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Allelopathic Association Between Weeds Extract and Rice (*Oryza sativa* L.) Seedlings

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Selection of weeds with greater allelopathic potential can be used as a tool in sustainable crop production. The study aims at exploring the allelopathic effects of four weed species on seed germination of the test crop (*O. sativa* L). Four weed species used are namely, *Cyperus esculentus* (Della), *Axonopus compressus* (Itsit), *Convulvulus arvensis* (Lehli) and *Parthenium hysterophorus* (Parthenium). A laboratory experiment using completely randomized design was conducted on ten local and exotic varieties of *O. sativa* by using filter-paper bioassay technique. The crude extracts were prepared in ethanol solvents (10:100 w/v) and was further used in germination experiments. Ten days old rice seedlings (including *A. compressus + C. arvensis* and *C. esculentus + P. hysterophorus*) were observed after treatments with crude extracts of weed leaves. A comparative study of the seedlings was conducted with control sets treated with distilled water. Germination % age was observed at an interval of 24hr up to 8 days and expressed as % seed germination. The interaction between *C. esculentus + P. hysterophorus* indicated a significant effect on germination rate, plumule length, radicle length, fresh weight and dry weight of seeds. Results showed that allelopathic effects caused a significant increase among rice varieties: Munji 220 (96%), Sabina (84%) and Gulfmonth (80%). Conversely, the seedling growth of Basmati 370, CB-42, B. Pak and B. 802 rice varieties were least effected by weed extracts as compare to control. Pre sowing soaking by weed extracts proved to be the most effective to germination and seedling growth of rice. The overall results indicated the possible supportive effect of allelo-chemicals present in tested weeds on rice seeds.

Key words: *Oryza sativa* seeds, allelopathy, germination, seedling growth, weed extracts.

Rice (*Oryza sativa* L.) occupies 11% of world's crop area supplying approximately 23% of the per capita energy for six billion people worldwide[15]. Rice is placed on second position in cereal cultivation around the globe and occupies an important position in the economy of Pakistan as an export item as well as staple food[5]. Pakistan is famous for producing and exporting long-grain aromatic basmati rice. In addition it also exports a substantial quantity of coarse rice. Rice exports are around 3 million tons per annum. The provincial shares of Punjab, Sindh, NWFP and Baluchistan in the overall rice area: 69, 22, 3 and 6 per cent, and in production 58, 30, 3 and 9 percent respectively[10]. In Punjab approximately 88% of the total rice area is under Basmati varieties. Major producing areas include Gujranwala, Hafizabad, Sheikhupura,

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Sialkot, Jhang and Okara of Punjab and Larkana, Jacobabad, Shikarpur, Badin, Dadu and Thatta districts of Sindh[3]. In Pakistan, rice is mainly grown in the Sindh and Punjab. The Sindh is specialized in producing the Long grain white rice IRRI-6 and IRRI-9, while Punjab is producing world class Basmati rice among IRRI-9 and other varieties. Punjab is the biggest producer of rice in the country and contributes 58 per cent to national production while the provinces of Sindh, Baluchistan and NWFP to 29, 3 and 10 per cent, respectively. Some of the important varieties grown in the country are Super Basmati, Kernel Basmati, Basmati 385, IRRI-6, IRRI-9, KS-282, DR-82 and DR-83[4].

The influences of weeds on crops and vice versa have important implications in agriculture, as these govern and direct the planning of suitable agricultural operations. An understanding of the effects of weeds on seed germination and seedling growth of crop plants is essential in exploiting the possible improvement of crop productivity[12]. The soluble allelochemicals from weeds which leach out come into direct contact with crop root. There are several reports, which indicate that allelopathic potentiality of weeds plays a major role by affecting the crop growth and nutrient status of soil. Chemicals with allelopathic potential exist in almost all plants and most tissues and can positively or negatively affect growth vegetation[20]. Some allelochemics are intermediates for lignification and can also activate plant defense after exposure to pathogens. Therefore, they have a structural or physiological role within plants. The recognized importance of allelopathy in agriculture has increased with the main objectives of using allelopathy in crop productivity[11]. Reports indicate that some allelochemicals delay germination. Besides most research on allelopathy has focused on the effect of interactions among weed species, weeds and crops, and crop species[13,22]. Therefore the main objective of present study was to evaluate the various physical parameters of local and exotic lines of rice varieties with reference to their allelo pathic effects. In addition, to check the allelochemicals effect, extracted and released in a natural way from *C. esculentus* (Della), *A. compressus* (Itsit), *C. arvensis* (Lehli) and *P. hysterophorus* (Parthenium), on the germinability of rice seeds.

MATERIALS AND METHODS

Procurement of Rice Germplasm

Sample of 15 local varieties of rice viz., Basmati-370, Shaheen-basmati, CB-36, CB-21, CB-39, Sufaida-154, CB-42, IRRI-6, Basmati-Pak, Basmati-2000, DHAN KASARWALA 108-31, CHAKLALA-214, KASARWALA & MUNDAR, JHONA 91-A, JHONA-153 were obtained from Rice Research Institute, Kala Shah Kaku, Lahore (Table 1) and stored in bags of 250 grams. Exotic rice germplasm of 15 different lines of rice viz., MARICH BATI, CYPRESS, RONDO, IRRI-IRGC-101524, IRRI-IRGS-101073, WW8/2290, B53R3540, IR-64, WAB462-10-3-1, Ru8803072, REXMONTH, W1193, STg-567989, SIERRA, PRESIDIO was obtained from United States Department of Agriculture, USA (Table 2).

Selection of Seeds

Seeds from each rice samples were randomly selected and tested for different physical traits (seed length, seed width, seed thickness, length/width, and 1000 seed weight) and germination tests.

Measurement of Physical Characteristics of Local and Exotic Germplasm lines

Physical characteristic of 15 local varieties and 15 exotic varieties of rice were measured. For this purpose, 1000 seed weight for each sample was measured on a digital weighing balance, and three readings were taken for each sample. Seed length, seed width and seed thickness of these local rice varieties was measured with the help of a digital Vernier Calliper. For each sample of rice, these measurements were taken in three replicates; in each replicate 5 seeds were measured. The seeds were randomly selected from the seed samples.

Collection of Weed Material

The dominant weed species of *C. esculentus*, *A. compressus*, *C. arvensis* and *P. hysterophorus* were collected (aerial parts at maturity) from different fields of Lahore. Then leaves of each weed were washed, shade dried and finely ground with mortal and pestle.

Preparation of Ethanol Extract of weeds

Powder of each shade dried weed materials (10 g) was put in the thimble and extracted successively with 100 mL ethanol solvent for 48 h by using a soxhlet extractor. Then extract was

concentrated using rotary flash evaporator, weighed and preserved in airtight bottles at 4ºC until further use[1]. The prepared extracts of *A. compressus* + *C. arvensis* and *C. esculentus* + *P. hysterophorus* are mixed in DMSO and made two concentrations.

Filter Paper BioAssays

Heathly, uniform and sterilized 30 seeds of Stg567989, New bonnet, Sabina, Gulf Mont, Basmati 802, Basmati Pak, IRRI-6, Basmati-370, CB-42 were selected. Ten seeds were dipped in each mixture (treatments) and sterilized distilled water (control) and placed on petri dish (9.0 cm dia.) containing two blotting papers for each variety with triplicates. The seeds were moistened daily either with water and weed mixtures. Petri dishes were kept under laboratory conditions and readings were observed at an interval of 24hr up to eight weeks.

Measurement of growth parameters

At the end of the experiment, germination rate was determined weekly by using the formula (Germinated seed/Total seed × 100) for each replication of the treatment[19]. The length of radicle and plumule were measured in centimeters from the point where the radicle and plumule joins together at the end of the radicle and to the top of the plumule, in addition fresh and dry weight were also measured by using the method of Tanveer *et al*.[23]. All measurements were done in three replicates and the mean calculated.

Statistical Analysis

The data were analyzed using Analysis of Variance (ANOVA) test. The means of treatments were grouped on the basis of least significant difference (LSD) at the 0.05 probability level.

Table 1. Plant Material

Sr.No	Name	Origin	Accession Number	Taxonomy
1	Presidio	United States	PI 633624	*Oryza sativa*
2	Sierra	United States	PI 633623	*Oryza sativa*
3	IRRI-IRGC-101524	India	GOSR311699	*Oryza nivara*
4	IRRI-IRGS-101073	Philoppines, Mindanan	GSOR311700	*Oryza officinalis*
5	W1193	Brazil, Amozonas	GSOR311703	*Oryza rufipogon*
6	B53R3540	Thailand	GSOR310024	*Oryza sativa*
7	WW8/2290	Netherlands	GSOR310494	*Oryza sativa*
8	Stg 567989	United States, Arkansas	GSOR311705	*Oryza sativa*
9	Marich Bati	Bangladesh	GSOR311729	*Oryza sativa*
10	Cypress	United States, Louisiana	GSOR311792	*Oryza sativa*
11	IR-64	Philippines, Luzon	GSOR311793	*Oryza sativa*
12	Rondo	United States	PI657830	*Oryza sativa*
13	Ru8803072	United States, Arkansas	PI590419	*Oryza sativa*
14	WAB462-10-3-1	Cote D'lvorie	GSOR311790	*Oryza sativa*
15	Rexmont	United States, Texas	PI502968	*Oryza sativa*
16	Basmati 370	RRI- Pakistan	Approved Variety	*Oryza sativa*
17	Shaheen-Basmati	SSRI- Pakistan	Approved Variety	*Oryza sativa*
18	Basmati-Pak	RRI- Pakistan	Approved Variety	*Oryza sativa*
19	Basmati 2000	RRI- Pakistan	Approved Variety	*Oryza sativa*
20	CB-36	RRI- Pakistan	4427	*Oryza sativa*
21	CB-21	RRI- Pakistan	4048-11	*Oryza sativa*
22	CB-39	RRI- Pakistan	33608	*Oryza sativa*
23	CB-42	RRI- Pakistan	33797-1	*Oryza sativa*
24	IRRI- 6	RRI- Pakistan	Approved Variety	*Oryza sativa*
25	Chaklala-214	RRI- Pakistan	0082	*Oryza sativa*
26	Dhan kasarwala 108-31	RRI- Pakistan	0112	*Oryza sativa*
27	Kasarwala & Mundar	RRI- Pakistan	0115	*Oryza sativa*
28	Sufaida-154	RRI- Pakistan	0175	*Oryza sativa*
29	Jhona 91-A	RRI- Pakistan	0109	*Oryza sativa*
30	Jhona -153	RRI- Pakistan	0129	*Oryza sativa*

RESULTS

In present study various physical parameters of local and exotic lines of rice varieties were showed. Moreover the allelopathic effect of different weeds (*C. esculentus*, *A. compressus*, *C. arvensis* and *P. hysterophorus*) were checked on the germination of rice seeds, these allelochemicals were extracted and released in a natural way. Results exhibited that the seed length, width, thickness, length/width and 1000 seed weight of the local and exotic varieties of rice were measured and it was clear that Stg-567989 (10.36mm) has the healthiest looking grain with husk among fine and coarse rice varieties used for study; since it has longest grain, equal in seed width and thickness with IRRI-6 (2.93mm and 2.92mm). Among fine grain 'Stg-567989' had highest 1000 seed weight (30.2g) whereas 'B53R3540' had the least weight of 17.2g. Exotic varieties have highest seed length to width ratio. Furthermore, finding of this study were demonstrated that allelopathic effects caused a significant increase among rice varieties: Munji 220 (96%), Sabina (84%) and Gulfmonth (80%). On the

Table 2. Physical Parameters of different lines of Rice seeds (*O. sativa* L

S.V	DF	SL	SW	ST	L/W	1000GW
Genotypes	29	1.0084**	0.13897**	0.05959**	0.6950**	34.1827**
Replications	2	0.0512	0.00275	0.00717	0.0425	0.0498
Error	58	0.0215	0.00315	0.00440	0.0142	0.0718

Level of Significance: P<0.05=* P<0.01=**
*= significance, **= highly significance

Table 3. Simple Correlation among different seed physical parameters of Rice seeds (*O. sativa* L.)

Traits	SL	SW	ST	L/W	1000GW
SL	1.00				
SW	-0.078ns	1.00			
ST	0.095*	0.390**	1.00		
L/W	0.245**	-0.236**	0.132**	1.00	
1000GW	0.293**	0.490**	0.489**	0.041ns	1.00

Level of Significance: P<0.05=* P<0.01=**
*= significance, **= highly significance, NS= Non significant

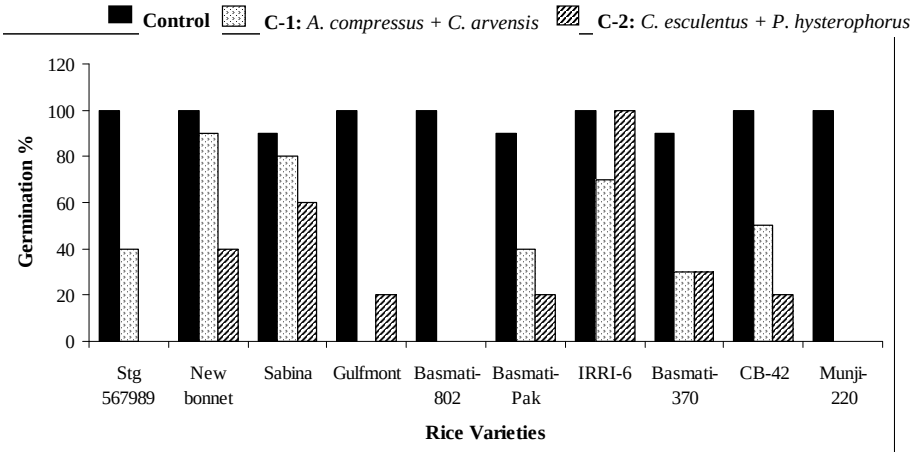

Fig. 1. Germination rate of different lines of Rice seeds after 3 days

Table 4. Two way anova of mean square values of various rice seedling traits at various concentrations

Sov	DF	Control				Concentration-1				Concentration-2			
		PL	RL	FW	DW	PL	RL	FW	DW	PL	RL	FW	DW
Genotypes	9	6.018**	3.618**	0.001ns	0.624 ns	5.622**	7.671**	0.002ns	0.442*	5.321**	0.376**	0.002 ns	0.00002ns
Replications	2	1.803	1.087	0.003	0.608	0.329	0.229	0.001	0.458	0.061	0.154	0.0007	0.00005
Error	18	0.967	0.438	0.002	0.637	0.973	0.915	0.003	0.445	0.334	0.073	0.002	0.00004

Level of significance $p<0.05=^{*}$, $p<0.01=^{**}$ and ns = non significant. PL; Plumule length, RL; Radical length, FW; Fresh weight, DW; Dry weight, Concentration1: *A. compressus* + *C. arvensis* and Concentration2: *C. esculentus* + *P. hysterophorus*

other hand the seedling growth of Basmati 370, CB-42, B. Pak and B. 802 rice varieties were least effected by weed extracts as compare to control. The different solvent extracts of weed used in this study produced negative and positive allelopathic effects on germination and seedling vigor of rice seeds (see tables). In the case of IRRI-6, the solvent extracts of weeds produced a significantly higher germination rate than the rest of the treatment combinations. Conversely the lowest germination rate was observed in Munji-220 variety while Gulfmont and CB-42 showed moderate rate of seed germination. Pre-sowing soaking by weed extracts proved to be the most effective to germination and seedling growth of rice. The overall results indicated the possible supportive effect of allelochemicals present in tested weeds on rice seeds. On the basis of these findings it is that these weed extracts has biomolecules in its organs in various concentrations; therefore it is necessary to keep these weed under check at the emergence stage in field trials.

DISCUSSIONS

The length, width, seed thickness, length width ratio and 1000 grain weight is one of the quantitative measures for grain size and grain shape. Grain morphology i.e. colour, size and shape having unique position for the breeders during the selection and evaluation process[14,9]. In the present study all the genotypes showed significant variations with respect to their desirable seed morphological traits. It is thought to relate the largest shape variation in small grain crops. On the other hand, length width ratio is the major genetic variation of rice grain shape and highly associated with the quantitative traits parameters and can be used in the breeding program for the improvement of the rice varieties[6,7]. The results exhibited that seed length, width, thickness, length/width and seed weight is highly significance because the values are less than 0.01 and 0.05 by using variance. It may also involve in seed health and vigour that leads to increase the germination rate and ultimately increase the yield potential and also important for the allelo chemicals studies that produce from the various rice varieties. It is also a sign of healthy crop and good seeing to the scientist and farmers community. Furthermore results demonstrated that

the seed width and seed length have negative correlation and non-significant. Positive and negative correlations both important for plant biology study. Although it was a preliminary laboratory study yet it provided encouraging results and basis for future research. This may be important in increasing the germination and seedling growth of rice varieties in field and establishment of sustainable agriculture.

Another aspect of allelopathy is a potential field of research all over the world. Few researchers consider only the deleterious interactions as allelopathy, while, the latest rational includes allelopathy to both harmful and beneficial interactions between the plants[19]. In agriculture, the inhibitory effect of weed species on germination and growth of crops has been attributed to phytotoxic chemicals released from the leaf litter and roots. On the contrary in this context, a study was undertaken to elucidate the effects of few common weed species on germination behavior, root and shoot growth of some of local and exotic lines of rice varieties[12]. Many allelochemicals e.g., parthenin, pcoumaric acid, caffeic acid, coronopillin, and sesquiterpene lactones from the aqueous extracts of *Parthenium* responsible for positive allelopathic effects have also been reported[16,8]. In this experiment, the effects of these

Table 5. Correlation studies of different rice genotypes seedling traits at various concentrations

Concen	Control				Concentration-1				Concentration-2			
Traits	PL	RL	FW	DW	PL	RL	FW	DW	PL	RL	FW	DW
PL	1.00											
RL	-0.072*	1.00										
FW	-0.067^{ns}	0.115*	1.00									
DW	-0.055^{ns}	-0.103^{ns}	0.031^{ns}	1.00								
PL	-0.036^{ns}	0.194**	-0.021^{ns}	-0.096^{ns}	1.00							
RL	-0.279**	0.063*	0.060*	-0.065^{ns}	0.382**	1.00						
FW	-0.084^{ns}	0.134*	-0.080*	-0.015^{ns}	0.064*	0.089*	1.00					
DW	-0.031^{ns}	-0.015^{ns}	0.080*	0.092*	0.106*	0.020^{ns}	0.074*	1.00				
PL	0.018^{ns}	0.035^{ns}	-0.018^{ns}	-0.015*	-0.453**	-0.393**	0.037*	-0.090*	1.00			
RL	-0.079*	-0.121*	-0.080^{ns}	-0.078*	-0.299**	0.097*	0.106*	-0.060^{ns}	0.535**	1.00		
FW	-0.063*	-0.014^{ns}	-0.180**	0.062^{ns}	-0.008^{ns}	0.074*	0.368**	0.045^{ns}	0.034^{ns}	0.060*	1.00	
DW	0.093*	-0.172**	0.055^{ns}	0.023*	0.020^{ns}	0.034^{ns}	-0.022^{ns}	-0.054^{ns}	-0.016^{ns}	0.033^{ns}	-0.027^{ns}	1.00

Level of significance p<0.05=*, p<0.01=** and ns = non significant. PL; Plumule length, RL; Radical length, FW; Fresh weight, DW; Dry weight, Concentration1: *A. compressus* + *C. arvensis* and Concentration2: *C. esculentus* + *P. hysterophorus*

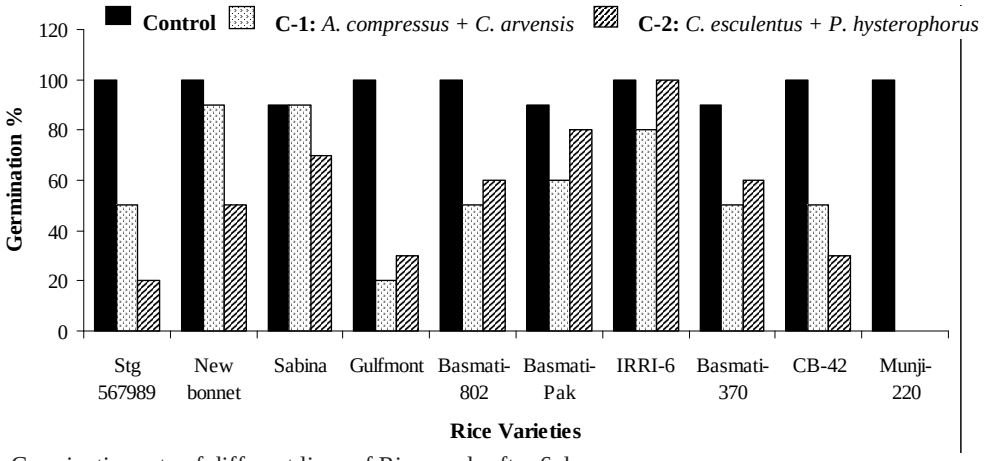

Fig. 2. Germination rate of different lines of Rice seeds after 6 days

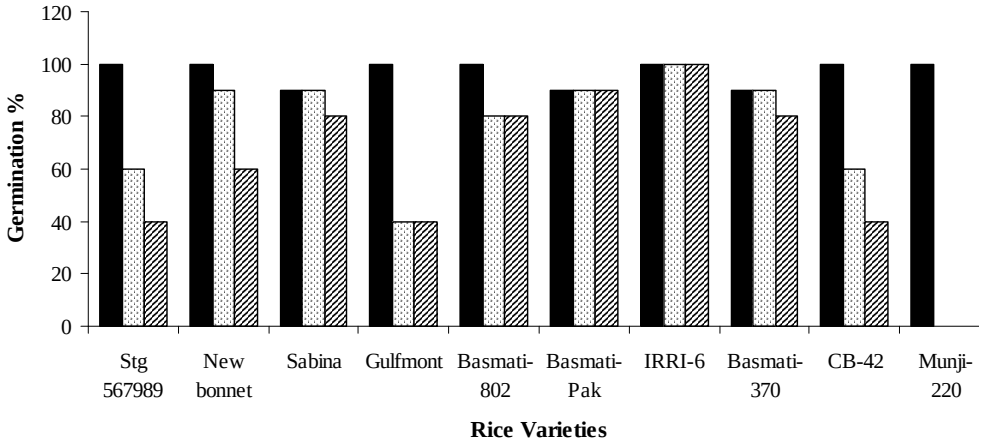

Fig. 3. Germination rate of different lines of Rice seeds after 10 days

allelochemicals were significantly showed while phytotoxic effects of these allelochemicals were not observed. Some growth-promoting substances such as glucose, galactose, and potassium chloride in the diluted aqueous extracts of *Parthenium* have also been reported[22]. This is probably why the aqueous extract of *Parthenium* generally produced germination comparable with that of the control, and higher roots and shoot elongation and dry matter accumulation[18]. Despite the fact that the negative (stimulatory) allelopathic effects of different aqueous extracts of *Parthenium* on field crops have been reported Oudhia[18], Oudhia and Tripathi[17]. The present study indicated the possibility of using different extracts of weeds (*C. esculentus*, *A. compressus*, *C. arvensis* and *P. hysterophorus*) to promote early germination and seedling growth of rice seeds through soaking treatments. Repetition of this work using different hybrid rice varieties and different concentrations would provide a better understanding of allelopathy[18]. Nevertheless Kalita and Dey[13] found that higher inhibitory activity was by shoot extract than root extract of some weeds viz. *Ageratum cenyzoides*, *Borreria hispida*, *Cynodon dactylon* and *Cyperus rotandus* on growth of rice[20]. On the other hand, different weed extracts used as a bio-control agents and very effective for conducting different experiments[2] (Amna et al., 2013). Allelopathic effects of different weeds on wheat and chickpea crops have been reported in the literature[7,14,21,23] but no research has yet been conducted on the allelopathic effects of weeds specifically on local and exotic lines of rice varieties.

CONCLUSION

The information generated from the study support the rational of positive allelopathic interaction between weed's extract and rice germination, plumule and radicle growth. This effect was possibly due to the presence of favourable allelochemicals in these weeds. The results could be used for the production of rice varieties on large scales which is equally beneficial to the plant biologist scientists and farmers community by applying solvent extracts of different weeds. Further investigations are also needed to identify the various active compounds and their composition that are involved in allelopathy.

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