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EFFECTS OF HERBICIDES ON SOIL ENZYME L-ASPARAGINASE ACTIVITY

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Abstract

To study the effect of new generation herbicides on soil enzyme L-asparaginase a field experiment was designed and carried out on maize as test crop. The weed management practices tested were two pre-emergence herbicides (pendimethalin and atrazine @ 1.0 kg a.i/ha each) and two post-emergence herbicides topramezone @ 25.2 g a.i/ha at 15 DAS and tembotrione @ 105 g a.i/ha at 15 DAS and combine application of pre- and post emergent herbicides topramezone + atrazine @ 25.2 + 250 g a.i/ha at 15 DAS, tembotrione + atrazine @ 105 + 250 g a.i/ha was applied at 15 DAS and in addition to these the unweeded control and hand weeding (twice at 20 and 40 days) treatments. Soil samples were collected at 15 days intervals and enzyme activity was assayed. In pre-emergence herbicides, control, and hand weeding, there was an increase in the activity of the enzyme from 0 to 60 days and then decrease till harvest, whereas in post emergence herbicide both stimulation and inhibition were observed.

In the process of becoming self-sufficient in food production, developing countries started concentrating in improving and sustaining agricultural productivity. Therefore, many new chemicals have been used, and particularly herbicides which are used to destroy weeds; weeds are plant species growing where it is not desired, or plant out of place, or plant that is more detrimental than beneficial. During the process of modernization of agriculture, the farmers shifted themselves from hand weeding to usage of chemicals, the herbicides. Pests and weeds can reduce yields of agricultural crops up to 20% in developed countries and up to 50% in undeveloped regions (Dobrovaljskiy and Grishina 1985). To overcome these problems, usage of herbicides and pesticides has increased enormously. Moreover, incorrect, and indiscriminate application of herbicides affects the soil health and also influence the soil ecosystem. Hazardous herbicides are poorly degradable, and also their persistence may lead to long-term accumulation that cause imbalance of soil fauna and interactions between soil-plant-herbicide-fauna-man relationships. Nowadays, much emphasis is given on sustainable agriculture systems which involves optimizing agricultural resources to satisfy human needs and at the same time maintaining the quality of the environment and sustaining natural resources. Soil enzyme plays a crucial role in nutrient transformation in soil; hence, it is considered as an important factor of crop productivity. All the transformations of nutrients occurring in soil are stimulated by the enzymes so, such conversions are available to plants and microorganisms. Enzymes are frequently referred to as markers of soil environment purity (Aon and Colaneri 2001). The use of herbicides has become a widely adopted practice in Southern Telangana zone of Telangana State and the herbicides like pendimethalin, atrazine, topramezone and tembotrione are being extensively used. Though much information is available concerning the effects of these herbicides on the agronomic traits i.e., weed control, phytotoxicity, dry-matter production, grain and stover yields but very little information is available concerning their interactions on soil biochemical properties particularly with reference to the activity of soil enzymes. L-asparaginase (L-asparagine amidohydrolase EC 3.5.1.1) plays an important role in N mineralization of soils. The chemical nature of N in soils is

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such that a large proportion (15 - 25%) of the total soil N is often released as NH_4^+ by acid hydrolysis (6N HCl). Sowden, (1958) suggested that a portion of the released NH_4^+ comes from the hydrolysis of amide (asparagine and glutamine) residues in soil organic matter. Bremner (1955) reported that hydrolysis of humic preparation released 7.3 to 12.6% of total nitrogen in the form of amide nitrogen. Sowden (1958) also reported that a percentage of the NH_4^+ released during acid hydrolysis was equal to or nearly equal to the sum of nitrogen released from asparatic acid - N plus glutamic acid - N derived from of asparagine and glutamine. Hence, a field experiment was conducted at College Farm to study the effect of two pre-emergence herbicides (pendimethalin and atrazine @ 1.0 kg a.i/ha each) and two post-emergence herbicides topramezone @ 25.2 g a.i/ha at 15 DAS, and tembotrione @ 105 g a.i/ha at 15 DAS and combine application of pre- and post emergent herbicides topramezone + atrazine @ 25.2 + 250 g a.i/ha at 15 DAS, tembotrione + atrazine @ 105 + 250 g a.i/ha was applied at 15 DAS and in addition to these the unweeded control and hand weeding (twice at 20 and 40 days) treatments. The soil samples were collected at 15 days intervals till harvest and were examined for the activity of enzyme L-asparaginase.

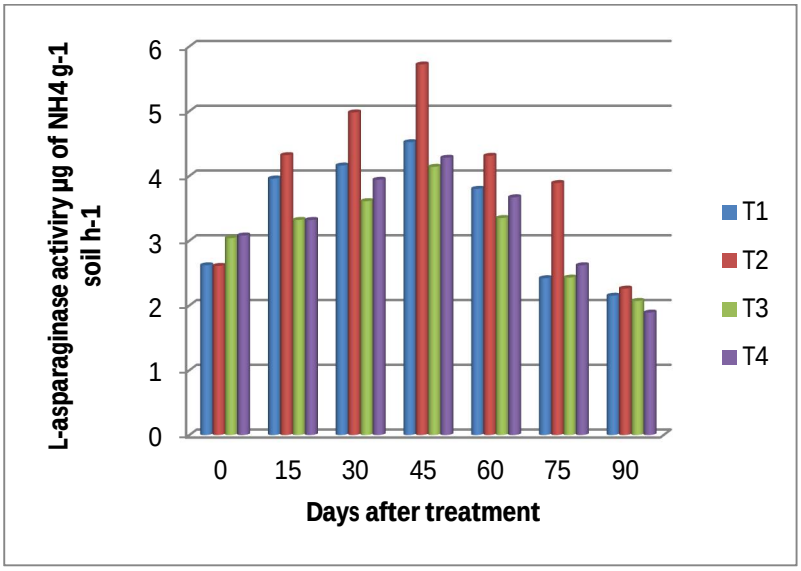
A field experiment was carried out in the College farm at College of Agriculture in Professor Jayashankar Telangana State Agriculture University during the Karif season 2014 . The climate of the experimental site was semi-arid during the growth period from 08.07.2014 to 20.10.2014. Initial soil samples were analyzed for physico-chemical and chemical properties by following standard procedures. Mechanical analysis Bouyoucos Hydrometer (Bouyoucos 1962) chemical analysis pH (Jackson 1973), EC (Jackson 1973), organic carbon (Walkley and Black method 1934) was used. Available nitrogen alkaline permanganate method (Subbaiah and Asija 1956), available phosphorus Olsen's method (Olsen *et al*. 1954) and available potassium neutral normal ammonium acetate method using Flame Photometer (Jackson 1973) were used. The pH of the experimental field was 7.68, EC 0.19 dS/m, CEC (c mol (p+)/kg) 39.38 and organic carbon was 0.20. The N status of the experimental field was low (255.4 kg/ha), available P (25.1 kg/ha) and available K status were in higher range (263.2 kg/ha). The soil texture was sandy loam with sand (70%), silt (12%) and clay (18%).

Treatments	
T_1	Atrazine 50% WP @ 1.0 kg a.i/ha as PE fb inter cultivation at 30 DAS
T_2	Intercropping of maize with cowpea and application of T_2pendimethalin 30 % EC @ 1.0 kg a.i/ha as PE
T_3	Tembotrione 42% SC @ 105 g a.i/ha as PoE at 15 DAS
T_4	Topramezone + atrazine @ 25.2 + 250 g a.i/ha as PoE at 15DAS
T_5	Tembotrione+ atrazine @ 105 + 250 g a.i/ha as PoE at 15DAS
T_6	Tembotrione 42% SC @ 105 g a.i/ha as PoE at 15 DAS
T_7	Handweeding at 20 and 40 DAS
T_8	Unweeded control.

Soil enzyme L-asparaginase activity was assayed by quantifying the rate of release of NH_4^+ from the hydrolysis of asparagine as described by Frankerberger, W.T., Jr., and Tabatabai, M.A., (1991). The activity of L-asparaginase was assayed by steam distillation method. In this method 30 ml of the supernatant with KCl-$AgSO_4$ extract was taken and transferred to Kjeldahl flask. To this a pinch of MgO was added and was kept at one end of the distillation unit. During steam distillation for 4 min, the solution containing MgO was heated and the ammonia was released into

boric acid containing mixed indicator through a tube dipped in the solution. The ammonia released would change the colour of the solution from pink to pale green at the end of the distillation. This was titrated against standardized 0.005 N H_2SO_4 and the amount released was calculated and expressed as µg of NH_4^+ released/g soil/h. Correlation study was carried out to find the relationship between soil properties and soil enzyme activities

The texture of experimental field was sandy loam with pH of 7.6. The soil was low in available nitrogen (255.4 kg/ha), medium in available phosphorus (25.1 kg/ha) and potassium (263.2 kg/ha). The activity of L-asparaginase (expressed as µg of NH_4^+ released/g soil/hr) as influenced by the herbicide treatments is presented in Figs 1 - 2. A close perusal of data indicates that significant difference exists between different herbicide treatments and periods of study. In case of pre-emergence treatments with herbicides atrazine, pendimethalin the enzyme activity of L-asparaginase (expressed as µg of NH_4^+ released/g soil/h) was observed to increase from at 0 to 60 DAT. At 0 DAT the L-asparaginase activity for atrazine was (1.71), pendimethalin (2.79), hand weeding (2.9) and control (2.79). At 60 DAT the L-asparaginase activity for atrazine was (4.52),

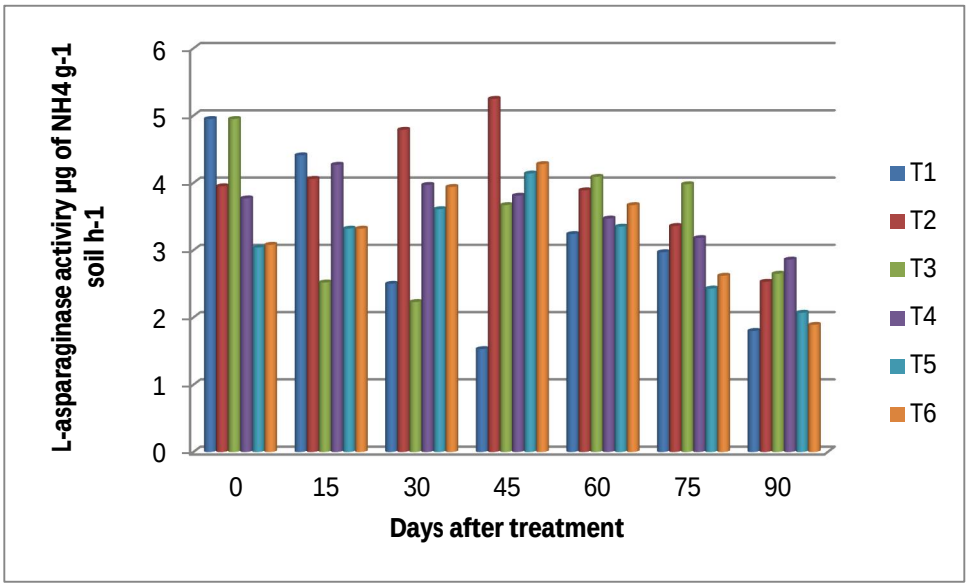


T_1 atrazine, T_2 pendimethalin, T_3 hand weeding and T_4 control
Fig. 1. Effect of pre-emergence herbicides on soil L-asparaginase activity.

pendimethalin (5.72), hand weeding (4.14) and control (4.28) and then decreased till harvest. The highest enzyme activity was observed at 60 DAT for all the four treatments. However, at the initial stages in case of atrazine inhibition was observed and these might be due to competitive inhibition of the enzyme later was restored due synthesis of fresh enzyme as well as more substrate. The decreasing order of L-asparaginase activity was pendimethalin (5.72)> atrazine (4.52) > control (4.28) > hand weeding (4.14).

The enzyme activity observed in case of post-emergence herbicides, tembotrione is as follows, the activity increased from 0 (3.95) to 45 DAT (5.25) and thereafter decreased till harvest, the activity was higher than that of control at each stage. On the other hand, the activity of the enzyme in case of topramezone increased from 0 to 15 DAT and later decreased during the process of decrease, the activity was lower than that of control. Here, it was evident that as the

period of incubation increased the rate of activity of the enzyme decreased with exception when new enzyme was synthesized by the plant roots and other fauna present in the soil. In the combined form of application, the topramezone and atrazine the activity decreased from 0 (4.95) to 30 DAT (2.23) and then increased till 60 (4.09) later decreased till harvest. In case of tembotrion and atrazine the enzyme activity increased at initial stage i.e.:0 DAT (3.77) when compared to that of control (3.08) to 15 DAT (4.27) and decreased latter till harvest, a clear comparison could be obtained between control and treatments because both stimulation and inhibition was observed in case of treatments. From the data, one can clearly observe that significant difference exists between different herbicide treatments and periods of study.



T_1 topramezone, T_2 tembotrione, T_3 topramezone + atrazine, T_4 tembotrione + atrazine T_5 hand weeding and T_6 control.

Fig. 2. Effect of post-emergence herbicides and combination of pre- and post emergence herbicides on soil L-asparaginase activity.

Table 1. Statistical analysis.

Analysis of variance	SE(m)	CD
Herbicides	0.030	0.084
L-asparaginase	0.031	0.089
Herbicide × L-asparaginase	0.090	0.252

Similar results were observed by (Latha and Gopal 2010). In the present study, there was a clear stimulation at the beginning as time of incubation increased, the rate of enzyme activity decreased and later the enzyme started recovering from initial inhibition and the enzyme activity was lower than control at all the stages of DAT. The herbicides affect microbial population indirectly causing physiological changes and increased enzymatic production by the microbial population after recovery and there by degrading the herbicide. The increased activity with the application of atrazine, pendimethalin at 60 DAS and the possible reason could be that soil

microbial population uses herbicides and their metabolites as source of nutrition and some groups of microbes get themselves involved in decomposing the herbicide a few days after their application. Similarly, secondary population of microbes induces enzymes which decompose herbicides while they are passing through a period of adaptation. Further the herbicides were found to affect the microbial population indirectly by changing their bio-synthetic mechanism, that is, a change in level of protein biosynthesis which is reflected on ratio of extra- and intra-cellular enzymes produced. Microbes are also affected in protein biosynthesis, thereby causing induction or repression of synthesis of certain enzymes (Milosevic and Govedarica 2002). Xiaohua *et al.* (2005) reported decrease in the activity of alkaline phosphatase due to acetamiprid application because this herbicide had altered the membrane permeability of phosphate solubilizers that release phosphatase enzyme.

Some workers (Martins *et al*. 2011, Bello *et al*. 2013, Nikoloff *et al*. 2013) opined that the decrease in activity of soil enzymes is due to biotic stress induced by herbicides in the soil. (Sofo *et al*. 2012, Vladoiu *et al*. 2015) reported that when herbicides were incubated for prolonged period under certain circumstance, can act as strong inhibitors of enzymes. Vandana *et al.* (2012) revealed that urease enzyme activity has increased from 0 to 60 days after transplantation. Tejada (2009) revealed that when more than one herbicide is used it can cause strong inhibitory effect on the microbiological activity of soil than individual application which is in turn effect soil enzyme activity.

Besides, certain groups of microorganisms start to decompose herbicides a few days after the application (Milosevic *et al*. 2002). Rasool and Reshi (2010) found that the influence of pesticides on activity of the enzyme differ variably, soil enzymes like urease and asparaginase were inhibited and others like dehydrogenase, protease and amidase activities were stimulated. Apart from these few enzyme activities differed drastically because other factors also play a role, pH effect phosphatase activity. Weaver *et al. (*2004) reported inactivation of most soil enzymes, because of herbicide attachment on the active site of enzyme and thus preventing substrate attachment to the enzyme. Increasing trend in the activity of urease was observed from day 15 to day 60. This reduction in inhibitory effect was due to soil microbe's recovery with the passage of time due to which urease activity increased. Singh (2014) reported that high dose of pendimethalin inhibited soil enzymes irreversibly by completely inactivating the enzyme hence decrease in enzyme activity during lag period of bacteria but the microbes were able to overcome this period and synthesis of new pool of enzymes by plant as well as microbes. Zahir *et al*. (2001) described mortality of weed roots by herbicide application as a result root exudate that contain auxin and gibberellins declined which might be involved in enzymes activity enhancement, so their suppression contributes toward this decline in dehydrogenase. Enzymes of extracellular origin and the death of some microorganisms may cause a decline in the production and excretion of enzyme which leads to the reduction in the soil activity. Riah *et al.* (2014) classified herbicides as stimulants with positive effect and inhibitors with negative effect, formesafen a class of herbicide has stimulatory effect on acid and alkaline phosphates and mixed effect on ureases was found by Zang *et al* (2014). Aruna *et al.* (2018) have observed similar effect with respect to urease activity. Pre-emergence herbicides have shown stimulation and post emergence herbicides mixed responses

From the present study, it is suggested that before using new herbicides, studies related to their adverse effects have to be studied and low concentration of herbicides may be beneficial some times to soil fauna and the herbicides having stimulation effect can be used for weed control after identifying their effect by incubation studies.

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