Indian Journal of Weed Science **49**(2): 159–164, 2017
DOI: 10.5958/0974-8164.2017.00041.7

Impact of herbicide mixtures on earth worm population, organic carbon content and β glucosidase enzyme activity in soil

Sheeja K Raj* and Elizabeth K Syriac[1]
Coconut Research Station, KAU, Balaramapuram, Thiruvananthapuram, Kerala 695 501

Received: 7 March 2017; Revised: 9 May 2017

ABSTRACT

Earthworm population, soil organic carbon content and β glucosidase enzyme activity in the soil are considered as the indicators of soil quality and biomarkers for toxicity and bioaccumulation assessment. Hence, field experiments were conducted during first crop season (*Kharif* 2014) and second crop season (*Rabi* 2014-15) to study the impact of pre- mix herbicide mixtures bispyribac-sodium + metamifop and penoxsulam + cyhalofop-butyl on earth worm population , soil organic carbon content and β glucosidase enzyme activity in soil. The experiments were conducted in randomized block design with 12 treatments and three replications at Upaniyoor Padashekaram in Thiruvananthapuram district of Kerala, India. The treatments were bispyribac-sodium + metamifop at 60, 70, 80 and 90 g/ha, penoxsulam + cyhalofop-butyl at 120, 125, 130 and 135 g/ha, bispyribac-sodium applied alone 25 g/ha, penoxsulam applied alone at 22.5 g/ha, hand weeding twice and weedy check. All the herbicide treated plots registered earthworm population at par to weedy check and hand weeding. The organic carbon content in soil was higher in herbicide treated plots as compared to weedy check at all the stages of crop growth. Herbicide treated plots were comparable to non-herbicide treated plots with respect to glucosidase activity. It was concluded that the pre-mix bispyriibac-sodium + metamifop and penoxsulam + cyhalofop are environmentally safe.

Key words: Earthworms, β glucosidase enzyme activity, Herbicide impact, Non-target organism

Earthworm population, soil organic carbon content and β glucosidase enzyme activity in the soil are used as bioindicators of soil fertility and biomarkers for toxicity and bioaccumulation assessment (Nusetti *et al.* 1999, Gobi *et al.* 2004). Since, earthworms play a major role in soil quality by shredding residues, stimulating microbial activity and decomposition, improving soil fertility and soil physical properties; they are of great significance in the recycling of carbon and nitrogen in the ecosystem. Soil organic carbon is the important constituent of soil providing energy to the microorganisms and releasing nutrients to the plants through mineralization process (Abbas *et al.* 2015). β glucosidase enzymes releases low molecular sugars from organic matter, the important energy sources of microorganisms (Tabatabai 1994, Bandick and Dick 1999).

Weeds are the major biological constraint in direct-seeded rice due to the simultaneous emergence of crop and weeds and absence of water at the time of seedling emergence. Herbicidal management of weeds is the only viable and economic option for the control of weeds. But the continuous use of same herbicide or herbicides with similar mode of action will lead to the development of herbicide resistance and shift in weed flora either slowly or rapidly. One of the recent effective ways to overcome this problem is the use of herbicide mixtures. Herbicide mixtures contain two or more herbicides with different mode of action. Penoxsulam + cyhalofop-butyl 6% OD and bispyribac-sodium + metamifop 14% SE are the two new generation pre-mix herbicide mixtures which are having broad-spectrum activity and are more effective in controlling weeds compared to the individual application (Raj 2016). Penoxsulam + cyhalofop-butyl 6% OD is a pre- mix herbicide mixture containing, penoxsulam, a post-emergence broad-spectrum herbicide of triazolopyrimidine group inhibiting the enzyme acetolactate synthase and cyhalofop-butyl, also a post-emergence grass effective herbicide of group aryloxyphenoxy-propionate, inhibiting acetyl CoA carboxylase enzyme. Similarly, bispyribac-sodium + metamifop 14% SE contains, bispyribac-sodium, a post-emergence herbicide of group pyrimidinylcar-boxate inhibiting acetolactate synthase and metamifop also a post-emergence grass effective herbicide of group aryloxyphenoxypropionate group inhibiting acetyl CoA carboxylase.

***Corresponding author:** sheejakraj70@gmail.com
[1]Department of Agronomy, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala 695 522

Latha and Gopal (2010) reported that large proportion of herbicides accumulate in the top 0-15 cm layer of soil due to the application of pre- and post-emergence herbicides. Several workers opined that herbicides have adverse effect on the survival of earthworms as well as its growth and reproduction and organic carbon content in soil (Zhou *et al*. 2007, Correia and Moreira 2010). Trimurtulu *et al*. (2015) pointed out that the herbicides, pendimethalin, oxyfluorfen and pretilachlor increased the organic carbon content in soil. However, Baboo *et al*. (2013) reported significant reduction in organic carbon level in soil after the application of herbicide. Mishra *et al*. (2013) revealed that significant quantity of organic matter accumulated in weedy check and hand weeded conditions compared to herbicides. β glucosidase enzyme is a soil quality indicator which gives the reflection of past biological activity and the capacity of soil to stabilize the soil organic matter (Bandick and Dick 1999, Ndiaye *et al*. 2000). Depending on the nature and concentration of herbicide, incubation period and soil condition, application of herbicide influence the β glucosidase activity in soil (Hussain *et al*. 2009).

According to Constenla *et al*. (1990) and Hoerlein (1994), preferred herbicides should not only have good efficacy in controlling weeds, but also pose minimum adverse effects to crop, ecology and environment. Hence the present investigation was undertaken to study the effect of herbicide mixtures, penoxsulam + cyhalofop-butyl and bispyribac-sodium + metamifop on earth worm population, soil organic carbon content and β glucosidase enzyme activity in soil, to ensure the environmental safety of these herbicide mixtures.

MATERIALS AND METHODS

Field experiments were conducted during the first (*Kharif* 2014) and second (*Rabi* 2014-15) crop seasons of 2014-15 at Upaniyoor Padashekaram (8°26.762' N latitude and 77°0.136'E longitude and 29 m above mean sea level) in Thiruvananthapuram district, Kerala, India. The experimental site has humid tropical climate. The average annual rainfall received during the period of experimentation was 875.5 mm during first crop season and 203.4 mm during second crop season.The mean maximum and minimum temperature recorded during first and second crop seasons were 30.1ºC and 24.4ºC and 30.8ºC and 22.6ºC, respectively. The total number of rainy days during first and second crop seasons were 46 and 21, respectively.

The soil was sandy clay loam, acidic in reaction, high in organic carbon and medium in N, P and K status.The experiment was laid out in a randomized block design with 12 treatments and three replications. The treatments were bispyribac-sodium + metamifop at 60, 70, 80 and 90 g/ha, penoxsulam + cyhalofop-butyl at 120, 125, 130 and 135 g/ha, bispyribac-sodium applied alone 25 g/ha, penoxsulam applied alone 22.5 g/ha, hand weeding twice and weedy check. The herbicides were applied on 15 DAS as per the treatment schedule using knapsack sprayer fitted with flat fan nozzle. The spray fluid used for the study was 500 l/ha. The variety used was '*Kanchana*', a short duration variety released from Regional Agricultural Research Station, Pattambi of Kerala Agricultural University. The crop was fertilized with 70:35:35 kg/ha N, P and K, with one third N and K and half P applied on 15 DAS (days after sowing), one third N and K and half P on 35th day and remaining one third N and K on 55th day of sowing.

Soil samples for soil organic carbon estimation and β glucosidaseenzyme activity were collected with the help of soil auger at 15 DAS (just before herbicide application), 30 DAS (15 days after herbicide application), 60 DAS (45 days after herbicide application) and at harvest stage. Four samples of soil were collected from each experimental plot to a depth of 15 cm, mixed thoroughly to form a composite sample and kept in a polythene bag and stored at 4°C. The enzyme assay was completed within a week. β glucosidaseenzyme assay was carried out by incubating the soil with buffered (pH 6.0) para nitrophenyl β glucopyranoside. Para nitrophenol released was determined and expressed as µg para nitro phenol g/soil/h (Eivasi and Tabatabai 1988). After the estimation of β glucosidaseenzyme activity in soil, soil samples were shade dried, sieved through a 0.2 mm sieve and analysed for organic carbon content by rapid titration method (Walkley and Black 1934).

Estimation of earthworms was carried out before the sowing and after the harvest of first and second crop. Two representative samples from each plot were collected for the estimation of earthworm population. Sampling area was plotted with one metre square wooden frame. Soil samples were drawn up to 10 cm depth (Bano and Kale 1991). The soil lumps were broken and the soil was passed through the fingers to sort out the worms. The smaller worms were collected by passing through a sieve of 3-4 mm size. The worms were then counted.The data generated were statistically analysed using analysis of variance technique (ANOVA) and difference between the treatment means was compared at 5% probability level.

RESULTS AND DISCUSSION

Effect on earth worm population

Data on earthworm population indicated that before herbicide spraying, the average number of earthworms present in the experimental field was 3.8/m^2 and 3.2/m^2 for the first and second crop seasons, respectively and no significant difference was observed among the treatments (**Table 1**). After the experiment, during both the seasons, an increase in earthworm population was observed irrespective of the treatments.The earthworm population recorded after the first and second crop was 3.9 and 7.3 no./m^2, respectively and no significant variation could be observed among the treatments (**Table 1**). Though not significant, all the herbicide treated plots registered the same number or more of earthworms per square metre compared to weedy check and hand weeding (**Table 1**). This implies that, the tested herbicide mixtures and their doses did not leave any toxic residue in soil to affect the earthworm population.The results of the experiment also conforms the findings of Scott and Pollak (2005). Zarea (2010) also reported that, herbicides in general showed low toxicity towards earthworms. Post experiment observations of second season indicated an increase in earthworm population, in all the treatments as compared to first crop season. This might be due to higher organic carbon content in the soil (**Table 2**) resulting from the higher glucosidase enzyme activity (**Table 3**). β glucosidase is an enzyme plays a major role in the microbial degradation of cellulose to glucose, an important source of carbon for the life of microorganisms in soil (Tabatabai 1994). Fonte *et al.* (2009) opined that earth worm population in the soil appears to be closely linked with total carbon and N content in the soil.

Effect on organic carbon content in soil

Data on organic carbon content indicated an increasing trend from 15 to 60 DAS and a decline at harvest stage (**Table 2**). The highest soil organic carbon content noticed at 60 DAS corresponds to booting stage of the crop. At 15 DAS (just before herbicide application), there was no significant difference among the treatments in organic carbon status of the soil during both the seasons.

Weed management treatments significantly influenced the organic carbon content of the soil at 30 and 60 DAS and at harvest stage during both the seasons (**Table 2**). It has been observed that compared to weedy check, the hand weeding treatment and herbicide treatments recorded comparable or higher organic carbon content in the soil at 30 DAS (15 days after herbicide application), 60 DAS (45 days after herbicide application) and at harvest stage. These results indicated that, applied herbicides at their tested doses did not have any adverse impact on the organic carbon content of the soil. This might be due to increased microbial activity resulting from the release of root exudates into the rhizosphere. Presence of herbicides in the rhizosphere of plant influence the physiological activities of the host plant root system which lead to the release of more quanta of exudates and indirectly result in higher level of organic carbon in the rhizosphere soil (Trimurtulu *et al.* 2015). Root exudates are organic substrates comprising of simple and complex sugars, amino acids, vitamins, proteins and phenolics which stimulate the microbial growth (Dakora and Philips 2002). The quantity of organic carbon released by plants to the rhizosphere may amount to 40% of the total dry matter produced by the plant (Lynch and Whips 1990). Sebiomo *et al.* (2011) reported an increase in organic matter content

Table 1. Effect of herbicide mixtures on earth worm population in soil

Treatment	Before the experiment (no./m^2)		After the experiment (no./m^2)	
	Kharif 2014	*Rabi* 2014-15	*Kharif* 2014	*Rabi* 2014-15
Bispyribac-sodium + metamifop 60 g/ha	1.65(2.7)	2.12(4.0)	2.7(1.65)	6.7(2.65)
Bispyribac-sodium + metamifop 70 g/ha	2.12(4.0)	2.7(1.65)	4.0(2.12)	6.7(2.65)
Bispyribac-sodium + metamifop 80 g/ha	1.92(4.0)	4.0(2.12)	2.7(1.65)	5.3(2.39)
Bispyribac-sodium + metamifop 90 g/ha	2.12(4.0)	2.7(1.65)	5.3(2.39)	8.0(2.86)
Penoxsulam + cyhalofop-butyl 120 g/ha	2.12(4.0)	2.7(1.65)	4.0(2.12)	8.0(2.86)
Penoxsulam + cyhalofop-butyl 125 g/ha	1.92(4.0)	5.3(2.39)	5.3(2.39)	10.7(3.24)
Penoxsulam + cyhalofop-butyl 130 g/ha	2.39(5.3)	4.0(2.12)	5.3(2.39)	9.3(3.07)
Penoxsulam + cyhalofop-butyl 135 g/ha	2.12(4.0)	2.7(1.65)	5.3(2.39)	9.3(3.07)
Bispyribac sodium 25 g/ha	1.65(2.7)	4.0(2.12)	2.7(1.65)	6.7(2.59)
Penoxsulam 22.5 g/ha	1.65(2.7)	1.3(1.18)	2.7(1.65)	6.7(2.59)
Hand weeding twice 20 and 40 DAS	2.12(4.0)	2.7(1.65)	2.7(1.65)	5.3(2.39)
Weedy check	4.0(2.12)	2.7(1.65)	4.0(2.12)	5.3(2.39)
LSD (p=0.05)	NS	NS	NS	NS

Values in parentheses are original values, Data are subjected to square root transformation -($\sqrt{x+0.5}$), NS - non significant of the soil from second week of herbicide application. Maximum organic carbon content in the soil was observed at 60 DAS, it might be due to the vigorous crop growth at the booting stage resulting in greater root exudation (Dotanita *et al*. 2014). Organic carbon showed a reduction at harvest stage might be due to the decline in the release of root exudates into the soil by plant roots due to senescence and continuous decomposition of organic matter.

Effect on β glucosidaseenzyme activity

β glucosidase enzyme plays a major role in organic matter mineralization in the soil ecosystem. Weed management treatments significantly influenced β glucosidase enzyme activity in soil at 30 and 60 DAS and at harvest stage during both the seasons; however at 15 DAS (just before herbicide application), no significant difference was observed among the treatments (**Table 3**). During both the seasons at 30 DAS (15 DAHA), a decline in β glucosidase enzyme activity was observed in all the treatments. The decline in β glucosidase enzyme activity observed at 30 DAS might be due to changes in soil pH. β glucosidase enzyme activity is very sensitive to pHchanges and soil management practices (Martinez and Tabatabai 2000). Soil pH affects the activity of soil enzymes through its controls on microbial enzyme production, ionization-induced conformational changes of enzymes, and availability of substrates and enzymatic co-factors (Tabatabai 1994). All the herbicide treated plots recorded higher or comparable glucosidase activity at 30 and 60 DAS and at harvest stage, as that of non-herbicide treated plots. This implies that, the applied

Table 2. Effect of weed management treatments on organic carbon content of soil

Treatment	Organic carbon (%)							
	Kharif 2014				*Rabi* 2014-15			
	15 DAS (JBHA)	30 DAS (15DAHA)	60 DAS (45DAHA)	Harvest	15 DAS (JBHA)	30 DAS (1DAHA)	60 DAS (45DAHA)	Harvest
Bispyribac-sodium + metamifop 60 g/ha	1.19	1.21	1.47	1.13	1.12	1.26	1.70	1.60
Bispyribac-sodium + metamifop 70 g/ha	1.20	1.23	1.46	1.19	1.05	1.29	1.67	1.62
Bispyribac-sodium + metamifop 80 g/ha	1.20	1.27	1.48	1.20	1.04	1.32	1.70	1.79
Bispyribac-sodium + metamifop 90 g/ha	1.20	1.22	1.55	1.26	1.15	1.28	1.89	1.64
Penoxsulam + cyhalofop-butyl 120 g/ha	1.21	1.35	1.55	1.27	1.18	1.32	1.99	1.81
Penoxsulam + cyhalofop-butyl 125 g/ha	1.23	1.37	1.56	1.23	1.20	1.56	1.97	1.63
Penoxsulam + cyhalofop-butyl 130 g/ha	1.12	1.23	1.58	1.20	1.13	1.36	1.98	1.60
Penoxsulam + cyhalofop-butyl 135 g/ha	1.22	1.29	1.55	1.20	1.13	1.31	1.93	1.84
Bispyribac sodium 25 g/ha	1.18	1.23	1.46	1.09	1.15	1.28	1.75	1.48
Penoxsulam 22.5 g/ha	1.21	1.23	1.45	1.11	1.13	1.32	1.76	1.64
Hand weeding twice 20 and 40 DAS	1.18	1.26	1.51	1.10	1.16	1.37	1.82	1.54
Weedy check	1.16	1.24	1.40	1.10	1.14	1.26	1.67	1.47
LSD (p=0.05)	NS	0.066	0.083	0.122	NS	0.121	0.069	0.117

DAS - Days after sowing, JBHA - Just before herbicide application, DAHA - Days after herbicide application, NS - non significant

Table 3. Effect of weed management treatments on β glucosidase activity in soil

Treatment	β glucosidase activity (µg para nitro phenolg/soil/h)							
	Kharif 2014				*Rabi* 2014-15			
	15 DAS (JBHA)	30 DAS (15 DAHA)	60 DAS (45 DAHA)	Harvest	15 DAS (JBHA)	30 DAS (15 DAHA)	60 DAS (45 DAHA)	Harvest
Bispyribac-sodium + metamifop 60 g/ha	34.71	20.10	37.16	54.71	51.57	34.41	58.82	57.35
Bispyribac-sodium + metamifop 70 g/ha	34.71	27.31	35.88	56.27	48.76	40.59	50.88	59.80
Bispyribac-sodium + metamifop 80 g/ha	40.78	30.20	39.31	56.27	44.90	40.59	57.24	69.21
Bispyribac-sodium + metamifop 90 g/ha	36.86	27.03	39.12	62.25	51.31	39.22	59.31	68.14
Penoxsulam + cyhalofop-butyl 120 g/ha	38.63	35.26	39.31	59.41	52.94	43.83	66.89	73.45
Penoxsulam + cyhalofop-butyl 125 g/ha	40.78	40.42	41.86	57.06	54.12	46.27	65.29	59.61
Penoxsulam + cyhalofop-butyl 130 g/ha	38.63	27.48	39.51	57.16	49.41	39.51	68.72	63.53
Penoxsulam + cyhalofop-butyl 135 g/ha	40.98	31.28	38.73	58.24	44.44	42.75	64.12	75.03
Bispyribac sodium 25 g/ha	39.90	22.74	32.85	52.55	46.07	32.22	51.08	63.61
Penoxsulam 22.5 g/ha	39.51	23.33	36.27	53.82	45.49	39.41	52.45	64.51
Hand weeding twice 20 and 40 DAS	37.85	33.43	38.24	55.68	44.83	42.06	56.47	59.31
Weedy check	37.55	27.16	35.40	54.02	44.98	32.16	49.12	55.28
LSD (p=0.05)	NS	3.436	3.465	5.030	NS	3.007	4.995	5.434

DAS- Days after sowing, JBHA- Just before herbicide application, DAHA- Days after herbicide application, NS - non significant herbicide mixtures did not have any adverse impact on β glucosidase activity in soil. Trimurtulu *et al*. (2015) reported that presence of herbicides in the rhizosphere stimulates the physiological activities of the host plant root system and enhanced the release of root exudates into the rhizosphere. The organic root exudates will provide a variety of liable carbon compounds to soil which will stimulate the enzyme activity by the microorganisms (Kotroczo *et al*. 2014). Several researchers have reported the enhanced β glucosidase activity followed by herbicide application (Saha *et al*. 2012, Santric *et al*. 2014). β glucosidase enzyme activity was found to increase from 30 DAS, and reached the maximum at harvest stage. The increased root activity at 60 DAS might have stimulated the bacterial and fungal population that are likely a significant source of β glucosidase enzyme. There is considerable evidence suggesting that β glucosidase is an extra cellular enzyme secreted mainly by bacteria and fungi (Sinsabaugh and Moorhead 1994, Veena *et al*. 2011). Maximum β glucosidase enzyme activity values observed at harvest stage might be due to an increase in the amount of decomposing litter in the soil, since the crop is in the senescence stage. Larson *et al*. (2002) reported that increased inputs of soluble organic constituents' increases the β glucosidase enzyme activity in soil.

It can be concluded that application of herbicide mixtures, bispyribac-sodium + metamifop at 60, 70, 80 and 90 g/ha and penoxsulam + cyhalofop-butyl at 120, 125, 130 and 135 g/ha at 15 DAS did not have any adverse effect on non-target organism, *viz*. earth worm, organic carbon content and β glucosidase enzyme activity in soil, the biological indicators of soil fertility and biomarkers of toxicity. Post-emergence application of herbicide mixtures recorded comparable or higher number of earth worms per square meter, β glucosidase enzyme activity and organic carbon content in the soil as compared to non-herbicide treatments, *viz*. weedy check and hand weeding twice. Hence the herbicide mixtures, bispyribac sodium + metamifop at the tested doses, *i.e*. 60, 70, 80 and 90 g/ha and penoxsulam + cyhalofop butyl at 120, 125, 130 and 135 g/ha are environmentally safe.

REFERENCES

Abbas Z, Akmal M, Khan KS and Hassan FU. 2015. Impact of long term application of buctril super (bromoxynil) herbicide on microbial population, enzymes activity, nitrate N, Olsen P and total carbon in soil. *Archives of Agronomy and Soil Science* **61**(5): 627-644.

Baboo M, Pasayat M, Samal A, Kujur M, Maharana JM and Patel AK. 2013. Effect of four herbicides on soil organic carbon microbial biomass-C, enzyme activity and microbial populations in agricultural soils. *International Journalof Research in Environmental Science and Technology* **3**(4): 100-112.

Bandick AK and Dick RP. 1999. Field management effects on soil enzyme activities. *Soil Biology and Biochem*istry **31**(11): 1471-1479.

Bano K and Kale RD. 1991.Earth worm fauna of Southern Karnataka, India. pp. 627-633 In: *Advances in Management and Conservation of Soil Fauna*, (Eds. Veeresh GK, Rajagopal D and Virakthamath CA). Association for Promotion of Organic Farming, Bangalore.

Constenla MA, Riley D, Kennedy, SH, Rojas CE, Mora LC and Stevans JE. 1990. Paraquat behavior in Costa Rican soils and residues in coffee. *Journal of Agricultural Food Chemistry* **38**(10): 1985-1988.

Correia FV and Moreira JC. 2010. Effects of Glyphosate and 2, 4-D on earthworm (*Eiseniafoetida*) in laboratory tests. *Bulletin of Environmental Contamination and Toxicol*ogy **85**(3): 264-268.

Dakora FD and Philips DA. 2002. Root exudates as mediators of mineral acquisition in low- nutrient environments. *Plant and Soil* **245**(1): 35-47.

Dotaniya ML, Kushwah SK, Rajendran S, Coumar M, Kundu S and Rao AS. 2014. Rhizosphere effect of *Kharif* crops on phosphatase and dehydrogenase activities in Typic Haplustert. *National Academy Science Letters* **37**(2): 103-106.

Eivazi F and Tabatabai MA. 1988. Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry* **20**(5): 601-606.

Fonte SJ, Winsome T and Six J. 2009. Earthworm population in relation to soil organic matter dynamics and management in California tomato cropping system. *Applied Soil Ecology* **41**: 206-214.

Gobi M, Suman J and Ganesan SV. 2004. Sub lethal toxicity of the herbicide butachlor on the earthworm *Perionyxsan-sibaricus* and its histological changes. *Journal of Soils and Sediments* **5**(2): 62-86.

Hoerlein G. 1994. Glufosinate (phosphinothricin)- a natural amino acid with unexpected herbicidal properties. *Reviews of Environmental Contamination and Toxicology* **138**: 73-145.

Hussain S, Siddique T, Saleem M, Arshad M and Khalid A. 2009. Impact of pesticides on soil microbial diversity, enzymes and biochemical reactions. *Advances in Agronomy* **102**: 160-200.

Kotroczo Z, Veres Z, Fekete I, Krakomperger Z, Toth JA, Lajtha K and Tothmerez B. 2014. Soil enzyme activity in response to long- term organic matter manipulation. *Soil Biology and Biochemistry* **70**: 237-243.

Larson JL, Zak, DR andSinsabaugh RL. 2002. Extracellular enzyme activity beneathtemperate trees growing under elevated carbon-dioxide and ozone. *Soil Science Society of America Journal* **66**: 1848-1856.

Latha PC and Gopal G. 2010. Influence of herbicides on cellulolytic, proteolytic and phosphate solubilizing bacteria. *International Journal of Plant Protection* **3**(1): 83-88.

Lynch JM and Whips JM. 1990.Substrate flow in the rhizosphere. *Plant and Soil* **129**: 1-10.

Martinez AV and Tabatabai MA. 2000. Enzymes activities in a limed agricultural soil. *Biology and Fertility of Soils* **31**: 85-91.

Mishra V, Chowdhary T, Singh, AP and Gupta SB. 2013. Changes in biochemical properties of rice rhizosphere as influenced by tillage and herbicide application. *Indian Journal of Weed Science* **45**(4): 231-234.

Ndiaye EL, Sandeno JM, McGrath D and Dick RP. 2000. Integrative biological indicators for detecting change in soil quality. *American Journal of Alternative Agriculture* **15**(1): 26-36.

Nusetti O, Parejo E, Esclapés MM, Rodríguez-Grau J and MarcanoL. 1999. Acute-sub lethal copper effects on phagocytosis and lysozyme activity in the earthworm *Amynthashawayanus*. *Bulletin of Environmental Contamination and Toxicology* **63**(3): 350-356

Raj SK. 2016. *Herbicide Mixtures for Weed Management in Direct-Seeded Puddled Rice (Oryza Sativa L.).* Ph. D thesis, Kerala Agricultural University, Thrissur, 308 p.

Saha S, Dutta D, Karmakar R and Ray PD. 2012. Structure-toxicity relationship of chloroacetanilide herbicides. Relative impact on soil microorganisms. *Environmental Toxicology and Pharmacology* **34**(2): 307-314.

Santric L, Radivojevic L, Umiljendic JG, Durovic-Pejcev R and Saric-Krsmanovic M. 2014. Assessment of microbial activity and biomass in different soils exposed to nicosulfuron. *Pesticides and Phytomedicines* **29**: 213-219.

Scott PM and Pollak LM. 2005. Transgenic maize. *Starch-Starke* **57**: 187-195.

Sebiomo A, Ogundero VW and Bankole SA. 2011. Effect of four herbicides on microbial population, soil organic matter and dehydrogenase activity. *African Journal of Biotechnology* **10**(**5**): 770-778.

Sinsabaugh RL and Moorhead DL. 1994. Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. *Soil Biology and Biochemistry* **26**: 1305-1311.

Tabatabai MA. 1994. Soil enzymes. pp. 775-833. In: *Methods of soil analysis, Part 2. Microbiological and biochemical properties. SSSA Book Series No. 5*. (Eds. Weaver RW, Angle JS and Bottomley PS). Soil Science Society of America. Madison, Wisconsin.

Trimurtulu N, Ashok S, Latha M and Rao AS. 2015. Influence of pre- emergence herbicides on the soil microflora during the crop growth of black gram *Vignamungo*. L. *International Journal for Current Microbiology and Applied Sciences* **4**(6): 539-546.

Veena V, Poornima P, Parvatham R, Sivapriyadharsini and Kalaiselvi K. 2011. Isolation and characterization of â glucosidase producing bacteria from different source. *African Journal of Biotechnology* **10**(66): 14907-14912.

Walkley A and Black IA. 1934. An estimation of the Degtijareff method of determining soil organic matter and proposed modification of the chromic and titration method.*Soil Science* **37**: 29-31.

Zarea MJ. 2010. Conservation tillage and sustainable agriculture in semi- arid dry land farming. pp. 195-232. In: *Biodiversity, Agroforestry and Conservation Agriculture*. (Ed Lichtfouse E). Springer Science + Business Media B.V, Netherlands.

Zhou SP, Duan CP, Fu H, Chen YH, Wang XH and Yu ZE. 2007. Toxicity assessment for chlorpyrifos contaminated soil with three different earthworm test methods. *Journal of Environmental Sciences* **19**(7): 854-858.