SWEET PEPPER SEED RESPONSES TO INOCULATION WITH MICROORGANISMS AND COATING WITH MICRONUTRIENTS, AMINOACIDS AND PLANT GROWTH REGULATORS

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ABSTRACT: Small sized seeds, such as the horticultural species, have limited quantities of reserves that can be balanced by coating then with essential nutrients for their initial development. In addition, inoculation of the seeds with microorganisms may protect the plants against phytopathogens, thus enhancing their growth. The present work had the objective of evaluate the physiological quality and seedling development of sweet pepper seeds and seedlings coated with several kind of films. Seeds were first coated with polymers and then with antagonistic microorganisms (*Trichoderma viride, Trichoderma polysporhum, Trichoderma stromaticum, Beauveria bassiana, Metarhizium anisopliae*), mycorrhizas, aminoacids, micronutrients and plant growth regulators. Evaluation was performed for percentage of germination and for seedling emergence, speed of emergence index, number of plants, dry mass of the aerial and root parts and height of the seedlings. Inoculation with *Trichoderma viride* increased the percentage and rate of the seedlings emergence Inoculation with *Trichoderma viride, Metarhizium anisopliae* and mycorrhizas promote better seedling development; seed microbiolization with microorganisms *Trichoderma viride, T. polysporhum, T. stromaticum, Beauveria bassiana, Metarhizium anisopliae*. Mycorrhizas mixture negatively affected seeds and seedling quality. Seed covering with plant growht regulator, at a 5 mL kg^{-1} dose increased the roots dry matter.
Key Words: *Capsicum annum*, film coating, microbiolization

TRATAMENTO DE SEMENTES DE PIMENTÃO COM MICRORGANISMOS, MICRONUTRIENTES, AMINOÁCIDOS E REGULADORES DE CRESCIMENTO

RESUMO: Em sementes pequenas, como as de espécies hortícolas, as limitadas quantidades de reservas podem ser equilibradas por meio do seu recobrimento com nutrientes essenciais para o seu desenvolvimento inicial. Além disso, a inoculação dessas sementes com microrganismos, além de proteger as plantas contra fitopatógenos, pode promover o seu crescimento. Assim, objetivo do presente trabalho foi avaliar o efeito de tipos de revestimentos na qualidade de sementes e mudas de pimentão (*Capsicum annum*). Para tanto, as sementes foram primeiro revestidas com polímeros e depois foram adicionados microrganismos antagônicos (*Trichoderma viride*, *Trichoderma polysporhum, Trichoderma stromaticum, Beauveria bassiana*, *Metarhizium anisopliae*), micorrizas, aminoácidos, micronutrientes e reguladores vegetais. Avaliaram-se a porcentagem de germinação e de emergência, o índice de velocidade de emergência, o número de plantas, a massa seca de parte aérea e de raiz e a altura da parte aérea das mudas. A inoculação de sementes com *Trichoderma viride* aumentou a porcentagem e o índice de velocidade de emergência das plântulas; *Trichoderma viride, Metarhizium anisopliae* e as micorrizas inoculadas às sementes dessa espécie promovem melhor desenvolvimento das mudas; a microbiolização de sementes com a mistura dos microrganismos *Trichoderma viride, T. polysporhum, T. stromaticum, Beauveria bassiana, Metarhizium anisopliae* e micorrizas afetou negativamente a qualidade das sementes e mudas de pimentão e o revestimento dessas sementes com regulador vegetal, na dose de 5 mL kg^{-1} de sementes proporciona aumento na massa seca das raízes das plantas.
Palavras-chave: *Capsicum annum*, peliculização, microbiolização

INTRODUCTION

Until the mid of 1990s sweet pepper (*Capsicum annum*), was a crop with small commercial significance in Brazil. Since then, there was an increase trading of then in the market, as reported by CEAGESP (Bordin, 2002). In Brazil, 13,000 tons were produced in 2003, which having, therefore, a great demand for seeds. Melo et al. (2004), reported that only the seed national marked generated US$ 1,5 million in 2003. On the other way, organic agriculture has become a growing important option for farmers, as it meets the consumer demands on both national and international scale. Consumer claims for food organic foods have created market niches that cannot be overlooked anymore, especially by family producers that need to enlarge their income. Organic production in Brazil is estimated to generate from US$ 45 million to US$ 75 million a year, which accounts for the need for technological implementation, researches and marketing with such kind of activity.

Within this context, seed treatment with microorganisms has been used in order to protect plants against phytopathogens and promote higher growth rates. This mechanism includes general plant development, beneficial effects both in seed germination and seedling emergence and development, plus grain and fruit production. Seeds incorporation with aminoacids, micronutrients, plant growth regulators and mycorrhizas has been investigated in a great number of horticulture plant species, in order to obtain higher productivities and lower costs. Hormones, vitamins, sugars and other substances effect have also been assessed, aiming to interfere in a different and positive ways on seed metabolism and initial seedlings development (Sampaio & Sampaio, 1994).

The success of the horticulture depends on many factors, among them the initial condition for seedlings development which is fundamental to get a good stand for the transplant stage. The uses of less vigorous seedlings decrease the population of the culture or decrease the general plant development. Thus, the use of higher quality seeds is essential for a good stand in the field, as well as a more vigorous and productive potential of seedlings.

The cycle of life of the horticultural plants is normally short, which is a relevant factor when studying their nutrition. Some authors demonstrated that an external nutritional approach, by means of the localized fertilizer additions in simple formularizations or combinations, frequently make the seedling grown faster and more vigorous. In small sized seeds from horticultural plants the limited amounts of reserves may be balanced by means of their recovering with those essential nutrients for their initial development (Sampaio & Sampaio, 1994).

Hence, the present study aimed to evaluate sweet pepper seed physiological quality and their seedling development after film coated with antagonistic microorganisms, mycorrhizas, aminoacids, micronutrients, and plant growth regulators.

MATERIAL AND METHODS

Experiments were performed in a greenhouse in Lavras, Minas Gerais State, Brazil. Sweet pepper seeds were coated with a polymer to be added to the antagonistic microorganisms (*Trichoderma viride, Trichoderma polysporhum, Trichoderma stromaticum, Beauveria bassiana, Metarhizium*), mycorrhizas, aminoacids, micronutrients and plant growht regulators. The experiment was divided into two different assays (I and II). For each 100 g of *inoculum*, a kilo of talc was added to make easer the application and homogenization of the product to the seeds. The polymer Disco Trichodermina® was used for microorganisms inoculation, and Disco Clear® was used for the others materials. A volume of 50 mL of polymers additives was diluted in 50 mL of water. Then, the seeds were covered with that mixture at the dose 50 mL kg^{-1} per seed.

In assay I, the seed film coats and the inoculation of antagonistic microorganism were performed at the doses of 100 g per 100 kg^{-1} seed. The mycorrhizas were inoculated at the dose of 500 g per 100 kg^{-1} seed. The assay comprised the following treatments: seeds without covering (control seeds), seeds with Disco Trichodermina®, seeds with Disco Trichodermina® + *Trichoderma viride*, seeds with Disco Trichodermina® + *T. viride/T. polysporhum/T. stromaticum*, seeds with Disco Trichodermina® + *Metarhizium anisopliae*, seeds with Disco Trichodermina® + *Beauveria bassiana*, seeds with Disco Trichodermina® + mycorrhizas, seeds with Disco Trichodermina + *T. viride/T. polysporhum/T. stromaticum* + *Metarhizium anisopliae* + *Beauveria bassiana* + mycorrhizas.

In assay II, the seeds were treated with aminoacids, micronutrients, plant growht regulator and *Trichoderma viride*, which included the following treatments: seeds without cover (control seeds), seeds with Disco Clear® + micronutrients (200 g 100 kg^{-1}), seeds with Disco Clear® + micronutrients (400 g 100 kg^{-1}), seeds with Disco Clear® + plant growth regulator (5 mL kg^{-1}), seeds with Disco Clear® + plant growht regulator (10 mL kg^{-1}), seeds with Disco Clear® + aminoacids (5 mL kg^{-1}), seeds with Disco Clear® + aminoacids (10 mL kg^{-1}), seeds with Disco

Trichodermina® + *Trichoderma viride* (200 g 100 kg^{-1}). For each treatment, a solution was prepared with the polymer and the product in the above mentioned doses.

The products used in each treatment were provided by INCOTEC® Company (Netherlands) under the trade names Bruxil (powdered micronutrients), Stimulate (90 mg L^{-1} of kinetin, 50 mg L^{-1} of IBA and 50 mg L^{-1} of GA_3 / L of product) and Aminoacids + Zn. Mixture and the methodology of application were performed according to the manufacturers' instructions. The supplier of the product also performed the test for the inoculum viability.

After seed treatment with each product, the following tests were carried out: **Germination test**: sowing in germination boxes over blotting paper moistened with distilled water, in an equivalent amount to 2.5 times the weight of the dry substratum. Afterwards, the boxes were transferred to the germination chamber (BOD), in an alternate pattern of light and darkness (12 hours), at 25ºC. Four replications of 100 seeds of each treatment were used and results were expressed in percentages of normal seedling, according to the Rules for Seed Analyses (Brasil, 1992).

Emergency test under controlled conditions - sowing was performed in the substratum (soil + sand in 1:2 rate) on plastic trays. Substrate moisture was adjusted to 60% of retention capacity. Four replications of 100 seeds were used for each treatment. After sowing, trays were kept in a plant growth chamber, previously regulated at 25ºC, in an alternate pattern of light and darkness (12 hours). Since emergency start, daily evaluations were accomplished and the number of seedlings emerged was computed until stabilization of their number. The percentage of seedling emergency and emergency rate were calculated according to the equation proposed by Maguire (1962).

Seedlings - sowing was carried out in the commercial substratum Plantmax in a 128-cell styrofoam tray. Four replications of 100 seeds were performed in a greenhouse with controlled temperature and an automated irrigation system. When seedlings reached the transplant stage (at 30 days) the seedlings were counted, along with measurement of the height of the aerial part. Afterwards, the seedlings were removed from the substrate and washed under running water; the root system was separated from the aerial part and placed in paper bags. Then, they were taken to a forced air circulation stove (60ºC) until the seedlings reached a constant weight. Afterwards, root dry matter was calculated and results were expressed in grams per plant.

The assays were performed in a random design with eight treatments and four replications, in a total of 32 portions. Statistical analysis was performed with the aid of SANEST program (Zonta & Machado, 1984). Averages between treatments were compared with the Tukey test ($p < 0.05$).

RESULTS AND DISCUSSION

The seeds inoculated with all microorganism mixtures presented the lowest results (Tables 1 and 2). The negative effect on plant development might be due to competition for water, space, nutrients and oxygen among microorganisms and the antagonistic effects. Diniz et al. (2007) verified that the microbiolization of seeds with the mixture of *Trichoderma viride*, *T. polysporhum*, *T. stromaticum*, *Bassiana Beauveria*, *Metarhizium Anisopliae* with mycorrhizas, negatively affect the physiological quality of the seeds and seedlings development of lettuce. *Trichoderma viride*, *Metarhizium anisopliae* and mycorrhizas produced better shoot dry matter results, although they have only

Table 1 - Germination Percentage of seed (G), emergence (E) emergence rate (ER) and number of plants originating from sweet pepper seeds covered with materials (Assay I).

Treatment	G	E	ER	Nr. plants
	------------------ %	------------------	rate	number
Control seed	98.87 a	93.17 a	5.058 a	48.50 a
Disco Trichodermina	98.53 a	89.81 a	5.398 a	47.50 a
Trichoderma. viride	95.67 ab	96.32 a	5.477 a	48.50 a
Trichoderma sp. Mixture	98.15 ab	94.31 a	5.729 a	46.00 a
Metarhizium anisopliae	98.51 a	87.82 a	5.118 a	48.50 a
Beauveria bassiana	90.58 ab	89.40 a	4.934 a	48.00 a
Mycorrhiza	98.53 a	89.70 a	5.254 a	48.75 a
Mixture	82.81 b	73.06 b	3.588 b	38.00 b
C.V. (%)	2.1	3.9	7.8	2.8

Averages followed by different letters differ (Tukey test, $p < 0.05$).

Table 2 - Shoot dry matter (SDM), root dry matter (RDM) and height of the seedling shoot from sweet pepper seeds which were covered with materials (Assay I).

Treatment	SDM	RDM	Height
	------------------ %	------------------	rate
Control seed	0.12 abc	0.0205 abc	10.41 abc
Disco Trichodermina	0.09 bc	0.0169 bc	8.28 cd
Trichoderma viride	0.15 a	0.0309 abc	11.70 a
Trichoderma sp. Mixture	0.14 ab	0.0307 abc	11.56 ab
Metarhizium anisopliae	0.16 a	0.0367 ab	11.55 ab
Beauveria bassiana	0.13 abc	0.0285 abc	8.73 bcd
Mycorrhiza	0.16 a	0.0396 a	11.40 ab
Mixture	0.08 c	0.0135 c	7.33 d
C.V. (%)	2.2	28.1	1.4

Averages followed by different letters differ (Tukey test, $p < 0.05$).

differed from those treated with Disco Trichodermina® and from the microorganism mixture (Table 2).

The highest seedling root dry matter content was also found in the ones originated from seeds inoculated with mycorrhizas, which have also differed from treatments with Disco Trichodermina® both isolately, and treated with the microorganism mixture. On the other hand, the highest values of seedling shoot height were found for seeds inoculated with *Trichoderma* and *Metarhizium anisopliae* genera, also differing from Disco Trichodermina® treatment both separated and from the microorganism mixture (Table 2). Such result may be due to the *Trichoderma* sp. and mycorrhizas capacity to solubilize insoluble microelements in soil (Altomare et al., 1999) and also to absorb and translocate minerals such as phosphorus and nitrogen (Brown, 1974). Similar results were obtained by Okleifeld & Chet (1992) working with *Trichoderma* sp., that verified increases in the seed germination and in the foliar area of sweet pepper. Further, by inoculating commercial varieties of tomato and sweet pepper with mycorrhizas Nemec & Datnoff (1993) observed higher shoot dry matter content, higher root fresh matter content and an increase in height of shoot of the seedlings, according to the cultivar.

Assay II variance analysis results revealed differences for germination percentage, seedlings shoot and root dry matter separately (Table 3). The lowest germination percentage was registered for treatment with micronutrients, at the highest dosage (Table 3). Louzada & Vieira (2005) verified that the application of very high doses of micronutrients in bean seeds caused an increase of abnormal and deceased seedlings due to the toxic action of the micronutrients. *Trichoderma viride* and plant growth regulators at the dose 10 mL for kilo/seed presented the better shoot dry matter results, only differing from aminoacids + Zn treatment in the same dosage. As for root dry matter, the best results were obtained for the plant growth regulator treatment (5 mL kg^{-1} of seeds), which differed from micronutrients (200 g kg^{-1} of seeds) and aminoacids + Zn treatments (10 mL kg^{-1} of seeds) (Table 3). Diniz et al. (2006) verified that the epithelization of lettuce seeds with vegetal regulator and aminoacids + Zn and the inoculation with *Trichoderma viride* increase the emergency of the seedlings. Diniz et al. (2007) concluded that the vegetal regulators increase the speed of seedling emergency index of when incorporated to the lettuce seeds mL kg^{-1} between the 20 mL kg^{-1} and 30 mL kg^{-1} doses per seeds and the enrichment of these seeds with aminoacids did not affect seed germination, seedling emergency and the speed of seedling emergency index.

Overall, the treatments applied to the seeds did not provided expressive increases in the variables studied, when compared to the control. Nevertheless, there was a small profit when the seeds were enriched with the vegetable regulators. This suggests that higher doses may give better results, mainly with seeds of low vigor. Aragão et al. (2003) studied the effect of the plant growth regulators in seed germination and vigor of corn seedlings and observed that the higher dosages provided the best germination percentage. These substances, which are mediators of the physiological germination processes, transform specific environmental signals in biochemistry answers, producing modifications in the physiological state of the seeds, through the differential transcription, repression or genetic disrepression or the activation of the RNA messenger or through changes in the membrane permeability (Davies, 1994). The modifications in the physical properties of the membranes directly affect the rate of moisturization, enzyme releases, ionic transportation, pH and inhibitor content, these situations intervene with seed germination.

Table 3 - Germination percentage of seeds (G), shoot dry matter (SDM) and root dry matter (RDM) of seedlings originating from sweet pepper seeds covered with materials (Assay II).

Treatment	G	SDM	RDM
	%	------------------------ g	-------------------------
Control seed	95.74 a	0.10 ab	0.0173 ab
Micronutrients (200 g)	81.85 ab	0.10 ab	0.0151 b
Micronutrients (400 g)	79.49 b	0.11 ab	0.0192 ab
Plant growth regulator (5 mL)	98.22 a	0.11 ab	0.0230 a
Plant growth regulator (10 mL)	99.50 a	0.12 a	0.0215 ab
Aminoacids + Zn (5 mL)	98.85 a	0.11 ab	0.0219 ab
Aminoacids + Zn (10 mL)	98.94 a	0.09 b	0.0154 b
Trichoderma viride	96.29 a	0.12 a	0.0218 ab
C.V. (%)	13.6	11.1	0.3

Averages followed by different letters differ (Tukey test, $p < 0.05$).

The use of micronutrients, aminoacids, mycorrhizas and antagonistic microorganism regulators in seeds has shown great potential for the increase of the productivity, even though its use is still not a practical routine in cultures that do not reached a high technological level. Results achieved either here or in other works have shown that the incorporation of such products in seeds is a promising technique, but still provides inconsistent results. Copeland & McDonald (1995) assert that, in many cases, the biological treatment of seeds is not persistent in soil, seed or plant, under natural conditions. Therefore, a deeper research concerning the methodology of application, doses, storage of coated seeds, responses from different species to the materials, types of products and polymers should be performed, so that such technology could be adopted by companies and producers with higher security, thus yielding the expected results.

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