Madras Agric. J., 2018; doi:10.29321/MAJ 2018.000209

RESEARCH ARTICLE

Effect of Glyphosate on Arbuscular Mycorrhizal Fungi in Soil and Growth of *Abelmoschus esculentus*

P.M. Brindhavani*, P. Janaki and J. Ejilane

Department of Soil Science and Agricultural Chemistry, Anbil Dharmalingam Agricultural College and Research Institute Tamil Nadu Agricultural University, Trichy, India.

Received : 13th July, 2018
Revised : 28th September, 2018
Accepted : 30th September, 2018

Abstract

A field experiment was carried out during Rabi 2017-18 to study the effect of glyphosate on Arbuscular mycorrhizal fungi (AM) activity in sodic soil and Okra *(A. esculentus*) growth at Anbil Dharmalingam Agricultural College & Research Institute, Tiruchirapalli. Experiment was conducted in Randomized Block Design (RBD) with seven treatments viz., control, AM 100 kg ha^{-1}, glyphosate 12 $ml.L^{-1}$, AM + different rates of glyphosate @ 6, 12, 18 and 24 $ml.L^{-1}$ and were replicated thrice. The glyphosate was applied as pre plant herbicide 20 days before sowing and AM bio fertilizer was applied as basal before sowing of *A. esculentus*. Results showed that the increased rates of glyphosate decreased the root colonization and AM spore was only 19.00 and 20.67 (no/100 g of soil) at flowering and harvest stages respectively in AM fungi plus double dose of glyphosate (24 $ml.L^{-1}$) applied plot. Significantly higher root colonization and AM spore was recorded by AM alone applied plot and also increased the soil available P status at harvest significantly. The AM application over control increased the colonization percentage as well as spore count (no/100 g of soil) to the tune of 66-69 and 55-59 per cent, respectively and showed that the native AM viability is very low in sodic soil. The higher rates of glyphosate plus AM application reduced the root and leaf efficiency of *A. esculentus* and also delayed the days to 1st and 50% flowering.

Keywords: *Abelmoschus esculentus, Glyphosate, AM fungi, Root colonization, Spores, Sodic soil*

Introduction

Glyphosate, an aminophosphonic analogue of the natural amino acid glycine is the world's largest selling herbicide due to low production cost and high weed killing efficiency. It is applied to both cropped and noncropped lands for controlling weeds due to broad spectrum of activity and low mammalian and ecological toxicity (Busse *et al*., 2001). Glyphosate controls the weeds by inhibiting the chloroplast-localized enzyme 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase in shikimate pathway (Bode *et al.*, 1984). Since the shikimate synthesis pathway is also occurring in microorganisms, it may also affect the growth and population of non targeted microorganisms in soil. Abundant research has reported the effect of glyphosate on the soil microbial communities, microbial biomass and microbial respiration and the results are vastly inconsistent and ambiguous. Negative effects of glyphosate on the nitrogen fixing symbiotic bacteria have been reported (Reddy *et al*., 2000). Studies have also reported the positive to negative or no effect of glyphosate on AM root colonization (Pasaribu *et al.,* 2013). Reduction of the AM fungal spore viability and root colonization due to glyphosate and its metabolite Amino methyl phosphonic acid (AMPA) was observed by Zaller *et al*. (2014).

The AM influence soil fertility and crop production by involving in nutrient cycling particularly cationic micronutrients and phosphorus, soil aggregate formation and organic matter turnover etc. However the type of microbial community and their behavior in sodic soil (ESP > 15 and pH > 8.5) is varying when compared to neutral cultivated soil. The impact of glyphosate on AM spores and root colonization was also influenced by soil characteristics. Influence of soil characteristics like soil P level, salinity (Abbott and Robson, 1991), hydrologic condition of the soil (Escudero and Mendoza, 2005), seasons and type of vegetations (García & Mendoza, 2008), herbicide particularly glyphosate (Druille *et al.,* 2013; Gomes *et al*., 2015) on the AM growth and distribution has been reported. No studies have been found in the pertinent literature referenced to glyphosate effects on AM colonization and spore viability in sodic soil. Hence it is hypothesized that the effect of glyphosate on nontargeted AM colonization and spores number in sodic soil is varying and was tested in the present study with *A. esculentus* as test crop.

Corresponding author's email: brindhamanohar94@gmail.com

Material and Methods

Experimental details

A field experiment was conducted at Anbil Dharmalingam Agricultural College and Research Institute, TNAU, Tiruchirapalli, Tamil Nadu, India during *Rabi*, 2017-18. The experimental site is geographically located in Cauvery Delta zone of Tamil Nadu at 10°45' N latitude and 78°36' E longitude and at an altitude of 85 MSL. The soil of the experimental fields is sandy clay loam in texture, low in available nitrogen, medium in available phosphorus and potassium. The experimental field soil have pH 8.90, EC is 0.37 dSm^{-1}, CEC is 20.90 c mol (P+) kg^{-1} and ESP 24.9%.The *A. esculentus* variety, Arka Anamika was grown as test crop in a spacing of 30 ×45 cm with seven treatments *viz.,* control, AM fungi 100 kg ha^{-1} alone, glyphosate 12 $ml.L^{-1}$ (recommended dose) alone, AM fungi + glyphosate 6$ml.L^{-1}$, AM fungi + glyphosate 12 $ml.L^{-1}$, AM fungi + glyphosate 18 $ml.L^{-1}$, and AM fungi + glyphosate 24 $ml.L^{-1}$. Experiment was conducted in Randomized Block Design (RBD) and each treatment were imposed in the plot size 20 m^2 (5 x 4 m) with three replications. Preplant application of glyphosate was done on 20 days before the sowing of *A. esculentus* seeds along with the adjuvant ammonium sulphate (15 g L^{-1}) as per the treatments. After 20 days of its application each plot was ploughed separately using power tiller and sowing of *A. esculentus* was done on ridges and furrows. The AM biofertilizer purchased from TNAU, Coimbatore was applied as basal by mixing with sand on a day before sowing of *A. esculentus* seeds.

Plant and soil sampling

Three plants from each plot were uprooted on 45th and 60th days after sowing corresponding to the time of flowering and harvest for studying the AM colonization in *A. esculentus* roots and growth parameters. Similarly the soil samples were also collected on the above days to observe the AM spores in sodic soil.

Measurement of AM colonization and spore number

The AM colonization was studied using root cleaning and staining method of Phillips and Hayman (1970). First the fresh root bits were cut into small pieces of less than 1 cm and immersed in 10 % KOH solution for clearing the host cytoplasm and nuclei for the penetration of stain. Then it was autoclaved at 15 lbs/sq. inch pressure for about 20 minutes. The root bits were taken out and washed with tap water for about 3 minutes or until no brown colour appeared in the rinsed water and later the roots were acidified with 2% HCl for proper staining. After acidification, it was rinsed with tap water and were stained by 0.05 M trypan blue in lactophenol solution and boiled for 2 minutes. The stained root bits were examined under stereomicroscope. About 80 root bits in each plot were used to determine the AM colonization. The colonization percent was calculated using the formula as given below.

$$\% \text{ of AM colonization} = \frac{\textit{No. of root bits with infection}}{\textit{No. of root bits examined}} \times 100$$

Enumeration of AM fungal spores in soil

AM spore population in soil was estimated by wet sieving and decantation method of Gerdemann and Nicolson (1963). About 100 gram of soil was collected from rhizosphere region of *A. esculentus* from each plot and mixed thoroughly in 1 litre of tap water and allowed to stand until the heavier particles settles down. Suspension was decanted through a coarse soil- sieve (500-800 µm sieve) to remove large pieces of organic debris and the liquid from sieve was collected separately and stirred to re-suspend all particles. The suspension was decanted through a sieve (38-250 µm sieves) fine enough to retain desired spores. Soil along with spores retained in petridish were collected and examined under stereomicroscope. The spore numbers from each treatment were counted and expressed as number per 100 g of soil.

Growth and yield parameters of A. esculentus

Root and leaf efficiency

After 45 days of herbicide application, the three plants from each plot were uprooted and the number of secondary roots and the number of leaves were counted to calculate the rooting efficiency and leaf efficiency as given below.

$$\text{Root efficiency } \% = \frac{\textit{No. of secondary roots in treatment plot}}{\textit{No. of secondary roots in control plot}} \times 100$$

$$\text{Leaf efficiency } \% = \frac{\textit{No. of leaves in treatment plot}}{\textit{No. of leaves in control plot}} \times 100$$

Days to flowering

The days to onset of first and 50 per cent flowering from the date of sowing was recorded from each plots on the five randomly selected plants and the mean values were calculated.

Results and Discussion

Effect of glyphosate rates on AM spores and colonization

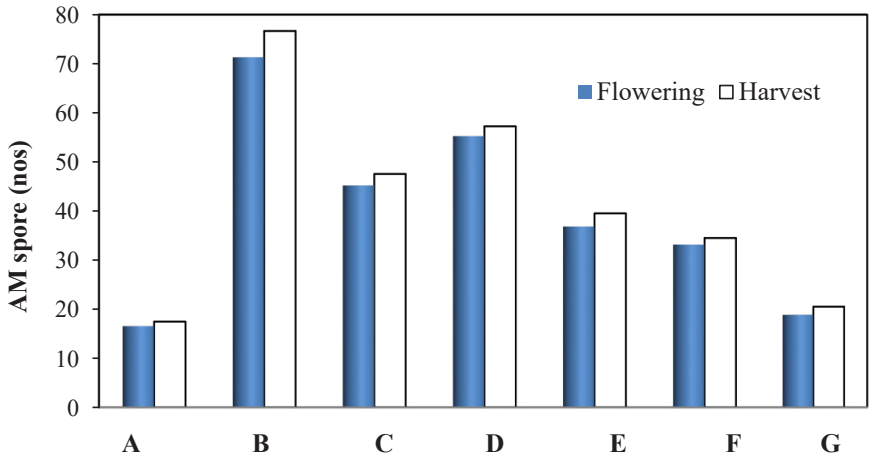
AM spores in sodic soil

Glyphosate rates had significant influence on AM spore population and root colonization measured during flowering and harvest stages of the *A. esculentus* crop (Fig. 1) in sodic soil. Significantly higher AM spores of 71.33 and 76.67 % at flowering and harvest stages, respectively, was recorded in AM fungi alone applied plot and was followed by AM fungi + glyphosate 6 ml L^{-1} applied plot. The AM spore was only 19.00 and 20.67 (no./100 g of soil) at flowering and harvest stages respectively in AM fungi plus double dose of glyphosate (24 ml L^{-1}) applied plots. Negative impact of glyphosate on non-target organisms like earthworms and its cocoon viability (Casabe *et al.*, 2007) and nodule biomass and leghemoglobin content in soybean (Reddy *et al.,* 2000) was also reported in literature.

Table 1. Effect of combined application of glyphosate (ml L^{-1}) and AM fungi (100 kg ha^{-1}) on available P (kg ha^{-1}) status in soil

Treatments	Available P status in soil	
	Flowering	Harvest
Control	12.2	7.4
AM Fungi alone	14.7	18.5
Glyphosate alone (12 ml L^{-1})	12.2	16.3
AM + Glyphosate (6 ml L^{-1})	12.0	13.2
AM + Glyphosate (12 ml L^{-1})	12.0	11.3
AM + Glyphosate (18 ml L^{-1})	12.0	11.0
AM + Glyphosate (24 ml L^{-1})	10.3	8.5
Mean	12.20	12.31
CD (P=0.05)	NS	2.91

Fig. 1. Effect of combined application of glyphosate (ml L^{-1}) and AM Fungi (100 kg ha^{-1}) on spore count (nos/100 g of soil)



A- Control; B- AM; C- Glyphosate (12 ml.L^{-1}); D- AM + Glyphosate (6 ml.L^{-1}); E- AM + Glyphosate (12 ml.L^{-1}); F- AM + Glyphosate (18 ml.L^{-1}); G- AM + Glyphosate (24 ml.L^{-1})

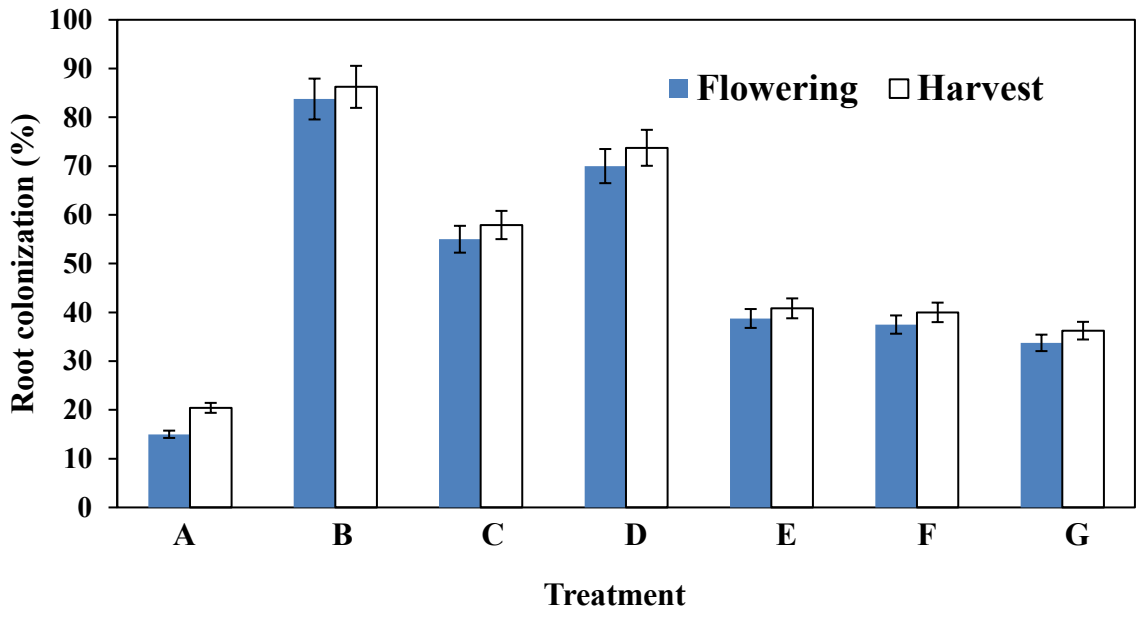
The decrease in AM spore was observed even at lower rate (6 ml.L^{-1}) of glyphosate application and decreased further with increase in glyphosate rate in the present study. It could be attributed to the changes in properties of the present experimental soil due to high pH (8.9) and ESP (24.9%) like destruction of soil structure, soil compaction, poor soil aeration, and reduction of macro pores might affected the AM spore population and the development of mycorrhizal colonization in soil. Malty *et al.* (2006) found that the glyphosate concentrations above the recommended rate of field use affected AM fungal spore germination and germ tube growth in the

Table 2. Effect of combined application of Glyphosate (ml.L^{-1}) and AM (100 kg ha^{-1}) on Growth and Yield parameters of *A. esculentus*

Treatments	Efficiency of root and leaf formation (%)		Days to first flowering	Days to 50 % flowering
	Root	Leaf		
Control	-	-	42	44
AM Fungi alone	91.33	90.61	40	43
Glyphosate alone (12 ml.L^{-1})	84.00	76.43	43	46
AM + Glyphosate (6 ml.L^{-1})	77.67	69.45	44	47
AM + Glyphosate (12 ml.L^{-1})	72.33	62.12	45	48
AM + Glyphosate (18 ml.L^{-1})	71.33	54.94	46	50
AM + Glyphosate (24 ml.L^{-1})	57.33	47.61	48	52
Mean	75.67	66.86	44	47
CD (P=0.05)	9.52	8.62	5.2	6.0

culture medium. However Pasaribu *et al.* (2013) reported that the glyphosate had no significant effects at all application rates on AM spore number in sterilized clayey soil with peanut crop. This showed that the soil type and properties influenced the AM spores population in soil (Nadian *et al.,* 1998).

Fig. 2. Effect of combined application of glyphosate (ml.L^{-1}) and AM fungi (100 kg ha^{-1}) on root colonization percentage



A-Control; B-AM; C-Glyphosate(12ml.L^{-1}); D-AM+Glyphosate(6ml.L^{-1}); E-AM Glyphosate (12ml.L^{-1}); F-AM+Glyphosate(18ml.L^{-1});G-AM+Glyphosate (24 ml.L^{-1})
Error bars indicates the SEd at 5% value

AM colonization in sodic soil

The effect of glyphosate rates and AM fungi on *A. esculentus* root colonization during flowering and harvest was studied (Fig. 2) in sodic soil. Root colonization of AM in *A. esculentus* roots was higher during harvest than at flowering stage. At both the stages, the significantly higher colonization per cent was recorded by AM fungi alone plot and was followed by the AM fungi plus glyphosate @ 6 ml.L^{-1} applied plot. Similar to AM spores count, the root colonization was very low at the higher rates of glyphosate plus AM applied plots. It is evident that, the higher doses of glyphosate decrease the root colonization drastically. The decrease in AM colonization of *Lolium multiflorum* roots with increased glyphosate rate was also observed by Druille *et al.* (2013). They reported that soil residence time of glyphosate and/or its degradation products was enough to reduce AM spore viability and their ability to colonize roots.

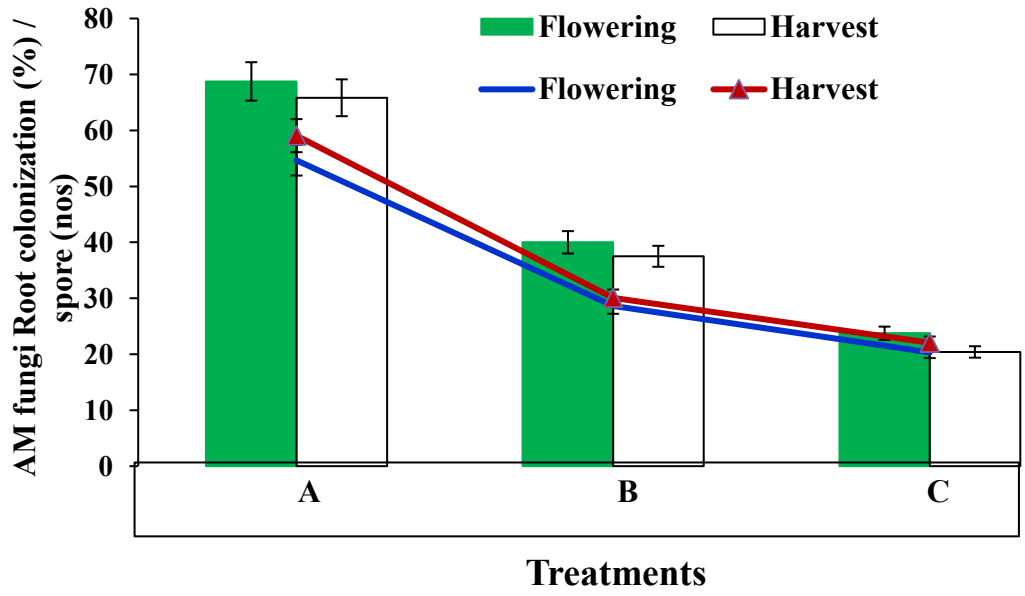
Table 3. Effect of combined application of glyphosate (ml.L^{-1}) and AM (100 kg ha^{-1}) on Yield of A. esculentus

Treatments	Fruit yield (q ha^{-1})
Control	36.88
AM fungi alone	50.03
Glyphosate alone 12 ml.L^{-1}	50.18
AM + Glyphosate 6 ml.L^{-1}	45.90
AM + Glyphosate 12 ml.L^{-1}	45.23
AM + Glyphosate 18 ml.L^{-1}	37.88
AM+ Glyphosate 24 ml.L^{-1}	28.95
Mean	42.15
CD (P=0.05)	9.71

Interaction of glyphosate and sodicity on AM

In the present study, AM colonization and spores count was very poor under control plot (Figs.1 and 2) where in the AM biofertilizer was not applied irrespective of the crop growth stages. This reflected the effect of soil conditions like sodicity, nutrient availability, poor soil structure etc on AM. However the AM application increased the colonization as well as spores count to the tune of 66-69 and 55-59 per cent respectively (Fig.3)

Fig. 3. Per cent increase in AM colonization (%) and spore numbers (no./100 g of soil) over control



A- AM (100 kg ha^{-1}); B- Glyphosate (12 ml.L^{-1}); C- AM + Glyphosate (12 ml.L^{-1}) *Bars denote the root colonization and Lines denotes the AM spore numbers; Error bars indicate the SEd at 5% value

over control which showed that the native AM viability is very low and due to poor physical conditions of the sodic soil. The destruction of soil structure by the dispersion of colloids or clay by higher Na^+ level causes soil compaction and poor soil aeration in the present sandy clay loam soil might have contributed to the decreased AM colonization and spore numbers. The decreased response of mycorrhizal growth with increasing soil compaction has been reported by Nadian *et al*. (1998) and Saif (1983) also stated that the optimum AM development needs good soil aeration. Further the accumulation of exchangeable Na^+ in the rhizosphere region may cause mechanical impedance to root penetration due to poor soil structure and thereby responsible for poor native AM mycorrhization and spore counts in soil.

The combined effect of glyphosate and different rates of glyphosate on P availability in soil at flowering and harvest stages was studied and found that the effect was significant only at harvest stage (Table 1). The P availability was significantly higher in the AM fungi alone and was decreased when both AM fungi and glyphosate was applied together. The increase in glyphosate rate plus AM fungi 100 kg/ha decreased the P availability in soil. In alkaline and high pH soils, the availability of P is low due to its adsorption on clay particles and the

formation of complexes with Ca^{2+} and H^{+} soil. Hence when AM fungi are applied it enhances P availability in soil by its mobilization through the formation mycorrhizal hyphae and the same is observed in the present study. The decrease in P availability with the combined application of AM fungi with different rates of glyphosate could be attributed to the reduction in AM fungi colonization and spores (Fig. 1 & 2) caused by the long residence time of glyphosate (Druille *et al.,* 2013).

This interaction of glyphosate with AM at recommended rates of application was studied. When comparing the recommended glyphosate 12 ml.L^{-1} alone and the combined application of glyphosate 12 ml.L^{-1} plus AM biofertilizer applied plots, the AM colonization and spores number decreased significantly irrespective of the *A. esculentus* growth stages (Fig. 1 and Fig. 2) over the AM alone applied plot. The per cent decrease was significantly higher (45%) in the plot which received combined application of AM plus glyphosate 12 ml.L^{-1} and was followed by glyphosate alone (28%) applied plot. Zaller *et al*. (2014) also reported about 40% reduction of mycorrhization of white clover after glyphosate (Roundup) application in soils amended with the mycorrhizal *G. Mosseae* in silt loam soil. This decreased root colonization and spore numbers in the present experimental sodic soil could be attributed to the increased bioavailability of glyphosate in soil solution owing to decreased adsorption and increased desorption on/from soil exchange sites. This elevated concentration of glyphosate might have reduced the mycorrhiza hyphae growth and spores number by inhibiting the shikimate pathway which is also present in the microorganisms. Gomes *et al*. (2014) reported that the chemical similarities, between PO_4^{3-} and glyphosate compete for soil adsorbing sites and higher PO_4^{3-} through fertilization increasing glyphosate bioavailability in the soil solution which increased glyphosate uptake by willow roots.

Effect of glyphosate and AM on A. esculentus growth parameters

The leaf and root forming efficiencies of the *A. esculentus* observed on 30 DAS was significantly influenced by AM application when compared to control (Table 2). Both leaf and root efficiency was significantly higher in AM fungi alone applied plot. However the combined application of glyphosate at different rates with AM biofertilizer decreased the leaf and root forming efficiency. Both 1st and 50 per cent flowering was first seen in AM alone applied plot as a result of the higher leaf and root formation. The days to 1st and 50 per cent flowering in *A. esculentus* was delayed with increase in glyphosate rates plus AM application. Both the root and leaf efficiency was significantly very poor in glyphosate applied plots, especially in the AM fungi + glyphosate 24 ml.L^{-1} treatment. This showed that the application of AM enhanced the glyphosate availability in soil and affects plant growth. This could also be due to the non availability of essential micronutrients to the crop (Eker *et al*. 2006).

Bhendi fruit yield

The application of AM fungi and different glyphosate rates had significant influence on the bhendi fruit yield. The fruit yield was higher in glyphosate alone 12 ml.L^{-1}, AM fungi alone, AM fungi + glyphosate 6 ml.L^{-1} applied treatments. This could be ascribed to the efficient formation of root which might facilitate the plant to take up more nutrients from the soil due to increased AM colonization of bhendi roots and spores in soil. Positive and significant correlation of bhendi fruit yield was observed with the root formation (0.867*) and leaf formation (0.786*).

Significantly lower fruit yield (28.95 q ha^{-1}) was recorded in the control plot and plots which received AM fungi + glyphosate 24 ml.L^{-1} and AM fungi + glyphosate 18 ml.L^{-1} treatments. Since the root and leaf efficiency were low and took more days for first flowering and the yield was very low. Lower yield in control plot could be attributed to the higher weed growth and its competition with bhendi for nutrients throughout its growth period. The increase in glyphosate dosage decreased the fruit yield. This was also reported by Abouziena *et al*. (2008) and Santos *et al*. (2007).

Conclusion

The present study concludes that the pre-plant application of glyphosate above the recommended rates @ 18 or 24 ml. L^{-1} plus AM fungi biofertilizer to sodic soil affected the AM spore numbers as well as its colonization in *A. esculentus* roots. It was also found that the native AM spore and mycorrhization is very low in sodic soil and could be enhanced by the application of AM biofertilizer for benefitting the *A. esculentus* growth. However detailed investigation is required to recognize the actual mechanism of interaction between the increased dose of glyphosate and AM activity in sodic soil and its effect on crop growth.

References

Abbott, L.K. and A.D. Robson. 1991. Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. *Agriculture, Ecosystems and Environment.*,**35(2–3):** 121–150.

Abouziena, H.F., Hafez, O.M., El-Metwally, I. M., Sharma, S.D. and M. Singh. 2008. Comparison of weed suppression and mandarin fruit yield and quality obtained with organic mulches, synthetic mulches, cultivation, and glyphosate. *Hort Science.,* **43(3)**: 795–799.

Bode, R., Melo, C. and D. Birnbaum. 1984. Mode of action of glyphosate in Candida maltosa. *Archives of Microbiology.*, **140(1)**: 83–85.

Busse, M.D., Ratcliff, A.W., Shestak, C.J. and R.F. Powers. 2001. Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. *Soil Biology and Biochemistry.*, **33(12–13)**: 1777–1789.

Casabé, N., Piola, L., Fuchs, J., Oneto, M. L., Pamparato, L., Basack, S. and E.Kesten. 2007. Ecotoxicological assessment of the effects of glyphosate and chlorpyrifos in an Argentine soya field. *Journal of Soils and Sediments.,* **7(4):** 232–239.

Druille, M., Cabello, M.N., Omacini, M. and R.A. Golluscio. 2013. Glyphosate reduces spore viability and root colonization of arbuscular mycorrhizal fungi. *Applied Soil Ecology.*, **64**: 99–103.

Eker, S., Ozturk, L., Yazici, A., Erenoglu, B., Romheld, V. and I. Cakmak. 2006.Foliar-applied glyphosate substatially reduced uptake and transport of iron and manganese in suflower (*Helianthus anuus* L.) plants. *Journal of Agricultural and Food Chemistry.,* **54:** 10019–10025.

Escudero, V. and R. Mendoza. 2005. Seasonal variation of arbuscular mycorrhizal fungi in temperate grasslands along a wide hydrologic gradient. *Mycorrhiza.*,15(4): 291–299.

García, I. V. and R. E. Mendoza. 2008. Relationships among soil properties, plant nutrition and arbuscular mycorrhizal fungi-plant symbioses in a temperate grassland along hydrologic, saline and sodic gradients. *FEMS Microbiology Ecology.,* **63(3)**: 359–371.

Gerdemann, J.W. and T.H. Nicolson. 1963. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society.*, **46(2)**: 235–244.

Gomes, M. P., Smedbol, E., Chalifour, A., Hénault-Ethier, L., Labrecque, M., Lepage, L. and P. Juneau. 2014. Alteration of plant physiology by glyphosate and its by- product aminomethylphosphonic acid: An overview. *Journal of Experimental Botany.*, **65(17)**: 4691–4703.

Malty, J.D.S., Siqueira, J.O. and F. M. De Souza Moreira. 2006. Efeitos do glifosato sobre microrganismos simbiotróficos de soja, em meio de cultura ecasa de vegetação. *Pesquisa Agropecuaria Brasileira.*, **41(2)**: 285–291.

Nadian.H, Smith. S.E, Alston. A.M. and R.S. Murray. 1998. Effects of soil compaction on plant growth, phosphorus uptake and morphological characteristics of vesicular–arbuscular mycorrhizal colonization of *Trifolium subterraneum. The New Phytologist.*, **135(2)**: 303–311.

Pasaribu, A., Mohamad, R. B., Hashim, A., Rahman, Z. A., Omar, D. and M.M. Morshed. 2013. Effect of herbicide on sporulation and infectivity of vesicular arbuscular mycorrhizal (*Glomus mosseae*) symbiosis with peanut plant. *Journal of Animal and Plant Sciences.*, **23(6)**: 1671–1678.

Phillips, J. M. and D. S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society.,* **55(1)**: 158-IN18.

Reddy, K. N. and K.Whiting. 2000. Weed Control and Economic Comparisons of Glyphosate-Resistant, Sulfonylurea-Tolerant and Conventional Soybean (*Glycine ma*)., **14(1)**: 204–211.

Ronco, M. G., Ruscitti, M. F., Arango, M.C. and J. Beltrano. 2008. Glyphosate and mycorrhization induce changes in plant growth and in root morphology and architecture in pepper plants (*Capsicum annuum* L.). *Journal of Horticultural Science and Biotechnology.*, **83(4)**: 497–505.

Saif, S. R. 1983. The Influence of Soil Aeration on the Efficiency of Vesicular- Arbuscular Mycorrhizas . II . Effect of Soil Oxygen on Growth and Mineral Uptake in *Eupatorium odoratum* L . *New Phytologist.*, **95(3)**: 405–417.

Santos, B. M., Gilreath, J.P., Esmel, C.E. and M. N. Siham. 2007. Effects of sublethal glyphosate rates on fresh market tomato. *Crop Protection.*, **26(2)**: 89–91.

Zaller, J. G., Heigl, F., Ruess, L. and A. Grabmaier. 2014. Glyphosate herbicide affects below ground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem. *Scientific Reports.*, **4:** 1–8.