PERSISTENCE OF SELECTIVE HERBICIDES IN RICE-RICE SYSTEM

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "Persistence of selective herbicides in rice-rice system" is a bonafide record of research work done by me during the course of research and that this thesis has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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We, the undersigned members of the Advisory Committee of **Mr. M. Muthu Kannan**, a candidate for the degree of Master of Science in Agriculture with major in Soil Science and Agricultural Chemistry agree, that the thesis entitled **"Persistence Of Selective Herbicides In Rice-Rice System"** may be submitted by **Mr. M. Muthu Kannan**, in partial fulfillment of the requirement for the degree.

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M.Muthu kannan.

Dedicated to my beloved parents And Teachers

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LIST OF ABBREVIATIONS

AEC	- Anion exchange capacity
a.i	- active ingredient
@	- at the rate of
°C	- degree Celsius
cfu	- colony forming unit
cm	- centimetre
CEC	- Cation exchange capacity
DAS	- Days after spraying
2,4-D	- 2,4-dichloro phenoxy acetic acid
fb	- followed by
Fig	- Figure
g	- gram
g m^{-2}	- gram per metre square
h	- hour
ha	- hectare
K	- Potassium
Kmph	- Kilometre per hour
kg	- kilogram
kg ha^{-1}	- kilogram per hectare
m	- metre
mm	- millimetre
min	- minute
mPa	- mega pascal
µg g^{-1}	- microgram per gram
mg g^{-1}	- milli gram per gram
ml	- millilitre
µl	- microlitre
N	- nitrogen

NS	- Non significant
ND	- Not detected
NA	- No application
%	- per cent
P	- phosphorus
t	- tonnes

Introduction

1. INTRODUCTION

Rice has been most companion crop of man since antiquity. It is the staple food of millions of people. Rice is grown in our country in an area of 43 million hectares with a production of 77.7 million tonnes (Anon., 2003). In the era of ever increasing population and shrinking resources, including land and input, it becomes necessary to adopt modern techniques of cultivation to raise the productivity as well as to supply rice to all.

Weed infestation is one of the serious negative factors in crop production because weeds compete with crop plant for nutrients, water, space, sunlight and carbondioxide. It has been estimated that weeds caused 5 per cent loss in agriculture production in most developed countries, while it caused 10 per cent in less developed countries and 25 per cent in least developing countries (Bhowmik, 1999). In India, weeds caused 33 per cent loss in agricultural production. Bhan and Mishra (1998) reported that weeds reduced rice production by 30-35 per cent.

Chemical weed control is indispensable in rice culture due to severity of weed problem, hike in labour wages and non availability of labour during peak periods of cultivation. Due to lack of knowledge on residual effect of herbicides, they are used indiscriminately in rice fields. This causes soil-plant-animal health hazards. Due to the continuous application of herbicides there is possibility for occurrence of residue related problems like shift in weed flora, appearance of resistant weeds and environmental pollution. Hence it is necessary to monitor the build up of herbicide residues in soil by detailed study.

Pre emergence herbicides butachlor and pretilachlor are recommended in rice ecosystem for selective control of grasses. Butachlor is primarily absorbed through germinating shoots and secondarily through roots, while pretilachlor is absorbed by the hypocotyls, mesocotyls and coleoptiles and to lesser extent by the roots of germinating weeds. 2,4-D is recommended as post emergence herbicide for the selective control of broad leaved weeds in rice. Salts of 2,4-D are readily absorbed by

roots, while esters are readily absorbed by the foliage. At low concentration 2,4-D acts as an auxin (plant growth regulator) but at high concentration acts as a herbicide.

An understanding of the persistence of each herbicide used in the rice crop is important for estimating their pollution potential. Persistence of a herbicide refers to the residence time of chemical in soil, before being completely removed by physical, chemical and biological degradation (Scheunert *et al.*, 1993). Residence time of a particular herbicide in soil is determined by balance between adsorption on soil colloids, uptake by plants (residue), transformation or degradation processes and losses in liquid or gaseous form. This balance is partly controlled by the type of herbicide, its amount and method of application and partly by soil characteristics such as texture and organic matter content.

Microbial degradation is one of the major processes responsible for dissipation of herbicides in soil. Because herbicides are simple organic molecules, microorganisms readily attack them and they can utilize the carbon for their energy requirements. Increasing the organic matter content of the soil would enhance the rate of degradation of herbicides by providing a favourable environment for the growth and multiplication of soil microflora. Soil organic matter plays important role in the adsorption of herbicide. Therefore, addition of surplus of organic matter in the form of FYM, compost or green manure is one of the management techniques for reducing the build up of herbicide residues in soil. This aspect has to be taken in to consideration in any weed management programme.

Research studies conducted in different parts of India with single application of butachlor at recommended rates of 1-2 kg ha^{-1} showed that the residues were below detectable limits in grain and straw (Saxena *et al.*, 2002). However, the effect of continuous application of butachlor in the rice-rice system has not been assessed. Pretilachlor is an alternative to butachlor and its DT_{50} (time required to disappear half the quantity applied) in the 0-1 cm soil layer range between 7-10 days (Fajardo *et al.*, 2000).

Studies on the persistence of 2,4-D in the major rice soils of Kerala has been conducted during 2002 with different concentrations of the herbicide. Effect of 2,4-D on the population of microorganisms in the wetland paddy was also investigated. Results of these studies revealed that the present recommended rate of application of 2,4-D @ 1.0 kg ha^{-1} do not have any adverse effect on rice soil or crop (Devi, 2002).

All of the above herbicide residue studies had been focusing on the evaluation of either a pre-emergence or a post-emergence herbicide. An understanding of the cumulative effect of all the herbicides used in the cropping system is essential to evolve environmentally safe recommendations for weed control in rice.

Under the circumstances present study was contemplated with the following objectives.

1. To estimate the persistence and degradation of common rice herbicides in the cropping system.
2. To study the effect of organic matter on herbicide residue build up in soil.
3. To compare the status of soil microflora and herbicide residues in soil under single and repeated application of same herbicide
4. To monitor the terminal residues of herbicides in rice grain and straw.
5. To study the changes in weed flora due to continuous application of herbicides

Review of Literature

2. REVIEW OF LITERATURE

Chemical weed control in India started with the introduction of 2,4-D. Since then many chemicals have been introduced for weed control in field crops. About 250 chemicals have been discovered for the control of weeds in the world. Whereas in India, 33 herbicides have been registered for use and out of which farmers use only 14. Isoproturon, 2,4-D, butachlor and anilofos constitute the major portion of herbicide consumption in our country. Among pests, only weeds emerge in all crop fields and continuous introduction of new herbicides is necessary to reduce the labour and tillage cost and also to avoid resistance problem (Chhokar, 2001).

Preemergent spray of butachlor @ 1.25 kg ha^{-1} or pretilachlor @ 0.75 kg ha^{-1} at 6-9 days after sowing followed by the application of 2,4-D @ 1.0 kg ha^{-1} at 20 days after sowing effectively controls grassy as well as broad leaved weeds in rice (KAU, 1996). The technical information and toxicity data for three herbicide under study are given in Table 1.

Once a herbicide reaches the soil it undergoes various interactions with soil as presented in Fig.1. Hurle and Walker (1980) suggested that the optimal period of persistence of herbicide in soil should be "long enough to give an acceptable period of weed control but not so long that herbicide residues, after crop harvest, limit the nature of subsequent crops which can be grown".

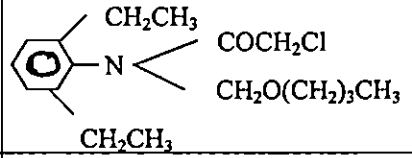
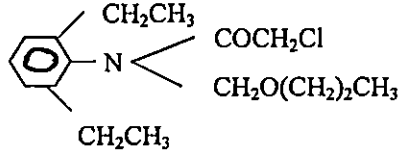
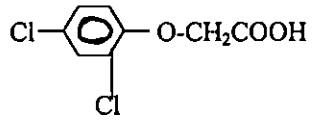
2.1. MECHANISMS OF HERBICIDE DISSIPATION

Applied herbicides become dissipated by physical, chemical and biological processes.

2.1.1. Physical processes

Leaching and volatilization are the two main physical processes leading to loss of herbicides from the soil (Scheunert *et al.*, 1993).

Table 1. Technical information and toxicity data on butachlor, pretilachlor and 2,4-D.

COMMMON NAME	BUTACHLOR	PRETILACHLOR	2,4-D
Chemical Name	**N-(butoxy methyl)-2 chloro-N-(2-6 diethyl phenyl)=acetamide**	**2-chloro-N-(2,6-diethyl phenyl)-N-(2-propoxy ethyl) acetamide**	**2,4-dichloro phenoxy acetic acid**
Chemical structure			
Molecular weight	313.5	299.5	221.0
Physical state, colour, odour	Yellow to purple liquid with a faint, sweet odour	Colourless liquid	White crystals, odourless when pure
Specific gravity	1.076 (25°C)	1.076 (20°C)	1.565
Melting point	-2.8°C to 1.7°C	-	140.5°C
Boiling point	156°C	135°C	160°C
Stability	Decomposes $\geq$ 165°C, stable indefinitely $\leq$ 45°C	At 20°C, 50% hydrolysis occurs in 14 days at pH 13.	Stable at its melting point
Vapour pressure	0.24 mPa (25°C)	0.133 mPa (20°C)	0.01 mPa (20°C)
Solubility: Water Organic Solvents	20 mg/l (20°C) Soluble in diethyl ether, acetone benzene, ethanol, ethyl acetate and hexane	50 mg/l (20°C) Soluble in benzene, hexane, methanol and dichloromethane	311 mg/l (25°C) Soluble in diethyl ether, ethanol heptane, toluene, xylene.

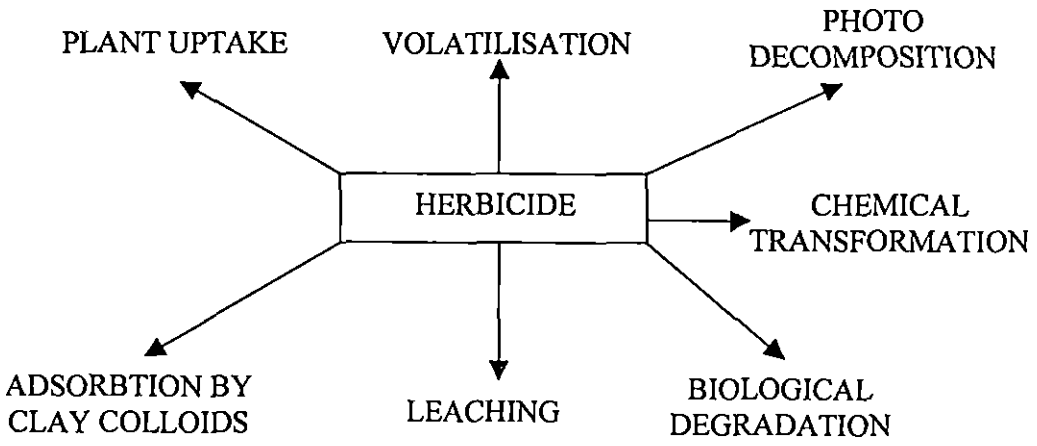
Contd.

Table 1. Continued

Chemical Name	N-(butoxy methyl)-2 chloro-N-(2-6 diethyl phenyl)=acetamide	2-chloro-N-(2,6-diethyl phenyl)-N-(2-propoxy ethyl) acetamide	2,4-dichloro phenoxy acetic acid
Acute toxicity Acute oral LD50) for rats	2000 mg/kg	6099 mg/kg	375 mg/kg
Acute percutaneous LD50	>13000 mg/kg (rabbit)	> 3100 mg/kg (rats)	>1600 mg/kg (rats)
Inhalation LD 50 (4h) for rats	>3.34 mg l^{-1} air	>2.8 mg l^{-1} air	>1.79 mg l^{-1} air
Acceptable daily intake	0.01mg/kg body weight	0.018 mg/kg body weight	0.01 mg/kg body weight
Environmental fate In soil	Degradation is principally by microbial activity. Persistance for 6-10 weeks. Converted to water soluble derivatives, with a slow evolution of CO_2.	Strongly absorbed to soil, DT_{50} (lab) 30d rapidly degraded.	Microbial degradation involves hydroxylation, decarboxylation, clevage of the acid fide chain and ring opening, half life <7 days.
In plant	Rapidly metabolized to water-soluble metabolites, leading eventually to mineralsation	Substitution of chlorine atom to form conjugate. Cleveage of ether bond to yield an ethyl alcohol derivative. Hydrolytic and reductive removal of the chlorine atom.	Hydroxylation, decarboxylation clevage of acid side chain and ring opening are metabolism involved.

Source: BCPC, 1979; RSC, 1987

Fig.1. Fate of applied herbicide in soil.



(Source: Harris, 1972)

2.1.1.1. Leaching

Leaching of herbicides refers to their downward movement in soils as solutes in soil water. Leaching is more influenced by soil properties than by the physico-chemical properties of the compounds. The degree of leaching of a pesticide from soil is correlated with its water solubility but this is also modified by the capacity of pesticides for adsorption on soil fraction (Edwards, 1973).

Beestman and Deming (1974) reported that leaching did not contribute to dissipation of the herbicide from soil, since no residue was found below the upper four centimetre layer of soil. Degree of leaching of chemicals through soil decides ground water contamination. Residues of 2,4-D can enter ponds and streams by direct application or accidental drift, by inflow of herbicide previously deposited in dry stream beds, pond bottoms or irrigation channels; run off from soils; or by leaching through soil columns (Norris, 1981).

Obrigawitch *et al.* (1981) in their studies using soil column and thin layer plate revealed that metolachlor was significantly mobile in soils low in organic matter due to lesser adsorption. Peter (1984) revealed that herbicide adsorption was generally inversely related to mobility and water solubility of the compound. Sabatini and Augustin (1990) concluded that adsorption and desorption are the major mechanisms affecting the pesticide movement in soil. In the soil with high clay content, as in sandy clay and sandy clay loam, the leachate contained less herbicide due to high adsorption. The movement of Simazine was more restricted in the laterite soil compared to that in red and black soils (Rajanna *et al.*, 1991).

The extent of leaching and run off of 2,4-D is influenced by the formulation, soil properties, slope, timing and intensity of rainfall. 2,4-D was found susceptible to run off if the rain event occurred shortly after the application, with run off concentration of 2,4-D decreased over the time (Stearman and Wells, 1997).

2.1.1.2. Volatilisation

Volatilisation of chemicals is an important pathway for their loss from treated agricultural lands. Volatilisation of a herbicide from soil depends mainly on the vapour pressure of the compound, its concentration, its adsorption to the soil and its solubility in water. It is also affected by air temperature, wind velocity above the soil surface, relative humidity, soil temperature and soil moisture. Measurement of vapour pressure of herbicides in soil at various water contents conclusively demonstrated that the greater vaporization from wet than from dry soils is mainly due to increased vapour pressure resulting from displacement of the chemical from the soil surfaces by water (Glotfelty *et al.*, 1984).

Beestman and Deming (1974) reported that volatilisation losses of butachlor from continuously moist soil under 21°C was three to six times greater than volatilisation from air dry soil. Chen and Chen (1979) reported that the loss of butachlor by volatilization from 0.05 M $CaCl_2$ was 45 per cent at 21.5°C and it increased to 30 per cent at 40°C.

2.1.2. Chemical processes

Chemical processes of herbicide dissipation include photodecomposition and chemical decomposition. Chemical decomposition is not considered to be of major importance as it usually begins as soon as the herbicide is applied to soil without any lag phase (Scheunert *et al.*, 1993).

2.1.2.1. Photodecomposition

Photodecomposition is one of the pathways of pesticide conversion in the environment and this process is induced mainly by ultra violet (UV) sunlight radiation. In the UV region the energy absorbed by organic molecules causes excitation of electrons and can induce such transformations as the breakage or formation of chemical bonds, fluorescence etc. It is observed that compounds of several classes of herbicides are decomposed by UV-light. Energy adsorption of herbicides is dependent upon their chemical structure. It is well known that compounds having an aromatic ring exhibit intense adsorption in the UV region.

Several herbicides absorb energy strongly at wave length between 220 and 380 nm, which ensures the basic requirements of sunlight for the photolysis process.

The major products of photolysis of 2,4-D were 2,4-dichlorophenol and 4-chloro catechol. 2,4-DB and 2,4-5 T also formed corresponding phenols during photo decomposition (Crosby and Tutass, 1966). Crosby (1976) suggested that hydroxylation of chlorinated aromatic groups is significant for disappearance of 2,4-D from the environment. Photodecomposition on soil surfaces play a very minor role in break down of 2,4-D. The effect of sunlight on 2,4-D degradation is similar to that of UV light (Rao, 1992).

The photodegradation of butachlor in aqueous solution is more rapid and more complicated than that of thin film on glass and silica gel plates (Chen and Chen, 1978). They also reported that only 20 per cent of butachlor was remained when 100 ml of field water containing 0.29 ppm of butachlor in petridish was exposed to sunlight at 25-35°C for one day.

2.1.2.2. Adsorption

Adsorption is the bonding of a chemical to sites on soil mineral or organic surfaces. Adsorption of herbicides can be physical or chemical. Physical adsorption is the result of Van der waal's forces interacting between neutral molecules and surface of soil colloids. Chemical sorption is the result of coulombic forces interacting between oppositely charged adsorbent and adsorbates (Harper, 1994).

Adsorption of the herbicide by soil is important in determining their environmental fate, biological activity and persistence in soil. Adsorption is influenced by organic matter, clay content, ion exchange capacity, bulk density, solution composition, pH, temperature and tillage practices, and structure and properties of the herbicides (Chesters *et al.*, 1989).

Nkedi-Kizza *et al.* (1983) measured the adsorption coefficient for diuron and 2,4,5-T on different particle sizes based on soil organic matter content and found that fine fraction adsorbed three times 2,4,5-T than coarse fraction by using a simple

regression analysis between adsorption capacities and soil properties. Hermosin and Cornejo (1991) reported that high organic matter and free ions in soils favoured the adsorption of 2,4-D, while high pH, large surface area and phyllo silicates as essential clay component decreased the adsorption.

The extent of adsorption of 2,4-D increased with increase in soil organic carbon and hence the rate of degradation of 2,4-D as measured by half life (t½) decreased with increase in soil organic carbon. With increase in adsorption, the rate of desorption decreased resulting in low concentration of 2,4-D in the soil solution for microbial degradation (Bolan and Baskaran, 1996). The adsorption isotherms of metolachlor, acetachlor, pretilachlor and butachlor fit the Fruendlich equation and the extent of adsorption increased in the order metalochlor < acetochlor < pretilachlor < butachlor (Quiquan *et al.*, 1999).

Prakash *et al.* (2000) reported that adsorption isotherm confirmed to the Freundlich equation. Butachlor adsorption increased with increasing organic carbon content. The adsorption isotherms of butachlor, oxadiazone, pretilachlor and thiobencarb were described by the Freundlich equation. The adsorption distribution coefficient (kd) of butachlor, oxadiazone, pretilachlor and thiobencarb were 1.34, 1.40, 2.86 and 0.61 respectively (Moon and Kim, 2000).

2.1.3. Biological processes

It includes microbial decomposition by soil microorganisms are also responsible for dissipation.

2.1.3.1. Microbial decomposition

Microbial action is a major mode of decomposition of herbicides in soil. The microbial decomposition is characterized by increased rates of CO_2 evolution, which marks rapid microbial activity called lag phase. During the lag phase the decomposition of herbicide structure happens. Microbes degrade herbicides predominantly through hydrolysis, alkylation, dealkylation, oxidation, reduction, hydroxylation, ring clevage and conjugation.

Microbial activity, and hence biodegradation of herbicides, is activated by 20 to 30°C soil temperatures and near field capacity soil moisture (Burnside, 1965). Rao (1992) pointed out that herbicide loss curves were typified by an initial lag phase in which decomposer organisms adapt to the herbicide substrate by producing adaptive enzymes. This lag phase was followed by a period of rapid loss as the 'substrate adapted' microbial population builds up, as found for the phenoxy acetic acid herbicides like 2,4-D and MCPA (Torstensson, 1975). The duration of lag phase is dependent on the herbicide under attack and the organisms present. The lag phase of 2,4-D is considerably shorter than that of 2,4,5-T and MCPA .

2.2. PERSISTENCE OF HERBICIDES

According to IUPAC, chemical persistence is defined as the residence time of the chemical species in a specifically defined compartment of the environment, which may be the parent compound or a derivative, but not both. According to Kearney *et al.* (1969) persistence is defined as the time required to reduce the pesticidal concentration in soils from 75 to 100 per cent of the amount initially added. The concept of half-life is widely used in persistence of pesticides in soil. It is the time required for one half of the pesticide to disappear.

Beestman and Deming (1974) reported the half-lives of butachlor in viable and sterilized soils as 11.4 ± 0.3 and 64.0 ± 8 days respectively. Jayakumar and Sreeramulu (1993) reported a half-life of 19 days for butachlor. According to Devi *et al.* (1997) half lives of butachlor varied from 12.3 to 16.2 days. Kulshrestha *et al.* (1981) observed that butachlor did not persist in a sandy loam soil for more than 38 days. At recommended rate of 1.5 kg ha^{-1} butachlor was degraded before harvest of rice. However at levels of 4.0 kg ha^{-1}, it could persist beyond harvest (Sankaran *et al.*, 1993). Deka and Gogoi (1993) reported that the persistence of butachlor at 2.0 kg ha^{-1} was 0.112 ppm at 15 days after transplanting. In upland rice, at 21 days after application of butachlor (1.0, 1.5 and 2.0 kg ha^{-1}) the herbicide got degraded to non-detectable limits.

The half-life of 2,4-D in soil was 59.3 days (Hermosin and Cornejo, 1991). Cox (1999) reported that persistence of 2,4-D is variable with half-lives ranging from 2-297 days. Devi (2002) reported that half-life of 2,4-D in the rice soils of Kerala varied from 3.44-10.76 days. Jayakumar and Sreeramulu (1993) found that 2,4-D at 0.4 kg ha^{-1} alone and in combination persisted up to harvest with half-lives of 18 to 22 days. Studies on persistence of 2,4-D conducted at Bangalore showed that 2,4-D persisted in red soil up to 3 months (Leela, 1986). At Faizabad, the 2,4-D sodium salt persisted only for 42 days (Sankaran *et al.*, 1993).

2.2.1. Factors affecting persistence of herbicides

The persistence of chemicals in soil is affected by physicochemical soil properties such as content of organic matter, kind of clay minerals, clay content, CEC, pH, moisture content, soil structure and soil texture. These properties affect the persistence of each herbicide in a different way.

2.2.1.1. Soil moisture

Both chemical and microbial degradation depends on moisture content.Mabbayad *et al.* (1986) found that faster dissipation of butachlor occurred at higher soil moisture and higher temperatures. Prakash and Devi (2000) reported that butachlor dissipation was faster under field capacity followed by submergence. Half lives of butachlor under airdry, field capacity and submergence ranged from 52.40 to 59.57, 12.35 to 20.58 and 28.48 to 39.20 days respectively.

2.2.1.2. Soil pH

Corbin and Upchurch (1967) investigated the rates of degradation of several herbicides in two high organic matter soils adjusted to four pH levels in the range of 4.3 to 7.5. They reported maximum degradation of 2,4-D and dicamba at pH 5.3. They suggested that maximum rates of loss should occur at pH levels favourable for growth of the specific microorganisms involved in degradation.

Three times more alachlor was adsorbed at pH 4.1 than at pH 12 in three soils with organic matter content less than 1.1 per cent (Sethi and Chopra, 1975).

Acidification of soils greatly enhanced their ability to retain 2,4-D (Grover, 1977). Weber *et al.* (1987) reported that persistence of herbicides in soil depend on the pH and organic matter content of soil.

2.2.1.3. Soil properties

Clay content is one of the principal factors influencing persistence of herbicides in soil, because clay and organic matter contents are themselves correlated (Edwards, 1973). Cations such as Al^{3+} and Fe^{3+} can form hydroxide on the clay surface. This results in a greater increase of the adsorption capacity of the clay mineral (Calvet, 1980). Weber and Peter (1982) reported that the adsorption and bioactivity of herbicides were correlated with clay content.

Miles and Moye (1986) confirmed that CEC played a significant role in the adsorption process of glyphosate on cation-saturated clays. They also reported that in the coarse fraction of soils, the added pesticides are deactivated more compared with finer fractions. Glass (1986) found that adsorption of glyphosate by soils was related to the clay content and CEC of the soil. High surface area and CEC increased the adsorption and decreased the herbicide activity. Inactivation occurs on organic and clay surfaces but is greater at lipophilic than hydrophilic sites (Chesters *et al.*, 1989).

2.2.1.4. Soil type

Beestman and Deming (1974) determined degradation of alachlor in different soils. Half life values of alachlor in silt, silty loam, silty clay, clay loam and sandy loam were 4.0, 11, 7.3, 9 and 11-24 days respectively. Stougaard *et al.* (1990) observed that adsorption was greater in silty clay loam and less in sandy loam soils.

2.2.1.5. Soil organic matter

Soil organic matter is another important factor influencing the adsorption of herbicides in soil (Karrie *et al.*, 1991). Slowest degradation was observed in samples containing little organic matter with least adsorption (Hance, 1974). Bolan and Baskaran (1996) reported that increasing organic matter content increased the rate of

degradation. It also increased the microbial activity and reduced the inhibitory effects on microbes.

Benoit *et al* (1996) reported that lignin from plant tissue or aliphatic compound from microbial origin contributed to increase sorption of 2,4-D. Organic matter is the main factor controlling the adsorption process of acetanilide herbicides (Quiquan *et al.*, 1999).

2.2.1.6. Soil microorganisms

Microorganisms are able to degrade a wide variety of chemicals, from simple polysaccharides, aminoacids, proteins, lipids etc. to more complex materials such as plant residues, waxes and rubbers. Many herbicides are retained in the surface layer of soil for a relatively long term and are degraded mostly by soil microorganisms.

Beestman and Deming (1974) reported that the half-life of butachlor in viable and sterilized soils were found to be 11.4 ± 0.3 and 64.0 ± 8 days respectively. Many scientists reported that degradation of butachlor is mainly microbial (Pionke and Chesters, 1973; Beestman and Deming, 1974; Chen and Wu, 1978). The degradation of butachlor by several microbial isolates in a medium and in soil was studied by Chen and Wu (1978). They found that seven species of fungi and *Bacillus* sp. had butachlor degrading activity. Chakraborty and Bhattacharya (1991) found that two soil fungi namely *Fusarium solanum* and *Fusarium oxysporum* effectively degraded butachlor in a 0.02M KH_2PO_4 buffer solution at pH 5.2.Walker *et al.* (1992) observed that the degradation rate was positively correlated with microbial biomass. Both microbial population and adsorption were positively correlated with soil organic matter.

Microbial degradation is considered to be the major route in the breakdown of 2,4-D in soil. Han and New (1994) found that sandy loam soil contain 2,4-D degrading single-celled bacteria, actinomycetes and fungi. They also reported that increase in water potential resulted in increased rates of breakdown up to an optimum

of -0.1 mPa. Gill *et al.* (1997) reported that greatest biodegradation of butachlor by *Pseudomonas alkaligenes* occurred at 40°C and at pH 7.0 after incubating for 48h.

2.3. HERBICIDE RESIDUES IN RICE ECOSYSTEM

Audus (1951) found that in general 2,4-D persisted for 2 to 4 weeks. The maximum residue limits for 2,4-D rice grain and straw are 0.01 ppm and 20.0 ppm respectively (FCN, 1982). Butachlor @ 1.5 kg ha^{-1} degraded before harvest but @ 4.0 kg ha^{-1} persisted beyond harvest. In the grain and straw butachlor residues were below the prescribed maximum residual limits (Sankaran *et al.*, 1993).

Kulshrestha (1987) reported that butachlor residues in the plants ranged from 0.001-0.16 ppm, after a month, depending on rate of application. Grain residue did not exceed 0.01 ppm. Sankaran *et al.* (1993) summarized the results of the herbicide residue studies conducted at different AICRP centres in India. At Bangalore, 2,4-D persisted in red soil up to 3 months. At Faizabad 2,4-D salt persisted only for 42 days. Deka and Gogoi (1993) reported that residues in grain (0.012 ppm) and straw (0.002 ppm) were recorded in low land rice treated with butachlor at 2.0 kg ha^{-1}. In soil the persistence of this herbicide was 0.112 ppm at 15 days after transplanting. Devi *et al.* (1997) reported that at 1.0 to 2.0 kg application rates butachlor residues were not detected in the soil or grain. At the application of 2.0 kg ha^{-1} residues of butachlor in rice, bran, straw and post harvest soil were far below the maximum permissible residue limit of 0.25 ppm.

Reddy *et al.* (1998) found that butachlor application @ 1.0 kg a.i ha^{-1} retained residues of 0.002, 0.009,0.006 mg kg^{-1} in rice bran, straw, and grain respectively. At 2.0 kg a.iha^{-1} the residue levels were 0.001, 0.005, 0.010 and 0.025 mg kg^{-1} in rice, bran, straw and grain respectively.

In a study on the possible contamination of pesticides in kuttanad ecosystem, 2,4-D residues were detected in 30% of the soil samples in the range of 0.001 to 0.12 µg g^{-1} Higher concentration was observed in the month of March. No residues of 2,4-D were detected in the rice grain samples. However 2,4-D was detected in 7 per cent of the straw samples up to a concentration of 0.0225 µg g^{-1} (Anon., 1999).

Devi (2002) found that 2,4-D residues persisted in soil for less than 60 days irrespective of the soil type. Residues of 2,4-D in the rice grain and straw were below the maximum residue limits at all the levels of application.

Bioassay studies conducted using herbicide combination of butachlor, 2,4-D and pretilachlor showed more residual effect of herbicides during initial phase of 10 to 20 days after spraying. But at 40 days after spraying, herbicides got degraded to safer levels. Persistence of butachlor in alfisols was observed beyond 35 days after application and 50 per cent of the applied herbicide was detected by 15 days after application (NRCWS, 2000). Residues of butachlor were not found in grain at harvest and were below the detectable limits in post harvest soils (NRCWS, 2002).

2.4. EFFECT OF HERBICIDES ON SOIL MICROBIAL ACTIVITY

According to Anderson (1978), herbicides generally appear to have no adverse effect on the population of total bacteria in soil except at concentrations exceeding recommended rates. Deshmukh and Khande (1977) reported that the ester derivatives showed pronounced inhibitory effect on the growth of bacteria throughout 45 days. Actinomycetes were also depressed during entire incubation period in the presence of high and low doses of herbicides. Herbicide excerted inhibitory effect on the growth of fungi in the first 8 days however the fungal population increased after 30 days. Baruah and Mishra (1986) reported that the application of butachlor at the manufacturer's recommended rates to a paddy soil stimulated carbondioxide evolution. It had initially stimulated but subsequently inhibited dehydrogenase activity, and did not affect urease activity over a 35 day period. Application of butachlor caused short term fluctuation in the actinomycete population and initially depressed bacteria, but no prolonged effects on soil microflora were observed (Mandal *et al.*, 1987).

Schuster and Schroder (1990a) observed only slight and short lived effects on microbial activity with successive applications of isoproturon and glyphosate. At high dosages, side effects were dominated by strong and irreversible inhibitions (Schuster

and Schroder, 1990b). Chauhan *et al.* (1994) opined that small concentration of pesticides did not alter the activity of organisms but at higher concentration most bacterial and fungal species were affected. Shukla (1997) found that application of butachlor increased fungal and bacterial population on application of the herbicides, but their population decreased during the later part of the study.

Application of herbicides in upland rice though reduced the microbial population during the initial stages of crop growth up to 25 days after spraying, no significant differences was recorded in their population in the later stages (OUAT, 2000). Devi (2002) found that population of microflora in rice soil varied with time after application of 2,4-D. Though a negative influence of 2,4-D on soil bacteria was observed in the early period their population was restored by 30 days after spraying and this period coincided with the persistence of 2,4-D in wetland paddy.

2.5. EFFECT OF HERBICIDES ON WEED CONTROL

Tosh (1975) reported that granular formulation of butachlor 20.0 $kgha^{-1}$ controlled grasses effectively but did not control sedges and broad leaves weeds. Subbian (1983) concluded that pre-emergence application of 2,4-D plus one hand weeding on 40^{th} day of the crop gave effective weed control and significant increase in yield.

Sawant and Judhar (1983) recorded better weed control efficiency with 2,4-D and suggested as an alternative for hand weeding. In the studies in NRCWS (1986) anilofos 0.3 kg mixed with 2,4-D (0.4 kg) was found to be usually effective in controlling weeds as butachlor (1.25 kg) mixed with 2,4-D (0.6 kg). Butachlor @ 1.5 $kgha^{-1}$ followed by hand weeding was found effective in reducing total number of weeds and reduction in biomass of grassy weeds (TNAU, 1987).

Janardhan *et al.* (1999) reported that pretilachlor @1.0 kg ha^{-1} gave good weed control. Rajendran *et al.* (1999) found that pretilachlor @ 0.3 kg ha^{-1} with one hand weeding effectively reduced the density of the dominant weeds. Pre-emergence

application of butachlor @ 1.0 kg ha^{-1} or pretilachlor @ 0.75 kg ha^{-1} in saturated soil were found effective in controlling weeds in transplanted rice (NRCWS, 2000).

2.6. HERBICIDE RESIDUE ANALYTICAL METHODS

Bioassay procedures have been used to investigate many practical aspects of herbicide behaviour in the environment. The bioassay procedure developed by crafts (1935) is followed by many researchers with little modification. Jayakumar (1987) standardised the bioassay technique for a number of herbicides in different soil types and brought out quadratic models for easy assessment. The instrumentation methods involved in herbicide residue analysis are visible and ultraviolet spectrophotometry, thin layer chromatography, gas chromatography and high performance liquid chromatography etc.

Among the various instrumental techniques available for herbicide residue analysis, gas chromatography is the most sensitive detection technique for the residue estimation of butachlor, 2,4-D and pretilachlor in soil and plant parts including grain (Sankaran *et al.*, 1993). James and Martin introduced the gas chromatographic (GC) technique in 1952 for the separation of volatile organic compounds. In pesticide analysis, GC has assumed a role of primary importance as compared to other methods. This is because GC technique is capable of rapidly resolving complex pesticide mixtures and provides qualitative identification and precise quantitative analysis of the components. In multi residue analysis, GLC is the only method of choice.

Chlorinated pesticides are analysed on stationary liquid phases on aluminium columns with an electron capture detector. The organo chlorine pesticide extract should be free of waxes, fats, pigments and other impurities. In the residue analysis of 2,4-D, gas chromatography is the most sensitive method (QueeHee and Sutherland, 1981). The phenoxy fatty acids are the class of herbicide widely used in formulations as salts as well as various esters. The free phenoxy acids and salts are too polar and non volatile to chromatograph easily. For gas liquid chromatography analysis, the phenoxy fatty acids have to be transformed in to esters (Das and Kulkarni, 1981). By

derivatization free acids have been usually converted to methyl esters, which are less polar and easier for analysis. The reagent of choice for producing a high yield of GC pure esters in the shortest time was the BF_3 or alcohol reagent. Procedure for esterification of 2,4-D with BF_3 or alcohol is well presented by QueeHee and Sutherland (1981). They proposed an alkaline and acid hydrolysis method for the extraction of 2,4-D from the plant tissue and the recovery was > 92 per cent.

Mabbayad *et al.* (1986) standardised the procedure, extraction, clean up, and operating conditions of gas chromatograph for estimation of butachlor residues. Xu and Liu (1990) described a method in which the samples were extracted with petroleum spirit acetone, concentrated and cleaned up on a column and then analysed by Gas chromatography with an electron capture detector. The detection limit and recovery of this method were 3-6 ppb and 78.6-98.3 per cent respectively.

Butachlor residues were extracted with acetone + high petroleum distillate, concentrated and purified on a chromatographic column containing aluminium oxide, silver-aluminium oxide and florisil, and quantified by GC using on electron capture detector. Detection limit was 0.001-0.015 mg kg^{-1} with average recoveries ranging from 79.4 to 104.6 per cent (Liu *et al.*, 1991). Pretilachlor residues in soil and plant materials were extracted with methanol and the residue in grain extracted with acetonitrile (Sankaran *et al.*, 1993).

Materials and Methods

3. MATERIALS AND METHODS

The research programme consisted of a series of field experiments and laboratory analysis. The field experiments were conducted during 2001-2002 at Agricultural Research Station, Mannuthy. Residue studies related to the field experiments were conducted at the Herbicide Residue Laboratory of All India Coordinated Research Programme on Weed Control, Thrissur Centre, located in the Radio Tracer Laboratory, College of Horticulture, Kerala Agricultural University, Vellanikkara. Microbiological studies and physico chemical analysis of the soil samples were conducted at College of Horticulture, Vellanikkara.

3.1. LOCATION

The Agricultural Research Station, Mannuthy located at 10°31' N latitude and 76°13' E longitude and at an altitude of 40.29 M above sea level, is situated about 6 km east of Thrissur town on the southern side of Trichur-Palakkad portion of the NH-47.

3.2. WEATHER AND CLIMATE

The area enjoys a typical humid tropical climate. During the crop period for the year 2001,weekly mean maximum, mean minimum temperature and mean rainfall were 29.3°C, 22.8°C and 63.6 mm respectively. Corresponding values for the year 2002 were 30.6°C, 23°C, 61.3 mm respectively.

3.3. CROPPING HISTORY

The experimental site was a double-cropped paddy land in which a semi dry crop (June – September) and wet crop (September – December) are regularly cultivated. The land is usually left fallow during summer season.

3.4. SOIL

The experimental field consists of soil type of laterite sandy clay loam of the Oxisol order. The soil is acidic in reaction with a pH of 4.5 to 5.6.

3.5. CROP VARIETY

An extra short duration rice variety, Hraswa (75-80 days) was used for the study.

3.6. LAY OUT

The experiment was laid out with six large sized (Fig.2) plots of 100 sq.m. each (20 m x 5 m). The bunds were kept permanent so as to apply the herbicides continuously or alternatively as per the treatments. The bunds between plots were not disturbed and the land preparation confined to individual plot only.

3.7. TREATMENTS

The study was a part of the "Permanent Herbicide Trial" of All India Co-ordinated Research Programme on Weed Control. Rice crop was raised in the puddled field during the first and second crop seasons of 2001 and 2002. The details of the treatments are given in Table 2.

3.8. FIELD EXPERIMENTS

3.8.1. Sowing and harvesting

	2001-2002			
	First crop	Second crop	First crop	Second crop
Date of sowing	19.06.01	21.09.01	19.06.02	26.09.02
Date of harvest	9.10.01	5.01.02	14.09.02	14.12.02

3.9. CULTIVATION OPERATIONS

3.9.1. Land preparation

The experimental area was ploughed thoroughly under moist condition, leveled and laid out as per the design. Soil samples were taken for determining the physicochemical characteristics before the experiment. The individual plots were perfectly leveled before sowing.

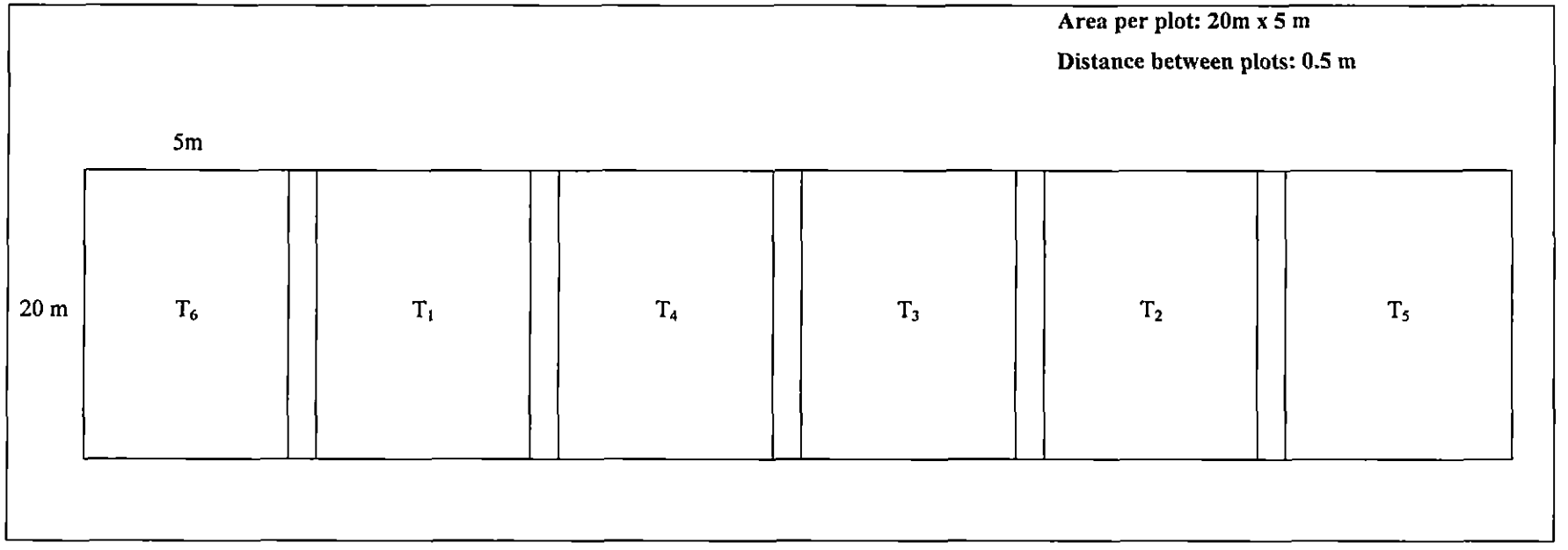


Fig. 2. LAYOUT PLAN

Table 2. Details of the treatments

Treatments	Year			
	2001		2002	
	First crop	Second crop	First crop	Second crop
T_1	Hand weeding (25 & 40 DAS)	Hand weeding (25 & 40 DAS)	Hand weeding (25 & 40 DAS)	Hand weeding (25 & 40 DAS)
T_2	Butachlor fb 2,4-D 100% NPK through fertilizer	Butachlor fb 2,4-D 100% NPK through fertilizer	Butachlor fb 2,4-D 100% NPK through fertilizer	Butachlor fb 2,4-D 100% NPK through fertilizer
T_3	Butachlor fb 2,4-D 100% NPK through fertilizer	Pretilachlor fb 2,4-D 100% NPK through fertilizer	Butachlor fb 2,4-D 100% NPK through fertilizer	Pretilachlor fb 2,4-D 100% NPK through fertilizer
T_4	Butachlor fb 2,4-D 75% NPK through fertilizer & 25% through FYM	Pretilachlor fb 2,4-D 100% NPK through fertilizer	Butachlor fb 2,4-D 75% NPK through fertilizer & 25% through FYM	Pretilachlor fb 2,4-D 100%NPK through fertilizer
T_5	Butachlor fb 2,4-D 100% NPK through fertilizer	Butachlor fb 2,4-D 100% NPK through fertilizer	Pretilachlor fb 2,4-D 100% NPK through fertilizer	Pretilachlor fb 2,4-D 100% NPK through fertilizer
T_6	Butachlor fb 2,4-D 75% NPK through fertilizer & 25% through FYM	Butachlor fb 2,4-D 100% NPK through fertilizer	Pretilachlor fb 2,4-D 75% NPK through fertilizer & 25% through FYM	Pretilachlor fb 2,4-D 100% NPK through fertilizer

Doses: Butachlor 1.25 kg a.i ha^{-1}, Pretilachlor 0.75 kg a.i ha^{-1}, 2,4-D 1.0 kg a.i ha^{-1}

3.9.2. Sowing

In the finely prepared soil, sowing was done by wet method. About 10 kg of rice seed was taken for 100 square meter and broadcasted. The soil moisture was optimum at the time of sowing with the receipt of pre-monsoon showers.

3.9.3.Herbicide application

Butachlor @ 1.25 kg a.i ha^{-1} and pretilachlor @ 0.75 kg a.i ha^{-1} were applied at 8 days after sowing. 2,4-D @ 1.00 kg a.i ha^{-1} was applied as post emergent at 20 days after sowing. Quantity of herbicide required for each plot was measured, mixed with water and sprayed using a spray volume of 500 litres per hectare. For spraying, an ASPEE knapsack sprayer fitted with a flood jet nozzle was used.

3.9.4. Manures and Fertilizers

Manures and Fertilizers were applied as per the treatments. In the hand weeded plot (T_1), continuous application of butachlor (T_2), butachlor alternated with pretilachlor between seasons (T_3) and butachlor alternated with pretilachlor between years (T_5) entire quantity of recommended NPK (70:35:35) was applied as inorganic fertilizers with half of N and K and full P as basal and the remaining half N and K as top dressing at 35 days after sowing. In the treatments T_4 and T_6, 25 per cent of the recommended NPK (70:35:35) was applied in the form of FYM at the time of land preparation and the remaining 75 per cent was applied as inorganic fertilizer with half of N and K and full P as basal and the rest as top dressing at 35 days after sowing.

3.9.5. Plant protection

According to the requirement, plant protection measures were carried out as per the package of practices recommendations of the Kerala Agricultural University (KAU, 1996).

3.9.6. Harvesting

Harvesting was done 80 days after sowing. Two border rows around the plot were harvested first and removed. The crop harvested from the net plot was threshed and weight was recorded for the separated grains and straw.

4.0. OBSERVATIONS

4.0.1. Physico chemical properties of soil

Major physico chemical properties of the soil viz., texture, pH, CEC, AEC, sesquioxide, organic carbon and the content of available N, P, and K before the experiment and at harvest of crop were determined. Methods adopted for the analysis are given in the Table 3.

4.0.2. Herbicide residues

Sampling

For the determination of herbicide residues in soil, three samples were collected randomly from each plot at 0-10cm depth. Sampling was done before spraying the herbicide, 1 and 30 DAS and at the time of harvest of the crop.

Grain and straw samples were collected at the time of harvest for the detection of herbicide terminal residues.

4.0.2.1. Estimation of residues of butachlor, pretilachlor and 2,4-D

4.0.2.1.1. Estimation of butachlor residue

4.0.2.1.1.1. From Soil

The method followed by Mabbayad *et al.* (1986) was used for extraction of butachlor residues from soil and plant samples. The clean up procedure and operating conditions of gas chromatograph with suitable column were standardised.

Extraction

Twenty five gram of the soil sample was placed in a 250 ml shaking bottle together with 100 ml acetone, the glass bottles with air tight caps were

Table 3. Methods for analysis of major physico-chemical properties of soil and plant samples

Sl.No.	Properties	Method	References
A. Soil			
1	Texture	Bouyoucos hydrometer method	Bouyoucos (1927)
2	pH	1:2.5 soil water suspension	Jackson (1958)
3	Cation exchange capacity	Sodium acetate method	Hesse (1972)
4	Anion exchange capacity	P fixing capacity	Hesse (1972)
5	Sesquioxides	AOAC method	Chopra and Kanwar (1976)
6	Organic carbon	Walkley-Black method	Jackson (1958)
7	Available N	Alkaline permanganate method	Subbiah and Asija (1956)
8	Available P_2O_5	Ascorbic acid reduced molybdophosphoric blue colour method	Watanabe and Olsen (1965)
9	Available K_2O	Neutral normal ammonium acetate extract using flame photometer	Jackson (1958)
B. Plant			
1	Total N	Microkjeldahl method	Jackson (1958)
2	Total P	Nitric-perchloric acid digest extract (2:1) estimated colorimetrically in spectrometer by yellow colour method	Jackson (1958)
3	Total K	Diacid digest extract method by using Flame photometer	Hesse (1971)

agitated for 30 min in a wrist action shaker. After the soil particles had settled the extract was passed through a whatman No.1 filter paper into a 250 ml round bottom flask. The soil was washed three times, each with 20 ml acetone and the funnel was washed once with 25 ml acetone.

Column clean up

The extract was concentrated to a moist residue in a water bath, with temperature maintained at 40°C. The moist residue was washed three times with 10ml dichloromethane. The residue and the dichloromethane washings were transferred to a 250 ml separating funnel. Fifty ml of one per cent sodium chloride was added to the funnel and the mixture was shaken manually for one min. The dichloromethane layer was passed through a funnel, lined with glass wool and sodium sulphate, into a round bottom flask. The residue was extracted twice with 20 ml dichloromethane from the aqueous phase. The sodium sulphate was washed with an additional 25 ml dichloromethane. The combined extract was passed through a column of activated neutral aluminium oxide (6g) packed along with anhydrous sodium sulphate (2g) at both ends. The column was washed twice with 25 ml dichloromethane. The clean eluate was kept in a water bath maintained at 40°C to evaporate dichloromethane. The residue was dissolved in n-hexane and the final volume was made up to 1 ml.

Estimation

One micro litre of the residue sample was injected into a gas chromatograph equipped with an electron capture detector and a 1.5 m x 4 mm i.d. glass column packed with 3% OV-1 on 80-100 mesh chromosorb W, AW. Carrier gas flow was 60 ml per min. Column, injection and detection temperatures were 210°, 240°and 270°C respectively. Quantification of the residue was done by comparing with the peak area of the standard.

4.0.2.1.1.2. From grain and straw

Powdered rice grain (40 g) and straw (20 g) samples were blended with 100 ml acetonitrile -water mixture (4:1 v/v) for 3 minutes. The samples were then filtered on a buchner funnel with suction and washed with 30 ml acetonitrile-water mixture twice. The filtrate was evaporated to dryness, using a rotary

evaporator at 50-60°C water bath. To the moist residue, 25 ml isopropanol-water (1:1 v/v) was added and the evaporation was continued. The moist residue was redissolved in dichloromethane after removing moisture through anhydrous sodium sulphate.

Column clean up

The extract was cleaned up by passing through selective absorbents, packed in a glass column (2 cm i.d. and 45 cm long). The absorbents are packed in the order of a small plug of glass wool at the tip, 0.5 cm layer of anhydrous sodium sulphate, 5 cm layer of activated florisil and 0.5 cm layer of anhydrous sodium sulphate. The column was prewashed with 25 ml dichloromethane. The dichloromethane extract of the rice grain or straw was transferred to the column and the eluate was collected. As the last portion of the extract solution entered the column, column was washed with 25 ml portion of dichloromethane twice to completely eluate out herbicide residue. The combined clean up extract collected in a beaker was kept in a water bath at a temperature of 50°C to evaporate dichloromethane. The dry residue was dissolved in n-hexane and the final volume was made up to 2 ml.

Estimation

Analysis was performed on a gas chromatograph as described in section (4.0.2.1.1.1)

4.0.2.1.2 Estimation of pretilachlor residues

4.0.2.1.2.1 From soil

The method proposed by Sankaran *et al.* (1993) was used for extraction of pretilachlor residues from soil and plant samples.

Extraction

The processed soil sample (25g) was moistened with 5 ml of distilled water and shaken for 2 h with 75 ml methanol and filtered.

Partitioning

The filtrate was transferred to a 250 ml separatory funnel and 100 ml of water and 10 ml of saturated sodium chloride solution were added. The aqueous solution was extracted thrice with 35 ml of hexane. The hexane phases

were collected, filtered through a plug of cotton and evaporated to dryness using a rotary vacuum flash evaporator.

Column clean up

The chromatographic column of 1.8 cm i.d. was filled to a height of 7 cm with alumina activity grade V and n-hexane was drained through the column. The residue dissolved in n-hexane was transferred to the chromatographic column, eluted with 50 ml of n-hexane and evaporated to dryness. The dry residue was dissolved in n-hexane and final volume was made up to 2 ml.

Estimation

Analysis was performed on a gas chromatograph with following conditions.

Detector: electron capture detector

Column: 5% SE 30 on chromosorb W

80-100 mesh

Temperature conditions

Injection: 220°C

Column: 210°C

Detector: 250°C

4.0.2.1.2.2. From grain and straw

To 50 g of milled grains or straw, 100 ml of acetonitrile was added and left over night. The contents were shaken for two hours in a mechanical shaker. The slurry was filtered through a fluted filter paper. The contents were rinsed with 30 ml acetonitrile and volume was adjusted to 100 ml. From this, 50 ml of aliquot corresponding to 25 g grain was taken and transferred to a 250 ml separatory funnel and the acetonitrile solution was extracted twice with 25 ml of hexane and the hexane was discarded. The active ingredient was re-extracted from the acetonitrile phase after addition of 125 ml water and 10 ml of sodium chloride solution with three 25 ml of portions of hexane by vigorously shaking the separatory funnel for two minutes during each extraction. The n-hexane phases

collected were pooled and filtered through a plug of cotton and evaporated to dryness using a rotary vacuum flash evaporator.

Column clean up and Estimation

The procedure followed was the same as that of soil sample presented in section 4.0.2.1.2.1

4.0.2.1.3. Estimation of 2,4-D residues

4.0.2.1.3.1. From soil

The method proposed by Sankaran *et al.* (1993) was used for extraction of 2,4-D residues from soil samples.

Extraction

To 25 g of the soil sample, 50 ml of solvent mixture (80:20:2.5 v/v) acetonitrile: distilled water: glacial acetic acid was added and shaken in a mechanical shaker for one hour. Later they were subjected to vacuum filtration using buchner funnel. The filtrate was transferred to a 250 ml separating funnel and 100 ml of sodium carbonate aqueous solution (50g of sodium carbonate in 1 litre of water) was added followed by 25 ml of hexane. After thorough shaking, the lower aqueous phase was separated and the organic phase (hexane phase) was collected after passing through sodium sulphate crystals. The process was repeated with aqueous phase with another 25 ml of hexane and the hexane portions were pooled.

The aqueous phase was acidified with 15 ml of conc. HCl and later 50 ml of diethyl ether was added. The ether portion was separated with another 50 ml portion of diethyl ether. The pooled ether was allowed to evaporate.

Derivatization

To the residue obtained after evaporation of diethyl ether 5 ml of boron trifluoride methanol mixture was added and placed in a water bath at 70°C for 45 min. Later 25 ml of saturated sodium chloride solution was added and the contents were transferred to 120 ml separatory funnel. To this 5 ml of hexane was added. After shaking the hexane portion was separated. The separation process was repeated 5 times, each with 5 ml hexane. Allowed the hexane portion to evaporate to 1 ml. This was passed through a column of 6 g silica and 2 g anhydrous sodium

sulphate using methylene chloride (30 ml) hexane mixture (70 ml). First 25 ml was rejected and collected the remaining 25 ml and allowed to evaporate. Then the residue was dissolved in n-hexane and 2 µl of the sample was injected into gas chromatograph.

Conditions for Gas chromatograph
Detector: Electron capture
Column: 1.5% OV 17 + 1.95% QF 1 *on chromosorb*
WCHP 100-120 mesh
Temperature conditions
Column: 210°C
Injector: 220°C
Detector: 240°C
Carrier gas flow: 30 ml/min

4.0.2.1.3.2 Grain and straw

The method suggested by QueeHee and Sutherland (1981) was used with suitable modifications.

Sampling

Five whole plants were collected randomly from each plot at harvest. Root portion was removed and the above ground portions were pooled. Grains were separated and the straw portions were pooled and chopped in to small pieces.

From grain

Ten gram of the grain sample was ground in a mixer grinder and macerated with 150 ml water. The extract was acidified with conc. HCl (15 ml) and kept over night. This was filtered and extracted five times each with 10 ml diethyl ether. The ether portion was allowed to evaporate. Esterification, clean up and estimation procedures were same as in the case of soil samples (Section 4.0.2.1.3.1).

From straw

Ten gram of the chopped sample was macerated with 100 ml water. Ten ml of 0.6 N NaOH was added and refluxed for two hours. This was filtered through an oilcloth and washed with 100 ml water. The filter cake was suspended in 50 ml of 2 M HCl, refluxed for two hours and extracted thrice each with 50 ml diethyl ether. The ether and alkaline extracts were combined and the ether portion was separated and allowed to evaporate Then 30 ml of 10 per cent NaOH was added so that the solution pH was greater than thirteen.

The aqueous alkaline solution was refluxed for 20 minutes, cooled and extracted thrice, each with 100 ml of diethyl ether. The caustic portion was acidified with concentrated HCl (pH=2) and extracted thrice each with 100 ml of diethyl ether. This procedure of acid and alkaline hydrolysis separates 2,4-D from conjugates and other compounds present in the plant material (QueeHee and Sutherland, 1981). The ether portions were mixed and allowed to evaporate. The separation, clean up and derivatization procedures were same as in case of soil samples.

4.0.3. Microbiological studies

During the first crop of 2001, enumeration of soil micro flora was done at 0, 1, 7 DAS and at the time of harvest. During second crop season of the same year observations were taken at 1, 7, 15, 30 and 45 DAS and at the time of harvest. During the first and second crop of 2002, samples were made at 0, 1, 7, 15, 30 and 45 DAS and at the time of harvest.

4.0.3.1. Quantitative estimation of microflora

The quantitative assay of microflora was carried out by serial dilution plate technique. The soil sample (10.0 g) was added to 100 ml sterile distilled water in 250 ml conical flask and shaken for 30 min in an orbital shaker. Ten ml of this soil dilution was then transferred to another flask containing 90 ml sterile distilled water to get 10^{-2} dilution. Later 10^{-3},10^{-4} and 10^{-6} dilutions were prepared from this by serial dilution. The composition of media used for assaying micro organisms are presented in Table 4.

4.0.3.2. Estimation of fungal population

One ml of 10^{-3} soil dilution was pipetted in to sterile petridishes to which 20 ml of melted and cooled Martin's rose Bengal streptomycin agar medium was poured (Table 4). Three petridishes were kept as replications for the sample. The petridishes with the medium were swirled thoroughly to get uniform distribution. After solidification, the dishes were incubated at room temperature for three days. The fungal colonies developed were counted using dark field colony counter and expressed as number of colonies per gram of dry soil.

4.0.3.4. Estimation of bacterial population

Bacterial population was estimated using 10^{-6} soil dilution in nutrient agar medium (Table 4). The method employed for the estimation of fungal population was followed. The dishes were incubated for 48 h at room temperature. The bacterial colonies developed were counted and expressed as number of colonies per gram of dry soil.

4.0.3.3. Estimation of actinomycetes population

The estimation of actinomycete population was done with a soil dilution of 10^{-4} using Kenknight's agar medium (Table 4) and the method followed was similar to that of the estimation of fungal population. The dishes were incubated for seven days at room temperature and the actinomycete colonies were counted, using dark field colony counter and expressed as number of colonies per gram of dry soil.

4.0.4. Biometric observations

4.0.4.1. Species wise weed count and dry matter production at 60 day after sowing

Quadrat of 0.25 m^2 was kept randomly at three places in each plot. The weeds coming under these areas were removed and their species wise counts were recorded. Then they were kept in an electric oven at 80°C and the total dry weight per quadrat was multiplied with four so as to express the data on square metre basis.

Table 4. Media composition for serial dilution plate technique

Micro organisms assayed	Name of the growth media	Composition of the growth media
Bacteria	Soil extract agar media	Glucose : 1 g KH_2PO_4 : 0.5 g Soil extract : 100 ml Distill water : 900 ml Agar : 20 g
Fungi	Martin's Rose Bengal agar media	Dextrose : 10 g Peptone : 5 g KH_2PO_4 : 1 g $MgSO_4$: 20g Agar : 20 g Rose Bengal : 1 Part in 3000 parts Distilled water : 1 litre
Actinomycetes	Kenknight's media	pH : 7.0 Glucose : 1g KH_2PO_4 : 0.1 g $NaNO_3$: 0.1 g KCl : 0.1 g $MgSO_4.7H_2O$: 0.1 g Agar : 20 g Distilled water : 1 litre

4.0.4.2. Height of plant and productive tillers at 60 days after sowing

Ten plants from each of the above quadrats were selected and their height and number of productive tillers were recorded and expressed on square metre basis.

4.0.4.2.3. Grain and straw yield at harvest

Total grain and straw yield from each plot was recorded and expressed on hectare basis.

4.0.5 Determination of major nutrients in the rice grain and straw

Grain and straw samples of the first and second crop of 2002 were analysed for total N, P and K as per the standard procedures mentioned in the table 3.

4.0.6. Statistical analysis.

Analysis of variance for one-way classification of data was done. Sampling variation within a plot (treatments) was considered as experimental error and comparison of treatments made accordingly.

Results

4. RESULTS

Field experiments and laboratory analysis were carried out during 2001 -2002 to investigate the persistence of common rice herbicides in rice-rice system. Residues of herbicides present in the soil, rice grain and straw were monitored during the two crop seasons of 2002. Changes in the soil microflora due to the herbicide application were analysed over a period of four crop seasons from 2001 to 2002. Effect of herbicide application on the population of major weed species in the rice field was also observed in the four crop seasons of the study period in 2001 and 2002. Results of the investigations are presented in this section.

4.1. PHYSICO CHEMICAL CHARACTERISTICS OF THE SOIL

The major physico chemical characteristics of the soil samples taken from the experimental field before the start of experiment are presented in Table 5. Soil samples taken from the six plots of the field were homogenous with respect to most of their properties.

Based on the sand, silt and clay content of the samples, the soil could be classified as sandy clay loam in texture. The sand, silt and clay content varied from 58.23 to 68.35 per cent, 4.00 to 13.90 per cent and 25.98 to 27.98 per cent respectively.

pH of the soil samples was in the range of 4.5 to 5.6. Cation and anion exchange capacities were more or less similar and these characteristics showed variation of 8.40 to 9.25 C mol (+) kg^{-1} and 7.30 to 12.40 C mol (-) kg^{-1} respectively. Silica content of the soil samples was very high and it ranged from 65.00 to 70.20 per cent and their sesquioxide content varied from 2.50 to 4.60 per cent.

The soil samples were medium in the organic carbon content (0.75 to 1.42%) and available nitrogen content was in the range of low to medium (209.05 to 324.05 kg ha^{-1}).

Table 5. Physico chemical characteristics of the soil

Soil characteristics	Range
Sand %	58.23 - 68.35
Silt %	4.00 - 13.90
Clay %	25.98 - 27.98
pH	4.5 - 5.6
CEC (C mol (+) kg^{-1})	8.40 - 9.25
AEC (C mol (-) kg^{-1})	7.30 – 12.40
Silica %	65.00– 70.20
Sesquioxides %	2.50 – 4.60
Organic carbon %	0.75 - 1.42
Available N, kg ha^{-1}	209.05 - 324.05
Available P, kg ha^{-1}	18.51 - 31.96
Available K, kg ha^{-1}	111.25 - 149.33

Available potassium status of the soil also ranged from low to medium (111.25 to 149.33 kg ha^{-1}) while the available phosphorus content ranged from medium to high (18.51 to 31.96 kg ha^{-1}).

4.2. EFFECT OF TREATMENTS ON SOIL CHARACTERISTICS

Studies on the persistence of herbicides in the rice-rice system were conducted in the first and second crop seasons of 2002. Therefore changes in important soil characteristics viz., organic carbon and available nutrients were monitored before the start of the experiment in 2002, after harvest of the first and second crop of 2002. The data are presented in Table 6.

4.2.1. Before the experiment (2002)

The organic carbon content of the soil ranged from 0.75 to 1.31 per cent and various treatments differed from each other. Among the treatments, butachlor applied with FYM (T_4) was found to be highest with respect to organic carbon content (1.31%). Hand weeded plot recorded the lowest value of 0.75 per cent. Butachlor applied without FYM (T_2 and T_3) and butachlor applied with FYM alternated with pretilachlor in the second year (T_6) recorded organic carbon content of 1.08, 1.24 and 1.02 per cent respectively.

Available nitrogen did not show any significant difference between treatments (294.21-303.63 kg ha^{-1}). Available phosphorous was in the range of 24.46-31.96 kg ha^{-1} and it showed significant variation among the treatments. The treatments T_1, T_2 and T_4 recorded higher values of 28.02, 31.96, 30.74 kg ha^{-1} respectively, while T_3, T_5 and T_6 recorded comparatively lower values of available P (24.73, 25.03, 24.46kg ha^{-1} respectively). Available potassium ranged from 125.43-149.33 kg ha^{-1} and the treatments T_4, T_5 and T_6 recorded higher values of available K (149.33, 144.85 and 144.11 kg ha^{-1} respectively). The hand weeded plot was very low in available K (125.43 kg ha^{-1}) while the T_2 and T_3 recorded comparable values of 137.39 and 134.40 kg ha^{-1} respectively.

Table 6. Changes in the organic carbon and nutrient status after application of herbicides over a period of two seasons.

Treatment No.	Before experiment				After harvest of first crop				After harvest of second crop			
	OC (%)	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})	OC (%)	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})	OC (%)	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})
T_1	0.75	296.85	28.02	125.43	0.93	291.07	25.36	124.69	0.89	324.05	25.68	128.43
T_2	1.08	297.33	31.96	137.39	0.90	292.10	23.23	132.15	1.00	209.05	24.79	111.25
T_3	1.24	297.33	24.73	134.40	1.06	292.13	26.85	138.11	0.87	240.43	25.98	117.97
T_4	1.31	303.63	30.74	149.33	1.16	291.90	19.41	147.83	0.93	245.65	20.90	121.71
T_5	0.99	294.21	25.03	144.11	0.87	290.53	24.73	143.36	1.08	256.11	29.12	119.47
T_6	1.02	300.00	24.46	144.85	1.42	294.97	20.67	146.33	0.77	266.58	18.51	114.24
CV %	8.31	1.12	9.33	2.73	14.78	1.03	15.43	1.98	16.10	39.36	11.59	6.60
CD(0.05)	0.159	NS	4.563	6.770	0.275	NS	NS	4.886	NS	NS	4.981	NS

4.2.2. After harvest of the first crop

Organic carbon content varied from 0.87 to 1.42 per cent between the treatments. The plots in which FYM was applied in the first crop season (T_6 and T_4) recorded higher values of 1.42 and 1.16 per cent respectively. The other four treatments were more or less similar in the organic carbon content and the variation was in the range of 0.87 to 1.06 per cent.

Available nitrogen was in the range of 290.53-294.97 kg ha^{-1}. No significant differences between treatments were observed. Available phosphorus ranged from 19.41-26.85 kg ha^{-1} and the treatments did not differ significantly.

Available potassium showed significant differences between treatments. Higher values have been shown by T_4, T_5 and T_6 (147.83, 143.36 and 146.33 kg ha^{-1} respectively) and lower values was recorded by hand weeded plot, T_2 and T_3 (124.69, 132.15 and 138.11 kg ha^{-1} respectively). The results were almost similar to that observed before the experiment.

4.2.3. After harvest of the second crop

Organic carbon content of the soil samples ranged from 0.77 to 1.08 per cent and there was no significant difference between the treatments.

Available nitrogen was in the range of 209.05-324.05 kg ha^{-1} and the treatments did not vary significantly. Available phosphorus showed significant differences between treatments. As recorded after the harvest of the first crop, hand weeded plot (T_1), butachlor applied without FYM alternated with pretilachlor in the second crop (T_3) and butachlor applied without FYM alternated with pretilachlor in the second year (T_5) showed higher values of available P (25.68, 25.98 and 29.12 kg ha^{-1} respectively). The treatments viz., butachlor applied continuously without FYM (T_2), butachlor applied with FYM alternated with pretilachlor in the second crop (T_4) and butachlor applied with FYM alternated with pretilachlor in the second year (T_6) recorded lower values (24.79, 20.90 and 18.51 respectively).

Available potassium ranged from 111.25-128.43 kg ha^{-1} and no significant differences between treatments were observed.

4.3. PERSISTENCE OF HERBICIDE RESIDUES IN SOIL AND PLANT

Herbicide residue analysis was conducted during the first and second crop seasons of 2002. Residues of herbicides remaining in the soil samples were estimated at 1 and 30 days after spraying and at harvest of the crop. Grain and straw samples were also analysed for their respective herbicide residue contents. The data are presented in Table 7 to 9

4.3.1. Butachlor residues

During the first crop of 2002, butachlor residues in the soil at 1 DAS ranged from 0.331 to 0.396 μg g^{-1}. The highest value (0.396 μg g^{-1}) was recorded in the treatment where FYM was incorporated (T_4). Lower values (0.331 and 0.389 μg g^{-1} respectively) were recorded by butachlor applied without FYM (T_2, T_3). At 30 DAS, residues of butachlor was in the range of 0.020 to 0.041 μg g^{-1} and treatment in which FYM was applied recorded the lowest value of 0.020 μg g^{-1}. Higher values of 0.024 and 0.041 μg g^{-1} were recorded by T_2 and T_3 respectively. At the time of harvest no butachlor residues were detected in the soil, grain and straw samples.

In the second crop season 2002, only one plot (T_2) received the spray of butachlor and the residues estimated at 1 and 30 DAS were 0.343 and 0.020 μg g^{-1} respectively. No residues were detected in the soil, grain or straw samples at the time of harvest.

4.3.2. Pretilachlor residues

During the first crop season of 2002, pretilachlor was sprayed in the two plots (T_5 and T_6), which received butachlor during the first and second crop of 2001. The soil samples registered residues of 0.215 and 0.200 μg g^{-1} at 1 DAS and 0.020 and 0.013 μg g^{-1} at 30 DAS respectively. At harvest stage, residues were not detected in soil, grain or straw.

Table.7. Butachlor residues in the soil and plant (µg g^{-1}) during the first and second crop seasons of 2002

Treatments	First crop					Second crop				
	Soil			Plant		Soil			Plant	
	1 DAS	30 DAS	Harvest	Grain	Straw	1 DAS	30 DAS	Harvest	Grain	Straw
T_1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
T_2	0.331	0.024	ND	ND	ND	0.343	0.020	ND	ND	ND
T_3	0.389	0.041	ND	ND	ND	NA	NA	NA	NA	NA
T_4	0.396	0.020	ND	ND	ND	NA	NA	NA	NA	NA
T_5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
T_6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

* ND - Not detected
* NA – No application of butachlor

Table.8.Pretilachlor residues in the soil and plant ($\mu g\ g^{-1}$) during the first and second crop seasons of 2002

Treatments	First crop					Second crop				
	Soil			Plant		Soil			Plant	
	1 DAS	30 DAS	Harvest	Grain	Straw	1 DAS	30 DAS	Harvest	Grain	Straw
T_1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
T_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
T_3	NA	NA	NA	NA	NA	0.227	0.030	ND	ND	ND
T_4	NA	NA	NA	NA	NA	0.199	0.010	ND	ND	ND
T_5	0.215	0.020	ND	ND	ND	0.231	0.025	ND	ND	ND
T_6	0.200	0.013	ND	ND	ND	0.250	0.010	ND	ND	ND

* ND - Not detected
* NA - No application of pretilachlor

In the second crop season, all the treatments except hand weeded control (T_1) and continuous application of butachlor without FYM (T_2) received pretilachlor spraying and the residues in soil at 1 DAS ranged from 0.199 to 0.250 μg g^{-1}. The highest value of 0.250 μg g^{-1} was registered by butachlor applied with FYM alternated with pretilachlor in the second year (T_6) and the lowest value of 0.199μg g^{-1} was registered by butachlor applied with FYM alternated with pretilachlor in the second crop (T_4). Pretilachlor residues in the soil samples at 30 DAS ranged from 0.010 to 0.030 μg g^{-1}. Higher values of 0.030 and 0.025 μg g^{-1} were recorded by butachlor applied without FYM alternated with pretilachlor in the second crop (T_3) and butachlor applied without FYM alternated with pretilachlor in the second year (T_5). The lowest value of 0.010 μg g^{-1} was recorded by both T_4 and T_6. When the soil, grain and straw samples were analysed at the time of harvest, no residues were detected in them.

4.3.3. 2,4-D residues

All the treatments except hand weeded plot received 2,4-D spraying. Residues of 2,4-D in soil at 1 DAS ranged from 0.310 to 0.502 μg g^{-1} . The plots in which FYM was applied (T_4 and T_6) registered higher residues and the corresponding values were 0.502 and 0.400 μg g^{-1}. Butachlor fb 2,4-D applied without FYM (T_2,T_3) and butachlor fb 2,4-D applied without FYM alternated with pretilachlor in the second year (T_5) recorded 2,4-D residues of 0.385, 0.310, 0.390 μg g^{-1} respectively.

At 30 DAS, butachlor fb 2,4-D applied with FYM (T_4), T_5 and butachlor fb 2,4-D applied with FYM alternated with pretilachlor in the second year (T_6) registered 2,4-D residues of 0.015, 0.015 and 0.010 μg g^{-1}respectively. The treatments T_2 and T_3 recorded higher value of 0.020 and 0.035 μg g^{-1} respectively.

At the time of harvest soil and plant samples (both grain and straw) did not contain detectable quantity of 2,4-D residues.

In the second crop season, 2,4-D residues at 1 DAS ranged from 0.395 to 0.480 μg g^{-1}. Butachlor fb 2,4-D applied continuously without FYM (T_2) recorded the

Table.9.2,4-D residues in the soil and plant ($\mu g\ g^{-1}$) during the first and second crop seasons of 2002

Treatments	First crop					Second crop				
	Soil			Plant		Soil			Plant	
	1 DAS	30 DAS	Harvest	Grain	Straw	1 DAS	30 DAS	Harvest	Grain	Straw
T_1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
T_2	0.385	0.020	ND	ND	ND	0.480	0.016	ND	ND	ND
T_3	0.310	0.035	ND	ND	ND	0.420	0.015	ND	ND	ND
T_4	0.502	0.015	ND	ND	ND	0.400	0.005	ND	ND	ND
T_5	0.390	0.015	ND	ND	ND	0.470	0.010	ND	ND	ND
T_6	0.400	0.010	ND	ND	ND	0.395	0.005	ND	ND	ND

* ND - *Not detected*
* NA - No applicaton of 2,4-D

highest value (0.480 μg g^{-1}) followed by T_3 and T_5 which had residues 0.420 and 0.470 μg g^{-1} respectively. The treatments T_4 and T_6 recorded comparatively lower residues (0.400 and 0.395 μg g^{-1} respectively). At harvest, 2,4-D residue was not detected in the soil, grain or straw.

4.4. EFFECT OF HERBICIDES ON THE POPULATION OF MICROORGANISMS IN SOIL

Effect of herbicides on the population of soil microflora (bacteria, fungi, actinomycetes) was studied over a period of two years and the data are presented in Table 10-18.

4.4.1. Soil bacteria

Effect of treatments on bacterial population for the year 2001 is presented in Table 10 and for the year 2002 in Tables 11&12.

During first crop of 2001, population of bacteria showed variation at one DAS and 7 DAS but did not show variation before spraying and at harvest. At 1 DAS, butachlor applied with FYM (T_4 and T_6) varied significantly from all other treatments. Highest count of bacteria (5.3 x 10^6 cfu g^{-1} soil) was recorded by T_6 followed by T_4 with a count of 4.6 x 10^6 cfu g^{-1} soil. The lowest count of 1.6 x 10^6 cfu g^{-1} was recorded by butachlor applied without FYM (T_3, T_5).

At 7 DAS, T_4 was on par with T_1 and T_6. The treatment T_4 recorded highest count (3.0 x 10^6 cfu g^{-1} soil) followed by T_1 and T_6 with a count of 2.6 x 10^6 cfu g^{-1} soil and the lowest count of 0.6 x 10^6 cfu g^{-1} was recorded by butachlor applied without FYM (T_5). During second crop of 2001, variation was observed in the population of bacteria at the time of harvest. Butachlor applied with FYM in first crop (T_6) showed the highest number (4.3 x 10^{-6} cfu g^{-1} soil) followed by hand weeded plot) and butachlor applied with FYM alternated with pretilachlor in the second crop (T_4) with a count of 3.0 x 10^6 cfu g^{-1} soil. The lowest count of 1.6 x 10^6 cfu $g^{-1}$was recorded by T_5 and T_2.

Table 10. Population of soil bacteria (x 10^6 g^{-1} of soil) during the first and second crop of 2001

Treatments	First crop				Second crop					
	0 DAS	1 DAS	7 DAS	Harvest	1 DAS	7 DAS	15 DAS	30 DAS	45 DAS	Harvest
T_1	2.24 (4.6)*	1.86 (3.0)	1.76 (2.6)	1.86 (3.0)	1.54 (2.3)	1.27 (1.3)	1.27 (1.3)	1.33 (1.3)	1.58 (2.0)	1.87 (3.0)
T_2	2.08 (4.0)	1.56 (2.0)	1.05 (0.6)	1.86 (3.0)	1.29 (1.3)	0.88 (0.3)	0.99 (0.6)	1.22 (1.0)	1.87 (3.0)	1.45 (1.6)
T_3	1.76 (2.6)	1.46 (1.6)	1.17 (1.0)	1.56 (2.0)	1.17 (1.0)	0.71 (0.0)	0.99 (0.6)	1.61 (0.6)	1.67 (2.3)	1.58 (2.0)
T_4	2.34 (5.0)	2.56 (4.6)	1.86 (3.0)	1.28 (4.3)	1.74 (3.3)	1.17 (1.0)	1.77 (2.6)	1.34 (1.3)	2.74 (7.3)	1.87 (3.0)
T_5	2.18 (4.3)	1.44 (1.6)	1.05 (0.6)	1.46 (1.6)	0.99 (0.6)	0.99 (0.6)	1.35 (1.6)	1.34 (1.3)	2.66 (3.3)	1.45 (1.6)
T_6	1.84 (3.0)	2.38 (5.3)	1.76 (2.6)	2.21 (4.6)	2.11 (4.0)	1.39 (1.6)	1.17 (1.0)	1.58 (2.0)	2.47 (5.6)	2.19 (4.3)
CD (0.05)	NS	0.631	0.155	NS	NS	NS	NS	NS	NS	0.110
CV %	17.04	19.42	22.63	20.44	45.20	42.62	40.75	45.52	41.00	15.89

*Values in parentheses indicate original values

Table 11. Population of soil bacteria ($x10^6$ g^{-1} of soil) during the first crop season of 2002

Treatments	Before spraying	1 DAS	7 DAS	15 DAS	30 DAS	45 DAS	Harvest
T_1	2.86 (8.0)*	5.14 (26.0)	3.39 (14.6)	3.32 (10.6)	2.90 (8.0)	2.67 (6.6)	3.23 (10.0)
T_2	3.61 (12.6)	2.53 (6.0)	0.10 (0.6)	1.27 (1.3)	1.73 (3.3)	1.64 (2.6)	2.60 (6.3)
T_3	3.80 (14.0)	3.41 (12.3)	1.18 (1.3)	1.47 (2.0)	0.10 (0.6)	1.47 (2.0)	3.50 (12.0)
T_4	4.81 (22.6)	2.46 (5.6)	4.35 (19.0)	2.73 (7.3)	2.04 (3.6)	2.64 (6.6)	3.54 (12.6)
T_5	3.04 (10.0)	2.89 (8.0)	1.17 (1.0)	0.10 (0.6)	1.64 (2.3)	2.44 (5.6)	3.37 (11.3)
T_6	4.02 (16.6)	1.56 (2.0)	1.35 (1.6)	2.35 (5.3)	1.93 (3.3)	2.67 (6.6)	3.20 (10.3)
CD(0.05)	NS	1.146	2.052	1.103	1.023	NS	NS
CV %	22.70	21.48	55.64	30.66	30.68	25.72	21.87

*Values in parentheses indicate original values

During first crop of 2002, population of bacteria did not show any variation among treatments before spraying, 45 DAS and at harvest. But it showed significant variations at 1 DAS, 7 DAS, 15 DAS and 30 DAS. At 1 DAS, hand weeded control showed the highest number (26.0 x 10^6 cfu g^{-1}soil) followed by butachlor applied without FYM (T_3) with a count of 12.3 x 10^6 cfu g^{-1}soil. The lowest count (2.0 x 10^6 cfu g^{-1}soil) was recorded by butachlor applied with FYM alternated with pretilachlor in the second year (T_6). Butachlor applied with FYM (T_4), butachlor applied without FYM alternated with pretilachlor in the second year (T_5) recorded a total count of 5.6 x 10^{-6} g^{-1} soil and 8.0 x 10^6 cfu g^{-1}soil respectively.

At 7 DAS, T_4 showed highest value of 19.0 x 10^6 cfu g^{-1}soil and this treatment was superior to all the other treatments. The lowest count of 0.6 x 10^6 cfu g^{-1}soil was recorded by T_2 followed by T_5, T_3 and T_6 with count of 1.0, 1.3 and 1.6 x 10^6 cfu g^{-1} soil respectively. At 15 DAS hand weeded control recorded the highest count of 10.6 x 10^6 cfu g^{-1}soil, which was on par with T_4 and T_6. The other treatments T_2, T_3 and T_5 recorded comparatively lower counts (1.3, 2.0 and 0.6 x 10^6 cfu g^{-1}soil respectively).

At 30 DAS, highest count of 8.0 x 10^6 cfu g^{-1}soil was recorded by hand weeded control, which was on par with T_4 (3.3 x 10^6 cfu g^{-1}soil) and T_6 (3.3 x 10^6 cfu g^{-1}soil). Similar to 15 DAS, the other treatments recorded smaller counts.

During second crop of 2002, population of bacteria did not vary significantly before spraying, 1DAS, 45 DAS and at harvest. Significant variation between treatments was observed at 7, 15 and 30 DAS. At 7 DAS, hand weeded plot showed the maximum count of 11.3 x 10^6 cfu g^{-1} soil, which was superior to all the other treatments. Butachlor applied with FYM alternated with pretilachlor in the second crop (T_4) and T_6 recorded similar counts (4.3 and 4.6 x 10^6 cfu g^{-1}soil respectively). Very low counts were recorded by butachlor applied continuously without FYM (T_2) and T_5.

Table 12. Population of soil bacteria (x 10^6 g^{-1} of soil) during the second crop season of 2002

Treatments	Before spraying	1 DAS	7 DAS	15 DAS	30 DAS	45 DAS	Harvest
T_1	2.55 (13.3)*	2.93 (8.6)	3.42 (11.3)	3.46 (11.6)	3.36 (11.0)	2.64 (6.6)	3.68 (13.3)
T_2	3.05 (9.3)	1.64 (2.6)	0.88 (0.3)	1.09 (1.0)	1.86 (3.0)	2.93 (8.3)	3.23 (10.0)
T_3	3.93 (15.0)	1.05 (0.6)	1.47 (2.0)	1.42 (1.0)	2.04 (4.0)	2.34 (5.3)	3.15 (9.6)
T_4	3.0 (8.6)	1.79 (3.3)	2.18 (4.3)	2.16 (4.3)	2.91 (8.3)	3.30 (10.6)	3.34 (11.0)
T_5	3.69 (13.3)	1.17 (1.0)	1.05 (0.6)	1.74 (2.6)	1.51 (2.3)	2.30 (5.0)	3.76 (13.6)
T_6	2.50 (7.0)	2.30 (5.0)	2.25 (4.6)	2.30 (5.0)	2.97 (8.6)	2.25 (6.0)	2.92 (9.0)
CD(0.05)	NS	NS	0.773	1.074	1.148	NS	NS
CV %	26.95	39.65	23.19	29785	26.34	31.66	20.77

*Values in parentheses indicate original values

At 15 DAS, hand weeded control varied significantly from all the herbicide applied treatments. The treatments T_6, T_4, T_5 and T_3 were on par with respect to their count of bacteria. Hand weeded plot recorded highest count (11.6 x 10^6 cfu g^{-1}soil) and T_2 recorded lowest count (1.0 x 10^6 cfu g^{-1}soil). At 30 DAS, hand weeded plot showed significantly highest count of 11.0 x 10^6 cfu g^{-1}soil. The treatments T_5, T_2, T_3 recorded the lowest count of 2.3, 3.0, 4.0 x 10^6 cfu g^{-1}soil.

4.4.2. Soil fungi

Effect of herbicide treatments on fungal population for the year 2001 presented in Table 13 and for the year 2002 in Tables 14&15.

During first crop of 2001, population of fungi did not show significant variation among treatments at any sampling interval. But in second crop, significant variation between treatments was observed only at 15 DAS. Hand weeded plot showed the highest number (5.3 x 10^3 cfu g^{-1}soil) varied significantly from all the other treatments. Butachlor applied with FYM alternated with pretilachlor in the second crop (T_4) registered comparatively higher number of fungi colonies than that of butachlor applied continuously without FYM (T_2 and T_5). The lowest count of 0.3 x 10^3 cfu g^{-1} soil was recorded by T_5.

In first crop of 2002, population of fungi did not show significant difference before spraying, 1 DAS, 45 DAS and at harvest. But their counts varied significantly at 15 DAS and 30 DAS. Similar observations were noticed in the second crop also.

During first crop at 15 DAS, butachlor applied with FYM alternated with pretilachlor in the second year (T_6) recorded very high counts (6.0 x 10^3 cfu g^{-1} soil) for soil fungi and it varied significantly from hand weeded plot. Butachlor applied with FYM varied significantly from butachlor applied without FYM. Lowest count of 0.6 x 10^3 cfu g^{-1}soil was recorded by T_2 followed by T_3 with a count of 1.0 x 10^3 cfu g^{-1}soil.

Table 13. Population of soil fungi (x 10^3 g^{-1} of soil) during first and second crop season of 2001

Treatments	First crop				Second crop					
	0 DAS	1 DAS	7 DAS	Harvest	1 DAS	7 DAS	15 DAS	30 DAS	45 DAS	Harvest
T_1	2.75 (10.0)*	2.20 (4.3)	2.30 (5.0)	1.61 (2.6)	1.68 (3.0)	1.84 (3.0)	2.36 (5.3)	2.12 (4.0)	1.67 (2.3)	1.67 (2.3)
T_2	2.13 (5.6)	1.46 (1.6)	1.56 (2.0)	1.56 (2.0)	1.05 (0.6)	0.88 (0.3)	1.17 (1.0)	1.76 (2.6)	2.12 (4.0)	1.58 (2.0)
T_3	2.67 (6.6)	1.64 (2.3)	1.34 (1.3)	1.68 (2.3)	1.17 (1.0)	1.10 (1.0)	1.18 (1.3)	1.58 (2.0)	1.22 (1.0)	1.45 (1.6)
T_4	2.86 (5.3)	1.91 (3.6)	2.04 (3.6)	1.91 (3.6)	1.67 (3.3)	1.72 (2.6)	1.86 (3.0)	2.02 (3.6)	2.74 (7.0)	3.02 (3.6)
T_5	2.46 (5.6)	1.74 (2.6)	1.64 (2.3)	1.47 (2.0)	1.56 (2.0)	0.88 (0.3)	0.88 (0.3)	1.76 (2.6)	1.45 (1.6)	1.34 (1.3)
T_6	2.41 (5.6)	2.11 (4.0)	2.08 (4.0)	1.64 (2.3)	1.94 (4.6)	1.18 (1.3)	1.44 (1.6)	3.02 (8.6)	2.26 (4.6)	1.87 (3.0)
CD (0.05)	NS	NS	NS	NS	NS	NS	0.381	NS	NS	NS
CV %	48.80	25.52	21.24	39.16	60.88	43.55	34.55	42.01	43.25	29.91

*Values in parentheses indicate original values

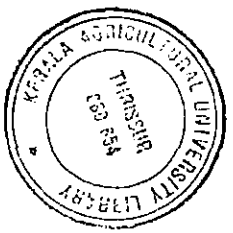


Table 14. Population of soil fungi (x 10^3 g^{-1} of soil) during the first crop season of 2002

Treatments	Before spraying	1 DAS	7 DAS	15 DAS	30 DAS	45 DAS	Harvest
T_1	2.58 (6.3)*	2.34 (5.0)	2.25 (4.6)	1.86 (3.0)	2.20 (4.3)	2.79 (7.3)	2.90 (8.0)
T_2	1.93 (3.3)	1.86 (3.0)	1.27 (1.3)	1.05 (0.6)	1.05 (0.6)	2.32 (5.0)	2.62 (6.6)
T_3	2.09 (4.0)	1.96 (3.6)	1.93 (3.0)	2.03 (1.0)	1.17 (1.0)	2.73 (7.0)	3.05 (9.0)
T_4	2.93 (8.3)	2.15 (4.3)	2.54 (6.0)	2.03 (3.6)	2.03 (3.6)	2.85 (7.6)	2.04 (7.3)
T_5	2.11 (4.0)	1.46 (1.6)	1.56 (2.0)	1.86 (3.0)	1.39 (1.6)	2.34 (5.0)	2.59 (6.3)
T_6	3.08 (9.0)	2.0 (3.6)	2.02 (4.6)	2.53 (6.0)	2.04 (3.6)	2.68 (7.0)	3.20 (10.0)
CD(0.05)	NS	NS	NS	0.590	0.634	NS	NS
CV %	20.59	22.80	32.53	18.98	21.66	14.79	20.90

*Values in parentheses indicate original values

Table 15. Population of soil fungi (x 10^3 g^{-1} of soil) during the second crop season of 2002

Treatments	Before spraying	1 DAS	7 DAS	15 DAS	30 DAS	45 DAS	Harvest
T_1	2.0 (3.6)*	2.60 (6.3)	2.76 (7.3)	2.58 (6.3)	2.89 (8.0)	1.78 (3.0)	2.04 (3.6)
T_2	2.16 (4.6)	1.96 (3.6)	1.64 (2.6)	1.34 (1.3)	1.56 (2.0)	2.59 (6.3)	2.40 (5.6)
T_3	2.18 (4.3)	2.08 (4.0)	1.46 (1.6)	1.27 (1.3)	1.17 (1.0)	2.89 (8.0)	1.91 (3.6)
T_4	2.10 (4.3)	2.11 (4.6)	1.81 (3.0)	1.81 (3.0)	2.67 (7.0)	2.18 (4.3)	2.83 (7.6)
T_5	1.86 (3.0)	1.86 (3.0)	1.17 (1.0)	0.10 (0.6)	2.18 (4.3)	1.91 (3.6)	2.37 (6.0)
T_6	1.94 (4.3)	2.60 (6.3)	2.08 (4.0)	2.02 (3.6)	2.66 (6.6)	2.53 (6.0)	2.11 (4.0)
CD(0.05)	NS	NS	NS	0.840	0.849	NS	NS
CV %	36.91	26.14	30.31	28.28	21.81	24.59	31.04

*Values in parentheses indicate original values

At 30 DAS hand weeded control showed significant variation for soil fungi with all herbicide treated plots without FYM. More or less similar counts were obtained in hand weeded control and the herbicide treated plots with FYM (4.3 and 3.6 x 10^3 cfu g^{-1}soil). The lowest value of 0.6 x 10^3 cfu g^{-1}soil was registered by T_2 followed by T_3 with a count of 1.0 x 10^3 cfu g^{-1}soil.

During the second crop at 15 DAS also hand weeded control showed significant differences from herbicide treated plots which received no FYM in the first crop season (T_2, T_3 and T_5). Butachlor applied without FYM alternated with pretilachlor in the second crop (T_4) and butachlor applied with FYM alternated with pretilachlor in the second year (T_6) varied significantly with butachlor applied without FYM alternated with pretilachlor in the second year (T_5). T_1 recorded highest count (6.3 x 10^3 cfu g^{-1}soil) and T_5 recorded the lowest count of 0.6 x 10^3 cfu g^{-1}soil.

At 30 DAS, hand weeded control, T_4 and T_6 showed higher counts of 8.0, 7.0 and 6.6 x 10^3 cfu g^{-1}soil respectively. The lower counts (2.0 and 1.0 x 10^3 cfu g^{-1} soil) were recorded by T_2 and T_3 respectively.

4.4.3. Soil actinomycetes

Effect of treatments on actinomycetes population for year 2001 presented in Table 16 and for the year 2002 presented in Tables 17 &18.

During first crop season of 2001, population of soil actinomycetes did not show significant variation between treatments at any interval. But in the second crop significant variation was observed at 1 DAS, 45 DAS and at harvest.

At 1 DAS, butachlor applied with FYM alternated with pretilachlor in the second year (T_6) recorded the highest count of 7.3 x 10^4 cfu g^{-1} for soil actinomycetes. Butachlor applied without FYM alternated with pretilachlor in the second crop (T_3) recorded lowest count of 1.3 x 10^4 cfu g^{-1}soil. At 45 DAS, both T_6 and butachlor applied with FYM alternated with pretilachlor in the second crop (T_4) recorded significantly higher count of actinomycetes (4.6 and 4.3 x 10^4 cfu g^{-1}soil respectively)

Table 16. Population of soil actinomycetes (x 10^4 g^{-1} of soil) during first and second crop season of 2001.

Treatments	First crop				Second crop					
	0 DAS	1 DAS	7 DAS	Harvest	1 DAS	7 DAS	15 DAS	30 DAS	45 DAS	Harvest
T_1	1.58 (1.3)*	2.11 (4.0)	1.68 (2.3)	2.18 (4.3)	1.43 (2.0)	1.17 (1.0)	1.39 (1.6)	2.02 (3.6)	1.45 (1.6)	1.94 (3.3)
T_2	1.74 (0.6)	1.17 (1.0)	1.34 (1.3)	2.08 (4.0)	1.25 (1.6)	0.88 (0.3)	1.05 (0.6)	2.12 (4.0)	1.34 (1.3)	1.87 (3.0)
T_3	1.34 (2.0)	1.05 (0.6)	1.10 (1.0)	1.86 (3.0)	1.18 (1.3)	1.17 (1.0)	0.99 (0.6)	1.58 (2.0)	1.05 (0.6)	1.45 (1.6)
T_4	1.23 (1.3)	1.64 (2.3)	1.44 (1.6)	2.35 (5.3)	1.79 (3.0)	1.39 (1.6)	1.47 (2.0)	1.87 (3.0)	2.19 (4.3)	2.55 (6.0)
T_5	1.34 (2.6)	1.44 (0.6)	1.05 (0.6)	1.39 (1.6)	1.72 (2.6)	0.88 (0.3)	1.39 (1.6)	1.67 (2.3)	1.76 (2.6)	0.89 (0.3)
T_6	1.35 (2.0)	1.64 (2.3)	1.52 (2.0)	2.09 (4.3)	4.19 (17.3)	1.18 (1.3)	1.68 (2.3)	1.67 (2.3)	2.26 (4.6)	1.67 (2.3)
CD (0.05)	NS	NS	NS	NS	1.346	NS	NS	NS	0.156	NS
CV %	22.70	25.44	30.39	28.89	39.29	46.46	39.06	43.55	19.64	19.35

*Values in parentheses indicate original values

Table 17. Population of soil actinomycetes (x 10^4 g^{-1} of soil) during the first crop season of 2002

Treatments	Before spraying	1 DAS	7 DAS	15 DAS	30 DAS	45 DAS	Harvest
T_1	1.95 (3.3)*	2.68 (7.0)	1.64 (2.3)	2.08 (4.3)	2.06 (4.0)	1.52 (2.0)	1.87 (3.6)
T_2	1.27 (1.3)	1.05 (3.0)	0.88 (0.3)	0.88 (0.3)	1.17 (1.0)	1.27 (1.3)	1.57 (2.3)
T_3	2.34 (5.0)	1.09 (3.6)	1.05 (0.6)	1.17 (1.0)	0.10 (0.6)	1.64 (2.3)	2.18 (4.3)
T_4	2.47 (5.6)	1.84 (4.3)	1.65 (2.3)	1.93 (3.3)	1.65 (2.3)	1.68 (2.3)	2.91 (8.0)
T_5	1.17 (1.0)	0.88 (0.3)	0.10 (0.6)	0.88 (0.3)	0.88 (0.3)	1.25 (1.5)	2.02 (3.6)
T_6	2.02 (3.6)	1.56 (3.6)	1.52 (2.0)	1.76 (2.6)	1.35 (1.6)	2.0 (3.6)	2.44 (5.3)
CD(0.05)	0.670	0.871	NS	0.670	NS	NS	NS
CV %	20.18	32.33	32.29	25.98	37.57	36.82	27.80

*Values in parentheses indicate original values

Table 18. Population of soil actinomycetes (x 10^4 g^{-1} of soil) during the second crop season of 2002

Treatments	Before spraying	1 DAS	7 DAS	15 DAS	30 DAS	45 DAS	Harvest
T_1	1.56 (2.0)*	2.53 (6.0)	1.72 (2.6)	1.74 (2.6)	2.18 (4.3)	2.53 (6.6)	1.64 (2.3)
T_2	1.47 (2.0)	1.56 (2.0)	1.17 (1.0)	0.88 (0.3)	1.17 (1.0)	2.34 (5.0)	1.87 (3.6)
T_3	1.05 (0.6)	1.47 (2.0)	1.18 (1.3)	0.10 (0.6)	1.86 (3.0)	2.68 (7.0)	2.88 (8.0)
T_4	2.60 (6.3)	2.16 (4.3)	2.25 (4.6)	1.18 (1.3)	2.53 (6.0)	1.90 (3.3)	2.17 (6.0)
T_5	1.72 (2.6)	1.05 (0.6)	0.88 (0.3)	1.27 (1.3)	2.55 (6.3)	2.62 (6.6)	2.36 (5.3)
T_6	2.18 (4.3)	2.18 (4.3)	1.17 (1.0)	1.78 (3.0)	2.79 (8.0)	2.0 (3.6)	1.91 (3.6)
CD(0.05)	0.805	0.809	NS	NS	NS	NS	NS
CV %	25.70	24.91	36.91	44.52	27.28	26.22	44.27

*Values in parentheses indicate original values

compared to all other treatments. At harvest T_4 showed the highest number 6.0 x 10^4 cfu g^{-1}soil followed by T_1 with a count of 3.3 x 10^4 cfu g^{-1}soil. The lowest count of 0.3 x 10^4 g^{-1}soil was recorded by butachlor applied continuously without FYM (T_5).

The population of actinomycetes varied significantly in first crop of 2002 before spraying, 1 DAS, 15 DAS and not showed any variation at 7 DAS, 30 DAS, 45 DAS and at harvest. Before spraying, hand weeded control, T_4 and butachlor applied with FYM alternated with pretilachlor in the second year (T_6) varied significantly from butachlor applied without FYM (T_2) and butachlor applied without FYM alternated with pretilachlor in the second year (T_5). The treatment T_4 recorded highest count (5.6 x 10^4 cfu g^{-1}soil) and lowest count (1.0 x 10^4 cfu g^{-1}soil) was recorded in T_5 for soil actinomycetes. At 1 DAS, hand weeded control varied significantly with all other herbicide treated plots. Hand weeded control recorded the highest count of 7.0 x 10^{-6} cfu g^{-1}soil and T_5 recorded the lowest count (0.3 x 10^4 cfu g^{-1}soil).

At 15 DAS, hand weeded control, T_4 and T_6 showed higher count of 4.3, 3.3 and 2.6 x 10^4 g^{-1}soil respectively. The other treatments T_2 and T_5 recorded lowest count of 0.3 x 10^4 cfu g^{-1}soil.

During second crop of 2002, population of actinomycetes did not show any variation at 7, 15, 30, 45 DAS and at harvest. But it showed significant variation before spraying and 1 DAS. Before spraying, butachlor applied with FYM alternated with pretilachlor in the second crop (T_4) and T_6 recorded higher counts (6.3 and 4.3 x 10^4 cfu g^{-1}soil respectively)an varied significantly with hand weeded plot and butachlor applied without FYM alternated with pretilachlor in the second crop (T_3) with a count of 2.0 and 0.6 x 10^4 cfu g^{-1}soil respectively.

At 1 DAS, hand weeded control, T_4 and T_6 varied significantly with T_2, T_3 and T_5. Hand weeded control recorded the highest count of 6.0 x 10^4 cfu g^{-1}soil and T_5 recorded the lowest count of 0.6 x 10^4 cfu g^{-1}soil.

4.5. EFFECT OF HERBICIDE APPLICATION ON WEED GROWTH

The species wise weed count and dry matter production during the year 2001 and 2002 at 60 DAS are presented in Table 19-22.

During the first crop of the year 2001, *Marsilea quadrifoliata, Sphenoclea zeylanica, Cyperus sp., Echinochloa crusgalli* and *Oryza rufipogon* were found in the experimental field. In second crop *Marsilea quadrifoliata, Echinochloa crusgalli, Echinochloa colona, Sphenoclea zeylanica* were the major weed species observed in the rice field.

During the year 2002, the weeds found in the first crop were *Marsilea quadrifoliata, Alternanthera echinata, Echinochloa crusgalli* and *Echinochloa glabrescens*. In the second crop *Ludwigia parviflora, Alternanthera echinata, Echinochloa crusgalli, Echinochloa colona* and *Echinochloa glabrescens* were identified in the experimental field.

During the year 2001, the total count and dry matter production of weeds were less in both the crop seasons compared to the year 2002. During the first crop of the year 2002, *Marsilea quadrifoliata* was found to be the dominant weed species. Large number of *Echinochloa* spp. was also seen in this season. However in the second crop *Marsilea quadrifoliata* was not found. Instead *Echinochloa* spp. was dominant in the rice field. Even though the total number of weeds was more in the first crop of 2002, their total dry weight was smaller. Lack of relationship between the total number and dry weight in the first crop season is attributed to the predominance of smaller weeds like *Marsilea quadrifoliata* during this season (Fig.12&13).

4.5.1. *Echinochloa* spp.

Effect of herbicides on weed count and dry matter production of *Echinochloa* spp. over a period of two years are presented in Table 23.

Table 19. Species wise weed count (m^{-2}) and dry matter production (g m^{-2}) at 60 DAS in the first crop, 2001

Treatments	Weed count (m^{-2})					Dry matter production (g m^{-2})				
	Marsilea quadrifoliata	*Spenochlea zeylanica*	*Cyperus sp.*	*Oryza rufipogon*	*Echinochloa crusgalli*	*Marsilea quadrifoliata*	*Spenochlea zeylanica*	*Cyperus sp.*	*Oryza rufipogon*	*Echinochloa crusgalli*
T_1	0.00	158.68	0.00	1.32	0.00	0.00	10.46	0.00	5.35	0.00
T_2	9.33	0.00	0.00	0.00	1.33	1.70	0.00	0.00	0.00	18.56
T_3	12.00	9.32	2.68	0.00	0.00	2.78	2.00	3.13	0.00	0.00
T_4	10.66	4.00	0.00	1.32	0.00	3.04	2.01	0.00	4.20	0.00
T_5	0.00	2.68	20.00	2.50	0.00	0.00	0.38	2.35	1.65	0.00
T_6	5.32	1.32	13.32	0.00	0.00	3.00	1.53	11.44	0.00	0.00

Table 20. Species wise weed count (m^{-2}) and dry matter production (g m^{-2}) at 60 DAS in the second crop, 2001

Treatments	Weed count (m^{-2})				Dry matter production (g m^{-2})			
	Marsilea quadrifoliata	*Echinochloa crusgalli*	*Echinochloa colona*	*Spenochlea zeylanica*	*Marsilea quadrifoliata*	*Echinochloa crusgalli*	*Echinochloa colona*	*Spenochlea zeylanica*
T_1	1.32	25.32	28.00	1.32	2.00	98.00	100.4	1.60
T_2	18.68	0.00	1.32	0.00	10.95	0.00	2.62	0.00
T_3	1.68	1.32	0.00	136.00	1.00	3.71	0.00	29.60
T_4	2.68	4.00	2.68	0.00	3.30	19.90	5.00	0.00
T_5	24.00	4.00	4.00	0.00	36.50	26.60	38.00	0.00
T_6	21.32	1.32	0.00	0.00	10.40	7.96	0.00	0.00

Table 21. Species-wise weed count (m^{-2}) and dry matter production (g m^{-2}) at 60 DAS in the first crop, 2002

Treatments	Weed count (m^{-2})				Dry matter production (g m^{-2})			
	Marsilea quadrifoliata	*Alternanthera echinata*	*Echinochloa crusgalli*	*Echinochloa glabrescens*	*Marsilea quadrifoliata*	*Alternanthera echinata*	*Echinochloa crusgalli*	*Echinochloa glabrescens*
T_1	5.30	0.00	4.00	2.60	0.38	0.00	6.01	3.24
T_2	108.00	5.20	9.32	25.28	8.14	2.98	28.29	49.69
T_3	208.00	0.00	6.40	13.60	18.32	0.00	7.82	37.20
T_4	157.20	1.20	8.00	8.00	8.57	0.95	17.92	30.80
T_5	193.20	5.20	8.00	14.50	14.93	2.27	16.17	46.33
T_6	166.40	4.00	9.30	0.00	11.01	2.93	16.29	0.00

Table 22. Species wise weed count (m^{-2}) and dry matter production (g m^{-2}) at 60 DAS in the second crop, 2002

Treatments	Weed count (m^{-2})					Dry matter production (g m^{-2})				
	Ludwigia parviflora	*Alternanthera echinata*	*Echinochloa crusgalli*	*Echinochloa glabrescens*	*Echinochloa colona*	*Ludwigia parviflora*	*Alternanthera echinata*	*Echinochloa crusgalli*	*Echinochloa glabrescens*	*Echinochloa colona*
T_1	21.40	34.60	10.40	4.00	1.60	11.20	25.70	45.38	2.90	1.70
T_2	2.67	0.00	10.40	0.00	6.90	1.62	0.00	24.73	0.00	21.15
T_3	5.33	2.66	12.00	2.40	8.20	3.53	1.89	33.62	1.97	13.59
T_4	2.66	32.00	12.00	0.00	2.70	1.25	18.57	36.18	0.00	4.00
T_5	0.00	0.00	57.20	9.20	14.90	0.00	0.00	133.45	21.50	32.05
T_6	13.33	13.30	6.40	0.00	1.60	10.84	10.81	21.06	0.00	2.11

4.5.1.1 Count

During the first crop of 2001, total population of *Echinochloa* spp. did not differ significantly between treatments. However, in the second crop hand weeded control registered the highest count (53.32 m^{-2}) followed by butachlor applied without FYM (T_5) and butachlor applied with FYM alternated with pretilachlor in the second crop (T_4) with counts of 8.00 and 6.68 m^{-2} per square metre respectively. The lower counts of *Echinochloa* spp. (1.32 m^{-2}) was recorded by butachlor applied without FYM (T_2), butachlor applied without FYM alternated with pretilachlor in the second crop (T_3) and butachlor applied with FYM in first crop (T_6).

During the first crop of 2002 also did not show any variation in the population of *Echinochloa* spp. between treatments. But in second crop of 2002, highest count of 81.30 per square metre was registered by butachlor applied without FYM alternated with pretilachlor in the second year (T_5) followed by butachlor applied without FYM alternated with pretilachlor in the second crop (T_3) with a count of 22.60 per square metre. Butachlor applied continuously without FYM (T_2), butachlor applied with FYM alternated with pretilachlor in the second crop (T_4), butachlor applied with FYM alternated with pretilachlor in the second year (T_6) recorded comparatively lower counts of *Echinochloa* spp.

4.5.1.2. Dry matter production

In the year 2001, total dry matter production of *Echinochloa* spp. during the second crop season showed significant variation. Hand weeded plot registered highest dry matter production (198.40 g m^{-2}) followed by T_5 and T_4 with dry weight of 64.60 and 24.90 gram per square metre respectively. The lowest dry matter production 7.96, 3.71 and 2.62 gram per square metre was registered by T_6, T_3 and T_2 respectively.

During the year 2002, first crop did not show variation in the total dry matter production of *Echinochloa* spp. butachlor applied without FYM (T_2) registered highest value (77.98 g m^{-2}) followed by butachlor applied without FYM alternated with pretilachlor in the second year (T_5) and butachlor applied with FYM (T_4), which recorded dry weight of 62.50 and 48.72 gram per square metre respectively.

Table 23. Changes in the count and dry matter production of *Echinochola* spp. at 60 DAS over a period of two years

Treatments	2001				2002			
	First crop		Second crop		First crop		Second crop	
	Weed count (m^{-2})	Dry matter production ($g\ m^{-2}$)	Weed count (m^{-2})	Dry matter production ($g\ m^{-2}$)	Weed count (m^{-2})	Dry matter production ($g\ m^{-2}$)	Weed count (m^{-2})	Dry matter production ($g\ m^{-2}$)
T_1	0.71 (0.00)*	0.71 (0.00)	7.20 (53.32)	13.90 (198.40)	2.39 (6.60)	2.76 (9.25)	3.97 (16.00)	5.98 (49.50)
T_2	1.18 (1.33)	2.97 (18.56)	1.18 (1.32)	1.43 (2.62)	4.39 (34.60)	8.81 (77.98)	4.10 (17.30)	6.45 (45.88)
T_3	0.71 (0.00)	0.71 (0.00)	1.18 (1.32)	1.61 (3.71)	4.53 (20.00)	6.63 (45.02)	4.70 (22.60)	6.63 (48.76)
T_4	0.71 (0.00)	0.71 (0.00)	2.30 (6.68)	4.06 (24.90)	4.01 (16.00)	6.69 (48.72)	3.83 (14.70)	5.86 (40.18)
T_5	0.71 (0.00)	0.71 (0:00)	2.56 (8.00)	6.81 (64.60)	4.44 (22.50)	6.92 (62.50)	8.97 (81.30)	13.51 (187.00)
T_6	0.71 (0.00)	0.71 (0.00)	1.18 (1.32)	2.12 (7.96)	2.71 (9.30)	3.46 (16.29)	2.56 (8.00)	4.15 (23.17)
CD(0.05)	NS	NS	0.793	1.872	NS	NS	0.760	1.932
CV %	42.43	147.57	51.50	63.26	43.09	53.24	27.32	45.92

*Values in parentheses indicate original values

During the second crop of 2002, variation was observed with total count as well as dry matter production of *Echinochloa* spp. T_5 registered highest dry weight of 187.00 per square metre followed by hand weeded plot, T_3 and T_2, which recorded dry weight of 49.50, 48.76 and 45.88 gram per square metre respectively.

4.5.2. Total broad leaved weeds

Effect of herbicides on weed count and dry matter production of broad leaved weeds over a period of two years are presented in Table 24.

4.5.2.1. Count

During the first crop of 2001, significant variation between treatments was observed in the total count of broad leaves weeds. Hand weeded plot registered highest count of 158.68 per square metre followed by treatment butachlor fb 2,4-D applied without FYM (T_3), which registered a count of 21.33 per square metre. In the second crop season when butachlor was alternated with pretilachlor highest count of 137.68 per square metre was recorded by T_3. This was followed by treatment butachlor fb 2,4-D applied continuously without FYM (T_5), which registered a value of 24.00 per square metre.

In the first crop of 2002, all the herbicide applied plots recorded higher counts compared to hand weeded control. All the treatments differed significantly. Higher count was recorded by butachlor fb 2,4-D applied without FYM alternated with pretilachlor in the second year (T_5) with a count of 198.60 per square metre. During the second crop season butachlor fb 2,4-D applied with FYM alternated with pretilachlor in the second crop (T_4) registered highest count of 61.30 per square metre followed by hand weeded plot and butachlor fb 2,4-D applied with FYM alternated with pretilachlor in the second year (T_6), which recorded values of 56.00 and 26.60 per square metre respectively.

4.5.2.2. Dry matter production

For the first crop 2001, hand weeded control recorded highest dry weight of 10.46 gram per metre square followed by butachlor fb 2,4-D applied with FYM (T_4)

Table 24. Changes in the count and dry matter production of total broad leaved weeds at 60 DAS over a period of two years

Treatments	2001				2002			
	First crop		Second crop		First crop		Second crop	
	Weed count (m^{-2})	Dry matter production (g m^{-2})	Weed count (m^{-2})	Dry matter production (g m^{-2})	Weed count ($n.m^{-2}$)	Dry matter production (g m^{-2})	Weed count (m^{-2})	Dry matter production (g m^{-2})
T_1	12.48 (158.68)*	3.29 (10.46)	1.44 (2.64)	1.59 (3.60)	1.82 (5.30)	0.90 (0.38)	7.49 (56.00)	5.99 (36.90)
T_2	2.77 (9.33)	1.40 (1.70)	4.26 (18.68)	3.24 (10.95)	8.26 (113.30)	3.05 (11.12)	1.44 (2.60)	1.24 (1.62)
T_3	4.61 (21.33)	2.29 (4.78)	10.74 (137.68)	5.31 (30.60)	14.43 (208.00)	4.34 (18.32)	2.86 (8.00)	2.41 (5.42)
T_4	3.39 (14.66)	2.13 (5.05)	1.44 (2.68)	1.54 (3.30)	12.14 (158.60)	3.12 (9.52)	7.63 (61.30)	3.95 (23.03)
T_5	1.44 (2.68)	0.90 (0.38)	3.31 (24.00)	3.97 (36.50)	13.96 (198.60)	4.14 (17.20)	0.71 (0.00)	0.71 (0.00)
T_6	1.98 (6.64)	1.72 (4.53)	4.49 (21.32)	3.28 (10.40)	12.16 (170.60)	3.76 (13.94)	4.48 (26.60)	4.06 (21.65)
CD(0.05)	1.120	0.562	1.894	NS	2.780	0.559	1.047	1.178
CV %	42.48	48.47	74.61	84.22	44.83	29.30	43.05	64.89

*Values in parentheses indicate original values

and T_3 with dry weight of 5.05 and 4.78 gram per metre square respectively. In the second crop dry weight of broad leaved weeds was more in T_5 followed by treatment butachlor fb 2,4-D applied without FYM alternated with pretilachlor the second crop (T_3) which recorded dry weight of 36.50 and 30.60 gram per metre square. Hand weeded plot, butachlor fb 2,4-D applied with FYM alternated with pretilachlor in the second crop (T_4) and butachlor fb 2,4-D applied with FYM alternated with pretilachlor in the second year (T_6) recorded lowest dry weight (3.60, 3.30 and 10.40 g m^{-2} respectively).

For the year 2002, first crop butachlor fb 2,4-D applied without FYM (T_3 and T_5) registered highest dry weight of 18.32 and 17.20 gram per metre square respectively. During the second crop season hand weeded plot, T_4 and T_6 recorded highest values of 36.90, 23.03, 21.65 gram per metre square respectively. The treatments T_2 and T_3 recorded lower dry weights of 1.62 and 5.42 gram per metre square respectively.

4.5.3. Total weeds

Effect of herbicide on weed count and dry matter production over a period of two years are presented in Table 25.

4.5.3.1. Count

During the first crop of 2001, hand weeded plot, butachlor fb 2,4-D applied without FYM (T_5, T_3) recorded higher counts of total weeds (160.00, 25.36, 24.00 per square metre respectively). No significant difference in the total weed count was observed between treatments in the second crop of 2001.

During the year 2002, no variation in the total weed count was observed between treatments.

4.5.3.2. Dry matter production

In the second crop of the year 2001, hand weeded plot registered the highest dry weight (202.37 g m^{-2}) followed by treatment butachlor fb 2,4-D applied without FYM (T_5) with a dry weight of 101.13 gram per metre square.

Table 25. Changes in the count and dry matter production of total weeds at 60 DAS over a period of two years

Treatments	2001				2002			
	First crop		Second crop		First crop		Second crop	
	Weed count (m^{-2})	Dry matter production ($g\ m^{-2}$)	Weed count (m^{-2})	Dry matter production ($g\ m^{-2}$)	Weed count (m^{-2})	Dry matter production ($g\ m^{-2}$)	Weed count (m^{-2})	Dry matter production ($g\ m^{-2}$)
T_1	12.52 (160.00)*	4.011 (15.81)	7.34 (55.96)	14.04 (202.37)	7.04 (56.00)	3.31 (11.41)	8.54 (73.32)	9.35 (88.19)
T_2	3.24 (10.64)	4.20 (20.31)	4.40 (20.00)	3.7 (13.57)	10.99 (150.60)	9.25 (89.97)	4.49 (20.00)	6.64 (47.51)
T_3	4.86 (24.00)	2.71 (7.91)	10.79 (138.64)	5.55 (34.31)	15.11 (228.00)	7.92 (63.34)	5.53 (30.40)	7.09 (54.16)
T_4	3.54 (16.00)	2.76 (9.25)	3.03 (9.36)	5.29 (28.29)	12.83 (174.60)	7.43 (58.24)	8.52 (76.00)	7.53 (63.29)
T_5	5.21 (25.36)	2.18 (4.38)	5.16 (32.00)	9.71 (101.13)	15.55 (244.00)	8.78 (83.88)	11.69 (81.20)	13.49 (187.00)
T_6	3.87 (20.00)	2.79 (15.94)	4.70 (22.64)	4.23 (18.36)	12.57 (178.40)	5.43 (30.24)	3.91 (34.66)	6.69 (44.80)
CD(0.05)	1.141	NS	NS	1.304	NS	1.230	NS	1.504
CV %	34.73	64.40	49.44	31.03	34.32	29.54	15.78	29.95

*Values in parentheses indicate original values

During the first crop of 2002, butachlor fb 2,4-D applied without FYM (T_2) and butachlor fb 2,4-D applied without FYM alternated with pretilachlor in the second year (T_5) recorded highest dry weight of 89.97 and 83.88 gram per square metre respectively. In second crop, T_5 recorded highest dry weight of 187 gram per square metre followed by hand weeded plot with a dry weight (88.19 g m^{-2}). The other treatments viz. butachlor fb 2,4-D applied without FYM (T_2), butachlor fb 2,4-D applied without FYM alternated with pretilachlor in the second crop (T_3), butachlor fb 2,4-D applied with FYM alternated with pretilachlor in the second crop (T_4) and butachlor fb 2,4-D applied with FYM alternated with pretilachlor in the second year (T_6) recorded comparatively lower counts.

4.6. EFFECT OF HERBICIDE APPLICATION ON RICE GROWTH AND YIELD

Effect of treatments on the rice yield attributes and yield are presented in Table 26.

4.6.1. Height of the plant

Herbicide application had no significant effect on the height of rice plant during the first crop season. Butachlor fb 2,4-D applied without FYM (T_2) and butachlor fb 2,4-D applied with FYM (T_4) recorded higher value compared to all other treatments.

However during the second crop season butachlor fb 2,4-D applied with FYM alternated with pretilachlor in the second year (T_4) recorded the lowest value of 69.92 cm. All the treatments differed from each other with respect to plant height. The highest value of 79.71 cm was shown by hand weeded control followed by butachlor fb 2,4-D applied without FYM alternated with pretilachlor in the second crop (T_3) of 78.22 cm. Butachlor fb 2,4-D applied without FYM (T_2), butachlor fb 2,4-D applied with FYM alternated with pretilachlor in the second year (T_6) and butachlor fb 2,4-D applied without FYM alternated with pretilachlor in the second year (T_5) recorded plant height of 72.50, 72.57 and 75.34 cm respectively.

Table 26. Effect of the treatments on the yield attributes and yield of rice during 2002

Treatments	First crop				Second crop			
	Plant height (cm)	No. of productive tillers ($No.m^{-2}$)	grain yield ($kg\ ha^{-1}$)	straw yield ($kg\ ha^{-1}$)	Plant height (cm)	No. of productive tillers ($No.m^{-2}$)	grain yield ($kg\ ha^{-1}$)	straw yield ($kg\ ha^{-1}$)
T_1	71.80	20.90	3200	5500	79.71	24.08	1700	3600
T_2	74.55	22.50	3000	5400	72.50	23.56	1800	5400
T_3	72.87	24.50	3300	5100	78.22	22.57	1500	3900
T_4	74.04	27.80	3200	4400	69.92	20.89	1500	3800
T_5	72.42	18.70	2200	4800	75.34	22.21	1400	4000
T_6	64.52	27.00	2800	4400	72.57	23.71	1400	4000
CD (0.05)	NS	NS	NA	NA	3.086	0.689	NA	NA
CV (%)	13.38	15.42	NA	NA	2.32	1.70	NA	NA

NS - Non significant, NA - Not analysed statistically

4.6.2. Number of productive tillers

During the first crop season variation was not obtained, T_4 and T_6 recorded higher value in the number of productive tillers per square metre.

In the second crop hand weeded plot, T_6, T_2 showed significantly higher values of (24.08, 23.71 and 23.56 per square metre respectively) compared to T_3, T_4 and T_5 which recorded 22.57, 20.89 and 22.21 per square metre respectively.

4.6.3. Grain yield

During the first crop of 2002 grain yield was maximum in the treatment butachlor fb 2,4-D applied without FYM (T_3), which recorded yield of 3300 kg ha^{-1}. This was closely followed by hand weeded plot and butachlor fb 2,4-D applied with FYM (T_4), both recorded grain yield of 3200 kg ha^{-1}. The lowest grain yield of 2200 kg ha^{-1} recorded in butachlor fb 2,4-D applied without FYM alternated with pretilachlor in the second year (T_5).

In the second crop of 2002, butachlor fb 2,4-D applied continuously without FYM (T_2) recorded highest yield of 1800 kg ha^{-1} closely followed by hand weeded plot (T_1). Both T_5 and T_6 recorded lower grain yield of 1400 kg ha^{-1}.

4.6.4. Straw yield

During the first crop straw yield was highest 5500 kg ha^{-1} in hand weeded plot compared to other treatments. Both T_4 and T_6 recorded the lowest value of 4400 kg ha^{-1}. Butachlor fb 2,4-D applied without FYM (T_2, T_3) and butachlor fb 2,4-D applied without FYM alternated with pretilachlor in the second year (T_5) recorded straw yields of 5400, 5100 and 4800 kg ha^{-1} respectively.

In the second crop, straw yield was more in butachlor fb 2,4-D applied continuously without FYM (T_2) 5400 kg ha^{-1} compared to all other treatments. A straw yield of 4000 kg ha^{-1} was recorded by both T_5 and T_6. Hand weeded plot control and the treatments by T_3 and T_4 recorded comparatively lower straw yield.

4.7. EFFECT OF HERBICIDE APPLICATION ON THE NUTRIENT CONTENT OF GRAIN AND STRAW

Effect of treatments on the nutrient content in rice grain and straw are presented in Table 27.

4.7.1. Grain

During the first crop nitrogen content was in the range of 0.97-1.53 per cent butachlor fb 2,4-D applied with FYM (T_4) and butachlor fb 2,4-D applied with FYM alternated with pretilachlor in the second year (T_6) showed significantly higher values (1.53 and 1.43 per cent respectively) compared to other four treatments. Phosphorus content was in the range of 0.12 to 0.27 per cent. Butachlor fb 2,4-D applied without FYM alternated with pretilachlor in the second year (T_5) and T_4 showed higher values of 0.24 and 0.27 respectively. Potassium content was in the range of 0.22 to 0.43 per cent. Butachlor fb 2,4-D applied without FYM (T_3) recorded higher content of potassium in the grain (0.43%), even though the variations between treatments was not significant.

In the second crop, nitrogen content in the grain samples ranged from 0.87 to1.53 per cent butachlor fb 2,4-D applied with FYM alternated with pretilachlor in the second crop (T_4) and butachlor fb 2,4-D applied with FYM alternated with pretilachlor in the second year (T_6) were significantly higher to the other four treatments and they recorded values of 1.41 and 1.53 per cent respectively. The lowest value of 0.87 per cent was registered by hand weeded plot which was on par with butachlor fb 2,4-D applied continuously without FYM (T_2, T_3) and butachlor fb 2,4-D applied without FYM alternated with pretilachlor in the second year (T_5). No significant variation between treatments was observed in phosphorus content, which ranged from 0.14 to 0.21 per cent. Potassium content ranged from 0.18 to 0.38 per cent T_5 recorded the lowest value of 0.18 per cent and the highest value of 0.38 per cent was recorded by T_6.

Table 27. Effect of the treatments on the nutrient status of grain and straw during 2002

Treatments	First crop						Second crop					
	N (%)		P (%)		K (%)		N (%)		P (%)		K (%)	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
T_1	1.26 (1.08)*	1.11 (0.74)	0.81 (0.16)	0.75 (0.06)	0.83 (0.25)	1.43 (1.55)	1.17 (0.87)	1.13 (0.78)	0.84 (0.21)	0.74 (0.04)	0.89 (0.30)	1.41 (1.50)
T_2	1.24 (1.04)	1.18 (0.90)	0.79 (0.12)	0.74 (0.05)	0.82 (0.23)	1.46 (1.65)	1.18 (0.90)	1.20 (0.93)	0.82 (0.17)	0.77 (0.09)	0.90 (0.32)	1.50 (1.75)
T_3	1.21 (0.97)	1.18 (0.89)	0.81 (0.16)	0.75 (0.07)	0.97 (0.43)	1.41 (1.50)	1.17 (0.88)	1.20 (0.93)	0.84 (0.21)	0.79 (0.12)	0.85 (0.22)	1.45 (1.62)
T_4	1.43 (1.53)	1.22 (0.98)	0.88 (0.27)	0.74 (0.05)	0.87 (0.25)	1.45 (1.60)	1.38 (1.41)	1.21 (0.97)	0.83 (0.19)	0.76 (0.08)	0.92 (0.35)	1.53 (1.83)
T_5	1.24 (1.07)	1.18 (0.90)	0.86 (0.24)	0.76 (0.08)	0.90 (0.32)	1.42 (1.53)	1.22 (0.98)	1.20 (0.93)	0.80 (0.14)	0.75 (0.07)	0.83 (0.18)	1.47 (1.65)
T_6	1.39 (1.43)	1.18 (0.89)	0.83 (0.19)	0.73 (0.03)	0.85 (0.22)	1.50 (1.78)	1.48 (1.53)	1.21 (0.97)	0.81 (0.16)	0.75 (0.06)	0.94 (0.38)	1.48 (1.70-)
CD (0.05)	0.056	NS	NS	NS	NS	NS	0.079	NS	NS	NS	NS	0.056
CV (%)	2.05	1.40	6.43	2.46	6.93	4.72	3.23	2.85	4.58	0.94	2.34	2.58

*Values in parentheses indicate original values

4.7.2. Straw

During the first crop, nitrogen content in the straw ranged from 0.74 to 0.98 per cent. The higher percent of nitrogen in the straw (0.98%) was registered by T_4. Hand weeded control registered the lowest value of 0.74 per cent.

No significant variation between treatments was observed either in phosphorus or potassium content of straw. In the case of phosphorus, the maximum value of 0.08 per cent was registered by T_5 and the minimum value of 0.03 per cent was recorded by T_6. Potassium content in the straw ranged from 1.50-1.78 per cent, even though the treatments did not differ significantly.

In the second crop, nitrogen content of straw ranged from 0.78 to 0.97 percent. The treatments T_4 and T_6 showed comparatively higher content of nitrogen (0.97%) than the other treatments. Hand weeded plot recorded the lowest value of 0.78 per cent. Phosphorus content of the straw ranged from 0.04 to 0.12 per cent. Butachlor fb 2,4-D applied without FYM alternated with pretilachlor in the second crop (T_3) showed higher value compared to all other treatments. There was significant variation between treatments in the potassium content which ranged from 1.50 to 1.83 per cent, Butachlor fb 2,4-D applied continuously without FYM (T_2), T_4 and T_6 were on par with respect to potassium content of straw which recorded values 1.75,1.83 and 1.70 per cent respectively. All the other treatments recorded significantly lower values.

Discussion

5. DISCUSSION

Investigations were carried out in the field and laboratory during 2001-2002 for monitoring the persistence of selective herbicides in a rice-rice system and the effect of herbicide application on soil microflora. Changes in the nutrient content of the soil and rice plant and dynamics of weed population consequent to herbicide application were also studied. The results are presented in sections 4.1 to 4.7 and the findings are discussed below.

5.1. PERSISTENCE OF HERBICIDES IN THE RICE-RICE SYSTEM

Persistence of a herbicide refers to the residence time of chemical in soil, before being completely removed by physical, chemical and biological degradation (Scheunert *et al.*, 1993). Ideally a herbicide should persist in soil until the critical period of crop weed competition is over. In rice, the critical period is 60 days from the date of sowing. Therefore, the ideal persistence for pre emergence herbicides like butachlor and pretilachlor would be 50 to 55 days. As 2,4-D is applied 20 days after sowing, it should persist in the soil up to 40 days only. However, in the case of extra short duration varieties like Hraswa, the optimum persistence would be 20 days less than the normal.

Both butachlor and pretilachlor are applied as pre emergence for the control of grassy weeds. They same were applied @ 1.25 and 0.75 kg a.i ha^{-1} respectively at 8 days after sowing. Therefore at the time of application, herbicide concentration in the upper 0-10 cm soil layer would be 0.856 and 0.514 $\mu g\ g^{-1}$ for butachlor and pretilachlor respectively. For controlling broad leaved weeds, 2,4-D was applied @ 1.00 kg ha^{-1} and the concentration of the chemical in the 0-10cm soil layer would be 0.685 $\mu g\ g^{-1}$.

5.1.1. Herbicide residues in soil.

Herbicides applied in the first and second crop season of 2002 were estimated for their residues by taking soil samples from the treatments at 1 and 30 days after spraying and at the time of harvest (Fig 3.)

At one day after spraying butachlor, its residue in the soil was in the range of 0.331 to 0.396 μg g^{-1} in the first crop season of 2002. The highest concentration of 0.396 μg g^{-1} was recorded in the plot were FYM was applied along with 75 per cent of the recommended NPK. Adsorption by soil organic matter would have played an important role in the quantity of herbicide in soil solution. Prakash et al (2000) reported that butachlor is highly adsorbed by FYM. In the second crop season, only one plot received butachlor application, which registered butachlor residues of 0.343 μg g^{-1} at one day after spraying. The results indicated that the initial residues remain more or less same in both the crop seasons. However the disappearance of the residue was faster in the second crop season.

More than 95 per cent of the applied herbicide had been dissipated from the soil by 30 days after spraying (Fig 4). Greater losses were observed in the FYM applied plots (97.66 per cent). Since soil microorganisms are involved in the degradation of butachlor, soil organic matter should influence their break down. Usually, the microbial activity is more in soils containing greater amounts of organic material than in mineral soils. Several scientists reported that degradation of butachlor is mainly microbial (Pionke and Chesters, 1973; Beestman and Deming, 1974; Chen and Wu, 1978). Beestman and Deming (1974) reported the half-lives of butachlor in viable and sterilized soils as 11.4 ± 0.3 and 64.0 ± 8 days respectively. Jayakumar and Sreeramulu (1993) reported a half-life of 19 days for butachlor. According to Devi *et al.* (1997) half lives of butachlor varied from 12.3 to 16.2 days. Deka and Gogoi (1993) reported that in upland rice, butachlor got degraded to non detectable levels by 21 days after application. Kulshrestha *et al.* (1981) reported that persistence of butachlor was less than 38 days.

Plots that did not receive FYM also had registered higher rate of dissipation during the second crop season, which could have resulted from the greater number of microorganisms present in the soil during this crop period. Chiang *et al.* (1987) also noticed higher dissipation rate in second crop than in the first crop. Weather data (Appendix III) indicated that the rice field was under submerged conditions during the first crop season, particularly during the initial stages of crop growth. In the flooded soil

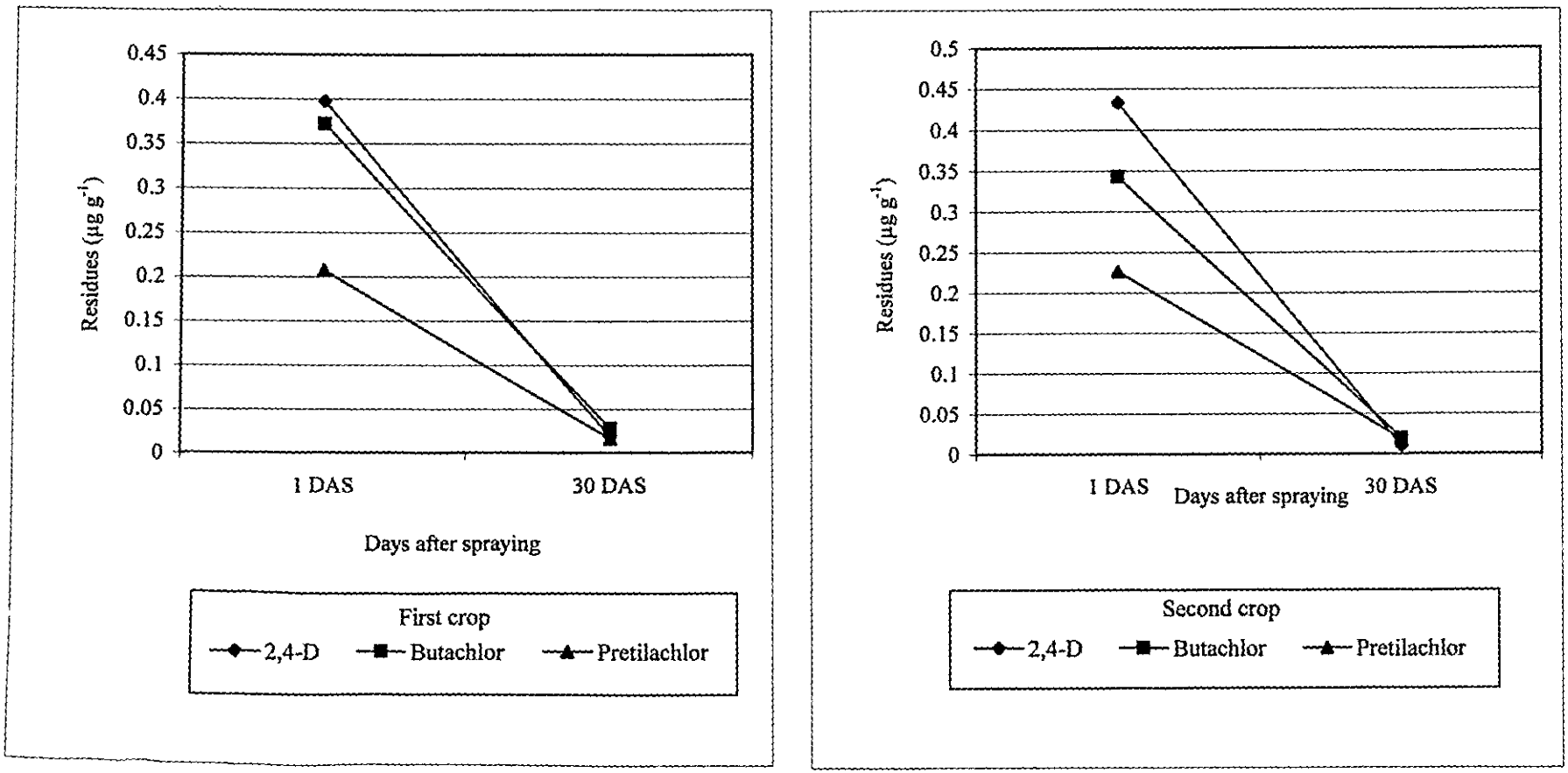


Fig. 3. Persistence of 2,4-D, butachlor and pretilachlor during the first and second crop season of 2002

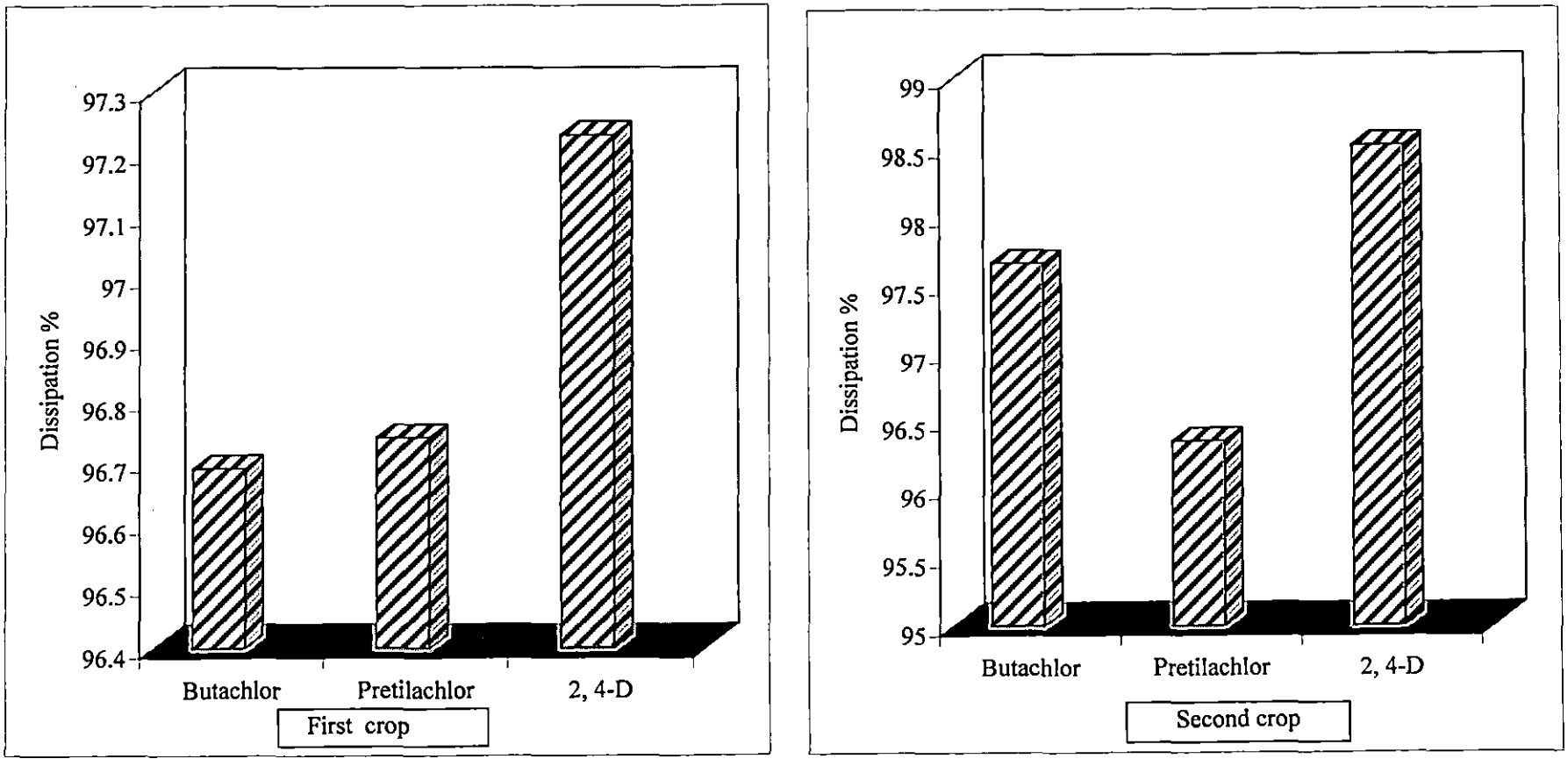


Fig. 4. Percentage dissipation of applied herbicides from the soil by 30 days after spraying

aerobic microorganisms would be comparatively fewer in number and hence the degradation would have been carried out by lesser number of microflora present in the soil, which would have resulted in greater persistence of the chemical.

During the second crop season, the rice field was under field capacity rather than submergence. Prakash and Devi (2000) also reported that disappearance of butachlor was more rapid under field capacity than under air dry and submergence in all the soils studied and irrespective of application rates. The half-lives of butachlor reported by Prakash and Devi (2000) was in the range of 52.40 to 59.27, 12.35 to 20.58 and 28.48 to 39.20 days for air dry, field capacity and submergence condition at 5.0 mg kg^{-1} application.

The initial residue levels (one day after spraying) of pretilachlor in the soil was in the range of 0.200 to 0.215 μg g^{-1} in the first crop season and 0.199 to 0.250 μg g^{-1} in the second crop season. At 30 days after spraying the residues declined to 0.020 to 0.013 μg g^{-1} in the first crop and 0.010 to 0.030 μg g^{-1} in the second crop season. About 94 to 98 per cent of the applied pretilachlor was lost from the soil by 30 days after spraying (Fig. 4). Higher losses of pretilachlor were observed in the plots, which received FYM (97.47 to 98.05 per cent). As in the case of butachlor, higher degree of dissipation was noticed in the second crop season. However, the plots which received pretilachlor spraying (without FYM) continuously during the first and second crop seasons registered comparatively lower rates of dissipation which revealed that this herbicide had some inhibitory effect on soil microflora. Pretilachlor also belongs to chloro acetanilide group of herbicides (similar to butachlor) and its degradation would have been mainly microbial. The contribution of organic matter towards degradation may mask other factors (Stearman *et al.*, 1989) and hence the plot which received FYM during first crop season and pretilachlor spraying during the first and second crop seasons registered higher dissipation rate. DT_{50} (time required to disappear half the quantity applied) for pretilachlor in the 0-1 cm soil layer was between 7-10 days (Fajardo *et al.*, 2000) and faster degradation was noticed in the reductive soil layers. They also observed that

pretilachlor leached from the upper layer within the first two weeks and then quickly disappeared. The results of the present investigation indicated similarities in the pattern of dissipation of butachlor and pretilachlor in the paddy field under a particular soil and climatic conditions.

Degradation of 2,4-D in the soil was faster than butachlor and pretilachlor (Fig.3). Up to 97.81 to 99.27 per cent of the applied herbicide had been dissipated from the plots by 30DAS, which received FYM. Except in T_3 (Butachlor fb 2,4-D with 100 per cent NPK) 2,4-D disappeared from the other plots to the extent of 97.08 to 97.81 per cent (Fig 4). In T_3, only 94.89 per cent of the applied 2,4-D had been disappeared fom the soils during first crop season. Hermosin and Cornejo(1991) reported half-life of 2,4-D in the soil as 59.3 days. According to Cox (1999) the persistence of 2,4-D is variable with half-lives ranging from 2-297 days. Devi (2002) reported that degradation of 2,4-D in the rice soils of Kerala followed first order rate equation with half-lives ranging from 3.44-10.76 days.

All the three herbicides namely, butachlor, pretilachlor and 2,4-D had been dissipated from the soil to non-detectable level by the time of harvest of each crop.

5.1.2. Herbicides residues in the crop.

None of the herbicides was detected in the rice grain or straw. Kulshrestha *et al.* (1981) reported that butachlor degraded in the soil and plant with formation of 2-chloro 2,6 diethyl acetanilide, the only metabolite that was detected in trace quantities. Chen and Chen (1979) reported that loss of butachlor was mainly the result of dilution due to crop growth.

In the soil, the main degradation pathways of both chloro acetanilides and phenoxy acids include dechlorination and dehydroxylation. The degraded products are finally converted to CO_2. Since the pH of the soil under the field study was in the neutral range and the organic carbon content was medium, both bacteria and fungi would have been

involved in the degradation of the herbicides. Devi (2002) observed an increase in the population of soil fungi due to the application of 2,4-D at higher rates. Although there was reduction in bacterial population at higher rates of application of 2,4-D, their total population was higher than that of fungi, which revealed that the inhibitory effect on bacteria was not due to the effect of 2,4-D alone. Enhancement of fungal population would have suppressed the bacterial population to a certain extent.

In the plant system butachlor, pretilachlor and 2,4-D form conjugates with amino acids. Conjugation with reduced glutathione is supposed to be the major deactivation pathway of these herbicides in the rice plant. Butachlor was absorbed and translocated in rice and showed half-life of 2.3-3.5 days (Kulshrestha, 1987). Conjugation of chloro acetanilides with glutathione takes place non enzymatically and enzymatically by the action of glutathione-S transferase (GSH). The non enzymatic conjugation rate varied in the order of propachlor > pretilachlor > alachlor > acetachlor > dimethachlor > metolachlor > butachlor (Shimabukuro, 1985). Pretilachlor and its glutathione conjugates were identified as major metabolites of *in vitro* reaction with GSH, pretilachlor and extracts of rice (*Oryza sativa*) seedlings. Time sequentially monitored with GC analysis, pretilachlor content was rapidly reduced on initiation of the reaction (Ma *et al.*, 1999).

Based on the above discussion, effect of the treatments on herbicide residues is summarized below.

All the plots except hand weeded control received 2,4-D spraying at 30 days after sowing. Therefore it is difficult to discuss the unique effect of 2,4-D in the four crop seasons and the discussion was concentrated on the effect of treatments namely. (i) continuous application of butachlor (ii) butachlor alternated with pretilachlor between seasons, (iii) butachlor alternated with pretilachlor between years, (iv) effect of organic matter and (v) difference between herbicides.

(i) Continuous application of butachlor

Irrespective of the seasons and treatments, dissipation of butachlor was at a faster rate and the residues were not detected at the time of harvest. Even though butachlor was

sprayed continuously in the four crop seasons without addition of organic matter, more than 96.20 per cent of the applied herbicide had been disappeared from the soil by 30DAS. Slightly higher rate of dissipation (97.66 per cent) observed during the second crop season could be attributed to the higher temperature and optimum moisture content of the soil.

(ii) Butachlor alternated with pretilachlor between seasons

Since butachlor had been completely dissipated before the harvest of first crop, its application during the preceeding season could not exert any influence on the persistence of pretilachlor sprayed in the succeeding season. Compared to butachlor, dissipation of pretilachlor took place at a slower rate. Only 94.16 per cent of the applied pretilachlor had been disappeared from the soil by 30DAS.

(iii) Butachlor alternated with pretilachlor between years

When butachlor was replaced with pretilachlor (after the application of butachlor for two seasons of the previous year), extent of disappearence of residue from the soil by 30DAS was 96.01 per cent in the first crop and 95.14 per cent during the second crop season respectively. The results indicate that lag phase of pretilachlor is comparatively higher than that of butachlor. However at the time of harvest no detectable amount of pretilachlor residues was recorded in this treatment even after its application for two seasons.

(iv) Effect of organic matter

Application of FYM enchanced the rate of degradation of butachlor, pretilachlor and 2,4-D in the soil. In the first crop season, percent dissipation of butachlor within a period of 30DAS from the plots with and without FYM was 97.66 and 96.20 per cent respectively. The corresponding values for pretilachlor were 97.47 and 96.01 per cent respectively and that of 2,4-D were 98.17 and 96.59 per cent respectively.

(v) Difference between herbicides

The per cent dissipation of applied herbicide followed the sequence 2,4-D> butachlor > pretilachlor.

5.2. EFFECT OF HERBICIDES ON THE POPULATION OF MICROORGANISMS IN SOIL

Dynamics of population of soil microflora consequent to the application of butachlor, pretilachlor and 2,4-D was monitored by conducting soil plate dilution study on the soil samples taken from the treatments at varying intervals. The study was conducted in the four crop seasons during 2001-2002.

During the first crop of 2001, changes in soil micro flora was studied at 0, 1, 7 DAS and at the time of harvest. While during second crop season, changes were observed at 1, 7, 15, 30 and 45 DAS and at the time of harvest. During the first and second crop of 2002, samples were taken at 0, 1, 7, 15, 30 and 45 DAS and at the time of harvest and observations were recorded.

The results presented in section 4.4 indicated that considerable variation in the microbial population would result from the application of herbicides. However the change was observed only for a short period particularly up to 15DAS. Results obtained during the year 2001 gave some insight on the dynamics of microflora in the rice field up on spraying herbicides butachlor, pretilachlor and 2,4-D. The detailed study conducted during the year 2002 gave much more information on this aspect. Graphs were plotted using the data for the year 2002. In order to see variations in the population of soil bacteria, fungi and actinomycetes due to various weed control treatments in the rice-rice cropping system, their percentage variation over the normal population worked out. Population before spraying (0 DAS) was taken as normal population (100) for each treatment. By subtracting the percentage change from control, their population was worked out and those values were taken on the (y) axis for plotting the graphs (Fig. 5-7).

5.2.1. Effect on soil bacteria

The results indicated that population of bacteria in the soil did not remain static throughout the entire crop period even in the hand weeded plot (Fig.5). There were variations in their population over varying time intervals. There was an increase in the population of soil bacteria from 0 to 15 DAS in the hand weeded plot. The population came down and attained initial level at the time of harvest.

A drastic decline in the population of soil bacteria was noticed after the application of herbicides, which extended upto 45 days after spraying in the case of first crop and to a period of 30 days in the second crop season. Maximum reduction was noticed at 7 DAS in both the crop seasons.

In the plot where butachlor followed by 2,4-D was applied during the first and second crop seasons (T_2) percentage reduction in soil bacteria was 95.2 per cent for the first crop and 96.8 per cent during the second crop season. In both the seasons maximum percent reduction was at noticed at 7 DAS. In the treatment T_3 (Butachlor fb 2,4-D with 100 per cent NPK in first crop and pretilachlor fb 2,4-D with per cent NPK in second crop) also about 96 per cent of the reduction in population was noticed in both the seasons. In the first crop, maximum reduction was noticed at 30 DAS while in the second crop season drastic reduction was observed immediately after spraying (1 DAS) which prolonged up to 15DAS only. The result indicated that the effect of pretilachlor on the soil bacteria was comparatively less than that of butachlor.

In the FYM applied plot (T_4) when butachlor fb 2,4-D was alternated with pretilachlor fb 2,4-D in the second crop season, the percentage reduction was less (61.7 to 84.10 per cent).

In the plot where pretilachlor fb 2,4-D was applied during the two crop seasons without FYM application, percentage inhibition of bacterial growth during the first crop

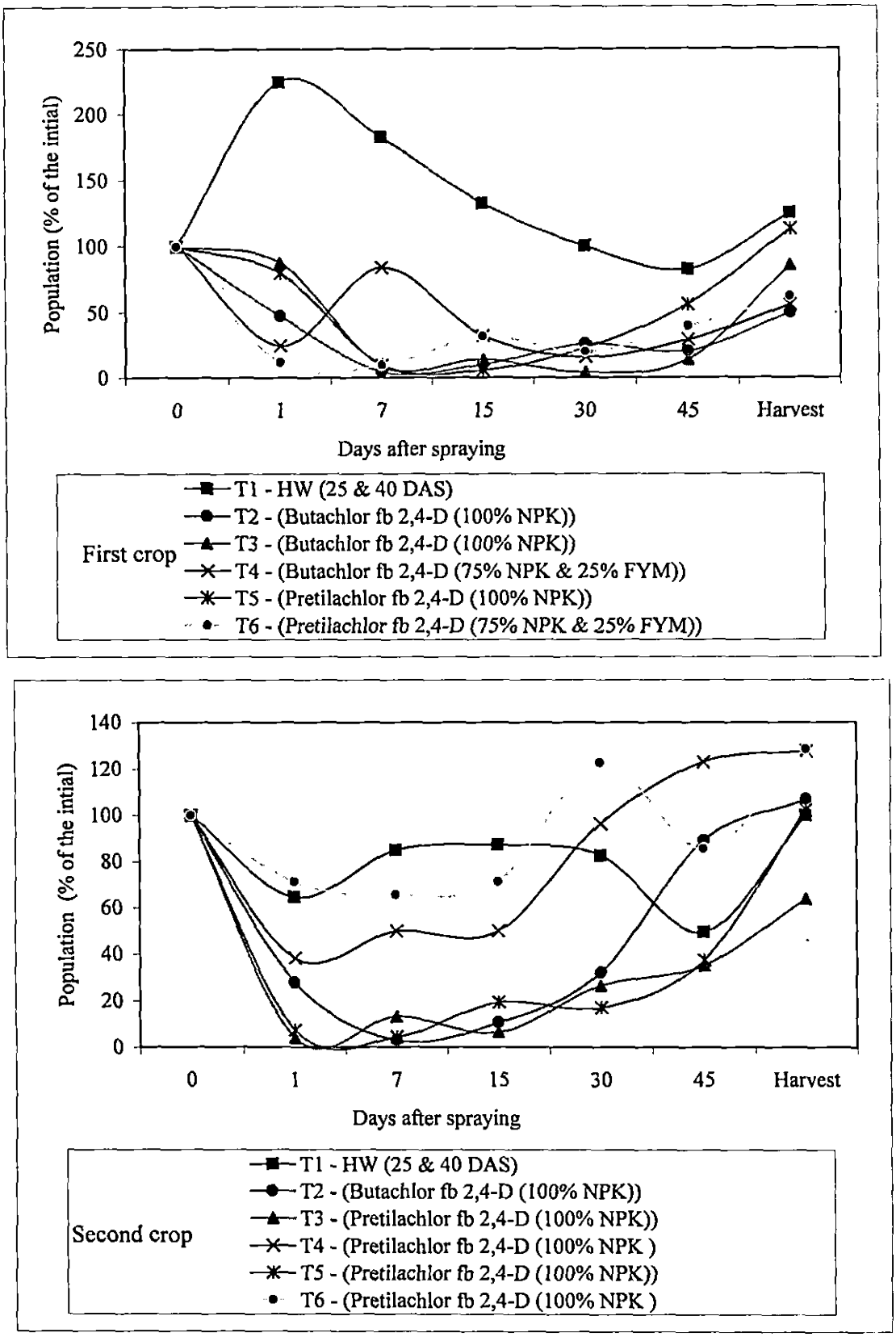


Fig. 5. Changes in the population of soil bacteria due to application of herbicides to wet land paddy

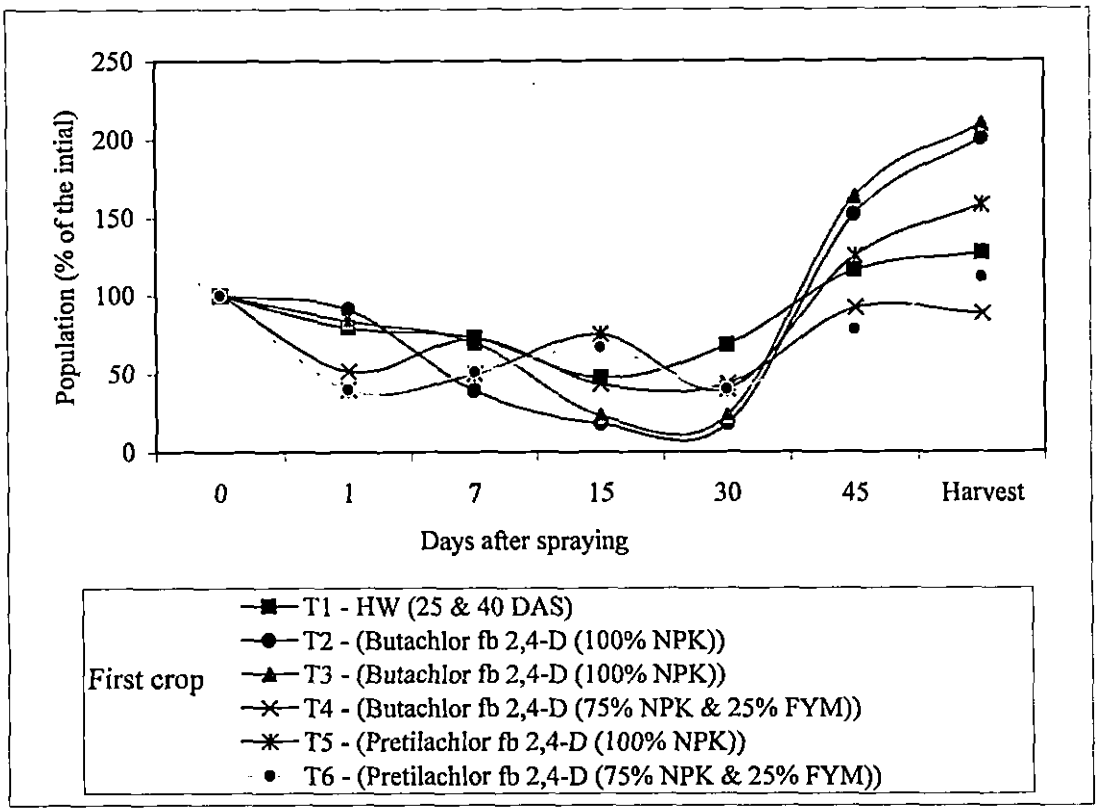
at 15 DAS was 96 per cent while in the second crop season percentage inhibition was 80.5 per cent. When pretilachlor was sprayed in the FYM treated plots the negative effect of herbicides was less compared to the plot, which received no FYM. Here also maximum reduction was noticed in the first crop season (90.4 per cent). Patel and Patel (1998) reported that application of FYM @ 5.0 t ha^{-1} significantly increased bacterial population over control on the same day and tenth day after application of fluchloralin, while fungal population was significantly higher up to 30 days. Microbial population was higher in FYM treated plots over control at all the intervals.

5.2.2. Effect on soil fungi

The result revealed that population of fungi in the soil was also not uniform during the entire crop period in the hand weeded plot. Compared to the second crop, their population was high in the first crop season. In all the treatments percentage inhibition was comparatively less for soil fungi than for soil bacteria (Fig. 6).

When butachlor fb 2,4-D was continuously applied during first and second crop seasons, maximum reduction in their population at 15 DAS for the first and second crop seasons were 81.9 and 71.8 per cent respectively. It was also noticed that both butachlor and pretilachlor (T_3) inhibited the fungal population to the same magnitude (76.8 per cent) in both the seasons. So, pretilachlor was equally harmful to soil fungi as butachlor, while in the case of bacteria the inhibitory effect of pretilachlor was less compared to that of butachlor.

As in the case of bacteria, the plots which received FYM when sprayed with butachlor during the first crop season and pretilachlor in the second crop season, variation in their population was occurred to a lesser magnitude (56.76 and 30.30 per cent for butachlor and pretilachlor respectively). When pretilachlor was applied continuously in first and second crop seasons without the application of FYM, fungal population had been retarded to greater extent during second crop season, which again is an indicative of



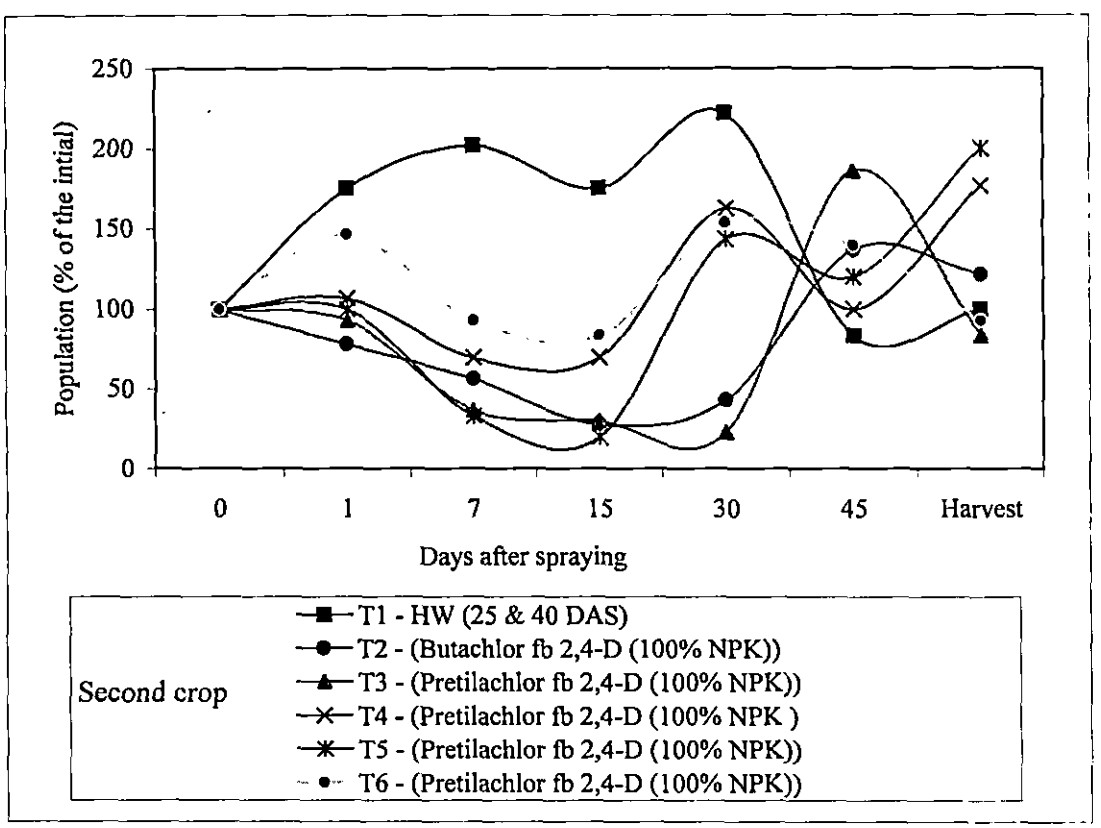


Fig. 6. Changes in the population of soil fungi due to application of herbicides to wet land paddy

the toxicity of pretilachlor to fungi. Observations recorded from T_6 (pretilachlor fb 2,4-D with 75 per cent NPK and 25 per cent FYM) made clear that the inhibitory effect of pretilachlor on soil fungi was less when sprayed after the application of FYM.

5.2.3. Effect on soil actinomycetes

As in the case of bacteria and fungi, population of actinomycetes showed varying trends at varying intervals in the hand weeded plot. However in the plots that were sprayed with butachlor (or) pretilachlor, registered lower count of actinomycetes up to 15 DAS and in certain treatments up to 30 DAS (Fig.7). When butachlor was continuously applied in two seasons, maximum reduction was noticed at 15 DAS in both the crop seasons. The extent of reduction for the first and second crop seasons was 77 and 88 per cent respectively. Hence the suppression effect of butachlor on actinomycetes was intermediary to bacteria and fungi.

In the treatment (T_3) when butachlor was sprayed during the first crop season, inhibitory effect of the herbicide was pronounced up to 45 DAS. In the second crop, there was no inhibitory effect on soil actinomycetes due to the application of pretilachlor. In the FYM treated plot butachlor inhibited the population of actinomycetes slightly (59 per cent), while during the second crop season degree of inhibition of actinomycetes population was more (79.4 per cent).

Continuous application of pretilachlor without FYM application reduced the population of actinomycetes to an extent of 70.0 per cent during first crop season and 88.5 per cent in the second crop season. FYM application reduced the negative effect of pretilachlor in both the seasons, which was evidenced from the observations taken on the samples from T_6 (Pretilachlor fb 2,4-D applied with 75per cent NPK and 25 per cent FYM).

Studies on the changes in microbial population due to the application of butachlor and pretilachlor revealed that inhibitory effect of these herbicides are more on soil

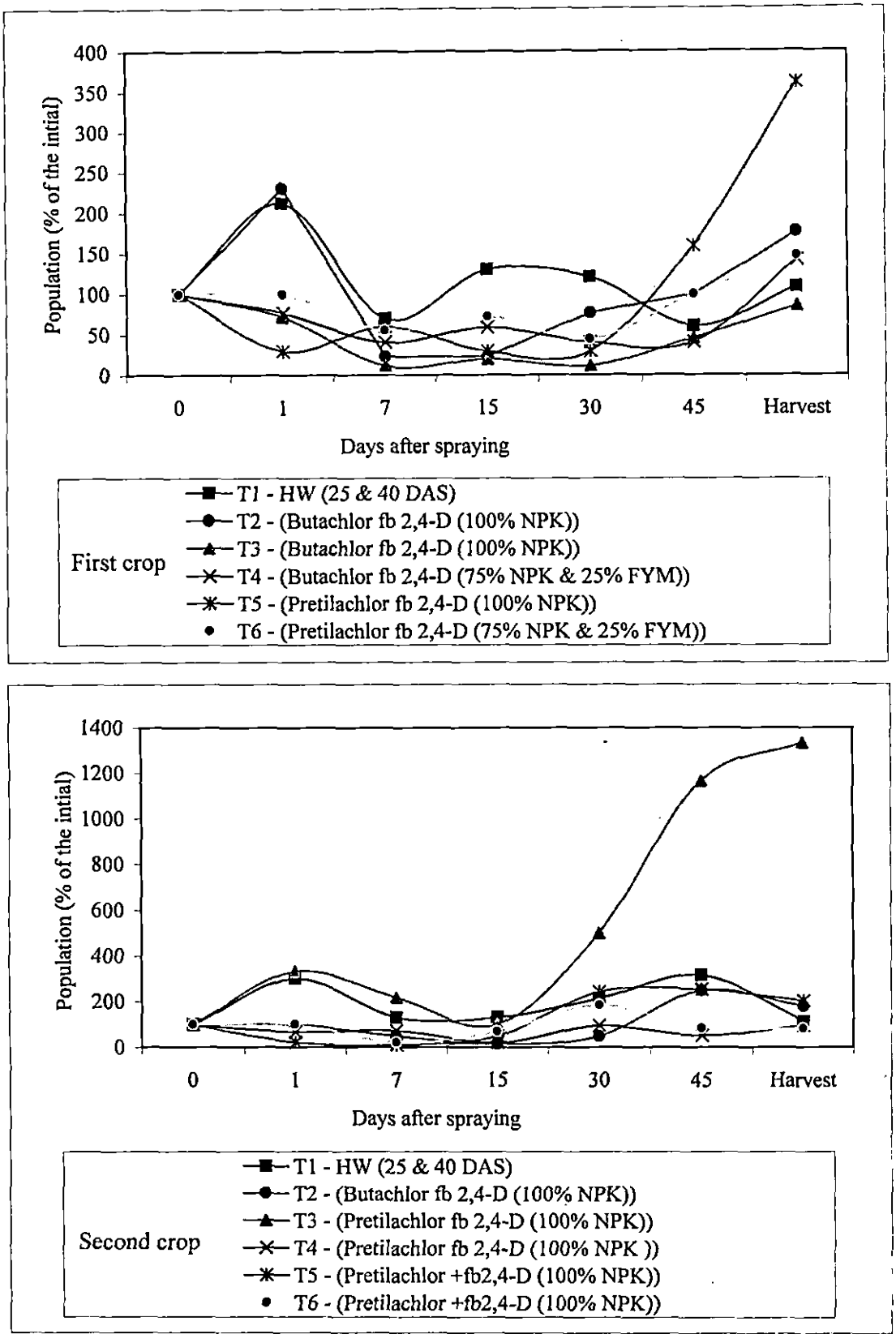


Fig. 7. Changes in the population of soil actinomycetes due to application of herbicides to wet land paddy

bacteria than soil fungi or actinomycetes. Both herbicides suppressed the population of soil bacteria to the same degree. However the effect of butachlor lasted for longer period in the first crop season particularly when FYM was not incorporated in the soil. Higher persistence of butachlor in the soil during the first crop season may be one of the reasons for this change. Devi (2002) also observed that inhibitory effect of herbicides was more on bacteria than on fungi.

Since 2,4-D was sprayed at 20 DAS in all the plots except hand weeded control, the time of 2,4-D application coincided with 12 days after application of butachlor or pretilachlor. More suppression of microflora during the 7-15 DAS period would have been resulted from the additive effect of butachlor, pretilachlor and 2,4-D.

Varying influences of butachlor on the bacteria, fungi and actinomycetes and enzyme activities in paddy soil was observed by many scientists (Hang *et al.*, 2001). Chauhan *et al.* (1994) opined that small concentration of pesticides did not alter the activity of organisms but at higher concentration most bacterial and fungal species are affected. In the studies conducted by Chakraborty and Bhattacharya (1991) it was found that two soil fungi namely *Fusarium solanum* and *Fusarium oxysporum* effectively degraded butachlor in a 0.02M KH_2PO_4 buffer solution at pH 5.2. The rice soil under the present investigation is having pH of 4.5 to 5.6 and the fungi would have exerted the key role in the degradation of herbicides.

Immediately after the application of herbicides, there is lag phase during which microbes try to adapt for the herbicides applied. Then they start multiplying and the degradation process proceeds (Rao, 1992). Therefore the period during which deficits of population of microorganisms noticed in the soil could be considered as the lag phase for a particular herbicide. A lag phase of 7-15 days was noticed for the herbicides namely butachlor and pretilachlor used in the present study. In the case of 2,4-D, the lag phase was less than 7 days as evidenced from the present investigation. When the soil was flooded, the gas exchange was limited and the temperature also deviated from the normal

and depression of population was noticed (Greaves and Malkomes, 1980). The same factors apply to the present study and more inhibitory effect on soil microflora was observed in the first crop season.

The above discussion on the effect of treatments on the soil microflora can be summarized in the following manner.

i) Use of butachlor continuously

Butachlor application drastically reduced the bacterial population within a period of 7 -15 DAS (95-97 per cent). Even at the time of harvest about 50 per cent reduction in their population was manifested during the first crop season. However, the population attained the original level at the time of harvest during the second crop season. Due to the difference in the soil moisture and temperature, bacterial species may vary between seasons. The result is indicative of the harmful effect of butachlor to certain species of bacteria. However, its continuous application did not make any drastic difference in the magnitude of inhibition.

Population of fungi or actinomycetes in the soil was not adversely effected by the continuous application of butachlor except for a very short period.

ii) Butachlor alternated with pretilachlor between seasons

The inhibitory effect of butachlor persisted for longer period than for butachlor,eventhough the degree of inhibition was smaller. Due to the spraying of pretilachlor in the second crop season, bacterial population did not come up to the original level by the time of harvest. When butachlor was sprayed in the second crop season, no adverse effect was noticed at the time of harvest in either 2001 or 2002.

As in the case of butachlor, pretilachlor application reduced the population of soil fungi and actinomycetes only for a short period.

iii) Butachlor alternated with pretilachlor between years

Application of pretilachlor, after two sprayings of butachlor in the previous year enhanced the bacterial population. Unlike butachlor, adverse effect of pretilachlor was only temporary in the first crop season.

Negative effect of pretilachlor on soil fungi and actinomycetes was also for a short period only.

iv) Effect of organic matter

Farmyard manure influenced the activity of soil bacteria only during first crop season. The adverse effect of butachlor on soil bacteria was reduced by the application of FYM but enhanced that of pretilachlor. The total number of soil bacteria at the time of harvest was very much less than that of before spraying. The adverse effect of butachlor on soil fungi was also more in the presence of organic matter. However, in the case of pretilachlor application of FYM reduced adverse effect. In the case of actinomycetes adverse effect of butachlor has been reduced by FYM application.

v) Difference between herbicides

The adverse effect of pretilachlor on soil bacteria was less than that of butachlor. However, pretilachlor was equally harmful for soil fungi as butachlor. Suppression effect of butachlor on soil actinomycetes was intermediary to soil bacteria and fungi. Effect of butachlor on soil actinomycetes was more during the first crop season compared to pretilachlor. During the second crop season both the herbicides inhibited the population in similar magnitude. It was very difficult to separate out the effect of 2,4-D on the population of soil microorganisms because both the butachlor and pretilachlor persisted in the soil up to 30 DAS.

5.3. BIOEFFICACY OF SELECTIVE HERBICIDES IN RICE-RICE SYSTEM

Efficacy of a herbicide in controlling weeds in the cropping system depends on the activity as well as selectivity of the chemical. Butachlor @ 1.25 kg a.i ha^{-1} and pretilachlor @ 0.75 kg a.i ha^{-1} are recommended at 0-6 days after sowing for control of grassy weeds in rice. Broad leaved weeds are effectively controlled by application of 2,4-D @ 1.00 kg a.i ha^{-1} at 3-4 weeks after sowing.

Selectivity is the greatest single factor that enabled the use of butachlor, pretilachlor and 2,4-D in rice. The efficacy of herbicides used in the experiment in controlling the weeds are presented in Fig.8 to 13. Observations on population and dry weight of different weed species indicated that predominant grassy weed in the experimental field was *Echinochloa spp, Marsilea quadrifoliata, Alternanthera echinata, Sphenoclea zeylanica* and *Ludwigia parviflora* were the major broad leaved weeds. The population and dry matter production varied between treatments.

Among the different weed species, *Echinochloa spp* contributed very much to the total weed population and total dry weight because of its more aggressive nature. Dynamics of weed population over the four crop seasons (Fig.8) gave an indication that the number of *Echinochloa spp* is increasing with continuous use of chloroacetanilides.There are many reports stating that *Echinochloa crusgalli* is resistant to pretilachlor (Chungi and Ramteke, 1987). At the end of four seasons total number of Echinochloa as well as total weeds was more in the treatment T_5 where pretilachlor fb 2,4-D was applied in first and second crop season of 2002, while in the case of broad leaved weeds, the population as well as dry matter production was more in T_6 where pretilachlor was sprayed after the incorporation of FYM in soil.

In the first crop of 2002, *Marsilea quadrifoliata* was the major broadleaf weed, which contributed less dry matter production. During the second crop, Echinochloa *spp* dominated all the other weeds, which contributed to more dry matter production during this season.

Except in the hand weeded plot, 2,4-D was applied in all the other treatments in all the seasons and the total dry weight of broad leaved weeds was less during the period of experimentation. The results indicated the better efficiency of 2,4-D in controlling broad leaves weeds. None of the broad leaved species showed build up in the population.

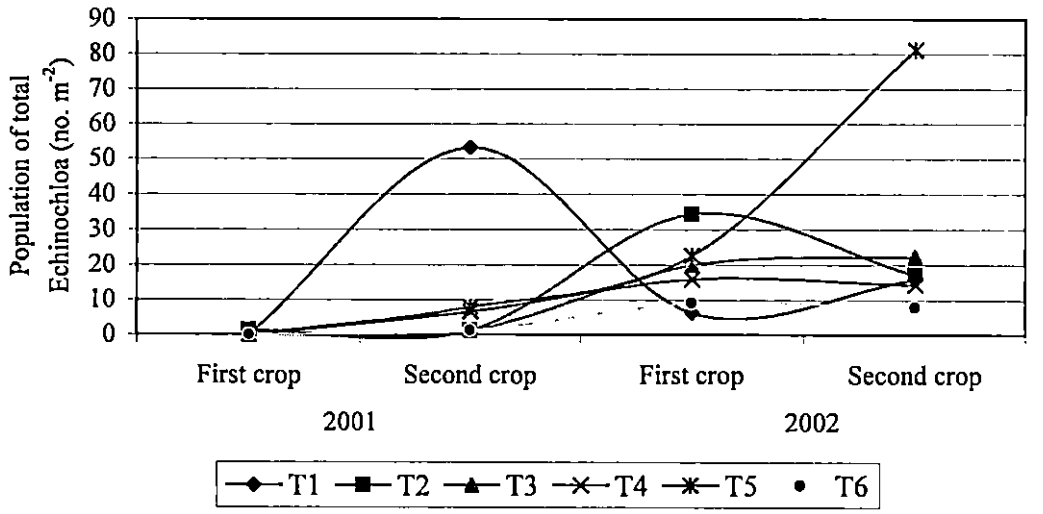


Fig. 8. Variation in the population of total *Echinochloa spp* due to the application of herbicides over a period of 2 years

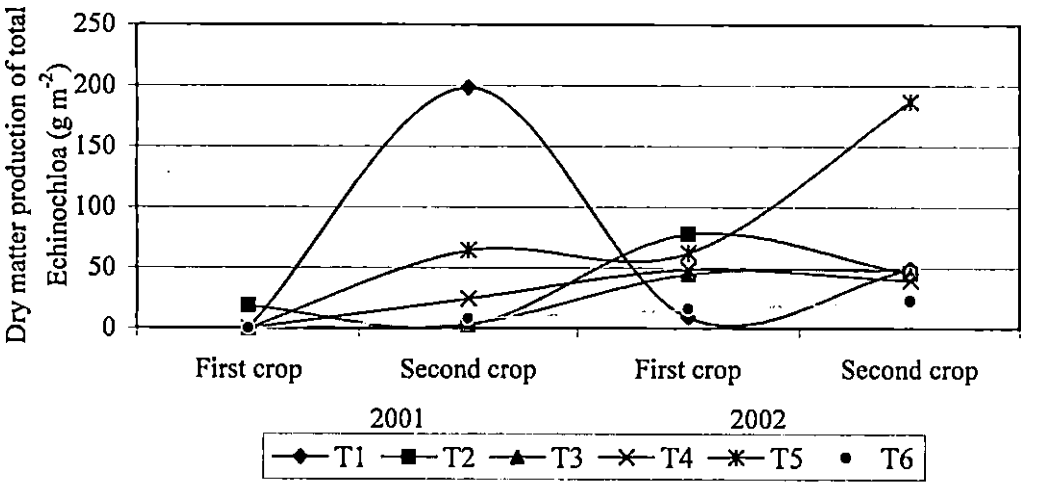


Fig.9. Variation in the dry matter production of total *Echinochloa spp* due to the application of herbicides over a period of 2 years

First crop

T1 - HW (25 & 40 DAS)

T2 - Butachlor fb 2,4-D (100% NPK)

T3 - Butachlor fb 2,4-D (100% NPK)

T4 - Butachlor fb 2,4-D (75% NPK & 25% FYM)

T5 - Butachlor fb 2,4-D (100% NPK) for 2001

T5 - Pretilachlor fb 2,4-D (100% NPK) for 2002

T6 - Butachlor fb 2,4-D (75% NPK & 25% FYM) for 2001

T6 - Pretilachlor fb 2,4-D (75% NPK & 25% FYM) for 2002

Second crop

T1 - HW (25 & 40 DAS)

T2 - Butachlor fb 2,4-D (100% NPK)

T3 - Pretilachlor fb 2,4-D (100% NPK)

T4 - Pretilachlor fb 2,4-D (100% NPK)

T5 - Butachlor fb 2,4-D (100% NPK) for 2001

T5 - Pretilachlor fb 2,4-D (100% NPK) for 2002

T6 - Butachlor fb 2,4-D (100% NPK) for 2001

T6 - Pretilachlor fb 2,4-D (100% NPK) for 2002

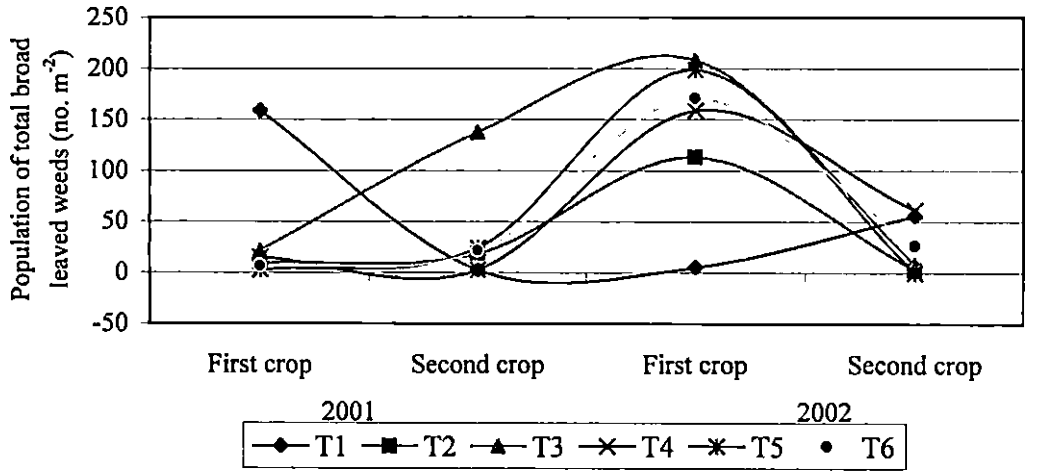


Fig.10. Variation in the population of total broad leaved weeds due to the application of herbicides over a period of 2 years

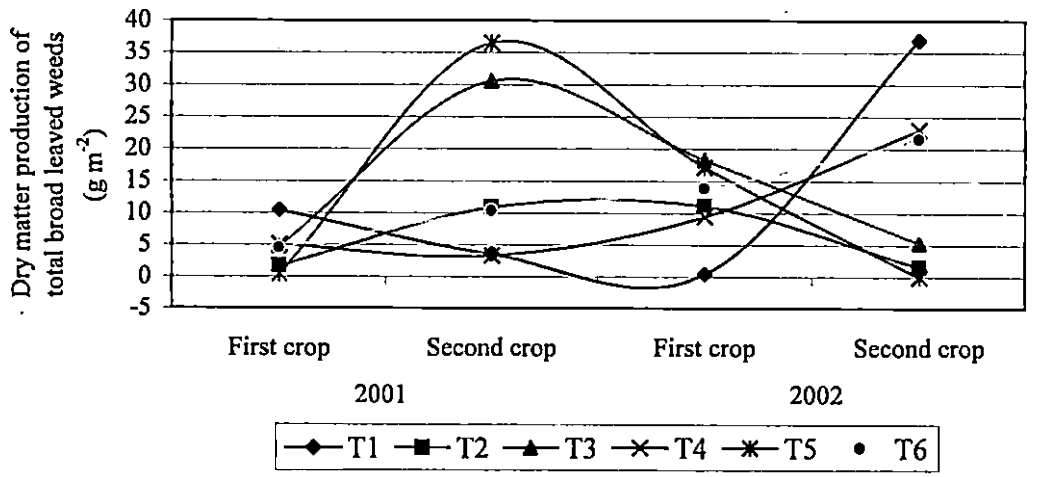


Fig. 11. Variation in the dry matter production of total broad leaved weeds due to the application of herbicides over a period of 2 years

First crop

T1 - HW (25 & 40 DAS)

T2 - Butachlor fb 2,4-D (100% NPK)

T3 - Butachlor fb 2,4-D (100% NPK)

T4 - Butachlor fb 2,4-D (75% NPK & 25% FYM)

T5 - Butachlor fb 2,4-D (100% NPK) for 2001

T5 - Pretilachlor fb 2,4-D (100% NPK) for 2002

T6 - Butachlor fb 2,4-D (75% NPK & 25% FYM) for 2001

T6 - Pretilachlor fb 2,4-D (75% NPK & 25% FYM) for 2002

Second crop

T1 - HW (25 & 40 DAS)

T2 - Butachlor fb 2,4-D (100% NPK)

T3 - Pretilachlor fb 2,4-D (100% NPK)

T4 - Pretilachlor fb 2,4-D (100% NPK)

T5 - Butachlor fb 2,4-D (100% NPK) for 2001

T5 - Pretilachlor fb 2,4-D (100% NPK) for 2002

T6 - Butachlor fb 2,4-D (100% NPK) for 2001

T6 - Pretilachlor fb 2,4-D (100% NPK) for 2002

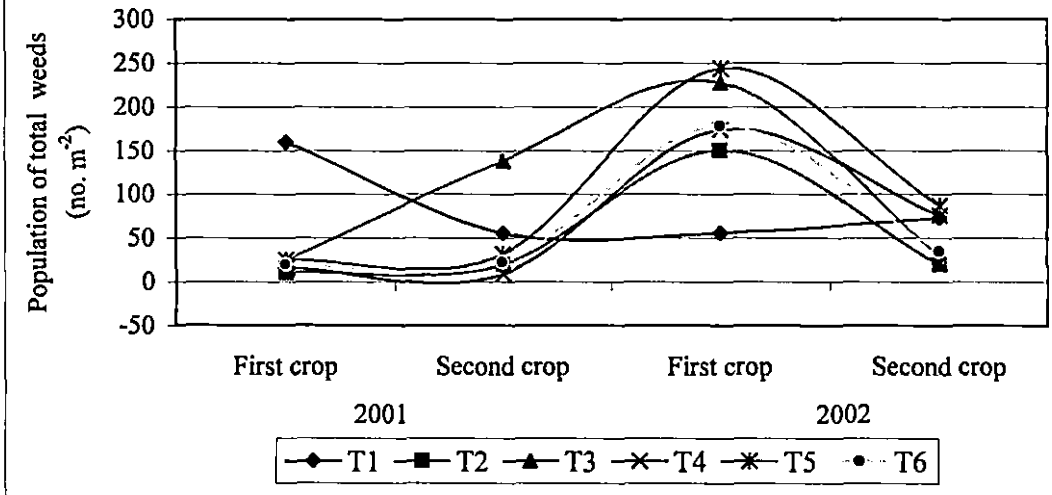


Fig.12. Variation in the population of total weeds due to the application of herbicides over a period of 2 years

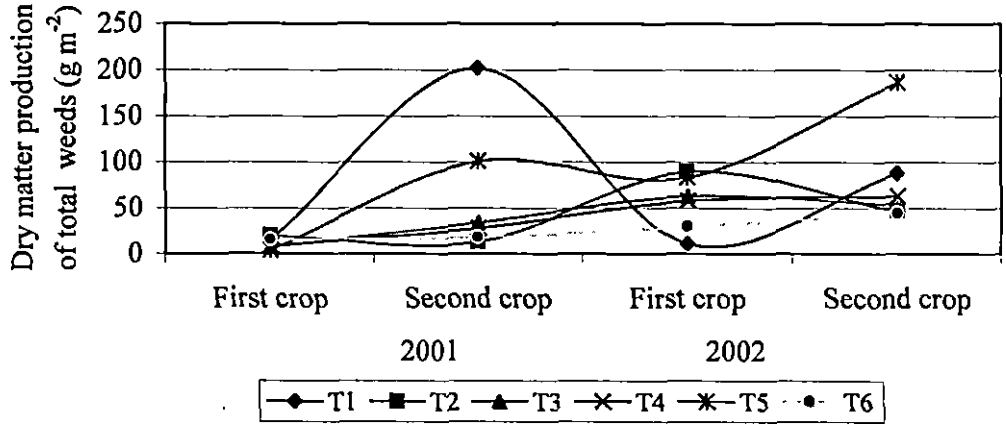


Fig. 13. Variation in the dry matter production of total weeds due to the application of herbicides over a period of 2 years

First crop

T1 - HW (25 & 40 DAS)

T2 - Butachlor fb 2,4-D (100% NPK)

T3 - Butachlor fb 2,4-D (100% NPK)

T4 - Butachlor fb 2,4-D (75% NPK & 25% FYM)

T5 - Butachlor fb 2,4-D (100% NPK) for 2001

T5 - Pretilachlor fb 2,4-D (100% NPK) for 2002

T6 - Butachlor fb 2,4-D (75% NPK & 25% FYM) for 2001

T6 - Pretilachlor fb 2,4-D (75% NPK & 25% FYM) for 2002

Second crop

T1 - HW (25 & 40 DAS)

T2 - Butachlor fb 2,4-D (100% NPK)

T3 - Pretilachlor fb 2,4-D (100% NPK)

T4 - Pretilachlor fb 2,4-D (100% NPK)

T5 - Butachlor fb 2,4-D (100% NPK) for 2001

T5 - Pretilachlor fb 2,4-D (100% NPK) for 2002

T6 - Butachlor fb 2,4-D (100 % NPK) for 2001

T6 - Pretilachlor fb 2,4-D (100% NPK) for 2002

Both butachlor and pretilachor are coming under chloroacetanilide group. Rice plant has the capacity to deactivate these herbicides by conjugation with glutathione (tripeptide containing glutamic acid,cysteine,glycine) non enzymatically and enzymatically by the action of glutathione S-transferase. The non enzymatic conjugation rate varied in the order propachlor > pretilachlor > alachlor > acetochlor > dimethachlor > metalochlor > butachlor (Shimabukuro, 1985). Hence the molecular structure of 2-chloroacetanilides, their concentration in protein bulks and the protein content in the plot and catalytic efficiency of GST enzyme are the crucial factors in determining the plant tolerance. Metabolism (deactivation) of pretilachlor has been reported in rice as well as *Echinochloa.* However the rate of metabolism of pretilachlor was rapid in rice and low in *Echinochloa crusgalli* (Usui *et al.*, 1999). Morphologically *Echinochloa* shows resemblance with rice plant. Similarities in the metabolic reaction would have contributed to the resistance of *Echinochloa spp* to the herbicides like butachlor and pretilachlor.

The resistance of graminaceous species to lower concentration of 2,4-D is due to their ability to metabolize the chemical to non herbicidal compounds. 2,4-D is detoxified by rice plants by hydroxylation and conjugation with plant constituents like glucose and aminoacids. Presence of greater amount of hydroxylase in resistant species has been reported by many scientists (Weintrub *et al.*, 1952).

One of the major factors determining the weed population in a field is the moisture content of the soil. During the first crop season the rice field was under submerged condition due to heavy rains and the weed population was comparatively lower. At field capacity emergence of weeds is more. Therefore higher population of weeds was observed in the second crop season. Civco and Moody (1979) reported that the growth of that *Echinochloa spp* was not affected by the time of flooding or moisture level and it could survive in both lowland and upland rice once it become established.

5.4. EFFECT OF HERBICIDE APPLICATION ON THE HEIGHT OF PLANT AND YIELD

The growth of plant is affected when there is reduction in nutrient availability, caused by competition of weeds. To some extent herbicide activity also inhibit plant growth. Crop weed competition lowered the panicle number by 37%, number of filled grains per panicle by 13% and 1000 grain weight by 4% (Ghobrial, 1981). In the present study, weeds were kept under control in all the plots received weed control treatments (either a herbicide or handweeding). Therefore, crop weed competition was not significant and there was not much variation in the plant height or in productive tillers. However, during first crop and second crop of 2002, the highest grain and straw yields were recorded in the plot where butachlor was applied.

Toxicity to the rice seedlings was less observed in butachlor sprayed plots compared to pretilachlor. In addition comparatively smaller number of weeds was present in the field favoured the crop to utilize all available resources efficiently. These two factors would have been responsible for the higher yield of rice by butachlor application. Similar results were also reported by Ghobrial (1981).

Joy *et al* (1991) reported that butachlor resulted in the greater benefit cost ratio (28.1) compared to hand weeding (5.2). Butachlor @ 1.5 kg proved to be most effective, giving 89.41% control of weeds and highest yield of 2047 kg ha^{-1} was achieved (Choudhary and Pradhan, 1989).

5.5. CHANGES IN NUTRIENT CONTENT OF THE SOIL AND RICE PLANT

The rice field in which the experiments were conducted consisted of laterite soil with a pH in the range of 4.5 to 5.6, which is conducive for the growth of rice. Texturally the soil of the area is sandy clay loam and the organic carbon content was in the medium range. Silica content of the soil was very high (65 to 70.2 per cent). Sesquioxide content and cation, anion exchange capacities of the soil under study were varying within a narrow range. Available nitrogen and potassium was in the range of low to medium,

while the available phosphorus content ranged from medium to high. The soil analysis data indicated that the plots in which different herbicide treatments applied were genetically homogenous. However their dissimilarities in the available nutrients within a narrow range led to conclusion that cropping system or their management practices followed in the previous crop seasons would have resulted in some changes in nutrient availability.

Effect of treatments

Even though available nitrogen content of the soil did not vary between treatments in any of the crop seasons studied, nitrogen content of the grain was significantly higher in the plots, which received FYM during first crop season (T_4 and T_6). One of the major factors contributing to this variation would be the smaller number of *Echinochloa spp* present in this plots. As the uptake of nitrogen by grassy weeds is generally higher than that of broad leaved weeds, crop competition for this nutrient would be much higher. The treatments containing organic matter (T_4 and T_6) might have experienced lesser crop weed competition for nitrogen, which would have resulted in the higher absorption and translocation of nitrogen from organic manure would have minimized nitrogen loss and made the plant to utilize more quantity of applied nitrogen.

After the harvest of the second crop in 2002, available P content of the soil varied significantly between treatments. The plots received FYM (T_4 and T_6) recorded lower content of available P in the soil after harvest of the first and second crop. One of the possible reasons for this difference would be the better growth of broad leaved weeds in this plots. In general broad leaved weeds are accumulators of phosphorus and potassium from soil. Since FYM application would increase the availability of phosphorus in the soil, absorption of P by the plants would be more, which would have resulted in depletion of available phosphorus from the soil. Due to the predominance of broad leaved species in the FYM applied plots, available potassium in the soil also declined gradually, though the differences between treatments was not significant. Even if broad leaved weeds were larger in number in the FYM treated plots, their contribution to the total dry matter

production was only small. Therefore rice plant would not have experienced much competition, which resulted in smaller differences in the phosphorus and potassium content of grain and straw.

The availability of nutrients in the soil to plant depends on many factors like soil properties, climatic conditions, nutrient losses and concentration of nutrient at particular stage. Actually when there is good weed control, the uptake of nutrients would be high. Due to the predominance of other factors as mentioned above, variation in the nutrient uptake was not attributed to the effect of herbicide application alone.

Summary

6. SUMMARY

The study entitled "Persistence of selective herbicides in rice-rice system" was the part of the "Permanent herbicide trial" of All India Coordinated Research Programme on Weed Control conducted during the year 2001 to 2002 with objective to determine the persistence of butachlor, pretilachlor and 2,4-D in the rice soil and plant. Effect of these herbicides on soil microflora, weed population and nutrient content of rice plant were also studied. The salient results of the present study are summarised below.

1. Among the three plots, which received butachlor spraying during first crop season of 2002, FYM applied plot recorded highest concentration of butachlor residues (0.3430 $\mu g\ g^{-1}$) at one day after spraying. Dissipation of butachlor was also observed higher in the FYM applied plot (97.66%) at 30 days after spraying.

2. About 94.00 to 98.00 per cent of the pretilachlor was disappeared from the soil by 30 days after spraying. Dissipation of pretilachlor was also higher in the plots, which received FYM at 30 days after spraying.

3. 2,4-D had been dissipated to an extent of 97.81 to 99.27 per cent in the plot, which received FYM while in the other treatments dissipation of 2,4-D ranged from 94.89 to 97.81%.

4. In the soil, all the three herbicides namely butachlor, pretilachlor and 2,4-D persisted up to 30 days after spraying and dissipated to non detectable level by the time of harvest. None of the herbicides was detected in the rice grain or straw.

5. All the three herbicides under study inhibited the population of bacteria in the soil. Maximum reduction of population was observed (95.24 to 96.80%) at 7 days after spraying in both the crop seasons.

6. Population of soil fungi was also reduced by the application of herbicides. However, the magnitude of reduction was less compared to that of soil bacteria. It was also noticed that butachlor and pretilachlor inhibited the fungal population to the same magnitude (76.80%).

7. About 59.00 to 85.00 per cent reduction was noticed in the population of actinomycetes with maximum inhibition at 15 days after spraying (77.00 to 85.00%) in both the crop seasons. Effect of butachlor on inhibition of soil actinomycetes found to be intermediary when compared to bacteria and fungi.

8. Application of FYM was found to be effective in enhancing the total population of microflora in the soil.

9. Observations on weed count and dry matter production of different weed species showed that *Echinochloa* spp. contributed very much to the total weed population and total dry weight because of its more aggressive nature.

10. Dynamics of weed population over the four crop seasons gave an indication that the number of *Echinochloa* spp. increased with continuous use of pre emergence herbicides. Among the treatments, pretilachlor and 2,4-D applied with 100 per cent NPK as inorganic fertilizer recorded higher count of *Echinochloa* spp.

11. 2,4-D was effective in controlling broad leaved weeds except *Marsilia quadrifoliata*. Build up in the population of broad leaved weeds was not observed in any plot.

12. There was not much variation in the plant height and productive tillers of rice plant due to alteration of herbicides.

13. Highest yield of grain and straw was obtained in the plots where butachlor and 2,4-D was applied with 100 per cent NPK as inorganic fertilizer.

14. Incorporation of FYM in to the soil improved organic carbon content of the soil as well as nitrogen content of grain and straw. However, the effect of herbicides on the uptake of P and K by the rice plant was not conspicous.

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Appendices

APPENDIX I

Weekly weather data for first crop of 2001 — 4th June, 2001 - 30th September, 2002

Date	Temperature		Relative humidity (%)		Wind speed (kmph)	Sunshine (hrs)	Rainfall (mm)	Evaporation (cm)
	Max. (°C)	Min. (°C)	Morning	Evening				
4/6 - 10/6	29/0	22.0	95	81	3.2	1.5	185.5	2.6
11/6 - 17/6	28.0	23.1	94	83	3.8	1.7	265.8	2.7
18/6 - 24/6	29.7	23.3	95	80	3.3	2.8	146.2	3.0
25/6 - 1/7	29.3	22.9	93	78	3.1	2.0	61.7	2.7
2/7 - 8/7	28.7	22.7	94	83	3.8	1.3	284.7	1.7
9/7 - 15/7	28.9	22.5	94	83	3.1	2.1	109.1	2.7
16/7 - 22/7	30.2	23.2	90	66	3.7	4.3	10.4	3.4
23/7 - 29/7	28.3	22.6	93	79	3.5	1.8	62.7	3.0
30/7 - 5/8	27.2	22.3	94	83	3.4	0.8	54.2	2.0
6/8 - 12/8	29.9	23.5	95	72	3.6	4.8	37.4	2.6
13/8 - 19/8	29.0	23.1	95	81	2.8	2.2	105.4	2.5
20/8 - 26/8	30.1	23.2	94	72	3.7	3.8	52.6	3.7
27/8 - 2/9	30.3	23.5	93	70	4.0	6.7	12.4	4.6
3/9 - 9/9	31.8	23.2	91	57	3.2	8.2	2.5	4.7
10/9 - 16/9	31.7	23.5	90	61	3.0	6.6	2.5	4.7
17/9 - 23/9	30.8	23.5	88	69	3.4	4.5	10.2	4.3
24/9 - 30/9	28.8	22.3	94	80	3.5	1.6	193.4	2.3

APPENDIX II

Weekly weather data for second crop of 2001 — 1st October, 2001 - 1st January, 2002

Date	Temperature		Relative humidity (%)		Wind speed (kmph)	Sunshine (hrs)	Rainfall (mm)	Evaporation (cm)
	Max. (°C)	Min. (°C)	Morning	Evening				
1/10 - 7/10	30.1	23.1	93	72	2.9	3.6	47.6	3.3
8/10 - 14/10	30.1	23.0	91	74	3.1	3.6	94.0	3.3
15/10 - 21/10	31.0	22.8	90	70	3.2	5.1	56.2	3.1
22/10 - 28/10	31.5	22.3	91	69	3.4	5.6	62.1	3.6
29/10 - 4/11	31.8	23.5	90	62	2.8	7.0	11.3	3.8
5/11 - 11/11	31.2	23.6	92	68	3.2	5.0	30.6	3.2
12/11 - 18/11	31.4	23.2	91	67	4.1	5.0	74.3	3.3
19/11 - 25/11	31.7	23.3	73	53	7.6	8.1	0.0	5.4
26/12 - 2/12	31.2	22.1	72	52	6.3	7.6	0.0	4.7
3/12 - 9/12	31.5	22.3	72	48	8.8	9.4	0.0	5.5
10/12 - 16/12	31.1	18.9	67	37	7.0	9.2	0.0	5.6
17/12 - 23/12	30.9	23.2	74	53	12.8	7.4	0.0	6.2
24/12 - 31/12	32.1	23.8	76	51	11.8	8.1	0.0	6.6
1/1 - 17/1	32.4	23.7	70	44	12.7	9.8	0.0	7.4

APPENDIX III

Weekly weather data for first crop of 2002 — 4th June, 2002 - 23rd September, 2002

Date	Temperature		Relative humidity (%)		Wind speed (kmph)	Sunshine (hrs)	Rainfall (mm)	Evaporation (cm)
	Max. (°C)	Min. (°C)	Morning	Evening				
4/6 - 10/6	30.7	23.4	94	73	4.0	5.0	64.2	3.5
11/6 - 17/6	28.9	22.5	94	83	3.7	0.6	219.1	2.5
18/6 - 24/6	29.5	23.3	93	81	4.5	1.8	109.8	2.8
25/6 - 1/7	30.5	23.7	94	75	3.7	3.5	74.6	3.1
2/7 - 8/7	30.3	23.6	94	72	3.7	5.2	57.0	3.4
9/7 - 15/7	29.4	23.1	94	77	4.0	3.0	126.1	3.1
16/7 - 22/7	29.7	22.7	95	73	3.8	2.7	58.0	3.1
23/7 - 29/7	29.9	22.9	93	73	3.7	3.8	70.4	2.9
30/7 - 5/8	28.1	22.5	95	86	4.0	0.73	83.6	2.4
6/8 - 12/8	28.6	22.2	95	79	3.8	0.9	94.0	2.8
13/8 - 19/8	27.9	22.8	94	83	3.7	2.6	337.0	2.1
20/8 - 26/8	30.1	23.4	93	72	3.7	5.4	13.8	3.8
27/8 - 2/9	30.9	24.1	93	65	3.8	7.3	3.8	4.3
3/9 - 9/9	29.8	23.2	94	71	3.7	5.5	98.7	3.4
10/9 - 17/9	30.7	22.9	92	63	3.7	8.7	0.0	4.4
17/9 - 23/9	31.3	22.8	91	59	3.6	8.3	0.0	4.3

APPENDIX IV

Weekly weather data for second crop of 2002 — 24th September, 2002 - 31st December, 2002

Date	Temperature		Relative humidity (%)		Wind speed (kmph)	Sunshine (hrs)	Rainfall (mm)	Evaporation (cm)
	Max. (°C)	Min. (°C)	Morning	Evening				
24/9 - 30/9	32.5	22.7	90	55	3.6	8.2	21.5	4.4
1/10 - 7/10	32.2	23.3	89	67	3.2	5.7	51.0	3.7
8/10 - 14/10	29.3	23.1	93	89	3.5	2.1	268.3	1.8
15/10 - 21/10	30.1	23.0	92	74	2.8	4.3	25.1	3.5
22/10 - 28/10	31.5	23.5	92	66	3.1	6.0	9.9	23.0
29/10 - 4/11	31.6	23.3	84	61	5.1	5.7	33.4	4.4
5/11 - 11/11	31.8	23.5	90	66	3.4	4.7	8.7	3.1
12/11 - 18/11	31.2	23.7	83	59	5.1	4.7	9.4	4.2
19/11 - 25/11	31.3	23.3	78	63	5.4	6.8	4.0	4.3
26/11 - 2/12	32.9	22.6	79	51	4.5	9.2	0.0	4.5
3/12 - 9/12	32.1	23.2	73	56	9.6	7.6	0.0	5.9
10/12 - 16/12	32.1	24.3	69	50	11.8	10.8	0.0	8.8
17/12 - 23/12	32.2	20.9	69	39	8.3	8.3	0.0	6.5
24/12 - 31/12	32.5	20.5	74	37	4.1	9.3	0.0	5.2

PERSISTENCE OF SELECTIVE HERBICIDES IN RICE-RICE SYSTEM

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ABSTRACT OF THE THESIS

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Master of Science in Agriculture

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ABSTRACT

The study entitled "Persistence of selective herbicides in rice-rice system" was the part of 'permanent herbicide trial' of All India Coordinated Research Programme on Weed Control conducted during the years 2001 and 2002 with the objective of determining the persistence of butachlor, pretilachlor and 2,4-D in the rice-rice cropping system. The effect of herbicides on soil microflora, weed growth and nutrient content were also studied. The field experiments were conducted during 2001 to 2002 at Agricultural Research Station, Mannuthy. Residue studies related to field experiments were conducted at Herbicide Residue Laboratory of All India Coordinated Research Programme on Weed Control, Thrissur Centre, located at Radio Tracer Laboratory, College of Horticulture, Vellanikkara. Microbiological studies and physico chemical analysis of the soil samples were conducted at College of Horticulture, Vellanikkara.

The technical programme consisted of six treatments namely (i) hand weeding twice (25 and 40 DAS); (ii) butachlor fb 2,4-D (with 100% NPK as inorganic fertilizer) in the first and second crop seasons of 2001 and 2002; (iii) butachlor fb 2,4-D (with 100% NPK as inorganic fertilizer) in the first crop season of 2001 and 2002, pretilachlor fb 2,4-D (with 100% NPK as inorganic fertilizer) in the second crop season of 2001 and 2002; (iv) butachlor fb 2,4-D (with 75% NPK as inorganic fertilizer and 25% through FYM) in first crop of 2001 and 2002, pretilachlor fb 2,4-D (with 100% NPK as inorganic fertilizer) in second crop of 2001 and 2002; (v) butachlor fb 2,4-D (with 100% NPK as inorganic fertilizer) in the first and second crop of 2001, pretilachlor fb 2,4-D (with 100% NPK as inorganic fertilizer) in the first and second crop of 2002 and (vi) butachlor fb 2,4-D in the first and second crop of 2001 (with 75% NPK as inorganic fertilizer and 25% through FYM in the first crop and 100% NPK as inorganic fertilizer in the second crop), pretilachlor fb 2,4-D in the first and second crop of 2002 (with 75% NPK as inorganic fertilizer and 25% through FYM in the first crop and 100% NPK as inorganic fertilizer in the second crop).

The pre-emergence herbicides butachlor @ 1.25 kg a.i ha^{-1} and pretilachlor @ 0.75 kg a.i ha^{-1} were applied at 8 days after sowing for the control of grassy weeds. 2,4-D @ 1.00 kg a.i ha^{-1} was applied at 20 days after sowing for the control of broad leaved weeds. Persistence of the above three herbicides applied in first and second crop of 2002 was estimated at one and 30 days after spraying and at the time of harvest.

Residues of butachlor at one day after spraying ranged from 0.331 to 0.396 μg g^{-1} in first crop of 2002. The highest concentration of 0.396 μg g^{-1} was recorded in the plot where butachlor was applied with 75 per cent NPK as inorganic fertilizer and 25 per cent through FYM. About 95.21 to 97.66% of the applied butachlor dissipated from the soil by 30 days after spraying. Greater dissipation was observed in the FYM applied plots (97.66%). The application of FYM enhanced the microbial degradation which resulted in higher dissipation of butachlor. In the second crop, only one plot received butachlor spraying which recorded residues of 0.343 μg g^{-1} at 1 day after spraying and 0.020 μg g^{-1} at 30 days after spraying.

Residues of pretilachlor at one day after spraying ranged from 0.200 to 0.215 μg g^{-1} in the first crop season and 0.199 to 0.250 μg g^{-I} in the second crop season. At 30 days after spraying residues ranged from 0.020 to 0.013 μg g^{-1} and 0.010 to 0.030 μg g^{-1} in the first and second crop season respectively. As in case of butachlor higher degree of dissipation was observed in FYM applied plots (97.47 to 98.05%).

Residues of 2,4-D at one day after spraying ranged from 0.310 to 0.502 μg g^{-1} in first crop season and 0.395 to 0.480 μg g^{-1} in second crop season of 2002. At 30 days after spraying the residues ranged from 0.035 to 0.010 μg g^{-1} in first crop and 0.016 to 0.005 μg g^{-1} in second crop season. Up to 97.81 to 99.27 per cent of 2,4-D had been dissipated from the plots which received FYM. In the other plots percent dissipation of 2,4-D from the soil was 94.89 to 97.81 per cent.

On comparing the extent dissipation of butachlor, pretilachlor and 2,4-D it was found that 2,4-D had been dissipated to a higher magnitude than butachlor and pretilachlor. At the time of harvest residues were not detected in soil, rice grain and straw.

Studies on the effect of herbicides on soil bacterial population showed that the total number of bacteria in the soil had been considerably reduced by spraying herbicides. The extent of reduction was maximum (95.24 to 96.80%) at 7 DAS. The inhibitory effect of pretilachlor on soil bacteria was comparitively less than that of butachlor. The herbicides reduced the population of soil fungi. However, the magnitude of reduction was less than that of soil bacteria. It was also observed that butachlor and pretilachlor inhibited fungal population to the same degree (76.80%). Actinomycetal population in the soil was also inhibited by the application of herbicide and the maximum percent inhibition (77.00 to 85.00%) was at 15 DAS. Effect of butachlor on supression of soil actinomycetes was found to be intermediary to bacteria and fungi. In the plot where FYM was applied, population of bacteria, fungi and actinomycetes were higher than that of other plots, which gave an indication that FYM could reduce the adverse effect of herbicides on soil microflora.

The data on weed count and dry matter production of different weed species showed that *Echinochloa* spp. contributed very much to total weed population and total dry weight. Build up in the population of *Echinochloa* spp. was observed with continuous use of pre emergence herbicides. The plot where pretilachlor fb 2,4-D was applied with 100 per cent NPK as inorganic fertilizer recorded higher counts of *Echinochloa* spp.

Application of 2,4-D was effective in controlling broad leaved weeds. None of the broad leaved weeds showed build up in the population. The plot which received butachlor fb 2,4-D with 100 per cent NPK as inorganic fertilizer recorded highest grain and straw yield.

From the study it could be concluded that at the present recommended rate of application, residues of herbicides butachlor, pretilachlor and 2,4-D do not persist in paddy soil to detectable level beyond 30 days. Residues were not detected in the grain and straw. Application of FYM enhanced microbial degradation of herbicides and reduced the adverse effect of herbicides on soil microflora. 2,4-D was highly efficient in controlling broad leaved weeds in the rice-rice system. Both pretilachlor and butachlor controlled grasses except *Echinochloa* spp. Butachlor had shown its superiority over pretilachlor in the weed management of rice-rice cropping system.