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Weed Management through Bio-herbicidal Activity of Rice Straw for Mitigating Herbicide Dependency

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ABSTRACT

Phytotoxicity related ecological significance of ten rice (*Oryza sativa* L.) varieties was evaluated under laboratory and greenhouse conditions against *Avena ludoviciana* Durieu. Basmati 370, Govind and VL Dhan 85 significantly ($P<0.01$) reduced seed germination and seedling dry weight in *A. ludoviciana*. Pot experiments exhibited a significant ($P<0.01$) reduction in height of *A. ludoviciana* with VL Dhan 85 and Govind. Proline accumulation was recorded with all varieties, whilst malondialdehyde with Govind and Pant Dhan 11. Ten phenolic acids *viz.,* cinnamic, chlorogenic, benzoic, gallic, ferulic, p-hydroxybenzoic, salicylic, syringic and vanillic were identified and quantified from straw extracts of all ten rice varieties. Maximum seven phenolics were identified in Govind and VL Dhan 85 followed by Basmati 370 (six) whereas minimum in Pant Dhan 16 and Tarori Basmati (three each). These findings indicated the involvement of phenolics in phytotoxicity of rice straw, which could be utilized for developing natural compound based bio-herbicides to achieve sustainability in agriculture.

Key Words: Allelopathy; Malondialdehyde; Proline; Phenolics; Phytotoxicity; Sustainable Agriculture.

INTRODUCTION

In Asia, the primary food security of more than 80 % of the people is entirely dependent on rice production (Kabir 2006). Uncoupling of population growth and cereal production has led India in a paradox where huge food surpluses at the aggregate national level coexist with large undernourished poor population. In order to enhance productivity and its further protection, globally about 3 million tons of herbicides are used annually (Shibayama 2001). Although synthetic herbicides have been successful in weed control, associated hazards against environment and public health have become great concern. To combat with these issues, it is necessary to develop sustainable weed management system. Utilizing allelopathic potential of plant/crop species could be one of the options for substituting synthetic herbicides (Xuan et al. 2005, Rajput and Rao 2013).

Some authors identified the chemicals released by rice root exudates or decomposing straw through HPLC or GC-MS and few exhibited allelopathic potential under laboratory, greenhouse and field conditions against different weed species (Khanh et al. 2007, Mattice et al. 2001, Wu et al. 2000). Lin et al. (2000) reported that the aqueous extracts of six rice lines significantly induced lipid peroxidation (MDA concentration) in barnyard grass. Inderjit et al. (2004) observed that the foliar proline content was increased in mustard with increasing concentration of rice straw over control. The extract of oat shoots containing ethyl ether, acetone and water-soluble fractions reduced the germination and growth of roots and hypocotyls of lettuce (*Lactuca sativa* L.) in which water-soluble fraction was highly inhibitory (Kato-Noguchi et al. 1994). Wu et al. (2000) reported p-hydroxybenzoic, trans-p-coumaric, cis-p-coumaric, syringic, vanillic, trans-ferulic and cis-ferulic acids and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBO) in both the shoots and roots of 17d old wheat seedlings. Dilday et al. (1994) documented that the allelochemicals present in rice straw exhibited allelopathic activity in the field around *Heteranthera limosa* (Sw.) Willd. The formulations of phyto products such as neem (*Azadirachta indica* A. Juss.), rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), tulsi (*Ocimum sanctum* L.), sorghum (*Sorghum bicolour* L.) and sunflower (*Helianthus annuus* L.) have also been reported to be effective as bio-herbicides (Khanh et al. 2007). In the present study, an attempt has been made to identify potential phenolic acids and examine the allelopathic effect of different varieties of rice straw on *Avena ludoviciana* Durieu. under laboratory and greenhouse conditions.

MATERIAL AND METHODS

The straw (stem+leaves) of ten rice (*Oryza sativa* L.) varieties was collected from Crop Research Centre (C.R.C.) fields, G.B. Pant University of Agriculture & Technology, Pantnagar, India in October 2011. The seeds of *A. ludoviciana* were collected in April 2011 and stored under laboratory conditions to conduct both pot and laboratory experiments. Pantnagar is located at 29º N latitude 79º N 30' E longitude and at an altitude of 243 m above mean sea level in the Terai belt of Shivalik range of Kumaun Himalaya.

Preparation of Aqueous Extracts

The straw of all ten rice varieties was ground in a Wiley mill. Aqueous straw extracts (10 %, w/v) were prepared by taking 10 g of powdered straw in 100 ml distilled water and kept for 24 h. The solutions were filtered through four layers of cheese cloth and centrifuged at 3000 rpm for 4 h and filtered by using Whatman No. 42 filter paper and finally made 100 ml by distilled water (Chung et al. 2001). Ten healthy seeds of *A. ludoviciana* were sterilized with mercuric chloride ($HgCl_2$), after thorough washing, placed on filter paper (Whatman No. 42) layered Petri dishes (9 cm dia.). The seeds were moistened with distilled water (control) or 10% (w/v) aqueous extracts (treatments) of all rice varieties *viz.,* Basmati 370, Govind, Pant Dhan 11, Pant Dhan 16, Pant Sugandh 17, Pusa Sugandh 4, Pusa RH 10, Super Basmati, Tarori Basmati and VL Dhan 85. The experiment was arranged in completely randomized design (CRD) with triplicates (temperature 25 ± 1ºC and diffused light). Germination was recorded at an interval of 24 h up to 9 days. After 9 days, three seedlings from each Petri dish (a total of 9 seedlings for each extract) were considered for dry weight (mg) of root and shoot.

Greenhouse Experiment

Seven more allelopathic varieties (Basmati 370, Govind, PD 11, Pusa S 4, Pusa RH 10, SB, and VL Dhan 85) were selected from laboratory bioassay. Powdered straw (5, 10, 20 g) of all seven rice varieties was mixed in the upper layer of 1.25 kg of soil (silty clay loam to clay loam) in each pot (6×6 cm) and without straw as control for each variety in completely randomized design (CRD) with triplicates. The seeds of *A. ludoviciana* were sown in soil (2 cm depth) and watered when required. Emergence of seedlings from the soil surface was considered as germination of seeds and recorded daily up to 15 days after sowing (DAS). The seedlings were thinned and three uniform seedlings were maintained per pot. Plant height (cm), proline content and malondialdehyde (MDA) content in *A. ludoviciana* were measured at 60 DAS. Proline was analyzed by Bates et al. (1973). MDA was determined by the method of Heath and Packer (1968).

Identification of Phenolics

Phenolic acids were extracted from the straw (stem+ leaves) of all ten rice varieties by acid hydrolysis as described by Harbourne (1977) and Charpentier and Cowles (1981).

Sample Preparation

Powdered straw (5 g) was heated with 100 ml 2 N HCl for 2 h in water bath at 40 ºC and filtered through Whatman No. 42 filter paper and then extracted with diethyl ether. Then, 5 g hot anhydrous sodium sulphate (Na_2SO_4) and after cooling 100 mg active charcoal was added in organic layer. The filtrate was evaporated to dryness on a rotary evaporator at 40 ºC and re-dissolved in 5 ml MeOH and finally filtered through Millipore filters (0.22 µ). The HPLC (Dionex 300), comprising LC -10 AT VP pumps, SCL - 10 A VP auto injector and Phenomenex Luna $C_{18,}$ 5 µm, (250 x 4.6 mm) column was used at ambient temperature. Isocratic elution was carried out with mobile phase, water:MeOH (77:23) consisting 2% glacial acetic acid at flow rate of 1 ml/min. A 20 µl sample was injected into column for analysis. The quantification was based on the method of

Chung et al. (2001). HPLC grade standards as cinnamic, chlorogenic, benzoic, gallic, ferulic, p-hydroxybenzoic, salicylic, syringic and vanillic were obtained from Sigma and Merck.

Statistical Analyses

All data were analysed by one way analysis of variance (ANOVA). The mean values were separated on the basis of least significant difference (LSD) at 0.01 probability level. The analysis of the phenolic compounds by HPLC was repeated twice for each rice variety.

RESULTS AND DISCUSSION

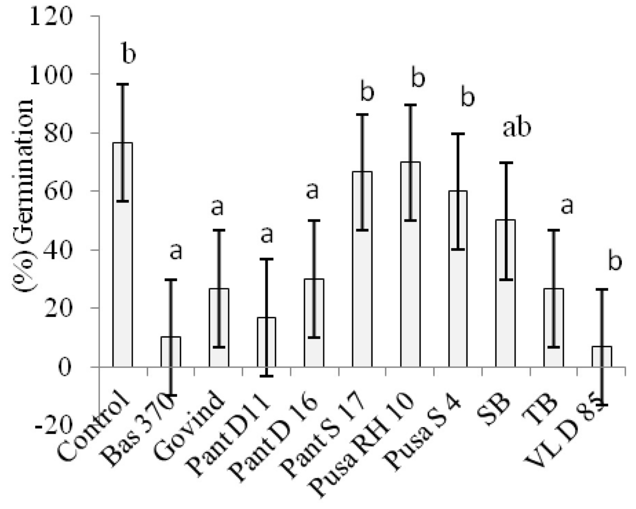
Laboratory Bioassay

The application of aqueous extracts of different rice varieties significantly ($P< 0.01$) inhibited seed germination of *A. ludoviciana*. The germination (%) was ranged from 6.66% with the extract of VL Dhan 85 to 70.00% with Pant S 17 among treatments (Figure 1a). Interestingly, none of the extracts showed positive effect on germination. Less than 50 % germination was observed with six out of ten extracts. The radicle and plumule dry weight (mg) was significantly ($P< 0.01$) inhibited by all extracts, except with Pant S 17, in which it was stimulatory. The extract of Basmati 370 was highly inhibitory on radicle and plumule dry weight compared to other treatments (Figure 1 b-c). The similar trend was also observed in total seedling dry weight (Figure 1d). Chon and Kim (2004) showed that the extracts of barley, oats, rice and wheat significantly reduced root growth of alfalfa (*Medicago sativa* L.), barnyard grass (*Echinochloa crus-galli* Beauv.) and eclipta (*Eclipta prostrata* L.). Seyyednejad et al. (2010) speculated that water hull extracts of 11 rice cultivars were inhibitory on germination, dry weight and root length of *Silybum marianum.* Recently, Rajput et al. (2010) observed inhibitory effect of rice straw extracts of Basmati 386, Govind and Kalanamak on seed germination, seedling growth (length and dry weight), total protein and protein profile of *Chenopodium album, Echinochloa crus-galli, Phalaris minor* and *Solanum nigrum* under laboratory.

Greenhouse Experiment

Incorporation of rice straw (5, 10, 20 g) significantly ($P< 0.01$) inhibited height of tested weed species as compared with control (Figure 2a). Generally, reduction increased proportionally with the amount of straw. The height at 60 DAS (cm) was minimum with 20 g of Govind (20.57 ± 0.38) while maximum with 5 g of Pusa RH 10 (47.00 ± 0.28). The plant height is often considered as one of the most important factors for total competitive ability of a crop (Garrity et al. 1992). All rice variety exerted reduction on height at 60 DAS compared to control, in which Govind was highly inhibitory. Similarly, Turk and Tawaha (2003) found that residue incorporation of *Brassica nigra* L. in soil reduced the height of *Avena fatua* L. Different rice varieties influenced the amount of endogenous proline in leaf tissues of *A. ludoviciana* (Figure 2b). All the rice varieties significantly reduced the proline content except 5 g straw of VL Dhan 85. Proline content (μ mol g^{-1} fw) ranged from 6.48±0.64 to +10.33±0.84 among treatments. Proline could also protect cellular activities against free radicals when plants are exposed to oxidative stress (Bohnert and Shen 1999) and its accumulation could be essential for osmotic adjustment in water stress (Lutts et al. 1996). Allelochemicals can sometimes induce accumulation of compatible solutes as proline. Interestingly, all rice varieties decline proline content compared to control which suggest the accumulation of free radicals in the leaf tissues of selected weed. Among rice varieties, the straw of Govind was more effective in terms of proline reduction. MDA production in leaf tissues of weed species increased proportionally with increase in straw application rates (Figure 2c). MDA accumulation was highest with Pant Dhan 11 (20.91±0.15) while lowest increase was recorded for Pusa RH 10 (4.91±0.20). Interestingly, the straw of Pusa S 4 showed reduction in MDA content.

In contrast, MDA (malondialdehyde), the main thiobarbituric acid-reactive species (TBARS), increased in the leaves of *A. ludoviciana* when treated with rice straw of different varieties. Accumulation of MDA, highest with PD 11, is an indicator of lipid peroxidation and membrane damage which ultimately leads to oxidative damage in leaf tissues of *A. ludoviciana*. Likewise, Sampietro et al. (2007) found that the sugar-cane straw leachates significantly enhanced the lipid peroxidation in arrowleaf sida (*Sida rhombifolia* L.) proportionally with the increase of leachate concentration. Zhang et al. (2010) observed that the exogenous application of both benzoic acid (BA) and cinnamic acid (CA) enhanced MDA contents, especially with BA.



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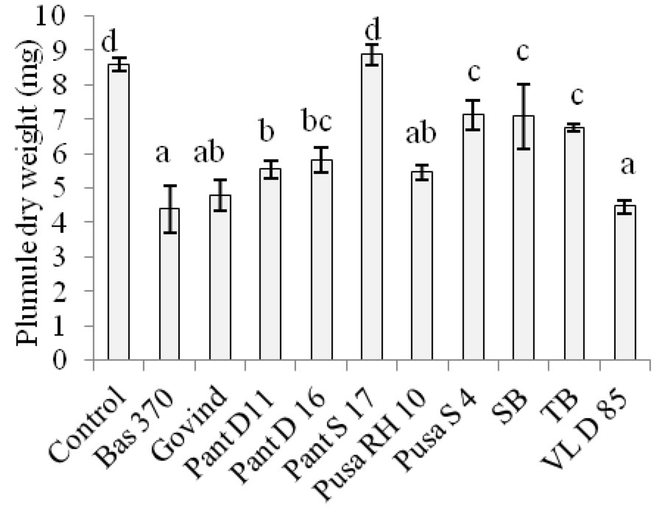

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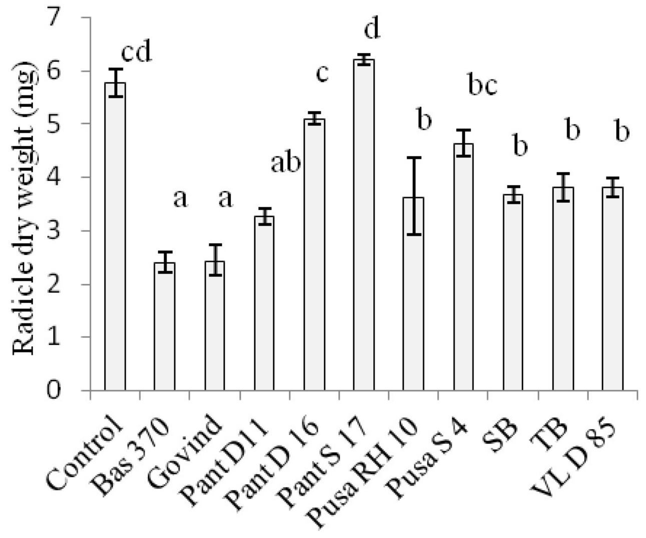

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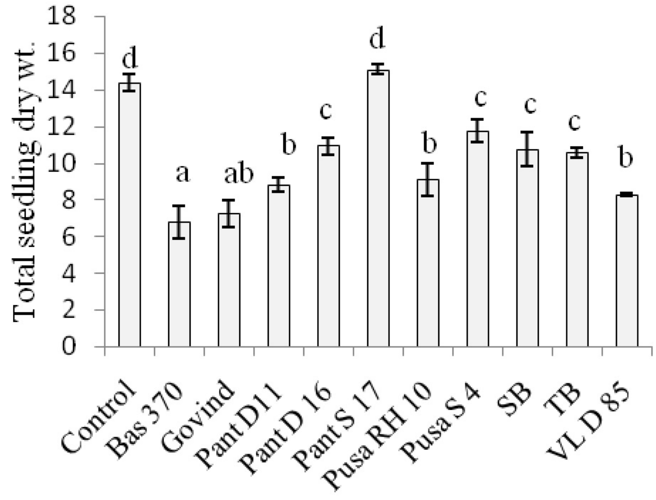

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Figure 1(a-d). Effect of different rice straw extracts on *A. ludoviciana* in different parameters under laboratory conditions. Bar indicates standard deviation. Different letters indicate significant differences with each other.

Identification of Phenolics

HPLC analysis clearly demonstrated the presence of phenolic compounds in straw extracts of all ten rice varieties. However, number and concentrations of phenolics varied from variety to variety (Table 1). The maximum numbers of phenolics were identified in Govind and VL Dhan 85 (seven each) followed by Basmati 370 (six) and minimum (three each) in PD 16 and TB. The former two varieties exhibited higher inhibitory effect and the later two were least effective/ positive against the assayed weed species. Similarly, Mattice et al. (2001) attributed the herbicidal activity of different allelopathic rice cultivars containing significant higher level of phenolic derivatives when compared with non-allelopathic species. Likewise, El-Shahawy et al. (2006) identified eight phenolics in rice straw on TLC: cinnamic, salicylic, vanillic, p-hydroxy-benzoic; 2,5 dihydroxybenzoic, ferulic, o-coumaric and p-coumaric acids. Chung et al. (2001) identified p-hydroxybenzoic acid (6.87 mg g^{-1}), p-coumaric acid (0.34 mg g^{-1}), ferulic acid (0.05 mg g^{-1}) in high allelopathic rice cultivars and reported the greatest inhibitory effect of p-hydroxy-benzoic acid on barnyard grass.

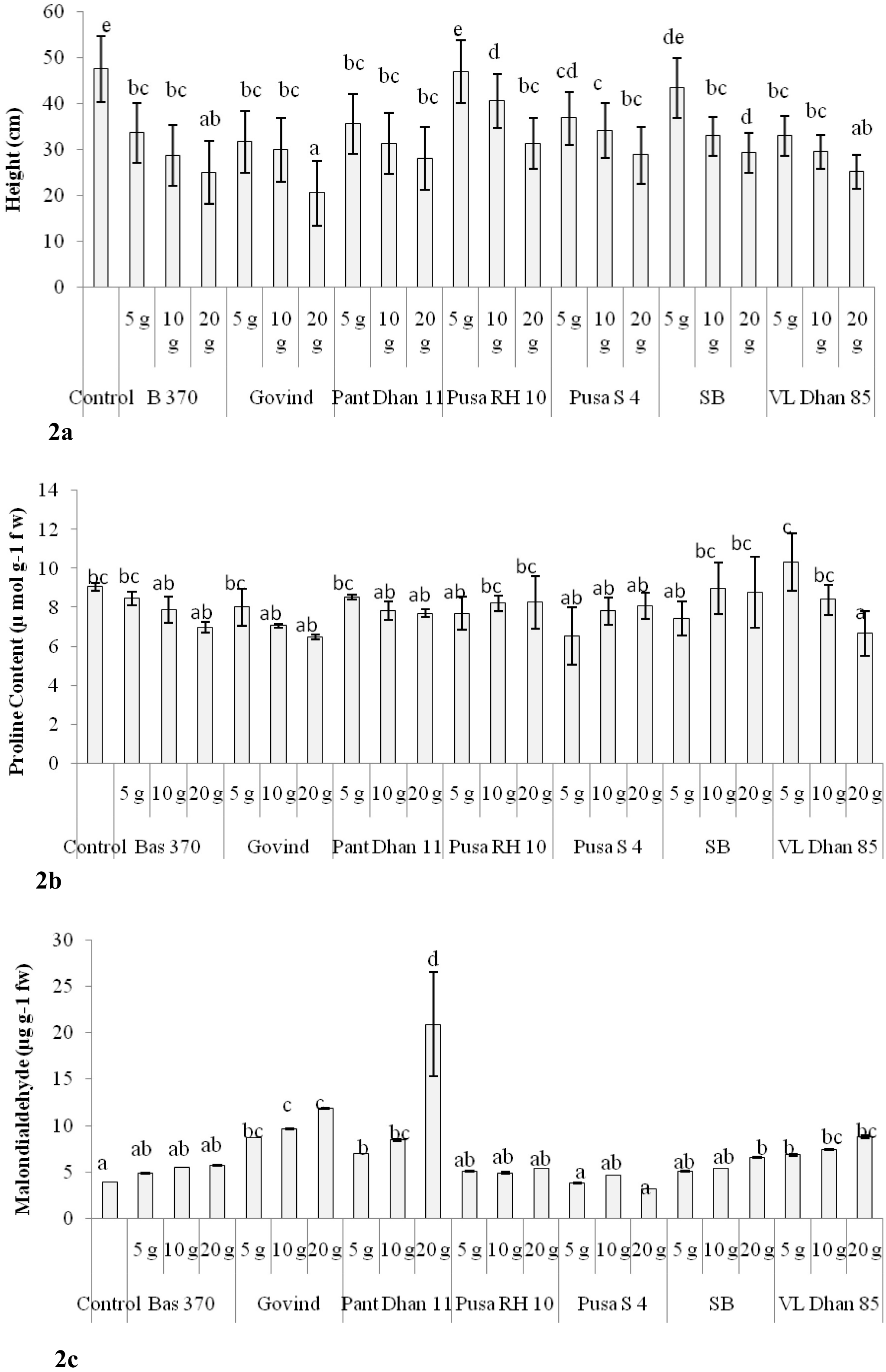

Figure 2 (a-c): Effect of different straw quantities of rice varieties on *A. ludoviciana* under pot experiment. Bar indicates standard deviation. Different letters indicate significant differences with each other.

Table 1. Number and quantity of phenolic acids identified in the straw extracts of ten different rice varieties by HPLC.

Rice Varieties	Number	Phenolic Acids Cinnamic	Gallic	Benzoic	Chlorogenic	p-HB*	Ferulic	Caffeic	Salicylic	Vanillic	Syringic	Total Amount (µg g^{-1} d.w.)
B 370	6	454.48	384.98	5.20	1.76	16.90	21.14	-	-	-	-	884.46
Govind	7	-	441.33	17.63	29.51	-	26.67	23.50	45.65	1998.45	-	2582.74
PD 11	5	-	253.33	7.03		1132.89	-	1035.92	-	373.05	-	2802.22
PD 16	3	-	386.62	-	-	93.69	-	-	-	706.72	-	1187.03
Pant S 17	3	195.48	-	-	-	2.15	-	2.86	-	-	-	200.49
Pusa RH 10	5	165.55	29.32	17.15	-	5.29	-	16.81	-	-	-	234.12
Pusa S4	4	45.25	-	1.02	-	1.52	-	3.19	-	-	-	50.98
SB	5	45.17	73.81	-	-	-	26.29	-	16.71	12.96	-	174.94
TB	3	-	-	68.00	-	-	-	-	188.62	-	38.75	295.37
VL Dhan 85	6	238.01	448.89	34.22	54.89	183.39	-		-	699.80	-	1659.20*

* p-HB= p-hydroxybenzoic acid. B 370= Basmati 370; PD 11= Pant Dhan 11; PD 16= Pant Dhan 16; Pant S 17= Pant Sugandh 17; Pusa S 4= Pusa Sugandh 4; SB= Super Basmati; TB= Tarori Basmati.

In the present study, among phenolic acids, gallic, benzoic, chlorogenic, ferulic, caffeic, salicylic and vanillic were detected in Govind; cinnamic, gallic, benzoic, chlorogenic, p-HB, caffeic and vanillic in VL Dhan 85; and cinnamic, gallic, benzoic, chlorogenic, p-HB and ferulic in Basmati 370. Quantitatively (µg g^{-1}dry wt.), cinnamic (454.48) was highest in Basmati 370; ferulic (26.67), vanillic (1998.45) in Govind; and gallic (448.88), chlorogenic (54.89) in VL Dhan 85 compared to other varieties (Table 1). However, syringic acid was present only in Tarori Basmati (38.75). TB extracts exhibited stimulatory effect on dry weight (radicle, plumule and total) while Govind and VL Dhan 85 exhibited inhibitory effect which contain higher quantity of different phenolics. Concentration of phenolics in different varieties of rice followed the order: vanillic > gallic > p-HB > caffeic > cinnamic > salicylic > benzoic >chlorogenic > ferulic > and syringic. Among phenolics, cinnamic acid was recorded in highest quantity (454.48 µg g^{-1} dry wt.) in Basmati 370; ferulic (26.67) and vanillic (1998.45) in Govind; p-HB (1132.89) and caffeic (1035.92) in PD 11; gallic (448.89) and chlorogenic (54.89) in VL Dhan 85 (Table 1). The above results were in accordance with Chon and Kim (2004) who analysed caffeic, hydro-cinnamic, ferulic, m-coumaric, p-coumaric and coumarin in extracts of wheat, oat, rice and barley by HPLC and reported hydro-cinnamic acid in highest amount. Similarly, Kuwatsuka and Shindo (1973) determined 13 phenolic acids in ether fraction of rice straw, cv. Kinmaze of these, p-coumaric, ferulic, vanillic and p-HB acids were present in the highest quantities. The maximum and minimum quantity (µg g-1 dry wt.) of total phenolics was found in PD 11 (2802.22) and Pusa S 4 (47.79), respectively. Many authors reported that these phenolics were inhibitory on germination, length (root, shoot), various physiological and biochemical reactions (Sampietro et al. 2007, Sampietro and Vattuone 2006, Esmaeili et al. 2012).

CONCLUSION

The reduction in proline was significantly correlated with number of phenolics in *A. ludoviciana* (Figure 3).

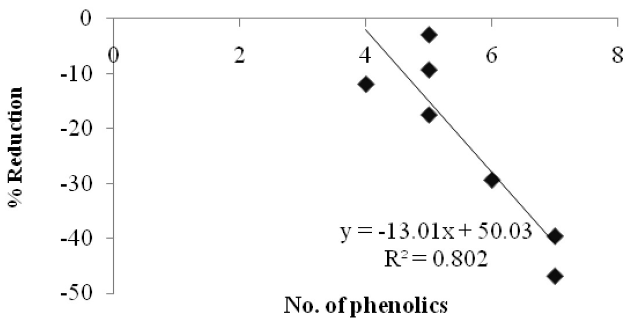

Figure 3: Relationship between number of phenolics and proline content in *A. ludoviciana*.

Among the rice straw, Basmati 370, Govind and VL Dhan 85 had high phytotoxic potential in different parameters examined against *A. ludoviciana.* In general, the effect increased with increasing amount of straw. This may be due to the release of water soluble allelochemicals (phenolics) and secondary metabolites (growth regulators, alkaloids, terpenoides, toxins, etc.) in growth medium and thus inhibiting the germination and growth of weeds (Chung et al. 2003, Rajput et al. 2010). On the basis of experimental results, the phenolic acids such as cinnamic, ferulic and vanillic might have allelopathic potential and serve as a key factor of rice allelopathy for suppressing a wide range of weeds in different crops (El-Shahawy et al. 2006). As such, the straw of rice varieties- Govind, VL Dhan 85 and Basmati 370 can be utilized to control one of the noxious weeds *A. ludoviciana*. Further investigations need to be carried out concerning the response of these effective rice varieties in field conditions, before recommending to the farmers.

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