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COMPATIBILITY OF FUNGICIDES WITH *Trichoderma asperelloides* AND *Azospirillum brasilense*

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ABSTRACT - The use of fungicides to control agricultural diseases has been increasing in agriculture. Thus, the biological control using fungus is an effective tool in the integrated diseases management. This study aimed to evaluate the compatibility of fungicides Certeza, Apron and Vitavax on the microorganisms *Trichoderma asperelloides* and *Azospirillum brasilense*. The experiments were conducted *in vitro* in a completely randomized design with a factorial arrangement 2 x 3 with three replications. The first factor composed of two doses and the second referred to the fungicides. The microorganisms were developed in PDA medium. After the medium has solidified, in a flow layer 10 µL of the fungal solution was added to each plate with the aid of an automatic pipette (D1, recommended dose; D2, double of recommended dose). Then, each Petri plate was centrally inoculated with a disc of *Trichoderma asperelloides*, in addition to the uninoculated control. For *Azospirillum brasilense*, the colony surface was scraped with the aid of a sterilized Drigalsky loop. Then, the filter of discs paper was dipped in the fungicides solution and placed on the medium surface. After this process, the plates were incubated in a BOD (Biochemical Oxygen Demand) at 26±2ºC and photoperiod 12 h. The parameters evaluated were a mycelial growth and sporulation. The results showed that there was no significant interaction between the factors. The fungicides Certeza (Tiofanato metílico + Fluazinam), Apron (Fludioxonil + Metalaxil-M) and Vitavax (Carboxina + Tiram) are considered compatible with *Trichoderma asperelloides* and *Azospirillum brasilense*. The fungicides can be used in an integrated disease management practices, priority must be given to use the products that have been shown to be less harmful.
Keywords: biological control, chemical combination, phytosanitary products, seed treatment.

COMPATIBILIDADE DE FUNGICIDAS COM Trichoderma asperelloides E Azospirillum brasilense

RESUMO - O uso de fungicidas para o controle de doenças agrícolas vem aumentando com frequência na agricultura. Assim, o controle biológico utilizando fungos representa uma ferramenta efetiva no manejo integrado de doenças. Este trabalho teve como objetivo avaliar a compatibilidade dos fungicidas Certeza, Apron e Vitavax sobre os microrganismos *Trichoderma asperelloides* e *Azospirillum brasilense*. Os experimentos foram desenvolvidos *in vitro*, em delineamento experimental inteiramente casualizado em esquema fatorial 2 x 3, contendo três repetições. O primeiro fator correspondeu às doses, o segundo se referiu aos fungicidas. Ambos os microrganismos foram repicados em meio de cultura BDA (Batata, Dextrose e Ágar). Após a solidificação do meio, em camada de fluxo adicionou-se em cada placa 10 µL da solução fúngica com o auxílio de uma pipeta automática (D1, dose recomenda; D2, dobro da dose recomendada). Em seguida, foi depositado no centro de cada placa um disco do *Trichoderma asperelloides*, além da testemunha sem inoculação. Para *Azospirillum brasilense*, foi realizada a raspagem da superfície da colônia com o auxílio de uma alça Drigalsky. Em seguida, foram utilizados discos de papel de filtro, os quais foram mergulhados nos fungicidas e colocados em contato com a superfície do meio de cultura. Após esse processo, as placas foram mantidas em câmara do tipo BOD à temperatura de 26±2 ºC e fotoperíodo de 12 h. Os parâmetros avaliados foram crescimento microbiano e esporulação. Os resultados mostraram que não houve interação significativa entre os fatores. Os fungicidas Certeza (Tiofanato metílico + Fluazinam), Apron (Fludioxonil + Metalaxil-M) e Vitavax (Carboxina + Tiram) são considerados compatíveis, tanto com *Trichoderma asperelloides*, quanto com *Azospirillum brasilense*. Os fungicidas podem ser usados em práticas do manejo integrado de doenças, porém, deve-se dar prioridade ao uso dos produtos que se mostraram menos prejudiciais.
Palavras-chave: controle biológico, combinação química, produtos fitossanitários, tratamento de sementes.

INTRODUCTION

Brazil is one of the important actors in world agricultural production, the expansion of new crop areas has caused a series of phytosanitary problems, especially regarding the control of diseases, which can cause damages in agriculture (SMANIOTTO et al., 2010; CABRAL et al., 2011; BALDIN et al., 2014).

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In fungal disease management programs, the use of fungicides has been increasingly used, but the intensive use for the control of soil borne diseases are costly and also produce environment and health hazards to men and also adversely affect the beneficial microorganisms in soil (MACÍAS-RODRÍGUEZ et al., 2020). Among the many alternatives for sustainable agriculture, biological control using microorganisms is a strategy of great interest to reduce or replace the use of pesticides. It is a natural and ecologically correct tool that can be successfully exploited in the integrated pests and diseases management (PATEL; SINGH, 2020).

The disease control through seed treatment is a widely used practice in crop management. In addition for controlling important pathogens and insect attacks, it is an efficient practice to ensure adequate plant populations (ESPINOSA et al., 2020). It promotes the initial development of plants.

Among the groups of microorganisms most studied for this purpose, the genus *Trichoderma* stands out. They are regarded as free-living microorganisms that can inhibit pathogens and increase plant immunity and growth (MUARYA et al., 2020). These fungi have emerged as the most powerful biological control agents for the management of soil-borne plant diseases and have become more valuable in controlling agricultural diseases. They act through several mechanisms, such as competition, antibiosis, resistance induction, and growth promoter (ZIN; BADALUDDIN, 2020; STEFFEN et al., 2019), capable of generating benefits in the germination processes and the initial development of plants through the treatment of seeds and their direct application to the soil. These genera of *trichoderma* have received more attention, several studies have reported their benefits in several crops of agronomic importance. In this sense, the use of the combination fungicides that are compatible with bioagents can protect the crops against soil borne diseases (VASQUEZ et al., 2018).

The *Azospirillum brasilense* represents a promising technology associated with several positive factors for crops such as phytohormone induction, nitrogen fixation, root growth and greater accumulation of nutrients (SANTOS et al., 2020). Considering the successful use of *Azospirillum brasilense* and the fact that the seeds are frequently exposed, it is very important to study the combination of this bacterium with fungicides.

Thus, the objective of this work was to evaluate the compatibility of fungicides Certeza (Tiofanato metílico + Fluazinam), Apron (Fludioxonil + Metalaxil-M) and Vitavax (Carboxina + Tiram) with *Trichoderma asperelloides* e *Azospirillum brasilense in vitro*.

MATERIAL AND METHODS

The experiment was conducted at the Applied Agromicrobiology and Biotechnology laboratory of the Federal University of Tocantins (UFT), Campus of Gurupi, located at the coordinates 11°43’45” S and 49°04’07” N, and 280 m of altitude. The local climate characterization is humid tropical, cerrado or tropical savannah vegetation according to Köppen-Geiger classification (CHAGAS JÚNIOR et al., 2021).

Three fungicides were used: Certeza, whose active ingredients are Tiofanato metílico + Fluazinam, Apron (fungicide for seed treatment, used to control soil diseases), constituted by Fludioxonil + Metalaxil-M) and Vitavax composed by Carboxina + Tiram. To obtain the products, the solutions were prepared with distilled water, filtered and packed in autoclaved falcon tubes, and stored in the refrigerator until the beginning of the experiment. The strains of *Azospirillum brasilense* and *Trichoderma asperelloides* were acquired from the bank collection of the Applied Agromicrobiology and Biotechnology laboratory of UFT.

The isolate of *Trichoderma* was developed in Petri plates, which were autoclaved at 120°C for 25 min. to eliminate existing microorganisms. Subsequently, the plates were filled with PDA culture medium (200 g of potatoes, 20 g of dextrose and 20 g of agar), supplemented with tetracycline hydrochloride (500 mg L^{-1}). The medium was subjected to the sterilization process by autoclaving during 25 min, and was allowed to solidify. After the medium solidification, in a flow layer, 10 µL of the fungal solution was added in each plate with an automatic pipette (D1, recommended dose; D2, double of recommended dose). This solution was spread on the surface of the medium through a sterilized Drigalsky loop and then each Petri plate was centrally inoculated with a disc of *trichoderma*, in addition to the control treatment without inoculation.

For the bacterium *Azospirillum brasilense*, the colony surface was scraped with a sterilized Drigalsky loop. Then, the sterile paper discs were dipped in the fungicides and placed on the surface of the medium. After this step, the plates were placed in a BOD (Biochemical Oxygen Demand) incubator at 26±2 ºC and 12 h photoperiod. All plates were observed daily for growth assessment.

After five days, were performed measurements of the size of the fungus colony. These measurements were performed with the aid of a manual caliper. The reading was realized until the fungus reached the surface of the medium. For the bacterium, the count has been carried out after 72 h. The quantification of sporulation was realized by washing each plate containing fungal structures and adding 10 mL of previously autoclaved distilled water. After this process, the solution was filtered through a double layer of sterile gauze. In sequence, the spores were placed in a Neubauer chamber and transferred to the test tubes to determine the spore concentration using an optical microscope.

The experimental design was completely randomized, with a factorial arrangement 2 x 3 with three replications. The first factor corresponded to the doses and the second composed of three fungicides. The normality test was realized and the data was fallowed a normal distribution. The analysis of variance followed by the Scott-Knott’s test was used to evaluate the effects of fungicides on the microorganisms growth. The means were subjected to ANOVA and compared using the Scott-Knott’s test at 5% of probability error using the SISVAR statistical software (FERREIRA, 2011). And the tables were performed using the excel software.

RESULTS AND DISCUSSION

According to the statistical analysis, the interaction between the factors (fungicides and doses) was not significant. Therefore, the results are presented independently for the assessed factors. It was observed that the growth of the *Trichoderma* isolate showed a statistical difference. Only the treatment with the fungicide whose active ingredients Tiofanato metílico + Fluazinam did not differ from the control. The other treatments did not differ from each other, but differed from the Certeza fungicide and the control. The fungicides Apron and Vitavax showed higher values compared to the other treatments (Table 1).

TABLE 1 - Means of mycelial growth of *Trichoderma* according to the fungicides application.

Fungicides	Mycelial growth
Control	1.64 b*
Certeza	3.42 b
Vitavax	6.77 a
Apron	9.89 a
CV(%)	16.55

*Means followed by the same letter do not differ at 5% level according to Scott-Knott's test.

The comparison of the effect of fungicide dosages showed that there were no statistical differences between them (Table 2). It was observed that all fungicides interfered in the growth process of the *Trichoderma* isolate. Despite presenting statistical differences, it was observed that there was an increase in growth. Based on this parameter, within the fungus compatibility classification, the tested fungicides are considered non-toxic to the fungus.

TABLE 2 - Mycelial growth of *Trichoderma* according to the fungicides application.

Fungicides	Mycelial growth
D1	4.44 a*
D2	5.53 a
CV(%)	16.55

*Means followed by the same letter do not differ at 5% level according to Scott-Knott's test. D1 = recommended dose of fungicides, D2 = double of fungicides recommended dose.

According Espinosa et al. (2020), the action of fungicides on the growth of Trichoderma is directly related to the chemical composition of the products. It is important to emphasize that the results found in this work not differ from most works developed with fungicides in laboratory conditions with microorganisms of the genus *Trichoderma*. Maheshwary et al. (2020) investigated the compatibility of fungicides Copper hydroxide, Copper oxychloride and Mancozeb for the *Trichoderma*, and observed that the fungicides did not affect the fungus growth. This work is in disagreement with the results obtained by Kumar et al. (2016), who studied the compatibility of the fungicides Carbendazim + Mancozeb and Carboxin + Tiram with *Trichoderma* and totally inhibited the growth because the active ingredients previously act by hindering the germination of spores of the fungus. In addition, the authors observed that on high concentrations, there was a lethal reaction.

Analyzing the data in table 2, it was observed that the applied doses did not show any difference in the mycelial growth of the fungus. Karpagavalli and Kurmar (2020) evaluated doses of fungicides (Carbendazim, Thiophanate methyl, Benomyl, Sodium hypochlorite) on the compatibility of the fungus *Trichoderma*, and observed that there was no statistically significant difference in the doses. Similar results were obtained by Santini et al. (2018), who found that there was no difference, and found that did not impair growth. According Freitas et al. (2011), the low doses of Native fungicides whose active ingredients (Trifloxixtrobina+ Tebuconazol) and Sphere (Trifloxistrobina + Ciproconazol) have no toxic effects.

Regarding the sporulation, the analyzed data showed that the sporulation did not present a significant difference between them, with the highest value found in the control treatment (Table 3). Then, the Certeza treatment provided a higher average than the other treatments with fungicides.

It was observed at the applied dosages that the means showed statistical differences among them (Table 4). Gassen et al. (2008) evaluated 12 fungicides in guava growing in laboratory conditions, obtaining results in which all treatments behaved similarly. Despite their compatibility, the fungicides caused a reduction in the sporulation when compared to the control.

TABLE 3 - Mean values of *Trichoderma* isolate sporulation, according to the fungicides application.

Fungicides	Sporulation (x 10^5)
Control	57.50 a*
Certeza	47.41 b
Vitavax	44.25 b
Apron	46.00 b
CV(%)	5.69

*Means followed by the same letter do not differ at 5% level according to Scott-Knott's test.

Maurya et al. (2020), studied the compatibility of *Trichoderma* with fungicides Tiram, Copper oxychlorride Mancozeb and Metalaxyl, observed that all fungicides were compatible at all concentrations. The Fungicides did not adversely affect the growth of *Trichoderma* except Tiram. For Karpagavalli and Kurmar (2020), the use of microorganisms with compatible fungicides in seed treatment not only protects the seed against soil borne plant disease, but also protects against seed-transmitted diseases.

TABLE 4 - Number of spores of the *Trichoderma* isolate, depending the fungicides application.

Fungicides	Sporulations (x 10^5)
D1	47.33 a*
D2	44.44 b
CV(%)	5.69

*Means followed by the same letter do not differ at 5% level according to Scott-Knott's test. D1 = recommended dose of fungicides, D2 = double of fungicides recommended dose.

The data in table 4 indicated that there was a difference in the dosages and the recommended dosage had a greater effect, compared to twice the dose. These results are in agreement with the report Silva et al. (2018), who developed a study on the compatibility of *Trichoderma* with fungicides. Similar results were found by Patel and Singh (2020), tested doses of fungicides Carbendazim, Carboxin and Tebuconazole on the compatibility of the *Trichoderma* fungus.

When comparing the effect of fungicides on sporulation, it can be said that even with sporulation in the fungus, there is an inhibition when compared to the control of the other treatments. Vitavax presented the lowest value, followed by Apron. This inhibition is probably due to the mode of action of the active ingredients of the product Carboxin + Thiram and Fludioxonil + Metalaxil-M. According to Bampi et al. (2013), the mode of action of active ingredients represents one of the main factors involved in the toxicity of phytosanitary products.

The statistical analyses indicated that there was no significant interaction between the factors dose and fungicide for *Azospirillum brasilense*. It was observed a difference between the treatments and the highest value of the colony-forming unit was obtained by the control (Table 5). The treatment with Certeza fungicide differed from the control and the other treatments. The other treatments did not differ from each other. Comparing the effect of the dosages of fungicides, statistical differences were observed (Table 6).

TABLE 5 - Mean of *Azospirillum brasilense* colony forming unit according to fungicides application.

Fungicides	UFC-*Azospirillum*
Control	2.58 a*
Certeza	0.36 c
Vitavax	1.02 b
Apron	1.32 b
CV(%)	9.31

*Means followed by the same letter do not differ at 5% level according to Scott-Knott's test.

TABLE 6 - Mean of *Azospirillum brasilense* colony forming unit according to fungicide application.

Fungicidas	UFC-*Azospirillum*
D1	0.92 a*
D2	0.88 b
CV(%)	9.31

*Means followed by the same letter do not differ at 5% level according to Scott-Knott's test. D1 = recommended dose of fungicides, D2 = double of fungicides recommended dose.

Munerato et al. (2018), observed that the fungicide Difenoconazole has an effect on the colony-forming unit of the bacterium *Azospirillum brasilense*. Similar results were obtained by Dartora et al. (2013) tested the compatibility of fungicide Fludioxonil-Metalaxyl on the *Azospirillum brasilense* growth. Among these products, Certeza was the one that most interfered in the colony-forming unit. This value is due to the effect of the mode of action of active ingredients such as Thiophanate Methyl + Fluazinam. According to Patel and Singh (2020), the action of phytosanitary products varied depending the chemical nature, and the molecules that compose the fungicides interfered in the vital processes of the fungus.

The data analysis in table 6 showed that there was a difference, the recommended dose showed the highest mean for the colony-forming unit. Similar results were obtained by Santos et al. (2020), studied doses of fungicides Pyraclostrobin and Thiophanate-methyl in corn. The authors observed that the doses of fungicides interfered in the colony-forming unit of *Azospirillum brasilense*, and the recommended dose presented a higher average compared to the double of recommended dose.

According to the literature, there are fungicides that can cause deleterious effects after being in contact with the bacterium. However, this work did not evaluate the viability of the bacterium. Thus, the combination of compatible fungicides with microorganisms can become a strategy that can be used in integrated disease management programs.

CONCLUSIONS

The fungicides Certeza (Tiofanato metílico + Fluazinam), Apron (Fludioxonil + Metalaxil-M) and Vitavax (Carboxina + Tiram) are considered compatible with *Trichoderma asperelloides* and *Azospirillum brasilense*.

The fungicides can be used in integrated disease management practices, but the priority should be given to the use of products that have been shown to be less harmful.

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