1. INTRODUCTION

Soil is a dynamic system that appears to be in a biological equilibrium. However, this equilibrium is precarious since any disturbance in the soil ecosystem has the potential to change the microbial populations and activities. To maintain soil health, it is important to avoid contamination of soils by agrochemicals that have deleterious impact on soil organisms and processes. In reality, soil microorganisms have primary role in the environment through degradation of plant and animal residues. They play a vital role in many soil biological processes, including nitrogen transformation, organic matter decomposition, and nutrient release and availability (Sparling, 1985; Lee and Pankhurst, 1992). Activities of microorganisms in soil are thus essential to the global cycling of nutrients. Since majority of biochemical transformations in soil result from microbial activity, any compound that alters the number or activity of microbes could affect soil biochemical process and ultimately soil fertility and plant growth (Cohen et al., 1984). In general, harmful effects are generally related to the concentrations of pesticide applied, and correct dosage is often the key to successful use without hazardous side effects.

Excessive use of natural resources and large-scale synthesis of xenobiotic compounds have generated a number of environmental problems such as contamination of air, water and terrestrial ecosystems, harmful effects on different biota, and disruption of biogeochemical cycling. It is apparent that crop protection by pesticides results in pesticide residues in the soil, which is ultimately the sink of all these anthropogenic compounds. Subsequently, accumulations of these chemicals occur as a result of deliberate use in agriculture. It is well documented that the agricultural chemicals especially pesticides are perhaps the largest group of poisonous substances being disseminated throughout our environment (El-Aswad et al., 2001).
The intensified agriculture in developing countries has, therefore, dictated the increasing use of agrochemicals to meet growing food demands. Numerous reports have indicated that at the present time, the most widely used pesticides belong to the organophosphorus (OP) group. Historically, during the last 60 years, approximately 150 different OP chemicals have been used to protect crops, livestock, and human health (Casida and Quistad, 2004). Thus, the use of pesticides has become an integral and economically essential part of modern agriculture. Pesticides may enter the soil either directly or indirectly (Burns, 1975). Crop protection preparations, especially when applied in excess of recommended amounts may cause a variety of negative environmental changes, reflected by yield decrease and inhibition of soil biological activity (Wyszkowska and Kucharski, 2004). These compounds are among the most common chemical classes used in crop and livestock protection and account for an estimated one-third of worldwide insecticide scales (Singh and Walker, 2006). As pesticides are designed to be biologically active, their continuous use might affect soil microflora either by changing their properties or their number which may lead to impairment of soil fertility. Ultimately, the primary role of agriculture is to produce a reliable supply of wholesome food to feed the burgeoning world population, safely and without adverse effects on the environment.

More broadly, the term “soil microbial activity” implies to the overall metabolic activity of all microorganisms inhabiting soil, including bacteria, fungi, protozoa, algae and microfauna (Nannipieri et al., 1990). In recent years, the role of soil microorganisms in affecting the persistence of agricultural pesticides has been the subject of two areas of study. First is the capacity for rapid elimination of highly persistent or toxic chemicals. And, second is the reduced pesticide efficacy attributed to enhanced biodegradation, particularly of chemicals applied under a continuous
cropping program. It has long been known that the abundance and activities of microorganisms reveal the degree of soil development (Powlson et al., 1987). About 90% of the energy in the soil environment flows through microorganisms (Heal and Lean, 1975). Dilly and Munch (1998) opined that the biomass-specific respiration and metabolic quotient which combines microbial activity and population is a more sensitive indication of soil pollution that is either activity or population measurements alone. Recent evidences (Ronnpagel et al., 1998; Brohon et al., 2001) have suggested the use of exogenous microorganisms or enzymes as bioassays activity measurement needs careful consideration of the season and prevailing weather conditions, because climatic factors often determine in situ variation of soil microbial activities (Insam, 1990).

The role of microbial activity in the development and functioning of soil ecosystem is, therefore, inevitable, and changes in soil microbial activity may be an indicative of, and extremely sensitive to changes in the soil health (Pankhurst et al., 1995). The essential point to remember about enzymes is that they are frequently referred to as markers of soil environment purity (Aon and Colaneri, 2001), because such activities may reflect the potential capacity of a soil to perform certain biological transformations important to soil fertility. It is now known that monitoring of the pedosphere using the methods based on enzymatic tests enables a complex assessment of changes in the soil environment under the influence of anthropogenic factors (Taylor et al., 2002).

Once present in the soil, pesticides may influence the growth and activity of microorganisms in soil essential for maintaining the soil fertility (Wainwright, 1978; Somerville and Greaves, 1987). There is good evidence that the side effect of pesticides on soil microflora could be investigated by studies of microbial respiration
and soil enzymes (Greaves and Malkones, 1980). As already indicated, enzymes participate in numerous biochemical processes occurring in the soil; as a result they are sensitive to all environmental changes caused by natural and anthropogenic factors (Trasar-Capeda et al., 2000). Also, chemical and biological methods are used to assess the effect of pesticide applications in a cotton agroecosystem under field conditions. Pesticides are often applied several times during one crop season and a part always reaches the soil, eventually affects soil enzyme activities. 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 (Venkateswarlu, 1993). Hence, soil enzyme activities are useful integrative indicators of soil health and have been used widely to assess the effects of management practices on soil biological functioning (Dick, 1997). Generally speaking, pesticide applications at recommended rates have little or no effect on enzyme activity in soils (Ladd, 1985; Schaffer, 1993; Nannipieri, 1994; Dick, 1997). In contrast, soil enzyme activities were shown to be significantly affected when pesticides are applied to soil at higher than recommended rates over long periods (Voets et al., 1974; Rai, 1992; Sannino and Gianfreda, 2001; Megharaj, 2002).

For some years now, measurements of microbial biomass and various enzyme systems have been widely used to diagnose the soil state and to describe the effect of different influences of pollutants, agricultural management, and land use. It is also worth considering that enzymatic activities as caused by soil microbial activities are sensitive indicators to detect changes occurring in soils (Gonzalez et al., 2007). Possible indicator value of the microbial parameters for environmental stress, in general, was investigated through microbial processes including soil enzyme activities (Bandick and Dick, 1999; Tscherko and Kandeler, 1999). Certainly, enzyme activity in
soil is regulated by pH and microbial biomass (Dick et al., 1988), which is correlated to soil organic matter, and soil moisture content (Harrison, 1983) as well as to soil compaction (Karaca et al., 2000). However, soil enzyme activity is variable in time and limited by available substrate supply (Tateno, 1988; Degens, 1998), and may provide useful linkage between microbial community composition and carbon processing (Waldrop et al., 2000). Thus, information of soil enzyme activities used to determine soil microbiological characteristics are very important for soil quality and healthy. One of the most remarkable features of soil microorganisms is that they collectively decompose various xenobiotic compounds and return elements to the mineral state for utilization by plants. They also play important roles in the dissipation of xenobiotic pesticides in the soil.

In fact, organophosphates have been extensively applied as alternatives to organochlorine compounds which are long-term persistent and highly toxic. In general, OP compounds rapidly undergo degradation by soil microorganisms, so they do not persist in the environment. However, repeated applications of degradable organophosphates occasionally cause a significant reduction of their pesticidal effect. This phenomenon which results from microbial adaptation to pesticide degradation, called enhanced biodegradation, has often been observed in degradable pesticides such as organophosphates and carbamates. Most enhanced degradation in the field occurs after pesticide applications for two or more consecutive years (Racke and Coats, 1990). Many of these insecticides are phosphorous thioesters, with limited aqueous solubility, in which the leaving group is attached to the phosphorous center via a sulfur atom, such as malathion, dementon-s, acephate, azinophos-ethyl, and phosalone.

Native environmental biodegradation has been observed to have the potential to provide an effective means of detoxifying modest levels of environmental pollutants.
(Landis and Frank, 1991; Harkness et al., 1993). For example, soil samples selected from contaminated environments have been reported to degrade various OP-thioates in the field following repeated application of these insecticides (Chapalamadugu and Chaudhry, 1992). In recent years, OP compounds have been the most widely used group of insecticides in India which include acephate, endosulfan, phosphomidon, and dimethoate. Approximately 35 tones of OP-active ingredients comprising about 4 distinct compounds have been used annually in Nandyal region, Andhra Pradesh, India for many years. On the other hand, buprofezin, a thiadiazine compound, is highly effective in controlling harmful insect pests including rice brown planthopper (*Nilaparvata lugens*) and greenhouse whitefly (*Trialeurodes vaporariorum*). This insecticide is being used at a level > 30,000 L y⁻¹ in Nandyal division alone on various crops including the two major crops, rice and cotton. However, little is known about the interaction between these widely used two insecticides, buprofezin and acephate, and microorganisms in agricultural soils. Also, a perusal of the literature on nontarget effects of pesticides indicates that the data available are predominantly concerned with individual compounds and single application, and in fact, little data are available for pesticide combinations as well as repeated applications of individual insecticides in fertilizer (NPK)-amended and unamended soils. In the present investigation, an attempt has, therefore, been made

- to assess the impact of single or repeated (two or three) applications of buprofezin or acephate on activities of soil enzymes implicated in carbon (cellulases, amylase, invertase), nitrogen (proteases, urease) and phosphorus (acid and alkaline phosphatases) microbial cyclings,
- to assess the influence of NPK fertilizer amendment to the soil, a common agricultural practice, on activities of soil enzymes,
➢ to assess the impact of combination of buprofezin and acephate and amendments of NPK fertilizers on activities of the selected soil enzymes,
➢ to isolate soil bacteria capable of utilizing buprofezin or acephate as sole source of carbon and nitrogen following selective enrichment, and
➢ to determine the growth of isolated soil bacteria in minimal salts liquid medium containing different carbon and nitrogen sources supplemented with buprofezin or acephate.