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Effect of few pre emergence herbicides on soil L-glutaminase activity

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ABSTRACT

A pot culture experiment was conducted to investigate the effect of a few pre-emergence herbicides on soil L-glutaminase activity (µg of NH_4^+ released g^{-1} soil $4h^{-1}$) in a Vertisol and in an Alfisol. The experiment was under taken with pre-emergence herbicides viz., pendimethalin 30% EC (1 kg a.i/ha) for groundnut, greengram, sunflower and bhendi, atrazine 50% WP (1 kg a.i/ha) for maize and bensulfuron methyl 0.6% + pretilachlor 6% GR (0.66 kg a.i/ha) for rice. The experiment was conducted in a completely randomized block design with three replications. The results shown that in pendimethalin treated Alfisol and Vertisols L-glutaminase activity slowly increased from 0 days after application (DAA) to 30 DAA but later significantly increased upto 60 DAA in all treatments. Activity of L-glutaminase in the pendimethalin treated pots is lower than control upto 45 DAA in Alfisol and 30 DAA in Vertisol. Similar type of pattern was observed in atrazine treated maize crop. But in case of Bensulfuron + Pretilachlor treated Alfisol and Vertisol pot cultures, soil L-glutaminase activity decreases from 0 to 15 DAA but later activity of enzyme significantly increased from 15 to 60 DAA. Activity of L-glutaminase in the herbicide treated pots is lower than control up to 15 DAA in both Alfisol and Vertisol pot cultures.

Keywords: L-glutaminase activity, *Alfisol*, *Vertisol*, pre-emergence herbicides

INTRODUCTION

Mineralization is the conversion of organically bound elements into mineral form, which is easily absorbed by plants and essential for plant nourishment. Soil microorganisms and abiontic enzymes both contribute to the mineralization process. There are three types of soil enzymes, which include: Enzymes those linked with live cells, internally or on cellular surfaces; expelled enzymes in soil solution; and extracellular enzymes stabilised on soil colloids (Burns, 1982). The enzymes in the second and third groups are known as "abiontic" enzymes. The term "abiontic enzymes", developed by Skujins (1978) to describe enzymes of biological origin but no longer linked with living cells, has been neatly constructed to describe enzymes of biological origin but no longer associated with living cells. The activity of accumulated enzymes and enzymes produced by proliferating microorganisms results in the activity of enzymes in soils. In soils, accumulated enzymes are defined as enzymes that are present and active in the absence of microbial proliferation (Kiss *et al.,* 1975). As a result, abiontic enzymes cover a wide range of enzymes, which may be found in (i) Soil fluids or bound to inorganic and organic soil elements (ii) Particle cell debris, and (iii) Dead cells or cells that are viable but not growing. Several biochemical processes involving plant nutrient conversions and organic matter breakdown have been proven to be catalysed mostly by extracellular enzymes of plant origin (Pallab *et al.,* 1990). L-glutaminase (L-glutamine amidohydrolase E.C. 3.5.1.2) is an aminohydrolase that operates on free amino acids in soils. L-glutaminase is an enzyme that catalyses the hydrolysis of L-glutamine to glutamic acid and ammonium ion, which is necessary for plants to get the amide form of nitrogen (Hojjati and Nourbakhsh, 2007).

The transformation of natural ecosystems into agricultural ecosystems characterised by low biodiversity, as well as the intensive development of farming systems, resulted in the large-scale application of crop protection chemicals. Pesticides are widely used in crop production and induce problems related to contamination of soil, water, air and the food chain. The use of herbicides for the control of weeds has become a part of intensive agriculture. Most of the herbicides applied to soils interact with soil components and influence the biochemical processes driven by microorganisms (Hernandez-Rodriguez *et al.,*

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2006; Ronhede *et al.,* 2007). Herbicides are involved in soil processes like retention, transformation and transport. These herbicides may influence more or less the biological activity of soil, which is the result of microbial and enzymatic transformations (Engelen *et al.,* 1998; Hussain *et al.,* 2009). The inhibition of soil enzymes by agrochemicals can be direct or indirect. Any action of the chemical that alters the life functions of soil organisms could directly affect soil enzyme activity. An agrochemical may also modify the inter relationship between a particular group of organisms and this influences the amount and type of enzyme produced (Cerevelli *et al.,* 1975). The influence of pesticides on the performance of soil enzymes is determined by the type of pesticides used and the state of the enzymes, i.e., whether they are free, immobilized, or in soil. In homogenous system, when free enzymes interact with pesticides, and the observed reactions could be attributed to direct molecular interactions between the two species. In heterogeneous system enzymes are present in an immobilized form, and numerous scenarios could be derived like: (1) The enzyme undergoes structural and conformational changes during immobiliztion, resulting in different catalytic features; (2) pesticides could be adsorbed on inorganic and organic supports, resulting in diminished or enhanced effects on the immobilized enzyme; (3) A competition between the immobilized enzyme and the pesticide could have occurred, resulting in the release of free enzymatic molecules from matrices; (4) When soil is the source of enzyme activity, all of the above scenarios may occur at the same time, making the system more difficult to understand. In India, herbicides like atrazine, pendimethalin, and bensulfuron methyl + pretilachlor are widely employed as selective pre-emergence herbicides. These herbicides' efficacy has been studied on a variety of crops, as well as their impact on agronomic techniques. However, there is a scarcity of data on herbicide interactions with soil biochemical characteristics, particularly in relation to L-glutaminase activity. To investigate the effect of pre-emergence herbicides on L-glutaminase activity, a pot culture experiment was conducted using pendimethalin 30 % EC (groundnut, greengram, sunflower, and bhendi), atrazine 50 % WP (maize), and bensulfuron methyl 0.6% + pretilachlor 6 % GR (rice).

MATERIALS AND METHODS

A pot culture experiment was conducted at, College of Agriculture, Rajendranagar, Hyderabad during *rabi* 2019 in a *Vertisol* and an *Alfisol*. *Alfisol* was sandy clay loam in texture, non saline (EC 0.08 dSm^{-1}) and with neutral pH (6.98). The available N, total N and organic carbon % found to be 190.53 kg/ha, 0.161 % and 0.90 % respectively. The *Vertisol* was also non saline (EC 0.152 dSm^{-1}) with alkaline pH (7.98). The available N, total N and percentage organic carbon found to be 200.70 kg/ha, 0.170 % and 1.25 %, respectively. Pendimethalin 30% EC (1kg a.i/ha) was applied to groundnut, greengram, sunflower and bhendi at 24 hours after sowing. Atrazine 50% WP (1kg a.i/ha) was applied to maize at 24 hours after sowing and bensulfuron methyl 0.6% + pretilachlor 6% GR (0.66 kg a.i/ha) was applied to rice at 3 DAT. Every crop has a respective control without herbicide application. The experiment was conducted in Completely Randomized Block design with three replications to study the effect of different pre emergence herbicides on L-glutaminase activity under various crop covers. The soil samples were collected at 15 days interval from 0 DAA to 90 DAA and at harvest and wereassayed for L-glutaminase activity. The activity of L-glutaminase and rate of NH_4^+ released was estimated by modified indophenol blue method as described by Frankenberger and Tabatabai (1991) and Dorich and Nelson (1983) as modified by Yadav *et al.* (2022).

RESULTS AND DISCUSSION

Since herbicides are used as pre-emergence, a high proportion of herbicide reaches the soil and accumulates in the microbiologically active top layer of 0 to 15 cm of soil. L-glutaminase activity was measured in soil samples taken at 15-day intervals between 0 and 90 DAA and at harvest. The effect of Pendimethalin 30 percent EC on the activity of L-glutaminase (µg of NH_4^+ released g^{-1} soil $4h^{-1}$) in groundnut, greengram, sunflower and bhendi crops have been shown in Tables 1, 2, 3, 4 and figures 1, 2, 3, 4, respectively. Groundnut, greengram, sunflower and bhendi pot cultures

Table 1: Effect of Pendimethalin on soil L-glutaminase activity in Groundnut (µg of NH_4^+ released g^{-1} soil $4h^{-1}$)

Crops	Days After application L-glutaminase activity (µg of NH_4^+ released g^{-1} soil $4h^{-1}$)							
	0	15	30	45	60	75	90	Harvest
Alfisol	6.72	7.27	7.93	11.54	16.28	12.37	9.29	7.13
Alfisol control	6.73	7.93	9.52	11.91	13.59	10.91	8.73	6.96
Vertisol	5.56	7.14	8.93	11.55	14.32	12.18	8.23	5.83
Vertisol control	5.56	7.25	9.50	11.06	12.17	10.11	7.35	5.82
Mean	6.14	7.40	8.97	11.51	14.09	11.39	8.4	6.44
					C.D. (5 %)			SE(d) ±
Herbicides(A)					0.455			0.227
L-glutaminase activity (B)					0.643			0.321
Factor (A X B)					1.287			0.643

were all treated with pendimethalin before they emerged. First, L-glutaminase activity rose slowly up to 30 DAA, but it continued to increase to 60 DAA and then declined until harvest in both types of soils. Pendimethalin-treated *Alfisol* and *Vertisol* showed greater enzyme activity than untreated *Alfisol* and *Vertisol* in all crops from 45 DAA onwards. Pre-emergence herbicide atrazine was applied in maize.

Table 2: Effect of Pendimethalin on soil L-glutaminase activity in Green gram (µg of NH_4^+ released g^{-1} soil $4h^{-1}$)

Crops	Days After application L-glutaminase activity (µg of NH_4^+ released g^{-1} soil $4h^{-1}$)							
	0	15	30	45	60	75	90	Harvest
Alfisol	6.68	7.21	7.78	11.13	14.68	11.90	9.04	6.98
Alfisol control	6.68	7.72	9.15	11.29	12.98	10.26	8.16	6.83
Vertisol	5.58	6.68	8.21	10.67	12.98	11.56	7.69	5.81
Vertisol control	5.58	6.92	8.47	10.09	11.27	9.41	6.95	5.76
Mean	6.13	7.13	8.4	10.79	12.98	10.78	7.96	6.35
					C.D. (5 %)			SE(d) ±
Herbicides(A)					0.43			0.215
L-glutaminase activity (B)					0.608			0.304
Factor (A X B)					1.216			0.607

The results pertaining to effect of atrazine on L-glutaminase activity are presented in table 5 and also depicted in figure 5. L-glutaminase activity in *Vertisol* (atrazine treated) increased gradually from 0 DAA to 30 DAA and then significantly from 30 DAA to 60 DAA, but again decreased until harvest. Its activity was higher in control, up to 15 DAA.

Table 3: Effect of Pendimethalin on soil L-glutaminase activity in Sunflower (µg of NH_4^+ released g^{-1} soil $4h^{-1}$)

Crops	Days After application L-glutaminase activity (µg of NH_4^+ released g^{-1} soil $4h^{-1}$)							
	0	15	30	45	60	75	90	Harvest
Alfisol	6.63	6.97	7.43	10.27	14.46	11.28	8.97	6.72
Alfisol control	6.63	7.59	8.86	10.78	11.96	9.39	7.88	6.81
Vertisol	5.43	6.51	7.94	10.61	12.53	10.97	7.24	5.79
Vertisol control	5.43	6.80	8.11	9.73	10.87	9.14	6.62	5.61
Mean	6.03	6.97	8.08	10.35	12.46	10.19	7.68	6.23
					C.D. (5 %)			SE(d) ±
Herbicides(A)					0.434			0.217
L-glutaminase activity (B)					0.614			0.307
Factor (A X B)					1.228			0.614

Table 4: Effect of Pendimethalin on soil L-glutaminase activity in Bhendi (µg of NH_4^+ released g^{-1} soil $4h^{-1}$)

Crops	Days After application L-glutaminase activity (µg of NH_4^+ released g^{-1} soil $4h^{-1}$)							
	0	15	30	45	60	75	90	Harvest
Alfisol	6.62	6.81	7.27	8.11	10.13	8.27	7.46	6.73
Alfisol control	6.62	7.16	7.69	8.21	9.26	7.83	7.16	6.71
Vertisol	5.31	5.57	5.82	7.91	8.87	8.21	6.78	5.52
Vertisol control	5.31	5.63	6.01	7.24	7.92	7.07	5.91	5.54
Mean	5.96	6.29	6.70	7.87	9.04	7.84	6.83	6.13
					C.D. (5 %)			SE(d) ±
Herbicides(A)					0.387			0.193
L-glutaminase activity (B)					0.547			0.273
Factor (A X B)					1.142			0.547

Later, atrazine-treated soils showed higher activity. In the case of atrazine-treated *Alfisol*, L-glutaminase activity decreased from 0 to 15 DAA and later increased from 15 to 60 DAA. Treated *Alfisol* showed lower activity than control up to 30 DAA, but later showed higher activity than control. In the case of rice, bensulfuron + pretilachlor were used as pre-emergence herbicides. The results pertaining to effect of bensulfuron + pretilachlor on L-glutaminase activity were presented in table 6 and also depicted in Figure 6. In the treated soils of *Vertisol* and *Alfisol*, the activity of L-glutaminase decreased from 0 to 15 DAA and later increased up to 60 DAA, showing higher activity compared to control. Herbicides' inhibitory effect on soil L-glutaminase with time and recovery during the later stages may differ for many reasons. With the passage of time, the herbicides may have been irreversibly adsorbed on the soil colloid, resulting in diminished inhibition. After some time, the herbicides' effects on the microbial population might have stabilised. Another aspect contributing to the decline in inhibition with time is the partial breakdown of these herbicides in the soil. Sireesha *et al.* (2019) speculated that enzymes released by plant roots may be responsible for the recovery from inhibition in later stages.

Table 5: Effect of Atrazine on soil L-glutaminase activity in Maize (µg of NH_4^+ released g^{-1} soil $4h^{-1}$)

Crops	Days After application L-glutaminase activity (µg of NH_4^+ released g^{-1} soil $4h^{-1}$)							
	0	15	30	45	60	75	90	Harvest
Alfisol	6.59	6.36	6.98	8.95	10.92	9.38	8.29	6.73
Alfisol control	6.59	7.06	7.75	8.61	9.46	8.21	7.27	6.72
Vertisol	5.39	5.61	6.29	7.92	9.25	8.56	7.46	5.58
Vertisol control	5.39	5.79	6.27	7.63	8.23	7.55	6.49	5.62
Mean	5.99	6.21	6.82	8.28	8.96	8.42	7.38	6.16
					C.D. (5 %)			SE(d) ±
Herbicides(A)					0.314			0.157
L-glutaminase activity (B)					0.445			0.222
Factor (A X B)					0.889			0.444

Another possible reason for the increased activity after inhibitory affect with the application of atrazine, pendimethalin and bensulfuron + pretilachlor up to 60 DAA is that the soil microbial population uses herbicides and their metabolites as a source of nutrition and some microbes get involved in decomposing the herbicide a few days after its application. It's essential to recall that in soil, owing to the relatively large biomass with respect to available substrate for growth; almost all microorganisms are living under starvation conditions.

The agrochemical-induced events can be imagined as capable of disrupting this conditions: (1) the death of sensitive organisms with the consequent utilization of the organic residue by the surviving populations; (2) the direct utilization of agrochemicals by the organisms which are able to degradation to metabolize them in this sense the behaviour of

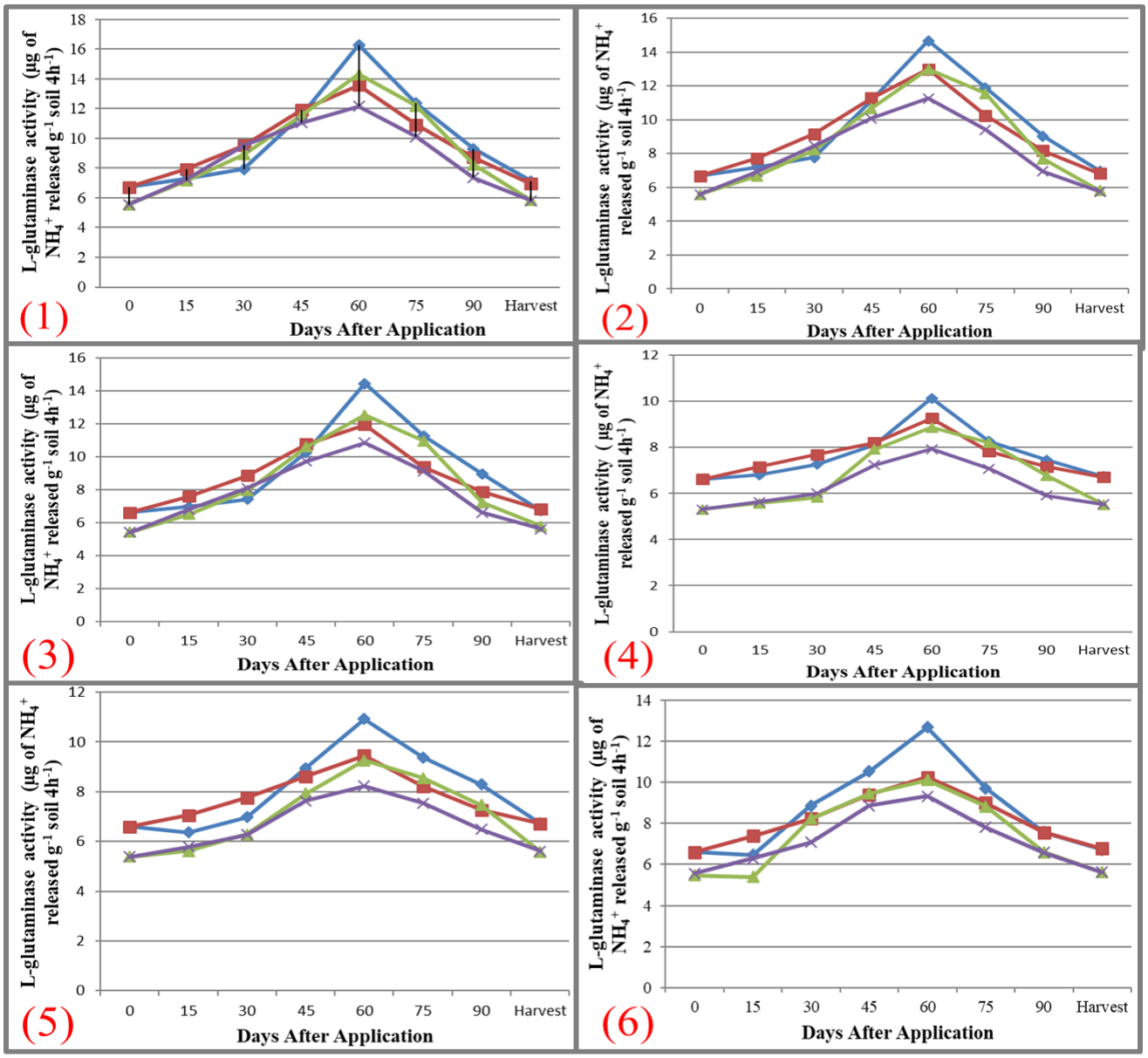

Fig. (1) Effect of Pendimethalin on soil L-glutaminase activity in Ground Nut; (2) Effect of Pendimethalin on soil L-glutaminase activity in Green gram; (3) Effect of Pendimethalin on soil L-glutaminase activity in Sunflower; (4) Effect of Pendimethalin on soil L-glutaminase activity in Bhendi; (5) Effect of Atrazine on soil L-glutaminase activity in Maize; (6) Effect of Pretilachlor + Bensulfuron on soil L-glutaminase activity in Rice

organic pesticides as a source of carbon may be the best example of agrochemicals influencing the dynamics of soil populations; (3) the development of microbial population which depends on secondary nutrient sources (Cerevelli *et al.,* 1978). Similarly, while passing through an adaptation period, a secondary population of microorganisms generates enzymes that breakdown pesticides (Milosevic and Govedarica, 2002). Herbicides are absorbed by organic soil amendments, forming bound or recalcitrant states, which decreases the potential of microbial attack and may also explain for the minimal effect or masking of herbicide effect on enzyme activity, according to Perucci *et al.* (2000).

Herbicides have detrimental effects on microorganisms, according to Ramalakshmi *et al.* (2017), lowering their abundance, activity, and thus the diversity of their communities. Herbicides, on the other hand, have the greatest damaging effects immediately after they were applied. Later, microorganisms participate in a breakdown process, and the decomposed organic pesticides give carbon-rich substrates, which boost the rhizosphere's microbial

Table 6: Effect of Pretilachlor + Bensulfuron on soil L-glutaminase activity in Rice (µg of NH_4^+ released g^{-1} soil $4h^{-1}$)

Crops	Days After application L-glutaminase activity (µg of NH_4^+ released g^{-1} soil $4h^{-1}$)							
	0	15	30	45	60	75	90	Harvest
Alfisol	6.61	6.47	8.87	10.53	12.69	9.71	7.58	6.71
Alfisol control	6.60	7.39	8.23	9.41	10.25	9.01	7.56	6.78
Vertisol	5.48	5.39	8.23	9.47	10.12	8.82	6.61	5.63
Vertisol control	5.56	6.29	7.08	8.87	9.32	7.81	6.58	5.63
Mean	6.06	6.38	8.10	9.57	10.59	8.84	7.08	6.17
					C.D. (5 %)			SE(d) ±
Herbicides(A)					0.35			0.175
L-glutaminase activity (B)					0.495			0.247
Factor (A X B)					0.991			0.495

population. This could also explain why L-glutaminase activity increased from 45 to 60 DAA as compared to 0 to 30 DAA. The recovery of microbial populations and enzyme activities after initial inhibition, according to Latha and Gopal (2010), was attributable to microbial tolerance to these compounds or their breakdown. It could also be owing to microbial proliferation stimulated by an increased supply of nutrients in the form of herbicide-killed microbes. According to Singh (2014), a high dose of pendimethalin was found to be toxic to soil enzymes when compared to low and medium concentrations. High doses inactivate enzymes irreversibly, lowering enzyme activity during the lag period until the bacteria recovered and a fresh pool of enzymes were synthesised by plants and microbes, which enhanced enzyme activity later. Herbicides cause osmotic stress, which decreases microbe survival, and soil enzyme activity is controlled by the native soil ecology as well as the type of herbicide used (Bharathi *et al.,* 2011). Herbicide applications disrupt and modify the biological balance in the soil. For example, 20 days after rice transplanting, a pre-emergent application of pretilachlor @ 0.75 kg a.i. ha^{-1} reduced microbial population and, as a result, enzyme activity. At subsequent stages, however, enzyme activity and microbial population increased, and unweeded control had the lowest enzyme activity at all development stages (Kavitha *et al.,* 2011). Interactions between agrochemicals and soil enzymes, are influenced by the physical and chemical environment of the soil. If an agrochemical is not adsorbed into the soil matrix, its fate is determined by other factors such as pH, soil water content, temperature, and so on. If the agrochemical is strongly adsorbed onto soil particles, on the other hand, its concentration will be lowered until no enzyme-catalyzed reaction occurs. In comparison to free enzymes in solution, enzymes adsorbed onto soil colloidal particles and those confined within humus particles behave differently (McLaren and Parker, 1970; Ladd and Butler, 1975). Influence of negative effect of the pre emergence herbicides on L-glutaminase activity was of short duration in *Vertisol* when compared to *Alfisol*. It is due to presence of Montmorillonite clay mineral as a dominant clay mineral in *Vertisol*. Due to higher surface area of the montmorillonite, pesticides adsorbed on the planar and edge surfaces rapidly and reduced slowly their impact.

CONCLUSION

There is an initial inhibitory effect of herbicides on soil L-glutaminase activity and later the enzyme activity started recovering from initial inhibition and increased its activity up to 60 DAA. The herbicides affected microbial population indirectly causing physiological changes and increased enzymatic production by the microbial population after recovery and there by degrading the herbicide.

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