Chemical and biological treatments of castor bean seeds: effects on germination, emergence and associated microorganisms[1]

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ABSTRACT – The effect of chemical and biological treatments on castor bean emergence, seedling vigor, dry matter production, and also the control of microorganisms associated with seeds of the AL Guarany 2002 and Lyra cultivars, was evaluated. The products tested were carbendazim + thiram, carboxin + thiram and a product based on *Trichoderma*. Total seed and seedling emergence were evaluated at 27 days after sowing whereas dry matter production was verified for plants removed 45 days after sowing. The Guarany 2002 AL cultivar had a higher incidence of microorganisms than the Lyra cultivar. The chemical treatment was 100% effective in controlling fungi but the biological treatment did not reduce microorganism incidence on the seeds. Chemical treatment resulted in plants with more dry matter and the best results were for carbendazim + thiram and carboxin + thiram at doses of 60 g + 140 g and 50 g + 50 g/100 kg of seeds, respectively. The carbendazim + thiram mixture was the only treatment which was statistically higher for total emergence whereas the biological treatment increased emergence only for the Lyra cultivar, thus demonstrating its lower efficiency. The importance of fungicides to control pathogens associated with seeds was discussed.

Index terms: *Ricinus communis,* blotter test, fungicides, *Trichoderma*.

Tratamentos químicos e biológicos de sementes de mamona: efeitos na germinação, emergência e microrganismos associados

RESUMO - Avaliou-se o efeito dos tratamentos químicos e biológicos sobre a emergência, vigor de plântulas de mamona, produção de matéria seca, além do controle de microrganismos associados às sementes das cultivares AL Guarany 2002 e Lyra. Os produtos testados foram carbendazim+thiram, carboxin+thiram e um à base de *Trichoderma*. A emergência total e de plântulas foram avaliadas aos 27 dias após a semeadura e a produção de matéria seca, com plantas retiradas 45 dias após a semeadura. A cv. AL Guarany 2002 apresentou maior incidência de microrganismos em relação à cv. Lyra. O tratamento químico foi 100% eficiente no controle de fungos e o tratamento biológico não reduziu a incidência de microrganismos nas sementes. O tratamento químico resultou em plantas com maior matéria seca e os melhores resultados foram para carbendazim + thiram e carboxin+ thiram, nas doses de 60 g + 140 g e 50 g + 50 g/100 kg de sementes, respectivamente. O carbendazim + thiram foi o único tratamento que se mostrou estatisticamente superior, quanto ao índice de emergência total, enquanto que o tratamento biológico aumentou os valores de emergência somente na cv. Lyra, evidenciando sua inferioridade. Destacou-se a importância dos fungicidas para o controle de patógenos associados às sementes.

Termos para indexação: *Ricinus communis*, fungicidas, teste de papel filtro, *Trichoderma*.

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Introduction

The castor bean (*Ricinus communis* L.) is grown for the oil contained in its seeds. The Brazilian Biodiesel Program has recently encouraged the planting of this crop due to its high oil yield and rusticity. Studies conducted by the Embrapa Temperate Climate Institute in Rio Grande do Sul have demonstrated the potential of castor bean in the region (Silva, 2005).

The slow and irregular germination of castor bean has numerous disadvantages, including initial weed competition, the attack of opportunistic microorganisms and vulnerability to drought during field emergence, since this can last up to 20 days in the main producing regions. Therefore, a rapid and uniform plant emergence is important since it results in suitable stands with well-developed plants, which will facilitate harvesting and processing management and have positive reflexes on crop productivity and oil production (Mendes et al., 2009).

The efficiency and economy of chemical treatments has already been demonstrated by various authors (Bittencourt et al., 2007). There are cases where fungicides are efficiently used to control phytobacteria (Lopez et al., 2008), as in the eradication of *Xanthomonas axonopodis* pv. *phaseoli* by the fungicide, tolylfluanid. Although chemicals can efficiently control plant diseases they can also have adverse effects, such as the development of fungicide-resistant species (Maketon et al., 2008). Some studies describe antagonists, such as *Pseudomonas* and *Bacillus*, in the protection and growth promotion of hydroponic lettuce (Corrêa et al., 2010).

The association of phytopathogens with seeds can severely affect their quality, reducing their germinative potential, vigor, emergence, storage period and even their yield. Therefore, concern about seed health quality is an important factor for reducing damage caused by these agents.

Many fungi associated with castor bean seeds have a phytopathogenic potential and species of *Fusarium* are the most important and easily found on seeds. *Fusarium* is a soil-inhabiting species and is often found on seeds, causing damage in various crops, such as basil (Reis et al., 2007), black pepper (Carnaúba et al., 2007), dry beans (Sala et al., 2006) and also castor bean, where it can cause severe damage (Silva, 2005). *Alternaria* is another fungus, associated with castor bean seeds, causing alternaria spot disease (Silva, 2005) and the fungus *Macrophomina* is responsible for one of the main diseases found in castor bean (stem or root rot) and various other crops via the seeds (Vanzolini et al., 2010).

According to Suassuna and Coutinho (2006), one of the visible damage symptoms caused by phytopathogens to castor bean seeds is damping-off in seedlings, which reduces the crop stand. Studies in Rio Grande do Sul state have indicated the presence of various genera of fungi (Silva et al., 2005). Mariotto et al., (1987) studied fungi and control methods in São Paulo around 20 years ago and studies to test new control methods are necessary.

According to Carvalho and Nakagawa (2000), knowledge of the pathogens associated with seeds is important since these can be disseminated and serve as an inoculant source, causing a disease epidemic in the subsequent crop. Therefore, it is important that phytosanitary measures for seeds be adopted to avoid losses in the new crop.

The objective of the present study was to evaluate the effect of chemical and biological treatment of castor bean seeds on germination, emergence, seedling vigor and dry matter production as well as the control of associated microorganisms.

Material and Methods

The experiments were carried out at the Embrapa Temperate Climate Institute, Pelotas-RS, with the Al Guarany 2002 (AL) and Lyra (HL) cultivars. The treatments are listed in Table 1 and included controls for both cultivars as well as the chemical and biological treatments.

Table 1. Chemical and biological treatments used in the trials with their respective dosages.

Technical name	Dosage/100kg of seeds
1) Carbendazim + Thiram	30 g + 70 g
2) Carbendazim + Thiram	60 g + 140 g
3) Carboxin + Thiram	50 g + 50 g
4) Carboxin + Thiram	100 g + 100 g
5) *Trichoderma**	200 mL
6) *Trichoderma*	400 mL
7) Control	distilled water

*Concentration 108 conidia/ mL.

The castor bean seeds were placed in a glass recipient, where the fungicides and the biological product, a formulation of *Trichoderma harzanium,*

came into contact with them. Distilled water was added at a 1:1 ratio and the recipient was shaken so that the seeds were covered by the treatments and showed the characteristic colors of the products used. The time taken to cover the seeds was the same for both the fungicides and biological control product.

The experimental design for the castor bean seedling emergence trial was a completely randomized factorial with seven treatments, two cultivars and three repetitions. Each block had 10 bags with untreated soil, placed in a plastic box. Two seeds were sown in each bag, totaling 60 seeds per treatment. The boxes were distributed at random on two tables under greenhouse conditions. Trial evaluations were made on the 27th day. The emergence index was according to a scale (i) of 0 to 5 (Figure 1).

Figure 1. Scale for evaluating seedling development to obtain emergence indices.

The formula ∑nxi(0~5)/20 was used to calculate the total emergence index per repetition, where n is the number of seedlings with the respective ranking i, obtained on the evaluation day (27th day) and 20 is the number of seedlings per repetition. The seedling emergence index was calculated by only considering the emerged seedlings, with the aim of comparing seedling vigor for different treatments. For the calculation of percentage emergence, only the number of emerged seedlings in relation to the total number of plants sown was considered. For the analysis of variance, the percentage data were transformed to arc sine root (x/100) and the emergence index data to root of x. The statistical analysis was done according to Banzato and Kronka (1995). Means were compared with the Tukey test at the 5% probability level.

The castor bean seed pathology trial was done using filter paper with the same treatments, repetitions and statistical design. The seeds, distributed in 42 gerbox type boxes, with 20 seeds per box, were incubated at 24 ºC and a 12 hour photoperiod, using the technique similar to Ferreira et al. (2010). Evaluations were made on the 7th day after incubation and microorganism structure was observed with a stereoscopic and optical microscope.

In the dry matter test, the plants were removed from their substrates 45 days after sowing and placed in paper bags to dry for a month under a glasshouse environment. They were then grouped by treatment, cleaned and the aerial portion was separated from the roots. Each portion was then weighed on a precision balance (Mettler PC 2000). The dry weight was calculated by using the weight of the plant portions of each treatment (aerial portion or roots), dividing it by the number of emerged seedlings and arriving at a mean weight for each sample.

Results and Discussion

There was no significant statistical difference for the total emergence index between the chemical and biological treatments, and the *Trichoderma*-based product did not differ from the control (Table 2). However, there was a statistical difference between the chemical treatments and the control. The only chemical treatment with a superior performance, which showed a statistical difference with the biological treatment, was carbendazim + thiram at the highest dosage rate (60 g + 140 g). There was no statistical difference between the biological treatment dosages but the dosage of 200 mL/100 kg seeds had the best mean (1.41). In general, there was a statistical difference between the cultivars, with AL Guarany 2002 performing better for all the treatments.

The chemical and biological treatments were statistically superior to the control for the seedling emergence index but no chemical treatment was better than the biological treatment for the production of more vigorous seedlings. AL Guarany 2002 also gave superior results compared to those of the Lyra cultivar (Table 2). The results for the seedling emergence index demonstrated, therefore, that the chemical and biological treatments resulted in more vigorous seedlings compared to the control. The best treatment was the highest dosage of carbendazim + thiram, since although it was not statistically different from the other chemical treatments for the seedling emergence index, it was superior for total emergence and was statistically different from the biological control products.

The chemical treatments were not statistically different among themselves for percentage emergence but the two dosages of the carbendazim + thiram applied were statistically different from the biological and control treatments and gave the highest percentage emergence for both cultivars (Table 3). The mean percentage emergence for AL Guarany 2002 was higher than for the Lyra cultivar.

Table 2. Effect of treatments on total emergence indices and those of seedlings for two castor bean cultivars: AL Guarany 2002 (AL) and Lyra Hybrid (LH). Evaluations made 27 days after sowing.

Treatment	Index of total emergence			Seedling emergence index		
	Cultivar			Cultivar		
	AL[1]	LH[2]	Mean[3]	AL	LH	Mean
Carbendazim + Thiram (30 g + 70 g)	2.62	1.52	2.07 ab	2.96	2.60	2.78 a
Carbendazim + Thiram (60 g + 140 g)	2.77	1.88	2.33 a	2.92	2.64	2.78 a
Carboxin + Thiram (50 g + 50 g)	2.55	1.52	2.03 abc	2.94	2.67	2.81 a
Carboxin + Thiram (100 g + 100 g)	2.35	1.63	1.99 abc	2.88	2.65	2.77 a
Trichoderma (200 mL)	1.78	1.03	1.41 bcd	2.98	2.66	2.82 a
Tichoderma (400 mL)	1.48	1.17	1.33 cd	2.80	2.75	2.77 a
Control	1.60	0.75	1.18 d	2.81	2.46	2.63 b
Mean[4]	2.62 A	1.52 B		2.96 A	2.60 B	

1) AL= cv. AL Guarany 2002. 2) LH= cv. Lyra. 3) Mean of treatments. 4) Mean of cultivars. 5) Values followed by the same small letter in the column and capital letter in the row show no statistical difference according to the Tukey test at 5%.

Table 3. Mean values of percentage emergence for cultivars evaluated for chemical and biological treatments.

Treatment	Percentage emergence		
	Cultivar		
	AL Guarany 2002	Lyra	Mean[1]
Carbendazim + Thiram (30 g + 70 g)	88.33	58.33	73.33 a[3]
Carbendazim + Thiram (60 g + 140 g)	95.00	71.67	83.33 a
Carboxin + Thiram (50 g + 50 g)	86.67	56.67	71.67 ab
Carboxin + Thiram (100 g + 100 g)	81.67	61.67	71.67 ab
Trichoderma (200 mL)	60.00	38.33	49.17 bc
Tichoderma (400 mL)	53.33	43.33	48.33 bc
Control	56.67	30.00	43.33 c
Mean[2]	74.52 A	51.43 B	

1) Mean of treatments. 2) Mean of cultivars. 3) Values followed by the same small letter in the column and capital letter in the row show no statistical difference according to the Tukey test at 5%.

Lazzaretti and Bettiol (1997) found that benomyl applied at 50 g/100 kg of seeds was statistically different from the treatments with *Bacillus* cells and metabolites for control of *Fusarium* spp. Medeiros et al. (2006) covered carrot seeds with carbendazim + thiram (200 g + 600 g), which did not affect germination and resulted in increased vigor. In spite of the lower mean values observed for the *Trichoderma*-based product, there are very interesting descriptions on the action of this mutant fungus, where the mortality of tomato plants in contact with *Rhizoctonia solani* fell 100% if *Trichoderma* antagonized the pathogen (Montealegre et al., 2010). Another idea defended by Figueirêdo et al. (2010) is that the period when the antagonist is applied to control soil pathogens significantly affects the result and that the application of *Trichoderma* at sowing may make a difference.

The highest dry matter production was from plants whose seeds were treated with chemical fungicides (Table 4). Carbendazim+thiram applied at a dosage rate of 30 g + 70 g resulted in higher values for the Lyra cultivar per treatment and per plant for both the aerial portion and the roots, compared to those observed for the AL Guarany cultivar. The higher dosage of 60 g + 140 g of carbendazim+thiram for AL Guarany 2002 was more efficient for dry matter production of the roots and the aerial portion for both the plant and the treatment. For treatments with *Trichoderma*-based products, the AL Guarany 2002 gave a better result for the aerial portion at the lowest dosage and was the treatment which gave the highest weight for the aerial portion of the plant when compared to the highest dosage. However, the roots of this cultivar developed better with the highest dosages. The *Trichoderma*-based treatments at lower dosages (200 mL) resulted in plants with a higher dry matter production and with roots having a higher dry weight. Although the biological treatment was less efficient than the chemical treatments, it was positive for plant growth.

Table 4. Mean values of dry weight production per treatment and per plant (in grams) of two castor bean cultivars : AL (AL Guarany 2002) and LH (Lyra).

Treatments	Per treatment		Per plant	
	Roots	Aerial portion	Roots	Aerial portion
AL Carbendazim + Thiram (30 g + 70 g)	94.04	60.18	1.74	1.10
LH Carbendazim + Thiram (30 g + 70 g)	126.70	60.31	3.50	1.68
AL Carbendazim + Thiram (60 g + 140 g)	106.10	66.83	1.80	1.20
LH Carbendazim + Thiram (60 g + 140 g)	97.22	68.45	1.90	1.30
AL Carboxin + Thiram (50 g + 50 g)	97.49	53.86	1.80	1.00
LH Carboxin + Thiram (50 g + 50 g)	50.01	47.03	0.80	0.90
AL Carboxin + Thiram (100 g + 100 g)	56.08	55.81	1.10	1.10
LH Carboxin + Thiram (100 g + 100 g)	62.69	61.00	1.65	1.61
AL *Trichoderma* (200 mL)	38.27	61.67	1.10	1.71
LH *Trichoderma* (200 mL)	53.38	50.86	2.00	1.90
AL *Trichoderma* (400 mL)	54.00	43.42	1.60	1.30
LH *Trichoderma* (400 mL)	42.69	51.90	1.30	1.60
AL Control	46.53	51.13	1.20	1.30
LH Control	22.49	31.50	1.00	1.40

The AL Guarany 2002 cultivar showed a greater incidence of microorganisms than the Lyra cultivar. The treatment with the two fungicides at both dosages gave 100% fungal control but not for other microorganisms. The use of the *Trichoderma*-based product (Ecotrich) also did not reduce the incidence of microorganisms on the seeds (Table 5).

The results of the present study demonstrated that fungicide use is important for reducing or eradicating phytopathogenic fungi associated with castor bean seeds. More detailed studies are necessary to show if the microorganisms detected in the seeds are important pathogens for castor bean. Various studies

describe the destructive action of pathogens of the same genera as found in this study, provoking diseases in other crops. They include *Bipolaris,* which causes foliar diseases in rice (Celmer et al., 2007) and damage in the grass, *Paspalum atratum* cv. Pojuca (Anjos et al., 2004) and fungi of the genus *Fusarium,,* which is a disease agent in various hosts (Sala et al., 2006; Dias and Toledo, 1993), including castor bean.

In general, the treatment with the biological product did not inhibit the growth of fungus species, including some, such as *Fusarium* (Silva et al., 2005), which are very important for castor bean, principally the AL Guarany 2002 cultivar (Table 5). Gomes et al. (2009) found *Fusarium* contaminating soybean seeds collected from different sites and this was related to the host cultivar. The biological product is composed of *Trichoderma*, a fungus much used for the biological control of other fungi (Patrício et al., 2007). It is interesting to note that *Trichoderma* was detected on only three Lyra cultivar seeds whereas a higher number of AL Guarany 2002 seeds had this fungus, suggesting that the Lyra seeds did not favor *Trichoderma* colonization. More detailed studies are needed to explain this difference.

Table 5. Effect of chemical and biological treatments on the incidence of microorganisms on seeds of two castor bean cultivars: AL Guarany 2002 (AL) and Lyra (LH).

Cv	Treatment	Microorganisms detected on castor bean seeds													
		FUS	ALT	ASP	PEN	CLA	RZP	TCD	ACT	BIP	BAC	PTL	GET	PHO	LEV
AL	Carbendazim + Thiram (30 g + 70 g)	0*	0	0	0	0	0	0	3	0	2	0	0	0	0
	Carbendazim + Thiram (60 g + 140 g)	0	0	0	0	0	0	0	1	0	12	0	0	0	0
	Carboxin + Thiram (50 g + 50 g)	0	0	0	0	0	0	0	1	0	8	0	0	0	0
	Carboxin + Thiram (100 g + 100 g)	0	0	0	0	0	0	0	0	0	7	0	0	0	0
	Trichoderma (200 mL)	23	0	23	21	12	0	46	35	3	1	0	0	0	0
	Trichoderma (400 mL)	28	0	14	15	8	3	40	33	2	1	0	0	0	0
	Control	24	0	4	7	9	13	0	37	3	1	0	0	5	0
LH	Carbendazim + Thiram (30 g + 70 g)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Carbendazim + Thiram (60 g + 140 g)	0	0	0	0	0	0	0	0	0	5	0	0	0	0
	Carboxin + Thiram (50 g + 50 g)	0	0	0	0	0	0	0	0	0	3	0	0	0	0
	Carboxin + Thiram (100 g + 100 g)	0	0	0	0	0	0	0	0	0	2	0	0	0	0
	Trichoderma (200 mL)	5	0	2	17	0	0	0	0	0	0	0	0	0	0
	Trichoderma (400 mL)	9	1	3	6	11	1	3	0	0	0	1	2	1	1
	Control	5	0	1	6	8	0	0	12	0	9	0	0	1	0

FUS (*Fusarium*); ALT (*Alternaria*); ASP (*Aspergillus*); PEN (*Penicillium*); CLA (*Cladosporium*); RZP (*Rhizopus*); TCD (*Trichoderma*); ACT (Actinomycetes); BIP (*Bipolaris*); BAC (bacteria); PTL (*Pestalotia*); GET (*Geotrichum*); PHO (*Phoma*); LEV (yeast).

*Number of seeds with the respective microorganisms.

Although the AL Guarany 2002 seeds showed a higher incidence of fungi compared to the Lyra cultivar, the control (untreated) gave better results for the total emergence tests, such as the seedling emergence index and percentage emergence (Tables 3 and 4). This suggests that besides the microorganisms associated with the seeds, those present

in the soil can also affect castor bean germination and seedling emergence.

Conclusions

This study shows that, under the study conditions, chemical treatment is the most recommended and is superior to the biological treatment. Besides the large difference between the chemical and biological treatments for the control of contaminating microorganisms, the chemical treatment produces plants with more dry matter and the products carbendazim + thiram and carboxin + thiram, at the dosages of 60 g + 140 g and 50 g + 50 g/ 100 kg, respectively, are the most recommended.

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