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EFFECT OF HERBICIDE ON SPORULATION AND INFECTIVITY OF VESICULAR ARBUSCULAR MYCORRHIZAL (*GLOMUS MOSSEAE)* SYMBIOSIS WITH PEANUT PLANT

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ABSTRACT

Arbuscular mycorrhizal fungi are of interest for their reported roles in preserving soil fertility in agroecosystem, which form mutualistic symbiosis with the roots of most agricultural plants. In the past, most research on VA mycorrhizae has been focused on possible responses to fungicides, rather than on the potential effects on mycorrhizal symbiosis following herbicide application. We studied the effect of three application rates of two widely used herbicides (1.8, 3.6 and 5.4 µg a.i. g^{-1} for alachlor and 1.08, 2.16 and 3.24 µg a.i. g^{-1} for glyphosate) on sporulation and infection of peanut plant by *G mosseae* under greenhouse conditions. The result of the study showed that the symbiont fungus *G mosseae* responds in a differential way to two different herbicides tested, and the fungus sensitivity to alachlor proved significantly higher than the glyphosate. However, none of the herbicide treatments affected the external hyphal length and SDH (Succinate dehydrogenase) activity. But the spore number, total and active infection intensity of internal hyphae was significantly reduced with the increasing rates of alachlor application, while glyphosate had no significant effects at all application rates. Consequently, P (phosphorus) inflow through mycorrhizal hyphae was significantly increased with the application rates of glyphosate, with the highest value (41.48 and 479.72 $x10^{-13}$ mol P m^{-1} s^{-1} hyphal inflow and hyphal uptake, respectively) obtained at field recommended glyphosate rate (2.16 µg a.i. g^{-1}). Therefore, symbiotic functions of *G mosseae* with host plant could be affected by the depressive effects of herbicides that are apparently related to the types of herbicide and their rates of application.

Key words: Herbicidal toxicity, mycorrhizae, spore germination, infection intensity, *G mosseae*, peanut plant.

INTRODUCTION

Mycorrhizal associations formed by *Glomeromycotan* fungi are known as arbuscular mycorrhizas or vesicular-arbuscular mycorrhizas, and abbreviated as VAM (Brundrett *et al*. 1996). VAMs are considered as endo-mycorrhiza as they produce arbuscules, hyphae and vesicles within the root cortex cell (Quilambo, 2000). VAM fungi form symbiotic associations with the roots of a wide variety of plant species, including many agricultural crop species (Kahiluoto *et al*., 2009). In this symbiosis, the host plant provides the fungus with soluble carbon sources, and the fungus facilitates the host plant with an increased access to water and nutrients from soil (Entry *et al*., 2002). This increased access of VAM fungi to nutrients is happened through their extraradical hypae that extend outside the host root up to several centimetres in the soil, allowing the fungi to absorb soil nutrients otherwise unavailable to the host plant (George *et al*., 1995). However, the relative abundance of VAM fungi within roots mostly depends on soil conditions that directly or indirectly affect the rapidity of fungal spore germination and plant root colonization such as growth and infectivity of both internal and external hyphae of the fungi (Smith and Read, 2008). Although VAM fungi are present in soils of all textures from sandy to those with high clay content and at a wide range of soil pH (Brundrett, 2002), but many soil factors such as conventional tillage, soil compaction, high fertilizer and pesticides applications have a negative impact on VAM functions (Entry *et al*., 2002).

The symbiotic relationship between mycorrhiza and host plant can be seriously hampered by the neglectful interference of human activities such as over application of pesticides mainly herbicides in modern intensive agricultural systems (Trappe *et al*., 1984). Herbicides, despite of their control on weeds, have the potential to affect beneficial non-targeted soil microbes including VAM fungi (Gupta *et al*., 2011). However, several authors have reported different effects of herbicides on VAM symbiosis, which ranges from no adverse effects to slightly or highly toxic effects (Burpee and Cole, 1978; Ocampo and Hayman, 1980; Smith *et al*., 1981; Nemec and Tucker, 1983; Trappe *et al*., 1984; Ocampo and Barea, 1985; Pellet and Sieverding, 1986; Dodd and Jefferies, 1989). However, their results showed that the types of herbicide and its rate of application are an important factor in mediating the toxic effects on mycorrhizae (Johnson and Pfleger, 1992; Hamel *et al*.,

1994). Direct effects of herbicides on root colonization and sporulation by VAM fungi have been found variable and often species- and dosage-dependent (Dehn *et al*., 1990). The beneficial effects of VAM were found to disappear when herbicides were applied at higher doses (Ocampo and Barea, 1985). Herbicides, when applied as foliar spray or *via* root system, showed the deleterious effects on the VAM-host interaction. Recent studies have shown that application of foliar-applied herbicides might affect the VA mycorrhizal symbiosis through affecting the photosynthesis of the host plant (Harley and Smith, 1983). On the other hand, the VAM-mediation uptake of soil-applied herbicides into herbicide-sensitive host plant may lead to added injury or stress that ultimately affects the growth and development of host plant (Nedumpara *et al*., 1999). Thus, the evaluation of the effects of herbicide application on VAM-plant association deserves increased attention. It is also important to identify herbicides that have toxic effects on the efficacy of VAM-plant symbiosis so that alternatives may be considered. Therefore, the present study was conducted to determine the effects of two widely-used herbicides on sporulation of VAM fungi and its infectivity in roots of peanut plants in response to herbicides application.

MATERIALS AND METHODS

Collection of 'Glomus mosseae' spore and inoculum preparation: Inoculums of *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe UK 118 obtained from INVAM (International Culture Collection of VA Mycorrhizal Fungi), which consisted of spores, external hyphae and infected root fragment. The inoculums were then propagated on *Sorghum bicolor* for 4 months in the glasshouse pot cultures using the method of Feldmann and Idczak (1991). Enumeration of the infective VAM propagules of fungi was determined by using most probable number (MPN) method as described by Sieverding (1991), resulting in 88.32 infective propagules/100 g inoculum. The spores of *G. mosseae* from above inoculums were isolated, and the number was 5.6 spores/g inoculum, as determined by the wet sieving method (Gerdemann and Nicolsom, 1963).

Herbicide treatments: The commercial herbicides used for treatments in this study were; Alachlor (Lasso®, Monsanto Sdn. Bhd.) and glyphosate (Roundup®, Monsanto). Three concentrations (rates) of each herbicide: 1.8, 3.6 and 5.4 µg a.i. g^{-1} dry soil for alachlor; and 1.08, 2.16 and 3.24 µg a.i. g^{-1} dry soil for glyphosate, were applied. These application rates represented 0.5, 1 and 1.5 times (x) their recommended field application rates (Alachlor 400 g a.i./ha, and glyphosate, 800 g a.i./ha). The treatments were calculated using the formula:

$$X\ mL/L\ media = \frac{Desired\ field\ rate\ (g\ a.i./ha)\ X\ 1000mL}{Amount\ of\ a.i.\ in\ formulation\ (g\ a.i./L)\ X\ Spray\ volume\ (L/ha)\ X\ 1L}$$

Experimental design and treatments application: The study was conducted in the greenhouse; treatments were arranged in a completely randomized design (CRD) with three replicates. The experimental pots (6 cm diameter and 20 cm height) were filled up with 1 kg of sterilized (121^0C for 1 hour) soil:sand mixture (1:3). The soil was bungor series soil (Classification: subactive typic paleudult) consisted of 34.9% sand, 16.4% silt, and 48.7% clay. The mycorrhizal inoculum was inoculated at 10% by weight per pot soil, before sowing of peanut in the surface soil layer of the pot, and then covered with a 5.0 cm soil. The herbicide treatments which consisted of three application rates of alachlor (1.8, 3.6 and 5.4 µg a.i. g^{-1}) and glyphosate (1.08, 2.16 and 3.24 µg a.i. g^{-1}) were applied as soil drench of 20 mL solution, before sowing of peanut. The control was treated with the same amount of sterile distilled water. Plants were harvested destructively at 4 and 6 weeks after planting (WAP).

Measurement of spore number in soil*:* Effect of herbicides on spore number of *G. mosseae* in the soil was determined by the wet sieving and decanting method as described by Gerdemann and Nicolson (1963). At each harvest, 20 g soil sample from each replicate was collected to a depth of 0-10 cm mycorrhizosphere soils. The soil sample was then wet sieved, and rinsed with 200 mL distilled water in a 500 mL beaker. The suspension was then poured through a series of sieves arranged in a decreasing mesh size from 300, 250, 106, and 45 µm. The trapped particles were then rinsed three times with 200 mL distilled water. The particles retained on the 106 and 45 µm sieves were transferred to a Petri dish, which was marked with parallel lines (±7 mm apart) to separate microscope fields for spore counting. Number of spores was counted by moving the dish systematically through all the lines under microscope.

Measurement of external hyphal length: The extraction and measurement of external hyphae were carried out using the method of Abbott and Robson (1995). At harvest time of peanut plant, five cores of moist soil were taken with a cork borer from each pot, and mixed well in a plastic bag. A 10 g subsamples of soil were agitated with 400 mL distilled water for 1 min and decanted through 250 and 53 µm sieves. These were then mixed together and agitated again with distilled water followed by vigorous steering on a magnetic stirrer for 5 min. A 50 mL aliquot was taken and passed through 1.2 µm Millipore filter. Estimation of the total hyphal length was done by staining the retained material on filter in trypan-blue (TB) in acidic glycerol. It (TB in acidic glycerol) was prepared by mixing 500 mL glycerol, 450 mL H_2O, 50 mL 1% HCL, and 0.05 % trypen blue (Koske and

Gemma, 1989). Total hyphal length was finally measured by using gridline-intersect method under a dissecting microscope at 250x magnification bearing a grid (10 x 10 -mm squares) in eyepiece micrometer as described by Newman (1966) and Tennant (1975). Hyphal length was calculated using the following formula:

Hyphal length/grid (cm/g soil) = c x n x g x a/b x 1/s

Where, c = constant (11/14)

n = no. of intersections

g = grid unit

a = area of filter covered by sample (mm^2)

b = area of grid (mm^2)

s = soil on filter (g)

Measurement of internal hyphal infectivity: A combined method of Phillips and Hayman (1970), and Koske and Gemma (1989) were used for observing the activity of internal hyphae of VAM fungi. Total infection intensity of the internal hyphae of *G. mosseae* was determined by staining the trypan blue (TB). At each harvest, roots were sampled and washed thoroughly on tap water, then placed into 50 mL McCartney bottles and kept overnight in 50% ethanol for fixing. The roots *ca* 3g (f. wt.) were fixed in 10% KOH, and heated in a water bath at 90^0C for 1 hour. The KOH solution was poured off and the roots was rinsed in two changes of 200 mL tap water, and finally acidified by soaking in 1% HCL for 5 min. The acidified roots were stained in 0.05% trypan blue mixed with an acidic glycerol solution. The stained roots were heated in a 90^0C water bath for 60 min. TB solution was then poured off, and the roots were distained in acidic glycerol. The distained roots were cut into 1 cm sections, and 10 cuttings among them were mounted in glycerol onto a glass slide under stereomicroscope to examine the presence or absence of VAM arbuscules, vesicles and/or internal hyphae.

The total and active infection intensity of the internal hyphae was calculated using the formulae given by Trouvelot *et al*. (1986):

$$M\% = (95n5 + 70n4 + 30n3 + 5n2 + 1n1)/N$$

Where, M= Intensity of infection

n5, n4, n3… n1 respectively designated number of fragments notes 5, 4,..,1

(0 = 0%, 1 = 1-5%, 2 = 6-10%, 3 = 11-50%, 4 = 51-90%, 5 = >90%)

N = Number of observed fragments

The active infection intensity of internal hyphae, however, was determined using the fungal SDH (Succinate dehydrogenase) staining for metabolically active fungus. The SDH activity was determined histo-chemically by the deposition of purple formazan following reduction of nitroblue tetrazolium (NBT in the presence of succinate (Smith and Gianinazzi-Pearson, 1990). Roots were cut into 0.5 cm length and incubated overnight in the reaction medium containing 50 mM Tris-HCL (pH 7.4), 0.5 mM $MgCl_2$, 1 mg mL-1 NBT, 0.25 M Na_2 Succinate. The stained roots were the rinsed with distilled water, and boiled in 20% choral hydrate for 10-15 min. The distained root cuttings were mounted in glycerol onto a glass slide under stereomicroscope to examine the presence of dark purple stain in the root fragments. The proportion of SDH activity of the internal hyphae was calculated by using the formulae as below:

$$\frac{\text{Active infection intensity of internal hyphae revealed by SDH stain}}{\text{Total infection intensity of internal hyphae revealed by TB staining}} \times 100$$

Measurement of P (phosphorus) inflow into peanut: The effect of herbicides on P inflow was measured by calculating the P inflow into plant root at 0-28 and 28-42 days after planting (DAP) of peanut. The P inflow into plant roots were calculated using the formula of Brewster and Tinker (1972) as below:

$$I = (p_2-p_1) \times In\ (L_2L_1)\ [t_2-t_1]^{-1}$$

Where, I = P inflow to roots (mol P m^{-1} root length s^{-1})

P = concentration of plant phosphorus, p_1 = first period, p_2 = second period

L = root length, L_1 = first period, L_2 = last period

T = plant age, t_1 = first period, t_2 = last period

P concentration in plant shoot was determined using the wet digestion method of concentrated sulfuric acid (H_2SO_4) and perchloric acid (Abdulhamid and Dynoodt, 1981). However, the contribution of the mycorrhizal hyphae to the root inflow (hyphal inflow) was calculated by subtracting the inflow in the control non-mycorrhizal plants (I_{nm}) from that of the mycorrhizal plants (I_m). Hyphal uptake was determined by the rate of P uptake by the external hyphae (mol P m^{-1} external hyphae length s^{-1}).

Statistical analysis: Data were analyzed by using the analysis of variance (ANOVA) and mean separation was done by Least Significance Difference (LSD) at 5% level. Some data were also analyzed by the contrast test.

RESULTS

External hyphae length and spore number: Application of different treatment rates of alachlor and glyphosate herbicides did not affect the length of external hyphae of *G. mosseae* at both 4 and 6 WAP (Table 1). Besides, contrast test for comparison between two herbicides also showed that the herbicidal effects on external hyphae length of *G mosseae* was non-significant at both 4 and 6 WAP (Table 2).

In case of sporulation, application of alachlor and glyphosate herbicides at 4 WAP showed insignificant effects on the mean spore numbers of *G. mosseae* (Table 1). However, alachlor herbicide at 6 WAP significantly decreased the mean spore numbers in soil with the increased application rates compared to the control. On the other hand, glyphosate herbicide exhibited the insignificant inhibitory effects on the mean spore

numbers among the application rates, and the lowest mean spore number (122.67 spores 10 g^{-1} soil) obtained at half of the glyphosate recommended rate (1.08 µg ai g^{-1}) was differed significantly from that of the control (229 spores $10g^{-1}$ soil). Contrast test for comparison between treatment rates of herbicides showed that at 4 WAP alachlor significantly decreased the mean spore number of *G mosseae* compared to glyphosate (Table 2). But at 6 WAP, however, both herbicides significantly decreased the mean spore number of the fungi, and clearly showed that alachlor was more toxic for *G mosseae* than glyphosate. It was also evident from table 2 that there was no correlation between external hyphae length and mean spore number of *G mosseae* at both 4 WAP and 6WAP.

Table 1. The effect of herbicides on external hyphae length and mean spore number of *G. mosseae* at 4 and 6 WAP

Herbicide	Rates (µg ai g^{-1})	External hyphae length (m)		Mean spore number	
		4 WAP	6 WAP	4 WAP	6 WAP
Control	0	1.79 (0.54) [a]	3.04 (0.51) [a]	63.00 (9.29) [a]	229.00 (26.46) [a]
Alachlor	1.8	2.02 (0.33) [a]	2.83 (0.58) [a]	58.33 (7.84) [a]	127.67 (19.67) [bc]
	3.6	1.50 (0.23) [a]	2.79 (0.38) [a]	40.67 (6.36) [a]	159.33 (25.44) [b]
	5.4	1.09 (0.51) [a]	2.62 (0.58) [a]	64.00 (5.69) [a]	87.33 (9.39) [c]
Glyphosate	1.08	1.38 (0.25) [a]	2.20 (0.16) [a]	72.00 (2.52) [a]	122.67 (8.74) [bc]
	2.16	1.58 (0.37) [a]	2.23 (0.22) [a]	76.00 (10.07)[a]	174.67 (10.91) [ab]
	3.24	1.97 (0.45) [a]	3.36 (1.28) [a]	65.67 (16.76)[a]	159.00 (13.61) [ab]

Means of data followed by the same letter in each column, are not significantly different using the LSD test at 5% level. Means in the parenthesis are standard errors

Table 2. Contrast test between treatment groups on the external hyphae and mean spore number of *G. mosseae* at 4 and 6 WAP.

Herbicide treatments	F – Value			
	External hyphae length		Mean spore number	
	4 WAP	6 WAP	4 WAP	6 WAP
Control vs Alachlor	0.39 ns	0.17 ns	0.67 ns	25.89 **
Control vs Glyphosate	0.13 ns	0.37 ns	0.58 ns	14.09 **
Alachlor vs Glyphosate	0.14 ns	0.08 ns	4.92 *	3.56 *

ns = non significant, * = significant (5%), ** = highly significant (1%)

Table 3. The effect of herbicides on total and active infection intensity of *G. mosseae* at 4 and 6 WAP

Herbicide	Rates (µg ai g^{-1})	Total infection intensity (%)		Active infection intensity (%)	
		4 WAP	6 WAP	4 WAP	6 WAP
Control	0	45.67 (7.54) [a]	55.67 (4.33) [ab]	31.93 (4.67) [a]	40.20 (3.12) [a]
Alachlor	1.8	46.00 (6.03) [a]	56.00 (5.29) [ab]	29.63 (2.91) [a]	39.60 (3.01) [a]
	3.6	30.67 (0.67) [bc]	45.67 (5.49) [b]	19.53 (2.77) [ab]	30.27 (4.22) [a]
	5.4	20.33 (1.76) [c]	25.67 (2.33) [c]	9.93 (0.29) [c]	13.27 (2.11) [b]
Glyphosate	1.08	50.00 (5.20) [a]	58.67 (3.76) [ab]	34.20 (4.23) [a]	40.60 (5.10) [a]
	2.16	40.33 (4.67) [ab]	60.33 (1.45) [a]	25.17 (3.15) [ab]	40.37 (3.10) [a]
	3.24	42.33 (4.67) [ab]	55.67 (2.96) [ab]	28.10 (3.32) [ab]	38.23 (3.16) [a]

Means of data followed by the same letter in each column, are not significantly different using the LSD test at 5% level. Means in the parenthesis are standard errors

Total and active infection intensity of internal hyphae: Application of herbicide affected the total as well as active infection intensity of internal hyphae of *G. mosseae* (Table 3). In fact, one and half of the recommended rate produced maximum deleterious effect as compared to recommended, and half of the recommended rate. Increasing rate of alachlor significantly decreased both total infection intensity (TII) and active infection intensity (AII) at sampling dates of 4 and 6 WAP; while, glyphosate showed no significant effects on both TII and AII at all application rates. On sampling date of 4 WAP, alachlor at the recommended, and one and half of the recommended rate (3.6 and 5.4 µg ai g^{-1}) showed 30.67% and 20.33% TII respectively, which was significantly varied with control (45.67%). At the same application rates of alachlor, AII was decreased

to 19.53% and 9.93% respectively, which significantly differed compared to control (31.93%). Besides, sampling on 6 WAP, the TII at rate recommended for field practice (3.6 µg ai g^{-1}) and at half of the recommended rate (1.8 µg ai g^{-1}) of alachlor application showed insignificant difference compared to control. However, the lowest TII (25.67%) obtained at one and half of the recommended alachlor rate (5.4 µg ai g^{-1}) was significantly differed compared to the control (55.67%). Similar trend was happened for AII when alachlor applied at recommended and at half of the recommended rates, whereas the AII (13.27%) at one and half of the recommended rate showed significant difference compared to control (40.20%). Contrast test for comparison between alachlor and glyphosate showed that alachlor significantly decreased both total and active infection intensity of internal hyphae of *G. mosseae* compared to glyphosate and control (Table 5).

SDH activity of internal hyphae: Proportion of SDH activity of internal hyphae of *G mosseae* was not affected by the application rates of both alachlor and glyphosate herbicides either at 4 WAP or 6 WAP (Table 4). Contrast test of comparison between alachlor and glyphosate showed that the effects of alachlor and glyphosate herbicides on SDH activity of internal hyphae of the fungi were not significant, i.e. SDH activity was not affected by both herbicides at either application rate (Table 5). **P inflow though hyphae:** Herbicides application significantly affected the P inflow through mycorrhizal hyphae. Application of alachlor decreased the P inflow into plant roots with the increase of application rates, which was however insignificant to the control (Table 6). On the other hand, glyphosate significantly increased the P inflow into roots, with maximum value of 65.31 x 10^{-13} mol P m^{-1} s^{-1} was found at recommended rate of glyphosate application (2.16 µg ai g^{-1}), which was significantly different to the control.

The hyphal inflow was substantially increased by glyphosate application in comparison to alachlor application and the control (Table 6). Consequently, hyphal uptake of P from soil was also increased substantially with glyphosate application compared to alachlor application as well as to the control plants.

Table 4. The effect of herbicides on the proportion of SDH activity of internal hyphae of *G. mosseae* at 4 and 6 WAP

Herbicide	Rates (µg ai g^{-1})	SDH activity (%)	
		4 WAP	6 WAP
Control	0	70.69 (3.57)[a]	72.63 (5.37) [a]
Alachlor	1.8	65.14 (4.48)[a]	70.99 (1.47) [a]
	3.6	63.42 (7.80)[a]	65.97 (3.03) [a]
	5.4	49.38 (3.04)[a]	51.06 (3.45) [a]
Glyphosate	1.08	68.18 (3.57)[a]	70.87 (13.06) [a]
	2.16	63.16 (7.72)[a]	66.73 (3.67) [a]
	3.24	66.26 (0.58)[a]	68.46 (2.10) [a]

Means of data followed by the same letter in each column, are not significantly different using the LSD test at 5% level. Means in the parenthesis are standard errors

Table 5. Contrast test between treatment groups on the total and active infection intensity and the proportion of SDH activity of *G. mosseae* at 4 and 6 WAP.

Herbicide treatments	F – Value					
	Total infection intensity		Active infection intensity		SDH activity	
	4 WAP	6 WAP	4 WAP	6 WAP	4 WAP	6 WAP
Control vs Alachlor	5.58 *	8.55 *	10.25 **	9.37 **	3.87 ns	2.16 ns
Control vs Glyphosate	0.07 ns	0.32 ns	0.53 ns	0.01 ns	0.70 ns	0.34 ns
Alachlor vs Glyphosate	8.88 **	24.35**	12.25 **	17.37 **	2.57 ns	1.58 ns

ns = non significant, * = significant (5%), ** = highly significant (1%)

Table 6. The effect of herbicides on P inflow into peanut roots, hyphal inflow, and hyphal uptake in symbiosis with *G. mosseae*.

Herbicide treatments	Rates (µg ai g^{-1})	P inflow into roots (x 10^{-13} mol P m^{-1} s^{-1})	Hyphal inflow (x 10^{-13} mol P m^{-1} s^{-1})	Hyphal uptake (x 10^{-13} mol P m^{-1} hyphae s^{-1})
Control	0	19.67 c	4.02bc	48.04bc
Alachlor	1.8	36.34 bc	-2.09cd	-25.96cd
	3.6	26.36 bc	5.67bc	66.82bc
	5.4	22.39 c	1.21c	12.09c
Glyphosate	1.08	36.32 bc	5.28b	74.35b
	2.16	65.31 a	41.48a	479.72a
	3.24	48.95 ab	32.58a	369.43a

Means of data sharing the same letter(s) in each column, are not significantly different using the LSD test at 5% level.

DISCUSSION

Results of the present study on interactions between herbicides and mycorrhizal association indicate that herbicides have profound effects on VAM symbiosis, However, these effects showed significant variation because of the variability in herbicide types and their rates of application. The influence of herbicides on VA mycorrhizal sporulation showed that reduction in mycorrhizal spore numbers appeared to be directly related with herbicidal application rates, and the number of spores was inversely proportional to the rates of herbicide application, as the rate increased the number of spores decreased. Some authors reported the effect of herbicide on VAM sporulation, which varied with species of VAM fungi and type of host plants (Sieverding and Leihner, 1984; Smith *et al*., 1981). The result of the present study showed that alachlor produced more inhibitory effects on the spores of *G. mosseae* than glyphosate that was in agreement with the findings of Pellet and Sieverding (1986) in which alachlor induced the maximum reduction of the spore number of *Glomus mosseae* in symbiosis with beans. However, in another study of Giovannetti *et al*. (2006) reported that glyphosate did not seem to affect the spore germination of *G. mosseae,* even at highest doses, but glyphosate added to culture medium reduced AM fungal spore germination and germ tube growth only at concentrations greater than those recommended for field use (Malty *et al*., 2006). This might be due to the differences in herbicide treatment sites in which alachlor is a soil-applied herbicide that could likely exert direct effects on nucleic acid metabolism and protein synthesis of VAM fungi than foliar-applied glyphosate (Audus, 1976). Decreased number of spores at the last observation (6WAP) might be due to the increase in number of germinated spores that already grown in the soil. This was similar to the findings of Smith *et al*. (1981) who concluded that reduce in number of spores of VAM fungi after 8 weeks of herbicide application was probably due to increased germination of spores.

The development of external hyphae of *G. mosseae* fungi was not affected by the application of both soil and foliage-applied herbicides (alachlor and glyphosate). This was due to the insensitiveness of the external hyphae of *G mosseae* to these herbicides. Similar findings were reported by Hamel *et al*. (1994) in which simazine herbicide did not affect the mycorrhizal hyphal elongation *in vitro*, but dichlobenil and paraquat herbicides, even at the lowest concentrations, significantly reduced hyphal elongation. The total and active infection intensity of internal hyaphe of *G. mosseae* was strongly decreased by the application of alachlor at the recommended and above recommended rates, which was explained by the herbicidal effects on host photosynthetate that ultimately affect the carbon requirements of VA mycorrhizal infectivity on host roots (Smith, 1980). In contrast, glyphosate had no adverse effect on the total and active infection intensity, although negative effects of glyphosate on AM fungal colonization of carrot roots were observed *in vitro* (Wan *et al*., 1998). However, in another study on different application rates of simazine, dichlobenil and paraquat herbicides showed no effects on mycorrhizal root colonization under greenhouse conditions (Hamel *et al*., 1994). This indicated that the application rate and type of herbicides are important factors for mediating the deleterious effects on mycorrhizal colonization with host root (Ocampo and Hayman, 1980; Smith *et al*., 1981; Ocampo and Barea, 1982, 1985; Nemec and Tucker, 1983). The proportion of SDH activity of internal hypae, calculated from the ratio of active and total infection intensity of internal hyphae, however, was unaffected by both alachlor and glyphosate applications. Some researchers who used vital stain assay on VAM fungi reported varying results from pesticides effects. Ocampo and Barea (1985) found decreased SDH activity in fungal metabolism due to the effects of phenmedipham herbicide. In contrast, Larsen *et al*. (1996) reported that benomyl did not inhibit fungal alkaline phosphatase activity (another vital stain) in a *Glomus*-cucumber symbiosis.

Phosphorus inflow through mycorrhizal hyphae was decreased by alachlor application, whereas application of glyphosate increased the P inflow. The increase in P inflow into the roots of plants was due to the increased P inflow and uptake by mycorrhizal hyphae after glyphosate application. This indicates that the symbiotic association of *G. mosseae* contributed significantly to the P inflow into roots peanut plants (Koide, 1991). This P increase was attributed from the enhanced mycorrhizal formation on host roots that increase the ability to absorb more nutrients through mycorrhizal hyphae (Gerdemann, 1968; Sukarno *et al*., 1996). This phenomenon can also be explained by the increased uptake of phosphorus from soil as described by Sander and Tinker (1971). As glyphosate is known as phosphate herbicides that contain P in its chemical composition ($C_3H_8NO_5P$), might contribute a great amount of phosphorus in the soil after its degradation by soil microorganism. It could be the fact that *G. mosseae* as soil fungi can take up the phosphorous released from the degraded glyphosate in the soil, which resulted in increasing P inflow into roots of peanut plants.

In the present study, foliar-applied glyphosate induces considerably less toxicity to *G. mosseae* symbiosis than soil-applied alachlor. Mycorrhizal sporulation and hyphal infectivity as well as P inflow to plant roots were significantly affected by the alachlor, but unaffected by glyphosate application rates compared to the control plant The results suggest that the depressive effects of herbicide application on mycorrhizal symbiosis

are variable, mostly depend on herbicide types and their field application rates.

REFERENCES

Abbott, L. K. and A. D. Robson (1995). Formation of external hyphae in soil by four species of vesicular-arbuscular mycorrhizal fungi. New Phytol, 99: 245-255.

Abdulhamid, S. and R. F. P. Dynoodt (1981). The fundamental manual of soil and plant analysis. Technical Bulletin. Universiti Putra Malaysia. 48 p.

Audus, L. J. (1976). Herbicides: Physiology, Biochemistry and Ecology, L. J. Audus (ed.).

Brewster, J. L. and P. B. H. Tinker (1972). Nutrient flow rates into roots. Soils and Fertilizers, 35: 355-359.

Brundrett, M., N. Bougher, B. Dell, T. Grove, and N. Malajczuk (1996). Working with Mycorrhizas in Forestry and Agriculture. ACIAR, Canberra.

Brundrett, M.C. (2002). Coevolution of roots and mycorrhizas of land plants. New Phytol, 154(2): 275-304.

Burpee, L. L. and H. Cole (1978). The influence of alachlor, trifluralin, and diazinon on the development of endogenous mycorrhizae in soybeans. B Environ Contam Tox, 19(1): 191-197.

Dehn, B., M. Bodmer, and H. Schuepp (1990). Influence of herbicides on VA mycorrhizal propagation in soil. Symbiosis, 9: 223-227.

Dodd, J. C. and P. Jeffries (1989). Effects of herbicides on three vesicular-arbuscular fungi associated with winter wheat (*Triticum aestivum* L.). Biol Fert Soils, 7: 113-119.

Entry, J. A., P. T. Rygiewicz, L. S. Watrud, and P. K. Donnelly (2002). Influence of adverse soil conditions on the formation and function of Arbuscular Mycorrhizas. Adv Environ Res, 7: 123-138.

Feldmann, F. and E. Idezac (1991). Inoculum production of VAM fungi for use in tropical nurseries. In: Methods in Microbiology, J.R. Norris, D.J. Read and A.K. Varma (eds.), 24, Techniques for the study of mycorrhiza. Academic Press, London. p. 339-357.

George, E., H. Marschner, and I. Jakobsen (1995). Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. Crit Rev Biotechnol, 1: 257- 270.

Gerdemann, J. W. (1968). Vesicular- Arbuscular mycorrhiza and plant growth. Annu Rev Phytopathol, 6: 397-418.

Gerdemann, J. H. and T. H. Nicolson (1963). Spores of mycorrhizal endogone extracted from soil by wet seiving and decanting. T Brit Mycol Soc, 46: 235-244.

Giovannetti, M., A. Turrini, P. Strani, C. Sbrana, L. Avio, and B. Pietrangeli (2006). Mycorrhizal fungi in Ecotoxicological Studies: Soil impact of fungicides, insecticides and Herbicides. Prevention Today, 2(1-2): 47-62.

Gupta, A., A. Aggarwal, C. Mangla, A. Kumar, and A. Tanwar (2011). Effect of Herbicides Fenoxaprop-P-ethyl and 2,4-D Ethyl-ester on Soil Mycoflora Including VAM Fungi in Wheat Crop. Indian J Weed Sci, 43 (1 and 2): 32-40.

Hamel, C., F. Morin, A. Fortin, R. L. Graner, and D. L. Smith (1994). Mycorrhizal colonization increases herbicide toxicity in apple. J Am Soc Hortic Sci, 119: 1255-1260.

Harley, J. L. and S. E. Smith (1983). Mycorrhizal Symbiosis. Academic Press, London. 483 p.

Johnson, N.C. and F.L. Pfleger (1992). Vesicular-arbuscular mycorrhizae and cultural stresses. In: *Mycorrhizae in sustainable agriculture*. Bethlenfalvay GJ, Linderman RG (eds). pp 71-100, ASA Special Publication No. 54. Agronomy Society of America, Madison, WI.

Kahiluoto, H., E. Ketoja, M. Vestberg (2009). Contribution of arbuscular mycorrhiza to soil quality in contrasting cropping systems. Agr Ecosyst Environ, 134(1-2): 36-45.

Koide, R. T. (1991). Nutrient supply, nutrient demand and plant response to mycorrhizal infection. New Phytol, 117: 365-386.

Koske, R. E. and J. N. Gemma (1989). A modified procedures for staining roots to detect VA mycorrhizas. Mycol Res, 92: 486-505.

Larsen, J., I. Thingstrup, and Jakobsen (1996). benomyl inhibits phosphorus transpost but not fungal alkaline phosphatise activity in a *Glomus*-cucumber symbiosis. New Phytol, 132: 127-133.

Malty, J.D., J.O. Siqueira, F.M.D. Moreira (2006). Effects of glyphosate on soybean symbiotic microorganisms, in culture media and in greenhouse. Pesqui. Agropecu. Bras., 41: 285–291.

Nedumpara, M. J., T. B. Moorman, and K. Jayachandran (1999). Effects of vesicular-arbuscular mycorrhizal fungus (*Glomus epigaeus*) on herbicide uptake by roots. Biol Fert Soils, 30:75-82.

Nemec, S. and D. Tucker (1983). Effects of herbicides on endomycorrhizal fungi in Florida citrus (Citrus spp.) soils. Weed Sci, 31: 427-431.

Newman, E. I. (1966). A method of estimating total length of root in a sample. J Appl Ecol, 3: 139-145.

Ocampo, J. A. and J. M. Barea (1982). Depressed metabolic activity of VA mycorrhizal fungi by

photosythesis inhibitor herbicides. In: Les mycorrhizas: bilogie et utilization, INRE (ed.), 13, Collq. Inst. Natl. Rech. Agron. Dijon, France, 5-6 May 1982. Institut National de la Recherche Agronomique, Cedex, France. p. 267-270.

Ocampo, J. A. and J. M. Barea (1985). Effect of carbamate herbicides on VA mycorrhizal infection and plant growth. Plant and Soil, 85: 375-383.

Ocampo, J. A. and D. S. Hayman (1980). Effects of pesticides on mycorrhiza in field-grown barley, maize and potatoes. T Brit Mycol Soc, 74: 413-416.

Pellet, D. and E. Sieverding (1986). Influence des traitements herbicides sur les mycorrhiza VA de *Glycine max* L. et *Phaseolus vulgaris*. In: Physiological and genetical aspects of mycorrhizae, Gianinazzi-Pearson, V. and Gianinazzi, S. (eds.), INRA, Paris. p. 707-712.

Philips, J. M. and D. S. Hayman (1970). Improved procedures for cleaning roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. T Brit Mycol Soc, 55: 158-160.

Quilambo, O. A. (2000). Functioning of peanut (*Arachis hypogaea*L.) under nutrient deficiency and drought stress in relation to symbioticassociations.PhD thesis. University of Groningen, the Netherlands.Van Denderen B.V., Groningen.

Sander, F. E. and P. B. Tinker (1971). Mechanism of absorption of phosphate from soil by Endogone mycorrhizas. Nature, 233: 278-279.

Sieverding, E. (1991). Vesicular-Arbuscular mycorrhiza management in tropical agrosystem. Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ) GmbH, Eschborn, Germany.32 p.

Sieverding, E. and D. E. Leihner (1984). Effect of herbicides on population dynamics of VA-mycorrhiza with cassava. Angew Bot, 58: 283-294.

Smith, S. E. (1980). Mycorrhizas of autotrophic higher plants. Biol Rev, 55: 475-510.

Smith, T. F., A. J. Noack, and S. M. Cosh (1981). The effect of some herbicides on vesicular-arbuscular endophyte abundance in soil and on infection of host roots. Pestic Sci, 12: 91-97.

Smith, S. E. and V. Gianinazzi-Pearson (1990). Phosphate uptake and arbuscular activity in mycorrhizal Allium cepa L. Effects of photon irradiance and phosphate nutrition. Aus J Plant Physiol, 17: 177–188.

Smith, S. E. and D. J. Read (2008). Mycorrhizal Symbiosis. Ed. 3, Academic Press, New York.

Sukarno, N., F. A. Smith, S. E. Smith, and E. S. Scott (1996). The effects of fungicides on vesicular-arbuscular mycorrhizal symbiosis. II. The effects on area of interface and efficiency of P uptake and transfer to plant. New Phytol, 132: 583-592.

Tennant, D. (1975). A test of a modified method of estimating root length, J Ecol, 63: 995-1001.

Trappe, J. M. R., R. Molina, and M. Castellano (1984). Reactions of mycorrhizal fungi and mycorrhiza formation to pesticides. Annu Rev Phytopathol, 22: 331-359.

Trouvelot, A., J. L. Kough and V. Gianinazzi-pearson (1986). Measure du taux de mycorrhization VA d'un systeme radiculaire. Recherche de methods d'estimation ayant une significantion fonctionnelle. In: Physiological and genetic aspects of mycorrhizae, Gianinazzi-Pearson and S. Gianinazzi (eds.), INRA, Paris. p. 217-221.

Wan, M. T., J. E. Rahe and R. G. Watts (1998). A new technique for determining the sublethal toxicity of pesticides to the vesicular-abuscular mycorrhizal fungus *Glomus intraradices.* Environ Toxicol Chem, 17: 1421-1428.