Adverse Effect of Buprofezin and Acephate on Enzymatic Activities in NPK Amended and Unamended Cotton Soils

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Abstract A thiadiazine compound, buprofezin, and an organophosphorous insecticide, acephate, singly, were assessed for their nontarget effects on activities of proteases, urease and acid phosphatase in NPK (nitrogen, phosphorous and potassium)-fertilizer amended and unamended cotton soils. The studied enzyme activities were adversely affected by the insecticides above 5 or 7.5 µg g⁻¹ concentrations. Additionally, activities were declined after three applications of insecticides. Therefore, the results of the present study clearly indicate that the soil application of buprofezin or acephate at higher rates and repeated applications greatly affected the activities of studied enzymes in unamended or NPK-amended soils.

Keywords Buprofezin, acephate, nontarget effects, soil enzymes, nutrient amendments

1. Introduction

In modern agriculture, it has become a common trend to apply different groups of pesticides, either simultaneously or in succession, for effective control of a variety of pests. Pesticide applications at recommended rates have little or no effect on enzyme activity in soils [1-5]. But, soil enzyme activities [6-9] and individual microorganisms [10] are usually affected when pesticides are applied to soil at higher than recommended rates over long periods. In contrast, soil application of insecticides stimulated soil microbiological activities [11]. Thus, monitoring of the pedosphere using the methods based on enzymatic tests enables a complex assessment of the changes in the soil environment under the influence of anthropogenic factors [12].

There is a serious concern about the vast economic damage caused to cotton by many insect pests such as *Amrasca biguttula* (Cotton Jassid), *Bemisia tabaci* (Cotton Whitefly), *Aphis gossypii* (Cotton Aphid), and *Thrips tabaci* (Cotton Thrips), because cotton is an important fiber yielding crop of global importance which is grown in tropical and subtropical regions of more than 80 countries the world over. For the effective control of these insect pests on cotton, several insecticides are used on need basis. In particular, the two insecticides, buprofezin (Applaud®) and acephate (Hythene®) are widely used in the recent years to combat major insect pests of cotton. More importantly, due to high hydrophobicity (log P = 4.31), buprofezin is easily adsorbed onto soil particles; strong soil adsorption is sometimes contributed to persistence of the compound in soils. Additionally, for improving soil fertility, the agricultural soils are generally amended with NPK-fertilizers. The average per-hectare use of NPK fertilizers on cotton is 120-80-60 kg ha⁻¹, respectively. In this direction, several studies were conducted to evaluate the effects many pesticides on soil populations of bacteria, fungi and actinomycetes, and soil enzymes after single or repeated applications [5, 13-16]. However, virtually no information is available in the literature on nontarget effects of the two widely used insecticides, buprofezin and acephate, towards microbial activities in soil. In the present investigation, an attempt has, therefore, been made to assess the impact of single or repeated applications of buprofezin and acephate on proteases, urease and acid phosphatase activities in unamended and NPK-fertilizer-amended soils.

2. Materials and Methods

2.1. Soil Collection

Soils with a known history of insecticide (buprofezin or acephate) use, were collected from fields (inherently very fertile) under cultivation of cotton at Nandyal, a semi-arid region of Andhra Pradesh, India, to a depth of 12 cm.

2.2. Insecticides and Fertilizers Selected in the Present Study

Two insecticides, buprofezin (2-tert-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one) and acephate (O,S-dimethyl acetyl phosphoramidothioate), were selected for the present investigation in view of their extensive and
intensive usage in Indian agriculture, in general, and Nandyal division, in particular, for control of major insect pests on cotton. Stock solutions of commercial formulations of buprofezin (Applaud, 25% SC, Rallis India Limited, Mumbai) and acephate (Hythene, 75% SP, Hyderabad Chemicals Limited, Hyderabad) were prepared in sterile distilled water for amendment to soil samples. The mineral fertilizer urea, calcium perphosphate and potassium were used at a rate of 120 kg h$^{-1}$ N, 80 kg h$^{-1}$ P$_2$O$_5$ and 60 kg h$^{-1}$ K$_2$O.

2.3. Effect of Selected Insecticides on Soil Enzyme Activities

Aliquots (0.5 mL) from stock solutions of the insecticides, prepared in water, were applied to the surface of 5 g soil samples contained in test tubes (25 × 200 mm) as followed by Lethbridge and Burns [17]. The final concentrations (on w/w basis) of each insecticide included 2.5, 5.0, 7.5 and 10.0 μg g$^{-1}$ soil, which correspond to 0.25, 0.5, 0.75 and 1 kg ha$^{-1}$, respectively [18]. These concentrations were chosen because of the fact that the field application dose of the selected insecticides range from ~0.3 to 0.6 kg ha$^{-1}$. Besides insecticide treatment, soil samples in one set were amended with N-P-K fertilizer at a rate of 120-80-60 kg ha$^{-1}$, respectively. But, soil samples in second set were treated with insecticides only. The soil samples receiving only 0.5 mL water served as controls. All the treatments including controls were maintained at 60% water-holding capacity, and incubated at 28 ± 4 °C. After 3 days of incubation (i.e., single application), triplicate soil samples were withdrawn for the assay of proteases [19], urease [20] and acid phosphatase [21] activities. Likewise, enzyme activities in soil samples were also determined after two (15 days incubation) and three (30 days incubation) repeated applications of insecticides separately.

2.4. Assay of Selected Soil Enzymes

2.4.1. Proteases

Activities of proteases in soil samples were determined by the method of Speir and Ross [19]. Soil samples (5 g) were incubated for 24 h at 30 °C with 10 mL of 0.1 M tris [2-amino-2[(hydroxyl methylmethyl)-propane-1:3-diol; pH 7.5] containing sodium caseinate (2% w/v). Four milliliters of aqueous solution of trichloro acetic acid was then added, and the mixture was centrifuged. A suitable aliquot of the supernatant was treated with 3 mL of enzyme buffer (100 mM sodium acetate of pH 5.5 containing 10 mM magnesium chloride) and 5 mL of 0.03 M $p$-nitrophenyl phosphate. The mixture was kept on ice for 20 min and centrifuged. A suitable aliquot of the supernatant was treated with 3 mL of enzyme buffer, and was kept on ice for 20 min. Then, 1 mL of aqueous solution of 5 mM calcium chloride and 4 mL of 0.5 M sodium hydroxide were added to the above mixture. The yellow color was read at 405 nm in an Elico digital spectrophotometer. $p$-nitrophenol (PNP) was used as a standard, and the acid phosphatase activity was expressed as milligrams of PNP released per g of soil per 30 min (mg PNP g$^{-1}$ 30 min$^{-1}$).

2.4.3. Acid Phosphatase

Acid phosphatase activity in soils was determined following the method of Tabatabai and Bremner [21]. Soil samples (5 g) were incubated at 37 °C for 30 min with 15 mL of enzyme buffer (100 mM sodium acetate of pH 5.5 containing 10 mM magnesium chloride) and 5 mL of 0.03 M $p$-nitrophenyl phosphate. The mixture was kept on ice for 20 min and centrifuged. A suitable aliquot of the supernatant was treated with 3 mL of enzyme buffer, and was kept on ice for 20 min. Then, 1 mL of aqueous solution of 5 mM calcium chloride and 4 mL of 0.5 M sodium hydroxide were added to the above mixture. The yellow color was read at 405 nm in an Elico digital spectrophotometer. $p$-nitrophenol (PNP) was used as a standard, and the acid phosphatase activity was expressed as milligrams of PNP released per g of soil per 30 m (mg PNP g$^{-1}$ 30 m$^{-1}$).

3. Results and Discussion

3.1. Nontarget Effects of Buprofezin and Acephate on Proteases

The data on protease activity in soil as influenced by buprofezin and acephate are shown in figures 1a and c, respectively. Concentration up to 7.5 μg of buprofezin g$^{-1}$ soil was either nontoxic or stimulatory to protease activity in soil after single application (Figure 1a). Furthermore, highest activity of the enzyme was noticed in soil that has received buprofezin at 5 μg g$^{-1}$ soil. However, activity was rapidly declined at the highest concentration of buprofezin used (10 μg g$^{-1}$). Interestingly, the stimulatory effect of buprofezin continued even after two repeated applications. But, the activity was slightly declined after three applications of the insecticide to soil. Thus, activities were increased over time for some extent, but later declined. On the other hand, the response of proteases to acephate was very similar to that of buprofezin (Figure 1c). Acephate, even at 7.5 μg g$^{-1}$ concentration, could stimulate (51%) protease activity in soil after single application. Similar effects have been noticed in many studies conducted earlier. According to Rangaswamy
et al. (1994), monocrotophos, cypermethrin and fenvalerate even at 10 kg ha\(^{-1}\) were stimulatory to protease activity; however, but the enzyme activity was negatively affected by the three insecticides at 12.5 kg ha\(^{-1}\). Among studies in which different xenobiotics were individually tested, those that generally caused the greatest inhibition in proteases activity in soil were fenthion [22], cartap hydrochloride [23], metsulfuron-methyl [24], manezeb [25] etc. Contrary to the results obtained in the present study, phenmedipham even at 10 mg kg\(^{-1}\) did not affect protease activity in soil [26]. Thus, the available literature and data of the present investigation clearly suggest that proteases respond negatively to most pesticides at concentrations above the field application rates.

The data on behavior of protease in soil that has received NPK-fertilizer upon insecticide treatment are shown in figures 1b and c. Single application of buprofezin, up to 7.5 µg g\(^{-1}\), was either nontoxic or stimulatory (17%) to protease in amended soil. The insecticide only at 10 µg g\(^{-1}\) soil could inhibit (27%) the activity of protease (Figure 1b). As with unamended soil, two applications of buprofezin also stimulated the protease activity in NPK-fertilizer amended soil. However, the enzyme activity markedly declined after three repeated applications. On the other hand, acephate could also influence the proteases similar to buprofezin (Figure 1d). Acephate, up to 7.5 µg g\(^{-1}\) and two repeated applications, could stimulate the protease activity. However, upon three repeated applications at 10 µg g\(^{-1}\) soil, the enzyme activity was adversely affected in amended soil. Renella et al. [27] performed long-term field experiments in which soil, contaminated with Mn-Zn- or Cd-Ni-rich sludge, was incorporated at two different rates. Protease activity was generally more pronounced in all the sludge-amended soils than in control soils.
3.2. Nontarget Effects of Buprofezin and Acephate on Urease

Soil urease, a vital enzyme in the nitrogen turnover, has been studied under the impact of single or repeated applications of buprofezin or acephate by determining the ammonical nitrogen released from urea, and the results are presented in figure 2. Buprofezin, at concentrations ranging from 2.5 to 7.5 µg g\(^{-1}\) soil was either stimulatory or nontoxic to urease (Figure 2a). Surprisingly, even 7.5 µg buprofezin g\(^{-1}\) soil did not affect urease in soil. However, optimum activity (40%) was recorded in soil that has received 5 µg g\(^{-1}\) of the tested insecticide. The activity was stimulated (25-45%) even after two applications of buprofezin to the soil. However, urease activity decreased by 30-50% after three repeated applications of the insecticide to soil. But, acephate was much more toxic to urease than buprofezin (Figure 2c). Though the activity was stimulated (7-53%) at 2.5 and 5 µg g\(^{-1}\), it was affected adversely (20-40%) at 7.5 and 10 µg acephate g\(^{-1}\) soil. Again, activity was stimulated after two repeated applications of acephate, but was inhibited after three applications of acephate to soil. Thus, two insecticides did stimulate the enzyme activity with time up to some extent, later did inhibit the enzyme activities. Available evidences also suggest that ureolytic microorganisms isolated from the soil were inhibited by the OP pesticides to a greater or lesser extent, but the development of tolerance was common [17, 28]. Even 100 mg fenamiphos kg\(^{-1}\) soil was not toxic to urease [29]. Small increases were measured for ureases in soils treated with glyphosate and paraquat [8] and chlorimuron-ethyl and Furadan [30]. Nevertheless, long-term treatment and higher rates of pesticides did adversely affect the urease activity in soils [6, 24, 31-34].

The response of urease was quite interesting and surprising in soil that has received both insecticides and NPK amendments. Urease activity was significantly enhanced (7-77%) by the single application of buprofezin at all four concentrations tested (2.5-10 µg g\(^{-1}\)) in NPK-amended soil (Figure 2b). Most pronounced activity was noticed in soil that was treated with the insecticide at 5 µg g\(^{-1}\) soil. Furthermore, repeated applications of selected insecticides to soil amended with NPK-fertilizer caused reduction (70-90%) in urease activity greatly. In fact, urease activity was decreased by more than 50% after two applications over the activity recorded after single application. Again, the lowest activity was noticed after three repeated applications. In addition, soil urease did respond to acephate very similar to that of buprofezin in amended soil (Figure 2d). These results are similar to the findings reported in the literature. Ingram et al. [35] applied diazinon and imidacloprid to lawns for insect control simultaneously with nitrogenous fertilizer such as urea and observed that diazinon briefly, but significantly, reduced urease activity in blue grass soil. Co-application of imidacloprid and urea appeared to increase urease activity in soil and sod. Likewise, soil urease activity was adversely affected in the presence of high level of mineral fertilization ([36], Mn-Zn- or Cd-Ni-rich sludge [27], Ag and Hg [37]. Also, decomposition rate of pesticides has been greatly declined by nitrogen and phosphorous [38] and Roundup Ultra [39] in soil. Studies by many authors [40-42] indicated that an increase in the concentration of hydrogen ions in soil has a negative effect on its enzyme activity.

3.3. Nontarget Effects of Buprofezin and Acephate on Acid Phosphatase

The nontarget effects of buprofezin and acephate on acid phosphatase activity in soils were determined by the release of PNP from p-nitrophenyl phosphate and results are shown in figure 3. It is evident from the result that after single application, buprofezin stimulated (29-186%) the enzyme activity at concentration ranging from 2.5 to 7.5 µg g\(^{-1}\) soils, with an optimum activity at 5 µg g\(^{-1}\) soil (Figure 3a). However, the insecticide is toxic (29% reduction in activity) to the enzyme at 10 µg g\(^{-1}\) soil. Also, activity of the enzyme was stimulated (11-65%) even after two applications of buprofezin to soil. But, after three applications, the enzyme activity declined (64-100%) drastically. Like other two enzymes, activities of acid phosphatase were also decreased with time. Similarly, acephate at field application rate was nontoxic or stimulatory to acid phosphatase activity in soil (Figure 3c). The accumulation of PNP was more striking (43% stimulation) at the 5 µg g\(^{-1}\) soil level. The highest level of 10 µg g\(^{-1}\) soil was inhibitory (29%) to the enzyme. Furthermore, activity was stimulated after two applications of acephate to the soil, but was declined after three repeated applications.

The extent of acid phosphatase activity in NPK-amended soil samples under the impact of buprofezin and acephate was also determined and the results were very similar to those observed with soil samples that not received the fertilizers (Figure 3b and d). Both the insecticides profusely stimulated (up to 233%) the enzyme activity to an optimal level at 5 µg g\(^{-1}\) soil. Nonetheless, the enzyme activity was inhibited greatly at higher rates of the two insecticides and, particularly, after three repeated applications. Similarly, Omar and Abdel-Sater [43] reported that brominal and selecron promoted acid phosphatase activity in soil at field application rates after some incubation periods, but the enzyme activity was less at the higher application doses.

Surprisingly, Yao et al. [44] found strong negative influence on phosphatase activity in soil treated with a new pesticide, acetamiprid, applied at normal field concentration (0.5 mg kg\(^{-1}\)) and at high concentrations (5 and 50 mg kg\(^{-1}\)). Furthermore, according to Sikora et al.[45], over 40% of the insecticide treated fence row soils which had no previous exposure to insecticides showed higher acid phosphatase activity. Likewise, a general inhibitory effect was observed for phosphatase in the presence glyphosate [8], propiconazole [46], chlorothalonil [28], chlorpyrifos [47], Ridomil Gold Plus copper [48], atrazine [6], a fungicide Swing Top 183 SC [33], benomyl and captan [49], fluchloralin, methabenzthiazuron, metoxuron, 2,4-D and isoproturon [50]. However, either fenamiphos [51] or flopet and captanf [52] did not influence the phosphatase activity in soil. In contrast, stimulation in phosphatase activity under the influence of paraquat, trifluralin, glyphosate and atrazine has been reported by Hazel and Greaves [53].
4. Conclusion

The results of the present investigation concerned with the insecticide–microflora interactions in cotton soils clearly reveal that two and three repeated applications of the insecticides caused adverse effects on measured enzymatic activities in NPK-amended and unamended soils. Also, soil application of buprofezin or acephate at higher rates greatly affected the activities of several enzymes in unamended or NPK-amended soils.

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