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Research Article

ACETAMIPRID IMPACT ON UREASE AND PHOSPHATASE ACTVITIY IN SELECTED SOILS OF SOUTHERN KARNATAKA

B.C. Punitha[1], T. H. Hanumantharaju[2], *R. Jayaprakash[3] and V. M. Shilpashree[4]
[1]Krishi Vignan Kendra, College Of Fishery, Manglore, Karnataka
[2]Department Of Soil Science and Agricultural Chemistry, UAS, GKVK, Bengaluru.
[3]Dept Of Soil Science And Agricultural Chemistry, College Of Agriculture, UAS, Dharwad, Dharwad
.[4]NBSS and LUP, Hebbal, Banglore
** Author for Correspondence*

ABSTRACT
The impact of acetamiprid on selected soil enzymes urease and phosphatase was studied using surface layer (0-15 cm) samples of movement studies. The samples were taken at different interval (10, 20, 30, 45 and 60 days) under field capacity and the enzyme activities were analyzed for Kodagu, Bangalore and Chamarajanagar soils of Karnataka. Highest inhibition was found on 10th day incubation compared to initial activity in all the enzymes. The increased activity of all the three enzymes was observed from 20th day incubation and reached maximum in 60th day incubation. The highest urease activity was recorded in Bangalore soil (356.66 µg urea g^{-1} soil h^{-1}) and the lowest activity was recorded in Kodagu soil (341.81 µg urea g^{-1} soil h^{-1}). The highest activity of acid phosphatase activity was recorded in Kodagu soil of pH 5.32 (6.23 µg *p*-nitrophenol hydrolyzed g^{-1} soil h^{-1}) followed by Bangalore (4.86 µg *p*-nitrophenol hydrolyzed g^{-1} soil h^{-1}) and Chamarajanagar (4.63 µg *p*-nitrophenol hydrolyzed g^{-1} soil h^{-1}) respectively. The alkaline phosphatase activity in soil was also high in Chamarajanagar soil of pH 8.1 (7.05 p-NP hydrolyzed μg^{-1} soil h^{-1}), followed by Bangalore soil (5.29 µg p-NP hydrolyzed g^{-1} soil h^{-1}) and Kodagu soil (3.85 µg p-NP hydrolyzed g^{-1} soil h^{-1}).

Key Words: *Phosphatase, Acetamaprid and Urease*

INTRODUCTION
Crop protection is an integral part of agriculture with pesticide application as a major component. It is estimated that one third of the world's food crop is destroyed by the pests annually. It is well known that enzymes in soil contribute to the total biological activities in the soil environment because they are intimately involved in catalyzing reactions necessary for organic matter decomposition, nutrient cycling, energy transfer, and environmental quality (Dick, 1994). Despite the beneficial impacts of pesticides in improving and stabilizing agricultural productivity by control of obnoxious weeds, fungi and insects, these organic chemicals are known to contaminate soil ecosystem and pose threat to balance equilibrium among various groups of microorganisms in soil, which play an important role in mineralization, nitrification and phosphorus recycling are dependent much on the balanced equilibrium existing among various groups of organisms in the soil. The neonicotinoid insecticide acetamiprid (N-[(6– chloro-3- pyridyl) methyl]-N-cyano-N- methyl-acetamidine is a new-generation insecticide with ground and aerial application against aphids, leafhoppers, whiteflies, thrips, leaf beetles, leafminer moth, termites etc. It is commonly used on leafy vegetables, fruiting vegetables, cole crops, citrus fruits, pome fruits, grapes, and ornamental plants and flowers. Acetamiprid poses low risks to the environment relative to most other insecticides and its use would pose minimal risk to non target plants (USEPA, 2002). With this in view, a study on effect of acetamiprid on enzyme activity in selected soils of Karnataka was conducted.

MATERIALS AND METHODS
The movement study was conducted in three soils of Karnataka, viz., Kodagu, Bangalore and Chamarajanagar. Poly vinyl chloride (PVC) pipes of 2.5 mm thickness and 6 cm diameter were employed for the movement studies (Austin and Briggs, 1976; Lindstorm *et al.*, 1968). The 50 cm length PVC pipes were cut longitudinally into halves and were again joined with adhesive tapes. The filter paper (Whatman No. 40) was placed at the bottom and covered with muslin cloth joined by the adhesive tapes. The columns were rested over the beakers using steel stand.

Each PVC column was filled with soil (Kodagu - 2020 g, Bangalore - 1880 g and Chamarajanagar - 1640 g) by tapping upto 45 cm to attain bulk density as found in the field situation in respective areas. Water (466, 390 and 756 mL respectively for Kodagu, Bangalore and Chamarajanagar soils) was added to columns to maintain the field capacity of soil and also to attain packing of the soil in the column as in the field conditions. The weight of each column (soil and water) was recorded. The acetamiprid formulation 2.0 g (0.4 g a.i column^{-1}) was mixed with 25 g of each soil and applied at the top of each soil column. Water (5.8, 5.0 and 11.5 mL for Kodagu, Bangalore and Chamarajanagar soils) was added to attain the field capacity, the loss in weight of the column was replenished by adding appropriate quantity of water. The acetamiprid treated columns were drawn at 10, 20, 30, 45 and 60 days after treatment. The soil samples of (0-15 cm depth) in each column at different intervals were taken out separately and subjected to enzymatic analysis using standard procedures. Three replications were maintained.

Urease activity: The urease activity was estimated by adopting the methodology as given by Watts and Crisp (1954). In this method, the unhydrolyzed urea was complexed with a coloring agent and the color intensity which is directly proportional to the urea present was measured using spectrophotometer. The quantity of urea hydrolyzed was computed based on the initial and final urea present and expressed as µg urea hydrolyzed g^{-1} h^{-1} at 37 ± 2 ^{0}C.

Pre-incubated treated and untreated soils with or without acetamiprid were taken in a 50 mL Erlenmeyer flask and treated with 0.2 mL of toluene and 9 mL of THAM buffer. The flasks were swirled for a few seconds to mix the contents. Then, 1 mL of 0.2 M urea was added as substrate and the flasks were swirled for few seconds and placed in an incubator at 37 ± 2 ^{0}C. After 2 hrs, about 35 mL of KCl-Ag_2SO_4 solution was added. The flasks were swirled again and allowed to stand until the contents have cooled to room temperature. The volume was then made up to 50 mL with addition of KCl-Ag_2SO_4. A known quantity of this filtrate was used for measuring the unhydrolyzed urea by colorimetric method. Nessler's reagent was used to develop the color and the intensity was measured at 510 nm using UV-Vis spectrophotometer (Shimadzu UV-Mini 1240).

Acid and alkaline phosphatase activity: The phosphatase activity was determined by adopting the procedure given by Tabatabai and Bremer (1969). In this method, *p*-nitrophenol phosphate (*p*-NP) was used as a substrate and the hydrolyzed *p*-nitrophenol (*p*-NP) was measured. The *p*-nitrophenol phosphate (*p*-NP) produced in the soil was extracted and quantified to assess the phosphatase activity. The activity was expressed as µg of *p*- nitrophenol hydrolyzed g^{-1} soil h^{-1} at 37 ± 2^0 C.

The pre-incubated with or without acetamiprid treated soils, were added with 0.2 mL toluene, 4 mL of modified universal buffer of pH 6.5 for an acid phosphatase; and pH 11 for alkaline phosphatase and 1 mL of *p*-nitrophenol phosphate solution (*p*-NP) made in the same buffer. Tubes were swirled for a few seconds to mix the contents. Tubes were then stoppered with rubber cork and placed in an incubator at 37 ± 2 ^{0}C. After one hour of incubation, 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were added. The suspension was centrifuged at 3000 rpm, filtered and the intensity of yellow color of the supernatant was measured in UV-Vis spectrophotometer (Shimadzu UV-Mini 1240) at 440 nm wave length.

RESULTS AND DISCUSSION

The data on the effect of acetamiprid on urease and phosphatase activity in Kodagu, Bangalore and Chamarajanagar soils of Karnataka is shown in Table 1, 2 and Fig 1, 2. Variation has been observed in all the enzyme activity with respect to acetamiprid application.

The initial urease activity in untreated soils were 385.23 µg urea g^{-1} soil h^{-1} in Kodagu soil, 493.54 µg urea g^{-1} soil h^{-1} in Bangalore soil and 479.47 µg urea g^{-1} soil h^{-1} in Chamarajanagar soil. There was a low urease activity in 10th day of incubation of 65.11, 114.45 and 127.91 µg urea g^{-1} soil h^{-1} in Kodagu, Bangalore and Chamarajanagar soils respectively. From 20th day, there was stimulation in the activity in all the three soils. The trend was 110.90, and 341.81 µg urea g^{-1} soil h^{-1} in Kodagu soil, 169.51 and 356.66 µg urea g^{-1} soil h^{-1} in Bangalore and 177.71 2and 351.09 µg urea g^{-1} soil hr^{-1} in Chamarajanagar soil in 20, and 60 days of incubation respectively. The inhibition in the urease activity was recorded in the 10th day of incubation with acetamiprid application. However, the

inhibitory effect was reduced upon further incubation due to the reduction in the concentraton and degradation of applied insecticide. Yao Xiaohua *et al*. (2005) noticed variance of urease and catalase showed no distinct relationship with the acetamiprid application rate. Elliot (1989) concluded that fungicides application will affect the urea hydrolysis in soil in the initial stages.

The soils without acetamiprid showed initial acid phosphatase activity of 10.47, 8.77 and 6.49 µg *p*-nitrophenol hydrolyzed g^{-1} soil h^{-1} in Kodagu, Bangalore and Chamarajanagar soils respectively. The reduced acid phosphatase activity on 10^{th} day was 1.45, 1.09 and 0.72 µg *p*-nitrophenol hydrolyzed g^{-1} soil h^{-1} in Kodagu, Bangalore and Chamarajanagar soils respectively. With the advancement in time there was an increase in the acid phosphatase activity of 2.65 and 6.23 µg *p*- nitrophenol hydrolyzed g^{-1} soil h^{-1} in 20 and 60 days of incubation respectively in Kodagu soil. The same trend was followed in Bangalore and Chamarajanagar soil with an activity of 2.53 and 4.86 μg^{-1} *p*-nitrophenol hydrolyzed g^{-1} soil h^{-1} (Bangalore), 1.48 and 4.63 μg^{-1} *p*-nitrophenol hydrolyzed g^{-1} soil h^{-1} (Chamarajanagar) in 20 and 60 days of incubation respectively.

Similarly, the soils without acetamiprid showed initial alkaline phosphatase activity of 5.08, 7.09 and 10.92 µg *p*-nitrophenol hydrolyzed g^{-1} soil h^{-1} in Kodagu, Bangalore and Chamarajanagar soils respectively. There was reduced activity of alkaline phosphatase of 0.49, 1.49 and 1.56 µg *p*-nitrophenol hydrolyzed g^{-1} soil h^{-1} in Kodagu, Bangalore and Chamarajanagar soils respectively in the 10^{th} day incubation.. The extent of increase in alkaline phosphatase activity in Kodagu soil was 0.98 and 3.85 µg *p*-nitrophenol hydrolyzed g^{-1} soil h^{-1}, in Bangalore soil was 2.31 and 5.29 µg *p*-nitrophenol hydrolyzed g^{-1} soil h^{-1} whereas in Chamarajanagar soil 2.84 and 7.05 µg *p*-nitrophenol hydrolyzed g^{-1} soil h^{-1} in 20 and 60 days of incubation respectively.

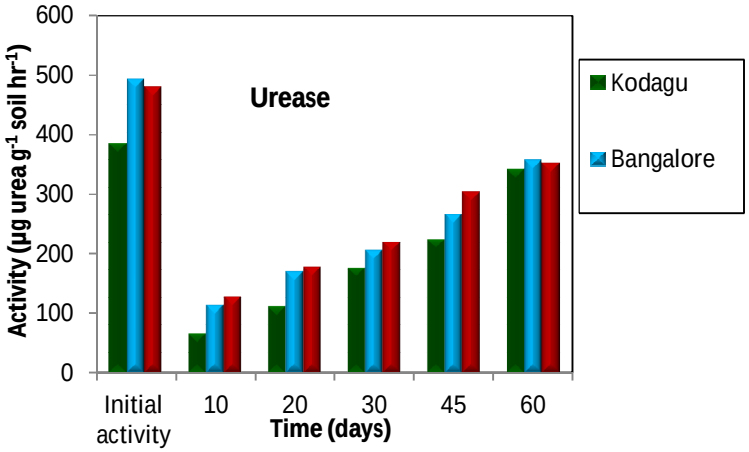

Figure 1: Effect of acetamiprid on dehydrogenase and urease activity in selected soils

Table 1: Effect of acetamiprid on soil dehydrogenase and urease activity in different soils

Soils	Urease activity in different days (µg urea g^{-1} soil hr^{-1})					
	Initial activity without acetamiprid	10	20	30	45	60
Kodagu	385.23	65.11	110.90	176.17	224.42	341.81
Bangalore	493.54	114.45	169.51	205.55	265.71	356.66
Chamarajanagar	479.47	127.91	177.71	220.40	303.92	351.09

Table 2: Effect of acetamiprid on soil phosphatase activity in different soils

Soils	Acid phosphatase activity in different days (µg *p*- nitrophenol hydrolyzed g^{-1} soil h^{-1})					
	Initial activity without acetamiprid	10	20	30	45	60
Kodagu	10.47	1.45	2.65	3.91	4.12	6.23
Bangalore	8.77	1.09	2.53	3.44	4.01	4.86
Chamarajanagar	6.49	0.72	1.48	2.95	3.50	4.63

Soils	Alkaline phosphatase activity in different days (µg *p*- nitrophenol hydrolyzed g^{-1} soil h^{-1})					
	Initial activity without acetamiprid	10	20	30	45	60
Kodagu	5.08	0.49	0.98	1.31	2.41	3.85
Bangalore	7.09	1.49	2.31	3.41	4.57	5.29
Chamarajanagar	10.92	1.56	2.84	3.65	4.81	7.05

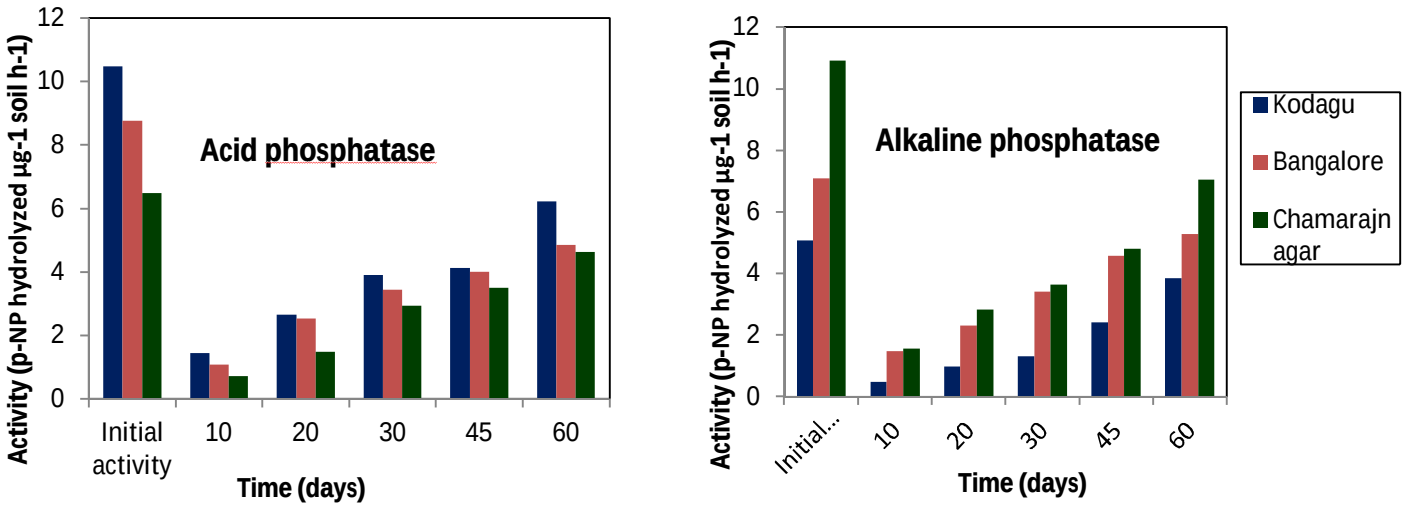

Figure 2: Effect of acetamiprid on acid and alkaline phosphatase activity in selected soils

Yao Xiaohua *et al.,* (2005) studied the effect of acetamiprid at higher concentration showed inhibitory effect on phosphatase activity. Initial reduction in the activity of phosphatase may be due to the lethal action of acetamiprid on P- solubilizers population which alter the membrane permeability of the microorganisms releasing Phosphatase enzymes. This statement is supported by Voets *et al*., (1974) in their study on the effect of atrazine on phosphatase activity in a forest soil who concluded that the inhibition of the phosphatase activity was upto 61.8 per cent. Similar results were also noticed by Krishnmurthy (1989) by employing fenvelerate and Kennedy *et al*. (1999) with carbofuran. Stimulation in phosphatase activity under the influence of paraquat, trifluralin, glyphosate and atrazine has been reported by Hazel and Greaves (1981).

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