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Biocidal potential of some organic by-products on sanitary and physiological quality of red and white fava beans seeds

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Abstract

Fava bean (*Phaseolus lunatus* L.), is a rustic species and has great adaptability to arid regions of Brazil and stands out for its social importance and high levels of protein. Even so, the culture presents low productivity due to several factors, such as the quality of the seed. In this sense, the objective of this study was to evaluate the fungitoxic action of organic products, namely vinasse, cassava wastewater and agave extract, isolated and mixed, for treatment of beans seeds. The experimental design was completely randomized in factorial 8 x 2 (eight byproducts x two seed groups) with five replications. The seeds were evaluated for hysiological and sanitary quality, properly incubated in Petri plates on a triple layer of filter paper, sterilized and moistened with distilled water, where the assessments of the incidence of plant pathogens were performed after 7 days of incubation. Through the results, it is observed that the seeds of red fava bean were the most tolerant to organic products, without compromising the physiological quality. In general, the byproducts have significantly reduced the incidence of fungi identified in red and white fava bean.

Keywords: Phaseolus lunatus, seed sanity, allelopathy.

Abbreviations: CCTA_Center for Agro-Food Science and Technology; UFCG_Federal University of Campina Grande, T1_terile distilled water (negative control); T2_Captan[®] equivalent to 1.0 g/kg of seeds (positive control); T3_Extract of Agave; T4_Cassava wastewater; T5_Vinasse; T6_Agave extract + cassava wastewater; T7_Agave extract + vinasse; T8_Cassava wastewater + vinasse; RF_ Red fava; WF_White fava; G_Germination; FC_First count; RL_Radicle Length; IVG_Germination Velocity Index; GCS_Germination Speed Index; RF_For seeds of Red Fava; WF_White Fava.

Introduction

Fava bean (*Phaseolus lunatus* L.) is one of the most exploited legumes adapted and nutritious in Brazil, with the planted area of 25,543 ha and average yield of productivity of 339 kg ha⁻¹ (lbge, 2013). Its economic and social importance has aroused greater interest in family farming (Lopes et al., 2010), which makes it an alternative source of income and livelihood of the population of the Brazilian Northeast, which is responsible for 98.24% of the national production (lbge, 2013; Penha, 2014).

The state of Paraíba, stands with productivity over 35.8% of the national production, being cultivated in almost all the microregions of the state with a planted area of 8,254 ha (lbge, 2017). However, the productivity of the region is below the reality of Rio Grande do Sul, which is a reference in the national scenario, with yield of 2,154 kg ha⁻¹, resulting from more adequate management techniques for the culture (lbge, 2017).

Among the challenges to the fava bean culture is the sanitary quality of the seeds, by the presence of pathogens, such as: *Fusarium solani, Aspergillus, Penicillium notatum, Phoma* sp. and *Rhizopus stolonifer, Botrytis* sp., which

compromise the physiological quality of the culture, due to the conditions of production and storage (Gomes et al., 2016).

Thus, the treatment of seeds constitutes one of the most important measures in agriculture, by the simplicity of implementation, low relative cost and effectiveness under various agronomic aspects (Flávio et al., 2014). However, the most common practice, relates to the use of chemical pesticides, however, there is no record of products recommended for the crop of beans, which in many cases, have to count on the use of products applied in *P. vulgaris*. Some natural active principles which have been used and marketed in Brazil, as oil of Geranium, Natualho, Pironat, Rotenat and Natuneem, all from plant species as the *Pelargonium graveolens, Allium sativum*, pyroligneous acid, *Ateleia glazioviana* and *Azadirachta indica*, with biocidal potential to the most varied pathogenic microorganisms (Lopes et al., 2005; Franke, 2005).

In this sense, the objective of this work was to evaluate the efficiency of organic byproducts such as vinasse, cassava wastewater and agave extract in reducing the incidence of fungi present in fava bean seeds, and possible interference in the physiological activities.

Results and Discussion

Effect of organic by-products on the sanitary quality of seeds of fava

Upon analyzing the results concerning the composition and the incidence of fungi in seeds of fava bean, the following species were observed and identified: *Aspergillus niger, A. flavus, Penicillium* sp., *P. variable* and *Fusarium verticillioides* (Table 2 and 3), as well as already observed by Araújo et al. (2009) studying the sanitary quality of seeds of d'anta fava beans.

Through the results of the analysis of variance presented in Table 2, there was a significant effect (p<0.01) of the interaction between the accessions of fava bean and the organic byproducts in the management of the fungi present in the fava beans.

The organic byproducts applied on the fava beans, showed variations among the different species of fungi. Regarding the incidence of the species *Aspergillus niger* in red fava bean seed, with the exception of the treatment T8 (cassava wastewater + vinasse), all treatments statistically differed from the negative control (water). Among the treatments with best neutralization in relation to the control, T6 stands out (agave + cassava wastewater) with a reduction of 98.60%, differing including from the treatment T2 (Captan) which reduced 92.60%, in addition to T3 (agave) which obtained 85.98% reduction in the incidence, with the result statistically equal to T2.

As for the white fava bean seeds, in addition to the treatment T8, there was also no significant effect for the treatment T5 (vinasse), demonstrating that these treatments had no effect on the reduction of the growth of the species (Table 3). The efficiency of chemical treatment observed in this study, is possibly related to the fact that the product used to be considered a broad spectrum of action in relation to the group of fungi found in the fava bean seeds. While the positive response of potentiality of efficiency of cassava wastewater+ agave extract, may be associated with the presence of the secondary metabolite of class of glycosides and hecogenin, respectively, with antimicrobial and bioinsecticide property, already observed in other studies (Santos et al., 2009; Fonseca et al., 2016; Fonseca et al., 2018).

For the fungus *Aspergillus flavus* (Table 3), the greatest efficiency of products applied on the variety of red fava bean, was observed with a reduction of 100% for treatments T5 (vinasse) and T6 (agave + cassava wastewater). However, the remaining treatments showed reduction between 44.68% T4 (cassava wastewater) and greater than 68.10% with the treatments T3 (agave); T7 (Agave + vinasse) and T8 (cassava wastewater + vinasse). The application of the treatments in seeds of fava white bean, maintained the efficiency of the by-products for treatments T3 (agave) and T6 (agave + cassava wastewater), with results statistically equal to T2 (chemical), demonstrating that the by-products have the same efficiency when compared to the application between the fava bean varieties (Table 3).

Among the products used in this study, the vinasse presents a high concentration of nutrients, which makes it, an alternative source in the fertilization, at the same time, promotes the induction of resistance against biotic and abiotic damages in the plants (Paulino et al., 2011). Whereas for the agave extract, the presence of secondary metabolites such as: steroidal saponins (Hart et al., 2007), tannins, alkaloids and coumarins (Morais et al., 2010), express different properties: antiulcer, anti-helminthic, larvicide and antifungal effect (Botura et al., 2013; Santos et al., 2009), which leverages it as a viable alternative in family farming.

In the accessions of fava and white fava beans, in the presence of the species *Penicillium* sp., with the exception of T8 (cassava wastewater + vinasse), when applied to the seeds of red fava bean, the others were significantly equal to T2 (chemical), preventing the emergence of the pathogen (Table 3). Among the byproducts, the presence of organic matter and total sugars in the vinasse, promotes the biological activity of the soil, which favors potential antagonistic microorganisms, in addition to promoting formation of organic substances, such as volatile fatty acids, which may present antimicrobial action (Tenório et al., 2000; Leite et al., 2019).

These results highlight the importance of seed treatment with products of this nature, particularly in view of the genera *Penicillium* sp. and *Aspergillus* sp. considered fungi of storages, being responsible for a reduction in seed vigor, preventing its germination and death of the embryo, also have the ability to deteriorate grains and seeds, as well as, can even produce mycotoxins (Riverberi et al., 2010).

For a variety of red fava bean the lower rate of incidence of the fungus Penicillium variable was observed in treatments T3 (agave), T5 (vinasse), T6 (agave + cassava wastewater), T7 (agave + vinasse) and T8 (cassava wastewater + vinasse), with a reduction of 100%, proving the efficiency of these organic products in pathogen control, however, when treated with cassava wastewater (T4) and the chemical product (T2), even favoring the emergence of P. variable, promoted reduction higher than 90.24% in relation to the negative control (T1). Whereas for the access of white fava bean, all treatments showed efficiency when compared to the negative control, highlighting just three products: T5 (vinasse), T6 (agave + cassava wastewater) and T7 (agave + vinasse), which did not differ from T2 (chemical), which promoted a reduction of 100%. The incidence of Fusarium verticillioides in variety of red fava bean, had a marked reduction (71.43%), in treatments T3 (agave); T6 (agave + cassava wastewater), T7 (agave + vinasse) and T8 (cassava wastewater + vinasse), did not differ statistically from the T2 (chemical). As for the seeds of white fava bean, all treatments showed to be efficient, where a smaller reduction was observed for T4 (cassava wastewater) with 62.22%, and for the others, reaching a reduction of 100% of the pathogen. It was also observed, little variation among the efficiency of treatments comparing between the varieties of fava beans. The species of the genus Fusarium in some cultures, in particular F. verticilioides in the culture of corn (Zea mays), considered as one of the main problems in seed germination, can be easily transmitted (Parsa et al., 2016). In this sense, it is possible that the fava beans seeds have been contaminated in the own production area, since the small producers have the habit of intercropped planting among different species of culture, including corn with fava bean. Therefore, studies using plant extracts as an alternative for the management of the sanitary quality of

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Physicochemical-parameters	Cassava	Agave extract	Vinasse		
Ashes	0.17% ± 0.01	0.60% ± 0.03	1.11% ± 0.09		
Proteins	0.65% ± 0.0	1.60% ± 0.13	1.02% ± 0.12		
Total sugars	1.07% ± 0.09	2.43% ± 0.18	-		
Phenolic	275.25μg/g ± 0.9	2403.38µg/g ± 0.8	472.41µg/g ± 0.9		
Brix	9.27 ± 0.06	11.90 ± 0.10	1.5 ± 0.0		
CDO	96000 mgO ₂ /L	128000 mgO ₂ /L	20800 mgO ₂ /L		
DBO	18130 mgO ₂ /L	18005.5 mgO ₂ /L	18106 mgO ₂ /L		
Total solids	86055 mg/L	117877.5 mg/L	6420 mg/L		
Fixed solids	11500 mg/L	6980 mg/L	2372.5 mg/L		
Volatile solids	74555 mg/L	110897.5 mg/L	4047.5 mg/L		

Source: Analysis Laboratory-LIEP/UFCG

Table 2. Summary of the analysis of variance of different species of fungi present in seeds of fava accessions, after treatments with organic by-products.

Source/variation	Df	Aspergillus	Aspergillus	Donicillium cn	Penicillium	Fusarium
Source/variation		niger	flavus	Penicillum sp	variable	verticillioides
Accessions	1	24.64**	0.41ns	0.66ns	11.93**	0.20ns
Products	7	122.77**	28.08**	6.90**	46.00**	9.85**
Accessions * Products	6	53.73**	7.63**	1.69**	3.01**	2.41**
Residue	48	2.19	0.64	0.18	0.41	0.27
CV (%)		27.17	34.06	34.13	35.05	33.95

^{ns} and **: not significant and significant at 1 %, by the F test; Df: degree of freedom.

Table 3. Incidence of pathogens in red fava (RF) and white fava (WF) seeds after treatments with organic by-products.

Accossions	Treatments (Organic by-Products)									
Accessions	T1	T2	Т3	T4	T5	Т6	Τ7	T8		
	Aspergillus n	iger								
RF	107.0 aA	20.5 aC	15.0 aC	46.0 aB	32.5 bB	1.5 aD	32.5 aB	89 aA		
WF	90.2 aA	6.0 bB	7.5 aB	9.5 bB	77.5 aA	5.5 aB	10.5 bB	67 bA		
	Aspergillus fl	avus								
RF	23.5 aA	1.5 aD	7.5 aC	13.0 aB	0.0 bD	0.0 aD	6.5 aC	4.0 bC		
WF	22.5 aA	0.5 aD	0.0 bD	7.5 bC	9.0 aB	0.0 aD	3.0 bC	8.5 aB		
	Penicillium sp									
RF	3.5 bA	0.0 aB	0.0 aB	0.0 aB	0.0 aB	0.0 aB	0.0 aB	2.0 aA		
WF	12.5 aA	0.5 aB	0.0 aB	0.0 aB	0.0 aB	0.0 aB	0.0 aB	0.0 bB		
	Penicillium variable									
RF	20.5 bA	2.0 aB	0.0 bC	2.0 aB	0.0 aC	0.0 aC	0.0 aC	0.0 bC		
WF	43.5 aA	3.0 aB	2.5 aB	2.0 aB	0.0 aC	0.0 aC	0.0 aC	4.5 aB		
	Fusarium verticillioides									
RF	3.5 bB	1.0 aC	0.5 aC	8.5 aA	2.5 aB	1.0 aC	0.0 aC	0.5 aC		
WF	11.5 aA	0.0 aC	0.0 aC	4.0 bB	1.0 aC	0.0 aC	0.5 aC	0.0 aC		

Averages followed by the same lowercase letter in the column and capitalized in the row do not differ from each other by the Scott-Knott test at 5% probability, original data is presented T1 (T2 (Captan), T3 (agave extract), T4 (Cassava), T5 (Vinasse), T6 (agave extract + Cassava), T7 (agave extract + vinasse), T8 (Cassava + vinasse).

Table 4. Summary of variance analysis for physiological variables: germination (G), first count (FC); Radicle Length (RL) and germination Velocity Index (IVG) for seeds of Fava accessions subjected to different treatments with organic by-products.

Source/variation	Df	GER (%)	FC (%)	RL (cm)	IVG (%)
Accessions	1	1369.00**	2889.06**	28.29*	14.48**
Products	7	311.68**	409.49**	15.09*	3.50**
Accessions * Products	7	271.00**	426.63**	11.56*	3.23**
Residue	48	41.29	44.65	5.01	0.35
CV (%)		7.54	8.43	20.58	7.09

^{ns}, ** and *: not significant and significant at 1 %, by the F test; Df: degree of freedom

Table 5. Mean values of germination (G), first count (FC), radicle length (RL) and germination Speed Index (GCS), and for seeds of
Red Fava (RF) and white Fava (WF) accessions, subjected to different treatments with by-products organic.

Accessions	Treatments (Organic by-Products)											
	T1	T2	Т3	T4	T5	Т6	Τ7	Т8				
	Germination - G (%)											
RF	94.0 aA	91.0 aA	93.5 aA	81.0 aA	91.5 aA	87.0 aA	92.5 aA	88.0 aA				
WF	89.5 aA	89.5 aA	81.5 bA	83.0 aA	65.0 bB	88.0 aA	87.0 aA	61.0 bB				
	First count - FC (%)											
RF	90.5 aA	89.5 aA	89.5 aA	69.5 aB	89.0 aA	85.5 aA	89.0 aA	85.0 aA				
WF	86.0 aA	81.0 aA	70.5 bB	76.5 aB	54.0 bC	83.5 aA	75.5 bB	53.0 bC				
	Radicle length - RL (cm)											
RF	9.04 aB	7.60 bB	12.03 aA	8.21 aB	11.54 aA	14.11 aA	9.78 aB	9.40 bB				
WF	9.38 aA	13.28 aA	12.14 aA	9.90 aA	12.23 aA	11.69 aA	10.90 aA	12.84 aA				
	Germination Speed Index - GCS (%)											
RF	9.33 aA	9.06 aA	9.27 aA	7.46 aB	9.08 aA	8.67 aA	9.18 aA	8.70 aA				
WF	8.87 aA	8.78 aA	7.95 bA	8.18 aA	6.28 bB	8.71 aA	8.44 aA	5.95 bB				

Averages followed by the same lowercase letter in the column and capitalized in the row do not differ from each other by the Scott-Knott test at 5% probability, original data is presented T1 (water), T2 (Captan), T3 (agave extract), T4 (Cassava), T5 (Vinasse), T6 (agave extract + Cassava), T7 (agave extract + vinasse), T8 (Cassava + vinasse).

seeds of different species, has been used by several authors, such as Silva et al. (2008), Almeida et al. (2012) and Almeida et al. (2016), who demonstrated that in addition to providing a reduction in the microflora, also provided an increase of seeds germination.

Effect of organic by-products on the physiological quality of seeds of fava

The results concerning the evaluation of the physiological quality of fava beans seeds, showed a significant difference (p<0.05) in the interaction among accessions of fava bean *versus* organic products for the variables: germination, first count, radicle length and speed of germination index (Table 4).

The organic byproducts behaved differently regarding the influence on the physiological characteristics of the fava beans seeds. For the red fava beans seeds, it was observed that there was no negative effect for germination, unlike what was observed in the white fava bean seeds, where treatments T5 (vinasse) and T8 (cassava wastewater + vinasse), decreased by 27.37% and 31.84%, respectively. These results corroborate with Smiderle et al. (2013), where a smaller percentage of germination was found in cowpea seeds when subjected to the presence of cassava wastewater applied in the planting furrow (Table 5). Voll et al. (2010), highlight that the vinasse presents in its composition the aconitic acid, a substance that has inhibitory properties on the germination, and this effect is recorded in weeds such as wild poinsettia (Euphorbia heterophylla), morning glory (Ipomoea grandifolia), beggar tick (Bidens pilosa) and soybean (Glycine max).

In analyzes of first count, a significant difference was observed only for the treatment T4 (cassava wastewater), in red fava bean seeds, influencing negatively, with a reduction of 23.20% in this variable. The opposite was observed for white fava bean seeds, where the seeds showed higher sensitivity to the by-products, with a reduction of 18.02% in T3 (agave); 11.04% with T4 (cassava wastewater), 37.20% with T5 (vinasse); 12.20 for T7 (agave + vinasse) and 38.37% with application of T8 (cassava wastewater + vinasse), in relation to the negative control. Moraes (2010), emphasizes

that the ethanolic agave extract, can inhibit the development of some seeds, in function of the presence of saponins, substance with allelopathic properties, which is confirmed in this study when applied alone or in mixture with vinasse (Table 5).

For the radicle length (CR), there was a gain of growth with the isolated application and mixtures of organic byproducts, with T3 (agave) of 58.3%, T5 (vinasse) with 51.84% and T6 (agave + vinasse) with 85.66%, in relation to the controls (T1 and T2), with the red fava bean seeds. As for the seeds of white fava beans, there was no variation among the treatments. Somehow, it was observed that the by-products effects may vary negatively or positively, according to the physiological stage of the seed.

As the index of germination speed, with the exception of T4 (cassava wastewater) responsible for the reduction of 20.04%, in the red fava bean, all the other treatments were statistically equal to the controls (T1 and T2). Unlike the white fava bean seeds, these demonstrate greater sensitivity to treatments T5 (vinasse) and T8 (cassava wastewater+ vinasse), where it is observed a reduction between 29.19% and 32.92%, respectively. According to Gonzaga (2007), the cassava wastewater may have a toxic effect on certain plants, including inhibiting the germination, due to the presence of toxic cyanogenic glycoside, however, it is of great importance with insecticide and nematicide action. Thus, it is possible that the presence of secondary metabolite has influenced the reduction of some of these variables studied, which signals the need to adapt the concentration to be employed for each intended use as an alternative.

Materials and Methods

Location and experimental procedures

The experiment was conducted in the Laboratory of Phytopathology, located in the center of Food Science and Technology, Federal University of Campina Grande, Campus of Pombal, UFCG. The geographical location is defined by the coordinates: $06^{\circ}46'13'$ south latitude, $37^{\circ}48'06'$ west longitude and altitude of approximately 184 m.

In this study, two seed lots of fava bean seed (*Phaseolus lunatus* L.) were used of the variety known as Lavanderia Vermelha and white fava, obtained from small producers in the municipality of Cajazeiras in Paraíba-PB.

For the completion of the experiment, three organic byproducts were used, consisting of vinasse and cassava wastewater, purchased at a distillery for the production of cachaça and cassava flour mill respectively, both located in the municipality of Areia-PB. In addition to these, agave extract was also used, obtained from the leaves fibers separation, collected in the municipality of Cuité-PB. It was verified in Table 1, the physicochemical composition of organic byproducts used in this experiment.

In the assembly of the experiment, a completely randomized experimental design was used in a factorial arrangement (8 X 2), being the first factor composed of the treatments: T1= sterile distilled water (negative control); T2= Captan® equivalent to 1.0 g/kg of seeds (positive control); T3= extract of Agave; T4= cassava wastewater; T5= vinasse; T6= agave extract + cassava wastewater; T7= agave extract + vinasse and T8= cassava wastewater + vinasse, and for the second factor, were employed two batches of fava beans (variety as Lavanderia Vermelha and white fava), with five replications.

Seed treatment

The byproducts, vinasse, cassava wastewater and agave extract, were diluted to 50% concentration in distilled water. In the application of the treatments in the seeds, these were treated with organic byproducts isolated and with the byproducts intermixed. Thus, for the byproducts when isolated, were used 50 mL of each treatment for each 100 g of seeds, while, in the by-products mixed, corresponded to 25 mL of each treatment for each 100 g of seeds. The controls were also used: the fungicide Captan $\$ (240 g i.a.100 kg⁻¹ of seeds), as recommended by the manufacturer, and the sterile distilled water, for both batches.

To achieve this, the seeds were distributed in Becker of 250 mL, where they were immersed in each treatment (organic products), homogenized by stirring with a glass rod for 3 minutes. In the end, they were placed to dry, under controlled conditions for 20 minutes, before being subjected to incubation period.

Sanitary Quality of seeds

The evaluations were carried out for quantification and identification of the seeds microflora, as well as to the characteristics of physiological variables of the seeds. For this reason, 400 seeds were used per treatment, previously disinfected in alcohol 70% for 30 seconds and sodium hypochlorite to 1%, for 3 minutes, leaving them under a temperature of 25 \pm 2 °C, for drying.

Subsequently, all variables were evaluated after the application of the treatments, the highlight: the seeds microflora, using the method of incubation on paper filter (Blotter test) proposed by Neergaard (1977). For this test, 400 seeds/treatment were used (five replicates of 80 units). The seeds were incubated in Petri plates on a triple layer of filter paper, previously sterilized and moistened with sterile distilled water. The plates were kept in the incubation chamber at $20^{\circ}C \pm 2^{\circ}C$ and photoperiod of 12 hours, where

they remained for seven days, and then to be evaluated for the presence of pathogens (Brasil, 2009). The identification and counting of fungi were performed in seeds with the aid of a stereoscopic and optical microscope. Confirmation of the fungi in the genus level was performed with the aid of an identification key (Barnett; Hunter, 1998), and then calculated the percentages of contaminated seeds.

Physiological quality of seeds

For the germination test, 200 seeds were used (5 replicates of 40 seeds for each treatment, distributed in germitest paper, previously sterilized and moistened with distilled water, in the amount equivalent to 2.5 times of the mass of the dry paper. Then, the rolls with 200 seeds were maintained in a germination chamber set at a constant temperature of 25°C under alternating photoperiod of 12 h. The counts were performed at the 3rd and 9th day after incubation, considering the germinated seeds, which show the root system with at least 2 cm in length (Brazil, 2009).

As the first count of germination, it was carried out jointly with the germination test, where the counts of normal seedlings were determined on the fifth day after the testes installation. To the radicle length, the test was conducted on germitest paper, using 200 seeds, after the ninth day, the normal seedlings obtained were measured with the aid of a graduated ruler.

As for the index of germination speed of seedlings in sand the same numbers of seeds per repetition was used, where they were sown in plastic trays containing washed and sterile sand, and moisturized daily, kept in a greenhouse for a period of nine days (Alves et al. 2014). This variable was represented by the number of emerged plants daily until the ninth day after installation. The evaluations of normal seedlings were performed daily, always in the morning schedule, from the first count of germination. The result was calculated according to the formula proposed by Maguire (1962) where the number of germinated seeds or seedlings was divided by the day of counting: IVG = $G_1/N_1 + G_2/N_2 + ...$



 $G_1, G_2, ..., G_n$ = number of sprouted diaspores

 N_1 , N_2 , ... N_n = number of days after sowing.

The data were subjected to analysis of variance for diagnosis of significant effects through the "F" test and when verified significant effect, the means were grouped by Scott-Knott test at 5% probability.

Conclusion

All organic by-products have potentiality in the reduction of fungi found in the two plots of bean seeds. The T6 treatment (agave extract + Cassava) has lower sensitivity in the physiological development of the seeds. The treatments submitted to the Vinasse and Cassava by-products negatively interfere with seed vigor. Red fava seeds are more tolerant to organic by-products.

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