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Combined inhibitory potential of Ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine on ureases activities.

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Abstract -Urea is used as a source of nitrogen, a key component of plant growth, to meet plant needs. Urease is a soil enzyme that degrades urea to carbon dioxide and ammonia and plays a role in the nitrogen and carbon cycles. However, its increased activity results in a decrease in the availability of nitrogen for plants. Different urease inhibitors are used to solve this problem, but they do not have significant potential individually. The present study investigated the combined inhibitory potential of Ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine in three different combination ratios (1:1, 0.25:0.75 and 0.75:0.25) and concentrations (0.1, 0.25 and 0.5 %). Inhibitors were applied to rice crops grown on soil samples collected from three different districts, Faisalabad, Gujranwala and Sheikhupura of Punjab. Results indicated that FSD and GUJ soil samples of combination 2 with a 0.5 % concentration showed the best potential while combination 1 with a 0.5 % concentration showed the best inhibitory effect in SKP soil samples. It is concluded that the combination of ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine has a significant potential to inhibit urease activity at lower concentrations.

Key Words- Ammonia, Ammonium thiosulphate, Fertilizers, Urea, Urease, Urease inhibitors, 2-chloro-6-(trichloromethyl) pyridine.

1 INTRODUCTION

Increasing the world's population and meeting their feed needs is a persistent challenge for developing countries [1]. Various fertilizers are used to overcome this problem and increase food production. Nitrogen fertilizers are one of them, as nitrogen is a mandatory component for the batter development of plant growth and is used in large quantities [2]. Urea, the most widely used N fertilizer, is low cost, highly soluble in water, has less corrosion capacity [3] and is five times more efficient than ammonium nitrate [4], [5].

Urease, a key enzyme for the nitrogen cycle, catalyzes urea hydrolysis within 24 to 48 h as Havlin et al. [4], Zaman et al. [6], Rochette et al. [7] and Dawar et al. [8] have suggested that urease enzyme activity increases with increasing amounts of surface residues. Ammonium, hydroxyl and carbonate ions are the end product of this hydrolysis, which results in an increase in the pH of the surrounding soil [6] and a loss of N resulting in a reduced availability of N for plants [9], i.e. a 30% loss from temperate climates and a 70% loss from tropical regions [10].

Stabilizers and slow release products are used to improve the efficiency of N fertilizers [11]. In stabilized N fertilizers, such stabilizers are combined with fertilizers that increase the availability of N in Urea-N or NH_4^+ soil [12]. Urease inhibitors (UIs) and nitrification inhibitors (NIs) are the two most commonly used N stabilizers. Use of urease inhibitors is one of the commonly used strategies to improve the performance of urea in agriculture and to reduce environmental pollution in the form of NH_3 [13], [14]. Urease inhibitors slow down the release of applied N by inhibiting the urease activity and slow the hydrolysis process up to 7 to 14 days [15], [16]. Various natural and synthetic urease inhibitors are present but only few are nontoxic [17].

The present study was designed to explore the combined inhibitory potential of the Ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine for urease activity with different combinations (ratio) and concentrations and also to investigate their effects on three different districts soil samples.

2 MATERIALS AND METHOD

The study was conducted at the Clinico-Medical Biochemistry Laboratory, Department of Biochemistry, University of Agriculture, Faisalabad, after the approval of the Director of Graduate Studies, University of Agriculture, Faisalabad. The study revealed the combined inhibitory effects of Ammonium thiosulphate (Sigma-Aldrich, UK) and 2-chloro-6-(trichloromethyl) pyridine; nitrapyrin (Sigma-Aldrich, UK) with three different combination ratios (1:1, 0.25:0.75 and 0.75:0.25) with blended urea. Their potential was investigated by monitoring urease activities in the rice crop. These inhibitors were applied at three different concentrations (0.1, 0.25 and 0.5%) to three different soil samples in the districts of Faisalabad, Gujranwala and Sheikhupura (FSD, GUJ, SKP).

2.1 Experimental design

The soil of the three districts (FSD, GUJ and SKP) of Punjab was collected. After soil collection and sowing of the rice crop, inhibitors with different combinations ratio and concentrations were applied with blended urea. For biochemical analysis soil samples were collected at different time periods and enzyme activity was measured.

2.2 Enzyme assay

For monitoring enzymatic activities of urease, method explained by May and Douglas [18] was used with some modifications. In order to determine enzymatic activity, in 1 gm soil samples 150 µL of toluene, 2 mL of citrate buffer (pH 6.7), 1 mL of urea (10% w / v) were added and the sample was incubated at 37 ° C for 3 hours. After incubation, 100 µL of incubated sample filtrate, 400 µL of sodium phenolate (12.5% w/v) and 300 µL of sodium hypochlorite were incubated for 15 min at room temperature and spectrophotometer absorption was recorded at 580 nm.

3 STATISTICAL ANALYSIS

Data was analyzed by two-way ANOVA using GraphPad prism [19].

4 RESULTS

Fig. 1 (a, b, c) shows the effect of ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine with three different combination ratios (1:1, 0.25:0.75, 0.75:0.25) and concentrations (0.1, 0.25 and 0.5%) on urease activities in rice crops grown in Faisalabad soil. Results showed that all combinations had maximum inhibitory potential until day 12, followed by an increase in urease activity. Fig. 1 (a, b, c) also shows that combination 2 (0.25:0.75) with a concentration of 0.5% gives maximum potential in Faisalabad soil samples.

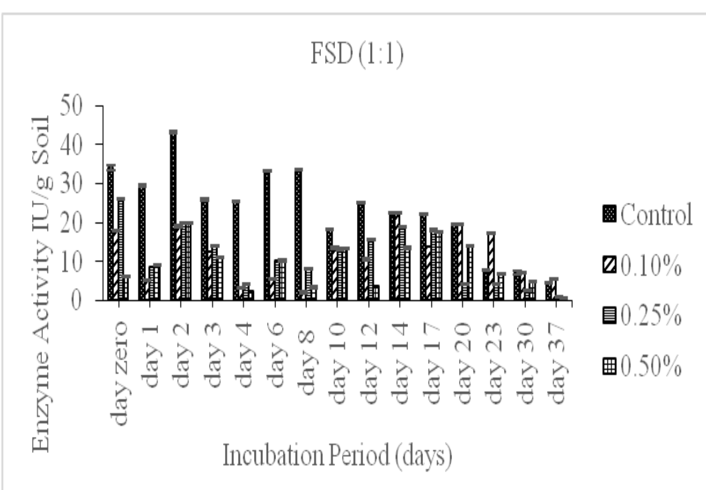

Fig. 1(a): Analysis of Ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine's inhibitory potential with combination ratio 1:1 with three different concentrations (0.1%, 0.25% & 0.5%) on FSD soil samples.

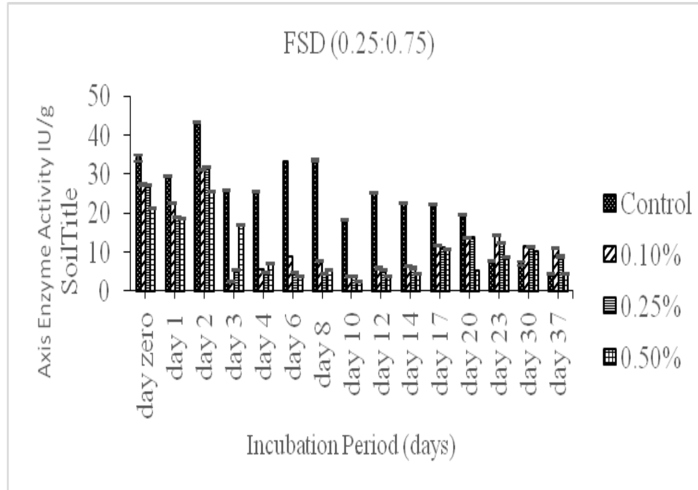

Fig. 1(b): Analysis of Ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine's inhibitory potential with combination ratio 0.25:0.75 with three different concentrations (0.1%, 0.25% & 0.5%) on FSD soil samples.

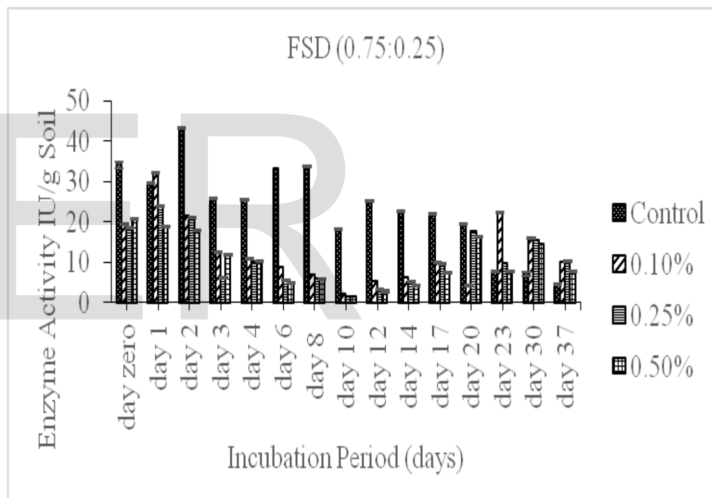

Fig. 1(c): Analysis of Ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine's inhibitory potential with combination ratio 0.75:0.25 with three different concentrations (0.1%, 0.25% & 0.5%) on FSD soil samples.

Fig. 2 (a, b, c) shows the inhibitory potential of ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine with three different combination ratios (1:1, 0.25:0.75, 0.75:0.25) and concentrations (0.1, 0.25 and 0.5%) on urease activity in rice cultivation in Gujranwala soil. In Gujranwala soil combination 1 (1:1) showed maximum potential until day 10, combination 2 (0.25:0.75) showed potential until day 12, while combination 3 showed potential until day 6 and then lost potential. Fig. 2 (a, b, c) also indicates that combination 2 (0.25:0.75) with a concentration of 0.5% showed a maximum inhibitory effect on Gujranwala soil samples.

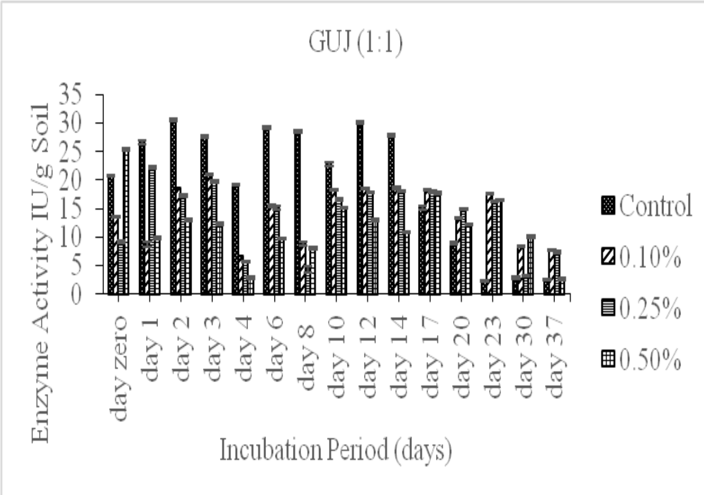

Fig. 2(a): Analysis of Ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine's inhibitory potential with combination ratio 1:1 with three different concentrations (0.1%, 0.25% & 0.5%) on GUJ soil samples.

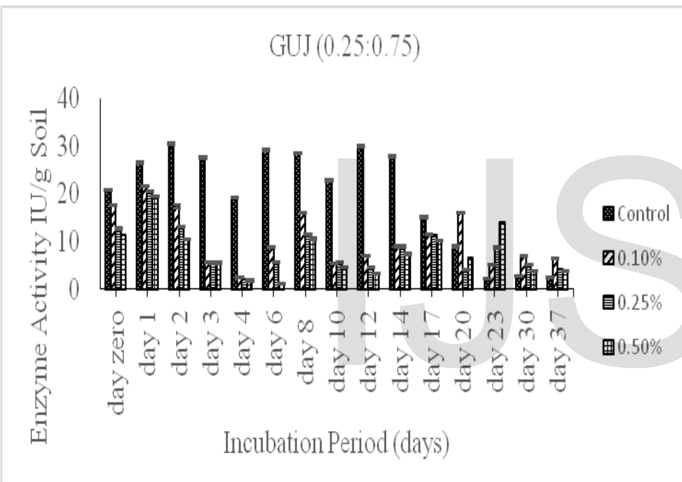

Fig. 2(b): Analysis of Ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine's inhibitory potential with combination ratio 0.25:0.75 with three different concentrations (0.1%, 0.25% & 0.5%) on GUJ soil samples.

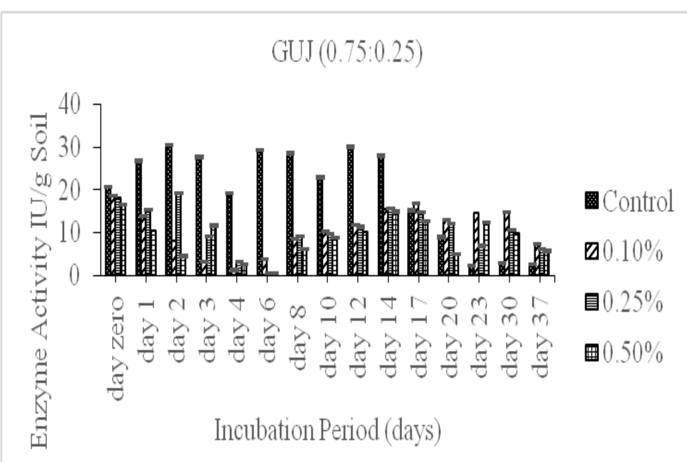

Fig. 2(c): Analysis of Ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine's inhibitory potential with combination ratio 0.75:0.25 with three different concentrations (0.1%, 0.25% & 0.5%) on GUJ soil samples.

Fig. 3 (a, b, c) demonstrates that when ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine were used as an urease inhibitor on Sheikhupura soil, combination 1 (1:1) showed its inhibitory potential until day 8 while combinations 2 and 3 (0.25:0.75 and 0.75:0.25) showed maximum inhibitory effects until day 6. Combination 1 (1:1) with a concentration of 0.5% showed a maximum potential for soil samples from Sheikhupura.

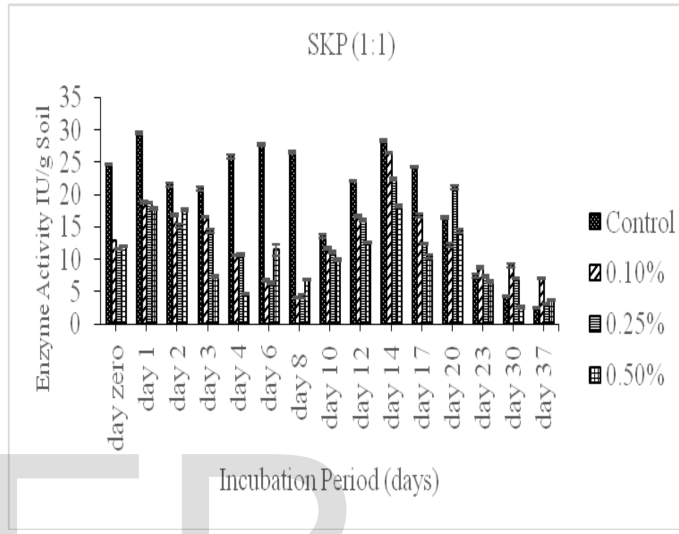

Fig. 3(a): Analysis of Ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine's inhibitory potential with combination ratio 1:1 with three different concentrations (0.1%, 0.25% & 0.5%) on SKP soil samples.

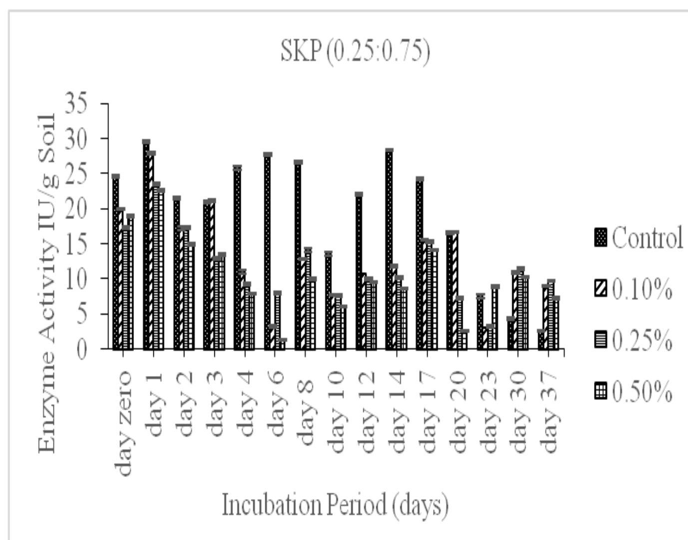

Fig. 3(b): Analysis of Ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine's inhibitory potential with combination ratio 0.25:0.75 with three different concentrations (0.1%, 0.25% & 0.5%) on SKP soil samples.

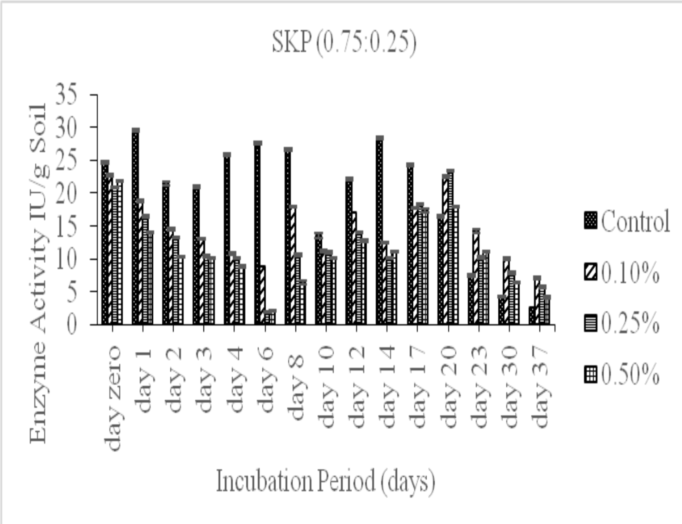

Fig. 3(c): Analysis of Ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine's inhibitory potential with combination ratio 0.75:0.25 with three different concentrations (0.1%, 0.25% & 0.5%) on SKP soil samples.

5 DISCUSSION

The current study was conducted to evaluate the combined inhibitory effects of ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine on urease activity. Urease hydrolyzes urea into CO2 and ammonia, volatilizes ammonia to the air and causes toxicity [20]. Various inhibitors are used individually to overcome this problem, but in this study two inhibitors with three different combinations and concentrations was used to investigate their efficacy. Activities of the urease enzyme were monitored from day 0 to 37 following the use of inhibitors and a significant change in urease activity was observed over time. Hagenkamp-Korth et al. [21] also found a change in urease activity over time. There was no significant decrease in urease activity in the first 2 to 3 days that inhibitors may act as substrates, or soil pH may affect inhibitor activity [22]. After day 3 to 14, urease activity decreased, followed by an increase in urease activity that may be due to unavailability of inhibitors [21].

In the first place, ammonium thiosulphate was used as a source of N and S fertilizer, but its inhibitory activity against urease was reported only when used in high concentrations. In our studies, in combination with 2-chloro-6-(trichloromethyl) pyridine, it was found to be involved in inhibiting urease activity and nitrification of urea at lower concentrations. Morgan et al. [23] found a significant decrease in urease activity for only 4-6 days when ATS was applied at high concentrations (2500 or 5000µg g-1) and lost its inhibition potential after day 10. Nitrapyrin (2-chloro-6-(trichloromethyl) pyridine) acts individually as a nitrification inhibitor and inhibits ammonium oxidation [24]. However, it is also found to be involved in inhibition of the urease enzyme in the nitrifying bacteria (Nitrosomonas) [25] and to prevent hydrolytic action on urea [15]. Patel et al. [26] observed a decrease in NO3-concentration and pH gradient on the 28th day following the use of nitrapyrin along with urea. In our studies, nitrapyrin and ammonium thiosulphate act as a urease inhibitor. Their combined effect has not yet been studied.

6 CONCLUSIONS

It is well known that only 30%-50% of applied N is used for crops. The unreported percentage represents N losses due to volatilization or leaching processes. N losses can therefore be deleterious for off-site ecosystems, especially given that the global use of N fertilizer has increased by about tenfold in the last half century and is expected to increase further by 2050, unless there is a significant increase in the efficiency of fertilizers. ATS show inhibition potential when used at a soil rates of 2500 mg·kg-1 soil. Nitrapyrin alone showed nitrification inhibition but application in combination with ATS reduced the activity of urease. This study indicates that urea was used in a blended form with ATS and Nitrapyrin to minimize N losses and increase crop growth. It is concluded that the combined inhibitors (ammonium thiosulphate and 2-chloro-6-(trichloromethyl) pyridine) can exhibit significant inhibition of urease activity even at lower concentrations after studying its effects on urea hydrolysis.

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