Plant Archives Vol. 25, Supplement 2, 2025 pp. 2168-2174 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-2.275

EFFECT OF TILLAGE AND HERBICIDE (PRETILACHLOR) APPLICATION ON SOIL BIOLOGICAL PROPERTIES IN WINTER RICE

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(Date of Receiving : 02-04-2025; Date of Acceptance : 10-06-2025)

ABSTRACT

The study on the "effect of tillage and herbicide (Pretilachlor) application on soil biological properties in winter rice" was conducted under long term trial of AICRP on weed management at ICR farm of Assam Agricultural University, Jorhat-13. The experiment was laid out in randomized block design replicating 3 times with 5 treatments *viz.*, T_1 -Conventional Tillage (Transplanted) ; T_2 -Conventional tillage (Transplanted + Herbicide), T_3 -Conventional Tillage (Direct-Seeded + Herbicide) ; T_4 -Minimum Tillage (Direct-Seeded + Herbicide), T_5 -Minimum Tillage (Direct-Seeded + Herbicide + Residue retained on the surface). Ranjit and Basundhara were used as the rice variety in transplanted and direct seeded rice respectively. Results of the study showed significant improvement in weed management through herbicides in turn significant improvement of soil microbial activity and microbial biomass carbon. Biological parameters were analyzed from surface soils collected periodically at 0, 3, 7, 15, 23, 30, 45, 60 days after application (DAA) of the herbicide and at harvest in winter rice. In the present study, herbicide application resulted in inhibition of beneficial microbes *viz.*, Bacteria, Fungi, *Azotobacter, Azospirillum* and phosphate solubilizing bacteria up to 7 DAA which it increased towards harvestings. Application of pretilachlor in winter rice showed a decline in dehydrogenase and β-glucosidase activity at initial stage while it increased the activity of acid phosphatase and microbial biomass carbon. Significantly higher enzyme activities were recorded in treatments receiving minimum tillage, herbicide along with residues retained on the surface.
***Keywords* :** Tillage, herbicide, dehydrogenase, acid phosphatase, microbial biomass carbon.

Introduction

As tillage systems influences the chemical, physical and biological properties of soil and have a great impact on soil productivity and sustainability. In Conventional agriculture, tillage practices may adversely effect on long-term soil productivity due to erosion and loss of organic matter in soils. On the other hand, Conservation tillage is one of the most important components of sustainable agriculture which involves reducing intensity of tillage operations and retaining plant residues on the soil surface. Conservation tillage may be defined as a tillage system in which at least 30% of crop residues are left in the field and is an important conservation practice to reduce soil erosion (Uri, 1999). The practice involved in conservation tillage like zero tillage (ZT), minimum tillage (MT) and permanent raised beds (PB). The advantages of conservation tillage practices over conventional tillage include reducing cultivation cost (Hobbs, 2008), allowing crop residues to act as an insulator (Alvarez, 2005) and reducing soil temperature fluctuation (Uri, 1999), building up soil organic matter (Schwab *et al.*, 2002) and conserving soil moisture (Schwab *et al.*, 2002; West and Post, 2002). Conventional tillage can lead to soil microbial communities dominated by aerobic microorganisms, while conservation tillage practices increase microbial population and activity (Staley, 1999) as well as microbial biomass (Balota *et al.,* 2003).

With widespread application of herbicides in rice, questions have often posed about the movement and length of time these herbicides remain toxic in soil. Herbicides may cause adverse effect on bacterial (Rajendran and Lourduraj, 1999), fungal (Shukla, 1997) and actinomycetes population (Rajendran and Lourduraj, 1999). At normal field recommended rates, herbicides are considered to have no major or long-term effect on microbial populations. Soil microorganisms like bacteria, fungi, algae, protozoa, actinomycetes and some nematodes have a vital role in maintaining the soil productivity. Herbicides have negative impact on soil microbial diversity which can affect the functional stability of the soil microbial community and soil health. In most of the cases herbicide accumulates in the top layer of the soil (0-15cm) where most of the microbial activity takes place. Application of chemical fertilizer, organic manure and pesticides has been reported to influence both size and function of microbial community. Pretilachlor [2-chloro-2'6'diethyl-N-(2- propoxyethyl) acetanilide] is recommended as selective herbicide for weed control in rice crop. Pretilachlor dissipates readily in rice fields by photodecomposition, microbial degradation and volatilization at recommended rate and pretilachlor does not affect soil properties or pose a serious problem for environmental pollution (Adachi *et al*., 2007). Pretilachlor, a member of the acetanilide group of herbicides, is used for selective control of annual weeds in rice fields (Saha *et al*. 2012). Pretilachlor application reduced weed density and increased grain and straw yields in rice (Faruk *et al*., 2013).

Microorganisms play a key role in decomposition, mineralization, nutrient cycling and improves various physico-chemical properties of the soil. It was reported by Carney & Matson (2005) that the decomposition of several organic compounds frequently used in agriculture, which directly affect the synthesis and decomposition of soil organic matter. Soil enzymes can be used as potential indicators of soil quality for sustainable management because they are sensitive to ecological stress and land management (Benedetti and Dilly, 2006). Enzymes may react to changes in soil more quickly than other physicochemical variables and therefore may be useful as early indicators of biochemical changes (Melero *et al*., 2008). Evidence of the stimulation effect of herbicides on soil biochemical properties has been reported (Garcı´a-Ruiz *et al*., 2008), even if herbicides are not designed to directly interact with soil enzymes (Speir and Ross, 1975). All the transformations of nutrients occurring in soil are stimulated by the enzymes that condition their conversion into forms available to plants and microorganisms. Major microbial enzymes involved in the mineralization of soil organic matter are cellulases, dehydrogenase, β-glucosidase, proteases, ureases and phosphatases (Kunito *et al*., 2001).

Since information about the effect of tillage and herbicide application on soil biological properties in acid soil is limited, and therefore it is felt essential to understand the changes in such indicators of soil quality as influenced by tillage and use of herbicide in rice-rice cropping system.

Materials and Methods

The study was carried out during 2020-2021 to monitor the "Effect of tillage and herbicide (Pretilachlor) application on soil biological properties in winter rice." The experiment was laid out in RBD replicating 3 times with 5 treatments *viz*., T_1 -Conventional Tillage (Transplanted); T_2 -Conventional tillage (Transplanted + Herbicide), T_3 -Conventional Tillage (Direct-Seeded + Herbicide); T_4 -Minimum Tillage (Direct-Seeded + Herbicide), T_5 -Minimum Tillage (Direct-Seeded+ Herbicide+ Residue retained on the surface). Ranjit and Basundhara were used as the rice variety in transplanted and direct seeded respectively. The direct seeding of Basundhara was done on 23rd June, 2020 and Ranjit was transplanted on 25th July, 2020. The monthly mean minimum and maximum temperatures during the crop season ranged from 10.3-26.2^{0}C and 23.9-32.6^{0}C respectively, total rainfall received was 51.1 mm during the entire crop season. Relative humidity and sunshine hours were found to varied from 90.3 to 99.1 *per cent*, and 2.3 to 6.4 hours, respectively. Prior to the experimentation the soil samples were collected from 0-15cm depth and various physico-chemicals parameters were analyzed. The soil was found sandy clay loam with pH value of 5.01, bulk density (1.34 gcm^{-3}), Porosity (44.56%) , Water Holding Capacity (42.05%), CEC (6.77 cmol (p+) 100 g-1), available N (302.54 Kg ha^{-1}), P (19.35 Kg ha^{-1}), K (150.23 Kg ha^{-1}), exchangeable Ca (0.68 meq 100g^{-1}), exchangeable Mg (0.33meq 100g^{-1}) and organic carbon (0.72%). All the above parameters were analyzed by standard procedure as described by Baruah and Borthakur (1997). Soil samples from all the treatments were collected on 0, 3, 7, 15, 23, 30, 45, 60 DAA of herbicide and after harvest of the winter rice and analyzed for soil microbial population, phosphatase activity, dehydrogenase activity, β-Glucosidase activity and Microbial biomass carbon.

For estimation soil microbial population serial dilution technique was used for isolation of total bacterial population, total fungal population, *Azotobacter*, *Azospirillum* and phosphate solubilizing bacteria (PSB) in appropriate media. The microbial count was done by taking one gram of the soil sample and was serially diluted. Hundred microlitres of it was plated separately for Total Bacteria (Agar Medium), Total Fungal (Rose Benal Agar Medium), *Azotobacter* (Burks Medium), *Azospirillum* (N-free bromothymol blue semisolid media-NFb) and PSB (Pikovskaya's medium) and the plates were then incubated at 28±2°C for 48-72 hours and the colony forming units were counted. Acid Phosphatase activity was detected colorimetrically following the method of Tabatabai and Bremner (1969) and expressed as µg p-nitrophenol g^{-1} dry soil h^{-1}. The p-nitrophenol (PNP) released after the incubation of the soil with p-nitrophenyl phosphate for one hour at 37±1^{0}C using modified universal buffer, MUB (pH 6.5) was estimated and expressed as µg p-nitrophenol g^{-1} dry soil h^{-1}. Dehydrogenase activity was determined by reduction of triphenyltetrazolium chloride (TTC) to triphenylformazone (TPF) as described by Casida (1968). The TTC reduction rate to TPF was estimated after incubation at 30°C ± 1°C for 24 hour. The dehydrogenase activity was expressed as µg TPF g dry soil^{-}1/7 days. β- Glucosidase activity of the soil was determined using the method of Eivazi and Tabatabai (1988). The p-nitrophenol (PNP) released after the incubation of the soil with p-nitrophenyl β-D-Glucosidase for one hour at 37±1^{0}C using MUB (pH 6.0) was estimated and expressed as µg p-nitrophenol g^{-1} dry soil h^{-1}. The soil microbial biomass carbon (MBC) was estimated through fumigation extraction method as described by Jenkinson and Powlson (1976), Vance *et al.* (1987). The technique of analysis of variance as described by Panse and Sukhatme (1967) was used in RBD for statistical analysis of data obtained from various treatments. For microbiological parameters, statistically significant difference between the treatment means was tested with DMRT test at 5 *per cent* probability level.

Results and Discussion

Soil microbial population

The result revealed that population count of total bacteria, fungi, Azotobacter, Azospirillum, phosphate solubilizing bacteria population and microbial biomass carbon varied significantly among the tillage and herbicide treatments (Fig. 1, 2, 3, 4 & 5). Significantly higher population count of soil microbes were observed in the treatment T_5 (MT+ Herbicide application+ Residue retained on the surface) at 60 DAA of pretilachlor (117.68 x 10^6cfu g^{-1} , 63.88 x 10^4cfu g^{-1}, 32.14 x 10^6cfu g^{-1}, 30.23 x10^6cfu $g^{-1}$and 18.44 x10^6 cfu g^{-1} respectively for total bacteria, fungi, *Azotobacter, Azospirillum* and PSB population) and significantly lower count recorded was 55.33 x 10^6cfu g^{-1}, 24.15 x 10^4cfu g^{-1}, 13.97 x 10^6 cfu g^{-1} , 10.36 x10^6 cfu g^{-1} and 6.09 x 10^6 cfu g^{-1} respectively for total bacteria, fungi, *Azotobacter, Azospirillum* and PSB population in treatment T_2 receiving CT at 7 DAA of pretilachlor. Irrespective of the treatments, temporary inhibition in total bacteria, fungi, *Azotobacter, Azospirillum* and PSB population were observed till 7 DAA of pretilachlor and followed by gradual increase in population count from 15 DAA to 60 DAA of pretilachlor. It was observed that a reduction in total population of total bacteria, fungi, *Azotobacter, Azospirillum* and PSB population at harvesting of the crops. The above results are similar with the findings of Ojah *et al.,* 2024, Singh *et al*., 2015 and Younesabadi *et al.* 2014. The reason of increased microbial population with MT might be due to the more accumulation of organic matter. A declining trend in microbial populations at initial stage up to 7 DAA and increased towards harvesting were also reported by Bera and Ghosh, 2013 and Ghosh *et al.*, 2012. The decrease in the population at initial stage might be due to competitive influence and the toxic effect as well as persistence periods of herbicides in different soil ecosystems (Barman *et al.,* 2009). The increased population towards harvesting might be due to commensalic or proto-cooperative influence of various microorganisms in the rhizosphere of rice (Chen *et al.,* 2009). This might be also due to degradation of herbicides residue by microorganisms (Radivojevic *et al*., 2004).

Microbial biomass carbon (MBC)

The study revealed that the MBC ranged from 226.51-465.45 µg C g^{-1} dry soil (fig.6). The result showed that MT significantly increased the MBC of soil as compared to CT while pretilachlor application tends to increase MBC significantly up to first 23 DAA, then decreased till 45 DAA and finally increased up to harvesting of the crop. A significantly higher MBC (465.45 µg C g^{-1} dry soil) was observed at 23 DAA in treatment T_5 and lower MBC (226.51 µg C g^{-1} dry soil) was recorded in treatment T_1 receiving CT and Pretilachlor application at 3 DAA. The increased MBC in MT practice was similar with the findings of Aziz *et al.* (2013), Ronanki *et al.* (2018), and Parihar *et al.* (2018). Increased soil MBC as a result of MT practice might be due to the fact that MT improves the substrate availability and the microbial nutrient in soil leading to more microbial population and thus more soil MBC (Roldan *et al.,* 2003). Araujo *et al.* (2003)

reported that application of herbicides stimulates the growth of bacteria, actinomycetes and fungi which ultimately leads to increase in the soil MBC.

Enzyme Activity

The enzyme activity ranged from 179.22 to 304.48 µg p-nitrophenol g^{-1} dry soil hr^{-1} for acid phosphatase, 82.44 to 165.81 µg TPF g^{-1} dry soil 7 $days^{-1}$ for dehydrogenase and 99.42 to 237.96 µg p-nitrophenol g^{-1} dry soil 2 hr^{-1} for β- Glucosidase. The MT (T_5) significantly increased the enzyme activity in soil as compared to CT (T_1) in winter rice (fig. 7, 8 & 9). Pretilachlor application showed an inclining trend in acid phosphatase activity at initial stage up to 3 DAA and then decreased up to 15 DAA, thereafter increased till harvesting and declining trend in dehydrogenase activity at initial stage up to 7 DAA, then increased up to 60 DAA and finally decreased at harvesting. The pretilachlor application tends to reduce the β- Glucosidase enzyme activity significantly up to first 15 DAA, then increased till 60 DAA and finally decreased at harvesting of the crop. Significantly higher enzyme activity was observed in the treatment T_5 at 60 DAA (304.48 µg p-nitrophenol g^{-1} dry soil hr^{-1}, 165.81 µg TPF g^{-1} dry soil 7$days^{-1}$ and 237.96 µg p-nitrophenol g^{-1} dry soil 2hr^{-1} respectively for acid phosphatase, dehydrogenase and β-Glucosidase enzyme activity. Significantly lower acid Phosphatase activity (179.22 µg p-nitrophenol g^{-1} dry soil hr^{-}1) was observed at 0 DAA in the treatment T_1. The lower dehydrogenase activity (82.44 µg TPF g^{-1} dry soil 7$days^{-1}$) was observed at 0 DAA in the treatment T_2. While significantly lower β- Glucosidase enzyme activity (99.42 µg p-nitrophenol g^{-1} dry soil 2hr^{-1}) was recorded in treatment T_2 at 15 DAA. The results are in accordance with the findings of Gajda *et al.,* 2013 who reported that with MT practice the enzyme activity was found to be higher significantly as compared to CT which might be the fact that MT might have increased the accumulation of organic-C and N in surface soils as well as greater accumulation of inorganic nutrients which in turn increased the microbial population and ultimately enzyme activity. Sahoo *et al.,* 2017 reported that application of pretilachlor significantly increased the acid phosphatase activity in soil up to initial 3 DAA and then decreased up to 15 DAA, thereafter again increased till harvesting which might be due to toxic effect of the herbicides on some soil microorganisms which probably unable to survive and consequently there was a temporary release of enzymes after cell diesis causing an immediate increase in enzyme activity. Decreased in the activity of phosphatase in later period (up to 15 DAA) might be due to proteolysis of non-stabilized extra-cellular enzymes (Das *et al.,* 2003). The recovery of the activities towards harvesting might be due to the fact that the growth of microbial population after adaptation to the existing environment or most probably due to increased availability of nutrients after complete degradation of herbicides (Ismail *et al.,* 1998). This higher enzyme activity in T_5 might be due to the retention of organic residue which on decomposition increased soil organic carbon and N content (Kadlag *et al*., 2008). Moreover, the organic acids produced during decomposition of organic residue tend to reduce soil reaction (pH) which in turn enhances the acid phosphatase enzyme activity in later period (Reddy and Reddy, 2009). The increased of dehydrogenase enzyme activity due to MT concurs with the findings of Parihar *et al.* (2018) and Rahman *et al.* (2010). Rasool *et al.,* 2014 also reported that Pretilachlor application showed a declining trend in dehydrogenase activity at initial stage up to 7 DAA, then increased up to 60 DAA and finally decreased at harvesting. Increased in the β-glucosidase activity with MT practice was reported by Chu *et al.,* (2016) which might be due to the increased in soil organic matter that are an important energy source for microbes.

Conclusion

In the present investigation, an attempt was made to evaluate the effect of tillage and herbicide (pretilachlor) application in winter rice on population dynamics of soil microbes and enzyme activities. Under winter rice, MT was found to improve the microbial population, enzymatic activities and soil microbial biomass carbon as compared to conventional tillage (CT). Application of pretilachlor in winter rice showed a decline in dehydrogenase and β-glucosidase activity in initial stage while it increased the activity of acid phosphatase and microbial biomass carbon in initial stage. All the biological parameters were found highest in the treatment T_5 receiving MT, herbicide along with residues retained on the surface. Hence it may be concluded that conservational tillage (MT) treatment helps in improving biological properties of soil.

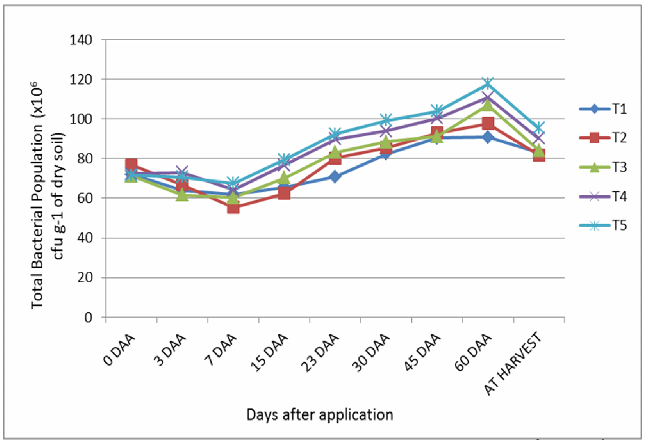

Fig. 1 : Population dynamics of total bacteria (x10^{6}cfu g^{-1} of dry soil) as affected by tillage and herbicide application in winter rice under conservation agriculture system

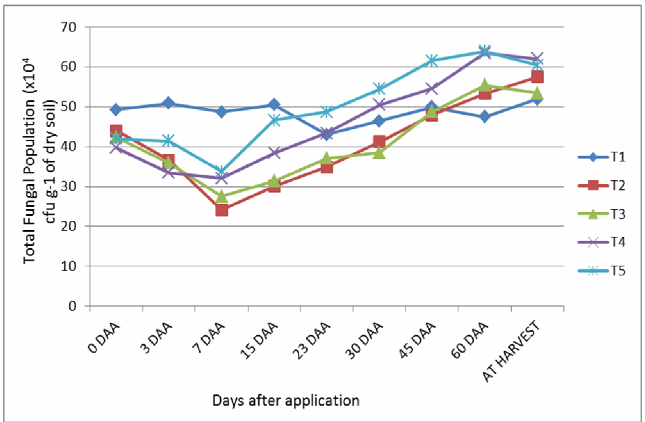

Fig. 2 : Population dynamics of total fungi (x10^{4}cfu g-1 of dry soil) as affected by tillage and herbicide application in winter rice under conservation agriculture system

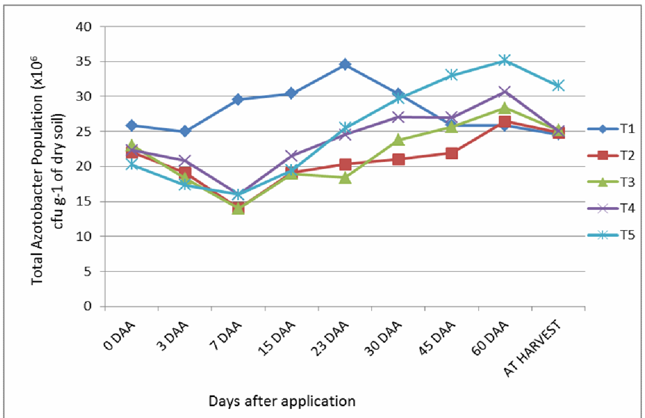

Fig. 3 : Population dynamics of Azotobacter (x10^6 cfu g-^{1} of dry soil) as affected by tillage and herbicide application in winter rice under conservation agriculture system

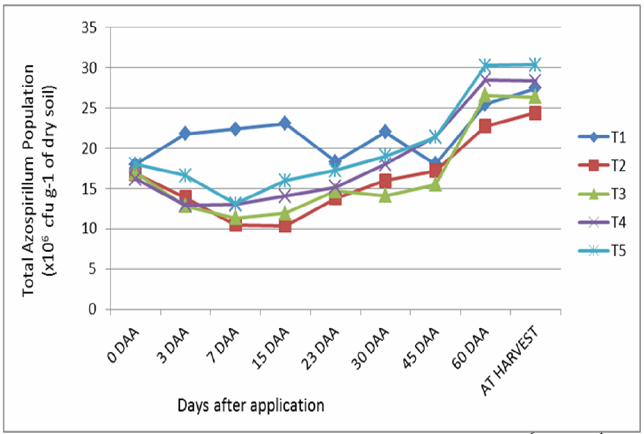

Fig. 4 : Population dynamics of Azospirillum(x10^6 cfu g^{-1} of dry soil) as affected by tillage and herbicide application in winter rice under conservation agriculture system

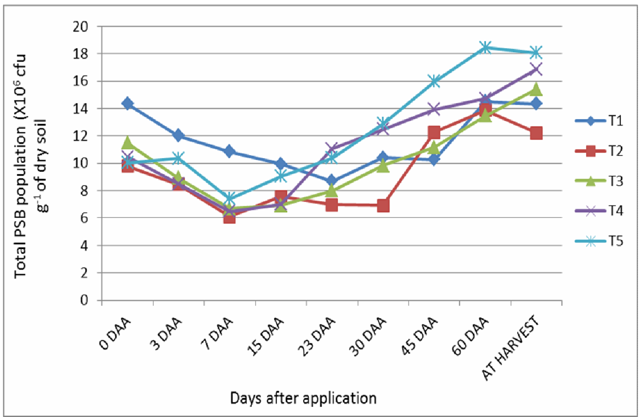

Fig. 5 : Population dynamics of PSB (x10^6 cfu g^{-1} of dry soil) as affected by tillage and herbicide application in winter rice under conservation agriculture system

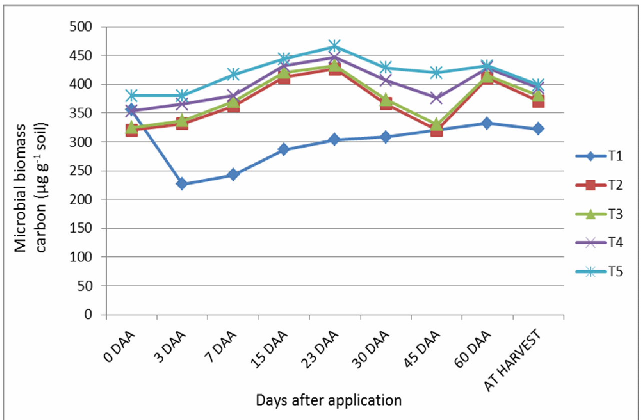

Fig. 6 : Microbial biomass carbon (µg g-^{1}soil) as affected by tillage and herbicide application in winter rice under conservation agriculture system

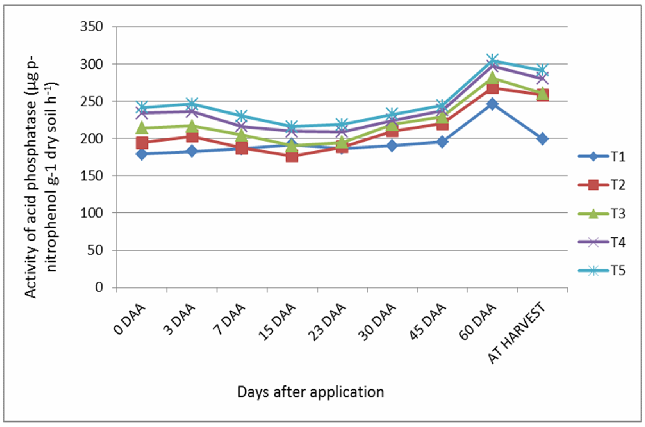

Fig. 7 : Activity of acid phosphatase enzyme (µg p-nitrophenol g-[1] dry soil h-[1]) as affected by tillage and herbicide application in winter rice under conservation agriculture system

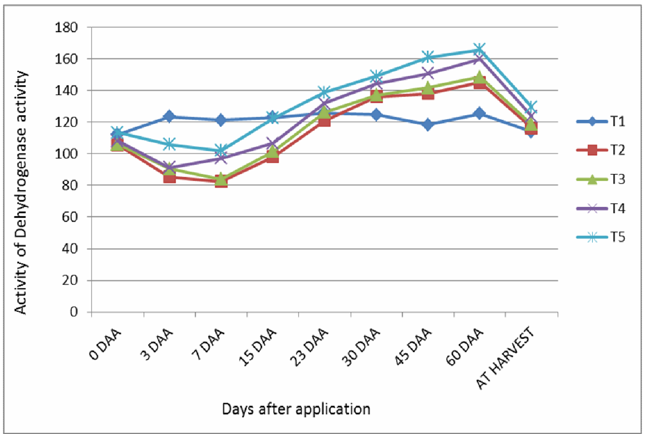

Fig. 8 : Activity of dehydrogenase enzyme (µgtpf g-[1] dry soil 7 days-[1]) in soil as affected by tillage and herbicide application in winter rice under conservation agriculture system

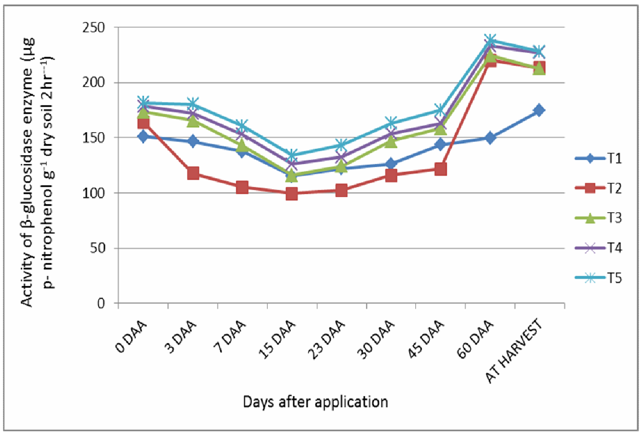

Fig. 9 : Activity of ß-glucosidase enzyme (µg p-nitrophenol g-[1] dry soil 2hr-[1]) as affected by tillage and herbicide application in winter rice under conservation agriculture system

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