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Cientific Paper

Yield parameters of maize crop in response to nitrogen fertilization, application of chemical fungicides and inoculation with *Trichoderma harzianum*

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Abstract

The aim of this study was to evaluate the effects of nitrogen fertilization on yield parameters of the maize crop in season and off-season, originated from the seed inoculated with *Trichoderma harzianum* or treated with chemical fungicides. The experiment was installed in RBD using subdivided plots. The factor of the plots was attributed to the application of nitrogen doses in cover (0, 60, 90 and 120 kg ha^{-1}). The subplot was characterized by the presence and absence of application of the fungicides Captan® and Maxim® and the presence and absence of inoculation with *T. harzianum* seed. The experimental design used in the off season was the same used in the season, changing only the factorial in the subplot. There were differences in the DKB747 hybrid yield through the application of nitrogen during the season. In off-season there were differences of the nitrogen doses in the characteristics of plant height and grain weight, because the fungicide factor altered the grain weight. As for the AG7575 hybrid, there was no effect of the nitrogen doses on yield and grain weight; the fungicide factor altered the yield characteristics, while the inoculum factor only changed the yield factor during the season. In general, the parameters of maize crop production are enhanced with inoculation of the seeds with *T. harzianum*. It is not recommended to use nitrogen doses above of the recommended for maximum yield potential of the hybrid.

Keywords: fertility; hybrids; microorganisms; yield; *Zea mays*.

Parametros de produção do milho em resposta a fertilização nitrogenada, aplicação de fungicidas químicos e inoculação com *Trichoderma harzianum*

Resumo

Objetivou-se verificar os efeitos da adubação nitrogenada em parâmetros produtivos da cultura do milho, em safra e safrinha, proveniente de sementes inoculadas com *T. harzianum* ou tratadas com fungicidas químicos. O experimento em safra foi instalado em DBC, utilizando-se parcelas subdivididas. O fator das parcelas foi atribuído à aplicação de doses de nitrogênio em cobertura (0, 60, 90 e 120 kg ha^{-1}). A subparcela foi caracterizada pela presença e ausência de aplicação dos fungicidas Captan® e Maxim® e presença e ausência de inoculação com *T. harzianum* das sementes. O delineamento experimental utilizado em safrinha foi o mesmo do plantio em safra, alterando-se apenas o fatorial na subparcela. Houve diferença na produtividade do híbrido DKB747 pela aplicação de doses de nitrogênio durante a safra. Em safrinha verificaram-se diferenças das doses de nitrogênio nas características de altura da planta e peso de grãos e o fator fungicida alterou o peso de grãos. Quanto ao híbrido AG7575, verificou-se efeito das doses de nitrogênio na produtividade e peso de grãos; o fator fungicida alterou as características de produtividade, enquanto o fator inóculo alterou apenas a produtividade durante a safra. De maneira geral, os parâmetros produtivos da cultura do milho são potencializados com a inoculação das sementes com *T. harzianum*. Não se recomenda utilizar doses de nitrogênio acima das preconizadas para o potencial máximo de produção do híbrido.

Palavras-chave: fertilidade; híbridos; microrganismos; produtividade; *Zea mays*.

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Parámetros productivos de maíz debido a la fertilización nitrogenada, aplicación de fungicidas químicos y inoculación con *Trichoderma harzianum*

Resumen

El presente estudio tuvo como objetivo investigar los efectos de la fertilización nitrogenada sobre los parámetros de producción del cultivo de maíz en cultivos tempranos (Brasil - zafra principal) y tardíos (zafra secundaria), a partir de semillas inoculadas con *T. harzianum* o tratadas con fungicidas químicos. El experimento en cultivo temprano fue instalado en DBC, utilizando parcelas divididas. El factor de las parcelas se atribuyó a la aplicación de dosis de nitrógeno en cobertura (0, 60, 90 y 120 kg ha^{-1}). La parcela secundaria se caracterizó por la presencia y ausencia de aplicación de fungicidas Captan® y Maxim® y la presencia y ausencia de la inoculación con *T. harzianum* en las semillas. En el experimento con cultivo tardío el diseño experimental fue el mismo, cambiando sólo el factor de la parcela secundaria. En el cultivo temprano hubo diferencias en la productividad del híbrido DKB747 debido la aplicación de nitrógeno. En cultivo tardío se han producido diferencias de las dosis de nitrógeno en las características de altura de la planta y peso del grano y el factor fungicida ha modificado el peso de los granos. Cuanto al híbrido AG7575, se verificó efecto de las dosis de nitrógeno en la productividad y el peso del grano, el factor fungicida alteró las características de productividad, mientras que el factor inóculo sólo cambió la productividad en lo experimento con cultivo temprano. En general, los parámetros productivos del maíz son potencializados con la inoculación de la semilla con *T. harzianum*. No se recomienda el uso de dosis de nitrógeno por encima de las ya recomendados para el máximo potencial de producción del híbrido.

Palabras clave: fertilidad; híbridos; microorganismos; productividad; *Zea mays*.

Introduction

Although Brazil is today the third larger producer of maize in the world, the average yield of the grains is low (3.000 kg ha^{-1}) when compared to China (4.700 kg ha^{-1}) and United States (8.670 kg ha^{-1}) (ARAÚJO et al., 2004). Among the responsible factors for the low yield of the maize crop in the country stand out the use of relatively low quantities of nitrogen fertilizer (60 kg ha^{-1}), and the low quality of the seeds used in the sowing (ANDREOLI et al., 2002).

Considering that the use of nitrogen fertilizers is not always economically viable due to its elevated cost and the low index of harnessing, there is the need to search more viable options in order to reduce the production cost and the risk of environmental pollution, like the fixation of atmospheric nitrogen by microorganisms (RESENDE et al., 2004).

Allied to this factor, the use of phytosanitary products applied through seeds in the maize crop has increased the demand for technologies which allow reducing the environmental contamination risks, without compromising the seeds quality (PEREIRA et al., 2005). In this sense, the use of benefic biological agents, via seeds treatment can be a viable alternative, because it does not harm the environment (RESENDE et al., 2005) and fixate atmospheric nitrogen, thus minimizing the costs of production for the maize crop.

Many microorganisms have been studied with these objectives, including fungus species of the genus *Trichoderma*, such as the *T. harzianum* (RESENDE et al., 2005). KLOEPPER and SCHROTH (1981) describe that this fungus is involved in the plants growth mechanisms, grains yield and in the control of secondary pathogens. These microorganisms have the capacity to control seeds pathogens, to protect the subterranean parts of plants against pathogens, improve the germination rate and the seeds vigor, fixate atmospheric nitrogen, improve absorption of nutrients and crop yield (HARMAN, 2000). Therefore, the aim was to verify the effects of nitrogen fertilization in yield parameters of the maize crop, in the season and off season, originated from seeds inoculated with *T. harzianum* or treated with chemical fungicide.

Material and Methods

The experiment was conducted in the Department of Plant Science of the Universidade Federal de Lavras, with seeds of the triple hybrid DKB747 and simple G7575, in a design of randomized blocks, using the split-plot design, with three repetitions. The plots factor was attributed to application of four doses of nitrogen in cover (0, 60, 90 and 120 kg ha^{-1}). The subplot was characterized by the presence/absence of application of the fungicides N-(trichloromethylthio) cyclohex-4-ene-1.2-dicarboximide (Captan®) and Fludioxonil and Metalaxyl-M (Maxim®) and presence/absence of seeds inoculation with *T. harzianum*. After this stage, the seeds were packed in multiwall paper bags and stored for a period of four months under ambient conditions. The temperature monitoring and the air relative moisture during the storing were done through a thermohygrograph (Figure 1). Each plot was composed by four lines with 5 m long, spaced in 0.8 m between lines, being considered as useful area the two central rows of the plot.

The planting of the DKB747 hybrid was done in the soil with the following chemical characterization (SILVA, 1999): pH in H_2O = 5.9; P-Mehlich = 21.1 mg dm^{-3}; K^+ = 33 mg dm^{-3}; Ca^{2+} = 2.1 $cmol_c$ dm^{-3}; Mg^{2+} = 1.1 $cmol_c$ dm^{-3}; H+Al = 2.6 $cmol_c$ dm^{-3}; sum of bases (SB) = 3.3 $cmol_c$ dm^{-3}; CTC potential = 5.9 $cmol_c$ dm^{-3}; base saturation (V%) = 55.5 and organic matter (M.O.) = 20 g kg^{-1}. The soil for the planting of the hybrid AG7575 presented: pH in H_2O = 6.2; P-Mehlich = 10.4 mg dm^{-3}; K^+ = 69 mg dm^{-3}; Ca^{2+} = 2.1 $cmol_c$ dm^{-3}; Mg^{2+} = 0.9 $cmol_c$ dm^{-3}; H+Al = 2.3 $cmol_c$ dm^{-3}; sum of bases (SB) = 3.2 $cmol_c$ dm^{-3}; CTC potential = 5.8 $cmol_c$ dm^{-3}; base saturation (V%) = 55.0 and organic matter (M.O.) = 24 g kg^{-1}. The soil was conventionally prepared and the sowing in season was done in the second fortnight of November, using 400 kg ha^{-1} of formulation 08-28-16 in the planting.

The sowing was done at 5 cm of depth, distributing seven seeds per meter. Thirty days after the planting, it was done the thinning leaving 5 plants per meter. The nitrogen fertilization in cover was done in two seasons, applying half of the corresponding dose to each treatment when the plants presented 4 to 6 leaves and 8 to 10 fully expanded leaves. The weed plants management was made with the herbicide application in post-emergence, and the control of weevils (*Spodoptera frugiperda*) with insecticide application. The climatic data referent to the conduction period of the experiment in season is presented in the Figure 2.

The maize sowing in the off season was done in February, and the water supply was done when necessary. The planting of the hybrid DKB747 was done in a soil with the following chemical characterization (SILVA, 1999): pH in H_2O = 5.4; P-Mehlich = 8.9 mg dm^{-3}; K^+ = 50 mg dm^{-3}; Ca^{2+} = 2.4 $cmol_c$ dm^{-3}; Mg^{2+} = 0.5 $cmol_c$ dm^{-3}; H+Al = 4.5 $cmol_c$ dm^{-3}; sum of bases (SB) = 3.0 $cmol_c$ dm^{-3}; CTC potential = 7.5 $cmol_c$ dm^{-3}; base saturation (V%) = 40.2 and organic matter (M.O.) = 29 g kg^{-1}. The soil used for the planting of the hybrid AG7575 presented: pH in H_2O = 4.9; P-Mehlich = 15.4 mg dm^{-3}; K^+ = 61 mg dm^{-3}; Ca^{2+} = 1.3 $cmol_c$ dm^{-3}; Mg^{2+} = 0.2 $cmol_c$ dm^{-3}; H+Al = 5.6 $cmol_c$ dm^{-3}; sum of bases (SB) = 1.7 $cmol_c$ dm^{-3}; CTC potential = 7.3 $cmol_c$ dm^{-3}; base saturation (V%) = 22.9 and organic matter (M.O.) = 24 g kg^{-1}.

The planting, fertilization and crop treats systems were the same adopted during the cultivation in season. The climatic data referent to the conduction period of the experiment in off season is presented in the Figure 3. The experimental design was the same used for the planting during the season, altering only

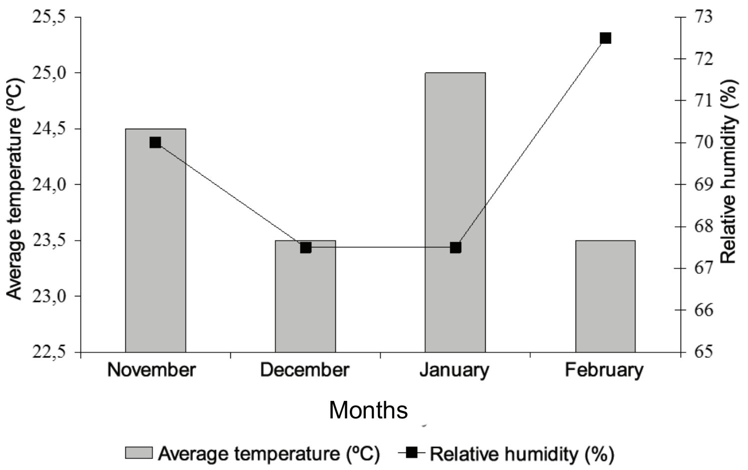

Figure 1. Relative temperature and humidity of the air during the period of seeds storage.

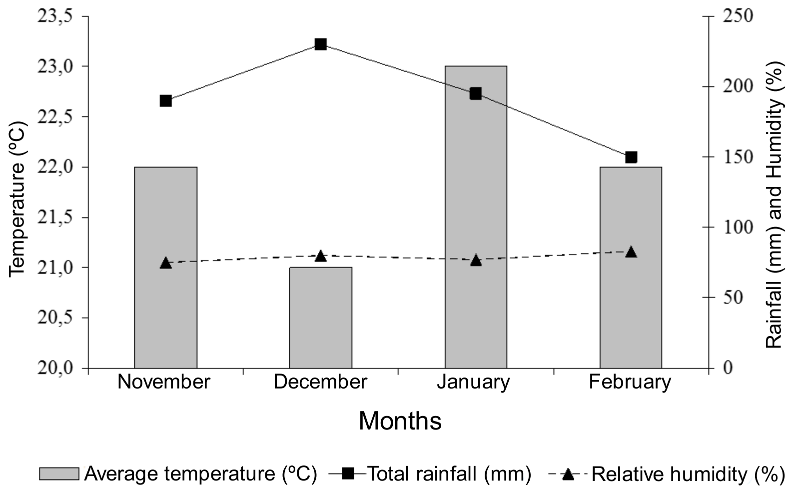

Figure 2. Average temperature, total rainfall and relative humidity of the air during the conduction of the experiment in season.

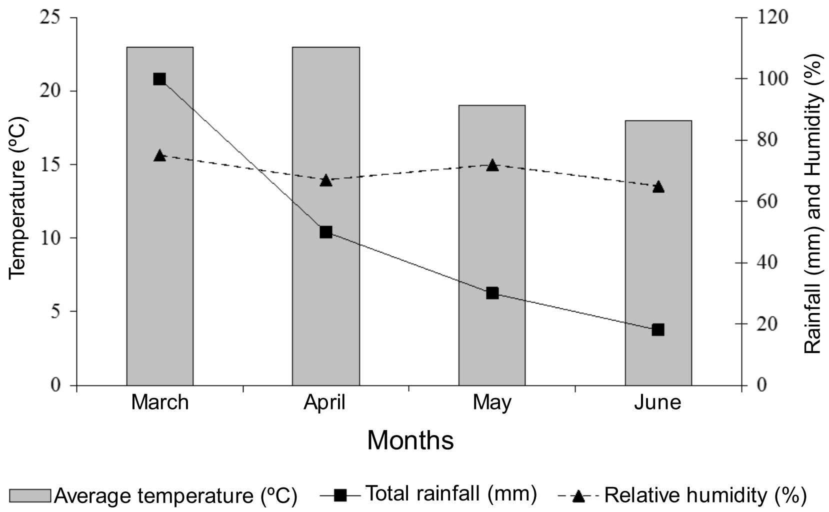

Figure 3. Average temperature, total rainfall and relative humidity of the air during the conduction of the experiment in off season

the factorial in the subplot, since that, in the off season cultivation, the fungicide N-(trichloromethylthio) cyclohex-4-ene-1.2-dicarboximide (Captan®) was not used. Therefore, the subplot was characterized by the presence/absence of application of the fungicide Fludioxonil and Metalaxyl-M (Maxim®) and presence/absence of seeds inoculation with *T. Harzianum*.

After 120 days of planting, in the season and off season, the maize harvest was done manually and individually per plot, assessing the following agronomical characteristics: plant height by taking the height of 10 plants of the useful plot, from the ground level to the point of insertion of flag leaf; height of insertion of the first ear, by taking the height of 10 plants of the useful plot, from the ground level to the point of insertion of the first ear; thousand grain weight obtained from the successive weights of 10 samples of 100 grains, with moisture corrected to 13%, agreeing with the recommendation of the Rule for seeds analysis (BRASIL, 1992) and grains yield obtained after the threshing, through the weighing of the grains of each plot, with moisture corrected to 13%, going beyond the result for kg ha^{-1}.

The data were submitted to the variance analysis using the statistical program Sisvar® (FERREIRA, 2011). The averages referent to application of fungicides and inoculation with *T. harzianum* were compared by the Tukey test ($p<0.01$), while it was used the regression analysis for the data obtained with the nitrogen doses.

Results and Discussion

There was positive response to the application of nitrogen doses between 60 and 90 kg ha^{-1} in the plants height and insertion of first ear of the hybrid DKB747, with reduction tendency when using 120 kg ha^{-1} (Figure 4). Similar results were reported by OLIVEIRA and CAIRES (2003), who found plants height of 2.06; 1.94; 2.00 and 1.98 m and ear insertion of of 0.91; 0.89; 0.94 and 0.91 m for the maize fertilized with 30, 60, 90 and 120 kg ha^{-1} of nitrogen in cover, respectively.

In Figure 5 it is found the results of plants height in function of the nitrogen doses and presence/ absence of inoculation with *T. harzianum*. There was linear increase in the plants height of the hybrid

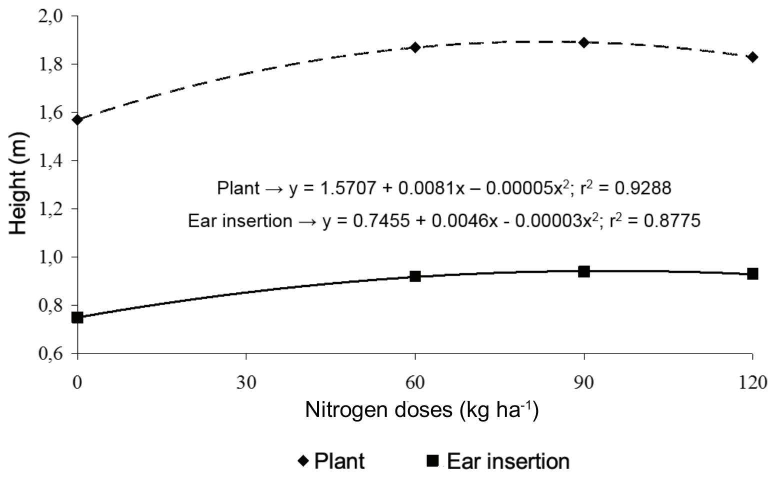

Figure 4. Plant height and insertion of first ear of the hybrid DKB747 in function of nitrogen doses in the season.

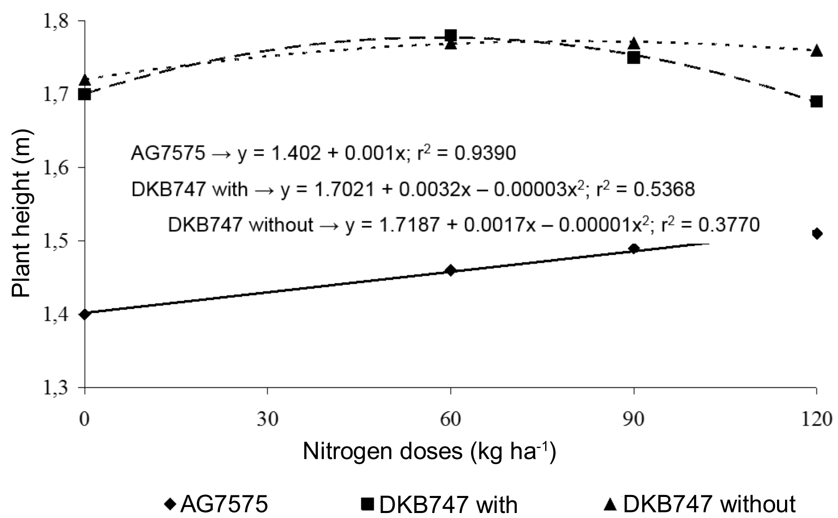

Figure 5. Plants height of the hybrid AG7575 in function of nitrogen doses, and of the hybrid DKB747 in the presence/ absence of inoculation with *T. harzianum*, with nitrogen doses during the off season cultivation.

AG7575, being the increase of 0.1 cm for each kg of nitrogen added. The response of the hybrid DKB747, inoculated and non inoculated with *T. harzianum*. was quadratic, noting increases in the height with the use of 60 kg ha^{-1} of nitrogen, and reduction when the doses were superior to this.

The hybrid AG7575 presented linear increase in the ears height in function of the nitrogen doses in the non inoculated seeds and responded quadratically when the seeds were inoculated, with increase in the insertion height when using up to 60 kg ha^{-1} (Figure 6). The hybrid DKB747 responded quadratically to the nitrogen application in inoculated and non inoculated seeds. There was increase in the insertion height of the first ear with application of doses close to 60 kg ha^{-1}.

Altogether, the characteristics of plants height and insertion height of the ear related to the nitrogen fertilization, adjusted better to the quadratic model. MAR et al. (2003) also reported responses of quadratic effect for the plants height and insertion height of the first ear, in response to the cover nitrogen fertilization in the off season. From these results, it can be stated that it is not recommend to use nitrogen doses above those prescribed for the maximal potential of production of the hybrid, because there is a limit for the nitrogen response, which may vary in function of cultivation season, soil and hybrids.

The thousand grain weight of the hybrid AG 7575 varied from 304.10 to 356.42 g in the doses of 0 to 120 kg ha^{-1}, respectively, with increase of 410 mg for each kg of nitrogen added in cover during the season (Figure 7). Similar results were found by AMARAL FILHO (2002), who assessed the nitrogen doses effect on maize hybrids and also verified linear increase in the thousand grain weight with the nitrogen dose increase. During the off season, the thousand grain weight of the hybrid AG7575 presented amplitude of 26 g, with gains of 133 mg for each kg of nitrogen added. In relation to the DKB747, it was observed quadratic effect with the nitrogen provision. It was also observed increases with the application of doses close to 90 kg ha^{-1}, followed by decrease with the use of 120 kg ha^{-1} (Figure 8). SORATTO et al. (2010) analyzed the maize cultivation during the off season in succession to soy, under doses of cover nitrogen (0 to 120 kg ha^{-1}) and also found quadratic response for the thousand grain weight.

It was verified that the grains yield of the hybrid AG7575 varied from 6783 kg ha^{-1} to 10332 kg ha^{-1} and of the hybrid DKB747 from 3568 kg ha^{-1} to 7949 kg ha^{-1} with the application of 0 to 120 kg ha^{-1} of nitrogen, respectively (Figure 9). Therefore, there was linear increase in the grains yield in response to nitrogen doses, with gains of 29.58 kg ha^{-1} and 36.50 kg ha^{-1} for each kg of nitrogen applied in the hybrids

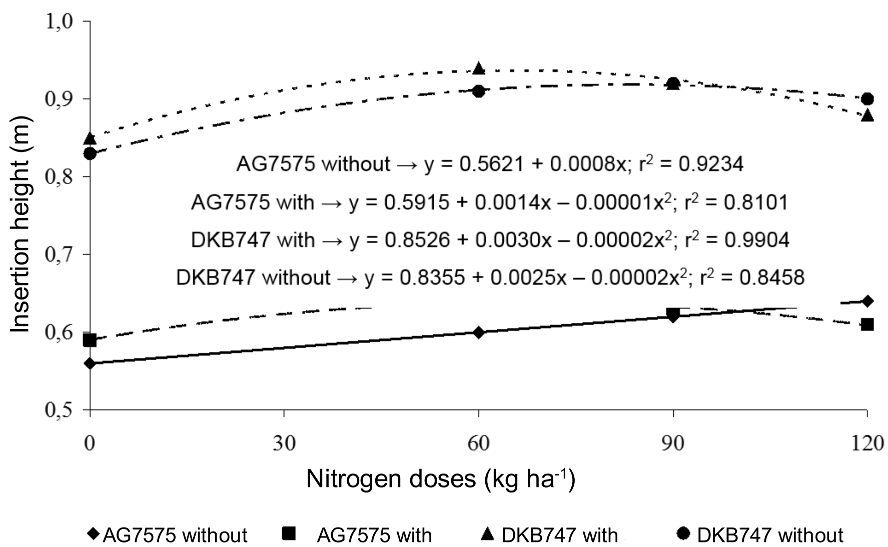

Figure 6. Insertion height of the first ear in the plants of the maize seeds originated from the hybrids AG7575 and DKB747, in the presence/ absence of fungicides application, in function of the nitrogen doses in the off season cultivation.

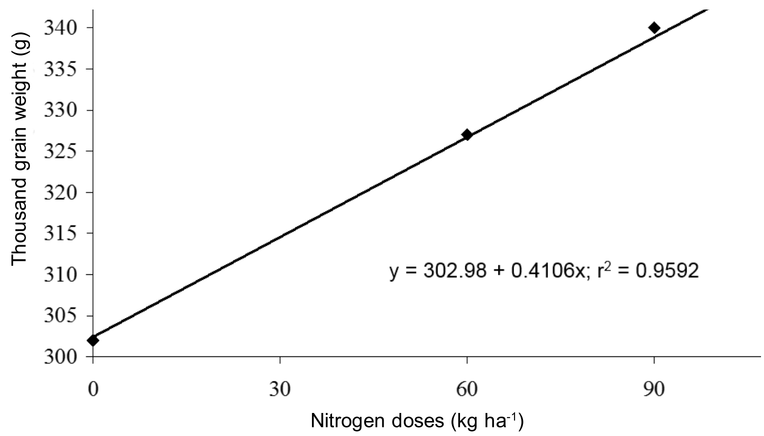

Figure 7. Thousand grains weight originated of the maize seeds originated from the hybrid AG7575 in function of the nitrogen doses in season.

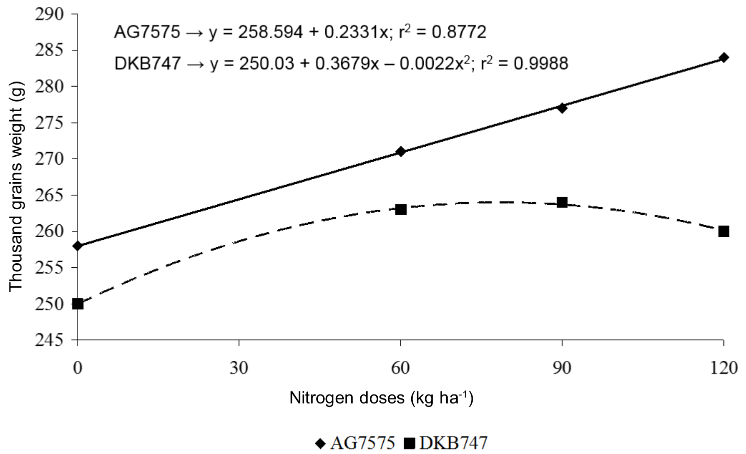

Figure 8. Thousand grains weight of the maize seeds originated from the hybrid AG7575 in function of the nitrogen doses in off season.

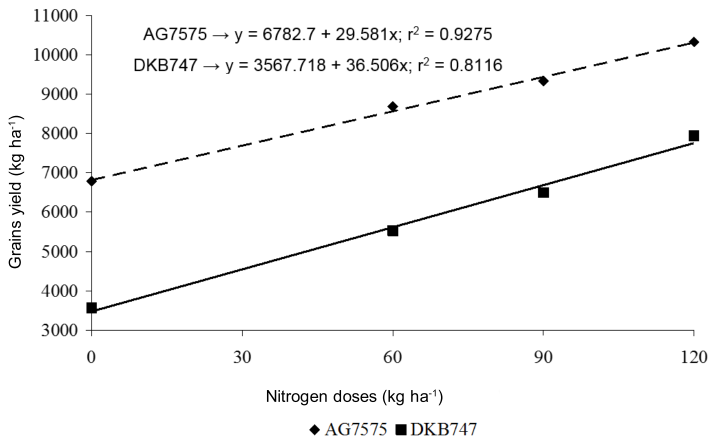

Figure 9. Grains yield of the maize seeds originated from the hybrid AG7575 and DKB747 in function of the nitrogen doses in season.

AG7575 and DKB747, respectively. OLIVEIRA and CAIRES (2003) reported yields varying from 7011 to 8101 kg ha^{-1} with application of 30 and 120 kg ha^{-1} of nitrogen, respectively.

The grains yield of the hybrid AG7575 without treatment with fungicide, increased linearly with the nitrogen application during the off season, noting increases of 14.16 kg ha^{-1} for each kg of nitrogen added. However, the plants from the seeds treated with the fungicide Maxim® presented yield increase with application of doses close to 60 kg ha^{-1} of nitrogen, followed by a decrease when using 90 and 120 kg ha^{-1}, conferring effect of quadratic response for the grains yield of this hybrid during the off season. There was linear response on the yield of the hybrid DKB747 treated with Maxim® with nitrogen application, and for the seeds without fungicide treatment, the response adjusted better to the quadratic function (Figure 10).

In the Table 1 are presented the results for plant height and grains yield of the maize seeds originated from the hybrid AG7575, in the presence/absence of fungicide application in the season. The treatment of seeds with the fungicide Maxim® provided the highest plant height (2.09 m) in comparison to the seeds which were not treated (2.03), however, it did not differ from those treated with Captan® (2.04 m).

It was observed that the seeds treated with the fungicide Captan® produced more when compared to the seeds treated with Maxim® and to the control treatment. These results differ from those found by VON PINHO (1991), where the seeds treatment with the fungicide Captan® did not influence in the grains yield, it is noteworthy that the fungicide response may vary according to the seeds quality, hybrid, soil and season of planting.

The height of plants and of insertion of the first ear, and the grains yield of the hybrid AG7575 were higher when using seeds inoculated with *T. harzianum* (Table 2). This fungus appears to have stimulated the plants growth through the production

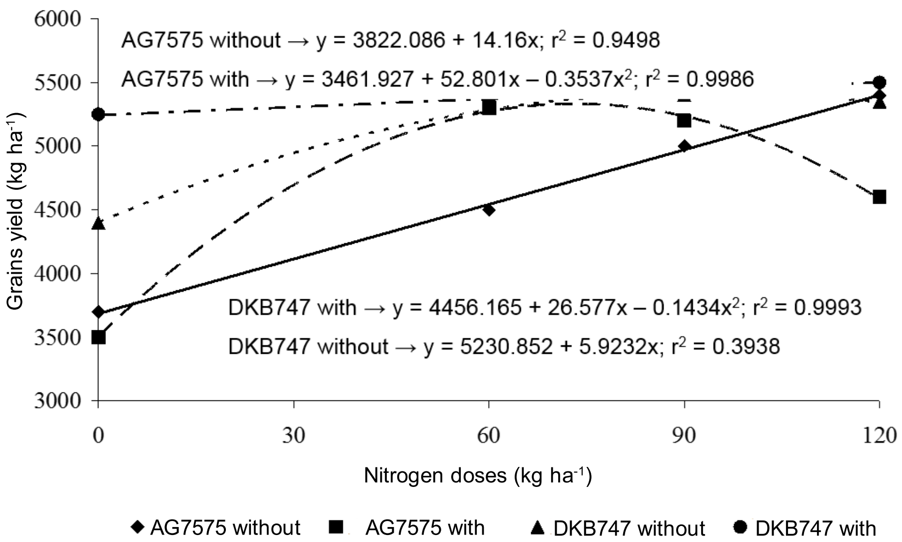

Figure 10. Grains yield originated from the maize seeds of the hybrid AG7575 and DKB747 in the presence/absence of fungicide application, in function of the nitrogen doses in off season.

Table 1. Plant height and grains yield of the maize seeds originated from the hybrid AG7575, in the presence/absence of fungicide application in the season.

Variables[1]	Fungicides		
	Control	**Captan®**	**Maxim®**
Plant height (m)	2.03[b]	2.04[ab]	2.09[a]
Grains yield (kg ha^{-1})	8627[b]	9220[a]	8492[b]

[1]*Averages followed by distinct letters in the lines differ, by the Tukey test (p<0.01).*

Table 2. Height of plants and of ears insertion of maize seeds originated from hybrid AG7575 with and without inoculation, during the off season, and grains yield with and without inoculation during the season.

Variables[1]	Hybrid	*T. harzianum*	
		Without	With
Plant height (m)	AG7575	1.45[b]	1.49[a]
Insertion height of the ear (m)	AG7575	0.60[b]	0.64[a]
Grains yield (kg ha^{-1})	AG7575	8610[b]	8849[a]

[1]*Averages followed by distinct letters in the lines differ, by the Tukey test (p<0.01).*

of the growth hormone (WINDHAN et al., 1986) and solubilized insoluble trace elements in the soil, because it absorbers and translocates minerals, such as phosphorus and nitrogen, which increases this crop yield (INBAR et al., 1994).

Conclusions

Altogether, the yield parameters of the maize crop are potentiated with inoculation of the seeds with *T. harzianum*.

The application of the fungicide Captan® increases the yield of the hybrid AG7575.

It can be said that it is not recommended to use nitrogen doses above those prescribed for the maximum potential for the hybrid yield, because there is a limit of response to nitrogen, which may vary in function of the cultivation season, soil and hybrid.

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