{,}41^{C}$	$4{,}70^{C}$	$3{,}91^{C}$	$2{,}85^{C}$	$2{,}88^{C}$
Control	2,24	3,71	--	--	--	2,67	3,34	--	--	--	2,18	3,79	--	--	--

*C = significant (P < 0,05) in relation to the control and M = significant (P < 0,05) in relation to the mancozeb and – the germination was zero, so was not possible to measure the root length.

The seedlings of seeds treated with garlic and trichodel presented higher length of roots than those originated from control and mancozeb at three months. Seeds treated with rosemary, trichodermil and mancozeb were higher only to the control in this period. At six months was not observed significant differences between the treatments evaluated.

For seeds conditioned in paper, was observed that the treatments with garlic and trichoplus were significant higher than the control and mancozeb, while the treatment with fennel was higher in comparison only to control at three months of storage. At sixth month of storage, the root length of basil treatment was the unique higher than the control and mancozeb. The most treatments, benzoate, cinnamon, horsetail, cloves, fennel, ginger, trichodermil, trichoplus and mancozeb were only superior to the control (Table 2).

When stored in packages of nylon with polyethylene and treated with mancozeb, these presented length of root superior than control during the period of evaluation. At three months, was observed that the trichodermil was the unique treatment where the roots length was superior than the seeds treated with mancozeb and control (Table 2).

In the same period, seeds treated with garlic, cinnamon, horsetail, cloves, trichodel and trichoplus were higher than the control. At six months, beyond the mancozeb, only the treatment with cinnamon presented roots length superior than the control. The same behavior was observed for trichodermil at ninth month.

Count of filamentous fungi and yeasts packed in flasks

Was verified high initial contamination of filamentous fungi and yeasts in coffee seeds of 7,8 x 10^5UFC/g. The filamentous fungi predominant in coffee seeds were in the sequence: *Penicillium*sp., *Aspergillus* sp., Trichoderma sp. e Fusarium sp. The higher reduction of fungi population was observed in treatments of seeds with rosemary, garlic, cloves, sorbate, trichodermil, trichoplus and mancozeb. These treatments, except the treatment with cloves, sorbate and trichodermil, preserved the germination power at six months of storage. In the other treatments, including the control, there was increase in the fungi population during the storage and a reduction of germination power of seeds (Table 3).

Table 3. Averages of decimal reductions (Y) of coffee seeds treated with biological products, chemical compounds and plants conditioned in paper flasks between 3 and 15 months of storage

Treatments	Monthsofstorage				
	3	6	9	12	15
	Y	Y	Y	Y	Y
Rosemary	0,69	0,51	0,81	--	--
Basil	-1,94	-2,11	-1,34	--	--
Garlic	1,29	1,66	0,69	--	--
Benzoate	1,99	1,89	-2,04	--	--
Cinnamon	1,40	-1,06	-0,95	--	--
Horsetail	-0,68	-2,02	-1,43	--	--
Clove	2,89	1,85	1,11	--	--
Fennel	-1,31	-2,65	-1,57	--	--
Ginger	-0,92	-2,22	-1,52	--	--
Basil	-2,28	-2,61	--	--	--
Sorbate	0,35	2,85	3,72	--	--
Trichodel®	3,90	-1,41	0,11	--	--
Trichodermil®	2,89	4,94	4,94	--	--
Trichoplus®	0,37	0,96	0,72	--	--
Mancozeb	1,66	0,69	2,21	--	--
Control	-1,01	-1,70	-2,43	--	--

-- the germination was zero, so was not possible to measure the decimal reduction.

Table 4. Averages of decimal reductions (Y) of coffee seeds treated with biological products, chemical compounds and plants conditioned in paper bags between 3 and 15 months of storage

Treatments	Monthsofstorage				
	3	6	9	12	15
	Y	Y	Y	Y	Y
Rosemary	0,66	0,85	0,57	--	--
Basil	-1,11	-0,01	0,48	--	--
Garlic	1,05	1,81	0,81	--	--
Benzoate	2,19	3,48	2,85	--	--
Cinnamon	0,85	-0,08	-0,54	--	--
Horsetail	0,27	-0,81	0,44	--	--
Clove	2,41	4,94	1,11	--	--
Fennel	-1,43	-0,47	--	--	--
Ginger	0,40	0,07	-1,47	--	--
Basil	-2,11	0,29	--	--	--
Sorbate	2,89	2,89	--	--	--
Trichodel®	3,90	1,05	1,11	--	--
Trichodermil®	2,89	4,94	4,94	--	--
Trichoplus®	0,78	0,39	0,37	--	--
Mancozeb	2,29	1,04	2,78	--	--
Control	0,43	-0,34	-0,15	--	--

-- the germination was zero, so was not possible to measure the decimal reduction

Table 5. Averages of decimal reductions (Y) of coffee seeds treated with biological products, chemical compounds and plants conditioned in naylon bags between 3 and 15 months of storage

Treatments	Monthsofstorage				
	3	6	9	12	15
	Y	Y	Y	Y	Y
Rosemary	0,59	0,10	0,81	--	--
Basil	-1,56	0,29	0,99	--	--
Garlic	0,94	1,85	0,75	--	--
Benzoate	2,05	0,81	0,75	--	--
Cinnamon	1,07	-0,28	--	--	--
Horsetail	-0,11	-1,57	-1,15	--	--
Clove	1,69	2,11	1,11	--	--
Fennel	-0,77	-1,52	--	--	--
Ginger	-1,57	-1,81	-2,19	--	--
Basil	-1,80	-1,94	--	--	--
Sorbate	2,89	2,14	--	--	--
Trichodel®	3,90	0,29	0,57	--	--
Trichodermil®	2,89	4,94	4,94	--	--
Trichoplus®	0,59	0,18	0,40	--	--
Mancozeb	2,44	1,53	2,05	0,97	0,69
Control	-0,60	1,11	0,72	--	--

-- the germination was zero, so was not emitted the decimal reduction.

In packages of nylon with polyethylene, seeds treated with garlic, rosemary and biological agents, also presented satisfactory results, above 70% of germination until the six months of storage. In control treatment, was observed the growth of higher diversity of fungi colonies, what can be associated to the absence of an inhibitory agent of microbial growth. In treatments of seeds with biological agents (trichodermil, trichoplus and trichodel) there was predominant growth of typical colonies of *Trichoderma*, which is the fungi presented in the composition of the evaluates products. The treatment with mancozeb reduced the fungal population and kept the germination power until the 15 months of storage.

Count of filamentous fungi and yeasts packed in kraft paper

In general, the treatments with rosemary, garlic and biological agents, presented good germinative development in this package until six months of storage, with germination above than70% and superior to the control (Table 4). Hightlightinghere, that the average of roots length in this package was better, with results significantly superiors when compared to the control (Table 2). The greater reductions on fungi populations were observed in treatments with rosemary, garlic, benzoate, clove, sorbate, trichodel, trichodermil, trichoplus and mancozeb. However, the treatments with benzoate, clove and trichodermil did not present the germinative powder of seeds at six months of storage. In the other treatments, including the control, there was increase in the fungal population and a reduction in the germinative powder of seeds (Table 1).

Count of filamentous fungi and yeasts packed in nylon bags

In these conditions the better results in controlling the fungi population were observed in treatments of seeds with rosemary, garlic, benzoate, clove, sorbate, trichodel, trichodermiltrichoplus and the mancozeb. These treatments, except the treatment with benzoate, clove and sorbate, preserved the germinative powder of seeds. In the others treatments, including the control, there was increase in the fungi population during the storage and reduction in the germinative powder of seeds (Table 5). The treatment with mancozeb reduced the fungi population and kept the germinative powder until the 15 months of storage.

DISCUSSION

According Silva *et al*. (2010), the behavior of seeds in relation to the water content during the storage varied independently of the specie. The minimum humidity relative of environment varied between 30 and 60% and the maximum remained next to 100%. The temperature achieved minimum valued of 5 ºC and maximum superiors to 30 ºC. Were observed higher longevity of seeds stored in nylon packages with polyethylene when compared to packages of flasks and paper (Table 1). The reduction of moisture content of seeds packaged in paper and the better maintenance of viability in plastic bags was also related by Andreoli *et al*. (1993), which observed that polyethylene packages provided better results than the sackcloth, conserving seeds with 83-90 % of germination and 84-86 % of vigor until the seventh month of storage.

These same authors report that, independently of culture, specie and the type of packing, the seeds germination decreases during the time of storage. This behavior has lower effect in seeds that was not stored in impermeable packages (Silva *et al*., 2010). Similar results were obtained by Monteiro and Silveira (1982) with bean seeds; by Braccini *et al*. (1999), with coffee seeds; by Padilha *et al*. (2001), with soybean seeds, and Carvalho *et al*. (2002), whose worked with seeds of wild lemon.

The reduction in germination of seeds is natural and varies in function of factors that affects their conservation like temperature, humidity relative of air, the moisture content of seeds and the type of package used (Guedes *et al*., 2010). In general, for all species is possible to verify the effect of different types of packages on germination of seeds after a long period of storage. In these cases, seeds stored in impermeable and semipermeable packages suffer lower losses of percentage of germination and vigor (Table 1 and 2). The development of root system is of huge importance to promote a good seedling in the germination test, beyond this, we highlight the importance of quantity of roots that have function of absorption of water and nutrients (Rena *et al*., 2000), facilitating their establishment on field.

The reduction in values of germination and vigor occurs due to the maintenance of metabolism which continue active during the storage. In this way, alterations in the permeability of membranes, degradation of the reserve compounds like lipids, of degradation of proteins, aminoacid leaching, alterations in DNA and enzymatic alterations during the time, affect the seeds quality and vigor. Other authors did not observe significate influence of the type of package on germination like Amaral and Baudet (1983), in seeds of soybean; Crochermore and Piza (1994), working with storage of oil radish seeds and Condé and Garcia (1995), for seeds of andropogon grass. The reduction that occurred on germination, even is seeds stored in package of flasks and nylon with polyethylene, is due mainly to the fact of these seeds been stored with high level of humidity for the period of storage of 15 months, cause according what it is known, for a period of time equal or superior to eight months, the water content must be reduce to values inferior than 10% of humidity base. Thus, the cellular metabolism will be reduced and lower damages will be caused in seeds in order to affect the vigor and the viability.

In treatment control, was observed the growth of higher diversity of colony of fungi, what can be associated to the absence of an inhibitor agent of the microbial growth (Tables 3, 4 and 5). In treatments of seeds with biological products (trichodel, trichodermil and trichoplus), we observed predominant growth of typical colonies of *Trichoderma*, fungi present in the composition of the evaluated products. Mata *et al*. (2008), using extracts aqueous and organics prepared from isolates of *Trichoderma* spp on the fungi*Fusarium* sp and *Sclerotium*sp., observed that all the extracts were efficient in the reduction of the initial growth.

The use of vegetal extracts is efficient in the microbial control cause having terpenoids, essential oils and alcaloids (Barrea-Necha *et al*., 2008), lectins, polypeptides and phenolic compounds and polyphenols, which are simple phenols, phenolic acids and quinones (Stern *et al*., 1996), and flavones, flavonols and flavanols. In this present study, the medicinal plants which allow the better control of fungal population during the storage period were: rosemary, garlic and clove in all packages (Tables 3, 4 and 5). The efficiency of garlic in the maintenance of viability of seeds can be related not only to pathogenic microorganisms, but also with their antioxidant properties (Queiroz *et al*., 2006), which were attributed to the organosulfur compounds and theirs precursors, just like the phenolic substances. The allicin, s-allyl cysteine, diallyl sulfide (DAS), diallyl disulfide (DADS) and the diallyltrisulfito are volatile compounds present in garlic with antioxidant activity (Queiroz *et al*., 2006). These compounds could influenced in the maintenance of seeds quality, not only controlling the proliferation of microorganisms.

In relation to the rosemary, results found by Tebaldi (2008) points out the α-pinene, 1,8 cineol and the camphor like majority constituents of the essential oil (Ribeiro *et al*., 2012). These compounds presents inhibitory effects on the growth of fungi and bacteria's, what can explain the efficiency of this treatment in the maintenance of germination and the roots length. When used their extract, autoclaved aqueous in dosage of 2,5%, Brand *et al*. (2010) verified that there was higher reduction in the growth of fungi and improvement of seeds viability.

Similar result in the control of microorganisms was observed by Souza *et al*. (2010) using extract of *A. sativum*in different concentrations in seeds of *Inga edulis*. The antifungal effect of the extract of *A. sativum*, according Abreu Júnior (1998), has relation on a lot of microorganisms (bacteria, fungi and nematode) demonstrating efficiency in the reduction of fungi. Souza *et al*. (2007), cited by Neto *et al*. (2012), verified reduction in the mycelial growth and germination of *Fusarium*spores with the use of garlic extracts in maize seeds.
In general, the contamination of seeds can affect harshly the physiological quality and, in some cases, completely inhibit the germination capacity of these seeds (Lopes *et al*., 2011; Neto *et al*., 2012). The use of medicinal plants not only influence the percentage of germination of seeds like also the speed of germination. Leite *et al*. (2012), evaluated the physiological quality of *Mimosa caesalpiniaefolia* seeds treated with vegetal extracts and verified that when treated with mint extract in concentration of 20 and 30%, reduced the incidence of the most part of pathogenic microorganisms.

Between the biological products, trichodemil and trichopolus were more efficient in the reduction of fungal population (Tables 3, 4 and 5) in all packages. This efficiency can be attributed to the various mechanisms of action used for these fungi, like the hyperparasitism and the competitions for nutrients (Harman *et al*., 2004), and like symbionts avirulent associated to plants (Carvalho *et al*., 2011). The positive effect on germination and root length can be attributed also to the possible effect of these as vegetal stimulators and also by the production of analogs of auxins (Harman *et al*., 2004 and Vinale *et al*., 2008).

Between the chemical products, mancozeb was the most efficient for the control of fungi than the others products used (Tables 1 and 2). This superiority can be attributed to the mechanism of action, which favors the inactivation of essential enzymes to the metabolism of the pathogenic microorganisms. The treatments with sorbate and benzoate compromised the seeds germination in the concentration evaluated, probably to caused phytotoxity to seeds.

According Goldbach (1979), the occurrence of fungi constituted one of the harmful factors to the conservation of recalcitrant seeds, in this case, intermediate. These pathogens are capable to penetrate in seeds during the development, the maturation, harvest and the post-harvest period, mainly when stored in unfavorable conditions. After infect the seeds, the most part of pathogens live in association or inside the cellular protoplasts, where they find the cellular contents like cytoplasm and nucleus. These pathogens feed of these contents which are rich in small molecules like sugars and aminoacids.
In nylon package with polyethylene, the results of medicinal plants like garlic, rosemary and biological agents, also

presented satisfactory results, above 70% until the six months of storage. This behavior can be due to the efficiency of the agents used to control the microorganisms (Chao and Young, 2000) (Table 5).

Beyond the seeds treated with medicinal plants and biological agents, those treated with mancozeb keep good performance until the end of the evaluated period. This product has broad spectrum of action in the control of microorganisms. The reduction in the water content of this treatment may have favored the good performance, once mancozeb forms a contact cover well uniform and with great adherence on seeds, conserving the viability of seeds for higher period of time. In flasks, in relation to the better treatments, there was the same tendency of the nylon packages with polyethylene, however occurs deterioration early of seeds, what can be due to the lower permeability of package and which may be due to lower permeability of package and which may have promoted higher accumulation of humidity and microorganisms proliferation.

Conclusion

When stored in natural environment, the better type of packing is the paper package which keep the seeds viable for six months independently of the control treatment of microorganisms. However, with treated with macozeb is indicate to store in nylon bags with polypropylene. The use of alternative products in the control of proliferation of microorganisms in coffee seeds is an alternative to the use of chemical agents. More researches must be realized in order to become this alternative viable economically.

Acknowledgements

Tothe Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG)

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