Australian Journal of Basic and Applied Sciences, 11(13) October 2017, Pages: 54-62

AUSTRALIAN JOURNAL OF BASIC AND APPLIED SCIENCES

ISSN:1991-8178 EISSN: 2309-8414
Journal home page: www.ajbasweb.com

Control of *Macrophominaphaseolina* and the Effect on Quality of Sesame Seeds (*Sesamumindicum*L.)

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ARTICLE INFO

Article history:
Received 10 August 2017
Accepted 1 October 2017
Available online 18 October 2017

Keywords:
biological inoculants, alternative control, physiological quality, infection.

ABSTRACT

Sesame is multiplied by means of botanical seeds, they are the strategic input for the crops, therefore, the quality of the seeds is of utmost importance to achieve optimal results within the agricultural production. One of the main limitations of the management of the sesame crop in Paraguay is the carbonaceous rot caused by the pathogen *Macrophominaphaseolina*. Taking into account that most of the current production in Paraguay is practically organic, the implementation of biological control in order to reduce inoculum density provides a control alternative applicable to the small producer, in order to achieve sustainable production, due to the null use of agrochemicals. Two experiments were carried out in the Biotechnology Laboratory of CEMIT/UNA, greenhouse and Laboratory of Seed Quality Analysis FCA/UNA, Paraguay in the 2016, with the objective of studying the biological inoculants effects in the treatment of sesame seeds, taking into account alternatives that can be used in said crop.**Experiment 1:**Effect of *Trichoderma* sp. and *Azospirillumbrasilense* on control of *Macrophominaphaseolina* in greenhouse. The percentage of the pathogen infection was evaluated and the methodology of Orrego*et al*. (2009) was used.The experimental design was completely randomized with 6 treatments: T 1: Sesame seeds, without biological or chemical treatment; T 2: Seeds inoculated only with *M.phaseolina*; T 3: Seeds treated with chemical fungicides; T4: Seeds inoculated with *Trichoderma*sp.; T5: Seeds inoculated with *A brasiliense*; T 6: Seeds inoculated with *Trichoderma*sp. plus *A. brasiliense*, with 4 replicates, making a total of 24 experimental units.**Experiment 2**The physiological quality of sesame seeds, treated with *Trichoderma*sp., and *A. brasiliense***.** The variables evaluated were germination and vigor, taking into account the methodologies described in ISTA(2011) and Krzyanowsky (1999). The design used was completely randomized with 4 treatments and 4 replicates. The results obtained evidenced that the inoculation with *Trichoderma*sp., alone orin combination with *A. brasiliense,* reduce the percentage of infection of *M. phaseolina*to 26.52%; the seeds treated with *Trichoderma*sp., *A. brasiliense*or the mixture of both do not affect the germination obtaining values higher than 95% for all the treatments and that *A. brasiliense*the growth of seedlings reaching up to 5.09 cm in length.The alternative control using biocontrollers would be very useful when including within the integrated management of carbonaceous rot, with emphasis on crops such as sesame which requires the minimum possible impact of agrochemicals, being able to open doors to other demanding markets in terms of quality of the product offered.

ToCite ThisArticle: Karen Coronel Acuña, YesminaLezcano Aquino, Líder Ayala Aguilera, Gerald Sormanti Valenzuela, Johana González Vera, Antonio SamudioOggero, Pamela Peña Alvarenga., Control of *Macrophominaphaseolina* and the Effect on Quality of Sesame Seeds (*Sesamumindicum* L.). ***Aust. J. Basic & Appl. Sci.,*** *11(13): 54-62, 2017*

INTRODUCTION

The cultivation of sesame seeds, is an area of great economy importance in Paraguay, because it is the smaller producers who are involved in it. It is highly valued in human feeding for its quality and taste, whose oil content in the seeds ranges from 45% to 50 %, 37% proteins and 10% carbohydrates (Valinotti, 2006).

The sesame is produced practically as an organic crop, with a minimal use of chemical products due to demands of the main import markets. Furthermore, the greatest demandis for the product in its natural form, it has a preference for the variety white Escoba(USAID, 2009).

For the agricultural year 2015/2016 the average national yieldswere 390 kg / ha, with a total production of 21,450 tons in 55,000 hectares of planted area, these values being lower than previous harvests. The most important departments are San Pedro, Concepción, and Boquerón (Ministerio de Agricultura y Ganadería - MAG 2016).

More than 95% of Paraguayan sesame production is destined to export, especially Middle East and Asia, countries like Japan are among the main importers of this raw material, being these the ones of the highest in demand, in addition to othersbelonging to the African continent and to a lesser extent to the American sector (USAID, 2009).

According to the same source named above, due to the increase on the planted surfacerecent years, combined with a poor management, lack of crop rotation, use of uncertified seeds, inadequate sanitation techniques and excessive rains. A lot of problems were generated, related especially to diseases, and which led to a low quality of seeds, creating a negative impact in the productivity.

Wulff and Pascholati (2005), emphasize that some of the most common fungal diseases are:Leaf Spot caused by the agent *Alternariasesami*, Cercosporiosis, caused by *Cercosporasesami*;Angular leaf spot caused by *Cercosporasesamicola*; Burning ofCorynespora by *Corynesporacassiicola;* The “Damping off” or root rot is caused by several fungu’s being the most common: *Macrophominaphaseolina*, *Fusariumoxysporium* f. sp. *sesami*, *Rhizoctoniasolani*, *Phytophthoraparasítica* f. sp. *sesami* and*Sclerotium (Corticum) rolfsii*.

As the fungus progresses through the plant, it produces sclerotia both on the surface and in the medullary region, which becomes dry and gray, for this reason the disease is known as carbonaceous rot (Mendoza and Laurantin, 2012).

The carbonic rot caused by the fungus *Macrophominaphaseolina*,mainly affects the root and vascular system of the plant, hindering the transport of water and nutrients. As a consequence, it obtains a reduction of the yield and also it attacks to a great diversity of crops,so thatits control without the help of an integrated management is difficult (Garcete, 2011).

However, there are chemical products used in the treatment of seeds to control or prevent diseases. In the sesame, this practice is very efficient but very difficult to implement, because the seeds, almost all of them, are exported in natura as a confectionery type, which requires that they present a high security as the absolute absence of phytosanitary(Ayala *et al*., 2011).

Cardona and Rodriguez, cited by Mendoza and Laurentin(2012), mention that many procedures have been studied to minimize the losses caused by *M. phaseolina* and in the search for alternative control biological is indicated, especially with the fungus *TrichodermaharzianumRifai*.

Infante*et al*. (2009), also mentioned that many studies have been carried out due to their effective control, reproductive capacity, ecological plasticity, stimulating effect on crops and recently their action as an inducer of systemic resistance in the plant to different pathogens.

In the same way, Vinale*et al*. cited by Grabowski*et al*. (2014), point out that their bioregulatory action is indirect, they also mention that it has been investigated for its ability to protect plants and control pathogens in different soil conditions by producing numerous biologically active compounds such as enzymes that degrade the cell wall and metabolites which has led to its commercialization as biopesticides and biofertilizers.

As a mechanism of alternative control, it is proposed the use of live microorganisms that act as a biocontrol agent for the management of diseases. Inside of the most studied biological controllers the genus*Trichoderma* stand out for its antagonistic potential a several pathogens that cause diseases of great importance in the agriculture in addition to the *Azospirillum*genus (Hernández *et al*., 2001; Garcete, 2011).

For all the above mentioned, this paper aims to determine the effect of biological inoculants on the control of *M.phaseolina*and the quality of seeds in terms of germination and vigor of sesame seeds.

MATERIALS ANDMETHODS

Two experiments were denominated: Experiment 1: Effect of *Trichoderma*sp. and *Azospirillumbrasiliense* on the control of *Macrophominaphaseolina* (Tassi) Goid. and Experiment 2: Effect of biological inoculants on the germination and vigor of sesame seeds.Thefirstexperimentwascarriedout inthe Laboratorio de Biotecnología del Centro Multidisciplinario de Investigaciones Tecnológicas (CEMIT/UNA), and thesecondin thegreenhouse of the experimental field of thearea of Protección Vegetal, and in the Laboratorio de Análisis de Calidad de

Semillas del Área de Producción Agrícola, bothareasbelonging to theFacultad de Ciencias Agrarias de la Universidad Nacional de Asunción-Paraguay (FCA-UNA).The experimental period covered the months of April and May of 2016.

The sesame seed used was the Escoba variety, obtained from the experimental field of the FCA/UNA produced in the agricultural period of 2014/2015, isolated from the pathogen *Macrophominaphaseolina,* in addition to commercial strains of *Trichoderma*sp. and *Azospirillumbrasiliense,* one with a Colombian origin and the other native. The chemical products used were purchased in the same way the form in the local market.

For the experiment 1, the treatments consisted in the application of biological inoculants in sesame seeds, with a commercial product based on *Trichoderma*spp., a product based of *Azospirillumbrasiliense,* a chemical fungicide with active ingredients Carbendazim plus Thiram, a positive control (seedsnot inoculated with *M. phaseolina*withoutany treatment), and a negative control (seeds inoculated with *M. phaseolina*without any treatment). The doseused were those recommended by the manufacturer. The Experiment consisted of 6 treatments (observed in the Table 1) and 4 replicates, with 24 experimental units, in which each experimental unit was composed of 10 pots, making a total of 240 seedlings.

Table 1: Description and dose of treatments applied in seeds. FCA-UNA. San Lorenzo, Paraguay. 2016.

TREATMENTS	DESCRIPTION	DOSAGE
T1	Positive control : Seeds without inoculation with the pathogen and no biological or chemical treatment	-
T2	Negative Control: Seeds inoculated only with *Macrophominaphaseolina*	-
T3	Seeds treated with *Carbendazim* 15% plus Thiram 35%	200 ml/100 kg of seeds
T4	Seeds inoculated with *Trichoderma*sp.	1 g /l
T5	Seeds inoculated with *Azospirillumbrasiliense*	250 ml/50 kg of seeds
T6	Seeds inoculated with *Trichoderma*sp. and *A. brasiliense*	1 g plus 250 ml

The *M. phaseolina*fungus was isolated from commercial sesame seeds of the Escoba variety, provided by the Laboratorio de Análisis de Calidad de Semillas of the FCA/UNA. The same, were seeded in Petri dishes on PDA cultivation. Later, they were introduced into an incubator at 25°C for five days. The seeds that showed formation of propagation structures of *M. phaseolina* were removed from the fungus hyphae until the obtention of pure colonies, following the methodology adapted by (Orrego *et al*., 2013).

The inoculum suspension of *M. phaseolina*, was prepared from 10 Petri dishes with pure colonies of the fungus, using the methodology cited by Orrego*et al*. (2009), which is described below:

- 20ml of distilled water were added to the Petri dishes with 10 days of incubation and the mycelial growth was scraped offwith a fine bristle brush.
- The supernatant of each Petri dish, was transferred to a beaker, adding sterilized distilled water to make up to 250 ml.
- Then, 100 grams of sesame seeds of the Escoba variety were weighted, conditioning them in a plastic tray and adding 100 ml of the suspension of the fungus.
- Finally, the seeds were soaked in the suspension for 10 minutes and dried up on a filter paper at room temperature.

The preparation of the suspension of commercial formulation of *Trichoderma*sp.consisted of weighing on a digital balance, 1 gram of the product, which was placed in a beaker, adding 500 ml of distilled water, followed by stirring with a glass rod in a manner to be diluted and homogenized.

For the suspension with *Azospirillumbrasiliense,* 0.4ml of the product was taken with a syringe and transferred to a test tube, adding 30 ml of distilled water, followed by stirring until obtaininga homogeneous suspension.

After the suspensions of the commercial formulations were prepared, 30ml of each dilution were added to the sesame seed previously infested with *M. phaseolina.*

In the greenhouse, 15x20 cm black polyethylene pots were used, loaded with substrate consisting of fat sand, bovine manure in a 2:1 proportion previously sterilized by the physical methods of heat, exposed to a temperature of 80°C for 30 minutes. Subsequently, 4 seeds were plantedequidistantly in pots and with the emergency, a thinning was made leaving only one seedling, the irrigation was performed as needed.

For the determination of the effect by the biological inoculants, the methodology of Orrego*et al*. (2009) was used. Where the percentage of infection in seeds was calculated by the quantification of colonies of *M. phaseolina.* For this purpose, roots were obtained from four seedlings for each treatment and were planted in Petri dishes containing PDA culture following the steps below:

- A cut (2 cm, from the stem to the neck) and a disinfestation by immersion in 70% alcohol solution for 30 seconds, passing to a sodium hypochlorite for 30 seconds. Subsequently a triple wash with distilled water was made.
- Dried on filter paper.
- Sowing the stems on PDA, in Petri dishes.

- Incubation at room temperature of 25 ° C for 4 days and subsequent counting of roots with mycelial growth of the fungus. Theresultswereexpressed as percentage of infection.
-

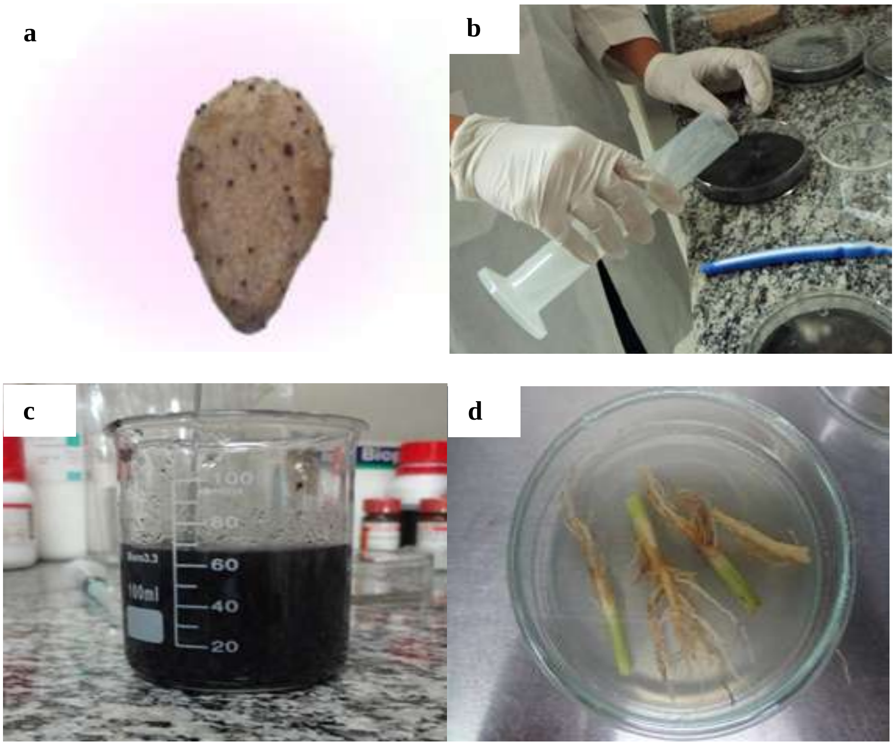

Fig. 1a, 1b, 1c, 1d: Procedures of isolating of the pathogen from sesame seeds, preparing the suspension to be used, regulating of the concentration and sesame roots previously inoculated.FCA-UNA. San Lorenzo,Paraguay. 2016.

The Experiment 2 consisted in the inoculation of sesame seeds with the commercial formulations of *Trichoderma*sp. and *A. brasiliense,* using the dose recommended by the manufacturer. The design used as shown in Table 2, was randomly selected with 4 treatments and 4 replicates, making a total of 16 units. Each experimental unit consisted of a Gerbox dishcontaining 100 seeds each.

Table 2: Description and dose of commercial products based on*Trichoderma*sp. and *A.brasilense* applied on Sesame seeds. FCA-UNA. San Lorenzo. Paraguay. 2016.

TREATMENTS	DESCRIPTION	DOSAGE
T1	Control (seeds without inoculation)	-
T2	Seeds inoculated with *Trichoderma*sp.	1 g/l
T3	Seeds inoculated with *A. brasilense*	250 ml/50 kg of seeds
T4	Seeds inoculated with *Trichoderma*sp.+ *A. brasilense*	250 plus 1 g

For the inoculation of the sesame seeds with the commercial products based on *Trichoderma*sp. and *A.brasiliense*was used the same method described in the Experiment 1 was used, differing only in that the seeds were not inoculated with *M. phaseolina.*

To determine the germination, it was carried out according to the ISTA (International Seed Testing Association, 2011) rules, using 4 replicates of 100 seeds for per treatment. First, the seeds were sowed in 11 x 3.5cm of Gerbox dishes, with double substrate of sterilized paper and then moistened with distilled water. Subsequently, the seeds were deposited in a germination chamber to an alternating temperature of 20-30°C. The reading was made 7 days after the sowing, it was considered normal seedlings those that did not presentevident damage and contained all its essentials structures. The results were expressed as percentage of normal seedlings.

The vigor test was performed according to the methodology proposed by Krzyzanowskiet al. (1999). It wasused 4 replicates of 10 seeds for each treatment. The seeds were sowed on Gerbox dishes, with double substrate of sterilized paper and then moistened with distilled water. It is worth to mention that the seeds were placed on the double substrate paper, the radical tip was directed to the floor, in order to orient the growth of the roots as straight as possible, to facilitate the measurement of the seedlings length. Subsequently, the plates were placed forming an angle of 45º with wall of the germination chamber, at an alternating temperature of 20-30°C for seven days.

At the end of this period, the root length of the seedling considered normal was evaluated with the aid of a centimetric ruler and it was determined the average of each treatment. The results were expressed in centimeters:

The results of both experiments were subjected to an analysis or variance (ANAVA) and a comparison of means was performed in all treatments, using the Tukey test with 5% probability of error.

RESULTS AND DISCUSSION

Experiment 1: Effect of Trichoderma sp. and A. brasilienseon M. phaseolina (Tassi) Goid.

The results obtained for the determination of the control of the pathogen, are observed in Table 3. They indicate that there are significant statistical differencesin respect to the studied variable (percentage of infection) among the different evaluated treatments. The coefficient of variation and mean values obtained were 12.66% and 83. 96%, respectively.

Table 3: Percentage of *M. phaseolina* infection in seedlings roots from seeds inoculated with biological and chemical agents. FCA-UNA. San Lorenzo, Paraguay. 2016.

TREATMENTS	DESCRIPTION	INCIDENCE (%)
T4	Seeds inoculated with *Trichoderma*sp.	69,38a
T6	Seeds treated with *Trichoderma*sp. and *A. brasilense*	75,63ab
T3	Seeds treated with Carbendazim 15 plusThiram 35%	82,08ab
T1	Positive control : Seeds without biological or chemical treatment	88,77ab
T5	Seeds inoculated with *A. brasilense*	92,56ab
T2	Negative control: Seeds inoculatedcon *M. phaseolina*	95,19b

*Means with the same letters in the columns do not differ between each other ($p > 0.05$) by the Tukey at 5% probability of error.

The treatment corresponding to the seeds inoculated with *Trichoderma*sp, presented the lowest percentage infection, 69.38% of root infection with *M.phaseolina*presence*;* differing statisticallywith the treatment of seeds inoculated only with *M.phaseolina,* which obtained a percentage of 95.19% of incidence in roots with the presence of the pathogen. The same did not present differences with the other treatments. These results agree with Garcete (2011) who, selecting isolates of *Trichoderma*sp and inoculating in sesame seeds, demonstrated that it presents great efficiency in the control of *M.phaseolina,* reducing 33% the incidence of the disease.

On the other hand, Orrego (2013), in a study of phytopathogen biocontrol fungi, mentions that the use of *Trichoderma*spp., reduced significantly the percentage of soybean seedlings infected with *M. phaseolina,* but the level was not less than 60%.

Garcete cited by Grabowski*et al*. (2016) mentions that, investigating carbonaceous rot in sesame cultivation in Paraguay, it was found that after a selection of native strains of *Trichoderma* spp., five reduced the incidence of the disease.

It is important to consider the percentage of incidence presented in the treatment of inoculated seeds with *Trichoderma*sspp., in relation to the negative control since the use of this biological organism would constitute a useful tool in the management of the pathogen USAID (2009), in that sense mentions that emerging diseases such as these produced by the fungus *M. phaseolina*in the sesame crop, can be controlled and combated with an adequate integrated management.

Orrego (2008), in relation to the previously exposed, mentions that it is fundamental to pay attention to *M. phaseolina*which in combination with other fungi may present asynergism, which added to favorable conditions for the pathogens could generate losses up to 100% ashappened in the agricultural cycle 2007/2008 in Paraguay.

However, Gonzalez *et al*. (2005), in a study about the effectiveness of *Trichoderma*spp. in the control of pathogenic of seeds and soil fungi in bean cultivation, mention that applying 2 isolates of the biocontrol in the seeds, it was obtained an average control of 87.7%. Likewise, Ezziyani*et al*. (2004), obtained a control for other pathogens such as *Phytophtoracapsici*in pepper with values of up to 100% inhibition, using *Trichoderma* spp.

As for the use of *Trichoderma* with *Azospirillumbrasiliense*, it shows a slight decrease in the incidence of *M. phaseolina*with a 75.63%o infected roots, this demonstrates that, the mixture of these microorganisms inhibit or reduce in a certain way the development of the fungus pathogen.

Bécquer *et al*. (2001), states that the bacteria and fungi of the rhizosphere, can produce allelochemical substances or antibiotics that prevent the development of diseases caused by edaphic pathogens in plants.

It is of vital importance to take into account the origin of the strains, because these being natives, are more adapted to the environment than those coming from areas with different climatic characteristics, and thus can reach their maximum potential.

Sanabria and Grabowski (2016), agree with the previously cited and mention that for an efficient control, this antagonist must be isolated from soils with ecological conditions, similar to those that are reintroduced, thus ensuring their antagonistic expression.

Following the analysis in the Table 3, it was observed that the treatment with fungicides did not significantly reduce the percentage of infection of *M. phaseolina*, having 82.08% of the roots infected by the pathogen, being superior to the seeds treated with the antagonist fungus *Trichoderma*sp.

Considering the requirements needed by the main demanding markets of this product and the certification companies that grant the quality of organic production, it is of vital importance to resort to biological alternatives that can be used in the management of pests and diseases, besides to guarantee the high safety levels of food, especially those that are of direct consumption (USAID, 2009). In this sense, the results obtained with the fungus *Trichoderma* sp. as a controlling agent are promising to meet these requirements.

Experiment 2:Quality of sesame seeds treated with biological inoculants Trichodermasp., and A.brasilense.

Germination

It is possible to observe in Table 5 the results corresponding to the analysis of germination to the different treatments evaluated. The data expressed as a percentage show the values of sesame seeds treated with biological inoculants based on *Trichoderma*sp. and *A.brasiliense.* The analysis of variance showed that there were no significant differences between the treatments. The coefficient of variation and mean values obtained were 1.85% and 97% respectively.

Table3: Germination of sesame seeds treated with biological inoculants *Trichoderma*sp. and *A. brasilense*. FCA-UNA. San Lorenzo, Paraguay. 2016.

TREATMENTS		GERMINATION (%)
T4	Biological treatment with *Trichoderma*sp. +*Azospirillumbrasiliense*	98a
T1	Control: Seeds without biological inoculation	97a
T3	Biological treatment with *A. brasiliense*	97a
T2	Biological treatment with *Trichoderma*sp.	96a

The SENAVE (Servicio Nacional de Calidad y Sanidad Vegetal y de Semillas, 2014), establishes within the compendium of the Paraguayan regulations on seeds, that for the commercialization of sesame, these must exceed the 90% of germination. The results obtained in this experiment showed percentages of germination above the minimum requirement.

Rodriguez *et al*. (2008), mentions that the temperature, humidity, oxygen, light and the adequate combination of these, are factors that propitiate the germination of seeds. These factors are artificially controlled during the germination tests, creating and ideal environment for all the seeds that demonstrate their germinating power.

However, it is important to note that, in the tests of germination in the laboratory, the presence of inhibit or pathogen substances may cause less real expression of the actual potential of the germination batch. Orrego and Garcete (2008), mention that the pathogens *M. phaseolina*, *Fusarium*sp., *Alternaria*spp., *Cercospora*spp., *Curvularia*spp. present in the seeds, can reduce the germination percentage.

The results of this experiments, evidenced that the use of, or the mixture of both, do not interfere in the expression of the germinating power of the seeds. It is demonstrated, in this way, that that the use of these inoculants in the sesame is feasible.

Vigor

The data obtained for the vigor variable, by the analysis of seedling root length, can be observed in Table 6. The results of the analysis of variance obtained and expressed in centimeters, show that there are significant differences between the evaluated treatments. The coefficient of variation and the means values obtained were 13.30% and 5.13 %, respectively.

Table 4: Root length of sesame seedlings (cm) obtained from sesames seeds treated with biological inoculants *Trichoderma*sp. and *A.brasiliense.* FCA-UNA. San Lorenzo, Paraguay. 2016.

	TREATMENTS	ROOT LENGTH (cm)
T3	Biological treatment with *Azospirillumbrasilense*	5,09a*
T4	Biological treatment with *Trichoderma*sp +*A. brasilense*	3,91b
T2	Biological treatment with *Trichoderma*sp.	3,73bc
T1	Control: Seeds without biological inoculation	2,68c

*Means with a common letter are not significantly different ($p > 0.05$) by the Tukey at 5% probability of error.

The treatment of seeds inoculated with *A.brasiliense*, was the one that presented the best result with an average length of 5.09cm, presenting significant differences in relation to the other treatments. On the other hand, the control was the treatment that presented a smaller length of seedlings with a mean of 2.68cm, without statistical differences in relation to the biological treatment with *Trichoderma*sp.

Acebo *et al*. (2007), in a study of the growth promoting effect with strains of *Azospirillum*sp., in rice cultivation mentioned to have obtained a remarkable increase in the parameters held for said variable in relation to the control.

On the other hand, Nuncio *et al*. (2004), reported increases in the root growth on pepper seedlings by inoculating seeds with *Azospirillum*sp.

Infante*et al*. 2009, emphasize that *Trichoderma* species have the ability to create an environment conducive to radical development which increases plant tolerance to stress.

Mangmang (2015), consider*Azospirillum* as one of the most studied plant growth promoter rhizobacteria genus due to its ability to improve the growth and development of numerous plant species of agricultural interest. Several authors (Canto, 2004; Parras and Cuevas, 2001; Pazos *et al*., 2000; Rives *et al*., 2007) claim that the bacteria of the genus *Azospirillum*, produce phytohormones such as a indoleacetic acid, gibberellins and cytokinins, that stimulate the growth of the plants in the aerial part and the roots, generating hairs and lateral roots.

The fungi of the genus *Trichoderma* are used as controlling agents of soil pathogens, however, studies performed by Camargo and Avila (2013), show that in addition to the biocontrol effect, they produce metabolites that directly stimulate the process of plant development.

Infante*et al*. (2009), points out that *Trichoderma* presents several mechanisms, whose bioregulatory action is of indirect form, among these can be mentioned those that induce mechanisms of physiological and biochemical defense as the activation in the plant of compounds related to the resistance, with the detoxification of toxins excreted by pathogens and the deactivation of enzymes from these during the infection process; the solubilization of nutrients, which in their original form are not accessible to plants.

The results of this work, put into evidence that the inoculation of sesame seeds with*A.brasiliense*and *Trichoderma*sp., alone or in mixture, favor a greater growth in relation to the seedlings coming from seeds without inoculation.

In addition, it was verified that the use of *Trichoderma*sp. with *Azospirilum*, did not allowed to match their highest potential reached individually for both experiments. Further research is suggested, in order to determine any antagonistic effects that may exist between the two agents.

Conclusions:

The inoculation of seeds with *Trichoderma*sp or in combination with *Azospirillumbrasiliense*decrease the percentage of infection of *Macrophominaphaseolina.*

The use of *Trichoderma*sp., *Azospirillumbrasiliense* or the mixture of both, does not interfere on the germination of the sesame seeds.

The use of these biocontrol agents, especially*Azospirillumbrasiliense*, promote the growth of the roots of sesame seedlings during the initial phase.

Taking into account the data obtained in the present study, it is of utmost importance to continue studying antagonists such as *Trichoderma*spp., its biocontrol effect in the sesame crop and to evaluate some benefits that would be determinant within an integrated management such as induction of resistance.

The various advantages of the use of *Trichoderma*spp., as a biological control agent, among which should be mentioned; a rapid growth and development, production of a large number of enzymes, inducible with the presence of phytopathogenic fungi, make it a viable alternative for family agriculture in which sesame is developed in Paraguay.

In addition, studies on its use in combination with other stimulant agents such as *Azospirillum* would provide important benefits taking into account crop performance under field conditions.

These bio-controllers are key to the development of bioproducts so we must encourage the interest of farmers for this type of control, which are very useful, perhaps not to eradicate diseases but, for the integrated and efficient management of systems sensitive to degradation or to conquer demanding markets with the use of phytosanitary products.

With the banned use of chemicals in the sesame crop, the use of biological microorganisms would play an important role in the management of the crop, since it is managed as organic and it handles demanding standards in terms of production.

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