

# Efficacy of rice-stubble allelochemicals on vegetative growth parameters of some oil-yielding crops

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## ABSTRACT

A laboratory experiment was conducted to investigate allelopathic potential of water extract of rice-stubble leachate on certain oil-yielding crops (mustard, sesamum and sunflower). A wide range of rice-stubble concentration (i.e. 5, 10, 15, 20, 25, 30 and 40) were prepared and used for testing of seedling growth parameters (Length, fresh and dry weight of shoot and root) of oil-yielding test crop seedlings. The experimental results found that the different seedling growth parameters were reduced with increased concentration of rice-stubble leachate in compared to control set. Root growth was more sensitive than shoot growth with increase of leachate concentration of rice-stubble. This experimental results show the existence of several bioactive compounds (i.e. allelochemicals) in rice-stubble. These bioactive compounds might serve as a source of natural herbicides.

**KEYWORDS:** Rice-stubble, seedling growth, leachate, allelopathic, bioactive compounds.

## INTRODUCTION

Allelopathy has been known for many years but it has only recently been accepted scientifically as a legitimate area of research. Until recently allelopathy has been thought of as mysticism and believes rather than science based on knowledge. Allelopathy may often be the most reasonable explanation for observations even in the light of legitimate criticism when researchers fail to isolate and identify causal agents. Though there are various definitions for allelopathy "The international Allelopathy society (IAS) suggested the following definitions for allelopathy to include in the IAS constitution. "Allelopathy may be defined (a) any process involves secondary metabolites produced by plants and micro-organism that influence the growth and development of agricultural and biological systems and (b) a study of the function of secondary metabolites, their significance in biological organization, their evolutionary origin and evolution of the mechanism involving plant - plant, plant micro-organisms, plant viruses, plant insects etc. which need further comments (Waller, 1998).

Recent research efforts have made it possible to use allelopathy for increasing crop production, worth quality food, reduce reliance on synthetics pesticides and improve the ecological environment (An, *et. al.* 1997). The current trends in agriculture production are to find a biological solution to reduce the apparent harmful impacts from herbicides and pesticides (Khanh, *et. al.* 2005). Plant allelopathy offers a great prospective to resolve this critical issue and may be used in different ways to manage weeds that include, the use of allelopathic crop residues as surface mulch (Jung *et. al.* 2004) and water extracts (Cheema *et. al.* 2013). Allelochemicals involve in autotoxicity of perennial crops, mulch stubble, crop rotations and direct interference by certain plants or weeds and thus cause replanting problem (Putnam and Weston, 1986; Weston, 1996). This problem of autotoxicity may be overcome through proper crops rotation that results in minimum crop suppression over time (Putnam and Weston, 1986; Weston and Duke, 2003).

In recent studies, intercropping that provides better weed suppression through resource competition/or allelochemical exudation into rhizosphere has been addressed as an option for integrated weed management (Iqbal *et. al.* 2007). Rice (*Oryza sativa* L.) is an allelopathic plant and many studies evaluate that rice cultivars act as a means of ecological weed control strategy (Dilday *et. al.* 1994 and Ma *et. al.* 2006). These scientists explored the allelopathic potential of rice varieties by evaluating leaf and straw extracts, decomposing straw and soil where rice was planted.

The existence of phytotoxins (coumaric acids, ferulic acid and vanillic acids) in the aqueous extracts of decomposing rice residues was first reported by Chou and Lin (1976). The radical growth of lettuce and coleoptiles of rice seeds, rice seedling growth and root initiation from hypocotyls cuttings of mung bean was inhibited by the presence of higher amounts of phytotoxic plant phenolics (p-hydroxybenzoic acid, p-coumaric acid, vanillic acid, ferulic and o-hydroxyphenyl acetic acids) in the water- extracts of decomposing rice residues in soil (Rimando and Duke, 2003). Chou *et. al.* (1981) evaluated that aqueous extract from soil and rice straw mixture was found to be more than twice phytotoxic compared with vanillic, p-coumaric, syringic and ferulic acids obtained from both the aqueous extract and residues incubated soil samples.

Rice plants possibly release allelochemicals into the neighboring environment and thus inhibit the growth of several plant species growing in their vicinity, Rice root exudates and decomposing rice residues contain a large number of putative allelochemicals compounds, such as phenolic acids, fatty acid, indoles and terpenes (Rimando and Duke, 2003). Water extracts from rice straw inhibited the germination and seedling growth of Chinese milk vetch (*Astragalus sinicus*) (Paramanik *et. al.*, 2001) and the vetch population density decreased when sown after the cultivation of rice (Nakano and Hari, 2000). Water soluble husk extracts inhibited the germination of barnyard grass more than leaf-extract (Chung *et. al.* 1997). Similarly in another study Ko *et. al.* (2005) determined that rice husk

showed the high allelopathic effect on barnyard grass and organic compounds found in husk were 9-octadecenoic acid, 7-octadecenoic acid; 5,8,11-heptadecatriynoic acid and androstan-17-one. Weed biomass was negatively correlated with rice root growth at early growth stages and with rice shoot and root growth at later growth stages (Fofana and Rayber, 2000). Water extracts of shoots and roots of rice varieties significantly inhibited root growth in *E. crus-galli* but the shoot extract gave a greater inhibitory effect on *E. crus-galli* than the root extract (Kim *et. al.* 2005). Rice residues of several varieties released similar concentrations and types of allelochemicals to inhibit successive plants (Kong *et. al.* 2006).

Though a lot of work has been carried out on allelopathic effect of rice residue (stubble, straw and husk), identification of an array of allelochemicals present there in and impact on different crops, weeds and soil characteristics, no literature are available on allelopathic effect of rice-stubble on oil-yielding crops which play vital role for human population on various ways. Hence, the present investigation is aimed to find out the allelopathic effect of rice-stubble on seedling growth parameters (Seedling length, fresh and dry weight) of three most popular oil-yielding crops viz., mustard, sesame and sunflower popularly cultivated by local cultivators for their economic, nutritive and pharmaceutical values, which will throw a beam of light on the path of agricultural as well as scientific researches for management of crop productivity.

## MATERIAL AND METHODS

In the present piece of investigation, three types of pure line seeds of oil-yielding crops namely on mustard, sesame and sunflower were used to study the effect of water-soluble allelochemicals leached out from the rice stubble of *Oryza sativa L.* plant on seed germination  $\alpha$ -amylase and protease activities were studied by pot-culture methods. The details of materials used and methods followed are described below.

The stubble of rice (*Oryza Sativa L. cv* Shamb Mashuri) were collected from fields soon after cutting of the crop, washed thoroughly with tap water to remove adhering soil particles, soaked on blotting paper, dried in shade and were chopped into pieces separately. Then 200 gm of such chopped materials were allowed to leach for 72 hr in 1 litre of distilled water at  $30 \pm 2^\circ\text{C}$  as per the method adopted by Padhy *et al.* (2002). The leachates were filtered through 2 layer gauge cloth and then watman No. 1 filter paper were considered as 20 % concentration and different diluted leachates (5, 10, 15, 20 and 25 %) with distilled water were prepared and used for seed germination studies.

Pure-line seeds of three test cultivars of oil-yielding (mustard, sesamum and sunflower) crops were procured from Regional Agricultural Research station of Odisha University of Agriculture and Technology (OUAT), Berhampur located at Ratnapur village, side of NH-59, near Berhampur, Dist.- Ganjam, Odisha. In order to study the percentage of seed germination of influenced by different concentration of leachate, visually selected seeds of test crop was surface sterilized with 0.03 % formalin solution for 10 minutes separately and then washed thoroughly with distilled water. The surface-sterilized seeds were allowed to germinate in plastic trays (3 x 9 x 12 cm size) at the rate of 20 seeds per tray containing equal volume of sterilized sand wetted with equal volume of 5, 10, 15, 20 and 25 % concentrations of leachate. The seeds were placed 0.5 cm

below from the top sand level. Trays with equal number of seed of test cultivar placed in sand, wetted with distilled water, equal to the volume of different leachate, were served as control set. For accuracy of the experiments, the trays of both treated and control sets were divided into five replicates with 3 trays in each set for each type of leachate. All the trays of both treated and control sets containing seeds were kept in a B.O.D. incubator maintaining  $30 \pm 1^\circ\text{C}$  for germination. To maintain the wetness of the sand care was taken to add distilled water and leachate as per experimental schedule. Appearance of sprouts from the seeds was considered as the criteria of germination.

All the trays of control and treated sets containing germinated seeds were transferred into the seedling growth chamber maintained at  $30 \pm 2^\circ\text{C}$  provided 12 hr photoperiod per day with illumination of  $2 \pm 0.8$  Klux light intensity from two florescent electric tube lights from top of the seedlings. The seedlings were provided with equal volume of respective test leachates and distilled water as per the experimental design at an interval of 24 hours. The seedling growth parameters such as shoot and root lengths and their fresh and dry weights were recorded at an interval of 5 days from 5 days after sowing (DAS) till 20 DAS in mustard and sesame respectively whereas in case of sunflower it was 7 days after sowing (DAS) till 22 DAS.

The shoot and root lengths of the seedlings were measured in cms, separately by collecting 10 seedlings at random from each tray on 5, 10, 15 and 20 DAS in mustard and sesame respectively but in case of sunflower it was 7, 12, 17 and 22 DAS and fresh weights of both shoot and root were taken separately excluding the cotyledons. Then the weighted shoot and root materials were kept in an oven maintained at  $50 \pm 2^\circ\text{C}$  provided  $2^\circ\text{C}$  for 48 hours and thereafter their dry weights were measured and expressed in mgs.

## SEEDLING GROWTH

After seed germination, establishment of seedling and their growth are considered as important parameters and play a vital role in the productivity of concerned crop. Changes in seedling growth parameters such as shoot and root lengths, fresh and dry weight of both shoot and roots influenced by different concentrations of shoot and root leachate of rice stubble on three test oil-yielding cultivars (mustard, sesame and sunflower) are described below:

### Seedling length

Seedling lengths, generally include the length of both shoot and root, are considered as important parameter as those are directly and indirectly influenced by both internal genetic factors and external environmental factors. Changes in seedling growth parameters shoot and root lengths influence by different concentration of shoot and root leachate of rice-stubble are described below.

### Impact of shoot leachate on shoot length of seedlings of test cultivars

It can be marked that shoot leachate exhibited adverse effect on seedling length of all oil-yielding cultivars studied. Minimum shoot lengths of  $2.6 \pm 0.03$  and  $2.0 \pm 0.3$  were recorded in seedling influenced by 30% leachate concentration on 20 day after sowing (DAS) whereas maximum shoot lengths of  $14.6 \pm 0.12$  and  $14.2 \pm 0.12$  cm in seedling of control sets of mustard and sesame respectively. In case of sunflower, maximum shoot length of  $15.6 \pm 0.12$  and minimum length of  $3.8 \pm 0.04$  cm were recorded on 22

DAS in seedling of control set and influenced by 30% leachate respectively. The germinated seeds could not establish into seedlings by the influence of 30% concentration of shoot leachate upto 10 day after sowing (DAS) in mustard and sesame cultivars whereas in case of sunflower it was 12 DAS. Shoot lengths of intermediate values between the above mentioned data were noticed in

seedlings of control and treated sets at different developmental stages. The shoot lengths of all test cultivars exhibited positive correlations with increase in the growth period and negative correlations with increase in the concentration of leachate throughout the period of observation [Fig.-3.3 (a-c)].

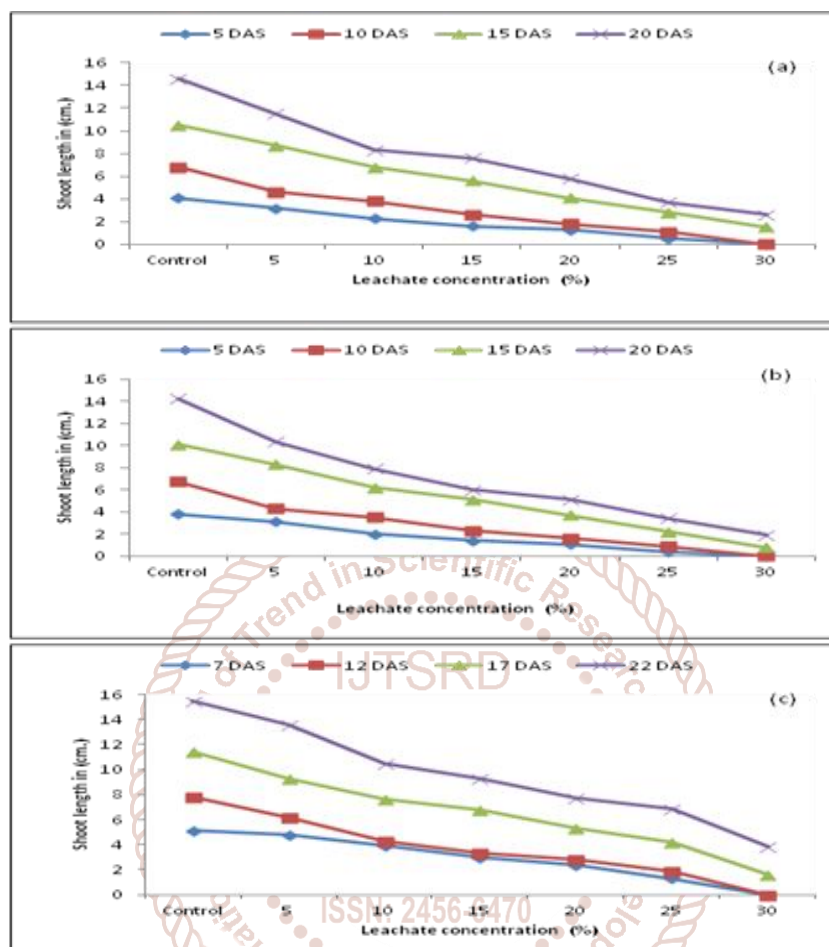


Fig.-3.3: Effect of different concentrations of aqueous leachate of rice stubble (shoot) on shoot length of (a) Mustard (*Brassica junica* L.), (b) Sesame (*Sesamum indicum* L.) and (c) Sunflower (*Helianthus annuus* L.) seedlings.

#### Impact of root leachate on shoot length of seedlings of test cultivars

All the concentrations of root leachate caused significant inhibition in seedling growth resulting decrease in shoot lengths of all test cultivars. Maximum shoot lengths of  $14.2 \pm 0.08$  and  $14.0 \pm 0.04$  cm were recorded in seedling of control set on 20 DAS whereas during the same period of growth, these were  $2.4 \pm 0.03$  and  $2.0 \pm 0.01$  cm in seedlings of mustard and sesame influenced by 25% leachate respectively. In case of sunflower seedlings, maximum shoot length of  $15.4 \pm 0.11$  and minimum length of  $3.0 \pm 0.05$  cm were observed in seedlings of control set and influenced by 25% leachate on 22 DAS respectively. No shoot growth was noticed in seeds treated with 30% root leachate as no seedlings were established in all test cultivars. Other concentrations exhibited, intermediate values. Like shoot leachate, the root leachate also exhibited positive correlations between shoot length of seedling with increase in growth period and negative correlations with increase in concentration of leachate throughout the period of observation. (Fig.-3.4(a-c)).

#### Impact of shoot leachate on root length of seedlings of test cultivars

The different concentrations of shoot leachate of rice-stubble considerably checked the root length of mustard, sesame and sunflower. In control set the seedlings exhibited root length of  $5.8 \pm 0.06$  and  $5.6 \pm 0.08$  cm on 20 days in mustard and sesame respectively. Whereas in sunflower the maximum and minimum values were  $6.8 \pm 0.08$  and  $2.2 \pm 0.02$  cm respectively on 22 DAS in seedling of control set and influenced by 30% leachate. However at concentration 30% seedling growth were not established on 5 DAS and 10 DAS in mustard and sesame respectively but after 15 DAS and 20 DAS their root length were  $0.6 \pm 0.01$  and  $0.08 \pm 0.02$  in mustard and  $0.4 \pm 0.01$  and  $0.6 \pm 0.01$  in sesame respectively. In case of sunflower, no seedlings were established upto 12 DAS and thereafter the root lengths were  $1.6 \pm 0.02$  and  $2.2 \pm 0.02$  on 17 and 22 DAS respectively. Seedlings grown in other concentration exhibited intermediate values. From Fig.-3.5 (a-c) it can be clearly marked the existence of positive correlations, between increase in the growth period and shoot length and negative correlation between increase in the shoot leachate concentrations and root length in all the test cultivars throughout the period of observation.

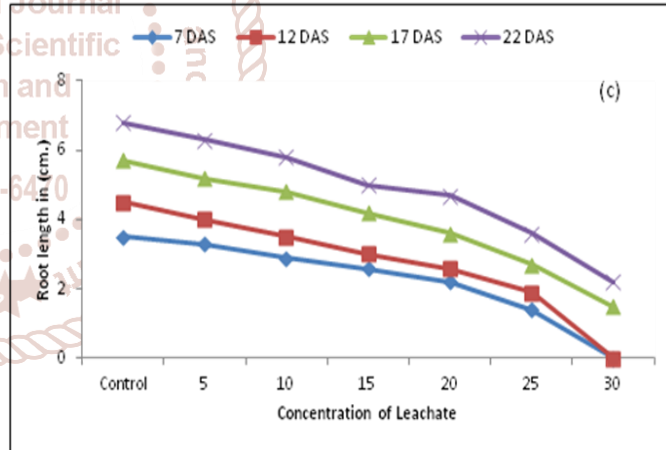
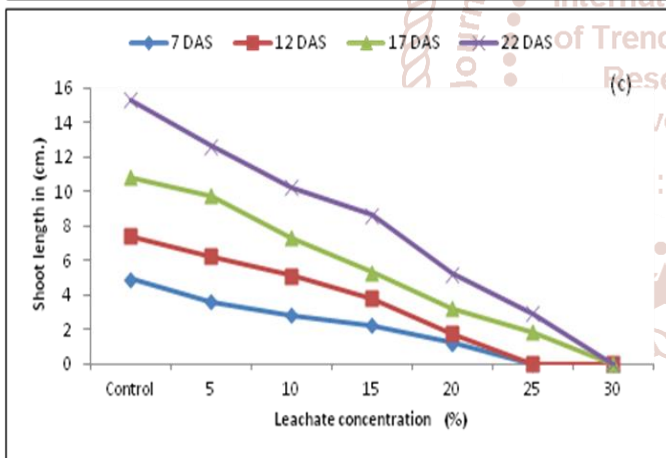
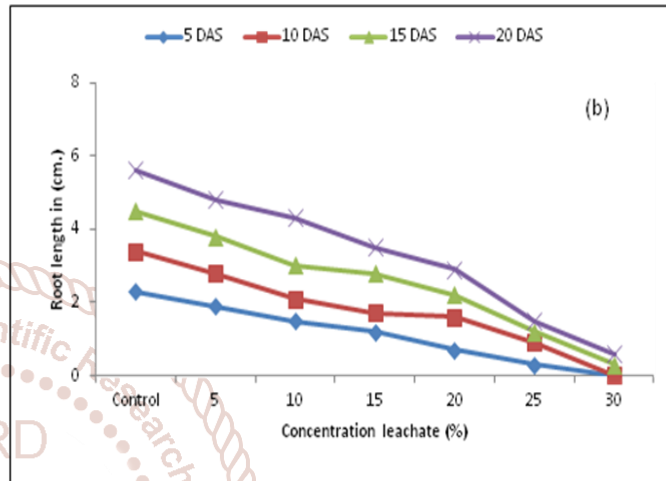
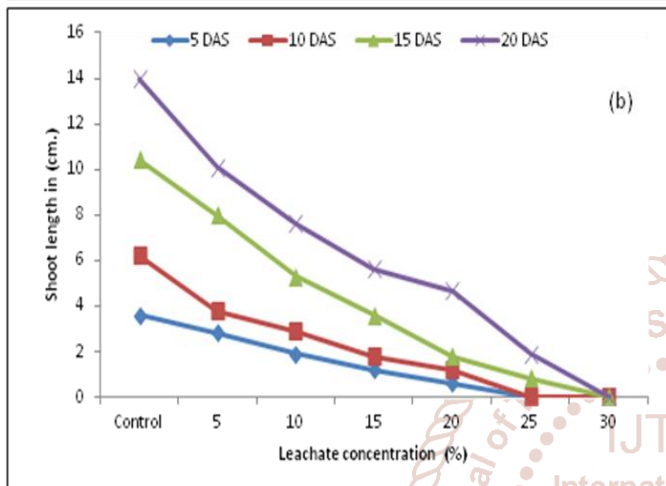
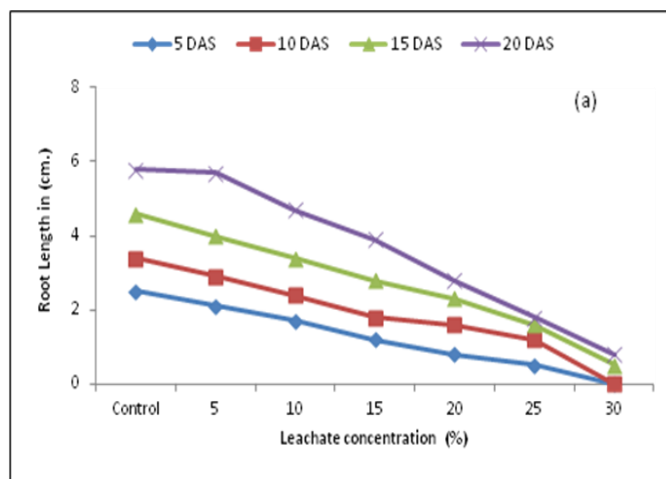
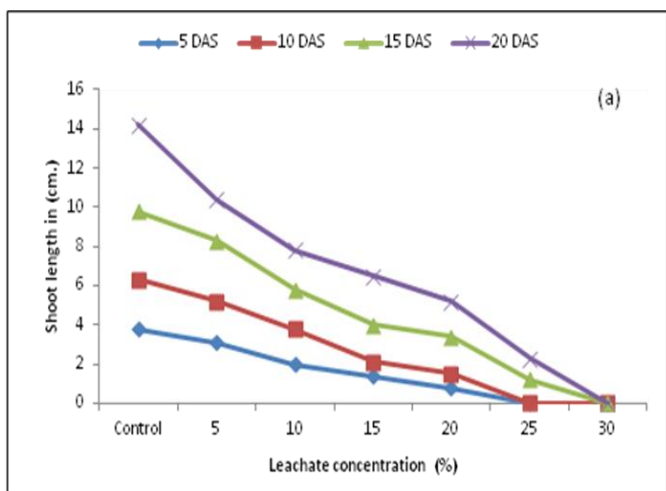


Fig.-3.4: Effect of different concentrations of aqueous leachate of rice- stubble (root) on shoot length of (a) Mustard (*Brassica junica* L.), (b) Sesame (*Sesamum indicum* L.) and (c) Sunflower (*Helianthus annuus* L.) seedlings.

Fig.-3.5: Effect of different concentrations of aqueous leachate of rice-stubble (shoot) on root length of (a) Mustard (*Brassica junica* L.), (b) Sesame (*Sesamum indicum* L.) and (c) Sunflower (*Helianthus annuus* L.) seedlings.

**Impact of root leachate on root length of seedling of test cultivars**

It can be noticed that all concentrations of root leachate of rice- stubble exhibited adverse effect on seedling growth resulting reduction of root length of the seedling. Minimum root length of  $0.6 \pm 0.01$  and  $0.4 \pm 0.01$  cm were recorded in seedlings grown in 25% leachate on 20 DAS in mustard and sesame respectively. During the same period of growth, the control sets exhibited root length of  $5.6 \pm 0.04$  and  $5.6 \pm 0.04$  in seedlings of mustard and sesame respectively. In case of sunflower more or less similar trends were noticed on 22 DAS in seedling of control set and treated with 25% concentration of root leachate. No root growths were noticed in seeds treated with 30% leachate as no seedlings were established in that concentration in all the test cultivars. Intermediate values were observed in other concentrations

under treatment. The root lengths exhibited positive correlations with increase in the seedlings growth period and negative correlations with increase in the concentration of leachate throughout the period of investigation (Fig.-3.6 (a-c)).

Among the two types of leachate i.e., shoot and root, root leachate was found to be more lethal to establish the seedling and seedling growths (shoot and root length). It can be concluded that root leachate was more susceptible and toxic for development of morphological characteristics of the seedlings.

### Fresh weight of seedling

After seed germination, seedling vigor and their establishment are considered as important parameters which include fresh and dry weight of the seedlings. The latter are directly and indirectly influenced by immediate environment and their induction or suppression of growth characteristics. The effects of different concentrations of rice stubble shoot and root aqueous leachates on shoot and root fresh weights of three oil-yielding crops are described below.

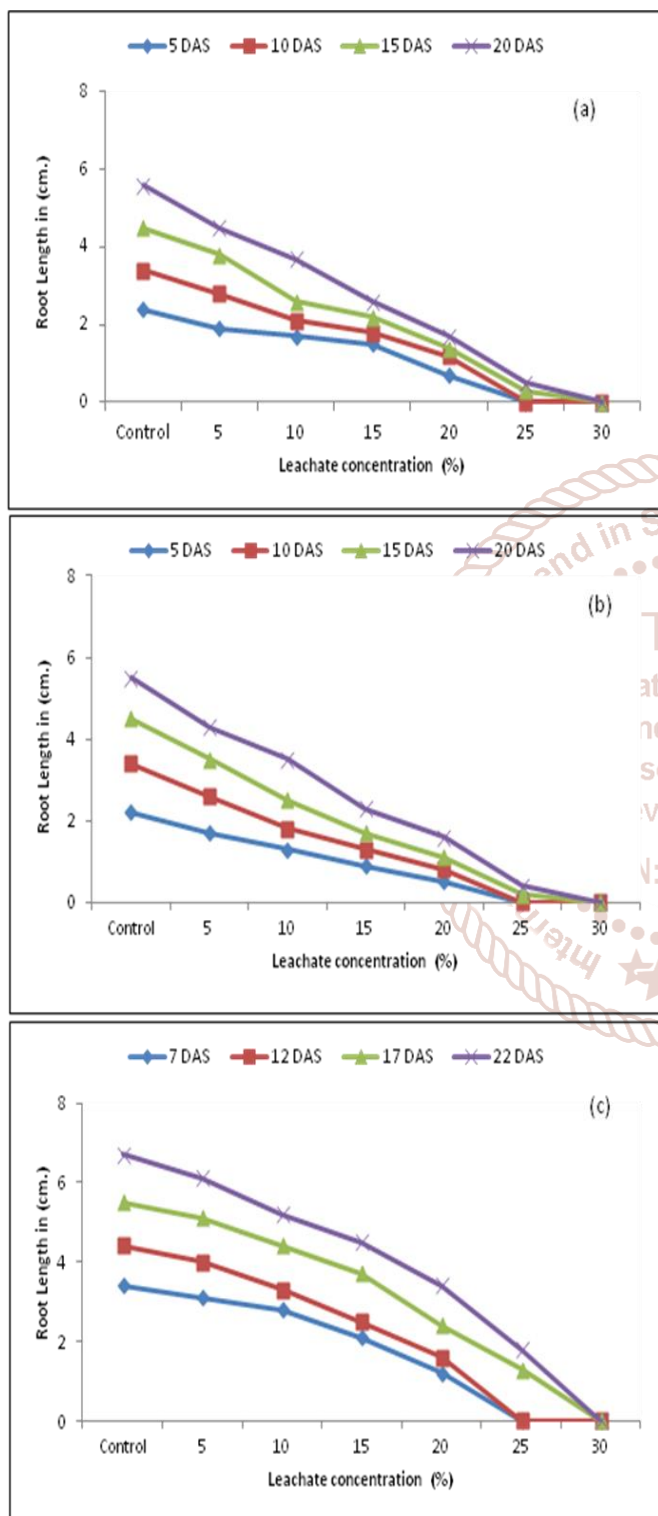


Fig.-3.6: Effect of different concentrations of aqueous leachate rice-stubble (root) on root length of (a) Mustard (*Brassica junica* L.), (b) Sesame (*Sesamum indicum* L.) and (c) Sunflower (*Helianthus annuus* L.) seedlings.

### Impact of shoot leachate on shoot fresh weight of seedling of test cultivars

All the concentrations of shoot leachate of rice-stubble considerably reduced the shoot fresh weight of all the seedlings of test cultivars compared with seedling of respective control sets. The maximum fresh weight of the shoot recorded in seedlings of control set on 20 DAS were  $32.6 \pm 0.11$  and  $30.6 \pm 0.12$  in mustard and sesame seedlings respectively and  $43.6 \pm 0.05$  mg in sunflower on 22 DAS. During the same period of growth, the least shoot fresh weight recorded in seedlings influenced by 30% leachate concentration were  $5.6 \pm 0.03$  and  $46 \pm 0.02$  mg in mustard and sesame whereas it was  $9.6 \pm 0.02$  mg sunflower seedlings. There was no seedling establishment influenced by 30% of shoot leachate upto 10 DAS in all test cultivars and thereafter there was poor seedling growth. Data of intermediate values were noticed in other seedlings affected by different concentrations of leachate during the period of observation. The shoot fresh weights exhibited negative correlations with increase of leachate concentrations and positive correlation with advancement of seedling growth period throughout the period of observation [Fig.-3.7 (a-c)].

### Impact of root leachate on shoot fresh weight of seedlings of test cultivars

The shoot fresh weights of all test cultivars seedlings influenced by different concentrations of root leachate of rice-stubble exhibited more or less same trends as were marked in case of shoot fresh weights affected by shoot leachate [Fig.-3.8 (a-c)]. Maximum shoot fresh weights recorded in seedlings of control sets on 20 DAS were  $32.0 \pm 0.06$  and  $29.6 \pm 0.05$  in mustard and sesame cultivars respectively while it was  $43.2 \pm 0.05$  mg in seedling of sunflower on 22 DAS; whereas minimum shoot fresh weight of  $7.8 \pm 0.02$  and  $6.8 \pm 0.02$  were recorded in seedling of mustard and sesame during same period of growth influenced by 25% leachate concentration and in case of sunflower the value was  $9.4 \pm 0.03$  mg on 22 DAS influenced by 25% concentration of leachate. No shoot growth was noticed in seeds treated with 30% root leachate, as no seedlings were established. Data of intermediate values were noticed in other seedling at different growth period influenced by varying concentrations of leachate.

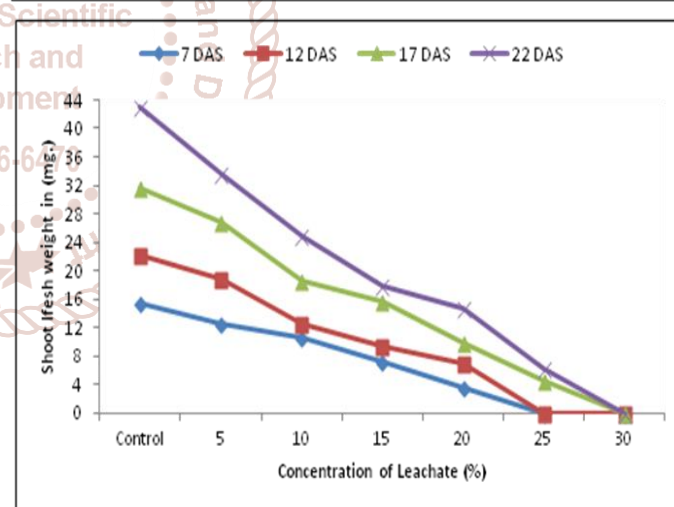
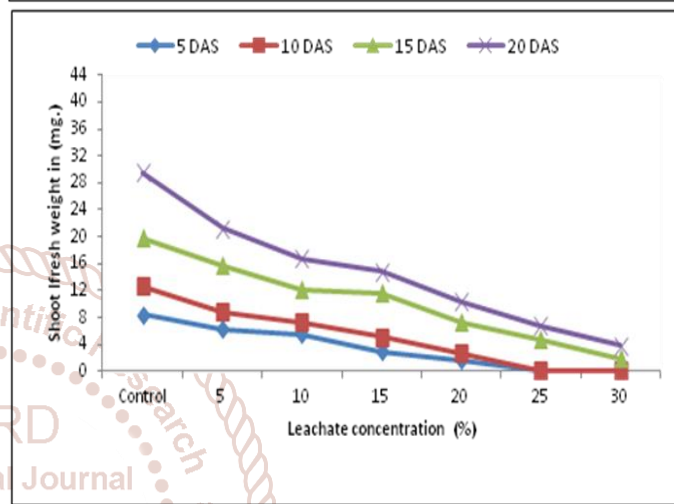
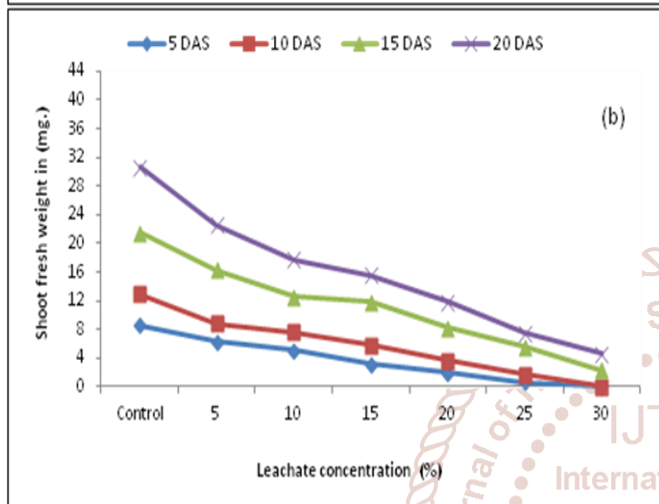
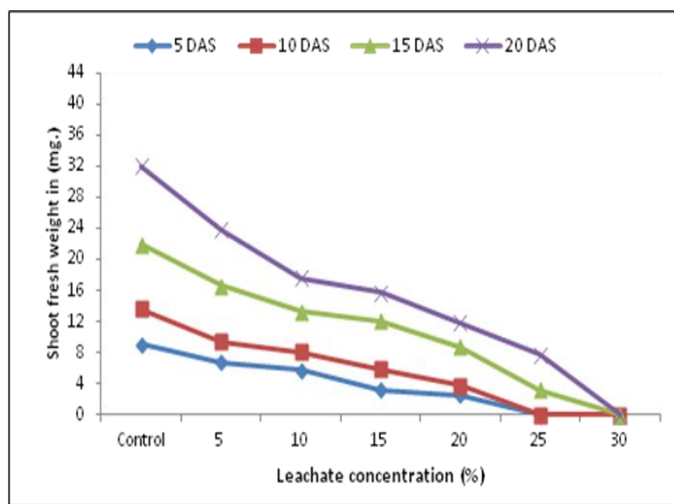
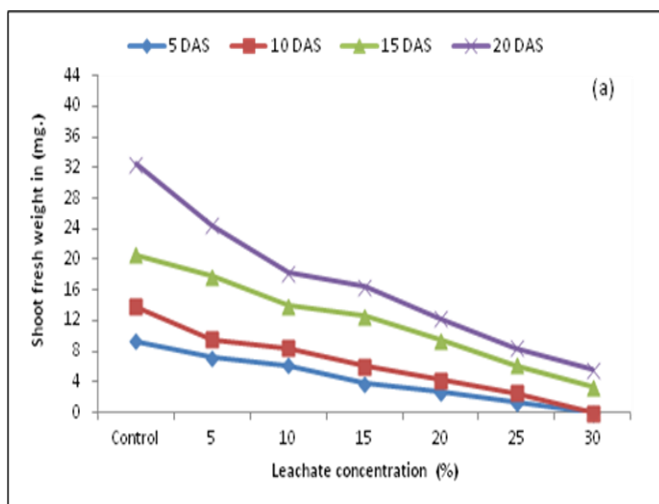


Fig.-3.7: Effect of different concentrations of aqueous leachate of rice stubble (shoot) on shoot fresh weight of (a) Mustard (*Brassica junica* L.), (b) Sesame (*Sesamum indicum* L.) and (c) Sunflower (*Helianthus annuus* L.) seedlings.

Fig.-3.8: Effect of different concentrations of aqueous leachate of rice-stubble (root) on shoot fresh weight of (a) Mustard (*Brassica junica* L.), (b) Sesame (*Sesamum indicum* L.) and (c) Sunflower (*Helianthus annuus* L.) seedlings.

**Impact of shoot leachate on root fresh weight of seedling of test cultivars**

All the concentrations of shoot leachate adversely affected the fresh weight of root, as a result the values of root fresh weights considerably decreased throughout the period of study. The maximum and minimum root fresh weights recorded on 20 DAS in seedling of control set and seedling grown in 30% shoot leachate were  $12.2 \pm 0.04$  and  $2.2 \pm 0.03$  in mustard;  $14.8 \pm 0.05$  and  $3.8 \pm 0.02$ , mg in sesame

respectively. In case of sunflower maximum and minimum values were  $18.6 \pm 0.05$  and  $8.8 \pm 0.03$  mg in seedling of control set and influenced by 25% leachate on 22 DAS. Other concentration showed intermediate values. The root fresh weights exhibited positive correlation with increase of seedling growth period and negative correlation with leachate concentration in all test cultivars as indicated from Fig.-3.9 (a-c).

### Impact of root leachate on root fresh weight of seedling of test cultivars

All concentration of leachate of root-stubble exhibited more or less similar trends and same values as were noted in case of shoot leachate. However, no seedling establishments were observed upto 10 DAS influenced by 25% leachate concentration in mustard and sesame and 12 DAS in case of sunflower. Leachate concentration of 30% totally suppressed the seedling growth in all test cultivars.

Maximum root fresh weights of  $12.0 \pm 0.05$  and  $14.8 \pm 0.05$  mg were recorded in seedling of control sets at 20 DAS whereas during the same period of growth the minimum values were  $2.2 \pm 0.02$  and  $4.8 \pm 0.04$  mg in seedling affected by 25% root leachate in mustard and sesame respectively. In case of sunflower, the maximum and minimum values on 22 DAS were  $18.0 \pm 0.05$  and  $5.0 \pm 0.04$  mg in seedling of control set and seedlings affected by 25% concentration of root-leachate of rice-stubble. Intermediate values between the above maximum and minimum data were recorded in seedling affected by other concentrations of leachate.

Significant negative correlations were noticed between root fresh weight and increase in concentrations of root leachate and a slow and steady positive correlation was noticed between root fresh weight and increase of growth period in all test cultivar seedlings [Fig.-3.10 (a-c)].

Among two types of leachates, root leachate exhibited more inhibitory effect than shoot leachate. Twenty per cent concentration of root-leachate exhibited no seedling establishment but in case of shoot leachate it was 30%. From this experiment results, it can be observed that root leachate was found to be more toxic compared with shoot leachate of rice-stubble.

### Dry weight of seedlings

The dry weights of the seedlings are considered as the real growth parameter as these do not contain water or moisture in their biomass. The change in shoot and root dry weights of the test crop seedlings influenced by different concentrations of shoot and root leachate of rice stubble are described below:

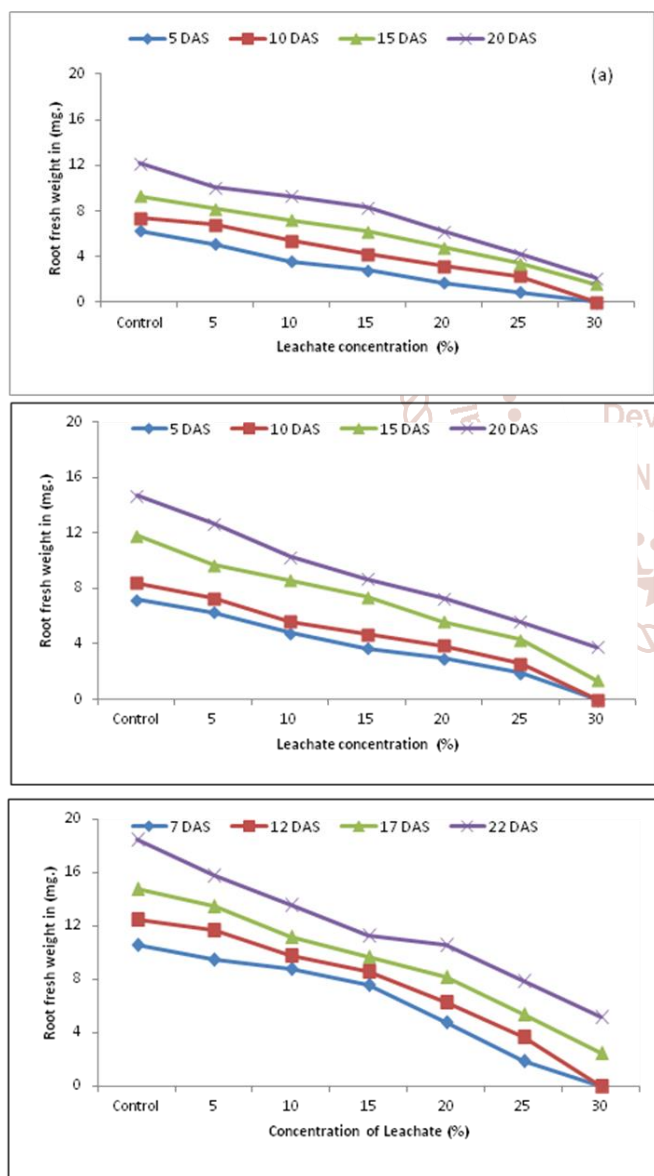


Fig.-3.9: Effect of different concentrations of aqueous leachate of rice-stubble (shoot) on root fresh weight of (a) Mustard (*Brassica junica* L.), (b) Sesame (*Sesamum indicum* L.) and (c) Sunflower (*Helianthus annuus* L.) seedlings.

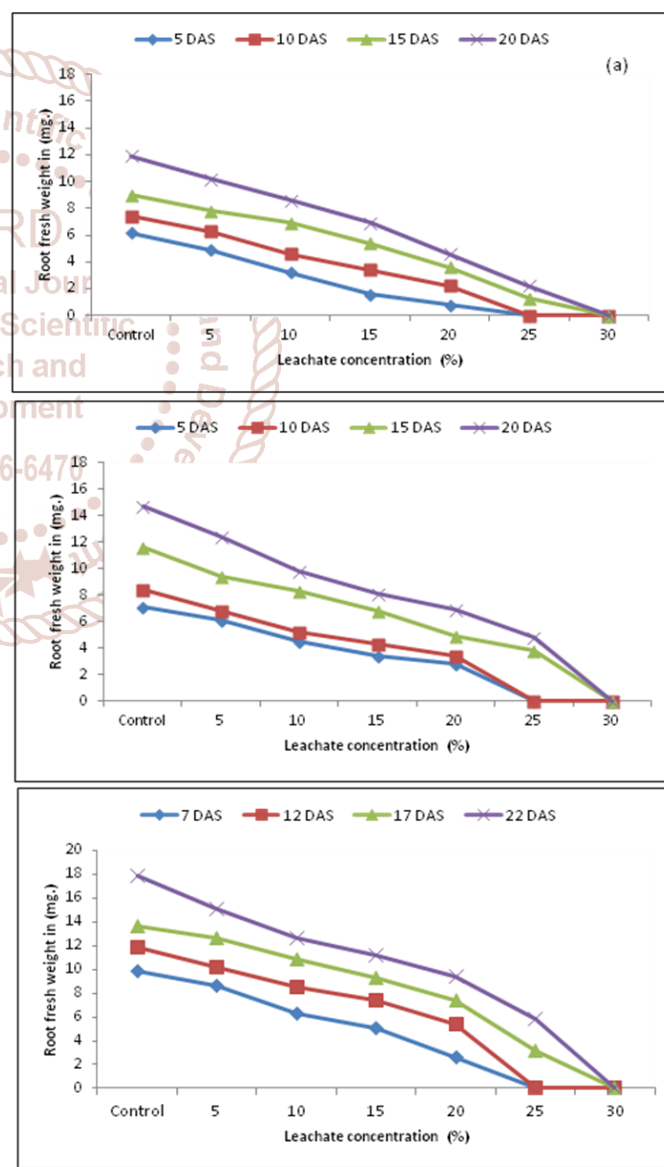


Fig.-3.10: Effect of different concentrations of aqueous leachate of rice-stubble (root) on root fresh weight of (a) Mustard (*Brassica junica* L.), (b) Sesame (*Sesamum indicum* L.) and (c) Sunflower (*Helianthus annuus* L.) seedlings.

**Effect of shoot leachate on shoot dry weight of seedling of test cultivars**

The shoot dry weights were considerably decreased by the influence of various concentrations of shoot leachate compared with respective control seedlings during the course of investigation. The maximum shoot dry weight of the control seedlings on 20 DAS were  $3.32 \pm 0.03$ ,  $3.12 \pm 0.03$  and  $43.6 \pm 0.04$  mg while the values were least ( $0.59 \pm 0.06$  and  $0.48 \pm 0.02$ ) in seedlings influenced of mustard and sesame by 30% leachate concentration respectively. In case of sunflower the shoot dry weights exhibited  $43.6 \pm 0.04$  mg (maximum) and  $9.6 \pm 0.02$  (minimum) mg on 22 DAS in seedling of control set and influenced by 30% concentration of shoot-leachate of rice-stubble. Data of intermediate values between the maximum and minimum values were recorded in other seedling of all test cultivar during the period of observation. Thus the shoot dry weights exhibited negative correlations with increase of leachate concentration and positive correlations with advancement of seedling growth period as evidence from Fig.-3.11(a-c).

**Effect of root leachate on shoot dry weight of seedling of test cultivars**

All the concentrations of root leachate of rice-stubble considerably checked the dry weight of the roots of test seedlings as was noticed in case of shoot dry weight. The maximum root dry weight recorded in seedling of control set on 20 DAS were  $3.24 \pm 0.03$  and  $2.98 \pm 0.04$  mg in seedling of mustard and sesame respectively whereas concentration of 25% caused dry weight of  $0.70 \pm 0.03$  and  $0.54 \pm 0.01$  mg on 20 DAS in mustard and sesame respectively. In case of sunflower, maximum and minimum values were  $43.2 \pm 0.03$  and  $6.4 \pm 0.01$  mg on 22 DAS in seedling of control set and influenced by 25% leachate. No seedling growth was established in 30% concentration of root leachate in all test cultivars. Data of intermediate values were recorded from seedling at different developmental stages influenced by different leachate concentrations. Fig.-3.12 (a-c) indicate that the shoot dry weights exhibits negative correlations with increase of leachate concentration and positive correlation with increase of seedling growth period of mustard, sesame and sunflower respectively.

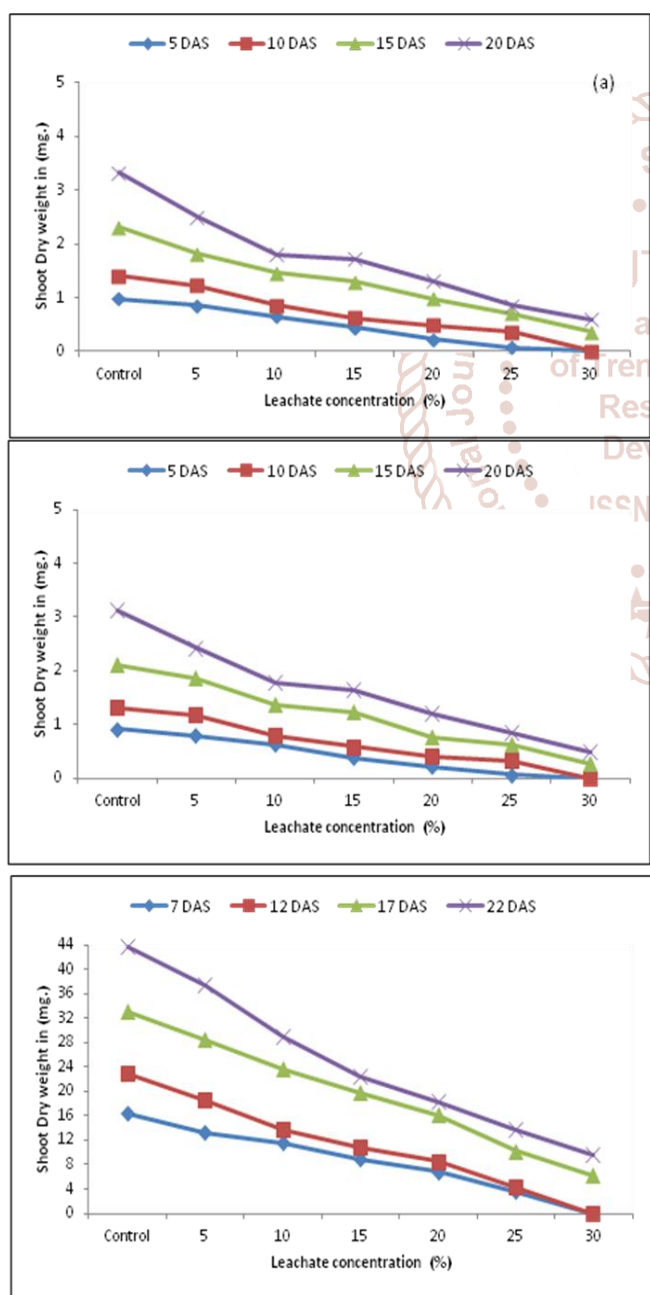


Fig. -3.11 : Effect of different concentrations of aqueous leachate of rice-stubble (shoot) on seedling shoot dry weight of (a) Mustard (*Brassica junica* L.), (b) Sesame (*Sesamum indicum* L.) and (c) Sunflower (*Helianthus annuus* L.) seedlings.

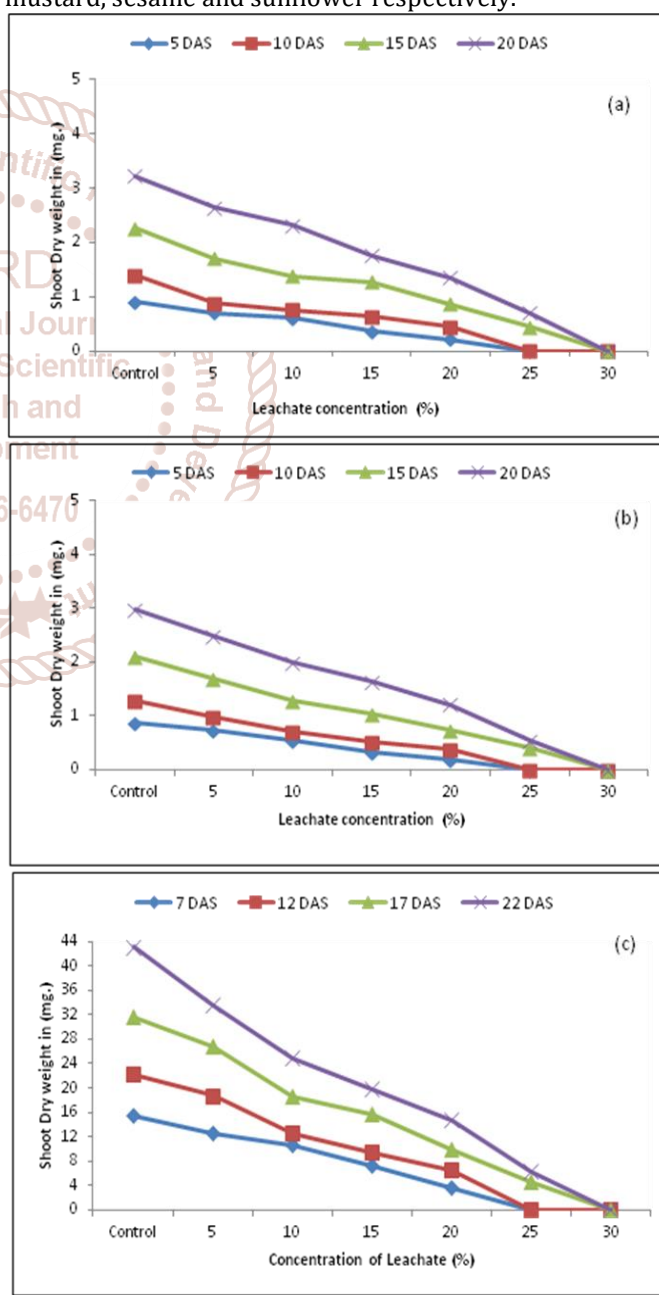


Fig. -3.12 : Effect of different concentrations of aqueous leachate of rice-stubble (root) on seedling shoot dry weight of (a) Mustard (*Brassica junica* L.), (b) Sesame (*Sesamum indicum* L.) and (c) Sunflower (*Helianthus annuus* L.) seedlings.



**Effect shoots leachate on root dry weight of seedling of test cultivars**

It can be marked that all concentrations of shoot leachate considerably checked the root dry weight of all test cultivars. Maximum root dry weight of  $1.32 \pm 0.02$  and  $1.46 \pm 0.04$  mg were recorded in seedlings of control set at 20 DAS in mustard and sesame respectively whereas in case of seedlings treated with 30% shoot leachate the values were  $0.22 \pm 0.02$  and  $0.38 \pm 0.03$  mg in mustard, sesame respectively. In case of sunflower seedling like shoot dry weight, the root dry weights also exhibited more or less same trends and values. Maximum root dry weight of  $1.80 \pm 0.03$  and minimum weight of  $0.46 \pm 0.01$  mg were recorded on 22 DAS for seedling of control set and influenced by 30% leachate concentration respectively. Seedling treated with other concentrations exhibited intermediate values. A positive correlation was observed between root dry weight and increase in growth period while negative correlations were noticed between root dry weight and increase of shoot leachate concentrations in all test cultivar tested [Fig.-3.13 (a-c)].

**Effect of root leachate on root dry weight of seedling of test cultivars**

It was observed that all the root leachate concentrations considerably checked the root dry weight throughout the

period of investigation. Dry weight of  $1.26 \pm 0.02$  and  $1.4 \pm 0.03$  mg were recorded in seedlings of control sets on 20 DAS which were reduced to  $0.32 \pm 0.02$  and  $0.42 \pm 0.02$  mg influenced by 25% concentration of root stubble leachate in mustard and sesame respectively. Like shoot stubble, the root leachate considerably reduced the root dry weight of sunflower seedlings resulting  $1.82 \pm 0.04$  (maximum) and  $0.54 \pm 0.01$  mg (minimum) mg on 22 DAS in seedling of control set and influenced by 25% concentration of leachate. Thirty per cent root leachate was found to be highly lethal to seedling growth, as no seedlings were established in all test cultivars under investigation. Other concentrations exhibited intermediate values between the maximum and minimum values of respective test cultivar seedlings. Like shoot dry weights, the root dry weights also exhibited more or less similar trends and same values with leachate concentrations and seedling growth periods [Fig.-3. 14 (a-c)].

From the above results, it can be marked that the root leachate of rice stubble comparatively caused lower values of seedling growth parameter than shoot leachate throughout the period of observation. Twenty five per cent of root leachate was found to be more toxic and lethal to all the test cultivars of oil-yielding crops whereas in case of shoot leachate it was 30%.

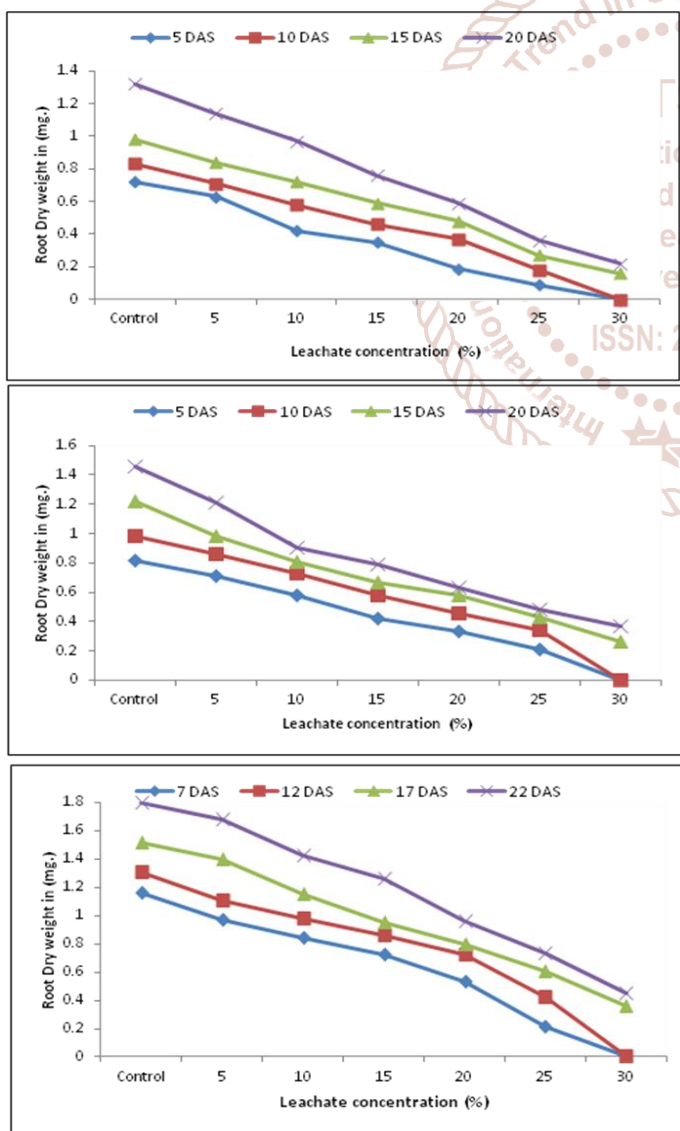


Fig. -3.13: Effect of different concentrations of aqueous leachate of rice-stubble (shoot) on seedling root dry weight of (a) Mustard (*Brassica junica* L.), (b) Sesame (*Sesamum indicum* L.) and (c) Sunflower (*Helianthus annuus* L.) seedlings.

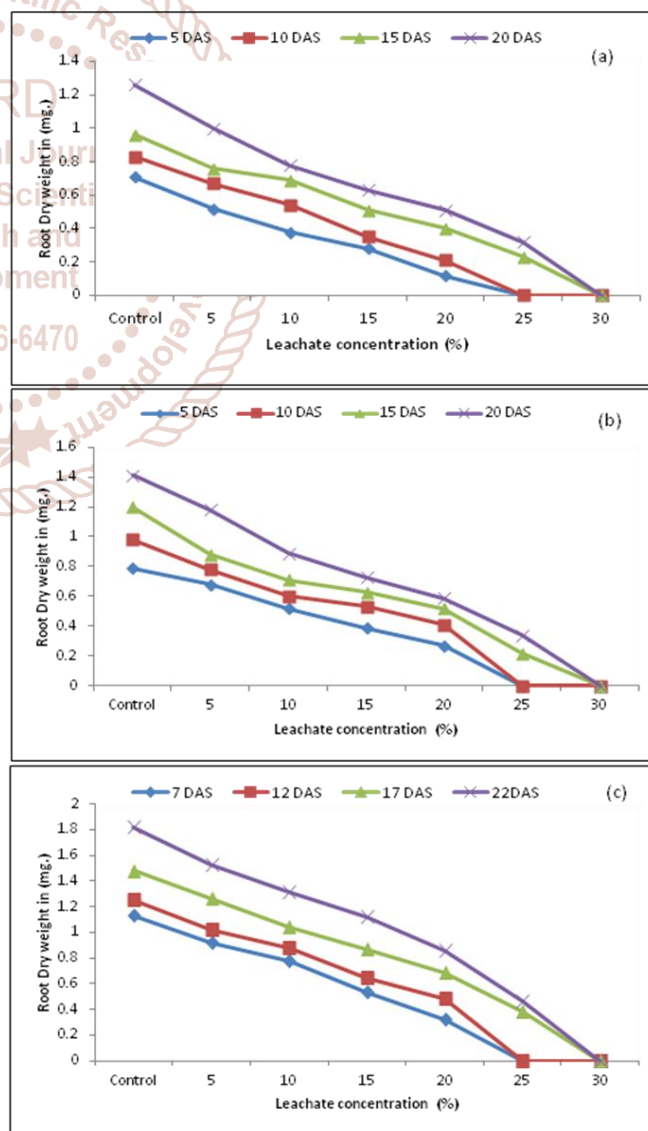


Fig. -3.14: Effect of different concentrations of aqueous leachate of rice-stubble (root) on seedling root dry weight of (a) Mustard (*Brassica junica* L.) (b) Sesame (*Sesamum indicum* L.) and (c) Sunflower (*Helianthus annuus* L.) seedlings.

## DISCUSSION

Seedling establishment is one of the most important and crucial stage after seed germination where various histological and morphological activities are established which are associated with changes in physiological and biological processes. During this process, newly developing areas occur where all necessary constituents of cell, cell wall and cell inclusions are synthesized and translocated from cotyledon to site of action. These complicated processes are also controlled by various environmental and/or internal factors. During this period, the seedlings become self established by developing their own shoot and root systems whose growth and development are controlled by various metabolic activities. The impact of different concentration of shoot and root leachate of test rice-stubble on different parameters of seedling growth such as seedling length (shoot and root) and seedling weight ( fresh and dry of both shoot and root) in seedlings of three oil-yielding test cultivars are discussed below.

### Seedling length

The meristematic tissues of the radicle and plumule undergo various morphological histological, cytological and biochemical changes through qualitative and quantitative growth which reflect on seedling vigour. The role of allelochemicals and other plant growth regulators on these processes cannot be ignored. Allelopathic chemicals act in a variety of ways. Some chemicals retard growth or inhibit germination by disrupting cell division and a large number of them interfere with respiration, nutrition, mineral and water absorption or translocation of mineral substances and food materials (Fisher, 1979). Generally, seedling growth is controlled by cell multiplication, cell differentiation and translocation of reserve food materials from cotyledon to various parts of the seedling at initial stage and from leaves to other organs at later stage.

In the present investigation, it was observed that all concentrations of shoot and root leachate of rice stubble reduced seedling growth (both shoot and root length) of all test crops viz. mustard, sesame and sunflower as evidenced from Figs.-3.3(a-c), Figs.-3.4(a-c), Figs.-3.5(a-c) and Figs.-3.6(a-c) respectively.

The results of this investigation agree with similar reports on other crop plants influenced by allelochemicals of various plants such as water extracts from rice straw exhibited reduced seedling growth of Chinese milk vetch (Paramanik *et. al.* 2001), extracts from the leave of rice seedlings inhibited root growth of duck salad and lettuce (Ebana *et. al.* 2001 and Okano and Ebana,2003), water extracts of shoots and roots of rice varieties significantly inhibited root growth of *E. crus-galli* (Kim *et. al.* 2005).

Rice germplasm was also found to be inhibitory to growth and development of aquatic weeds such as duck salad (*Heteranthera limosa*) (Mattice, 1998), rice callus co-cultured with soybean in vitro studies reduced the growth rate of soybean by more than 100 fold and it was demonstrated that growth inhibition was not due to ethylene produced by rice callus but due to volatile allelopathic-compounds (Yang and Fitsuha, 1991). Incorporating the residues of rice with high allelopathic activity minimized the growth of rice Barnyardgrass (*Echinochloa crus-galli*) (Lin and shin, 2000). Rice (1984) suggested that allelochemicals such as phenolics, terpenoids and flavonoids directly or indirectly

influence on various labels in cells physiology or biological activities such as (a) permeability of bio-membrane (b) ultra-structure of cells (c) uptake of mineral substances etc. (d) mechanism of movement of stomata (e) water translocation (f) photosynthesis and transpiration (g) protein synthesis (h) nitrogen balance and nitrification (i) lipid metabolism and inhibition or stimulation of specific enzyme activities and (k) hormone-induced growth. Baziramakenga *et. al.* (1997) suggested that interference with nucleic acids and protein metabolism by phenolic acid is one of the main mechanisms by which they influence plant growth and development. Chauhan and Chauhan, (2002 b), Mishra (2001) and Padhy *et.al.* (2006) have reported various mitoclastic abnormalities viz. mis-orientation, fragmentation, clumping, sticky and non- syndromous movement of chromosomes, chromosomal bridges at anaphase, nuclear vacuolation and polyploidy in onion root meristem due to influence of various allelochemicals present in different parts of many plants.

From the above facts, it is clear that the phytotoxic chemicals present in the shoot and root leachate of rice-stubble might have interfered with various metabolic activities, particularly synthesis of plant growth regulators which are controlled by enzymes, as a result of which the length the shoot and root were significantly reduced. Further the growth of seedlings depends upon amount of GA<sub>3</sub> synthesized and translocated from source to the site of action.

Papadakis (1981) suggested that the role of different allelochemicals in relation to seedling growth and development is not sufficient to draw any concrete conclusion. Hence, much more technological researches at molecular and sub-molecular level are needed for a better understanding of changes in physiological and biochemical processes influenced by allelochemicals during seedling growth of any crop plants.

### Seedling weight

Generally seedling weights (fresh and dry) are considered as the indices of seedling growth which are commonly reflected by seedling vigor. The weights of seedlings depend on the amount of metabolites, synthesized in shoot and root and retained in it. Any change in external environmental factors may influence the growth and development of seedlings which reflect on seedling weight.

In this investigation, it was observed that both fresh and dry weights of all seedlings of test cultivars were considerably reduced by the influence of allelochemicals present in aqueous leachate of both shoot and root of rice-stubble which might be due to inhibition or checking of synthesis of proteins, nucleic acids, carbohydrates and other bio-macromolecules. Further the phytotoxins present in the leachate might have controlled the activities of enzymes like protease, polyphenol oxidase, peroxidase, catalase,  $\alpha$  and  $\beta$ -amylase and others which have reflected on seedling growth parameters such as shoot and root growth and their fresh and dry weights.

The results of present investigation agree with similar reports of Chou and kim, (2004) on allelopathic effects of barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) extracts on root growth of alfalfa (*Medicago sativa* L.), barnyard grass (*Echinochloa crus-galli*) and eclipa (*Eclipta prostrata* L.).

Singh *et al.* (1999) reported that auto-toxicity in rice could provide an adaptive strategy to plants because they grow in adequately water-logged soils having sufficient oxygen and thus develop a negative radox potential in soil because of decomposing the rice residues. This induced the inhibition of root cells in order to capture more oxygen (Chou, 1995). Farooq *et al.* (2008) studied the allelopathic potential of different plant parts of rice plant against wheat, oat, barley and berseem and found that the seedling length and dry weights were reduced significantly. This sort of reduction of seedling weight influenced by Eucalyptus leaf-litter leachate on wheat, mustard and chick pea (Singh and Nandal, 1993); rice, ragi, sesamum and tobacco (Sahu, 1997, Pattanaik, 1998, Padhy *et al.* 2000 and Mohanty, 2015). Reports on reduction in seedling weight of rice (Nayak, 2000, Tripathy, 2000) and some legumes (Padhy *et al.* 2000, Tripathy, 2000 and Dash, 2012) influenced by phyllode litter leachate of *Acacia auriculaformis* corroborate with present finding. Since no adequate reports are available on the effect of individual allelochemicals of rice-stubble on any crop plants, no definite correlations and conclusions can be drawn for which in-depth research at molecular level is highly essential.

## CONCLUSION

In nutshell, it can be concluded that rice-stubble leachate reduced the shoot and root length, fresh and dry weight biomass of seedlings of all oil yielding tested crops. Rice-stubble leachate activity was due to the synergistic effect of various allelochemicals which inhibited/restricted the different seedling formation of test crops. The identification of rice-stubble leachate as an effective material for the growth inhibition of the test crops implies that it has the potential to be used as an environmental friendly bio herbicide for control of weeds. Rice-stubble leachate may be act as a natural weed inhibitor, which would reduce the need of chemical herbicides and provide economic benefits. Further research is suggested on the topic to screen out rice varieties for their Allelopathic potential and identification of allelochemicals involved in the allelopathic effects. In addition, studies will be conducted on purification, identification and testing such compounds under field condition.

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