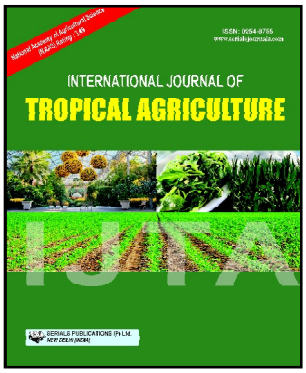

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Effect of Different Moisture Regimes on Persistence of Imazethapyr and Trifluralin

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Abstract: Persistence of any herbicide is vital for effective weed control and residual effect on succeeding sensitive crop which helps in the framing of crop plan for a field. Variable effect of ALS inhibitors has been observed on succeeding sensitive crops mediated by environmental conditions where moisture plays a significant role. An experiment was conducted in the lab and screen house to study the role of moisture on persistence of imazethapyr and trifluralin, using bioassay technique. Mustard and oat were used as bioassay plants for imazethapyr and trifluralin, respectively. Moisture incubation at 50%, 75% field capacity (FC) and FC at room temperature for one month was applied to soil treated with the above herbicides at different concentrations. Growth parameters *viz.* shoot and root growth, fresh and dry weight were measured to assess the level of persistence under screen house conditions. With increase in herbicide rates (imazethapyr 0, 10, 20, 40, 80 and160 g ha^{-1} and trifluralin 0, 0.125, 0.25, 0.5, 1.0 and 2.0 kg ha^{-1}), persistence increased across different moisture regimes. Soil moisture incubation at 75% FC had minimum persistence of imazethapyr whereas; maximum was recordedat FC, data averaged over different imazethapyr rates. Trifluralin persistence decreasedwith the increase in soil moisture incubation from 50% FC to FC as revealed by growth parameters of oat.

Key words: Shoot length; root length; fresh weight; dry weight; persistence; bioassay

1. INTRODUCTION

Knowledge of persistence of a herbicide is important because, on one hand, it determines the period of time in which weeds can be controlled, and on the other hand, it is related to the carryover effects which can damage subsequent sensitive crops. Persistence

of any herbicide molecule is determined by various factors *viz*. herbicide properties, agronomic practices, soil and climatic factors. Among these, climate is the main factor affecting herbicide degradation and among climatic factors, soil moisture has greater influence.

In dry land areas, moisture is the main factors of production and it significantly influences herbicide persistence. Moisture affects various reactions in soil involving physical, chemical and biological changes in the structure of herbicide molecule leading to degradation. With increase in available soil moisture, increased degradation of alachlor was recorded (Zimdahl and Clark, 1982). Similar results were reported by Jolley and Johnstone (1994) for degradation of trifluralin under field conditions whereas its persistence was more in the year of low rainfall when the soil was dry. Heiser *et al.* (2008) found that available concentration of imazethapyr decreased more rapidly in flooded soil as compared to corresponding non-flooded soils. The degradation rate of imazethapyr was found to increasewith increased soil moisture from -100 to -33 kPa (Goetz *et al.,*1990). It was more persistent in soil with the higher clay and organic matter content.Degradation of imazethapyr and imazaquin increased as soil moisture increased from 15 to 75% of FC (Flint and Witt, 1997). Saturated soil favored the dissipation of metolachlor and the formation of soil-bound residues as compared to unsaturated soil (Rice *et al.,* 2002). Joo *et al.* (2001) reported that degradation rate of imazamethabenz increased with increase in irrigation rate applied in wheat crop. Under irrigated conditions, atrazine at 1.2 kgha^{-1}, showed persistence only up to 1 year, but under dry condition it injured wheat and barley crops up to 2 years when applied at 1.5 kgha^{-1} (Moyer and Blackshaw, 1993).

Vischetti *et al.* (2002) reported that $T_{1/2}$ for imazethapyr increased more than two-fold, when incubated in soil held at 15 versus 75%FC. Yadav *et al.* (1997) concluded that after 120 days of metsulfuron application, persistence at low soil moisture was more than at high moisture level. Walker (1978) studied persistence of eight soil applied herbicides in sandy loam soils under controlled conditions and reported that moisture was a major factor for the degradation of metamitron and atrazine. Walker (1974) described a simulation model for prediction of persistence of napropamide as affected by soiltemperature and soil moisture and found that loss of activityfollowed first-order kinetics with half-lives of 54, 63, and90 days at 28^{O}C with soil moisture contents of 10, 7.5, and3.5%, respectively. Similarly, Walker (2006) developeda simulation model for simazine and linuron degradation and reported similar effect of moisture on their persistence.

Trifluralin is a selective, pre-sowing/pre-plant incorporated dinitroanaline herbicide used to control many annual grasses and broadleaf weeds in a large variety of arable and horticultural crops and inhibits root development through the interruption of mitosis whereas, imazethapyr, an acetolactate synthase (ALS) inhibiting herbicide is used mainly in soybean and other edible legumes for broad spectrum weed control. These herbicides have great potential of persistence under different conditions of soil and climate. Rahman (1977) reported that higher rates of trifluralin (2-4 kg ha^{-1}) caused residual toxicity on German millet; soil organic matter content had greater role on persistence than rainfall. In another study, residual toxicity of trifluralin on sorghum lasted longer under dry field conditions (Horowitz, et al. 1974). Sugar beet, turnip, oilseed rape and grain sorghum were found most sensitive to imazethapyr residues (Onofri, 1996). Intermediate tolerance was observed in corn to imazethapyr residues (Mallipudi *et al.,*1994), but significant sensitivity in some maize hybrids was reported by Jovanoviæ-Radovanov and Ibrahim (1994). Experiments conducted at research farm, CCSHaryanaAgriculturalUniversity, Hisar, India in clusterbean-mustard cropping system showed

persistence of imazethapyr in mustard crop (AICRPWC, 2007). In a similar experiment, Singh (2010) reported persistence of imazethapyr applied in clusterbean and its residues in subsequent mustard crop. Bioassay technique can help in deciding whether a potential problem exists in choosing the appropriate crop or variety for a particular herbicide. Screen house bioassays have been found reliable to predict field crop injuries to ALS-inhibitor herbicides residues. So, the present experiment was designed to study the effect of different moisture regimes on persistence of imazethapyr and trifluralin using bioassay techniques.

2. MATERIAL AND METHODS

Imazethapyr (Pursuit 10% SL**)** at0, 10, 20, 40, 80 and 160 g ha^{-1} and trifluralin (Treflan 48% EC**)** at 0, 0.125, 0.25, 0.5, 1.0 and 2.0 kg ha^{-1} rates were evaluated for their persistence mediated by different moisture levels.For both herbicides,four times of the highest experimentalrate i.e. 640 g ha^{-1}imazethapyr and 8.0 $kgha^{-1}$ trifluralin (for subsequent serial dilutions with soil) incubated to desired moisture levels using 8.0 kg field soil.The field soil was sandy loam (63.1% sand, 18% silt and 18.9%clay) in texture with a pH of 8.2. Chemical analysis showed it was low in carbon (0.41%) deficient in available N (102 kg ha^{-1}) and P (6 $kgha^{-1}$) and sufficient in K (252 kg ha^{-1}).

On weight basis, field capacity of the soil determined was 20%. Amount of water for maintaining the soil at moisture regimes of 50%, 75% and field capacity (FC) was calculated on this determination. Calculated herbicide was mixed in this amount of water to treat the soil. Plastic pots without holes at bottom were filled with treated soil and weighed. These pots were placed in a screen house at room temperature (40±5^{O}C) and the weight corresponding to each moisture regime was maintained for 30 days (d) by adding required amount of water. After 30 d the soil samples were put in a deep freezer, maintained at -5^{O}C to -10^{O}C to arrest all the reactions responsible for herbicide degradation.

At the start of winter season, treated soil samples were removedfrom the deep freezer and serially diluted with soil having soil: sand: vermi-compost: 3:1:1 ratio to get the desired concentration of herbicides. Twenty seeds each of mustard and oat (test plants for imazethapyr and trifluralin, respectively) were planted in each pot, replicated four times and arranged in a CRD design in a screen house. Pots were watered according to evapo-transpiration demand to avoid leaching of herbicides.

Observations were recorded for each test plant for shoot/root growth and fresh/dry weight 7 weeks after sowing (WAS). For the measurement of root length, each pot was saturated with water, soil was loosened and then plantswere rooted out gently under running tap with mild water pressure by ensuring no damage to the root system. Fresh weight per pot of rooted plants was measuredsoon after soil removal(soaking in tissue papers) and the plants of each pot were placed in paper bags for recording dry weight. The paper bags were first sun dried and then in an oven at 70^{O}C till constant weight was achieved. All bioassay studies were carried out in plastics pots (20 cm diameter) and repeated twice.

Similar trends were observed in both sets, hence data was pooled for analysisusing SPSS version 7.5. Data was subjected to arcsine and angular transformation for percent values and numbers, respectively. Significance of various treatments was tested by using F test. The significant difference among treatments was tested by calculating CD against 5% level of significance and One-Way ANOVA to separate the influence of moisture levels and dose rates. Histograms were prepared from analyzed data and curve fitting was done on the basis of "best fit" approach.

3. RESULTS AND DISCUSSION

3.1. Effect of Different Moisture Regime on Imazethapyr Persistence

All the four growth parameters of mustard were significantly influenced by different imazethapyr application rates, when averaged over different moisture incubations. Maximum root length of mustard was recorded with imazethapyr incubated at 75% FC and minimum at FC which was reduced by 24% compared to 75% FC,when data was averaged over different imazethapyr rates (Fig. 1a) and regressed by polynomial model {FC ($y = 1.97x^2 - 18.43x + 41.79$, $R^2 = 0.871$), 75% FC ($y = 1.670x^2 - 15.84x + 39.55$, $R^2 = 0.844$) and 50% FC ($y = 1.929x^2 - 17.95x + 41.33$, $R^2 = 0.861$)}. Imazethapyr 160 gha^{-1} incubated at 75% FC reduced root length significantly which was statistically similar to 40 gha^{-1} incubated at FC and 40 and 80 gha^{-1} incubated at 50% FC indicating that there was 4 and 2-4 times more persistence at FC and 50% FC compared to 75% FC (Fig. 1a).

Similarly, shoot lengthwas reduced by 8 and 26% at 50% FC and FC, respectively over 75% FC, when data averaged over imazethapyr rates indicating maximum degradation at 75% FC and minimum at field capacity (Fig.1b) as represented by exponential model {($y = 65.46e^{-0.68x}$, $R^2 = 0.976$ for FC), ($y = 70.63e^{-0.54x}$, $R^2 = 0.990$ for 75% FC) and ($y = 81.54e^{-0.65x}$, $R^2 = 0.981$ for 50% FC)}. Shoot length recorded with imazethapyr 40 g ha^{-1} incubated at FC was statistically similar to 80 gha^{-1} incubated at 75 or 50% FC showing two times more persistence of imazethapyr at FC than lower moisture incubations (Fig.1b).

Maximum reduction in fresh weight of root and shootwas recorded at FC (25%) followed by 50% FC (8%) compared to 75% FC, which recorded highest fresh weight, data averaged over different imazethapyr rates and regressed by polynomial model {($y = 2.718x^2 - 27.09x + 66.56$, $R^2 = 0.976$ for FC), ($y = 2.010x^2 - 22.38x + 65.19$, $R^2 = 0.966$ for 75% FC) and ($y = 2.373x^2 - 25.15x + 67.35$, $R^2 = 0.991$ for 50% FC)}(Fig. 2a). Imazethapyr 80 gha^{-1} incubated at 75% FC had the similar reduction in fresh weight to that of 20 and 40 gha^{-1} imazethapyr incubated at FC and 40 gha^{-1} at 50% FC suggesting 2-4 and 2 times more persistence of imazethapyr at FC and 50% FC compared to 75% FC (Fig. 2a).

Similar to fresh weight, highest dry matter of root and shoot recorded in pots with imazethapyr incubated at 75% FC, whereas lowest dry weight was recorded with FC which varied significantly with 50% FC, when data averaged over different imazethapyr rates (Fig. 2b) and regressed by logarithmic model {FC ($y = 0.263x^2 - 3.488x + 11.42$, $R^2 = 0.952$), 75% FC ($y = 0.191x^2 - 2.835x + 10.89$, $R^2 = 0.971$) and FC ($y = 0.364x^2 - 4.256x + 12.27$, $R^2 = 0.933$)}. Imazethapyr 10 gha^{-1} had similar reduction in dry weight at three moisture regimes, but increasing the rates further resulted in significant variations. There was complete mortality of mustard plants in pots treated with 160 gha^{-1} imazethapyr; hence no dry matter was recorded at FC and 50% FC moisture incubation. Reduction in dry weight of root and shoot at 80 gha^{-1}imazethapyr incubated at FC was more than its 160 gha^{-1}dose incubated at 75% FC (Fig. 2b).

Light plays an important role in the degradation of imazethapyr (Anonymous, 2007). But, in the present experiment effect of light can be ruled out because treated soil was packed in polythene bags and placed in shade. Aerobic microbial degradation is the major pathway for imazethapyr, but no degradation occurs under anaerobic conditions (Anonymous, 2007). Cantwell *et al.* (1989) reported that microbial degradation of imidazolinones was regulated by the amount of herbicide in soil solution and soil characteristics.Microbial degradation in soil depends upon the herbicide chemical structure, availability of herbicide to the microorganisms or enzyme systems that degrade them, and the quantity

and activity level of the microorganism or enzyme system (Anderson, 1984; Hance, 1973; Wagner, 1975; Wolt *et al.,* 1989). These variables are regulated by water content, temperature, oxygen content, pH and nutrient status of soil (Anderson, 1984; Hance, 1973; Morill *et al.,* 1982; Walker, 1987). All these parameters were similar in the present study with imazethapyr except moisture.

Soil water has been found to influence the activity and number of microorganisms surviving in soil which can alter the rate of herbicide degradation (Anderson, 1981, 1984; Hamaker and Goring, 1976). Rapid degradation of imazaquin was observed under hot and dry field conditions, whereas dissipation decreased under irrigated conditions (Basham *et al.,* 1987). Herbicide concentration in soil solution increases as the ratio of water to soil colloids increases (Anderson, 1984; Goetz *et al.,* 1986; Wolt *et al.,*1989). Also, herbicide availability for degradation is determined by relative rate of adsorption and desorption from colloids (Anderson, 1981; Hamaker and Goring, 1976; Morill *et al.,* 1982).

Significant effect of rainfall on imazethapyr activity in three soils was observed by Ayeni *et al.* (1998). Higher rates and post-emergence application has been found to provoke slow degradation of imazethapyr compared to its pre-emergence application and lower rates (Jovanoviæ-Radovanov and Ibrahim, 1994). They reported that carryover effect of imazethapyr was more prominent on root length and fresh weight of sensitive maize hybrids which also corroborates our results on mustard (Fig. 1& 2). Pre-plant incorporation of imazethapyr in clusterbean or sesame was less injurious to mustard planted after clusterbean harvest compared to its pre-emergence or early post applications (Anonymous 2011)

Moisture incubation at FC resulted in maximum mustard injury due to persistence of imazethapyr as showed by different growth parameters. This could be due to more anaerobic conditions because it degrades faster at aerobic conditions (Anonymous, 2007). Lower soil moisture due to delayed irrigation for 2 wk resulted in reduced bioactivity of imazethapyr (Ayeni *et al.,* 1998). In the present study, degradation at 50% FC was also slow compared to 75% FC because of less moisture which is necessary for the activity of microorganisms which are responsible for imazethapyr degradation (Anderson, 1981, 1984; Hamaker and Goring, 1976). Maximum degradation was at 75% FC due to moist aerobic conditions which were optimum for imazethapyr degradation.

3.2. Effect of Different Moisture Regimes on TrifluralinPersistence

Shoot and root length, fresh and dry weight decreased significantly with increase in trifluralin application rates, when averaged over moisture incubations. Maximum plant height was recorded when trifluralin was incubated at FC which varied significantly to 75 % FC and 50% FC moisture incubation (Fig. 3a) and was best described by regression equation {($y = -0.040x^2 - 8.985x + 66.72$, $R^2 = 0.972$ for 50% FC), ($y = 0.331x^2 - 10.45x + 68.27$, $R^2 = 0.971$ for 75% FC) and ($y = 0.454x^2 - 9.925x + 67.48$, $R^2 = 0.963$ for FC)}.Shoot length averaged over different trifluralin rates showed minimum persistence at FC moisture incubation and increased with decrease in moisture incubation levels. At 75% FC incubation of trifluralin 1.0 kg ha^{-1}, shoot length inhibition was similar to 2.0 kgha^{-1} trifluralin incubated at FC indicating 2 times more degradation at FC incubation than 75% FC (Fig. 3a).

Mean root length of oat was maximum at FC incubation of trifluralin which was significantly higher than 75 and 50% FC, when data was averaged over different herbicide rates supporting the observations of shoot length and data regressed to the exponential model {($y = 26.09e^{-0.46x}$, $R^2 = 0.807$ for 50% FC), ($y = 24.90e^{-0.39x}$, $R^2 = 0.801$ for 75% FC) and ($y = 25.72e^{-0.36x}$, $R^2 = 0.798$ for FC)} (Fig.

3b). Root length recorded in pots treated with trifluralin 1.0 kgha^{-1}incubated at FC was statistically similar to 0.25 and 0.5 kgha^{-1} incubated at 50% FC and 0.5 kgha^{-1}incubated at 75% FC which showed that trifluralin degradation was 2-4 time less at 50% FC and 2 times less at 75% FC compared to FC (Fig. 3b).Effect of trifluralin was more pronounced on root than shoot at all moisture regimes (Fig. 3 and 4). The soil applied trifluralin is not mobile within the plant. Therefore, primary injury symptoms are mostly confined to the site of uptake i.e. roots.

Fresh weight at different moisture regimes when averaged over different trifluralin rates was highest at FC and lowest with 50% FC which were statistically significant with the fresh weight recorded at 75% FC (Fig. 4a) indicating increase in degradation of trifluralin with increase in moisture level. Data regressed to the logarithmic model {($y = -35.4\ln(x) + 76.40$, $R^2 = 0.947$ for 50% FC), ($y = -30.5\ln(x) + 80.08$, $R^2 = 0.958$ for 75% FC) and ($y = -22.6\ln(x) + 80.32$, $R^2 = 0.891$for FC)} also support the above results. Trifluralin 0.25 and 0.5 kg ha^{-1} incubated at 75% FC and 2.0 kg ha^{-1} incubated at FC showed non-significant effect on fresh weight demonstrates that there was 4-8 times more persistence of trifluralin at 75% FC compared to FC moisture incubation (Fig. 4a).

Similar results were recorded in case of dry matter also(Fig. 4b). Effect of trifluralin incubated at different moisture regimes on dry weight of oat was regressed by logistic model {50% FC ($y = -11.0\ln(x) + 21.37$, $R^2 = 0.918$), 75% FC ($y = -10.1\ln(x) + 20.95$, $R^2 = 0.896$) and FC ($y = -8.32\ln(x) + 21.59$, $R^2 = 0.835$)}.

Soil moisture, temperature, soil type, organic carbon content and length of time before incorporation takes place are the several factors which determine the persistence of trifluralin (Horowitz *et al.,* 1974; Kennedy and Talbert, 1977; Probst *et al*., 1967; Savage, 1978). Leaching losses of trifluralin can be ruled out because it is strongly adsorbed to clay and organic colloids (Anonymous, 2007). Although, photodecomposition is major pathway for trifluralin degradation (Grover *et al.,* 1997), but in the present study, it can be overruled because the herbicide was well mixed with soil under lab conditions, keeping away from direct sunlight and was incubated under dark conditions in a deep freezer. Chemical degradation in trifluralin has not been reported (Anonymous, 2007) hence, biological pathways can be responsible for its degradation. Increased persistence of trifluralin in sterilized compared to unsterilized soil indicates the role of microorganisms in trifluralin degradation (Pahwa and Bajaj, 1997). Aerobic microorganisms have little role as compared to anaerobes (Anonymous, 2007). Under aerobic condition primarily N-dealkylation, hydroxylation and oxidation is major pathway, whereas under anaerobic conditions, nitro reduction and N-dealkylation are major degradation pathways.

Wheeler *et al.* (1979) reported that "bound" trifluralin on soil colloids increased with time. Herbicide availability for degradation is determined by relative rate of adsorption and desorption from colloids (Anderson, 1981; Hamaker and Goring, 1976; Morill *et al.,* 1982). Sometimes an adsorption process results in chemical change of adsorbed material. The changes are of such nature that desorption is inhibited and the process is called pseudo-adsorption (Tam, 1998). So, adsorption from soil solution to soil colloids and biological reactions could be the main pathways for degradation of trifluralin. Moisture is the main factor influencing adsorption reactions in soils thus, affect the bioavailability of trifluralin. Also it controls the activity of microorganisms, hence can control the degradation of trifluralin. Savage (1978) reported that flooding the soil significantly increased the dissipation rate of trifluralin.

Different growth parameters of oat revealed that with increase in soil moisture there was increase in degradation rate of trifluralin with minimum at

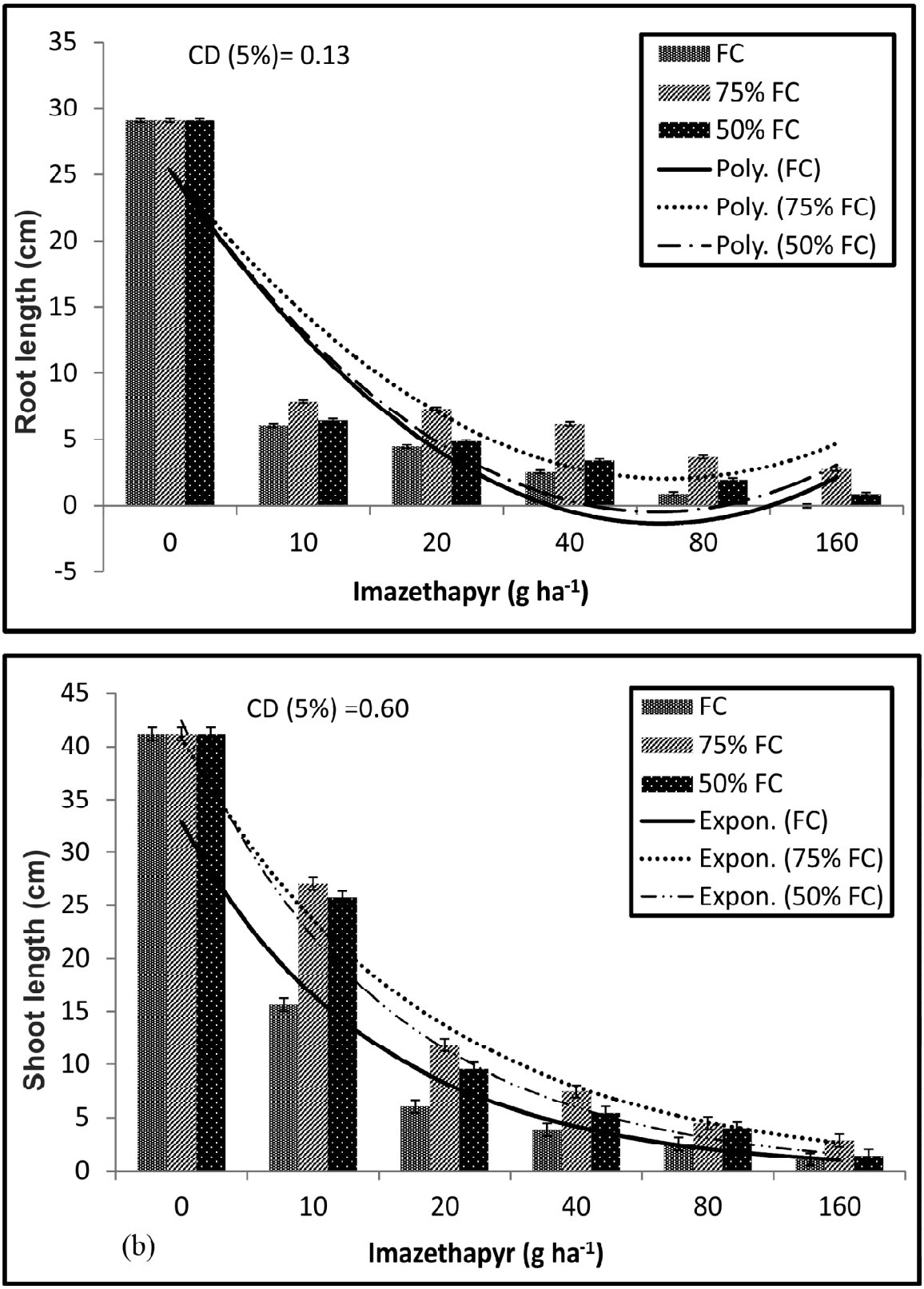
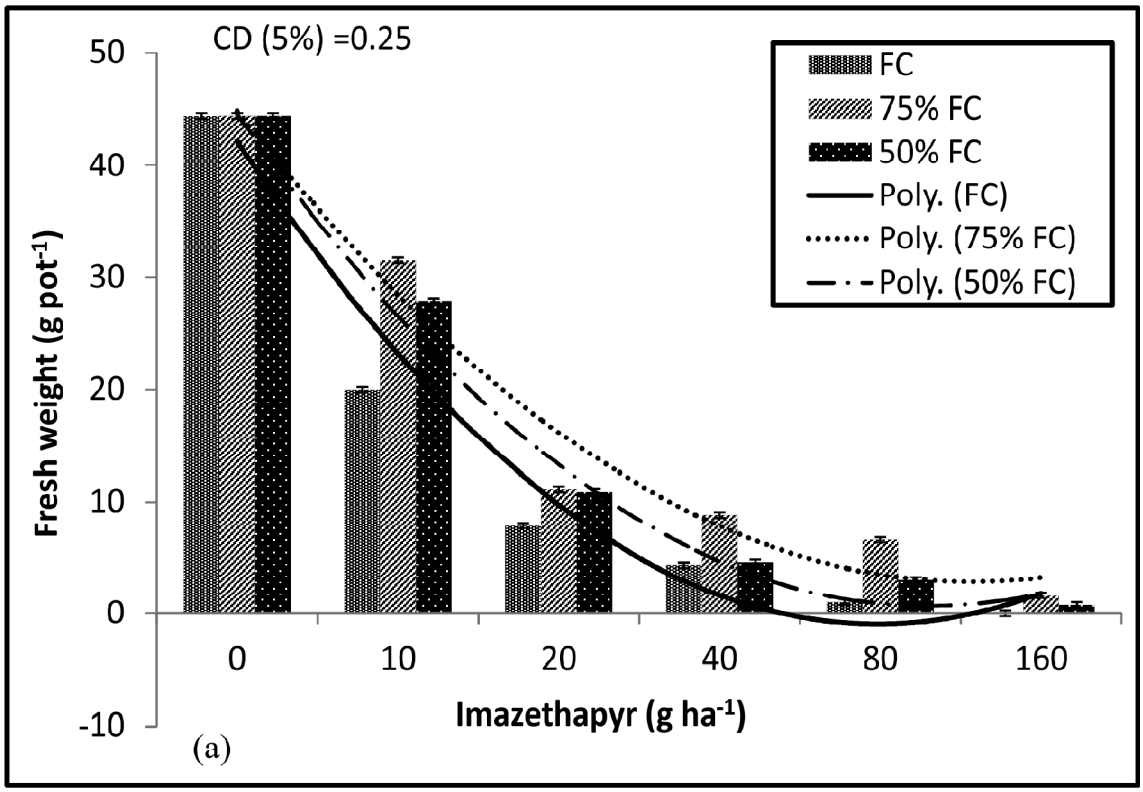


Figure 1: Effect of imazethapyr incubated at different moisture regimes on shoot and root length (cm) of mustard, 7 WAS



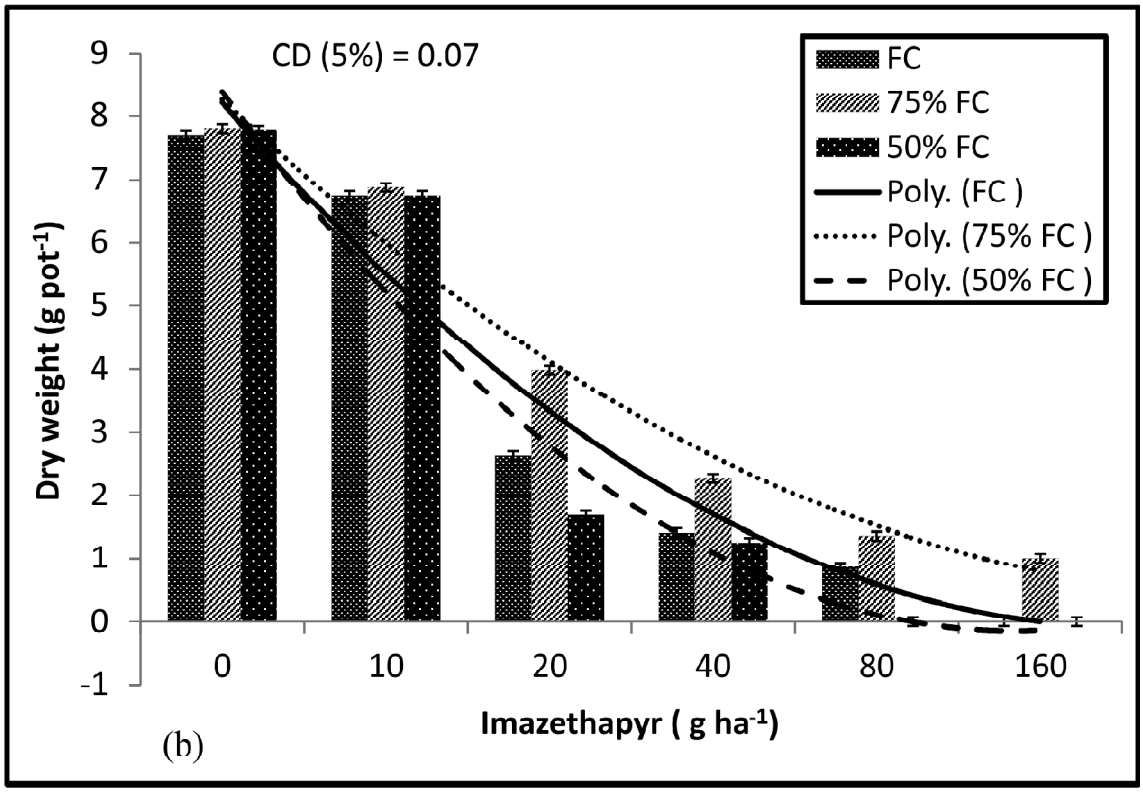
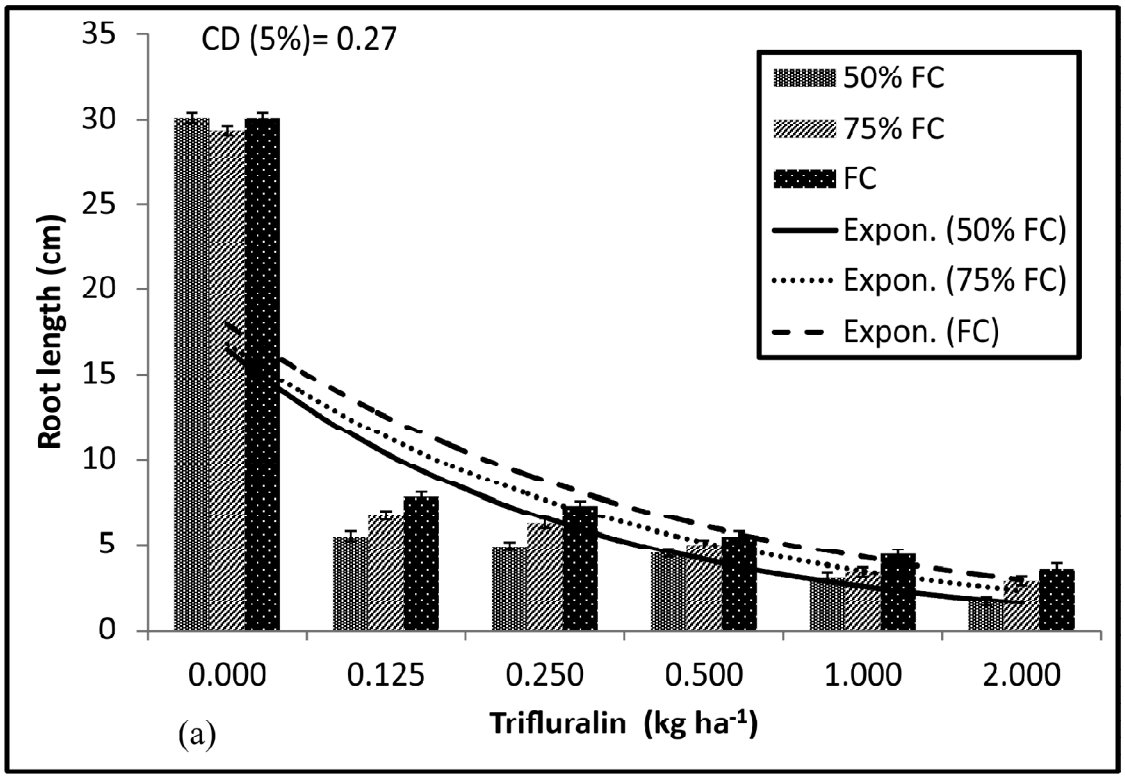


Figure 2: Effect of imazethapyr incubated at different moisture regimes on fresh and dry weight (g/pot) of mustard, 7 WAS



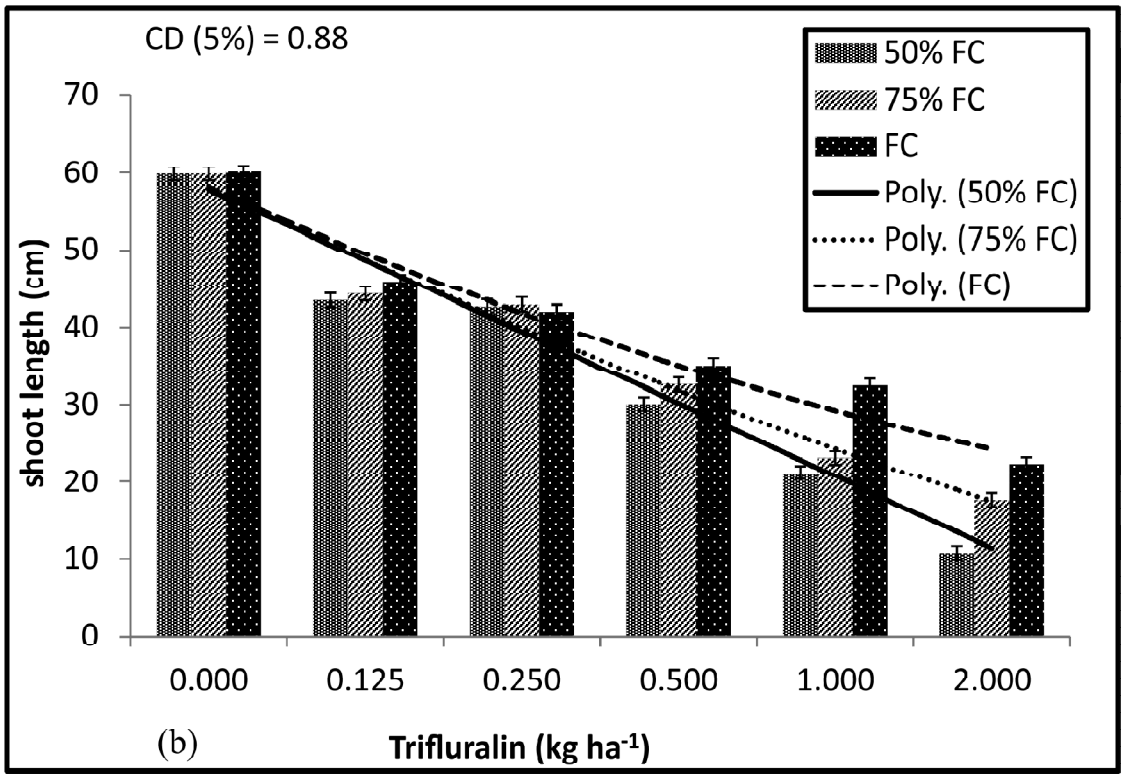


Figure 3: Effect of trifluralin incubated at different moisture regimes on shoot and root length (cm) of oat,7 WAS

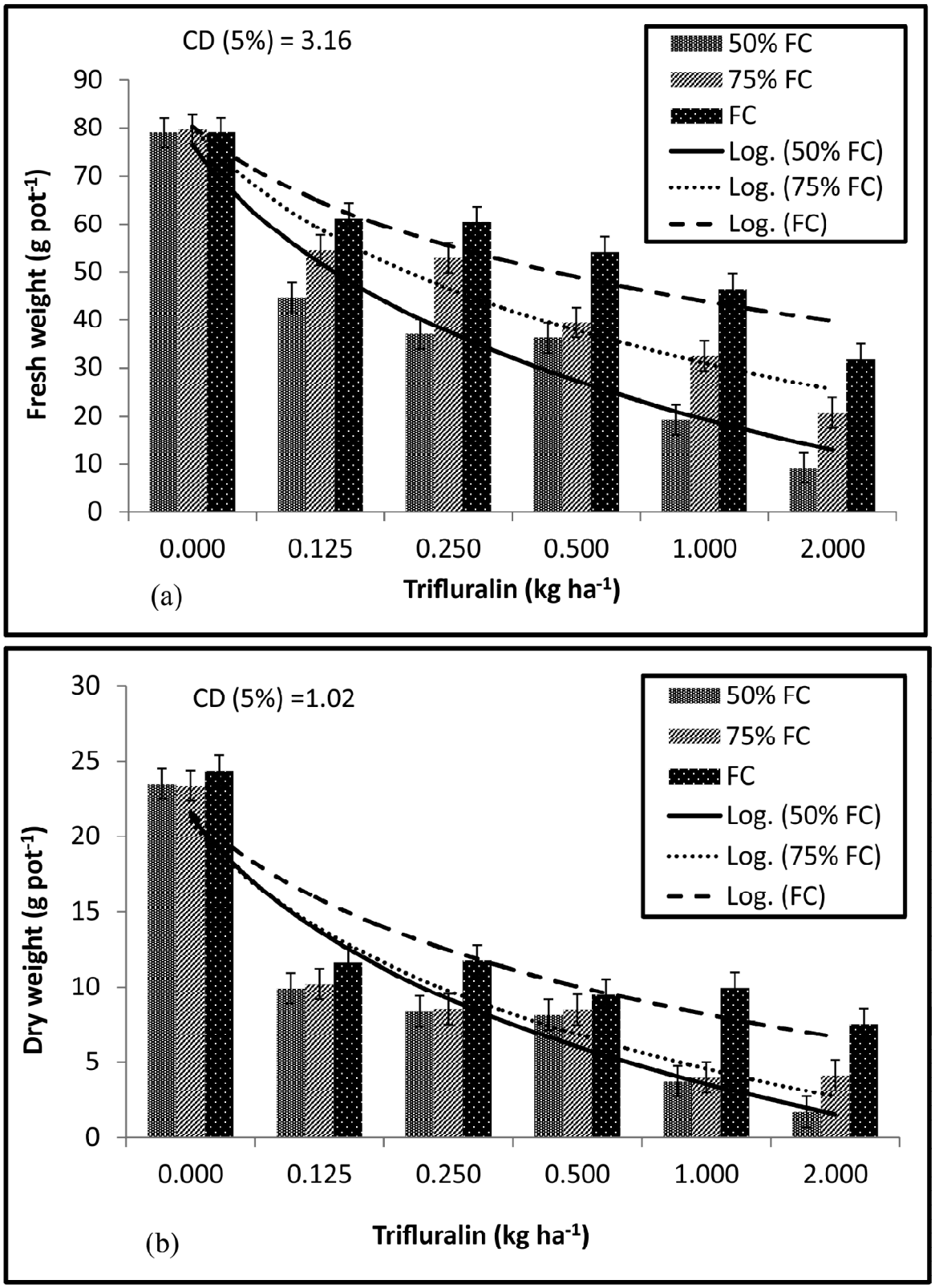


Figure 4: Effect of trifluralin incubated at different moisture regimes on fresh and dry weight (g/pot) of oat,7 WAS

50% FC and maximum at FC moisture level. With increase in soil moisture the activity of microorganisms responsible for trifluralin degradation might have increased thus increased rate of trifluralin dissipation. Chemical structure of herbicide determines the degree to which it is partitioned between soil solid and water solution (Anderson, 1984; Wolt *et al.,*1989), and the concentration of herbicide in soil solution increases as the ratio of water to soil colloids increases (Anderson, 1984; Goetz *et al.,* 1986; Wolt *et al.,*1989). Also, adsorption reaction could be positively influenced by soil moisture, hence decreased bioavailability of trifluralin with increase in moisture content.

Similarly, extent of trifluralin degradation was positively correlated with soil moisture content (Zimdahl and Gwynn, 1977; Solbakken *et al.,* 1982). Zimdahl and Clark (1982) reported similar role of moisture in degradation rate of alachlor. Jolley and Johnstone (1994) reported higher degradation of trifluralin under moist conditions and increased persistence due to decrease in soil moisture. Saturated soil favors dissipation of metolachlor and the formation of soil-bound residues compared to unsaturated soil (Rice *et al.,* 2002). Under irrigated conditions, atrazine showed less persistence than under dry conditions (Moyer and Blackshaw, 1993).

Root length of oat was severely affected by trifluralin incubation compared to shoot growth. Singh *et al.,* (1994) reported that root length and root weight of sorghum had greater reduction over shoot length and shoot weight due to residual effect of pendimethalin which persisted up to 240 days at 4 ppm concentration. Present results also corroborated those of Chauhan *et al.* (2006) who found that oat roots were more sensitive to trifluralin inhibition than oat shoots specifically with lower variability. Increase in moisture level (FC) resulted in lower persistence of trifluralin compared to lower soil moisture contents (75 and 50% FC). This information can be of great help in planning the succeeding crops where trifluralin has been applied and the residual effect can also be lowered by increasing soil moisture. On the other hand, imazethapyr degradation was maximum at 75% FC compared to field capacity and need a different approach to minimize residual effect on succeeding sensitive crops.

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