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2 **Original Research Article**

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4 **Spore density and arbuscularmycorrhizal**
5 **colonization in sunflower**

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11 **ABSTRACT**
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The objective of this study was to evaluate the number of spores and mycorrhizal root colonization in Cerrado soil, under sunflower cultivation. Sampling of rhizospheric soil occurred in three periods: sowing, flowering and sunflower harvest. The experimental design was a randomized complete block design with four replications. The evaluated parameters were number of total spores in 50 g of soil and arbuscularmycorrhizal colonization. The mean number of spores was 247 and 232 in 2009 and 2010, respectively. For root colonization there was a difference between years. The maximum spore production occurred during the flowering period and mycorrhizal colonization was not influenced by the genotype. To accomplish, mycorrhiza favors the development of sunflower in lower levels of phosphorus in the soil.

13
14 *Keywords: Helianthus annuus L.; soil; arbuscularmycorrhizal fungi.*

15
16 **1. INTRODUCTION**

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18 Soil quality and the viability of improvements through chemical, physical and biological
19 management are essential factors for success in agricultural production. In this context, the
20 study and the use of soil microbial population has shown the way to link sustainability to
21 efficiency.

22 The symbiotic association between plant and arbuscularmycorrhizal fungi (AMF) is called
23 mycorrhiza. Root colonization by arbuscularmycorrhizal fungi generates several
24 improvements; the plant provides photosynthates to the fungus, and this, through the
25 branching and extension of the mycelium, increases the area of nutrient absorption for the
26 plant [1]. Thus, AMs can be used as an alternative to reduce the use of agricultural inputs,
27 mainly fertilizers of chemical synthesis.

28 The influence of arbuscularmycorrhizal fungi acts not only on soil particles aggregation but
29 also on plant growth, providing essential nutrients [2] and improving their ability to withstand
30 adverse conditions.

31 The sunflower cultivation (*Helianthus annuus* L.) has aroused interest, especially in Brazilian
32 Midwest, due to the broad adaptability to edaphoclimatic conditions, suitability for crop
33 rotation and uses in the production of edible oil, biodiesel, ornamentation, animal food,
34 among others [3,4].

35 Considering that in the soils of the Cerrado Biome, for the optimization of the agricultural
 36 production, is necessary the use of a high amount of inputs, and that the agronomic
 37 efficiency is tied to the good indexes of soil quality, the present work aimed to evaluate the
 38 number of spores in different times and mycorrhizal colonization in Cerrado Biome soil,
 39 under cultivation of three sunflower genotypes.

40 41 2. MATERIAL AND METHODS

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 43 The experiment was carried out at Santa Luzia Farm, in Campo Verde (MT-Brazil), latitude
 44 15°45'12"S and longitude 55°22'44"W. The farm soil has clayed texture, with acid pH,
 45 average bases saturation of 50%, absence of aluminum and high content of organic matter
 46 (Table 1).

47
 48 **Table 1. Chemical and physical properties of soil under sunflower cultivation in the**
 49 **2009 and 2010 harvests at Farm Santa Luzia, Campo Verde – MT, Brazil**

Year	pH CaCl ₂	P	K	Ca	Mg	Al	H	MO	CTC
		mg dm ⁻³			cmol _c dm ⁻³		gdm ⁻³		cmol _c dm ⁻³
2009	5,1	21,8	76	3,2	0,9	0	4,4	37,8	8,7
2010	4,9	8,0	80	3,3	0,7	0	5,5	39,9	9,7
Bases saturation (V%)		Sand	Silt	Clay	Saturation (%)				
		g kg ⁻¹			Ca	Mg	K	H	
2009	49,3	196	133	671	36,7	10,5	2,3	50,7	
2010	43,3	172	200	628	33,9	6,8	2,1	56,7	

50
 51 The experimental design applied in the field was randomized blocks, with four replications.
 52 The plots were formed by four rows of 6.0 meters, spaced in 0.8 meters, between rows, and
 53 0.3 meters, between plants, considering as useful area the two central rows. Fertilization
 54 was carried out with 60-80-80 kg ha⁻¹ of N-P-K and 2.0 kg ha⁻¹ of boron.

55 The genotypes were M 734, Agrobela 960 and Helio 358, sown in 2009, and M 734, Embrapa
 56 122 and HLA 860 H.O. in 2010. Rhizospheric soil sampling was obtained at 0-20 cm depth,
 57 in three periods: sowing (first half of March), flowering (60 days after sowing) and harvesting
 58 (after maturation). The rainfall distribution in the region, during the experimente, is shown in
 59 Table 2.

60
 61 **Table 2. Rainfall (mm month⁻¹) in Campo Verde - MT, from February to July, in 2009**
 62 **and 2010**

Year	February	March (S)	April	May (F)	June	July (H)	Total
2009	262	132	16	10	22	0,2	442,4
2010	385	206	325	55	3	2	974,0

63 *S: sowing; F: flowering; H: harvest.*

64
 65 The evaluated parameters were total number of spores in soil, and arbuscularmycorrhizal
 66 colonization, whose root sampling occurred during crop harvest. The spore extraction was
 67 carried out by the wet sift methodology [5], in which the soil was processed in a sieving
 68 systems (0.42 and 0.053 mm mesh) and centrifuged with water at 2800 rpm for 4 minutes.
 69 Subsequently, the samples were resuspended in 50% sucrose solution, centrifuged and
 70 washed. The spores were counted in a stereomicroscope in a petri dishes with vessels.

71 For mycorrhizal colonization, the roots were washed, clarified with KOH (10%), acidified with
 72 diluted HCl and stained with trypan blue [7]. Ten segments of 1-2 cm in length were selected for
 73 slide assembly and quantification of colonization percentage under optical microscope.

74 Analysis of variance were preceded and the significant means were compared by Tukey test
 75 with 5% of significance.

76 3. RESULTS AND DISCUSSION

77

78 For the factor year, there was no difference in the number of spores of AMF (Table 3). This
 79 may occurred since the studied area adopted the minimum cropping system for more than
 80 10 years. According to the authors [8], the association and mycorrhizal propagules
 81 dissemination is more affected in the initial phases of the occupation and use of the soil, with
 82 later stabilization.

83

84 **Table 3. Quantification of spores of arbuscularmycorrhizal fungi in CerradoBiome**
 85 **soil, under sunflower cultivation, in two years and three periods, in Campo**
 86 **Verde – MT, Brazil**

Year	Genotype	Sowing			Flowering			Harvest			Average
		n° spores 50 g soil ⁻¹									
2009	M 734	153	b	B	296	a	A	267	a	A	247 a
	Agrobel 960	185	ab	B	342	a	A	233	ab	B	
	Helio 358	262	ab	AB	311	a	A	174	a	B	
	Média	200	a	B	317	a	A	225	a	B	
2010	M 734	234	ab	AB	270	a	A	147	b	B	232 a
	Embrapa 122	191	ab	A	254	a	A	216	ab	A	
	HLA 860 H.O.	271	a	AB	320	a	A	184	ab	B	
	Average	232	AB		281	A		182	B		
CV (%)					11,60						

87 *Means followed by different letters in the column differ from each other, by the Tukey test (P =.05).*
 88 *CV:coefficient of variation.*

89 For the periods, spore density in flowering was higher in the two years of study, with a
 90 general average of 317 in 2009 and 281 in 2010 (Table 3). The authors cited in the
 91 reference [9] confirm that maximum spore production can occur in the flowering period and
 92 in the final growth stage of the host.

93 According to the authors cited in the reference [2],the spore density of AMFs is generally
 94 higher in agricultural systems, and variations may occur due to edaphoclimatic factors,
 95 growing time, agricultural practices as well as the implanted crop.

96 The authors cited in the reference [10] studyingCerrado biome verified that the
 97 arbuscularmycorrhizal fungi contribute to the growth of cultivated plants in annual cropping
 98 ans pasture systems and the number of spores of the native fungi varies, being the crop and
 99 the cultivation system determinant for the enrichment of mycorrhizal fauna.

100 The interaction between the genetic factors and the period was significant, demonstrating
 101 that the genetic material influence the sporulation process. However, thevariations were low
 102 indicating the stabilization of the mycorrhizal fungi sporulation.

103 In a carried study was verified that spore densities varies from 301 to 608 for maize crop,
 104 whereas in soybean cultivated soil the values were between 239 and 287 [11], similar to
 105 those obtained in the present work with sunflower. Mycorrhizal dynamics involving root
 106 colonization and sporulation occur in different ways in different crops due to the compatibility
 107 between AMF and the genetic characteristics of plants [12]. In addition, environmental,
 108 climatic and edaphic factors generate changes in the symbiotic process [13].

109 In sugarcane the occurrence of AMF increase when the crop was preceded by sunflower
 110 [14]. Likewise, sunflower favored the inoculum potential of AMF in the soil, and subsequent
 111 corn growth [15].

112 Annual crops, green manures and forage species have a high degree of mycorrhizal
 113 dependency, acting as a soil conditioning, multiplying the native mycorrhizal community
 114 [16,13]. In this sense, sunflower is an option to benefit the soil mycorrhizal population in crop
 115 rotation / succession systems.

116 For the mycorrhizal colonization rate, it was observed a variation from 21 to 28% in 2009
 117 and 28 to 48% in 2010 (Table 4), with no difference between genotypes. According to the
 118 authors cited in the reference [17], mycorrhizal dependence can be defined as the plant's
 119 responsiveness to mycorrhization through increased growth, which may be related to the
 120 fertility and amount of phosphorus, present in the soil.

121 About the factor year, in 2010 there was a higher mycorrhizal colonization, which can be
 122 explained by the lower phosphorus content in the soil (Table 1). The correlation between the
 123 phosphorus content and mycorrhizal colonization is negative [18] so, the reduction in the P
 124 content may lead to an increase in plant colonization. Studing sunflower hybrids, it was
 125 verified that higher doses of P decreased sporulation and AMF colonization [19].

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Table 4. Average percentage of AMF colonization in soil under sunflower cultivation, in Campo Verde - MT, Brazil, in 2009 and 2010

Year	Genotype	Mycorrhizal colonization (%)	Average
2009	M 734	28 a	24 b
	Agrobel 960	21 a	
	Helio 358	22 a	
2010	M 734	38 a	38 a
	Embrapa 122	48 a	
	HLA 860 H.O.	28 a	
CV (%)		16,24	

129 *Means followed by different letters in the column differ from each other, by the Tukey test (P =.05).*
 130 *CV:coefficient of variation.*

131 In general, the relationship AMF-plant can be mediated by nutrient levels, present in the soil,
 132 since these fungi increase root exploration area, contributing to a greater absorption of
 133 nutrients for the plant. As the increase in soil phosphorus decreases the root mycorrhizal
 134 colonization and the plant dependence to mycorrhization [20], in soils with low levels of
 135 phosphorus, typical of the Cerrado biome, the AMF favors sunflower cultivation [21].

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Studying, AMF inoculation in sunflower, it was observed an increase in chapter diameter,
 thousand achenes weight and achenes yield, parameters that were related to the better
 development of the plants through the association with AMFs, due the higher absorption of
 nutrients as P, K and Fe.

142 In addition, there is evidences that mycorrhizal-sunflower ratio enables greater plant
143 resistance to heat, showing an interesting impact in Cerrado production systems, which is
144 characterized by high temperatures [23].

145
146 Moreover, the potential of AMFs as biofertilizer for oleaginous crops is reforced, especially
147 for soils with low fertility, since the practice allows to reach adequate levels of production,
148 with less use of synthetic fertilizers making the productive system more sustainable [19].

149
150 Therefore, colonization and mycorrhizal sporulation vary according to the sunflower
151 genotype and the evaluation period. On flowering period there were intense AMFs activity,
152 moment that is required to the plant a high nutritional supply for grain production.

153 154 **4. CONCLUSION**

155
156 The number of spores has low variation, demonstrating that the system is stable. The
157 maximum production occurs in flowering period.

158 Mycorrhizal colonization in sunflower is not influenced by the genotype.

159 Lower soil phosphorus levels favors arbuscular mycorrhizal colonization.

160 **COMPETING INTERESTS**

161
162 We declare that no competing interests exist.

163 164 **REFERENCES**

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