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Influence of arbuscular mycorrhizal fungi on glyphosate dissipation rate in okra cultivated sodic soil of Tamil Nadu

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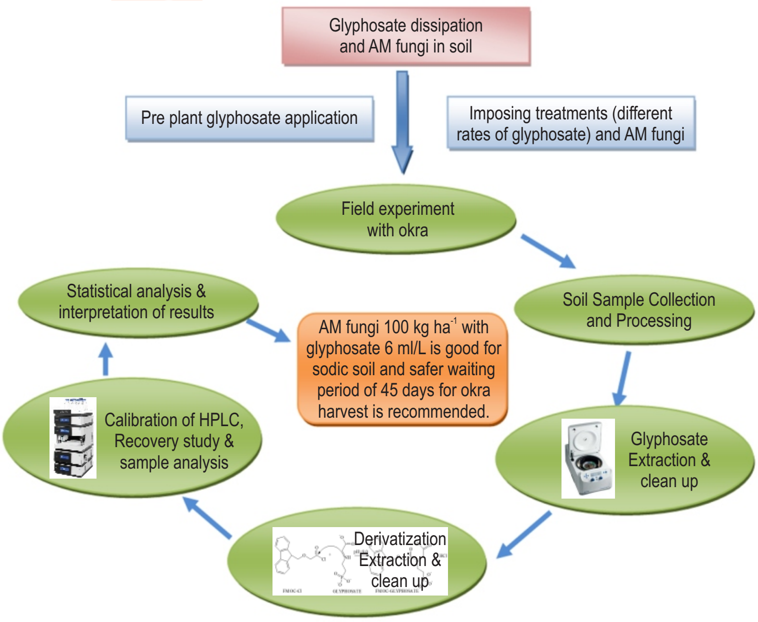
Abstract

Aim: To elucidate the influence of non target Arbuscular Mycorrhizal (AM) fungi on glyphosate dissipation in okra cultivated sodic soil of Tamil Nadu.

Methodology: A field experiment was carried out during Rabi 2017-18 to study the interactive effect of glyphosate and Arbuscular mycorrhizal fungi (AM) on glyphosate dissipation in sodic soil from okra (*A. esculentus*) grown field. Experiment was laid adopting Randomized Block Design (RBD) with different rates of glyphosate and with and without AM fungi. Glyphosate was applied as pre-plant herbicide 20 days before sowing, and AM biofertilizer was applied as basal before sowing of okra. Soil samples were collected from the field at different time interval after glyphosate application and analysed for glyphosate and Amino Methyl Phosphonic Acid (AMPA) residues.



Results: Glyphosate persistence decreased with time and degraded with the mean half-life ranging from 5.09 to 10.35 days by following first order reaction kinetics. AMPA was witnessed from day 1 and increased up to 15th day and followed first order reaction kinetics dissipation with the mean half-life of 8.62 to 13.06 days. Glyphosate and AMPA residues were recorded below detection limit (0.01 mg kg^{-1}) on 45th and 60th days, respectively, in soil.

Interpretation: The higher rates of glyphosate showed enhanced persistence of glyphosate and its metabolite AMPA in soil, hence application of AM fungi 100 kg ha^{-1} with lower glyphosate rate of 6 ml/L can be recommended for okra cultivated sodic soil with the safer waiting period of after 45 days harvest.

Key words: AM fungi, *A. esculentus*, Glyphosate, Sodic soil

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Introduction

The herbicides consumption in India during current decade is in ascending trend to surmount labour scarcity and achieve timely weed management. Since 2005, the value of herbicide market in India has doubled and is expected to grow about 40% annually over next 5 years (Frabotta, 2011). Out of 6704 tonnes of pesticides formulation consumed in India, 1697 tonnes was glyphosate during 2009-10 (Choudhury *et al*., 2018). Out of 66 herbicides registered in India, 12 are predominantly used in Tamil Nadu under different situations with the major consumption of 30.56% glyphosate (Janaki *et al.,* 2019). It is mainly applied for the management of weeds in non cropped situations or as pre plant application to craft field preparation trouble-free.

Glyphosate is immobile and moderately persistent in soil environment and resistant to chemical and photodegradation due to its strong adsorption to soil particles. Its degradation by microorganisms is the major mechanism found in soil and dissipated to either aminomethylphosphonic acid (AMPA), a primary metabolite (Peruzzo *et al.,* 2008) or sarcosine (Tu *et al.,* 2000). Glyphosate half-life in soil varies from 2 - 197 days with the field values of 47 days (Albers *et al.,* 2009; Yamada *et al.,* 2009) and is also reported in years too (Carlisle and Trevors, 1988). Glyphosate sorption, release and dissipation in soil are influenced by the soil properties like mineral content and type, pH, soil redox conditions, phosphate content or compounds that compete with sorption sites and organic matter (Borggaard and Gimsing, 2008; Todorovic *et al.,* 2013; Okada *et al.,* 2017). The intensity and frequency of rainfall and poor state of soils also influences the loss of glyphosate through erosion (Screpanti *et al.,* 2005). Herbicides, apart from controlling the weeds may also affect the crop plant and beneficial non-target soil microbes (Pasaribu *et al.,* 2013). Recent studies have reported that glyphosate not only destructs the shikimic acid pathway but it ceases the ability of plants to defend against pathogens that inhabit the rhizosphere (Trappe *et al.,* 1984). Hence, it is important to study the possible interactions among AM fungi, crops and glyphosate. Similarly, dissipation of glyphosate in sodic soil has not been deliberated which has poor physico-chemical and biological conditions due to deflocculating clays when compared to neutral or near neutral soils. Next to rice, vegetables like cluster bean, okra etc., are the recommended tolerant crops to be grown in sodic soil with suitable management practices. Okra (*Abelmoschus esculentus* L.) is consumer preferred vegetable cultivated by the farmers as it grows moderately well in sodic environment (Ghai and Arora, 2007). Normally the fibrous root system of okra favours growth of AM fungi and glyphosate uptake from soil. Hence, the present study has been conceded to comprehend the influence of AM fungi on glyphosate dissipation in okra rhizosphere under sodic soil environment of Tamil Nadu.

Materials and Methods

Field experiment: 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. 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 has reaction (pH) 8.90, Electrical conductivity (EC) 0.37 dS m^{-1}, Cation exchange capacity (CEC) 20.90 c mol (P^{+}) kg^{-1} and Exchangeable sodium percent (ESP) 24.9%. The okra variety, Arka Anamika was grown as test crop at a spacing of 30 × 45 cm with seven treatments viz., control, AMF 100 kg ha^{-1} alone, glyphosate 12 ml/L (recommended dose) alone, AMF 100 kg ha^{-1} + glyphosate 6 ml/L, AMF 100 kg ha^{-1} + glyphosate 12 ml/L, AMF 100 kg ha^{-1} + glyphosate 18 ml/L and AMF 100 kg ha^{-1}+ glyphosate 24 ml/L. Experiment was conducted in Randomized Block Design (RBD) and each treatment was imposed in 20 m^2 (5 x 4 m) sized plots with three replications. Glyphosate was applied as pre-plant herbicide before 20 days of okra sowing along with the adjuvant ammonium sulphate (15 g/L) as per treatments. Each plot was ploughed separately using power tiller after 15 days of its application and okra was sown in ridges and furrows. The AMF biofertilizer purchased from TNAU, Coimbatore was applied as basal by mixing with sand, a day before sowing of okra seeds.

Soil sampling and glyphosate extraction from soil: Soil samples were collected randomly from each treatment (5 samples / plot) on 3, 5, 7, 15, 25, 30, 45 days after glyphosate application and also at the time of okra 1st harvest. The collected soil samples from each plot was pooled, air dried and processed for homogenization and sieved through 2 mm sieve before sub sampling for analysis. Glyphosate and AMPA was extracted from a known weight of soil sample using 0.05 M sodium tetra borate (Islas *et al.,* 2014) by shaking in a rotary shaker for 1 hr. The soil suspension was centrifuged at 3200 rpm for 15 min and the residues of glyphosate in the supernatant were derivatized using FMOC-Cl (Catrinck *et al.,* 2014) before injecting into HPLC for estimation.

HPLC conditions: Glyphosate and its metabolite AMPA was analyzed using the Thermo fisher HPLC model UHPLC 3000 equipped with Diode Array Detector (DAD), quartnary pump and auto sampler having rheodyne injection system. Both the compounds were separated using the Thermo Fisher Scientific C18 column of 3 µm, 120Å, 4.6 × 150 mm size. The column temperature was maintained at 40°C and the pressure was around 130 bars. The compounds were eluted using acetonitrile: 0.1 % formic acid (40/60, v/v) as mobile phase. The flow rate of mobile phase was kept at 1.00 mL/min^{-1} and 20.00 µl of the sample was injected for analysis. The different concentrations of glyphosate and AMPA standards (0.01, 0.05, 0.1, 0.5, 1.0 mg l^{-1}) were prepared from the 99% technical material of glyphosate and AMPA purchased from Sigma Aldrich (Mumbai, India). In the HPLC-DAD signals were recorded using the Chromeleon software from 190 to 750 nm wavelength to optimize the suitable conditions for detecting the above compounds. After optimization, the samples run were performed at 266 nm for all the spiked and unknown samples.

Method validation and recovery of glyphosate and AMPA: Before analyzing the unknown samples, recovery studies were carried out to establish the reliability of the analytical method employed for the present study. The control soil sample was fortified with known concentrations of glyphosate and AMPA standards from 0.001 to 0.50 mg l^{-1}. After 1 hour of fortification, different substrates were subjected to an extraction and clean up as done for soil sample to determine the recovery percent of the glyphosate and its metabolite. Using the results of fortified samples, the estimated method detection limit (EMDL) was arrived by repeated injection of the lowest concentration fortified samples. Quantification of glyphosate and its metabolite residues concentration was accomplished by comparing the peak height response for samples with peak height of the standard. Precision standard deviation of replicate analysis of standards of different concentrations was used to calculate the limit of detection (LOD) and limit of quantification (LOQ).

Statistical analysis: Mathematical models to depict herbicide degradation curves were worked out as described by Timme *et al.* (1986). Linearization of degradation curves was performed by transformation of residue concentration (R) and time (t) using general regression equation y = a+bx. Based on the decline curves, the intercept (a), slope (b) and half-life ($T_{1/2}$) were arrived using first order reaction kinetics function.

Results and Discussion

The working standards of each compound were injected individually and as mixture to HPLC-DAD. The optimized mobile phase of 0.1% formic acid (60/40 v/v) at a flow rate of 0.5 ml/minute with the C_{18} column for elution and separation, allowed short chromatographic runs with the retention times of 2.71 and 11.62 minutes, respectively for glyphosate and AMPA (Fig. 1). The retention times were comparable with Sancho *et al.* (1994) who obtained 2.0 and 12.0 minutes for the elution of glyphosate and AMPA, respectively. The analytical curves for a concentration range between 0.01 to 1.0 mg l^{-1}, showed r^2 values higher than 0.98 for both glyphosate and AMPA. The detection and quantification limit of both the compounds by HPLC-DAD was found to be 0.01 and 0.05 mg l^{-1} irrespective of the matrices analyzed. The average recoveries of both the compounds from soil were 80.37 and 56.01 percent, respectively, across different concentrations and compounds. Since the replication standard deviation was less than 2.0 per cent and an average recovery was more than 80 percent, the present method of analysis was found to be sensitive enough to the international standards. Glyphosate was applied to sodic soil field as pre plant herbicide 20 days before sowing of okra at four rates *viz.,* 6, 12, 18 and 24 ml l^{-1} with AM fungi @ 100 kg ha^{-1} and at two rates *viz.,* 0 and 6 ml l^{-1} without AM fungi. In addition to

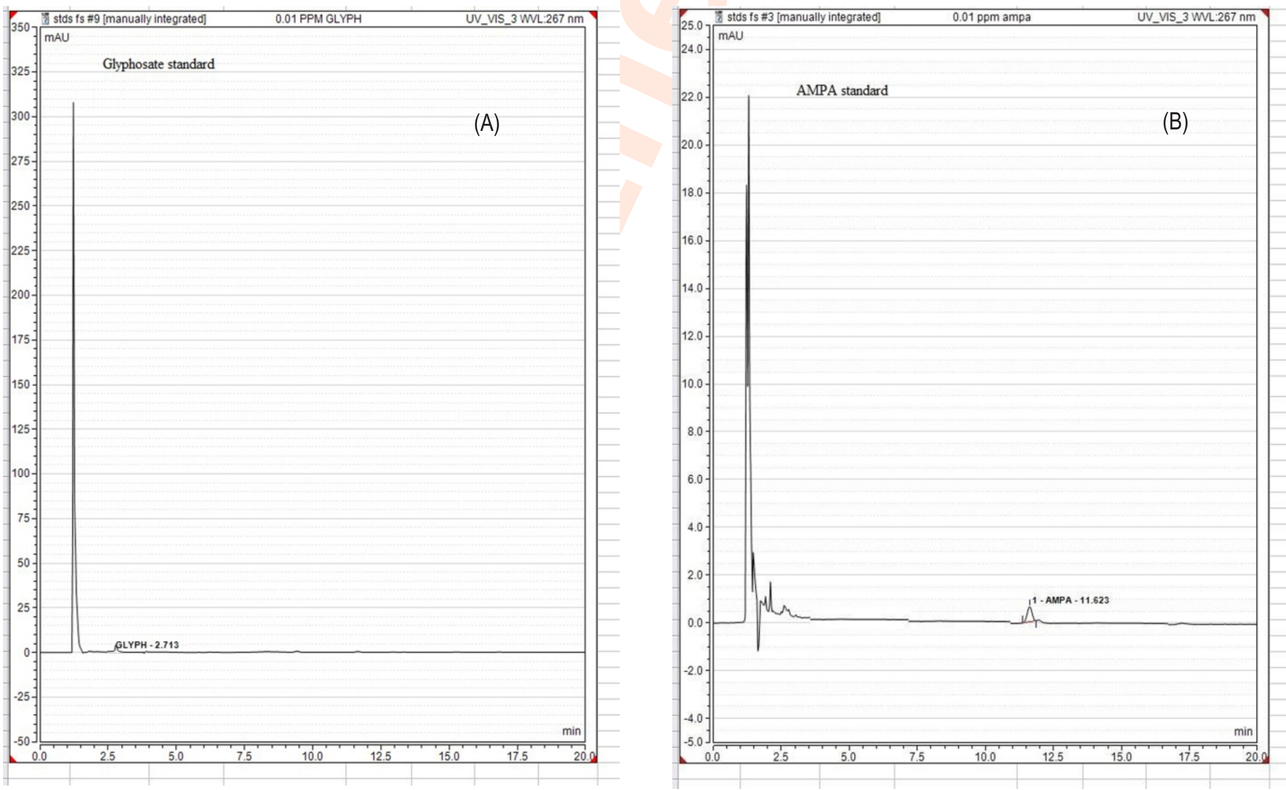

Fig. 1: HPLC – DAD detection of glyphosate (A) and AMPA (B) standards at 0.01 mg l^{-1} concentration.

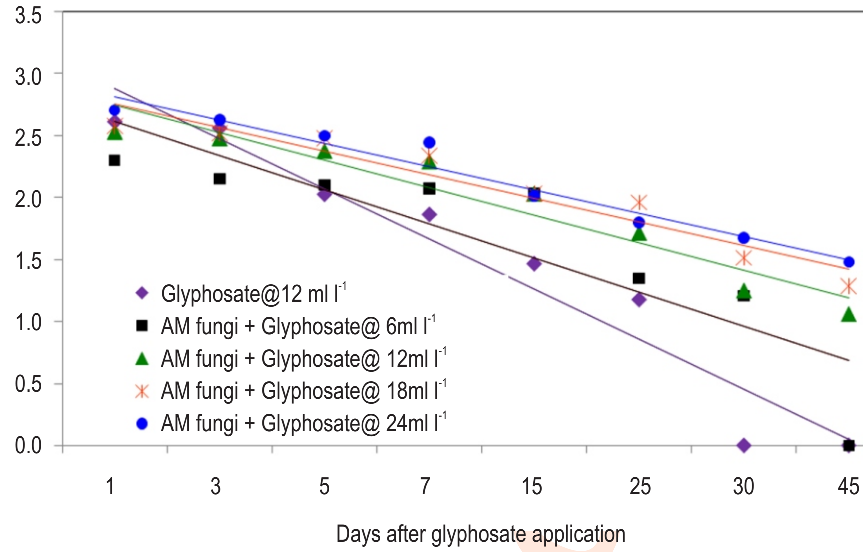

Fig. 2: Persistence of pre-plant applied glyphosate in okra cultivated sodic soil : Effect of AM fungi and glyphosate rates.

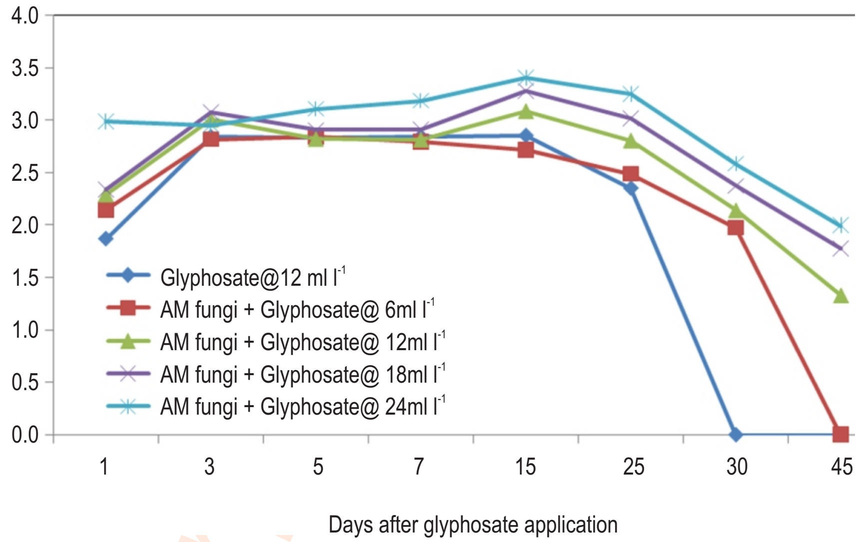

Fig. 3: Persistence of AMPA residue in okra cultivated sodic soil : Effect of AM fungi and glyphosate rates.

these, AM fungi alone @ 100 kg ha^{-1} was applied and maintained to have comparison with control and other treatments. Glyphosate residue deposited in soil on 1 day after application ranged from 0.1982 to 0.5019 mg kg^{-1} across different rates with and without AM fungi. Glyphosate applied at 24 ml l^{-1} and 6 ml l^{-1} plus AM fungi 100 kg ha^{-1} recorded higher and lower residue respectively on 1st day and an increased dose of glyphosate application increased the initial deposition. Glyphosate alone applied at 12 ml l^{-1} deposited more residues than its combined application with AM fungi 100 kg ha^{-1} (Fig 2). Residue concentration decreased with time and persisted in soil up to 45 days after application in all treatments, except in the plots which received glyphosate alone12 ml l^{-1} and lower dose of 6 ml/L glyphosate with AM fungi. Then the residues became below the quantification limit. Increased glyphosate residue degradation in the soil with time was observed in the present study and similar results were reported by Bandana *et al.* (2015) that the glyphosate persisted in the soil for 30 to 60 days, following its

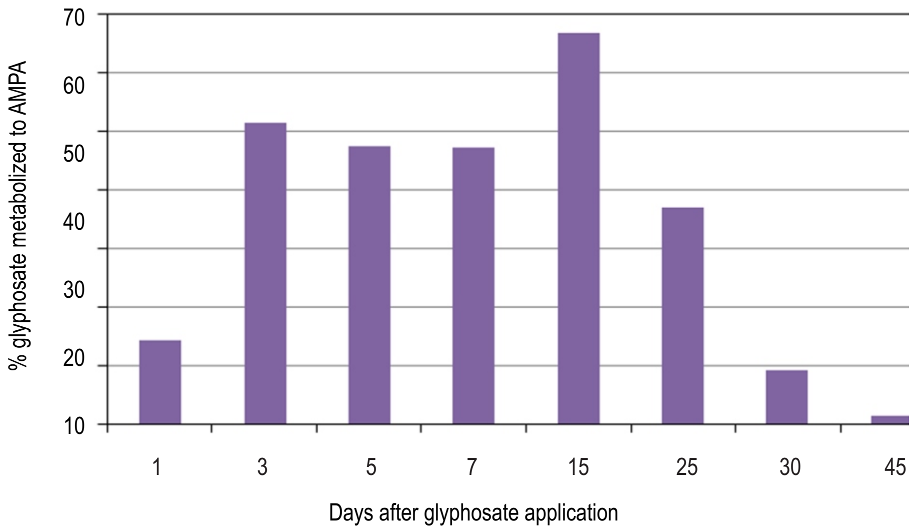

Fig. 4: Graph showing percent of glyphosate metabolized to AMPA with time in okra cultivated sodic soil.

Table 1: Effect of glyphosate rates and AM fungi on dissipation of glyphosate and AMPA (%) in okra cultivated sodic soil

	Days after glyphosate application						
Treatments	**3**	**5**	**7**	**15**	**25**	**30**	**45**
Glyphosate							
Control	-	-	-	-	-	-	-
AM fungi alone 100 kg ha^{-1}	-	-	-	-	-	-	-
Glyphosate 12 ml l^{-1}	11.1	74.0	82.1	92.8	96.3	-	-
AM fungi + Glyphosate 6 ml l^{-1}	29.1	36.1	40.6	45.5	88.8	91.9	-
AM fungi + Glyphosate 12 ml l^{-1}	11.2	29.8	42.4	68.3	84.7	94.8	96.6
AM fungi + Glyphosate 18 ml l^{-1}	13.3	20.9	42.6	71.7	76.3	91.4	94.9
AM fungi + Glyphosate 24 ml l^{-1}	16.0	37.3	44.8	79.5	87.5	90.5	93.92
AMPA							
Control	-	-	-	-	-	-	-
AM fungi alone 100 kg ha^{-1}	-	-	-	-	-	-	-
Glyphosate 12 ml l^{-1}	-	-	-	-	68.5	89.6	-
AM fungi + Glyphosate 6 ml l^{-1}	-	-	11.2	25.5	56.0	86.5	-
AM fungi + Glyphosate 12 ml l^{-1}	-	-	-	-	47.6	88.7	98.2
AM fungi + Glyphosate 18 ml l^{-1}	-	-	-	-	44.9	87.5	96.8
AM fungi + Glyphosate 24 ml l^{-1}	-	-	-	-	30.	84.9	96.1

applications at 0.5 to 2.0 kg/ha in tea cultivated field and its residue was found below detectable level at 0.5 kg ha^{-1}, 0.002-0.003 µg g^{-1} at 1.0 kg ha^{-1} and 0.011- 0.012 µg g^{-1} at 2.0 kg ha^{-1} application levels. In the present study, the residue of glyphosate was below the detection limit in control and AM fungi 100 kg ha^{-1} imposed plots throughout the crop period. More than 90 percent glyphosate residue was dissipated on 15th day itself when it was applied alone (Table 1). When glyphosate applied along with AM fungi, more than 50 percent residue dissipated on 15th day in all plots, except at lower dose (6 ml l^{-1}) and 90 percent dissipated on 25th day onwards. Slow dissipation of glyphosate in the AM fungi applied plots particularly after 15 days could be ascribed to the release of sorbed glyphosate molecules from soil particles due to phosphate fertilization for okra crop. Glyphosate remobilization due to phosphate fertilization in soils has been reported by Bott *et al.* (2011) and found competitive desorption of glyphosate with P fertilizers as both compounds are concentrated in the top surface soil. Further states that the increased root growth into the soil

Table 2: Regression coefficient (r^2), half-life and predicted equation for glyphosate and AMPA degradation in okra cultivated sodic soil

Treatments	r^2	T ½ (days)	Predicted equation
Glyphosate			
Control		-	-
AM fungi alone 100 kg ha^{-1}		-	-
Glyphosate alone 12 ml l^{-1}	0.8716	5.0851	Y=1.1468 + 0.1363x
AM fungi + Glyphosate 6 ml l^{-1}	0.9306	8.3205	Y=1.5407 + 0.0833x
AM fungi + Glyphosate 12 ml l^{-1}	0.9683	8.4216	Y= 1.0280 + 0.0764x
AM fungi + Glyphosate 18 ml l^{-1}	0.9598	9.9298	Y=0.9552 + 0.0698x
AM fungi + Glyphosate 24 ml l^{-1}	0.9392	10.3448	Y=0.8463 + 0.0670x
AMPA			
Control		-	-
AM fungi alone 100 kg ha^{-1}		-	-
Glyphosate alone 12 ml l^{-1}	0.8140	9.1923	Y=4.7440 - 0.0754x
AM fungi + Glyphosate 6 ml l^{-1}	0.8256	11.0896	Y=4.5732 - 0.0625x
AM fungi + Glyphosate 12 ml l^{-1}	0.9563	4.9261	Y=2.5299 - 0.0490x
AM fungi + Glyphosate 18 ml l^{-1}	0.9432	5.7566	Y=2.5699 -0.1204x
AM fungi + Glyphosate 24 ml l^{-1}	0.9229	6.0322	Y=2.8614 - 0.1149x

zones with the highest *P accumulation* might also remobilize higher glyphosate residues and is depends on the rhizosphere chemistry of the plant species. Since glyphosate molecules are adsorbed to the soil particles through phosphonic acid moiety (Sprankle *et al.* 1975), increased inorganic phosphate in soil solution due to P fertilization (50 kg P_2O_5 ha^{-1}) might compete with soil binding sites (Ahrens 1994) and desorbed the glyphosate residue slowly into solution phase which then degraded by the microbes. Miles and Moye (1988) and Gimsing *et al.* (2004) reported that the adsorbed glyphosate can readily be replaced by phosphate group but the glyphosate group cannot replace the adsorbed phosphate group easily. Application of AM fungi also enhanced the persistence of glyphosate in soil and could be the result of increased immobilization by uptake and binding which is related to the volume of root system as suggested by Nedumpara *et al.* (1999) for atrazine. This could also be attributed to the increased foraging capacity of AM fungi in soil due to increased fungal hyphae and AMF population. However, the decrease in AM fungi population with increased rate of glyphosate was reported by Brindhavani *et al.* (2018) who have reported that the poor native AM fungi mycorrhization and spore counts in sodic soil could be the result of poor root penetration and dispersed soil structure caused by the accumulation of exchangeable Na^+ in the rhizosphere region.

In the present investigation, the glyphosate dissipation profile fitted well with first-order and 1.5th order reaction kinetics irrespective of glyphosate rates (Table 2). The coefficient of determination (R^2) between log residues in sodic soil and time was computed using the first order reaction kinetics and the values varied from 0.8716 – 0.9683 (significant at P = 0.05), indicating that the dissipation of glyphosate could be accounted by first-order kinetics. Highly significant fit (r^2>0.979**) of the order function was observed for the combined application of AM fungi 100 kg ha^{-1} plus glyphosate 18 ml/L applied plot. Mean half-life of glyphosate ranged from 5.08 to 10.34 days across all the treatments and increased with increase in glyphosate rates irrespective of AM application. Similar result was observed by Bandana *et al.* (2015) across the glyphosate application levels of 0.5 to 2.0 kg ha^{-1} with the mean half lives of 5.80 to 19.10 days in the tea cultivated soil.

Characterization and information relating to transformation of the parent glyphosate compound to its metabolite is important for understanding the fate of glyphosate in sodic soil as well as for determining potential risks for soil and ground water contamination, as the degradation products are nearly as toxic as main compound. Hence, transformation of glyphosate to its degradation product namely AMPA in okra grown sodic soil environment was studied as influenced by the AM fungi application with different rates of glyphosate application. From day 1 onwards the AMPA, a metabolite of glyphosate was found to present in the soil irrespective of rates of glyphosate and AM fungi application (Fig. 3). The AMPA concentration increased up to 15th day in all the treatments except low glyphosate dose of 6 ml/L along with AM fungi 100 kg ha^{-1} applied plot. This showed the continuous mineralization of glyphosate to AMPA in sodic soil. On day 60, the AMPA residues were below the detection limit of 0.01 mg kg^{-1}. This could be ascribed to the fast mineralization of glyphosate to AMPA in sodic soil and similar results were reported by Al-rajab and Hakami (2014). In the present study, increase in AMPA concentration was observed up to 15 DAHA as against a decrease of glyphosate level (Fig. 4) in all the treatments and similar result was reported by Cheah *et al.* (1998) who reported that the rate of AMPA in the extracts of a sandy loam soil increased over the incubation time. The proportion of applied glyphosate converted to AMPA is about 14% on day 1 and increased upto day 15 with 66.6% and then decreased with time (Fig. 4). Similar AMPA occurrence of 19.7 to 53.8% was reported by EFSA (2015) under field conditions on glyphosate molar equivalents basis. Since the glyphosate mostly present as zwitterions at pH values of 6-9 due to protonation of amino-nitrogen, the AMPA conversion might have more in the present experimental soil with a pH of 8.9. A moderate positive correlation between soil pH and the glyphosate mineralization was also reported in literature (EFSA, 2015).

The dissipation rate for AMPA was calculated using the highest residue concentration detected on 15th day and found that more than 90% AMPA dissipated in soil after 30 days of glyphosate application (Table 1). The AMPA dissipation followed first order reaction kinetics with the mean half-life ranged from 4.92 to 11.09 days across different treatments (Table 2). Though the half-life was low for AMPA as against the reported half-life of 10.0 -36.9 days (Zhang *et al.,* 2015), it was detected in soil up to 45 days after glyphosate application. Such a low half-life could be attributed to the calculation of half-life from the highest AMPA concentration detected on 15th day. AMPA residue was low on 1st day, since glyphosate was applied as preplant herbicide on foliage and the large quantity of applied glyphosate has been absorbed by the weeds allowed lesser volume to reach the soil surface. AMPA residue increased upto 15 days and could be due to the release of glyhposate and its metabolites from weeds into the soil through root exudates and precipitation.

Further, the incorporation of dried weeds into the soil during field preparation for okra sowing, the absorbed glyphosate or its metabolized products like AMPA by the weed plants might have been added into the soil. The half-life of glyphosate and AMPA, are highly cariable, ranging from few days to one or two years, depending on edaphic and environmental conditions, namely temperature and soil moisture, soil binding extent, microbial breakdown and phosphate levels (Bento *et al.,* 2016) and also its significance depends on the quantity occurred in soils. It is concluded that the application of AM fungi (100 kg ha^{-1}) with lower glyphosate rate of 6 ml/L can be recommended for sodic soil with the safer waiting period of 45 days for okra harvest. It is inferred from the study that the continuous or frequent application of glyphosate might also affect the non-target organisms like AM fungi in sodic soil.

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