EVALUATION OF FLUFENACET FOR THE CONTROL OF HERBICIDE RESISTANT *Phalaris minor* Retz. AND ITS PERSISTENCE IN SOIL AND WHEAT CROP

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By

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CERTIFICATE

This is to certify that the thesis entitled "**Evaluation of flufenacet for the control of herbicide resistant *Phalaris minor* and its persistence in soil and wheat crop**", Submitted in partial fulfillment of the requirements of the degree of **DOCTOR OF PHILOSOPHY** in **AGRONOMY** of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi is a record of *bona-fide* research carried out by **Mr. MOHAMMAD BAZOOBANDI**, Under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigation have been duly acknowledged.

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1. INTRODUCTION

In recent decades the predominant weed control method in many parts of the world has been the use of effective and reliable chemical herbicide. Although herbicides are major factor contributing to world crop production the intensive use of herbicides is held responsible for environmental pollution, shift in weed flora and evolution of resistant weed biotypes, which jeopardize herbicide utility, availability and longevity and impose the threat to productivity of world agriculture (Duary and Yaduraju, 1999).

The increase in the number of new herbicide-resistant weeds has remained relatively constant since 1978 (on average 9 cases/year Worldwide). A total of 61 weed species have evolved resistance to triazine herbicide, but they have been controlled successfully by the use of alternative herbicides. Acetolactate synthase (ALS) and acetyl coenzyme A carboxylase (ACCase) inhibitor-resistant weeds will cause great problems in the future. A total of 33 weed species (mainly in rice, cereals, maize and soybeans) have evolved resistance to ALS-inhibitor herbicides in 11 countries. A total of 13 weed species have evolved resistance to ACCase inhibitors in 11 countries. ACCase inhibitor resistance in Lolium and *Avena* spp. threatens cereal production in Australia, Canada, Chile, France, South Africa, Spain, UK and USA. Fourteen weed species have evolved resistance to urea herbicides (Heap, 1997).

Indian farmers have enjoyed the benefits of rice-wheat cropping system since long ago but many problems have arisen in the system in the recent past, which has lowered the profitability of the system. One of the reasons is the poor control of *Phalaris minor* in wheat crop. The poor control is due to genetic resistance of some *Phalaris minor* biotypes to isoproturon. The herbicide resistant biotypes have been reported from many parts of Punjab, Haryana and U.P. (Walia *et al.* 1997).

Isoproturon-resistant *Phalaris minor* infesting wheat fields in north West India is of significant economic importance. The lack of alternative herbicides to control this weed with multiple herbicide resistance makes this the most challenging resistance problem (Heap, 1997).

Reports of the development of resistance in *Phalaris minor* to isoproturon in India also have been confirmed using pot experiments. Resistance has been more evident following post-emergence compared with pre-emergence application of isoproturon (Yaduraju and Ahuja, 1995).

The increase in the importance of *P minor* in NW India is associated with an increase in the use of wheat in crop rotations. Although among 3 recommended herbicides for use against the weed, isoproturon has been the most effective and was used almost exclusively; however, efficacy has fallen as resistance has developed and crop failure is now being reported (Malik. 1995).

Though the exact mechanism of resistance is not yet known, it is increasingly believed that the enhanced metabolism of herbicide might be

involved (Singh, *et. al.* 1997). Herbicides with different mechanism (s) of action when applied in sequence (Herbicide rotation) or as mixture delay/prevent the development of resistance in weeds (Hold *et. al.* 1993, Wrubel and Gressel 1994). Priority areas for action are resistance-monitoring, research into the mechanism of resistance and the development of integrated resistance management. In view of this, it is imperative to look for herbicides of different mechanism of action which are not only effective in controlling herbicide resistant *Phalaris minor* but are selective to wheat crop.

New herbicides such as clodinofop, fenoxaprop, sulfosulfuron and tralkoxydim are being tested in different centers for the control of herbicide resistant *Phalaris minor* (Brar and Walia, 1997). Lately flufenacent (foe 5043) has also been reported to be very effective against *Phalaris minor.* Flufenacet, with application possibilities in many crops around the world, represents a major new herbicide. The compound first synthesized in 1988 and registration submission in the US was made to EPA in 1995, with European submissions following in 1996. First product launched in 1999 (Reynolds J). Flufenacet belongs to oxiacitamide group of herbicides (Forester *et al.* 1997). It acts through inhibiting cell division. This herbicide can be used in many crops including wheat at a very low doses (Deege *et al.* 1995), which is very important from environmental pollution point of view. It is active against a wide range of grass weeds. And can be applied to a wide range of crops including corn, soybeans, potatoes, cereals, rice and peanuts. Flufenacet has undergone field-testing in many

countries around the world and will be commercialized on a wide scale. The compound will be marketed primarily in mixture with other products, which are acting mainly against broad leaf weeds. In Europe the first compound is flufenacet + metosulam (Diplome®, Terano®) for maize and flufenacet + diflufenican (Herold®) for cereals. Flufenacet + sencor was introduced for potatoes and now the most poular combination is Aximon® (flufenacet + metribuzin). It can be applied as pre-planting, pre-emergence or early post emergence, thus offering a wider window of application. This herbicide however has not been tested widely in India and therefore there is a need for optimizing the dose and time of application in wheat. As this herbicide is to be fit into rice-wheat cropping system, its suitability in terms of persistence in soil and crop need to be studied. As this herbicide is less effective against broad leave weeds, it is important to use this herbicide in combination with other herbicides for broad–spectrum weed control. Keeping these points in view, the present investigation has been proposed with the following objectives:

1. To optimize the time and dose of application of flufenacet for control of herbicide resistant *Phalaris minor*.
2. To study the performance of flufenacet in combination with other herbicides for broad–spectrum weed control.
3. To investigate the cross-resistance, if any, of *Phalaris minor* biotypes to flufenacet.
4. To study the persistence of flufenacet in soil and plant.

2. REVIEW OF LITERATURE

"In all human affairs ... there is a single dominant factor – time. To make sense of the present state of science we need to know how it got like that: we can not avoid historical account ... To extrapolate into the future we must look backwards a little into past." Z. M. Ziman.

Georgehiou (1986) may be the first one who has notably reported cases of herbicide resistance worldwide. Pesticide resistance began after DDT was discovered. Herbicides resistance in weeds is a relatively recent one in comparison to other pesticides. However, since the detection of a triazine resistance weed *Senecio vulgaris* in 1968 (Ryan, 1970), number of resistant weed species has notably increased as well as classes of herbicides to which resistance evolved. Since 1978, number of new cases of resistant weed biotypes has been relatively constant, averaging nine new cases per year (Duary and Yaduraju, 1999).
Multiple resistance has evolved in some populations of *Alopecurus myosuroides* in Europe and numerous population of *Lolium rigidum* in Australia (Hall *et al.* 1994).

Malik *et al.* (1996) gave an account to herbicide resistant weed problems in plantation and cereal crops (mainly wheat and rice) in developing countries. They mentioned that the main resistance problems include against isoproturon (India), *P. minor* and *P. paradoxa* against diclofop (Mexico), *Echinochloa colona* (*E. colonum*) against propanil (Costa Rica, Colombia and Nicaragua), *Echinochloa crus-galli* against butachlor and thiobencarb (China and Egypt), *Fimbristylis miliacea* against 2,4-D (Malaysia), *Bidens pilosa, Erigeron bonariensis* and *E. sumatrensis* against paraquat (Kénya, Egypt and Malaysia), *Avena fatua* against

diclofop-methyl (South Africa) and *Rottboellia cochinchinensis* against atrazine (Nicaragua).

2.1. Herbicide resistance development

Intensive agriculture involving monoculture systems using herbicide(s) having same mechanism of action continuously and minimum tillage have been the major causes of occurrence of herbicide resistance. The following factors are generally responsible for the development of herbicide resistance.

2.1.1. Initial frequency of resistant mutations in the gene pool

Unlike other pesticides, there is no evidence of developing herbicide resistance mutations due to herbicide application. It has been suggested that resistance genotypes are present in natural plant population in varying frequency and development of resistance in a field scale depends on the rate of increase in the proportion of the resistance genotypes within a population. Continuous application of the same herbicide or herbicides having same mechanism of action, results in killing the susceptible biotypes, allowing resistant individuals to multiply and produce seed. After a while plant population is dominated by resistant biotypes (Gressel, 1993).

2.1.2. Selection pressure

Selection pressure is the relative ability exerted by a herbicide to decimate the wild type and leave resistant individuals. The totality of factors included in selection pressure is measured as the ratio of survival of resistant to susceptible propagules over a growing season. The longer a herbicide remains active, the

greater its selection pressure when weeds germinate throughout the season. (Gressel, 1993).

Bourdot *et al.* 1989, have reported the first well-verified cases of 2,4-D resistance from New Zealand, not in wheat but in pastures, as there is better herbicide coverage. In pastures fewer seeds enter the soil seed bank.

2.1.3. Fitness

A measurement of competition between resistant mutant and susceptible wild types is another factor involved in development of herbicide resistance. Gressle and Segal (1990) reported that triazine-resistant mutant yield 10-50% of the seed yield of the susceptible wild type. Triazine and other resistant biotypes are often even less productive when grown alone. However, other studies have shown that the photosynthetic potential and growth are similar to those found in susceptible population. However, isoproturon resistant biotypes of *P. minor* have shown to be superior in respect of height and dry matter accumulation (Malik and Singh, 1995).

2.1.4. Seed bank in the soil

The soil seed bank may have a strong buffering influence in delaying the rate of evolution of resistance. Watson *et al.* (1987) reported that *Senecio vulgaris* has evolved triazine resistance in orchards, nurseries and roadsides where there was no mechanical cultivation, but not in cultivated maize field. The *S. vulgaris* seed is incorporated into the soil seed bank in cultivated fields, where it is viable for many years. All seed falling on undisturbed soil on roadsides or orchards either

germinate or die during the following season (Putwain *et al.,* 1982). Many other species such as *Lolium rigidium* do not have a seed bank under specific agronomic situation, i.e. in minimum agriculture, partly explaining propensity for rapid *Lolium rigidium* evolution in Australia (Gressel, 1993).

2.1.5. Models integrating factors involving in resistance

Maxwell *et al.* (1990) Mortimer *et al.* (1990) and Gressel (1993) have elaborated various models, involving various factors in herbicide resistance, based on a series of complicated equations. However field epidemiology has clearly shown that these models work only in mono-herbicide culture and yet to be improved (Gressel, 1988).

2.2. Mechanisms of resistance

The followings are reported to be the most important mechanisms of herbicide resistance.

2.2.1. Altered site of action

When the herbicide-binding site of action is modified due to genetic changes, weeds remain unaffected. This trait is inherited. Such a mechanism is known to be responsible in case of many triazines, acetolactate synthase (ALS) inhibitors and dinitroaniline herbicide resistance (Gronwald, 1994 and Yaduraju, 1999).

Chaleff and Ray (1984) reported that resistance to ALS inhibitors such as sulfonylureas is due to an alter site of action by alternation of the gene encoding

for ALS accompanied by production of ALS enzyme that is insensitive to inhibition.

2.2.2. Enhanced metabolism

In Australia in some populations of *L. rigidum*, resistance has been found to be due to increased metabolism in the plants (Christopher *et al.* 1991). Grownwald *et al.* (1989) reported that enhanced metabolism is involved in mechanism of triazine resistance in *Abutilon theophrasti* .

2.2.3. Sequestration and compartmentation

Holtum *et al.* (1994) found sequestration of herbicide by storage of the herbicide cells or tissue, far from site of action in some resistant biotypes of *L. rigidium* in Australia. In case of paraquat, resistance is precisely due to the rapid sequestration followed by reduction of toxic level at the site of action in the chloroplast and the rapid enzymatic detoxification of superoxide and other form of oxygen (Duary, 1999).

2.3. Herbicide resistance management

Since the herbicide resistance problem is accelerating, therefore the management of weeds could become more difficult in future. Malik *et al.* (1996) suggested that resistant weeds in cereals could be controlled by exhausting the existing seed bank of resistant populations through changes in crop sequences and new agronomic techniques. In plantation crops, an integrated approach involving alternate herbicides, herbicide mixtures and mechanical weeding can help to contain the spread of resistant populations.

Several strategies have been proposed to prevent or delay the evolution of resistant weed populations (Wruble and Gressel, 1994). These strategies are briefly reviewed here.

2.3.1. Herbicide management

2.3.1.1. Alternative herbicides

Use of alternative herbicides having different chemistry and mechanism of action is recommended as a short-term measure provided cost effective alternative herbicides are usually available. Yaduraju and Ahuja (1995) reported effective control of the resistant *P. minor* in India by pre-emergence application of pendimethalin @ 1.0 $kg.ha^{-1}$ and post-emergence application of diclofopmethyl and tralkoxydim.

2.3.1.2. Herbicide mixture and rotation

Wrubel and Gressel (1994) reviewed the herbicide mixtures as a strategy to prevent or delay herbicide resistance and concluded that while mixing strategy have some value for general weed control, not all the mixtures meet the criteria for resistance management. Mixtures have already broken in Europe. Atrazine-resistant weeds appeared when fenuron, a short residual herbicide was used together with atrazine.

2.3.1.3. Herbicide selection

Few weeds have evolved resistance to chloroacetamides, diphenylether and glyphosate despite extensive use of these herbicides. Therefore, they are considered as low risk for the selection of herbicide resistant weeds. On the other

hand weeds have readily evolved resistance to triazine. ALS inhibitors, bipyridylims. phenylureas and ACCase inhibitors (Powles and Holtum. 1994).

2.3.2. Threshold density of weed

The use of herbicide can be minimized. if prediction of threshold infestation of weed. that is severe enough to warrant herbicide use. is possible. This will help to maintain a proportion of both susceptible and resistant biotypes in the population and thus help to delay the rate of evolution of resistance (Duary, 1999).

2.3.3. Crop rotation

The introduction of alternative crops into a crop rotation may give the opportunity to change herbicide. to alter the herbicide application rate. or change other weed control techniques. Such tactics may be effective in reducing the population of resistant weeds (Matthews. 1994). Yaduraju (1999) reported that inclusion of sugarcane. or maize in *kharif* and or berseem. barley or oat in *rabi* reduces problem of *P. minor*.

2.3.4. Integrated weed management (IWM)

To have a long-term approach to weed control. we have to incorporate the use of any appropriate method of weed control with new developments in methods of population containment combined with the continued use of any effective herbicides in an IWM program. (Matthews. 1994 and Duary. 1999).

2.4. Development of resistance in *p. minor*

2.4.1. General

Singh *et al.* (1993), examined susceptibility of Wild canarygrass biotypes from different part of India. Amin (Kurukshetra district) and Lalodha (Hisar district) were found to be highly resistant to isoproturon, whereas biotypes collected from Garhi Gujran (Karnal district) and Balana (Ambala district) were found to be partially resistant to isoproturon.

Singh, *et al.* (1995), reported that the continuous use of isoproturon for more than a decade has resulted in the evolution of resistant biotypes of *P. minor* in India. The biotypes vary greatly in the extent of resistance and are a serious threat to the efficiency of wheat production in the affected areas.

Walia *et al.* (1997) studied the occurrence of resistance in *P. minor* to isoproturon and tried to find effective alternative herbicides to control the resistant biotypes and reported that most of the biotypes were not controlled by isoproturon even at double the recommended dose (1.88 kg. ha^{-1}).

Reports of the development of resistance in *P. minor* to isoproturon in India were also confirmed using pot experiments (Yaduraju and Ahuja, 1995). Resistance was more evident following post-emergence compared with pre-emergence application of isoproturon.

Pot experiments were conducted by Dixit *et al.* (1995) in *Rabi* 1994-96 at Jabalpur (India) to assess the effects of 1-2 $kgha^{-1}$ isoproturon applied 35 days after planting on 5 resistant biotypes of *P. minor* collected from Haryana and

compared to a susceptible biotype. Results indicated that although the 2 kg dose controlled (90-95%) all biotypes, mortality for the resistant biotypes was only 54-75% with the 1 kg dose. Assessed by measurements of shoot growth, the lower dose allowed significant plant growth in some of the resistant biotypes.

Field surveys of the affected areas by Malik *et al.* (1995) revealed that resistance in littleseed canarygrass was more prevalent in rice-wheat rotations compared to other crop sequences. Control of littleseed canarygrass with isoproturon has dropped form 78 to 21% from 1990 to 1993.

2.4.2. Level of resistance

Singh, *et al.* (1997) treated Wheat (co. WH-147) and five biotypes of *P. minor* (KR-1, H-4,K-2, H-2 and J-1) with isoproturon in controlled environmental conditions to assess their level of resistance. Resistance of *P. minor* to isoproturon was found in the order of KR-1 > H-4 > K-2 >H-2 = J-1. Compared with the susceptible (S) biotype (H-2), the resistant (R) biotypes (KR-1, H-4 and K-2) of *P. minor* required 13.0,4.5 and 2.7 times higher concentrations of isoproturon for a 50% reduction in growth (GR_{50}) and 2.4 times that of the S biotype (H-2) by wheat. The corresponding figures for KR-1, H-4, K-2 biotypes and wheat were 18, 4.1, 2.4 and 4.6 times based on dry weight reduction.

Tal *et al.* (1996) identified a population of *P. minor*, putatively resistant to fenoxaprop in a wheat field in Israel during 1993, showed 20-fold resistance in the laboratory when compared to a susceptible biotype. The resistant biotype also had enhanced resistance (1.1- to 3.0-fold) to ACCase inhibitors such as diclofop,

clodinafop, sethoxydim and tralkoxydim, but was equally susceptible to propanil, isoproturon and methabenzthiazuron, which did not inhibit this enzyme. ACCase from the resistant biotype was 19-fold less sensitive to fenoxaprop-P than that obtained from the susceptible biotype, and 1.5- to 5-fold less sensitive to clodinafop, tralkoxydim and cycloxydim.

Dhawan and Malik (1995) observed differences in the sensitivity of 2 biotypes of *P. minor* to isoproturon (applied at 1 $kgha^{-1}$ at the 2-leaf stage in screenhouse trials). The biotype obtained from Karnal showed less of a decline in reducing and non-reducing sugars than did the Hisar biotype, indicating their different resistance capacities to this herbicide.

2.5. Mechanism of resistance

Singh, *et al.* 1996 compared the uptake, translocation and metabolism of isoproturon in wheat (cv. WH-147) and susceptible (S) and resistant (R) biotypes of *P. minor*, using ^{14}C radiolabelled isoproturon (0-2 $kgha^{-1}$). They observed that uptake and translocation increased significantly from 0 to 7 days, but no significant differences were observed between S and R biotypes. Metabolic work (using thin layer chromatography subjected to bioimage analysis) indicated greater degradation of the parent compound by the R biotype. Since no target site alteration was observed, the cause of resistance was probably due to enhanced metabolic detoxification.

Tal *et al.* (1996) studied resistant and susceptible biotypes of *P. minor*. All biotypes had similar rates of absorption, translocation and metabolism based on

studies using ^{14}C-fenoxaprop-P, suggesting resistance was due to another mechanism. As there was a close association between the concentration-response at the whole plant level and ACCase sensitivity to fenoxaprop-P and other ACCase-inhibiting herbicides, resistance to fenoxaprop-P conferred by a modified ACCase was suggested.

The effect of isoproturon on photosynthesis was studied by Singh, *et al.* (1997) *in vitro* using five biotypes of *P. minor* and *in vivo* with wheat, KR-1 (R) and H-2 (S) biotypes of *P. minor*. Under *in vitro* treatment conditions, isoproturon inhibited the photosynthesis of all five *P. minor* biotypes, whereas *in vivo* the recovery was greater in the R biotype than in the wheat and the S biotype. Effects on chlorophyll fluorescence were also measured in wheat and the KR-1 (R) and H-2 (S) biotypes of *P. minor*. A 4-h treatment of excised leaves incubated in isoproturon solution (0.025 and 0.05 mM concentration) resulted in a decreased fluorescence coefficient (Fv/Fm ratio, in which Fv = variable fluorescence (Fm - Fo); Fm = the maximum fluorescence and Fo = initial fluorescence) in wheat and both biotypes of *P. minor*. The recovery was, however, greater in the R biotype than in wheat and it was completely recovered within 24 h. No recovery was recorded in the case of the S biotype of *P. minor*, and a greater recovery time was required for wheat than the R biotype.

2.6. Molecular characteristics of Biotypes of *P. minor*

There is no documented report on molecular characteristics of different biotypes of *P. minor* but many molecular characterization have been done in many crop and weeds.

The development of molecular techniques has ushered a new era of chemotaxonomy, which is mainly concerned with DNA and RNA (Hills & Moritz, 1990; Kohn, 1992) and to a lesser extent lipids, carbohydrates, proteins and cell wall membrane components (Bartnicki -Garcia, 1968; De Hoog *et al.*,1987). The significance of these biochemical characteristics may help in delimiting the species at the taxonomic level, whereas molecular methods play a major role in modern taxonomy as they are based on the analysis of chromosomes, genes, and their translation products (Frisvad *et al.*,1998). The Random amplified polymorphic DNA (RAPD) method uses Polyerase Chain Reaction (PCR) to amplify genomic DNA fragments with nonspecific oligonucleotide primers (Welsh & McCelland, 1990; Williams *et al.*, 1990). RAPDs polymorphisms have the ability to prove differentiation between closely related individuals (Bova, 1997). This feature combined with their easy identification, makes it a valid type of marker, potentially useful in many areas of genetic research such as gene mapping and individual identification (Bova, 1997).

2.6.1. Molecular markers

The realization that DNA sequence polymorphisms between individuals can be used for genetic mapping changed the practice of genetics. Several new

marker systems that promise to meet the requirements of an automated genetic diagnostic assay are available; many of these are based on DNA amplification (Rafalski and Tingey, 1993). Polymerease Chain Reaction (PCR) based amplification of target nucleic acids was conceived by Kary Mullis in California in 1984 (Williams *et al.*, 1990). Over the last decade PCR has become one of the most important and powerful tools in molecular biology (Bridge *et al.*, 1998). RAPD provide researchers with a quick and efficient screening for DNA sequence based polymorphisms at a very large number of loci

2.6.2. Molecular Analysis of Biotypes of *P. minor*

Characteristic fingerprints can be generated by amplifying genomic DNA at low stringency with short primers. This method is based on PCR amplification of random DNA fragments using a single primer with an arbitrary nucleotide sequence. PCR products are separated electrophoretically in agarose gel. The analysis of random amplified polymorphic DNA has been proposed to resolve genetic variations between plant biotypes.

2.7. Control

Earlier in this chapter different methods, which can prevent or delay herbicide resistance have been reviewed in general. In this session those alternative herbicides and herbicide mixtures that have been reported to be effective in control of herbicide resistant *P. minor* are mentioned specifically.

The use of surfactants and mixtures of herbicides with different modes of action has been postulated to delay the onset of resistance and to enhance herbicide

activity; the lower dose rates are desirable from both economic and environmental viewpoints (Singh, *et al.* 1995).

Walia *et al.*, (1997) noted good control all resistant biotypes of *P. minor* by the post-emergence application of tralkoxydim at 0.35 kg.ha-1 and diclofop-methyl 0.90 kg.ha-1, applied at the 2 to 3 leaf stage of *Phalaris.* These studies clearly show that *Phalaris* populations have developed resistance to isoproturon. All (resistant and susceptible) biotypes, when grown in association with wheat, suffered severe competition, reflected in greatly reduced shoot biomass as compared to *Phalaris* monocultures.

Singh, *et al.* (1995) have reported that addition of surfactant to isoproturon greatly enhanced its activity against pristine (PR-1) and susceptible (H-2) biotypes of *Phalaris*, but had little effect on the resistant (KR-1) biotype; wheat was damaged at higher doses. The tank mix application of isoproturon and fenoxaprop-P-ethyl was more effective than application of either alone but the effect was more pronounced on the susceptible as compared to resistant biotype, but can be used to control the Resistant biotype effectively.

Yaduraju and Ahuja (1995) exposed two resistant (Amin and Lalodha) and one susceptible (Pusa) populations of *P. minor* to pendimethalin, metribuzin, atrazine, diuron and oxyfluorfen, applied pre-emergence either singly or in combination with isoproturon. All treatments except those involving diuron were highly effective in controlling all the populations of *P. minor*. There was no evidence of cross-resistance to pendimethalin in populations resistant to

isoproturon. Of the post-emergence herbicides, tralkoxydim gave adequate control of resistant *P. minor*. Diclofop-methyl was not effective at the doses tried (0.4 and 0.6 $kgha^{-1}$). Isoproturon + tralkoxydim (0.50 + 0.20 $kgha^{-1}$) provided better control than other mixtures. Adjuvant 'Agral' at 0.3 or 0.5% significantly enhanced the activity of isoproturon against the resistant population Lalodha.

The response of littleseed canarygrass biotypes to isoproturon, pendimethalin, and diclofop-methyl was evaluated in India, in pot studies and in the field during the winters of 1991 to 1992 and 1992 to 1993 (Malik, *et al.* 1995). Some biotypes of littleseed canarygrass were resistant to isoproturon, but cross-resistance to pendimethalin and diclofop-methyl was not confirmed. The resistant biotype required a higher dose of diclofop-methyl for control than the susceptible biotype. Variations in the response of littleseed canarygrass biotypes were not due to isoproturon formulation. Resistant biotypes required 2 to 8 times more isoproturon than a susceptible biotype for the same level of control.

2.8. Flufenacet as a herbicide

2.8.1. Properties

Eleven new active substances were flagged for presentation at the December 1998 COLUMA conference. Flufenacet (proposed name flufenacet) was one of them for applying in cereal, corn (maize), rice, soybean, peanut (groundnut) and potato crops. (Michel, 1998)

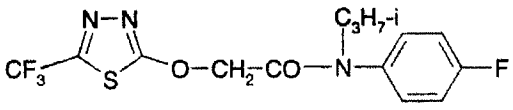
Machefer *et al.* (2000) reported that isoproturon has been used in Germany to control weeds in cereals for over 20 years. However, the fate of isoproturon in

the environment has become a subject for discussion. This is due to the occurrence of a number of cases of surface water contamination at the time of application. As a result severe restrictions have been placed on isoproturon treatments and the possibilities for its use have been substantially reduced. The use of 2 alternative herbicides, Bacara (flurtamone + diflufenican) and Herold (flufenacet + diflufenican), for autumn weed control is discussed.

Flufenacet (Bay foe 5043) is a new oxyacetamide herbicide being developed on an international basis within the Bayer organization having following physical, chemical and biological properties (Deege, 1995).

Chemical Name: (4'-fluoro-N-isopropyl-2-(5-trifluoromethyl-1,3,4-thidiazol-2-yloxy)acetanilide)

Structural Formula:



Chemical Formula:	$C_{14}H_{13}F_4N_3O_2S$
Common Name:	Flufenacet
Code Name:	BAY FOE 5043
Molecular Weight:	363.68
Appearance:	white to tan solid
Melting Point:	75-77°C
Dissociation Constant:	does not dissociate
Vapor Pressure @ 20°C:	9.0×10^{-5} Pa

Water Solubility @ 25°C:	pH	4	7	9
	mg/l	56	56	54

partition Coefficient: $\log K_{ow} = 3.2$

octanol/Water

Toxicology & Ecobiology of Technical Material

Acute Toxicity:	Oral LD_{50} rat	1617 mg/Kg
	Dermal LD_{50} rat	>2000 mg/Kg
	Inhalation LC_{50} rat	>3740 mg/m^3
Fish Toxicity:	LC_{50} blue gill	2.4 mg/l
	LC_{50} rainbow trout	3.5 mg/l
	LC_{50} Daphina	39.4mg/l
Irritation:	Eye rabbit	non-irritating
	Dermal rabbit	non-irritating
Mutagenicity:	Ames Test	non-irritating
Teratogenicity:	rabbit & rat	non-irritating
Hydrolysis:	pH = 5,7,9	stable
Photolysis:	Aqueous pH = 5	stable
	Soil	stable

Soil Metabolism:	aerobic DT_{50}	34 days
Soil mobility:	Sandy Loam	$K_{oc} = 354$

Deege, *et al.* (1995) reported that Bay foe 5043, has demonstrated excellent activity against many major annual grasses and certain small-seeded dicotyledonous weeds when applied to the soil at rates significantly lower than the current commercial standards. Field tests conducted in the United States, Europe, South Africa, Asia and South America from 1988 through 1994 validated efficacy when applied early pre-plant, pre-plant surface, pre-plant incorporated, pre-emergence as well as early post-emergence. Applied at suggested use rates, Flufenacet controlled a wide variety of economically relevant weed species in corn (maize), cereals, cotton, peanuts (groundnuts), potatoes, rice, soybeans, sunflowers, tomatoes and other crops. The major grass species controlled included foxtails (*Setaria spp.*), barnyard grass (*Echinochloa crus-galli*), fall panicum (*Panicum dichotomiflorum*) and crabgrass (*Digitaria spp.*). Flufenacet exhibited excellent properties as a mix partner for herbicides controlling dicotyledonous weeds. (Deege *et al.* 1995)

The environmental safety of the herbicide flufenacet was investigated by Hall *et al.* (1997). In view of its very low toxicity to mammals, birds, beneficial insects, earthworms, fish and aquatic invertebrates, flufenacet has a very low risk of causing damage to these species under normal conditions of use. Terrestrial and aquatic plants are very sensitive to direct treatment with flufenacet. However, if

the precautions customary in agricultural practice are taken when using the product to prevent drift or surface run-off, the risk to these species will be minimized.

Forster, *et al.* (1997) introduced Mefenacet (formulated as Hinochloa R and Rancho R) as the first commercial product from the Bayer oxyacetamide group of herbicides, being used for the control of barnyardgrass (*Echinochloa crus-galli*) in irrigated rice. They conducted studies to find an oxyacetamide which was effective as a herbicide in the absence of irrigation water, and which can be used in maize, soyabeans, and other crops. The trifluorothiadiazolyl oxyacetamides were very effective, and their efficacy and crop compatibility were optimized on the basis of structure-activity correlations. Only one compound (flufenacet) satisfied the required criteria of a good level of efficacy against grasses, and suitability for use in maize and soybeans. Flufenacet is particularly effective when applied before emergence or in the early post-emergence stage, and can be used selectively in maize, soybeans, cereals, and other crops. Flufenacet is listed in the K3 group of herbicides (inhibitors of cell division) as defined by the Herbicide Resistance Action Committee (HRAC).

Jobling *et al.* (1999) showed very good control of *G. aparine* using flufenacet + metribuzin (480 + 350 g a.i./ha and 600 + 437.5 g a.i./ha) alone and in tank mixture with metribuzin (0.375 and 0.5 $kgha^{-1}$) in field trials in potato crops in the UK. Applied as a pre-emergence treatment, the combination provided 84.8 and 95.2% mean reduction in *G. aparine* at the lower and higher doses,

respectively. Flufenacet + metribuzin also controlled various broadleaf weeds (84.5% *Aethusa cynapium*, 96.9% *Chenopodium album*, 84.8% *Fallopia convolvulus*, 100.0% *Matricaria spp.*, 97.3% *Polygonum persicaria* and 100.0% *Senecio vulgaris* at the higher dose) as well as, or more effectively than, metribuzin (1.0 $kgha^{-1}$) alone. *Elymus repens* and *Poa annua* were controlled by 91.5 and 90.2%, respectively. No phytotoxicity symptoms were observed on potato varieties when treated with flufenacet + metribuzin.

Brinkmann *et al.* (1997), showed that the anilide herbicide flufenacet suitable for mixing with Sencor R (metribuzin) for use in soybeans and transplanted tomatoes grown in Italy. These two herbicides represent the ideal combination for the control of all weeds (the main weed grasses and the most important dicotyledonous weeds) in soybeans and tomatoes. In particular, the mixture offers outstanding possibilities for the control of *Solanum nigrum* in tomatoes - a problem weed for which there is, at present, no satisfactory herbicide for control. A ready-to-use combination of flufenacet and metribuzin is being developed as a 56 WG formulation containing 42% flufenacet and 14% metribuzin, and is to be registered in Italy, France and Portugal.

2.8.2. Mode of action and selectivity

The mode of action of flufenacet at the molecular level, as with other oxyacetamides, has yet to be identified. Studies with the only commercially available oxyacetamide herbicide, mefanacet have shown a similarity in mode of action at the cellular and tissue levels to the chlroacetanilides (e.g. metolachlor).

Although the molecular mode of action of chloroacetanilides is also unknown, both class of compounds inhibit both cell division and growth. This inhibition results in a complete arrest of cell division in the root and shoot meristematic regions. New growth is halted and elongated tissues may become distorted. Detailed studies with mefannacet and metolachlor have shown that cells no longer enter the division cycle although progress through the individual phases of cell division (pro-, meta-, ana- and telophase) is not affected. The mitotic index is accordingly reduced.

Herbicide selectivity is a major factor in agricultural weed control and results from the differential detoxification ability of plant species (Zajc *et al.* 1999). The special agronomic value of plant glutathione S-transferases (GSTs) originates mainly from their metabolic herbicide detoxification properties that enhance the herbicide tolerance of crops. GSTs are a ubiquitous family of multifunctional enzymes involved in the metabolization of a broad variety of xenobiotics (e.g., herbicides in plants) and reactive endogenous compounds through covalent linkage to glutathione. In their investigations they found the metabolization of flufenacet resulted in a GSH conjugate that was subsequently degraded by release of the thiadiazole moiety. The comparison of crystal structures of different GST classes, including plant GSTs, provided a model system to understand active-site interactions on a molecular level. Additional protein structures of three plant GSTs (*Arabidopsis thaliana* GST, and GST I and III from maize) in complex with several ligands (S-hexylglutathione, S-

lactoylglutathione, and FOE 5043) may be tools to supply detailed knowledge for the rational design of new herbicides and GSTs for selectivity optimization in crops.

2.8.3. Persistence and mobility

Although there are several reports on the efficacy of flufenacet against various weeds in cereal crops, there are very few reports on the method of analysis and its environmental fate in agro ecosystems.

Investigations of Bieseler *et al.* (1997) on the metabolism of herbicide flufenacet in immature corn (maize) and soybean seedlings exposed to ^{14}C- flufenacet showed that it was degraded rapidly within plants. Although a number of metabolites were isolated, conjugation with glutathione is the first step in the degradation pathway, and therefore seems to be an important metabolite in tolerant plants. The chemical structure of the FOE-conjugate, which was determined by NMR-spectroscopy, showed that the nucleophilic addition of glutathione resulted in the release of the thiadiazole moiety. The enzymatic activity of glutathione S-transferases (GSTs) using flufenacet as substrate was 3-4 times higher in maize seedlings compared to some selected crops and weeds. Three GSTs, which catalyze the conjugation of flufenacet with glutathione, were identified. For protein X-ray crystallography, GST from maize and *Arabidopsis thaliana* was isolated by a PCR-based strategy, and heterologous expression of the proteins was performed in *Escherichia coli*. Proteins were crystallized by vapor diffusion using the hanging drop method. The *Arabidopsis* isoenzyme is the first plant GST for

which the structure has been solved. Using Arabidopsis GST as a search model, the structures of unligated and glutathione-ligated GST III and GST I from maize were determined. The isoenzymes were dimeric proteins consisting of identical subunits with a prominent large cavity formed between the two subunits. Each subunit is composed of two distinct domains, and the active site is located in a cleft situated between domains I and II. The structural information of three plant GSTs in complex with several ligands (FOE-glutathione, S-hexylglutathione and S-lactoylglutathione) indicated molecular interactions of the protein moiety and herbicide conjugates. It was concluded that structure-based information on substrate recognition and reaction mechanisms may prove useful for demonstrating the feasibility of rational selectivity optimization.

Gould *et al.* (1997) developed an analytical method for measuring residues of flufenacet and its metabolites in crops. The residues are briefly oxidized and hydrolyzed to fluoroaniline by digesting the crop mixture with sulfuric acid. The fluoroaniline is separated from the crop matrix by steam distillation after making the crop digest basic. The fluoroaniline is extracted from the steam distillate and derivatized. The derivative is measured by gas chromatography/mass spectroscopy-selected ion monitoring (GC/MS-SIM). The method showed recoveries of 67-116% of flufenacet and its metabolites at the 0.10 ppm level and 65-91% at the 0.05 ppm level. The limit of quantification was 0.05-0.1 ppm of flufenacet equivalents, depending on the plant matrix. The minimum detectable level was 0.01-0.05 ppm equivalents, depending on the plant matrix. The

magnitude of flufenacet residue levels in field-grown corn (maize) were 0.36 ppm in milk stage green forage, <0.05 ppm in late milk stage sweet corn, <0.05 ppm in dry harvest grain and 0.15 ppm in dry fodder. In soyabeans, the maximum flufenacet residues detected were 0.10 ppm in seeds, 1.20 ppm in green forage and 9.75 ppm in dry hay. Limited field rotational crop studies were conducted using wheat, turnips and mustard greens (*Brassica juncea*). Residues were detected in wheat hay (0.09 ppm) and grain (0.09 ppm) at the 10-month plant-back interval. Residues were <0.05 ppm at the 4-month old for turnips and mustard greens. Processing studies were conducted on corn and soyabean. The results showed no concentration of residues in any of the tested soyabean commodities. The only corn processed product that showed a concentration factor was corn grits (1.6X).

Rouchaud *et al.* (1999) studied soil persistence and mobility of flufenacet in spring and summer maize crops on sandy loam soil and after application in the autumn in wheat in Belgium during 1997-98. They applied flufenacet at 600 g/ha before sowing the maize crops and at 240 g/ha to winter wheat. During the main crop period of the spring and summer maize crops, there was a linear relationship between the naperian logarithms of the flufenacet soil concentrations in the 0-10 cm surface soil layer and time elapsed since application. When flufenacet was applied in autumn, first order kinetics were followed during the 7 months after treatment. During the last month of each of the 3 trials, the rate of dissipation of the residue remaining in the soil became greater than predicted by first order kinetics. At the wheat and maize harvests, flufenacet was not detected in the 0-10

and 10-15 cm surface soil layers. In wheat and in the spring and summer maize crops, the soils half-lives of flufenacet were 98, 74 and 56 days, respectively. During the maize crops, flufenacet remained in the 0-10 cm soil layer and was never detected in the 15-20 cm layer. After application to wheat, flufenacet was mainly in the 0-2 cm soil layer, moving to the 2-4 cm layer one month after treatment, indicating low mobility in the soil.

The environmental fate of flufenacet was studied in soil, under anaerobic and aerobic conditions using labled (phenyl - U - ^{14}C) flufenacet by Pangilinan and Smith (1997). It was relatively stable under anaerobic compared to aerobic conditions and mainly dissipated by binding to the soil matrix. . The half-life of flufenacet ranged between 33 - 64 days under aerobic conditions.

2.8.4. Performance

Diehl and Benz (1998) gave a brief description of three flufenacet-containing herbicides. The broad-spectrum maize herbicide "Terano R" combines the active ingredients flufenacet and metosulam, and is free of terbuthylazine. It is a soil-acting basic herbicide, which can be used from pre-emergence up to and during the post-emergence phase. "Terano" offers good opportunities for combination with major leaf-acting grass herbicides, with the timing of its application being tailored to particular weed infestations or weather conditions. The broad-spectrum herbicide "Herold R" represents a new solution for the control of weed grasses, which grow in profusion in winter cereals such as slender foxtail and congress. It combines flufenacet with diflufenican, and can be used

from the pre-emergence to early post-emergence phase. The broad-spectrum herbicide "Artist R" is expected to be available to farmers from 1999. It can be used prior to emergence in potatoes and is a combination of flufenacet and metribuzin. All these herbicides come in a user-friendly low-dust WG formulation.

Mittnacht (1998) reported that Monocotyledonous weed species occur in about 85% of the maize growing area in Baden-Wurttemberg. Herbicides that kill grasses, applied after emergence, are therefore the predominant form of control. Terbuthylazine-free maize herbicides are of great importance, because of regional restrictions on use (in "damaged" areas) and bans on use (in water-protection areas). The development of new herbicidal active ingredients for the control of grass weeds and other weeds in maize is opening up new perspectives. New developments of this kind were tested out in a state-wide research programme carried out over several years by the official crop protection service of Baden-Wurttemberg in collaboration with the Herbology Department of Hohenheim University. The results obtained with the active ingredient flufenacet in a ready-to-use formulation with metosulam "Terano R" and various combinations with additives "Rako-Binol" or sulcotrione "Mikado" are reported and evaluated, in a comparison with the usual methods employed in practice. With the new maize herbicide "Terano", maize can be kept free from weeds even in the early growth phase. Suitable combination partners or spray programmes involving other active ingredients are required, depending on the conditions of use and the weed composition.

Gehring (1998) found reliable efficacy of the new winter cereals herbicide "Herold R" (flufenacet + diflufenican) against corngrass (*Apera spica-venti*) and good efficacy against foxtail (*Alopecurus myosuroides*) in trials in Bavaria, Germany. Its spectrum of action against dicotyledons is sufficient for the usual mixture of weeds encountered in winter cereals. "Herold" allows reliable control of major weeds. Provided that the application conditions are good and that the infestation pressure is not excessive, there is no need for any further treatment measure to combat cleavers (*Galium aparine*) in the spring. "Herold" stands out as a modern cereals herbicide by virtue of its good crop tolerance characteristics and favourable ecotoxicological profile. The new graminicidal active ingredient flufenacet is a suitable agent for the practical implementation of herbicide treatment strategies, which aim to reduce the risk of resistance formation in foxtail.

Hoppe (1998) showed that the basic herbicide "Terano R" (flufenacet + metosulam) is characterized by good tolerability, exerts its action via the soil and leaves, and has a reliable efficacy against nightshade *Solanum nigrum* and white goosefoot (*Chenopodium album*), but under the described conditions it requires a suitable mixing partner. In the damage threshold approach Terano can be incorporated very well as part of a split-treatment programme applied at an early stage. "Terano", a WG formulation, is easy to work with, generally reduces the cost of control per hectare and, in view of its good physical and chemical properties, should help to defuse the discussion on active ingredients in water.

3. MATERIAL AND METHODS

The details of the materials used and methodologies adopted during the course of investigation are described in this chapter. This study was conducted in collaboration with Division of Agricultural Chemicals, Indian Agricultural Research Institute (IARI), New Delhi. The general climate and soil condition of the experimental field have also been given herein.

3.1. Experimental sites

The experiments were conducted during the *Rabi* seasons (November – April) of 1998-99 and 1999-2000, in the Block Mid B (b) at the farm and net house of Division of Agronomy, IARI, to evaluate flufenacet for the control of herbicide resistant *Phalaris minor* and its persistence in soil and wheat crop.

3.2. Climate and weather

Delhi is situated at a latitude of 28° 35' N and a longitude of 77° 12' E with an elevation of 228 meters above mean sea level. The climate is sub-tropical semi-arid with hot and dry summer and cold winter. May and June are the hottest months with temperature ranging between 41°C and 46°C while January is the coldest month with minimum temperature approaching 2°C to 5°C. The mean annual rainfall is about 652 mm, of which 547 mm (84%)is received during a short span of three months between July and September. The annual pan evaporation is about 850 mm. The mean daily open pan evaporation reaches as high as 16 mm in June and as low as 2.2 mm in January. The relative humidity reaches maximum in

January and minimum in March. The important weather parameters during cropping period are presented in Appendix 2.

3.3. Soil of experimental field

The mechanical and chemical composition of soil (0-30 cm) of the experimental area is given in Table 1. The soil was sandy loam texture in upper 30 cm layer and loam below that. Data in Table 1. show that soil was medium in fertility with the respect to available nitrogen, phosphorus and potassium with pH 8.3.

3.4. Field experimental details

3.4.1 Details of treatments

The main field experiment was laid out in a Randomized Complete Block Design with 14 treatments. Duncan's Multiple Range Test (DMRT) was used for all mean comparison because for experiments that require evaluation of all possible pairs of treatment means, the LSD test is usually not suitable. This is especially true when the total number of treatments is large i.e. more than six (Gomez and Gomez 1984). Unlike the LSD test in witch only a single value is required for any pair comparison at a prescribed level of significance, the DMRT requires computation of a series of values and, each corresponding to a specific set of comparisons. Treatments were combination of three levels of flufenacet doses and two times of application including flufenacet combination with other herbicides. Weed free check and unweeded control were included. These

Table 1. Mechanical and chemical analysis of soil.

Particular		Values	Method employed
(A)	Mechanical analysis		
	(i) Coarse sand (%)	0.6	International pipette method
	(ii) Find sand (%)	68.3	(Piper, 1966)
	(iii) Silt (%)	15.9	
	(iv) Clay (%)	15.2	
(B)	Chemical analysis		
	(i) Organic carbon (%)	0.51	Rapid titration method Walkley and Black, 1934
	(ii) Total Nitrogen ($Kg.ha^{-1}$)	1160.3	Modified Kjeldahl method Jackson, 1973
	(iii) Available phosphorus ($Kg.ha^{-1}$)	19.5	Olsen's method (Jackson, 1973)
	(iv) Available potassium ($Kg.ha^{-1}$)	231	Flame photometer (Jackson, 1973)
(C)	Other characteristics		
	(i) pH (Soil:Water, 1:2.5)	8.3	Beckman glass electrode (Jackson, 1973)
	(ii) EC (dSm^{-1} at 25°C)	0.33	Solubridge method, (Jackson, 1973)
	(iii) Bulk density ($g.cm^3$)		Core sampling (Piper, 1966)

treatments were replicated thrice. The details of the treatments are given in next page.

3.4.2. Details of experimental layout

The plan of the experimental layout is given in fig.

01) Flufenacet 180 gha^{-1} PE

02) Flufenacet 240 gha^{-1} PE

03) Flufenacet 300 gha^{-1} PE

04) Flufenacet 180 gha^{-1} 2WAS

05) Flufenacet 240 gha^{-1} 2WAS

06) Flufenacet 300 gha^{-1} 2 WAS

07) Flufenacet 180 gha^{-1} + Isoproturon 0.5 $kgha^{-1}$ PE

08) Flufenacet 180 $g.ha^{-1}$ + Metribuzin 150 gha^{-1} 4WAS

09) Flufenacet 180 $g.ha^{-1}$ 2WAS fb. Isoproturon 0.5 $kgha^{-1}$ 4WAS

10) Flufenacet 180 $g.ha^{-1}$ 2WAS fb. Metribuzin 150gha^{-1} 4WAS

11) Flufenacet 180 $g.ha^{-1}$ 2WAS f.b. 2,4-D 0.4 $kgha^{-1}$ 5WAS

12) Isoproturon alone 0.75 $kgha^{-1}$ 4WAS

13) HW-2, 4 and 6 WAS

14) UWC

PE: Pre-emergence application fb: Followed by

POE: Post-emergence application WAS: Weeks after sowing

3.5. Crop cultivar

The cultivar HD2329 that is dwarf wheat was used

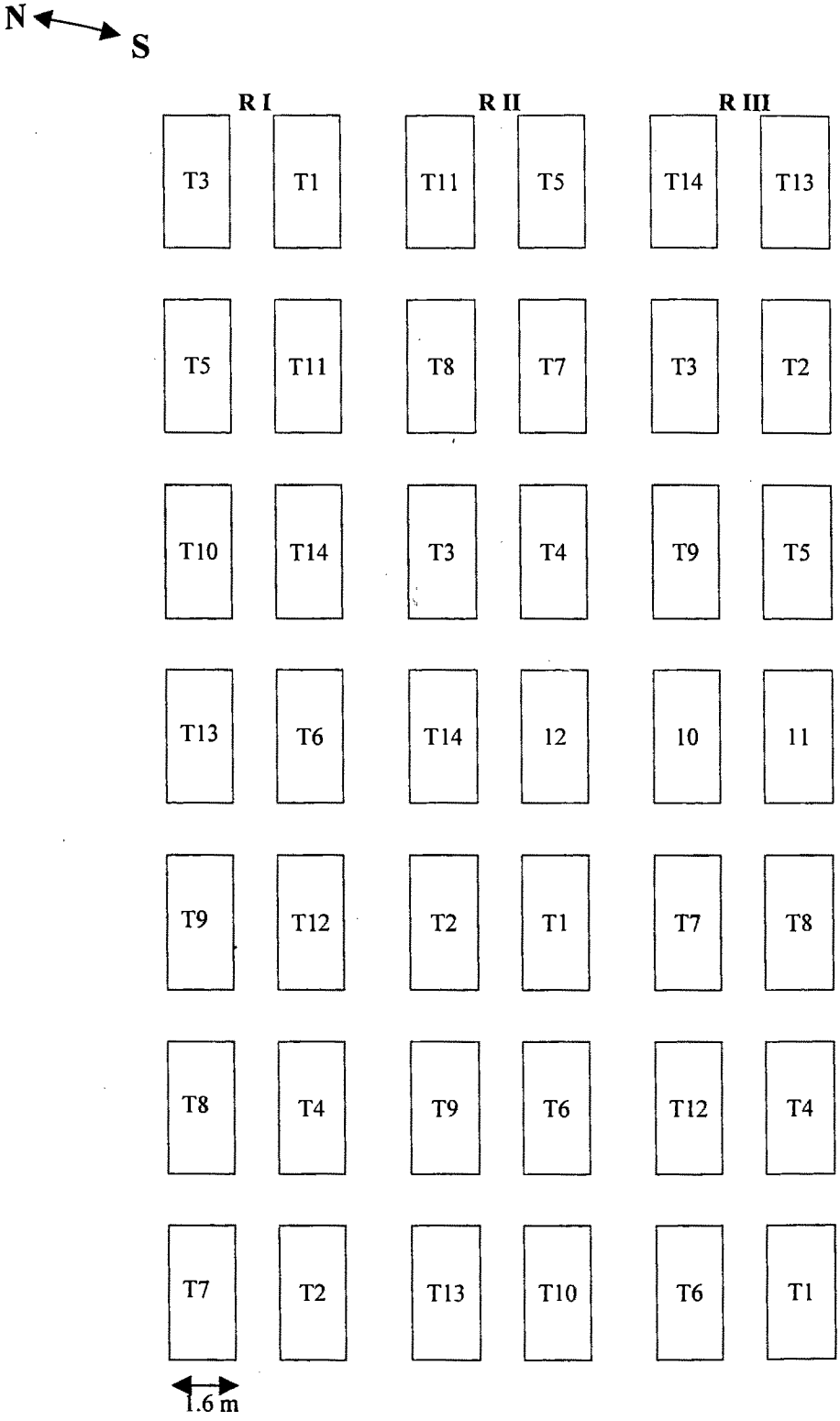


FIGURE 1. PLAN OF EXPERIMENTAL LAYOUT

Plot size (gross): 5 m x 1.6 m = 8 m^2
Plot size (net): 2 m x 1.15 = 2.3 m^2

3.6. Cultural operation

The details of all cultural operation from preparatory tillage to harvesting of wheat in both the years are given in Appendix 1.

3.6.1. Field preparation

The field was ploughed, disked and leveled well to a good tilth. Pre-sowing irrigation was applied before land was finally prepared by giving two disc harrowings followed by planting.

3.6.2. Fertilizer application

The recommended dose of 120 Kg N, 60 Kg P_2O_5 and 40 Kg K_2O ha^{-1} were applied uniformly as basal by broadcasting before sowing of wheat. Nitrogen however applied as pre-treatment, 50 per cent as basal along with P and K and rest at the time of first irrigation. Nitrogen, phosphorus and potassium were applied in the form of urea, single super phosphate and muriate of potash, respectively.

3.6.3. Sowing

In both the years, sowing was done by tractor drawn seed drill calibrated for the recommended seed rate of 100 $Kg.ha^{-1}$ and adjusted for the spacing of 22.5 cm between rows.

3.6.4. Hand weeding

Two hand weedings were given in weed free treatment with *Khurpi*. Besides, weeds were removed as and when seen emerging.

3.6.5. Irrigation

The first irrigation was given 21days after sowing at crown root initiation

(CRI). The other 4 irrigation were given at critical stages of growth (details in Appendix 1).

3.6.6. Plant protection

Except herbicide treatments no pesticides was used in either of the years, as crop was free from insect pests or diseases.

3.6.7. Harvesting

The crop in the net plot area was harvested using sickle. Central five rows from all the plots were taken for determining yield of the crop.

3.7. Observations recorded

3.7.1. Observation on weeds

Weed population counts and weed sample for dry matter accumulation were taken to assess the effect of various treatments on weed growth. These observations were recorded at 30, 60 and 100 days after sowing.

3.7.1.1. Weed population count

An area of 0.25 m^2 was selected randomly by throwing a quadrat of 50 cm x 50 cm. Species-wise weed population count was made from the quadrat area at 30, 60, 100 days after sowing and at harvest in both years. However, the dry weight was recorded by summing up as grass and broad-leaved weeds.

3.7.1.2. Dry matter accumulation

Weeds collected from 0.25 m^2 area at 30, 60 and 100 DAS were first dried in the sun separately as grass and broad-leaved weeds and then in an electric oven at 70°C for 48 hours and dry weight was recorded and expressed as $g.m^{-2}$.

3.7.1.3. Nitrogen uptake by weeds

The oven dried composite weed samples were ground in Wiley Mill and analysed for nitrogen. The Nitrogen uptake by weeds was calculated as $Kg.ha^{-1}$. The observation was made at harvest time.

3.7.2. Observation on crop

Observations on crop were taken at 30, 60 and 100 DAS and at the time of harvest in both the years of study.

3.8. Bioassay experiment

Soil samples drawn from field experiment 40 days after flufenacet application were used for determining herbicide residues through bioassay method in pots using *Avena sativa* L. as test plant. Five seeds of *A. sativa* were planted in each pot containing 100 g of soil sample in 5 replications. A standard curve plotting fresh weight/length of the test plant against different quantities of flufenacet was needed to determine the quantity of flufenacet residue in soil, Therefore known amounts of untreated soil was fortified with flufenacet to bring about 0.10, 0.08, 0.06, 0.04, 0.02 $\mu g.g^{-1}$ concentrations of flufenacet in soil. A herbicide-free control was included. To avoid herbicide leaching, pots were placed in a tray containing water to enable absorption of water through drainage holes at

the bottom of the pots. Height and dry matter of *A. sativa* was measured at 30 DAS. The treatments were replicated 5 times in a Randomized Block Design.

After harvesting the wheat crop in April 1999, a field bioassay was carried out by planting maize and in same layout in July 1999. Dry weight of these two crops were measured at 30 DAS to find out adverse effect of residues of flufenacet if any on these crops.

3.9. POT EXPERIMENT ON CROSS RESISTANCE IN ISOPROTURON RESISTANT BIOTYPES OF *P. MINOR*

To study the possibility of cross resistance to flufenacet among biotypes of *P. minor* a pot experiment was laid out using Six biotypes of *Phalaris minor* collected from different areas in September November 1999. Young plants were harvested 30 days after germination to record dry weight and height of plants for finding out the existence of cross resistance among collected biotypes.

3.9.1. Treatment:

Biotypes

Biotype	Place of collection	Susceptibility
B12	Asand	Resistant
B25	Kurukshetra	Resistant
B34	Karnal	Resistant
B57	Kithal	Sensitive
B59	Kithal	Sensitive
B95	Pusa	Sensitive

B) Doses of flufenacet

0, 60, 120, 240, 300 gha^{-1}. Applied at 2WAS

3.9.2. Design

Randomized Complete Block Design. Each treatment was replicated 5 times.

Considering dry matter production in control pots equal to 100 percent, regression equations were established between doses of flufenacet and scaled dry matter production for all biotypes of *P. minor* using Curve fit computer software. GR_{50} was predicted using the above mention equations for all biotypes

3.9.3. Details of experiment

Date of sowing:	01-12-98
Date of herbicide application:	02-12-98
Date of germination	15-12-98

3.10. POT EXPERIMENT ON PHYTOTOXICITY OF WHEAT CULTIVARS

A pot experiment using 20 different varieties of wheat crop was carried out to study the possible phytotoxic effects of flufenacet during November to December 1999. Young seedlings were harvested 30 days after germination. Dry and fresh weight and height of seedlings were recorded. Combinations of treatments were replicated 4 times in a RCBD.

3.10.1. Treatments

Doses of flufenacet (gha^{-1})

i) 240

ii) 480

Wheat varieties

HD2285	DL1014-2	DL153-2	DL7843
DL788-2	DL803-3	HD2329	HD2687
HD1744	HW3038	IND 99-33	IND 99-83
NIAW34	PBW343	PS 503	PS 504
UP2338	UP2339	WH542	WR904

3.11. Studies on persistence of flufenacet in soil and wheat crop

Nine sets of soil samples were drawn randomly from 0-15 cm depth with the help of a tube auger from a minimum of 7 spots in each plot following 300 $g.ha^{-1}$ pre-emergence application of flufenacet in main experimental field. Approximately 600 g soil was collected from each plot. The samples were drawn at 2h, 5, 10, 15, 20, 30, 60 and 90 days after treatment (DAT) and at harvest (150 DAT). Each of the collected samples was thoroughly mixed; air dried, ground in pestle and mortar and was passed through 2 mm sieve.

3.11.1. Chemicals

The analytical grade flufenacet (99.6% purity) was supplied by M/S Bayer India Ltd. All solvents were distilled before use.

3.11.2. Preparation of standards

A stock solution of flufenacet (1000 μg ml^{-1}) was prepared by dissolving 25 mg of analytical grade herbicide in 25 ml of acetone. Further flufenacet solutions (1, 0.5, 0.1, 0.05, 0.01 μg ml^{-1}) were prepared from the stock solution by diluting with hexane.

3.11.3. Instrument

The method employed a Hewlet Packard gas chromatograph instrument, model HP 5890, equipped with ^{63}Ni electron capture detector, column (10m x 0.53 mm i.d.) containing HP-1 as stationary phase and nitrogen as carrier gas at a flow rate of 15 ml min^{-1}. The operating temperatures for oven, injector and detector were 190, 210 and 270° C respectively. A 3 μl volume of sample was injected on column by auto-injector and chromatograms were visualized on computer. Instrument was connected to a computer having software able to compute detector response in terms of peak area.

3.11.4. Extraction and Cleanup

Extraction from soil

A soil containing 0.33% organic matter with a pH of 7.1 and a sandy loam texture consisting of 19% clay, 21% silt and 60% sand was used. Soil was dried in

shade and sieved. Soil samples (50g) in triplicate were fortified with different concentrations (0.1, 0.25 and 0.5 μg g^{-1}) of flufenacet and extracted using two methods namely shaking on a horizontal shaker for ½ h and soxhlet extraction.

Two solvent systems (50ml) viz. acetone:hexane (10:90) and acetone : 0.2 M HCl (95:5) were separately used to check efficiency of extraction by shaking on a horizontal mechanical shaker for ½ hr. The contents were filtered through büchner funnel. The extraction was repeated twice more (50+25 ml). The filtrates were combined and solvent evaporated on a rotary evaporator to about 10 ml.

Extraction from wheat straw and grain

Powdered wheat grain and straw samples (50 g) spiked with known quantities (0.1, 0.25 and 0.05 μg g^{-1}) of flufenacet were separately placed in a thimble contained in a soxhlet apparatus. To this was added 150 ml of solvent systems (acetone : 0.2 M HCl (95:5)) and extraction carried out for 4h. The contents were collected and solvent evaporated to about 10 ml on a rotary evaporator.

3.11.5. Cleanup

The first solvent mixture (Acetone:hexane (10:90)) concentrate did not require any clean up. The acidic concentrate was transfered to a separating funnel, diluted with water (70 ml) and extracted three times with methylene chloride (25+10+5 ml). The combined extract was dried by passing through anhydrous sodium sulfate and evaporated to dryness on a rotary evaporator. The residues were dissolved in hexane prior analysis.

3.12. Studies on mobility of flufenacet in soil

Another field experiment was laid out in 1999-2000 to determine the mobility of flufenacet in soil. The wheat was sown on 15 November 1999 and was raised as detailed in main experimental field. The herbicide was applied at 120 and 240 $g.ha^{-1}$ as post-emergence under two irrigation levels. One set received normal irrigation as already described and the other was given weekly irrigation. About 60 mm water was given for each irrigation. Soil samples were collected from three different depths; 0-10, 10-20 and 20-30 cm at 2h, 7,15 and 30 DAT from plots treated with higher dose of herbicide only. Collected samples were thoroughly mixed; air dried, ground in pestle and mortar and was passed through 2 mm sieve. Finally a 50 g representative sample was taken for quantitative analysis by gas chromatography (GC) method as detailed before.

3.13. Molecular analysis of P. minor biotypes resistant to isoproturon

3.13.1. DNA Extraction

DNA extraction of the *P. minor* was performed using the micro extraction method (Prabhu et al., 1998). The lyophilized seedlings were ground in sterile mortar and pestle by using liquid nitrogen to a fine frozen powder. The fine powder was transferred to sterile centrifuge tube. Approximately 50 mg of tissue was measured in 1.5 ml micro tubes. DNA extraction buffer [1 M Tris-HCl (pH 8.0), 3 M KCl, 0.5 M EDTA (pH 8.0) and sterile water] was prepared and pre-heated to 90-95 °C. Micro tubes containing tissue were placed on preheated (95°C) hot block with caps open. 1 ml of hot extraction buffer was added to each tube

using a disposable Pasteur pipet. This mixture incubated for 10 min and vortexed every 2 min. The tubes were removed and placed on ice for 2 min following which, the micro tubes were centrifuged for 10 min. at 12000 rpm. Approximately 800 µl of supernatant was removed from each tube to new tubes already containing 5 µl of $mg.ml^{-1}$ RNAse and incubated for 20 min at 37°C. 480 µl (.6 vol.) cold isopropanol was added to this solution and mixed by rocking. This step was followed by spinning for 5 at 10000 rpm). The supernatant was poured of into hazardous waste container. 300 µl of sterile water was added to redissolve DNA, heating briefly at 50°C. DNA was precipitated with 30 µl of 3 M sodium oxaloacetate and 600 µl of 95% ethyl alcohol, centrifuged 5 min (12000 rpm). 400 µl of 70% Ethyl alcohol was added and tubes were vortexed, centrifuged for 3 min (12000 rpm). Ethyl alcohol was removed and tubes allowed to dry for 40 min. pellets resuspended in 75 µl sterile water, kept in a preheated oven at 37°C for 20-30 min then flicked to disperse DNA. Concentrated DNA was stored at 4°C.

3.13.2. Polymerase Chain Reaction materials and optimization

The random primers having double stranded 10-mer oligonucleotide sequences, in Kits of 20 such sequences are supplied as lyophilized salts having a concentration 33 µl/ml (Operon Technologies, INC., USA). Each primer sample tube was resuspended in sterile Tris-EDTA buffer pH 8.0. Then this was subdivided into several aliquots (250 µl), and stored at - 20^{O}C. Thirty-four 10-mer oligonucleotides from sets Operon OP- B, OP- D and OP- J were used as single primers for the amplification of sequences.

Taq DNA polymerase together with 10X concentrated PCR buffer, was supplied by Genei, India. Protocol for the PCR was optimised by varying the concentration of template DNA, Taq DNA polymerase and $MgCl_2$ salt. Primer survey was carried out and only B and J primers that gave reproducible and scoreable amplifications were used in the analysis of all biotypes

3.13.3. Amplification Reaction conditions

The procedure of Williams *et al.* (1990) with certain modifications was used for detecting RAPD products. The PCR reaction mixture was prepared in disposable microamp tubes of 0.2 ml capacity. The amplification reaction was performed in a peltier based thermocycler MJ Research PTC 200 (MJ-Research, USA). All the transfers of PCR ingredients were done with sterile disposable micropipette tips.

Each 25 µl reaction mixture contained 4 µl of 1X reaction buffer (10mM Tris - HCl, pH 8.3 and 50 mM KCl), 5 mM $MgCl_2$, 0.5 unit of Taq DNA polymerase, 100 µM each of dATP, dCTP, dGTP and dTTP (all reagents from GENEI, Bangalore,India), 1 µM of 10-mer primer and approximately 25 ng of template DNA. There was no requirement for mineral oil overlay because of the provision in the thermocycler that makes micoamp tubes airtight and the reaction starts only after the cover temperature reaches $95^{o}C$. A control reaction containing all the components except genomic DNA was run for each primer to check for purity of different reaction mixtures used and also to check for any contamination.

The PCR amplification conditions were used as follows: First cycle, initial extended step of denaturation 1.5 min at 95^{O}C followed by second cycle of 3 steps, denaturation at 95^{O}C for 0.5 min, the annealing step at 36^{O}C for 1 min and 1 min at 72^{O}C for template extension followed 44 cycles and last cycle was extension step of 7 min at 72^{O}C. Amplified products (25 µl) were mixed with 3 µl of 10X loading dye (0.25% bromophenol blue, 0.25% Xylene cyanol and 40% sucrose, W/V) and the electrophoresis was carried out at a constant voltage of 100 V for two hours and stained with ethidium bromide for 30 min. visualized under ultraviolet transilluminator and photographed under UV light.

3.13.4. Scoring and data analysis

Each amplification product was considered as a potential RAPD marker. Gels were scored on the basis of the presence (1) or absence (0) of each band for all samples. PCR amplification for used primers were repeated at least twice and only reproducible bands were considered for analyses.

4. RESULTS

The data generated from the investigation " Evaluation of flufenacet for the control of herbicide-resistant *Phalaris minor* Retz. and its persistence in soil and wheat crop" in both years of experimentation i.e. 1998-99 and 1999-2000, are presented and described here in appropriate tables and figures after having them statistically analyzed. The effects on growth, yield and various other characters of both wheat and *P. minor* are depicted experiment-wise in this chapter. Unlike LSD test in witch only a single critical difference (CD) is computed for comparison of all the means, the DMRT requires computation of a series of CD values, each corresponding to a specific set of comparisons (14 CDs for 14 treatments). Illustrating all CDs is not required; therefore line or alphabetical notation is used (Gomez and Gomez 1984).

4.1. Weeds

4.1.1. Weed growth

4.1.1.1. *P. minor* population

Number of *P. minor* per m^2 at different stages of growth has been shown in Table 2 along with their rank computed by DMRT. Interaction between years and treatments was not significant but general mean of experiment in first year was lower than in second year.

It was observed that increasing dose of flufenacet from 180 gha^{-1} to 300 gha^{-1} significantly decreased population of *P. minor* at 30 DAS in both years.

Table 2. Density of *Phalaris minor* (No. m^{-2}) at different stages of growth

Treatment (gha^{-1})	30 DAS		60 DAS		100 DAS	
	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000
Flufenacet 180 PE	425.2 b	492.0 b	90.6 c	110.7 cd	200.0 a-d	186.7 bc
Flufenacet 240 PE	310.5 c	350.7 c	29.2 e	42.7 e	100.0 c-f	122.8 c-e
Flufenacet 300 PE	197.3 d	244.0 d	14.8 g	22.7 g	82.7 ef	111.0 de
Flufenacet 180 2WAS	436.0 b	517.2 b	93.2 c	113.3 c	146.7 b-e	173.2 b-d
Flufenacet 240 2WAS	310.7 c	350.8 c	65.2 d	85.3 d	90.7 c-f	108.0 c-e
Flufenacet 300 2WAS	206.7 d	242.7 d	22.8 ef	37.2 e	88.0 d-f	106.8 c-e
Flufenacet (180 PE) + Isoproturon (500 PE)	445.2 b	526.8 b	100.0cd	100.0 cd	246.7 a-c	294.4 ab
Flufenacet (180 4WAS) + Metribuzin (150 4WAS)	112.0 e	132.0 e	24.0 ef	38.9 ef	49.5 f	66.8 e
Flufenacet (180 2WAS) *fb.Isoproturon (500 4WAS)	450.8 b	530.8 b	100.0 cd	100.0 cd	166.7 a-d	190.2 bc
Flufenacet (180 2WAS) *fb. Metribuzin (150 4WAS)	89.3 e	109.3 e	22.8 ef	38.8 ef	88.0 d-f	106.7 c-e
Flufenacet (180 2WAS) *fb. 2,4-D (400 5WAS)	93.2 e	113.3 e	20.0 f	30.8 f	84.0 d-f	104.0 c-e
Isoproturon 750 4WAS	613.2 a	692.0 a	204 .0 b	238.7 b	262.8 ab	302.4 ab
Two hand weedings (4 and 6WAS)	20.0 f	30.8 f	25.2 ef	43.0 e	136.9 f	66.8 e
Unweeded control	666.8 a	753.2 a	312.0 a	352.0 a	358.8 a	422.8 a

*fb. : Followed by ** Any two means having a common letter are not significantly different at 5% level of significance

Insignificant difference was observed between times of application of the herbicides. Combination of 180 gha^{-1} of flufenacet with metribuzin and 2,4-D recorded the best control. It decreased *Phalaris* population down to about 85% compared to hand weeded plot. Lowest dose of flufenacet significantly reduced population of *P. minor* as compared to hand weeding treatment at this stage. Application of isoproturon (500 gha^{-1}) alone was as good as unweeded control treatment and its combination with flufenacet was however comparable with the lowest dose of flufenacet alone at 30 DAS.

Phalaris minor density decreased from 30 DAS to 60 DAS but it increased later till maturity of wheat. The pattern of reduction in *P. minor* population over the treatments at 60 DAS was similar to 30 DAS. However combinations of 2,4-D and metribuzin with flufenacet and post-emergence application of 300 gha^{-1} of flufenacet remained at par with two hand weedings treatment.

Difference between the doses of flufenacet disappeared for both times of application at 100 DAS. Unweeded control recorded the highest *P. minor* population while hand weeding treatment and combination of flufenacet with metribuzin recorded the lowest population.

4.1.1.2. *P. minor* dry matter

Total dry matter production of *P. minor* has been presented in Table 3. Although *P. minor* produced higher dry matter in second year but no interaction was found between years of experiment and treatments. The higher the dose of

Table 3. Total dry matter production (gm^{-2}) of *P. minor*

Treatment (gha^{-1})	30 DAS		60 DAS		100 DAS	
	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000
Flufenacet 180 PE	3.8 a-c	4.3 a-c	7.6 a-d	8.3 a-c	72.1 b-d	82.3 bc
Flufenacet 240 PE	2.9 d	3.9 bd	2.4 ef	2.9 de	63.2 b-e	70.3 bd
Flufenacet 300 PE	0.8 d	1.0 d	1.6 f	2.0 e	40.9 ef	45.8 de
Flufenacet 180 2WAS	2.5 a-c	3.0 cd	10.2 a-c	12.1 ab	68.4 b-e	76.0 bd
Flufenacet 240 2WAS	1.6 cd	1.8 cd	4.8 b-e	5.2 a-d	44.7 d-f	49.6 ce
Flufenacet 300 2WAS	0.8 d	1.0 d	2.1 ef	2.4 de	40.9 e-f	45.6 ce
Flufenacet (180 PE) + Isoproturon (500 PE)	6.3 ab	6.8 ab	6.1 a-d	6.8 a-c	90.1 a-c	103.2 ab
Flufenacet (180 4WAS) + Metribuzin (150 4WAS)	0.8 d	0.9 d	2.8 b-f	3.2 b-e	34.0 ef	38.0 ce
Flufenacet (180 2WAS) *fb.Isoproturon (500 4WAS)	6.2 ab	6.8 ab	3.7 b-f	4.3 be	73.5 b-d	81.4 bc
Flufenacet (180 2WAS) *fb. Metribuzin (150 4WAS)	0.6 d	0.8 d	2.0 ef	2.3 ce	36.0 e-f	40.0 c-e
Flufenacet (180 2WAS) *fb. 2,4-D (400 5WAS)	0.7 d	0.9 d	1.8 f	2.1 ce	32.1 f	37.2 c-e
Isoproturon 750 4WAS	7.2 a	8.2 a	20.2 a	24.9 a	104.8 ab	124.0 ab
Two hand weedings (4 and 6WAS)	0.0 e	0.0 e	0.4 g	0.4 e	23.2 f	27.6 e
Unweeded control	6.1 a	7.0 a	26.1 a	29.2 ab	168.2 a	197.6 a

*fb. : Followed by ** Any two means having a common letter are not significantly different at 5% level of significance

flufenacet the lower was the dry mater accumulation by *P. minor*. Time of application did not affect *Phalaris* dry matter production at 30 DAS. Combinations of isoproturon with flufenacet and isoproturon alone had no effect on *Phalaris* in respect of dry matter accumulation while combinations of metribuzin and 2,4-D with flufenacet regardless of time of application significantly decreased dry matter at 30 DAS. Unweeded control and isoproturon alone recorded higher dry matter in all stages. Difference between treatments was significant at 60 DAS in first year but the general trend was same as 30 DAS. Difference between 180 gha^{-1} and 300 gha^{-1} remained significant even at 100 DAS. Lower dry matter accumulations were recorded in hand weeded plots and combination of 2,4-D as well as metribuzin with 180 gha^{-1} flufenacet as compared to unweeded plots and isoproturon alone.

4.1.2. Broad-leaved weed population

Mean range comparison on number of broad-leaved weeds mostly *Chenopodium album, Melilotus indica* and *Cannabis sativa* has been illustrated in Table 4. Similar result was obtained in both years. In the other word, no interaction between years and treatments was significant. However, the population of broad-leaved weeds in first year remained significantly higher as compared to second year. Since 2,4-D had not been applied at 30 days after sowing the results obtained from this plots at 30 DAS did not reflect their effects but later at 60 DAS and at harvest this treatment made significant reduction in number of broad-leaved weeds. Metribuzin being applied as 4 WAS had the similar effect as 2,4-D had on

Table 4. Population of broad-leaved weeds (No.m^{-2}) at different stages of growth

Treatment (gha^{-1})	30 DAS		60 DAS		Harvest	
	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000
Flufenacet 180 PE	59.7 a	53.1 a	133.0 a	92.4 a	78.2 ab	70.0 a
Flufenacet 240 PE	47.5 a	37.2 a	101.2 a	81.2 a	78.0 ab	63.2 ab
Flufenacet 300 PE	84.0 a	58.1 a	166.3 a	150.0 a	57.0 a-c	41.2 a-c
Flufenacet 180 2WAS	65.3 a	43.5 a	189.9 a	172.1 a	40.3 a-c	29.6 a-d
Flufenacet 240 2WAS	56.0 a	74.4 a	253.5 a	202.8 a	69.1 a-c	56.0 a-c
Flufenacet 300 2WAS	56.1 a	49.6 a	152.0 a	105.2 a	88.0 a	80.0 a
Flufenacet (180 PE) + Isoproturon (500 PE)	34.4 a	31.2 a	72.5 a	51.3 ab	36.5 a-c	30.8 a-c
Flufenacet (180 4WAS) + Metribuzin (150 4WAS)	46.5 a	36.8 a	3.0 c	4.0 d	26.4 cd	24.0 b-d
Flufenacet (180 2WAS) *fb.Isoproturon (500 4WAS)	56.6 a	38.8 a	63.5 a	56.0 ab	34.2 a-c	32.0 a-c
Flufenacet (180 2WAS) *fb. Metribuzin (150 4WAS)	72.0 a	50.8 a	25.2 bc	22.8 cd	3.0 d	5.0 d
Flufenacet (180 2WAS) *fb. 2,4-D (400 5WAS)	60.0 a	48.0 a	29.4 b	25.6 bc	4.0 d	4.5 d
Isoproturon 750 4WAS	81.2 a	73.2 a	60.0 a	50.0 a	38.1 a-c	61.3 ab
Two hand weedings (4 and 6WAS)	5.1 b	5.0 b	13.3 bc	10.1 cd	21.1 cd	10.0 cd
Unweeded control	57.3 a	46.2 a	112.0 a	89.6 a	278.2 a	19.6 a-d

*fb. : Followed by ** Any two means having a common letter are not significantly different at 5% level of significance

broad-leaved weeds at 30 and 60 DAS. These treatments reduced population of broad-leaved weeds to the same level as hand-weeding treatment did. Combination of 2,4-D with flufenacet reduced weed population even higher than hand weeding treatment. Neither dose nor time of application of flufenacet was effective on broad-leaved weeds at any stages of growth. There was no significant difference in broad-leaved weed population between hand weeded plot and the plots received isoproturon alone at all stages of growth.

4.1.3. Effect on nutrient uptake by weeds

Table 5 show mean uptake of N, P and K by weeds at harvest during both years of experimentation.

4.1.3.1. Uptake of N by weeds

Analysis of variance revealed that there was significant difference between treatments in respect of N uptake by weeds in both years (Table 5). Increasing dose of flufenacet decreased N uptake while its time of application did not make any difference. Lower uptake of N was recorded in plots received flufenacet in combinations with 2,4-D and metribuzin whereas the highest uptake recorded in unweeded control. Difference N uptake by unweeded control and isoproturon alone was significant in both years. Uptake of N was significantly lower in plots received flufenacet in combination with isoproturon as compared to isoproturon alone.

4.1.3.2. Uptake of P by weeds

Uptake of P (Table 5) followed almost the same pattern as N uptake but unlike N, uptake of P by unweeded control and isoproturon alone was at par in these two treatments in second year. Uptake of P in plots received flufenacet in combination with isoproturon was at par with isoproturon alone in second year.

4.1.3.3. Uptake of K by weeds

Analysis of variance revealed that there was no significant difference between treatments in respect of K (Table 5) uptake. However unweeded control recorded the highest K uptake in both years.

4.2. Wheat

4.2.1. Effect on wheat growth

4.2.1.1. Wheat population

The average wheat population established was noted at 15 DAS in both the years. In the first year, the average number of wheat seedling established was 189 m^{-2} and in the second year it was 210 m^{-2}. No significant different was observed among the treatments and the years of study.

4.2.1.2. Plant height

The height of wheat plants at different stages of growth is presented in Table 6 along with their rank computed by DMRT. Height of wheat crop was significantly more in first year as compared to second year but no interaction was found significant between years and treatments. The difference in plant height between hand weeding and other treatments was significant except unweeded

Table 5. Uptake of N, P and K ($kgha^{-1}$) by weeds at harvest

Treatment (gha^{-1})	N		P		K	
	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000
Flufenacet 180 PE	6.8 de	6.9 de	1.2 c	1.1 e	4.0 a	4.1 a
Flufenacet 240 PE	6.5 e	6.6 e	1.1 cd	1.1 e	4.5 a	4.7 a
Flufenacet 300 PE	5.4 f	5.5 f	0.8 g	0.9 g	4.1 a	4.4 a
Flufenacet 180 2WAS	7.3 c	7.5 c	1.3 b	1.3 bc	4.2 a	4.4 a
Flufenacet 240 2WAS	6.5 e	6.7 e	1.2 c	1.3 bc	4.6 a	4.6 a
Flufenacet 300 2WAS	5.0 g	5.2 f	1.1 de	1.2 d	3.9 a	4.2 a
Flufenacet (180 PE) + Isoproturon (500 PE)	7.1 cd	7.3 cd	1.4 b	1.3 bc	4.6 a	4.8 a
Flufenacet (180 4WAS) + Metribuzin (150 4WAS)	4.0 h	4.1 g	0.8 g	0.8 h	4.3 a	4.5 a
Flufenacet (180 2WAS) *fb.Isoproturon (500 4WAS)	7.3 c	7.4 c	1.2 c	1.4 b	4.0 a	4.1 a
Flufenacet (180 2WAS) *fb. Metribuzin (150 4WAS)	3.9 h	4.0 g	0.9 f	1.0 f	4.0 a	4.9 a
Flufenacet (180 2WAS) *fb. 2,4-D (400 5WAS)	3.7 h	3.8 g	1.0 e	0.8 g	4.5 a	4.3 a
Isoproturon 750 4WAS	7.9 b	8.1 b	1.4 b	1.5 a	4.1 a	4.3 a
Two hand weedings (4 and 6WAS)	0.0 i	0.0 h	0.1 g	0.1 i	0.4 b	0.3 b
Unweeded control	8.5 a	8.7 a	1.5 a	1.5 a	4.9 a	5.0 a

*fb. : Followed by ** Any two means having a common letter are not significantly different at 5% level of significance

Table 6. Wheat plant height (cm) at different stages of growth

Treatment (gha^{-1})	30 DAS		60 DAS		100 DAS		Harvest	
	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000
ufenacet 180 PE	16.6 b	15.7 b	46.3 bc	43.6 bc	72.3 cd	68.9 cd	92.9	92.0
lufenacet 240 PE	14.0 d	13.2 d	40.7 d-f	38.6 de	76.9 a-c	72.6 a-c	93.4	91.3
lufenacet 300 PE	13.2 e	12.7 e	37.0 ef	35.2 e	71.3 cd	68.5 cd	91.3	91.0
lufenacet 180 2WAS	15.7 c	14.8 c	46.7 bc	43.4 bc	73.9 b-d	69.5 b-d	93.8	92.5
lufenacet 240 2WAS	14.3 d	13.6 d	43.3 c-e	41.0 cd	69.7 de	66.3 de	91.4	92.1
lufenacet 300 2WAS	14.0 d	13.4 d	35.3 ef	33.2 ef	72.1 cd	69.2 cd	92.6	91.6
lufenacet (180 PE) + Isoproturon (500 PE)	16.3 bc	15.0 bc	44.8 b-d	42.1 cd	69.9 de	65.0 de	91.7	90.6
lufenacet (180 4WAS) + Metribuzin (150 4WAS)	16.1 bc	15.5 bc	45.1 df	44.1 bc	75.7 b-d	72.9 a-c	92.5	91.4
lufenacet (180 2WAS) *fb.Isoproturon (500 4WAS)	16.0 bc	15.3 bc	40.5 d-f	38.0 de	64.9 e	62.6 e	93.1	92.0
lufenacet (180 2WAS) *fb. Metribuzin (150 4WAS)	16.5 b	15.4 b	43.3 c-e	40.0 d	77.1 a-c	71.7 a-c	91.8	91.3
lufenacet (180 2WAS) *fb. 2,4-D (400 5WAS)	16.1 bc	15.4 bc	42.6 c-e	40.7 d	72.6 cd	69.5 cd	92.6	92.1
soproturon 750 4WAS	19.0 a	18.1 a	49.7 bc	46.7 b	72.3 a-c	69.0 cd	92.7	91.8
Two hand weedings (4 and 6WAS)	19.2 a	18.1 a	56.0 a	52.9 a	79.7 a-c	75.2 a-c	93.1	92.1
Unweeded control	19.1 a	18.3 a	53.3 ab	50.8 a	82.2 ab	78.9 ab	92.4	93.1

*fb. : Followed by ** Any two means having a common letter are not significantly different at 5% level of significance

control and isoproturon alone at 30 DAS. Significant differences were observed between different doses of flufenacet in early stages of growth. Treatments received higher level of flufenacet showed lesser height. Combination of flufenacet with all other herbicides recorded similar crop height as 180 gha^{-1} flufenacet at 30 DAS. Wheat plants had maximum height in hand weeded plots and unweeded control whereas the minimum height in 300 gha^{-1} flufenacet at 60 DAS. There was no difference in plant height in response to the dose of application of flufenacet at 100 DAS. However unweeded control still could hold maximum plant height in this stage. No significant difference was found between times of application of flufenacet in respect to plant height at all stages.

4.2.1.3. Dry matter production

The data pertaining to the dry matter production (DM) of wheat presented in Table 7 have clearly shown the competitive effects of *P. minor* as well as phytotoxic effect of flufenacet at the highest dose on wheat biomass. At all the stages, in both the years, hand weeded plots showed maximum dry matter production. A significantly higher dry matter production was recorded in first year without any interaction between years and treatments.

Although two hand weedings, unweeded control and isoproturon alone did not significantly differ with 180 gha^{-1} flufenacet and its combination with other herbicides except combination with 2,4-D in second year, they held maximum dry matter at 30 DAS. Minimum dry matter was obtained in plots under 300 gha^{-1} flufenacet. Observation at 60 DAS showed almost a similar trend as at 30 DAS but

Table 7. Dry matter production by wheat (gm^{-2}) at different stages of growth

Treatment (gha^{-1})	30 DAS		60 DAS		100 DAS		Harvest	
	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000
Flufenacet 180 PE	28.0 ab	26.4 ab	293.6 ab	277.0 ab	510.8 de	486.6 de	735.0 b-d	693.4 b-d
Flufenacet 240 PE	21.8 b-d	20.6 bc	223.6 bc	209.8 c-e	779.2 ab	735.0 ab	753.6 a-d	718.4 a-c
Flufenacet 300 PE	16.4 d	15.8 cd	134.4 e	127.8 e	610.4 b-e	586.2 b-e	678.6 e	647.0 e
Flufenacet 180 2WAS	24.4 a-c	22.8 bc	223.0 bc	207.8 c-e	545.6 c-e	514.0 c-e	733.2 b-d	685.2 c-e
Flufenacet 240 2WAS	19.8 cd	18.8 cd	199.0 cd	188.4 de	569.2 c-e	539.6 c-e	740.4 a-d	706.4 b-d
Flufenacet 300 2WAS	16.4 d	15.6 d	154.8 de	145.0 e	486.8 de	467.8 de	725.8 c-e	683.4 c-e
Flufenacet (180 PE) + Isoproturon (500 PE)	25.0 a-c	23.4 a-c	238.0 bc	223.4 b-d	619.2 a-e	575.8 a-e	700.4 de	656.4 de
Flufenacet (180 4WAS) + Metribuzin (150 4WAS)	24.8 a-c	23.8 a-c	230.1 bc	221.4 b-d	734.0 a-c	706.8 a-c	748.0 a-d	720.2 b-d
Flufenacet (180 2WAS) *fb.Isoproturon (500 4WAS)	24.7 a-c	23.2 a-c	190.4 cd	179.4 de	612.0 b-e	589.6 b-e	733.6 b-d	696.8 b-d
Flufenacet (180 2WAS) *fb. Metribuzin (150 4WAS)	25.8 a-c	24.0 a-c	193.6 cd	178.2 de	688.0 a-d	649.0 a-d	786.6 ab	740.2 a-b
Flufenacet (180 2WAS) *fb. 2,4-D (400 5WAS)	23.6 a-d	22.6 b-d	189.9 cd	181.8 de	666.8 a-e	637.2 a-e	776.6 a-c	740.2 a-b
Isoproturon 750 4WAS	29.8 ab	28.2 ab	248.6 bc	234.2 cd	462.0 e	439.8 e	743.0 a-d	710.6 b-d
Two hand weedings (4 and 6WAS)	30.8 a	29.0 a	346.0 a	325.0 a	820.0 a	787.2 a	789.6 a	753.0 a
Unweeded control	30.0 a	28.8 a	278.0 ab	264.8 bc	596.0 b-e	554.2 b-e	728.8 cd	672.6 c-d

*fb. : Followed by ** Any two means having a common letter are not significantly different at 5% level of significance

dry matter was reduced with post-emergence application of 180 gha^{-1} flufenacet, its combination with other herbicides and isoproturon alone as compared to hand weeding treatment. Mean comparison of treatments at 100 DAS revealed that dry matter was its highest level in plots treated with pre-emergence application of 240 gha^{-1} flufenacet and in hand weeded one. Unlike plant height, difference in dry matter between highest dose of flufenacet and other treatments was observed even at harvest. Besides hand weeded plots the higher dry matter accumulation was observed on 240 gha^{-1} flufenacet and also combination of the lowest dose of flufenacet with 2,4-D and metribuzin. Difference between pre-emergence and post emergence application of flufenacet was not significant.

4.2.2. Effect on nutrient uptake

4.2.2.1. Uptake of nitrogen by wheat grain

Weeds in unweeded control plots posed serious competition to wheat for nitrogen utilization resulting in significant decrease in nitrogen uptake by wheat crop in this treatment compared to hand weeded plots (Table 8) in both years. Time of application of flufenacet did not influence nitrogen uptake by wheat at harvest time. Uptake of nitrogen in plots receiving a combination of 180 gha^{-1} flufenacet and metribuzin or 2,4-D was at par with hand weeded plots, which recorded maximum uptake of nitrogen in both years. Dose of flufenacet affected nitrogen uptake significantly, although 240 gha^{-1} recorded a higher rank in second year, the rank was less in first year. The highest dose of flufenacet adversely

affected nitrogen uptake. Application of isoproturon alone and its combination with flufenacet recorded an uptake of nitrogen as low as lowest dose of flufenacet.

4.2.2.2. Uptake of phosphorus by wheat grain

Dose of flufenacet significantly influenced uptake of phosphorous in both years. Interaction between years was not significant. Time of application of flufenacet did not make any difference in uptake of phosphorous. Plots received 240 gha^{-1} flufenacet recorded higher phosphorous uptake as compared to 180 and 300 gha^{-1} (Table 8). Even 180 gha^{-1} flufenacet recorded higher uptake of P compared to 300 gha^{-1}. Hand weeded plots with the highest uptake had shown a highly significant difference with unweeded control plots in this respect. Results revealed that lowest dose (180 gha^{-1}) of flufenacet in combination with other herbicides except isoproturon resulted in significant uptake as high as two hand weedings and/or middle dose (240 gha^{-1}) of flufenacet. Uptake of phosphorous was significantly higher in second year.

4.2.2.3. Uptake of potassium by wheat grain

Analysis of variance did not show any significant difference in the uptake of K between the treatments in both years (Table 8). However two-hand weeding recorded the highest K uptake by wheat grains in both years.

4.2.3. Effects on wheat yield attributes

4.2.3.1. Mean number of ear bearing tillers (EBT)

The mean number of ear bearing tillers has been shown in Table 9. In second year number of ear bearing tillers was significantly lower than in the first

Table 8. Uptake of N, P and K ($kgha^{-1}$) by wheat grain

Treatment (gha^{-1})	N		P		K	
	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000
Flufenacet 180 PE	32.7 d-f	33.8 c-e	21.6 d	22.0 d	13.5	13.1
Flufenacet 240 PE	34.2 b-d	35.6 a-c	23.7 a	24.2 b	13.7	14.0
Flufenacet 300 PE	29.0 h	30.1 f	20.9 e	21.3 e	13.3	13.7
Flufenacet 180 2WAS	33.2 c-e	34.4 b-d	21.6 d	22.3 d	13.4	13.9
Flufenacet 240 2WAS	35.3 ab	36.5 ab	23.3 ab	24.5 ab	13.7	14.2
Flufenacet 300 2WAS	29.7 gh	30.7 f	20.9 e	21.7 de	13.5	14.0
Flufenacet (180 PE) + Isoproturon (500 PE)	33.0 de	34.0 cd	22.5 c	23.2 c	14.1	14.5
Flufenacet (180 4WAS) + Metribuzin (150 4WAS)	34.9 ac	36.1 ab	23.9 a	24.6 ab	13.6	14.0
Flufenacet (180 2WAS) *fb.Isoproturon (500 4WAS)	32.2 ef	33.4 de	22.9 bc	23.4 c	13.8	14.3
Flufenacet (180 2WAS) *fb. Metribuzin (150 4WAS)	34.8 a-c	36.2 ab	23.6 a	24.1 b	14.2	14.1
Flufenacet (180 2WAS) *fb. 2,4-D (400 5WAS)	34.7 a-c	36.1 ab	23.9 a	24.6 ab	13.7	14.5
Isoproturon 750 4WAS	32.0 ef	33.2 de	22.3 c	23.4 c	14.1	14.4
Two hand weedings (4 and 6WAS)	36.0 a	37.2 a	24.0 a	25.0 a	14.2	14.5
Unweeded control	31.0 fg	31.9 ef	21.6 d	22.0 d	13.7	14.1

*fb. : Followed by ** Any two means having a common letter are not significantly different at 5% level of significance

Table 9. Yield attributes of wheat crop

Treatment (gha^{-1})	Ear bearing tillers (m^{-2})		Length of earhead (cm)		Grains per earhead		1000- grain weight (g)	
	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000
Iufenacet 180 PE	232 c	215 b	9.2 a	9.5 a	34.4	31.8	40.0 a	38.9 ab
Iufenacet 240 PE	283 a	269 a	8.7 ab	9.1 a	33.8	32.1	43.4 a	41.3 a
Iufenacet 300 PE	197 d	183 c	7.4 b	7.7 b	34.2	31.8	37.3 cd	34.7 d
Iufenacet 180 2WAS	237 c	224 b	9.1 a	9.6 a	35.1	33.1	40.0 ab	39.2 ab
Iufenacet 240 2WAS	277 b	258 a	8.8 ab	9.1 a	33.2	31	43.6 a	40.6 a
Iufenacet 300 2WAS	192 f	182 c	7.4 b	7.8 b	34.1	32.4	36.9 d	35.0 cd
Iufenacet (180 PE) + Isoproturon (500 PE)	197 d	184 c	9.1 a	9.5 a	34.8	32.6	38.8 c	36.3 cd
Iufenacet (180 4WAS) + Metribuzin (150 4WAS)	288 a	271 a	9.0 a	9.4 a	35.5	33.5	42.4 a	40.0 ab
Iufenacet (180 2WAS) *fb.Isoproturon (500 4WAS)	195 f	181 c	9.2 a	9.5 a	33.7	31.3	38.6 c	35.9 cd
Iufenacet (180 2WAS) *fb. Metribuzin (150 4WAS)	282 a	268 a	8.9 ab	9.3 a	33.9	32.2	41.9 a	39.8 ab
Iufenacet (180 2WAS) *fb. 2,4-D (400 5WAS)	283 a	265 a	8.8 ab	9.1 a	34	31.9	42.2 a	39.5 ab
Isoproturon 750 4WAS	237 c	224 b	9.0 a	9.4 a	34.5	32.6	35.5 cd	35.5 cd
Two hand weedings (4 and 6WAS)	282 a	267 a	9.4 a	9.7 a	35	33.7	43.9 a	42.3 a
Unweeded control	229 c	220 b	9.1 a	9.6 a	35.2	33.2	37.6 cd	36.0 cd

*fb. : Followed by ** Any two means having a common letter are not significantly different at 5% level of significance

year. Two hand weeding, application of 240 gha^{-1} of flufenacet and metribuzin/2,4-D in combination with flufenacet irrespective of its time of application had recorded significantly higher values. Isoproturon alone and its combination with 180 gha^{-1} flufenacet and also the highest dose of flufenacet recorded the lower number of ear bearing tillers in both years without any significant interaction. Unweeded plots had shown higher mean number of ear-bearing tillers as compared to highest dose (300 gha^{-1}) of flufenacet.

4.2.3.2. Length of earhead

Unlike EBT, only 300 gha^{-1} flufenacet (Table 9) significantly differed with all other treatments except 240 gha^{-1} of flufenacet irrespective of its time of application in both years of experimentation. Also significant difference between this dose of flufenacet and post-emergence application of metribuzin and 2,4-D was observed in first year. Flufenacet 300 gha^{-1} recorded shortest earhead irrespective of its time of application (Table 9). Time of application of all the herbicides did not make any statistically significant difference. Hand weeding and unweeded control held same rank in mean comparison. Length of earhead in plots received 240 gha^{-1} flufenacet obtained a lower rank in the first year compared to the second year.

4.2.3.3. Grain per earhead

Analysis of variance revealed no significant difference existed between all treatments in respect of grains per earhead in both years (Table 9).

4.2.3.4. Test weight

In first year hand weeded plot, plots with lowest dose (180 gha^{-1}) of flufenacet, its combination with metribuzin or 2,4-D and medium (240 gha^{-1}) dose of flufenacet recorded the greater test weight of wheat (Table 9) while highest dose of flufenacet, its combination with isoproturon, application of isoproturon alone and unweeded control had significantly lower test weight. Test weight was more in first year. Results obtained in second year have shown almost the same pattern as first year. Difference between pre- and post-emergence application of flufenacet along with post-emergence application of metribuzin at 4 WAS was not significant in both years.

4.2.4. Effect on wheat yield

4.2.4.1. Grain yield

General mean of grain yield was significantly higher in second year while interaction between years and treatments was not significant (Table 10). Two hand weedings treatment, 240 gha^{-1} flufenacet and its lowest dose (180 gha^{-1}) in combination with 2,4-D and metribuzin produced the higher grain yield compared to all other treatments in both years. On the other hand, 180 and 300 gha^{-1} of flufenacet alone, isoproturon alone and its combination with 180 gha^{-1} flufenacet and unweeded control treatments recorded minimum grain yield. Time of application of flufenacet did not make any difference in grain yield of wheat.

4.2.4.2. Straw yield

Mean comparison of straw production over different treatments has been shown in Table10. It was observed that there was not a sharp difference between the treatments. Weight of straw in unweeded control and hand weeded plots was statistically same. Although most of the treatments did not make any significant difference, 240 and 300 gha^{-1} of flufenacet drastically reduced the weight of straw in comparison to other treatments in first year. No significant difference was observed between treatments in second year.

4.2.4.3. Biological yield

Biological yield has shown almost the same pattern of grain yield in both years of experimentation (Table 10). Biological yield in second year was significantly more. Time of application could not make any difference and highest yield belonged to hand weeded plots, unweeded control, lowest dose of flufenacet in combinations with metribuzin. Difference between highest (300 gha^{-1}) and lowest dose (180 gha^{-1}) of flufenacet irrespective of time of application was significant. No difference was recorded between medium dose (240 gha^{-1}) of flufenacet with 180 and 300 $gha^{-1}$of this herbicide.

4.2.4.4. Harvest index

Application of flufenacet 240 gha^{-1} recorded the highest harvest index both in pre as well as post-emergence application in both years. The minimum harvest index was registered in unweeded control and application of isoproturon alone. Other treatments hold an intermediate mean rank (Table10).

Table 10. Grain, straw, total biological yield (qha^{-1}) and harvest index (%) of wheat

Treatment (gha^{-1})	Grain		Straw		Total		HI	
	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000
Flufenacet 180 PE	29.44 b	30.67 b	59.47 ab	53.41	88.92 ab	84.1 ac	33.1 de	36.6 bd
Flufenacet 240 PE	37.11 a	38.22 a	46.61 e	42.40	83.72 cd	80.6 ad	44.3 a	47.6 a
Flufenacet 300 PE	29.78 b	30.89 b	46.63 e	42.87	76.40 f	73.8 cd	39.0 a-d	42.0 ac
Flufenacet 180 2WAS	29.11 b	30.11 b	54.32 b-d	53.67	83.43 cd	83.8 ac	35 bc	36.0 cd
Flufenacet 240 2WAS	36.89 a	38.00 a	47.52 e	45.18	84.41 c	83.2 ad	43.8 bc	45.7 ab
Flufenacet 300 2WAS	29.67 b	30.56 b	48.74 de	46.07	78.41 ef	76.6 ad	37.8 a-d	40.0 ac
Flufenacet (180 PE) + Isoproturon (500 PE)	30.00 b	29.56 b	60.54 a	42.26	90.54 a	71.8 d	33.1 de	42.9 ac
Flufenacet (180 4WAS) + Metribuzin (150 4WAS)	35.89 a	37.22 a	55.24 a-c	48.60	91.13 a	85.8 ab	39.4 ad	43.4 ac
Flufenacet (180 2WAS) *fb.Isoproturon (500 4WAS)	29.11 b	30.33 b	55.71 a-c	51.93	84.82 c	82.3 a-d	34.4 ce	37.0 bd
Flufenacet (180 2WAS) *fb. Metribuzin (150 4WAS)	36.56 a	37.67 a	51.97 c-e	48.66	88.52 ab	86.3 ab	41.4 ab	43.8 ac
Flufenacet (180 2WAS) *fb. 2,4-D (400 5WAS)	36.44 a	37.56 a	50.08 c-e	49.26	86.53 bc	86.8 ab	42.3 a	43.3 ac
Isoproturon 750 4WAS	29.90 b	30.34 b	55.71 a-c	53.22	85.71 bc	83.6 a-d	35.0 bc	36.3 bd
Two hand weedings (4 and 6WAS)	37.00 a	38.45 a	54.5 b-d	49.99	91.15 a	88.4 a	40.7 ac	43.5 ac
Unweeded control	30.50 b	31.22 b	59.2 ab	53.11	89.70 ab	84.3 b-d	34.1 ce	37.0 bd

*fb. : Followed by ** Any two means having a common letter are not significantly different at 5% level of significance

4.2.5. Response to flufenacet doses

Since there was not any significant difference between time of application of flufenacet alone regarding grain yield and yield attributes therefore responses of these traits to the different doses of flufenacet alone were obtained using Curvefit computer software. The responses were quadratic.

It can be understood from Table 11. That except grain number per head all other yield attributes indicated high correlation with grain yield at different dose of flufenacet.

4.3. Pot experiments

4.3.1. Cross-resistance

Analysis of variance of data derived from this experiment showed that there were significant differences between various biotypes of *P. minor* and applied doses of flufenacet from resistance point of view (Table 12 and Table 14). Increasing dose of flufenacet beyond 60 gha^{-1} drastically decreased number, height and consequently dry matter in all biotypes of *P. minor*.

General model, coefficients of regression equations, which were established between doses of flufenacet and scaled dry matter production for all biotypes of *P. minor,* have been shown in Table 13. Estimated GR_{50} from these equations has been shown in Table 15.

4.3.2. Phytotoxicity to wheat

Results obtained from pøt experiment have been summarized in Table 16 and 17. These results although were in the same line with field data but higher

Table 11. Quadratic responses of grain yield and yield attributes to dose of flufenacet.

Coefficient of regression	Grain yield (qha^{-1})	EBT (m^2)	Earhead length (cm)	Grain No. per head	1000- grain weight (g)
a	-83.671	-675.25	5.075	53.625	-29.25
b	1.007	8.216	0.0469	-0.1756	0.6175
c	-0.0021	-0.0178	-0.0001	0.0004	-0.0013
R^2	0.9967	0.9845	0.9993	0.701	0.9945

Table 12. Mean effect of dose of flufenacet on plant number, height and dry matter of *P. minor*

Dose gha^{-1}	Number per pot	Height (cm)	Dry matter per pot (mg)	Dry matter per plant (mg)
0	24.7 a	15.0 a	541 a	22 a
60	15.2 b	8.1 b	244 b	16 b
120	12.7 c	4.5 c	164 c	12 c
240	11.7 d	2.7 d	126 d	9 d
300	9.6 e	1.8 e	96 e	8 e

Any two means having a common letter are not significantly different at 5% level of significance

Table 13. Model fitting dry matter response of *P. minor* to dose of flufenacet

Biotype	coefficients			coefficient of determination
	a	b	c	
B59	2.0619	1230.53	0.1213	0.9947
B95	-2.9049	1729.52	0.173	0.9938
B57	-3.0943	1941.14	0.1941	0.9985
B12	16.0785	3004.80	0.3005	0.9528
B25	41.4555	2043.01	0.2043	0.9781
B34	85.7958	0.9984	-0.0166	0.9812

In all biotypes $Y=a+b/x-c/x^2$ was the best fitting model except B34 in which $Y=a.b^X+X^c$ was applied.

Table 14. Dry weight of biotypes of *P .minor* at different dose of flufenacet

Dose gha^{-1}	B59	B95	B57	B12	B25	B34
0	581	651	643	483	418	471
60	134	172	188	315	310	342
120	63	64	83	206	257	312
240	47	37	40	170	216	247
300	36	17	15	92	187	231

Table 15. Estimated GR_{50} (g flufenacet.ha^{-1}) for different biotypes of *P. minor*

B59	B95	B57	B12	B25	B34
25	31	36	88	240	350

phytotoxic effects of flufenacet were observed in pot experiments. Since germination ability and growth of varieties were significantly different therefore all data expressed as percentage of growth in control pots. Three varieties viz. PS503, PS504 and HD2285 showed poor germination in control plots, therefore these varieties were not included in statistical analysis. Both doses of flufenacet resulted in significant decrease of dry matter production of wheat varieties. Differences between varieties and doses were significant.

UP2338 has shown higher dry matter production in both doses (240 and 480 gha^{-1}) of application. Based on DMRT there is no significant difference between all cultivars at the lowest (240 gha^{-1}) dose of flufenacet except IND 99-33, which had significantly less dry matter, compared to UP2338. However 9 (UP2338, HD1744, NIAW34, DL803-3, DL1014-2, PBW343, HD2329, HD2285 and WR904) out of 17 varieties have recorded less than 40 per cent reduction in dry matter, 7 cultivars (IND99-83, HD2687, HW3038, WH542, DL7843, DL153-2 and DL788-2) 40 to 60 percent reduction whereas only one variety viz. IND99-33 recorded more than 70 per cent decline in dry matter at 240 gha^{-1} of flufenacet.

Variation among wheat cultivars was more when treated with 480 gha^{-1} flufenacet. HD1744 held highest dry matter while WH542 and DL803-3 recorded the lowest value. UP2338 and HD1744 obtained highest rank in both doses.

Table 16. Mean effect of doses of flufenacet on wheat crop at 30DAS

Doses gha^{-1}	Dry matter mg pot-1	Dry matter reduction (%)
0	822 a	100
240	479 b	58.27
480	188 c	22.87

Any two means having a common letter are not significantly different at 5% level of significance

Table 17. Percent dry matter production of wheat cultivars in flufenacet treatment compared to control

Cultivar	Control	240 gha^{-1}	480 gha^{-1}
UP2338	100 (1050)*	79.17 a	55.28 ab
HD1744	100 (1025)	75.00 ab	59.53 a
NIAW34	100 (1000)	72.92 ab	22.42 bd
DL803-3	100 (1000)	71.94 ab	3.125 d
DL1014-2	100 (950)	70.83 ab	16.67 cd
PBW343	100 (850)	66.46 ab	21.88 bd
HD2329	100 (850)	65.83 ab	19.82 bd
HD2285	100 (725)	62.36 abc	20.56 bd
WR904	100 (825)	61.90 abc	19.84 bd
IND 99-83	100 (725)	59.38 abc	35.76 ad
HD2687	100 (725)	59.11 abc	42.72 abc
HW3038	100 (725)	58.03 abc	27.68 ad
WH542	100 (725)	55.71 abc	4.18 d
DL7843	100 (700)	52.50 abc	30.72 ad
DL153-2	100 (675)	43.13 abc	12.81 cd
DL788-2	100 (700)	40.65 bc	8.93 cd
IND 99-33	100 (600)	29.76 c	14.58 cd

Any two means having a common letter are not significantly different at 5% level of significance.

*Data quoted in ( ) show original weight of wheat young plants (mg).

Figure 2. Peak of standard solution (0.1 µg/g)

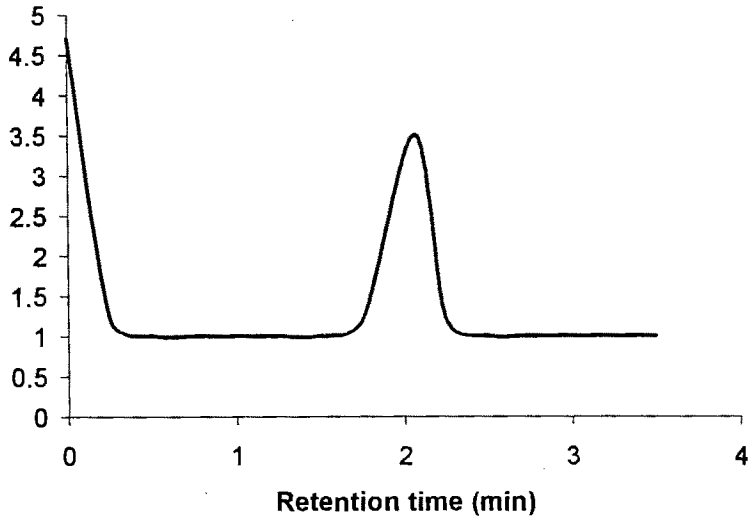
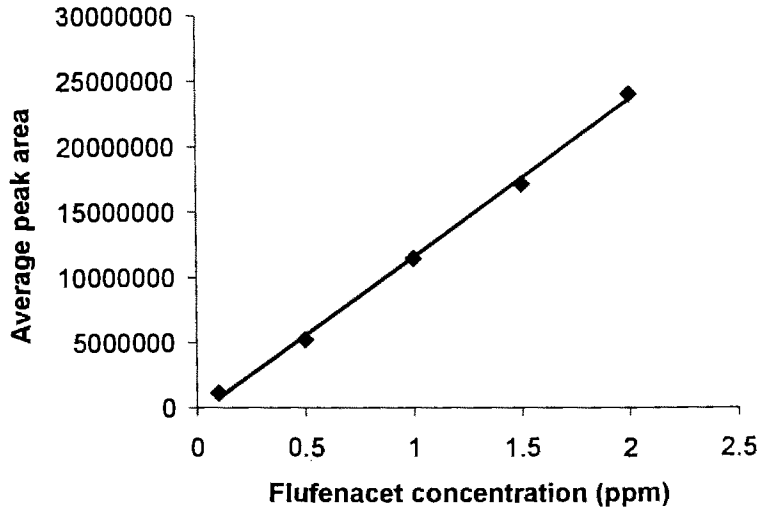


Figure 3. Standard curve of flufenacet by GLC technique



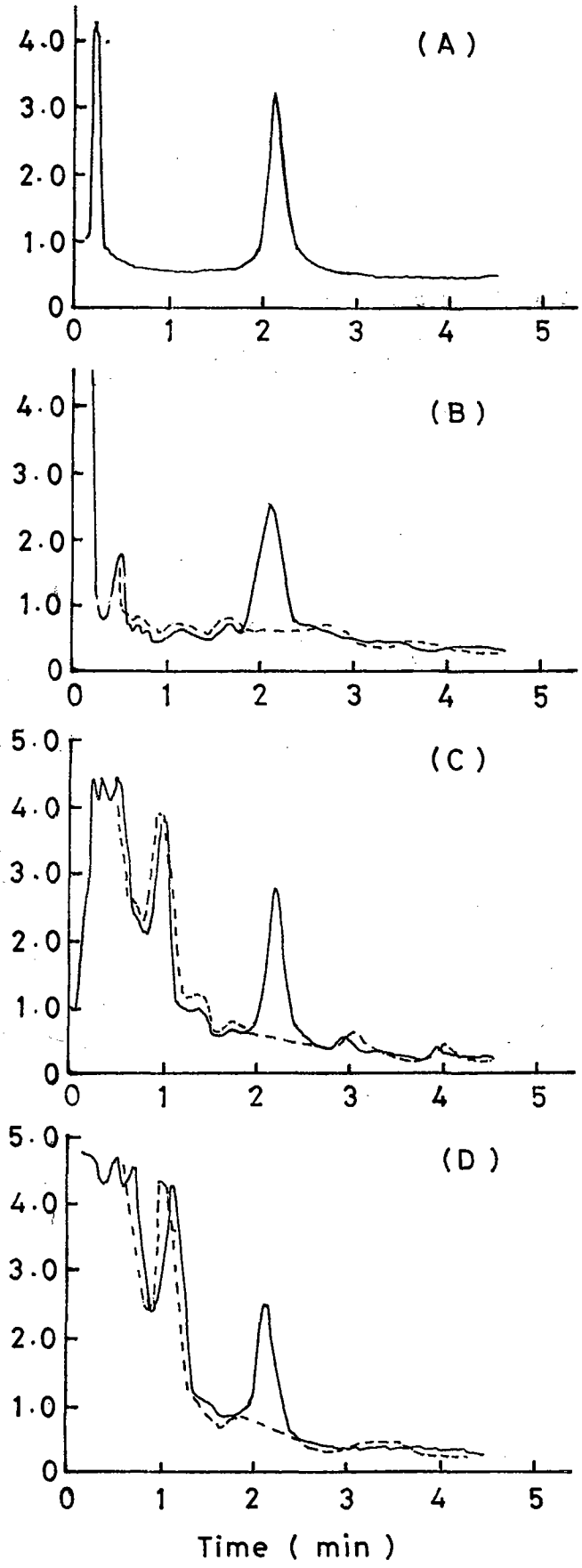


Fig.4 : Gas chromatograms of flufenacet

(A) Standard solution (0.1 µg ml^{-1}), (B) Soil (C) Grain

(D) Straw (- - - - Blank sample, —— Treated sample)

4.4. Results from persistence experiments

4.4.1. Calibration of flufenacet by GLC technique

Flufenacet was resolved as a single peak in gas chromatograph and had a retention time (R_t) of 2.07 min (Fig. 2). The method showed linearity over a range from 0.01 to 1.0 μg ml^{-1} (Fig. 3). The limit of determination of the technique was 0.03 ng of flufenacet.

Three-fold injections of flufenacet (0.01 to 1 μg ml^{-1}) were used to determine the standard deviation. It was observed that triplicate injections of each sample were optimum for operating in a 95% confidence interval (Table 18). It was found that limit of detection was also satisfactory at level down to 0.01 ppm.

Following optimization of instrument operation conditions, method was extended for analysis of flufenacet in soil, straw and grain. Although there was no interference in any extract in the gas chromatogram (Fig. 4B,C, D), the extraction efficiency of the herbicide from soil (Table 19) in acetone:hexane mixture by shaking was rather low (Av. 56.4%) as compared to acetone:0.2 M HCl (Av. 95.2%). The extraction from soil matrix was quantitative only when acetone:0.2 M HCl was used as an extraction solvent. While mechanical shaking was found satisfactory for soil, soxhlet gave quantitative recovery (81-100%) for wheat grain and straw (Table 20).

Table 18. Calibration of flufenacet by Gas Chromatographic method

Concentration ppm	Area	Average
1	11185245 12001653 11042452	11409783 ± 517523
0.5	5043141 5267263 5350011	5220138 ± 158770
0.1	1053767 1001026 1108398	1054397 ± 53689
0.05	592197 533492 566461	564050 ± 29427
0.01	108701 102112 114697	108503 ± 6295

Table 19. Percent recovery of flufenacet from fortified soil

Method of extraction	Solvent	Amount fortified ($\mu g\ g^{-1}$)	Amount * recovered ($\mu g\ g^{-1}$)	Recovery %
Shaking	Hexane:Acetone	0.10	0.04 ± 0.002	44.8
Shaking	Hexane:Acetone	0.50	0.34 ± 0.016	68.0
Soxhlet	Hexane:Acetone	0.10	0.07 ± 0.004	69.2
Soxhlet	Hexane:Acetone	0.50	0.34 ± 0.015	67.9
Shaking	acidified acetone	0.10	0.09 ± 0.005	91.5
Shaking	acidified acetone	0.25	0.24 ± 0.011	95.3
Shaking	acidified acetone	0.50	0.49 ± 0.023	98.7

* Average of three replicates

Figure 5. Residues of flufenacet in soil at different time intervals following pre-emergence application

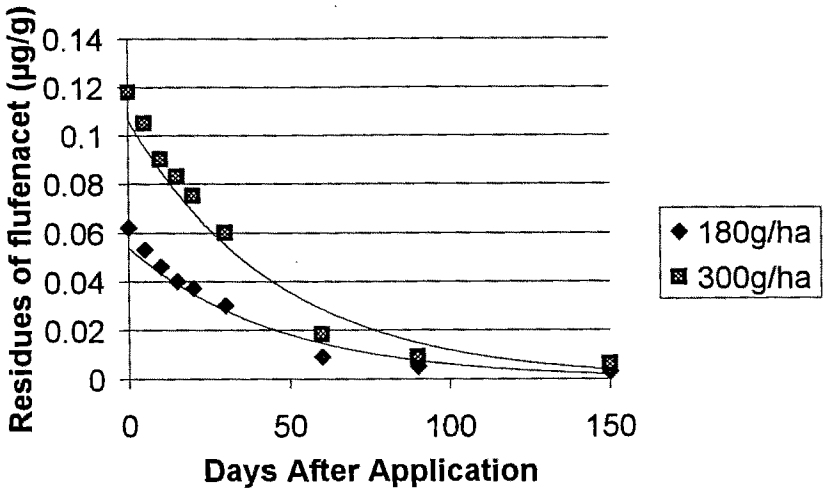
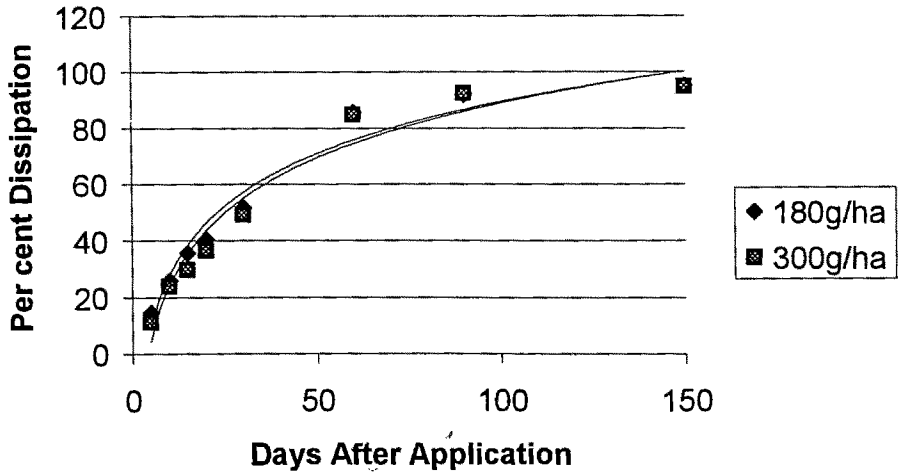


Figure 6. Dissipation of flufenacet in soil at different time intervals following pre-emergence application



The peak areas corresponding to various concentrations from 0.1 to 2.0 μg ml^{-1} solution of flufenacet have been illustrated in Fig. 3. It showed linearity over this range (r^2 =0.9881).

4.4.2. Persistence in soil

Results of analysis of samples drawn 2h after application and subsequently at different intervals up to 150 days (harvest time) showed that the initial concentration of flufenacet in soil (0-15) from low and high application rate were 0.062 and 0.118 $\mu g.g^{-1}$ respectively declining as a function of time (Fig. 5).

Dissipation fitted curves (r^2>0.99) have been shown in Fig. 6. It can be seen that the rate of dissipation was rapid during initial period but slowed down as the time proceeded and it is almost same for both rates of application. About 50 percent of flufenacet was dissipated in the first thirty days after application.

The logarithmic residues of herbicide were calculated and the regression equations of these logarithmic values against time were obtained. It was found that flufenacet degradation rate in soil was not constant, a rapid degradation during the first 30 days followed by slower degradation during the rest of the season. The slope of the curve, coefficient of determination (r^2) and half-life of the herbicide as obtained from the equations are summarized in Table 21.

The half-life ($T_{½}$) of the compound in soil under field condition was 28.90 and 31.09 days for low and high dose of application respectively in the first phase but increased to 59.36 and 60.68 in second phase of growing season (60-150 DAS).

Table 20. Percent recovery of flufenacet from fortified wheat grain and straw

Matrix	Amount added ($\mu g\ g^{-1}$)	Amount found ($\mu g\ g^{-1}$)	Recovery %
Wheat grain	0.10	0.10 ± 0.005	100.5
	0.25	0.23 ± 0.010	92.1
	0.50	0.42 ± 0.021	85.0
Wheat straw	0.10	0.09 ± 0.004	92.7
	0.25	0.22 ± 0.010	87.4
	0.50	0.40 ± 0.020	81.2

*Average of three replicates

Table 21. Slope, correlation coefficient and half-life of flufenacet

Dose (g a.i. ha^{-1})	Days After Application	Slope (b)	Coefficient of Determination (r^2)	Half life (Days)
180	0-30	-0.0104	98.67	28.90
180	60-150	-0.00968	99.48	59.36
300	0-30	-0.00507	94.77	31.09
300	60-150	-0.00496	88.87	60.68

Figure 7. Residues of flufenacet in different soil depths under weekly irrigation (M1) and normal irrigation (M2)

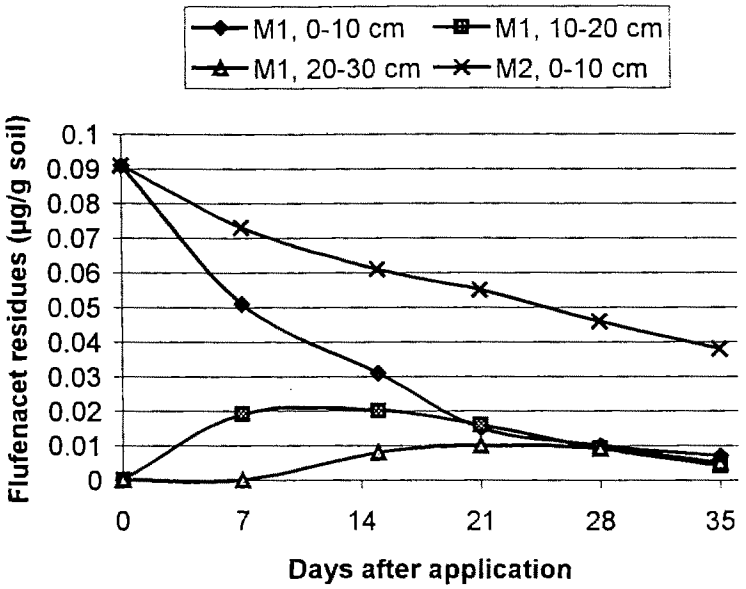
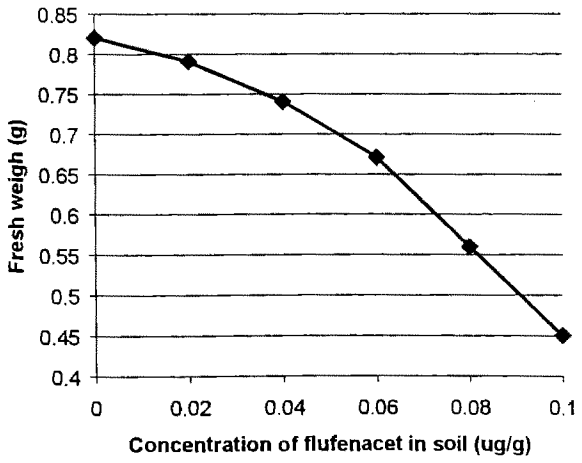
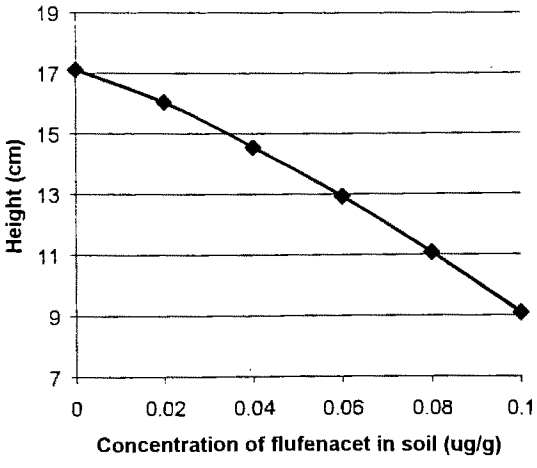


Figure 8. Changes of fresh weigh (A) and height (B) of test plant as a function of concentratio of flufenacet in soil

A)



B)



4.4.3. Flufenacet residues in wheat crop

No detectable residue of the compound was found in wheat grain and straw at the harvest time. Data collected from field bioassay test with maize and field (data not given) showed that there was no adverse effect of flufenacet residue on these crops as no significant difference was found in dry weight of the both crops. Maximum and minimum dry matters of maize were 47.6 and 28.4 gm^{-2} respectively without any certain trend in respect of treatments were involved in the experiment.

4.5. Movement of flufenacet in soil profile

Results obtained from field irrigation experiment (Fig. 7) revealed that flufenacet was highly mobile in soil. The rate of dissipation was more with weekly (M1) as compared to normal irrigation (M2). Herbicide residues (0.02 $\mu g.g^{-1}$) were detected in 10-20 cm depth 7 DAT in case of weekly irrigation, which increased to about 0.04 µg.g-1 on 15 DAT followed, by a declining pattern toward the end of study. The first detectable residues in 20-30 cm soil depth were observed 14 Days After Application. No residue was found below 10 cm soil depth up to 28 DAA in case of normal irrigation.

A high degree of correlation was found between height and fresh weight of test plant with concentrations of flufenacet in soil (Fig. 8). The regression equations were established between these two plant growth parameters and herbicide residues to estimate residues base on them.

Model fitting results for residue quantity (Y) as a function of height (H) and fresh weight (Fw) of test plant were significant at 5% level of error.

$Y=0.1351-0.0004 \times H - 0.0005 \times H^2$ $r^2=0.9983$

$Y = 0.0235 + 0.3951 \times Fw - 0.5101 \times Fw^2$ $r^2=0.9842$

As it can be seen in figure 9, residues estimated to be 0.0347, 0.0674 for 180, 300 gha^{-1} respectively. Results produced from GC method confirm estimations of residues generated through bioassay.

4.6. Results from molecular analysis of biotypes

For final molecular analysis a set of four selected biotypes namely B25, B34, B57, and B95 were scored using 60 primers from OPJ, OPD, and OPB primer sets. Polymorphisms were observed for few primers for example J7 (Fig. 10).

Figure 9. Estimated residues of flufenacet by bioaasy 26 days after application following post-emergence application

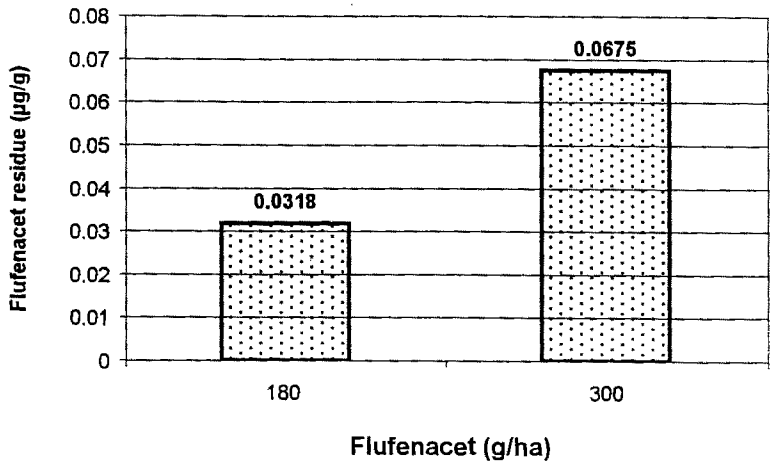
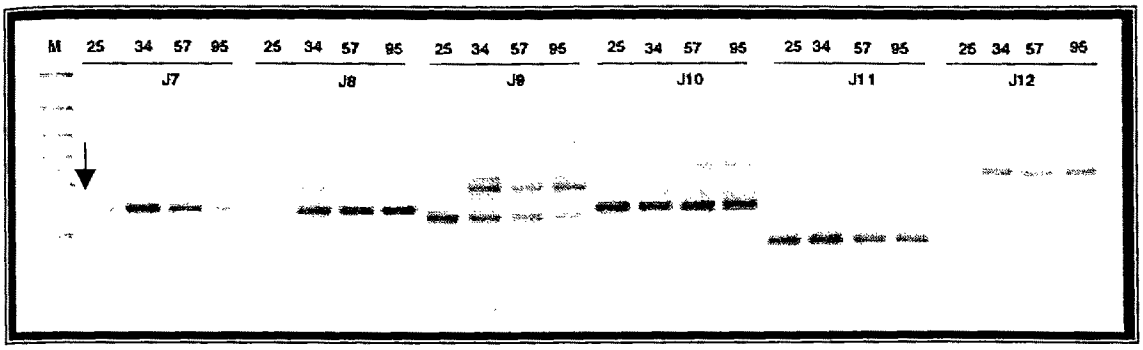


Fig. 10. Fingerprints of selected biotypes of *P. minor*



5. Discussion

The investigation carried out and reported herein was designed primarily to evaluate flufenacet a new chemical from oxyacetamide group of herbicide for controlling herbicide resistant *Phalaris minor*.

Flufenacet 180, 240 and 300 gha^{-1} applied alone as pre-emergence at 1 day after sowing (DAS) as well as post-emergence at 2 Weeks after sowing (WAS) along with combination of 180 gha^{-1} with 2,4-D, metribuzin and isoproturon. Weedy check and hand weeding were also included. Behavior of weeds as influenced by the treatments was studied in terms of weed population, dry matter accumulation and N, P and K uptake. Observations on wheat crop including dry matter production, yield and yield attributes were also recorded. In addition residues of flufenacet in soil and wheat crop were worked out.

Mobility of flufenacet in soil was studied in a separate experiment. Two doses of flufenacet viz., 120 and 240 gha^{-1} were applied 2 WAS under two irrigation levels. One set received normal irrigation as already described and the other was given weekly irrigation. Soil samples were taken for quantitative analysis by gas chromatography (GC) method. An analytical procedure for detecting residues of a new herbicide, flufenacet in soil, wheat grain and straw by gas chromatographic method using various solvents and extraction methods was standardized.

A pot experiment using 20 wheat varieties and 2 doses (240 and 480 gha^{-1}) of flufenacet was laid out to investigate the phytotoxic effects of flufenacet to wheat crop.

Another one pot experiment was also carried out in order to study the existence of cross-resistance in *P. minor* biotypes. Six selected biotypes of *P. minor* with different origin and susceptibility to isoproturon were exposed to two doses of flufenacet.

5.1. Field study

5.1.1. Effects on weeds

The dominant weed flora in the experimental wheat field comprised of *Phalaris minor, Melilotus indica* and *Chenopedium album.* However, occurrence of other weeds viz. *A. ludoviciana* and *Cannabis sativa* was very rare. In general population of *P. minor* was higher than those of broad-leaved weeds (Tables 2 and 4). Amongst broad-leaved weeds, *M. Indica* had higher population but lesser dry mater than *C. album.*

In almost all observations, interaction between years and treatments were not significant while mean effect of years and treatments found to be statistically significant. Since difference between treatments were too much Therefore lack of interaction was expected. Differences between years might be attributed to climatic conditions prevailing on these years. Higher monthly mean temperature and rainfall during early stages of growth in first year of study (Appendix 2) could

favored wheat crop resulting a better growth and damping off weeds compared to second year.

5.1.1.1. Effect of flufenacet alone

Neither dose nor time of application of flufenacet effected broad-leaved weeds (Table 4) while flufenacet could take care of all grassy weeds including isoproturon-resistant *P. minor* (Tables 2,3) as revealed from decreased population as well as dry matter accumulation. This was mainly due to the fact that flufenacet is basically a grass killer. Even application of 180 gha^{-1} flufenacet was effective against *P. minor* and could decline competition between the weed and wheat crop considerably. However better results were achieved by application of 240 gha^{-1} of the herbicide. Increasing dose beyond 240 gha^{-1} of this herbicide did not make any significant bearing (Table 2). Thus it was obvious that the lowest dose (180 gha^{-1}) of flufenacet has not been enough to cause a reasonable level of control while highest dose of flufenacet has been quiet excessive.

Significant difference with regard to resistant *P. minor* control was not found between times of flufenacet application. This may be due to mechanism of action of flufenacet as it interferes in growth and development in early stage of growth. In both years of experiment *Phalaris minor* started germinating about 15 days after sowing and continued for a long time due to subsequent flushes from soil seed bank. Pre-emergence application had therefore interfered in smooth germination while post-emergence application (14 DAS) interrupted subsequent growth of this weed as well as the newer flushes. Such a results can be considered

as an advantage of this chemical because it provides a wider window of application time, which from point of view of farm operation management offers greater choice to farmers.

5.1.1.2. Effect of flufenacet in combination with other herbicides

Application of isoproturon (500gha^{-1}) alone that was supposed to control grassy weeds, was not found effective (Tables 3 and 4). This confirmed that P. minor biotypes present in the field were really isoproturon-resistant. Although a combination of flufenacet with isoproturon reduced *P. minor* growth but the level of control was at par with application of flufenacet alone at a same dose.

A combination of metribuzin or 2,4-D with the lower dose of flufenacet (180 gha^{-1}) resulted in a very significant control of all weed flora. Such a result was due to the fact that flufenacet affected grassy weeds and the others viz. metrobiuzine/2,4-D declined broad-leaved weeds growth (Table 4). Metribuzin itself also affected both grassy and broad-leaved weeds. Broad-leaved weeds however were not as competitive as grassy weeds as vigorous growth of wheat at the early stages provided with enough growth factor mainly nitrogen can smother Broad leaved weeds.

The population of *Phalaris minor* also increased at third sampling (100 DAS) due to new flushes of this weed. It may be due to low residual effect and persistence of flufenacet in soil. However new flushes could not compete with well established wheat crop.

Except potassium uptake, nitrogen and phosphorous uptake by weeds at different treatments were almost in same line as dry matter accumulation by weeds (Table 5).

5.1.2. Effects on wheat crop

5.1.2.1. Effect of flufenacet alone

Observations at 30 DAS (Table 6) showed that flufenacet irrespective of its time of application resulted in stunting of wheat plants compared to control plots. Higher the dose of application, shorter was the wheat plants height, it however recovered later and as a result no significant difference was observed among treatments at next sampling (60 DAS) and onward. This may be due to mechanism of action of flufenacet which interferers in cell division, elongation and development in early stages of growth. It can also be interpreted as a phytotoxic effect of flufenacet on wheat crop. However as it has already been mentioned that this side effect did not remain for whole growing season and wheat crop was able to detoxify this chemical, most probably through enhanced metabolism system. Anyhow it may be noticed that in spite of apparent recovery even in highest dose of flufenacet in respect of plant height, there has been a permanent damage to global growth of wheat crop at highest level of flufenacet that will be discussed later on this chapter. No significant difference was observed between times of application of flufenacet. It might be due to little gap between them i.e. two weeks only.

Except the early stage of growth, behavior of dry matter accumulation was quite different from height of wheat crop (Table 7). The dry weight of wheat decreased as dose of flufenacet increased at 30 and 60 DAS. This was expected because growth was already damped off as it is reflected in height of plant, while an increment of dry matter was recorded at 240 gha^{-1} flufenacet with a secondary decline at 300 gha^{-1} flufenacet 100 DAS and onward. It may be assumed that in lowest dose, weeds were not adequately controlled and it was competition, which was severe while in highest dose competition was negligible but it was phytotoxicity that reduced dry matter accumulation by wheat. Dry weight has been more in plots received 240 gha^{-1} because of better control of grassy weeds and therefore less competition compared to 180 gha^{-1}. It, however, had less phytotoxic effects compared to highest dose (300 gha^{-1}).

Ear bearing tillers; one of the yield components was affected, in the same way the dry mater accumulation (Table 9). Results derived from mean comparison among mean length of earheads and test weight of wheat grain, showed that competition has posed little adverse effects. It is the reason why the first two dose of flufenacet located in a same group while the highest dose attributed to a lower rank in this regard (Table 9). Lack of significant differences between numbers of grain per earhead in both years of study (Table 9) indicated genetic features of plant rather any effect of flufenacet.

Grain yield is resultant of above mentioned attributes. Higher grain yield achieved in plots with 240 gha^{-1} (Table 10) was definitely due to higher test

weight, more ear bearing tillers and larger earhead. Grain yield obtained under this treatment was at par with weed free plots. Plots received 180 and 300 gha^{-1} in a same group of mean rank produced less grain yield compared to medium dose of flufenacet. Although these two treatments recorded similar grain yield, the causative factors were different. Competition in lowest dose while phytotoxicity in higher dose played the role.

Dry matter accumulation in straw was however, different from grain yield. The higher the dose of flufenacet the lesser was the dry matter accumulation in both times of application. It may be due to reducing vegetative growth ability of flufenacet. Greatest HI was recorded in medium dose (240 gha^{-1}) due to highest grain yield and least straw production. Harvest index was numerically more in plots received 300 gha^{-1} flufenacet compared to those received 180 gha^{-1}.
Unlike weeds, nitrogen and phosphorous uptake were higher in the medium dose of flufenacet (Table 8). It is due to higher dry matter accumulation in grains at harvest in this treatment. However as it has already mentioned in case of weeds; pattern of nitrogen and phosphorous uptake by wheat crop at different treatments were almost as same as dry matter accumulation in this crop. Since uptake is a resultant of nutrient concentration in plant into dry weight, therefore it may be concluded that dry weight had more influence on uptake than concentration.

5.1.2.2. Effect of flufenacet in combination with other herbicides

Insignificant differences between various combinations of flufenacet with other herbicides over the growing season may be due to this fact that all

combinations contained equal dose of flufenacet. It also indicated that other herbicides did not pose any growth inhibiting effect on crop plant. However all these combinations held a rank less than weed free control at 60 DAS but as it has already been mentioned, no difference existed by the end of growing season.

In case of dry matter accumulation the reason why difference between treatments appeared at latest stages of growth was that in all combinations grassy weeds was controlled equally and the only difference came from broad-leaved weeds competition. These weeds especially *C. album* although was the dominant one were controlled with 2,4-D and metribuzin included in mixture while isoproturon did not have any suppressing effect on broad-leaved weeds. Metribuzin had inhibiting effects on both grassy and broad-leaved weeds therefore a higher dry mater accumulation was recorded in this treatment.

Length of earhead and number of grains per head did not significantly differed. In former trait, lack of variation may be due to containing equal dose of flufenacet while in case of second characteristic; it may be dominant genetic features of plant, which decide number of grains rather than dose of flufenacet. Application of isoproturon and its combinations with flufenacet recorded lowest number of ear bearing tillers and test weight. It may be concluded that this herbicide has not been effective in control of weed community.

Grain yield at final harvest were influenced by number of ear bearing tillers and test weight and therefore followed same results and same justification may be hold true.

5.1.3. Effect of weeds on wheat

In the present research *P. minor, C. album* and *M. indica* were the major weeds in order of importance based on their population density. However, considering dry matter accumulation, *P. minor* was more competitive.

Weeds in general compete with crops for nutrients, moisture, light and thereby affect crop growth and final yield. As expected, in the present study weeds affected growth of wheat in terms of tillering and dry matter accumulation. In order to study the detailed influence of weeds on crop growth and yield, a comparison was made between weed free and weed infested crop. In the following paragraphs an attempt is made to discuss the effects of weeds in association with the other treatments under study.

In the present investigation weed competition adversely affected wheat crop in terms of reduced ear bearing tillers per plant, dry matter accumulation, N and P uptake by grains. Several workers have reported adverse effect of weeds on various growth parameters of wheat such as tillering (Godle, 1935. Burrows and Olson, 1955. Wright and Wilson, 1992.), dry matter accumulation (Thurston, 1959. Morishita and Thill, 1988), N uptake (Brar and chopra, 1987 Johri *et. al.* 1992).

Weeds also reduced ear head length and test weight of 1000 grains. On similar lines weed competition was reported to reduce ear bearing tillers (Farahbaksh et al., 1988. Rooney 1991), length of ear head (Godle, 1935. Burrows and Olson, 1955) , test weight of grain (Godle, 1935. Burrows and Olson, 1955.

Singh and Bajpai, 1992) and grain yield (Gautam, 1992. Balayan and Malik, 1989 and Yaduraju and Ahuja, 1992).

The effect of weeds on crop plant height was little inconsistent in terms of statistical significance, but there was a trend of plant height being more in weedy check (Table 6). This could have resulted due to competition for light.

5.2. Pot-culture study

5.2.1. Cross-resistant

As it has been shown in Table 12, not only number of *P. minor* per plot but also its height got affected adversely by increasing dose of flufenacet. It indicated that isoproturon-resistant *P. minor* biotypes could not resist flufenacet or in other word there was not any cross-resistance. Although level of resistance found to be varying among the biotypes included in the study (Table 14) the most resistant biotype even showed 50% reduction in growth. GR_{50} value equal to 30 g (flufenacet) for susceptible biotypes and 226 g for resistant ones (Table 15) compared to estimated GR_{50} values equal to 103 for susceptible and 310 for resistant biotypes of *P. minor* (Amin, Lalodha, Garhi Gujran and Balana as resistant vs. HAU, Hisar district as susceptible) in response to application of isoproturon (Singh and Malik , 1993) emphasizing the ability of flufenacet for the control of herbicide-resistant and its better efficiency as compared to isoproturon. It may be concluded that using new chemical may be one of the solution to over come the herbicide resistance problem as Singh and Malik (1993) found that isoproturon resistant biotypes of *P. minor* which treated with pendimethalin were

found to be highly sensitive to this herbicide, their GR_{50} values being only 76, 57 and 57 g, respectively

Such a vast variation among biotypes of *P. minor* in respect of resistance may be a good evidence for the fact that resistance in this plant is due to enhanced metabolism. Singh *et. al.* (1997) also found that the higher concentration of isoproturon was required for growth inhibition in the resistant biotypes of *P. minor*. Based on this observations and rapid recovery of oxygen evolution and fluorescence coefficient under *in vivo* conditions, together with the absence of selectivity *in vitro,* they suggested that the target site was unaffected. They concluded that resistance to isoproturon is most probably because of enhanced metabolism or sequestration of isoproturon, resulting in decreased target site delivery. It may also be concluded that biotypes collected from different places had different level of exposure to herbicides.

Tal *et. al.* (1996) identified a population of *P. minor*, putatively resistant to fenoxaprop-P in a wheat field in Israel during 1993, showed 20-fold resistance in the laboratory when compared to a susceptible biotype. The resistant biotype also had enhanced resistance (1.1- to 3.0-fold) to acetyl-coenzyme A carboxylase (ACCase) inhibitors.

GR_{50} was calculated based on best possible fitting regression equations with percentage of growth (compared with control as 100 percent) as a function of dose of flufenacet. Therefore the method could provide higher precision than the

method where GR_{50} is expressed as percentage of the untreated control plants, against logarithm of herbicide concentration (Yaduraju 2000).

It can be noticed that in the pot experiment the growth attributes of *P. minor* has shown higher reduction as compared to field condition. It is in the same line with findings of Singh and Malik (1993) who found that the GR_{50} of resistant biotypes of *P. minor*, as calculated in pot experiments, were about 3- to 5-fold less than those found in field trials at Lalodha, Amin and Balana (all in Haryana). It may be due to restricted growth environment i.e. controlled watering with no or less runoff from pots with a given volume of soil for root growth and development.

5.2.2. Phytotoxic effects of flufenacet on wheat crop

Herbicides not only have to control weeds but also must be selective to field crop i.e. it should not cause any adverse effect (phytotoxicity) on the field crop at the recommended dose.

Although about 50 percent reduction in dry matter of wheat cultivars were observed due to application of 240 gha^{-1} flufenacet (Table 16) but it should be remembered that adverse effects of chemicals on biological systems in pot experiments are always different than those of real field conditions and mostly are magnified due to restricted growth environment. Such a conclusion was confirmed from field observations as there was only mild growth delay and only during early stages of growth due to application of 240 gha^{-1} flufenacet.

Lack of significant differences among 15 out of 17 wheat cultivars with a growth between 43 to 79 percent of control (Table 17) may be interpreted as a

good profile for this herbicide (with application of 240 gha^{-1}) specially keeping in view this point that percentage of growth as compared to control would be more under field conditions. Although susceptibility of wheat cultivars has been expressed under application of 240 gha^{-1} flufenacet, however those cultivars that has resisted double dose of the herbicide may be considered as reliable ones. It can be seen from Table 17 that higher the dry weight in control pots, greater the tolerance has been in treated pots. Therefore it may be concluded that those cultivars having superior growth under normal conditions can tolerate flufenacet better.

Knobel *et .al.* (1992) also found different results from field as compare to pot experiments. He determined the susceptibility of wheat cultivars to, bromoxynil and tralkoxydim, applied both at recommended and double the recommended dosage (400 g a.i./ha). In the greenhouse, tralkoxydim was phytotoxic to 16 wheat cultivars and 2 promising lines. In a 2nd greenhouse trial, tralkoxydim, with the same adjuvant, was applied to 36 wheat cultivars and 4 promising lines in combination with bromoxynil all at double the recommended rate (400 and 900 g a.i., respectively). 26 of the 40 cultivars were susceptible to the herbicide combination. Those cultivars which were susceptible in the previous trials and for which tralkoxydim is recommended, were sown in the field and treated with tralkoxydim, bromoxynil and a tank mixture of these 2 herbicides at the recommended and double the recommended rates. Only grain yield of one

cultivar was significantly decreased when the mixture of both the herbicides was applied at double the recommended rate.

5.3. Standardization of gas chromatography method

Due to short retention time of the herbicide (< 3 min) and absence of interfering peaks in the area of herbicide for the matrixes analyzed (Fig. 2), each sample injection could be completed within 5-8 minutes and it was possible to analyze large number of samples in a short time. In other words, method was suitable for batch analyses.

It may be concluded that the described extraction procedure is simple and has given good recoveries for the fortified samples compared to a recent method developed by Gajbhiye *et. al.* (2000) using gas chromatography with a different way of extraction. They have reported recoveries of flufenacet residues about 80-90% from fortified soil and cauliflower samples. The gas chromatographic method is thus sensitive, specific, quick and can be successfully used to quantitatively estimate the residues of flufenacet in soil as well as crop samples. Higher level of detection in this experiment may be due to method of extraction.

5.4. Persistence of flufenacet in soil and crop

Rapid degradation during the first 30 days after application, which followed, by slower degradation during the rest of the season (Fig. 5 and 6) can explain how phytotoxicity symptoms on wheat were disappeared gradually by the end of growing season. It can also explicate why new generation of *P. minor* were appeared even till final stage of growth.

These results confirm observations of Pangilinan and Smith (1997) who in their investigation with radio labeled (phenyl-U-^{14}C) flufenacet, found that it had a shorter half-life under aerobic condition. Flufenacet degradation in soil under aerobic condition was biphasic, characterized by rapid degradation during the first 28 days followed by slower degradation during the rest of study. Assuming 1st order kinetics, the half-life of flufenacet was calculated to be 33-64 days. In another study flufenacet has been reported to have half-lives 248 days for the irradiated samples and 167 days for the dark control samples. Persistence of 3-4 percent of flufenacet at harvest time could be due to adsorption on soil particles. Keeping in view the short half-life time of flufenacet (Table 21), it was expected to find no or small quantity of flufenacet in plant parts at harvest time. This results are in the same line with finding of Krolski *et. al.* (1998) who defined the metabolic fate of flufenacet in plants grown in soil treated with (fluorophenyl-UL-^{14}C) flufenacet. He could not detect parent compound in no case. Investigations of Bieseler *et. al.* (1998) also revealed that in immature corn and soy been seedlings exposed to ^{14}C-flufenacet, the compound was degraded rapidly within plants. However Gould *et. al.* (1997) could detect only small quantities of flufenacet residues in wheat hay and grain (0.09 ppm) at the 10-month old plant.

Lack of adverse effect of flufenacet residues on succeeding maize and confirms the low residual activity of this herbicide.

5.5. Mobility of flufenacet in soil

Reduction of flufenacet residues from 0.09 to 0.052 $\mu g.g^{-1}$soil (42% decline), with one irrigation as compared to 16.7% decline without irrigation during the first week after herbicide application (Fig. 7) indicates that this chemical is highly mobile in soil but it may not be assumed as a threat for under ground water resources because of its quiet short half-life. In the other hand in real field conditions wheat crops never is irrigated weekly. It is the reason why Rouchaud *et. al.* (1999) who studied soil persistence and mobility of flufenacet reported that flufenacet remained in the 0-10 cm soil layer and was never detected in the 15-20 cm layer in spring and summer maize crops on sandy loam soil and after application in the autumn in wheat in Belgium during 1997-98.

5.6. Molecular Analysis of *P. minor*

All biotypes used for molecular analysis were isoproturon resistant except B95. However, the most of markers were monomorphic, but some of the primers such as J7 could differentiate B25 from other three biotypes. This molecular differentiation was not concordance with biotypes reaction to isoproturon resistance. This indicates that for molecular characterization of resistibility to isoproturon in *P. minor* biotypes further studies using specific markers is required.

6. SUMMARY AND CONCLUSION

6.1. Summary

The investigation "Evaluation of flufenacet for control of herbicide-resistant *Phalaris minor* and its persistence in soil and wheat crop" was undertaken during the *Rabi* season of 1998-99 and 1999-2000 at the farm of Division of Agronomy, Indian Agricultural Research Institute (IARI), New Delhi. Residue studies were carried out at the Division of Agricultural Chemicals and molecular characterization of *P. minor* at the National Phytotron Facility, IARI, New Delhi. The investigation consisted of two field and two pot experiments. In the field experiment I, the effects of different doses of flufenacet, viz., 180, 240 and 300 gha^{-1}, its time of application viz., pre-emergence and two weeks after sowing and its lowest dose in combination with isoproturon, metribuzin and 2,4-D on behavior of *P. minor* and wheat growth and yield were studied. In the second experiment, effects of two irrigation levels (one received normal irrigation and the other was given weekly irrigation of about 60 mm each) on mobility of flufenacet in soil were studied. Flufenacet was applied at 120 and 240 $g.ha^{-1}$ as post-emergence. In the first pot experiment cross-resistance to flufenacet among six biotypes of *P. minor* was investigated. In the second pot experiment, twenty different wheat cultivars were exposed to both recommended and double the recommended dose of flufenacet in order to evaluate herbicide tolerance in wheat

crop. Additional laboratory and pot experiment were also conducted to determine herbicide residues through gas chromatography as well as by bioassay method.

The objectives were: (i) To optimize the time and dose of application of flufenacet for control of herbicide-resistant *Phalaris minor,* (ii) To study the performance of flufenacet in combination with other herbicides, (iii) To investigate the cross-resistance, if any, of *Phalaris minor* biotypes to flufenacet. (iv) To study the persistence of flufenacet in soil and wheat plant.

Duncan multiple range test was used for all mean comparison because experiments that required evaluation of all possible pairs of treatment means, the LSD test is usually not suitable. This is especially true when the total number of treatments is large i.e. more than six.

In most of the observations interaction between years and treatments was not significant. Also insignificant difference was observed between times of application.

Increasing dose of flufenacet from 180 gha^{-1} to 300 gha^{-1} significantly decreased dry matter and population of *P. minor* at 30 DAS. Combination of 180 gha^{-1} of flufenacet with metribuzin and 2,4-D recorded broad-spectrum control weeds. It decreased *Phalaris* population down to about 85% compared to hand weeded plot. Isoproturon alone was no way better than unweeded control and its combination with flufenacet was as good as lowest dose of flufenacet alone at 30 DAS. Neither dose nor time of application of flufenacet did pose any effect on broad-leaved weeds at any stage of growth.

Increasing dose of flufenacet decreased N uptake as well as P uptake by weeds but exerted no effect on K uptake. Uptake of N and P by wheat grain was at its highest level in plots treated with 240 gha^{-1} but no significant difference existed in K uptake.

The average number of wheat seedling established was 189 m^{-2} and in the second year it was 210 m^{-2}. The higher the dose of flufenacet shorter was the wheat tillers during early stage of growth. Difference in height of tillers was insignificant at harvest. Highest dry matter accumulation in wheat crop was recorded 240 gha^{-1} flufenacet and 2,4-D and metribuzin in combination with lowest dose of flufenacet. Similar trend was also observed in grain yield and yield components.

The biotypes of *P. minor* showed significant difference in resistance to flufenacet. Number, height and consequently dry matter in all biotypes *of P. minor* notably decreased with increasing dose of flufenacet beyond 60 gha^{-1}. GR_{50} calculated for all biotypes showed large variation.

Higher phytotoxic effect of flufenacet on wheat crop was observed in pot experiments compared to field condition. Different wheat cultivars showed different behavior in response to different dose of flufenacet. It indicted that interaction between variety and dose was significant. Cultivar UP2338 has shown higher dry matter in both doses of application.

An analytical procedure for detecting residues of a new herbicide, flufenacet, in soil, wheat grain and straw by gas chromatographic method using

various solvents and extraction methods was standardized. The herbicide recoveries ranged between 81 to 100 percent. Flufenacet had a retention time (R_t) of 2.07 min. The method showed linearity over a range from 0.01 to 1.0 μg ml^{-1}. The limit of determination of the technique was 0.03 ng of flufenacet.

During initial period of study the rate of dissipation of flufenacet was rapid but slowed down as the time proceeded. About 50 percent of flufenacet was dissipated in the first 30 days after application. The half-life ($T_{½}$) of the compound in soil under field condition was 28.90 and 31.09 days for low (180 gha^{-1}) and high dose (300 gha^{-1}) of application respectively in the first phase but increased to 59.36 and 60.68 days in second phase of growing season (60-150 DAS).

Wheat grain and straw analyzed with gas chromatography indicated no detectable residue of the compound. No adverse effect of flufenacet residue was noticed on maize and green gram (based on dry weight and height of the plants) sown after harvesting of wheat in the same layout.

For estimating residues of flufenacet in soil through bioassay method, a standard curve plotting fresh weight/length of the test plant against different quantities of flufenacet was established. High degree of correlation was found between height and fresh weight of test plant (*Avena fatua*) with concentrations of flufenacet in soil in bioassay pot experiment.

In experiment II, Soil samples were collected from different depths at different times from plots treated with higher dose of herbicide only. Representative soil sample was taken for quantitative analysis by gas

chromatography (GC) method. No residue was found below 10 cm soil depth up to 28 days after application in case of normal irrigation. Herbicide residue (0.02 μgg^{-1}) was detected in 10-20 cm depth 7 days after treatment in case of weekly irrigation, which increased to about 0.04 µgg-1 on 15 DAT followed by a declining pattern towards the end. The first detectable residue in 20-30 cm soil depth was observed at 14 DAS.

To investigate molecular characterization of *P. minor*, their DNA was extracted based on the micro extraction method and then amplified using PCR machine. Each amplification product was considered as a RAPD marker. Gels were scored on the basis of the presence (1) or absence (0) of each band for all biotypes.

6.2. Conclusion

1. The present investigation was undertaken with the prime objective of determining suitability of a new selective herbicide flufenacet for application in wheat crop infested with herbicide-resistant *P. minor* including its persistence in soil and crop.

2. Increasing dose of flufenacet from 180 gha^{-1} to 300 gha^{-1} although severely decreased population and dry matter accumulation of *P. minor,* it did not cause any effect on broad-leaved weeds. On the other hand highest dry matter in wheat, yield and yield components were recorded with application of medium dose of flufenacet i.e. 240 gha^{-1} due to low competition by *P. minor* and very less/negligible phytotoxicity to wheat. Mild retarding growth symptoms evolved

with application of 240 gha^{-1} at the early stage, completely disappeared overtime. In the highest dose (300 gha^{-1}) of flufenacet, it was phytotoxicity, which reduced crop growth, and in lowest dose, it was competition by *P. minor*, which caused reduction in yield.

3. Time of application of flufenacet did not make any significant difference in all the observations made on *P. minor*, BLWs and wheat crop.

4. Recommended dose of isoproturon alone was quiet ineffective on resistant *P. minor* while its combination with flufenacet was as good as flufenacet alone at the same dose. Lowest dose of flufenacet followed 2,4-D and metribuzin resulted in the best control of weed flora.

5. Fifty per cent reduction in growth even in the most resistant biotypes by flufenacet, highlighted the ability of flufenacet for the control of herbicide-resistant *P. minor* .

6. Lack of significant difference in growth among 15 out of 17 wheat cultivars due to application of flufenacet reflected response stability of wheat crop to this herbicide.

7. The gas chromatographic method was sensitive, specific, quick and can be successfully used to quantitatively estimate the residues of flufenacet in soil as well as in crop sample.

8. Detection of residues of flufenacet in lower layer (20-30 cm) of soil under weekly irrigation forwarded that this chemical is highly mobile but half-life was quiet short (30 days).

9. The availability of RAPD polymorphism per se with random primers is a strong indicator that elaborated studies employing larger number of primers or AFLPs could provide molecular markers for resistance to isoproturon in *P. minor*.

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* Original not seen.

Appendix 1. Details of field operation

Operations	Date	
	1998-1999	1999-200
1. Preparatory tillage		
a) Ploughing	20.10.98	15.10.99
b) Discing and leveling	10.11.98	08.11.99
2. Layout	12.11.98	10.11.99
3. Sowing	15.11.98	12.11.99
4. Fertilizer application		
a) Basal	15.11.98	12.11.99
b) Top dressing (N only)	05.12.98	07.12.99
5. Thinning	26.11.98	25.11.99
6. Hand weeding treatment		
a) First	13.12.98	10.12.99
b) Second	28.12.98	24.12.99
7. Irrigation		
a) First	07.12.98	03.12.99
b) Second	28.12.98	23.12.99
c) Third	18.01.99	12.1.2000
d) Fourth	08.02.99	1.2.2000
e) Fifth	02.03.99	26.2.2000
8. Sampling		
a) First	15.12.98	10.12.99
b) Second	15.01.99	9.1.2000
c) Third	13.02.99	9.2.2000
9. Harvesting	03.04.99	2.4.2000

Appendix 2. Monthly meteorological data for crop season *Rabi* (1998-1999, 1999-2000)

A. Monthly mean temperature, relative humidity (RH) and rainfall (RF) during growing season.

	1998-1999			1999-2000		
	Temperature (°C)	RH (%)	RF (mm)	Temperature (°C)	RH (%)	RF (mm)
November	20.5	55.3	0.0	19.8	64.0	0.0
December	19.8	67.0	0.0	14.4	75.8	0.0
January	12.0	77.6	72.9	13.4	68.0	25.0
February	16.2	68.0	4.0	14.6	70.0	53.5
March	20.6	54.0	0.0	20.9	56.0	6.7
April	28.5	41.0	0.0	28.3	48.0	2.5

RH: Relative humidity RF: Rainfall

B. Weakly mean temperature and relative humidity (RH) during the first six weeks after treatment (WAT).

	1998-1999			1999-2000		
WAT	Temperature (°C)	RH (%)	RF (mm)	Temperature (°C)	RH (%)	RF (mm)
1	19.11	54.14	0.0	19.51	65.85	0.0
2	18.24	54.71	0.0	18.07	56.42	0.0
3	17.60	61.00	0.0	17.10	55.28	0.0
4	15.80	66.14	0.0	16.92	66.28	0.0
5	16.94	61.43	0.0	13.00	75.28	0.0
6	13.98	70.85	0.0	12.40	85.42	0.0

RH: Relative humidity RF: Rainfall WAT: Weeks after treatment