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Evaluation of intra-cultivar interaction of rice (*Oryza sativa* L.) In presence of aqueous testa extract of cashew-nut (*Anacardium occidentale* L.) Under NaCl salinity during early seedling growth

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Abstract
Intra-cultivar interaction of basmati (cv. Kasturi) and non-basmati (cv. Pantdhan 11) mixed cultures of rice (*Oryza sativa* L.) in presence of aqueous extracts of testa of cashew-nut (*Anacardium occidentale* L.) under NaCl salinity during seedling stage was studied in laboratory conditions. Basmati and non-basmati seeds as mono or mixed cultures were sown at 0, 0.5, 1.0% (w/v) NaCl concentrations along with 2.5 and 5% aqueous testa extracts (TE=1.5gm/100mL) at 29±2ºC under dark for 10 days. Results showed that, significant competitive interaction in mixed-culture of rice cultivars under salinity stress in presence of aqueous testa extracts. In 0.5% salinity+TE treatments of basmati/non-basmati mixed cultures, AS (speed of accumulated germination) data indicates sensitivity to germination in basmati and stimulation in non-basmati cultivars. SVI (seedling vigour index) values showed mixed culture had positive impact on basmati and negative impact on non-basmati during early days of seedling emergence. RCI (relative competitive intensity) values for root length, seedling height under 1.0% (w/v) salinity+5% TE treatment showed competitive facilitation in basmati and competitive inhibition in non-basmati and competitive facilitation for seedling dry weight in both the cultivars. CR (competitive ratio) values suggest under 0.5% (w/v) NaCl+2.5% TE and 1.0% (w/v) NaCl+5% TE treatments basmati was better competitor for root length, seedling height and dry weight than non-basmati cultivar. The relative interaction index (RII) showed competitive interaction for accumulating both shoot and root biomass. Root and shoot biomass increased under 0.5% (w/v) NaCl+TE and 0.5% NaCl + 5% TE respectively in basmati and decreased in non-basmati. Shoot biomass increased in 1.0% (w/v) Na Cl + 5%TE in both the cultivars. Presence of allele chemical (TE), competitive ability of basmati (cv. Kasturi) increased under Na Cl salinity stress.

Keywords: Competition, interaction, cultiver, mixed, salinity, stress, testa, cashew-nut

Introduction
Studies show that evaluation of allele chemicals strongly depends on germination responses [1]. Plant growth and root to shoot ratio is outcome of cultivar (genotype) interaction with environment under the control of ambiguous (unknown) factors [Biomass distribution theory]. Germination and seedling development determine the final density of the plant. Germination rate is the index of tolerance to stress [2]. Salinity affects the germination of seeds [3] and reduces the elongation of root and shoot [4] by primarily affection water uptake. Studies showed that dry matter allocation increased with increased cultivar diversity [5]. Stress affects root, shoot ratio, length and dry matter allocation [6]. Variation in root and shoot length and dry matter is cultivar dependent [7]. Reports show that under competitive environment, stem allocation less increased over its length [8]. The stimulatory or inhibitory effect of allelochemical depends on the amount released by the plant and genotype [9-10]. Allelochemicals are either single or mixture secreted in secondary metabolic pathway [11]. Allelochemicals act as natural herbicides [12] and has short life period [13]. In nature, plants secrete allelochemicals in response to different factors includes stress [14-16] helps in defense [17-18] to suppress the neighbor [19].

Materials and Methods:
Testa of cashew nut seed was procured from cashew processing unit located around Chirala-Andhra Pradesh, India. Freshly harvested seeds of cv. Kasturi [20] and cv. Pantdhan 11 (hence onwards cv. Pant 11) collected from agriculture field located near Selaqui region, Dehradun,

Uttarakhand, India. Testa was powdered and passed through 150 µm sieve. 1.5 Gm of 150 µm size powder soaked in 100 ml water for 24 hours at 29±1°C [21] and the aqueous extracts collected by filtering through What man filter paper 1 and used for preparing 2.5 & 5% dilutions and passed through 0.2 µm membrane filter before applying on rice seeds. Rice seeds were soaked for 24 hours in distilled water. Seeds were surface sterilized with 0.1% (w/v) $HgCl_2$ for 10 minutes and washed with sterile distilled water for 5 times. Petri dish (9 cm diameter in size) lined with a thin layer of cotton and What man filter paper 1 moistened with 6 mL of sterile double distilled water (as control treatments) or treatments. Salinity treatments of 0.5% & 1.0% (w/v) Na Cl with or without aqueous test extract (2.5 or 5% TE) and 8 rice seeds were disposed [22-23] either mono or mixed culture. There were duplicates for each treatment in completely randomized block design. Petri dishes were incubated in incubator with 95% humidity and temperature (29±1°C) in dark for 10 days.

Germination test was performed as per ISTA rules, 1999. Germination of seeds was recorded for seven days after every 24 hours. Seedling growth was measured on 7th and 10th day. Shoot and root length (SL&RL) and seedling height (SH), fresh and dry weight of shoot and root were measured. Germination percentage [24], Speed of germination [25], seedling vigor index [25] and seedling growth parameters were used for evaluation of effect of test aqueous extract on Na Cl salinity tolerance. Relative competition intensity (RCI), competitive ratio (CR), inhibitory rate (IR %) and relative interaction index (RII) were calculated to evaluate the effect of testa extract on intra-cultivar interaction and competitive ability of two rice cultivars under Na Cl salinity.

Performance indices (root length, shoot height and seedling dry weight) of two rice cultivars were used to calculate relative competition intensity (RCI) [26].

$$RCI = (P_{mono} - P_{mix})/P_{mono}$$

Here P_{mono} represents the performance indices of plant in mono culture and P_{mix} is performance indices of a plant in a mixed- cultures (treatments). RCI was calculated and used to evaluate the competition between two rice cultivars. Positive RCI value indicates competitive inhibition; negative RCI value indicates competitive facilitation.

Competitive ratio was calculated to compare competitive ability of two cultivars in different treatments. It was calculated [13].

$$CR_{ab} = (P_{mix,}\, a / P_{mono,}\, a)/ (P_{mix}\, b / P_{mono,}\, b)$$

Here CR_{ab} is competitive ratio of one cultivar on other cultivar. CR value indicates the ratio by which one cultivar is more competitive than the other.

Inhibitory rate (IR) was used to assess inhibition of one cultivar on other cultivar in presence of testa extract. The IR was calculated [13].

$$IR\ (\%) = (1\text{-treatment/control}) \times 100$$

IR>0 and IR<0 indicate inhibitory effect and stimulatory effect respectively.

Each target seedling was measured on shoot, root growth and shoot and root were separated and dried at 100°C in oven for 1 hour and both parts were weighed to measure the biomass. Seedling biomass in presence of competition in relation to with and without competition was measured as relative interaction index (RII) [27].

$$RII = (P_{mix} - P_{mono})/ (P_{mix} + P_{mono})$$

Here P_{mono} is the biomass of target cultivar in monoculture and P_{mix} is the biomass of target cultivar in mixed-culture. Combination of shoot and root competition may be negative, positive or zero value.

All the experimental data was subjected to one way ANOVA [28] followed by the least significant difference (LSD) at a 5% level of probability [29]. The statistical analysis was performed using excel 2007 and preparing graphs and tables.

Results and Discussion

Speed of germination was fast in mixed cultures over mono cultures in both the cultivars. In cv. Kasturi, in the presence of 2.5% TE, germination speed was increased in both mono and mixed cultures in control and in presence of 0.5% (w/v) NaCl.

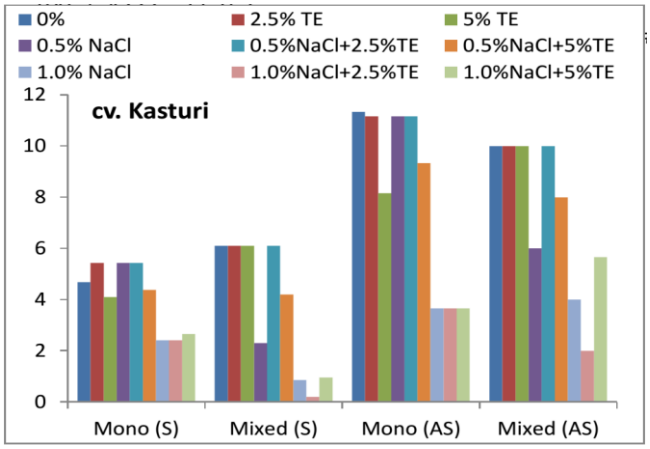

Fig 1: S and AS of cv. Kasturi at 7 days in presence of aqueous testa extract (TE) under NaCl salinity stress, Mono= cv. Kasturi, Mixed= cv. Kasturi & cv. Pant 11, P=0.01.

Accumulated speed of germination was higher in mono over mixed cultures in cv. Kasturi and accumulated speed of germination was higher in 2.5% TE treatment in presence or absence of 0.5% (w/v) NaCl in mono culture.

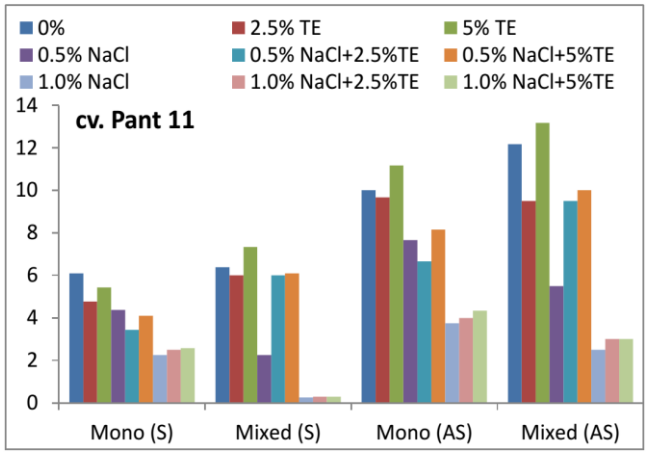

Fig 2: S and AS of cv. Pant 11 at 7 days in presence of aqueous testa extracts (TE) under NaCl salinity stress, Mono= cv. Pant 11, Mixed= Pant 11& cv. Kasturi, P=0.01.

In cv. Pant 11, speed of germination was fast in presence of 5% TE without salt treatments in mixed cultures. Accumulated speed of germination was higher in mixed over mono cultures in cv. Pant 11 and accumulated speed of

germination was higher in 5% TE treatment in presence or absence of 0.5% (w/v) NaCl in both mono and mixed cultures.

Seedling Vigour Index

SVI results indicate mixed culture had positive impact on basmati and negative impact on non-basmati during early days of seedling emergence.

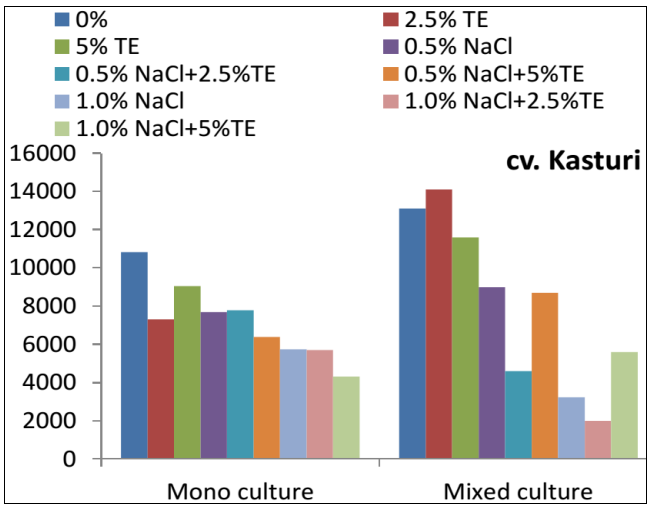

Fig 3: SVI of cv. Kasturi at 7 days in presence of aqueous testa extract (TE) under NaCl salinity stress, Mono= cv. Kasturi, Mixed= cv. Kasturi & cv. Pant 11, P=0.01.

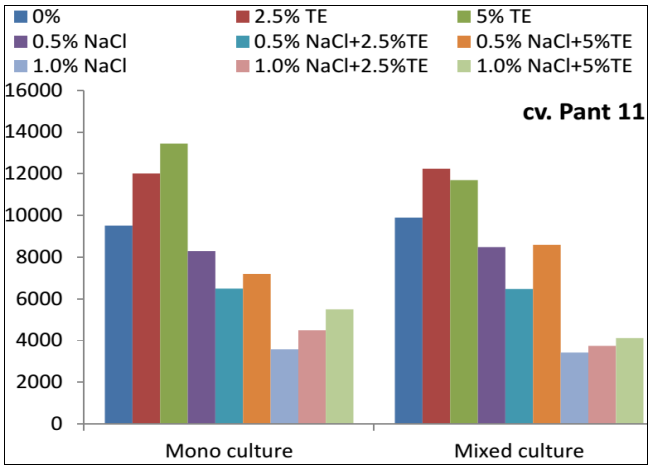

Fig 4: SVI of cv. Pant 11at 7 days in presence of aqueous testa extract (TE) under NaCl salinity stress, Mono= cv. Kasturi, Mixed= cv. Kasturi & cv. Pant 11, P=0.01.

Morphological Parameters

In cv. Kasturi, for rl, all treatments were less growth over control in both mono and mixed cultures at 7th day. However, 0.5%NaCl, 2.5% TE, 1.0% NaCl+2.5% TE, 5% TE, 1.0% NaCl+5% TE rl is better in mixed cultures over mono cultures. 0.5%NaCl+2.5% TE treatment in mixed cultures exhibited least growth of root in cv. Kasturi. SH is increased in mixed culture over mono culture. 0, 2.5% TE, 5% TE showed SH more over control and mono cultures both on 7th and 10th day. In 0.5%, 1.0% NaCl+2.5%TE & 1.0% NaCl+5%TE SH was more than mono cultures.

Table 1: Morphological parameters in mono and mixed cultures of cv. Kasturi at 7th and 10th Day; RL= Root length, SH= Seedling height, SdDW= Seedling dry weight, *** P=0.001, ns= not significant.

cv. Kasturi		7th Day						10th Day					
Morphological Parameters		RL (mm)		SH (mm)		SdDW (mg)		RL (mm)		SH (mm)		SdDW (mg)	
NaCl (%)	TE (%)	Mono	Mixed	Mono	Mixed	Mono	Mixed	Mono	Mixed	Mono	Mixed	Mono	Mixed
0	0	71	64	108	131	4.55	3.8	76	75	133	145	7.7	5.4
	2.5	42***	69***	73***	141***	3.6***	5.3***	48***	77**	112***	151^{ns}	6.8***	6.7***
	5	52***	59***	90***	116***	3.7***	4.6***	55***	65***	123***	136**	6.9***	7.7***
0.5	0	43***	63^{ns}	77***	90***	3.1***	1.8***	72***	66***	135^{ns}	103***	5.7***	5.5*
	2.5	44***	26***	77.7***	46***	3.3***	4.3***	45***	52***	87***	93***	6***	5.5*
	5	37***	30***	57***	40***	1.9***	1.5***	40***	40***	62***	57***	1.9***	2.4***
1	0	48***	32***	57.5***	43***	1.2***	1.5***	51***	34***	62***	48***	2.8***	2.4***
	2.5	41***	51***	64***	87***	2.7***	2.6***	50***	52***	118***	97***	5.5***	5.5*
	5	29***	35***	43***	56***	1.6***	1.4***	30***	36***	59***	71***	3.5***	4***

0.5%NaCl+5% TE treatment in mixed cultures exhibited least growth of seedling height in cv. Kasturi. SdDW increased in treatments with 2.5 & 5% TE in mixed culture over control and treatments in mono culture at 7th day. However, 5%TE with salt treatments showed increased value over its mono culture. 0.5%NaCl+2.5% TE in mixed culture was showed better over mono culture. %NaCl+2.5% TE treatment in mixed cultures exhibited least growth of root in cv. Kasturi. At 10th day, root growth increased in 2.5%TE in mixed culture over control and all other treatments. NaCl in presence of TE in mixed culture was showed growth better over mono cultures. SH in 0.5% NaCl+2.5%TE was showed increased in seedling height at 10th day.

Table 2: Morphological parameters in mono and mixed cultures of cv. Pant 11 at 7th and 10th Day; RL= Root length, SH= Seedling height, SdDW= Seedling dry weight, *, ** and *** are P=0.05, 0.01 and 0.001 respectively, ns= not significant.

cv. Pant 11		7th Day						10th Day					
Morphological Parameters		RL (mm)		SH (mm)		SdDW (mg)		RL (mm)		SH (mm)		SdDW (mg)	
NaCl (%)	TE (%)	Mono	Mixed	Mono	Mixed	Mono	Mixed	Mono	Mixed	Mono	Mixed	Mono	Mixed
0	0	43	47	95	99	4.5	4	74	58	150	128	7.3	6.6
	2.5	69***	76***	120***	140***	4.6***	2.2***	76***	78***	146***	155***	5***	6.8***
	5	64***	56***	134***	117***	5.95***	4.2**	66***	59*	139***	123***	6.6***	7.4*
0.5	0	45ns	65***	83***	97ns	2.9***	4.1*	50***	67***	104***	120***	4.5***	4.5***
	2.5	37***	40***	65***	74***	3.1***	2.56***	50***	48***	110***	105***	6.2***	3***
	5	30***	35***	45***	50***	1.26***	0.95***	32***	39***	72***	60***	5.2***	2.4***
1	0	32***	40***	41***	55***	1.79***	2***	39***	41***	63***	56***	3***	2.4***
	2.5	37***	43*	72***	86***	5.2***	3.8***	40***	44***	104***	99***	5.2***	4.5***
	5	37***	35***	55***	55***	1.35***	3.2***	42***	39***	82***	70***	4.1***	4.5***

Root/shoot ratio

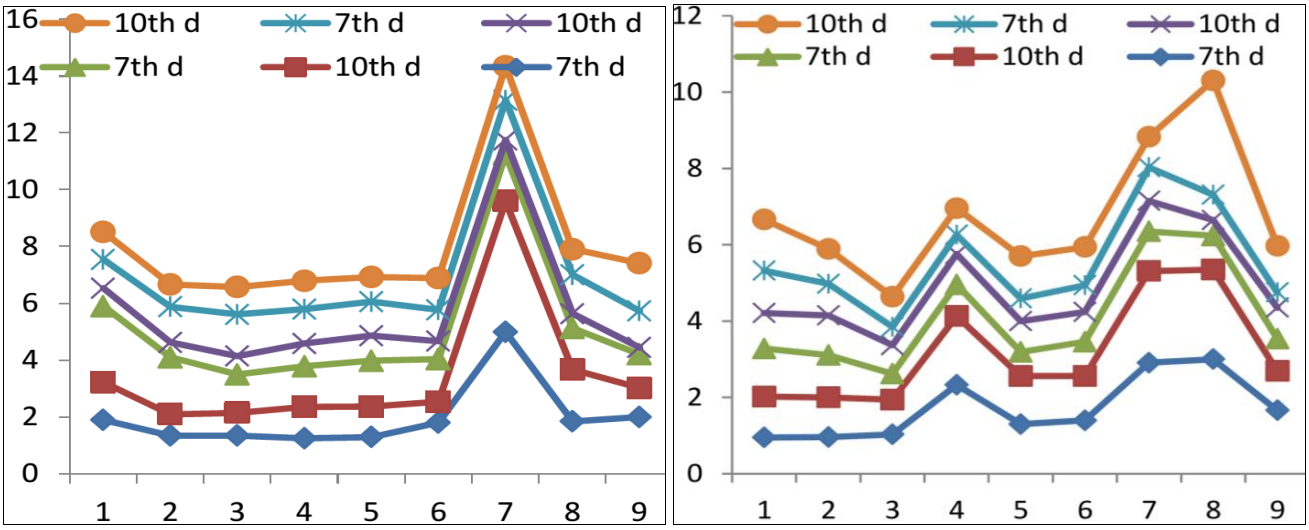

Fig 5: Root/shoot ratio of cv. Kasturi at 7 & 10 days in presence of aqueous testa extract (TE) under NaCl salinity stress, Mono= cv. Kasturi, Mixed= cv. Kasturi & cv. Pant 11, P=0.01.

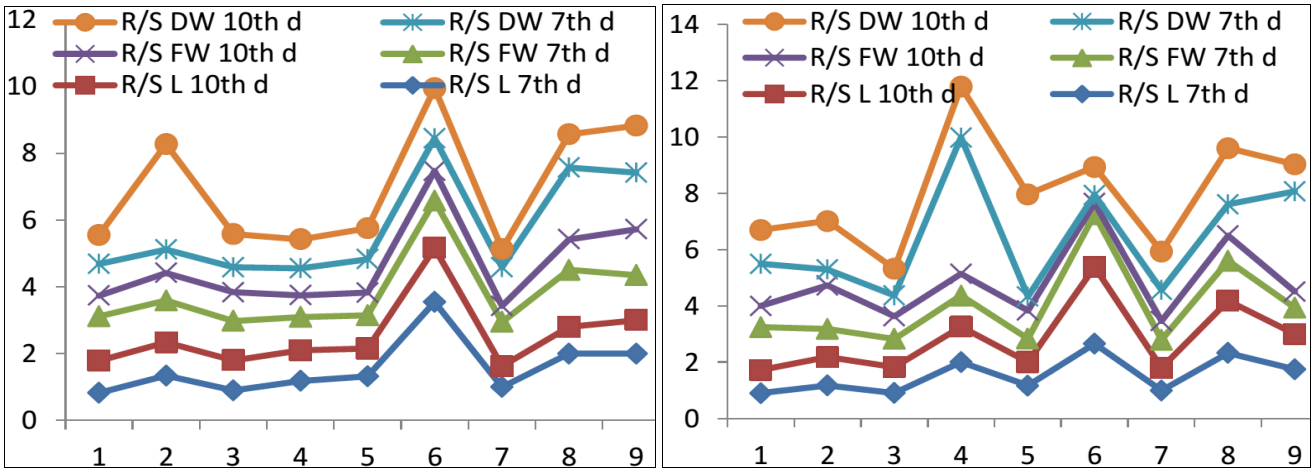

Fig 6: Root/shoot ratio of cv. Pant 11 at 7 & 10 days in presence of aqueous testa extract (TE) under NaCl salinity stress, Mono= cv. Pant 11, Mixed= cv. Pant 11& cv. Kasturi, P=0.01.

Relative Competitive Intensity

RCI values under salinity+ TE treatments for seedling height showed competitive facilitation in basmati and competitive inhibition in non-basmati and competitive facilitation in both the cultivars for seedling dry weight. RCI values for root length, seedling height under 1.0% (w/v) salinity+5% TE treatment showed competitive facilitation in basmati and competitive inhibition in non-basmati and competitive facilitation for seedling dry weight in both the cultivars.

Table 3: Relative competitive intensity of rice cultivars at 7th and 10th Day, RL=Root length, SH= Seedling height, SdDW= Seedling dry weight, P=0.01.

RCI		cv. Kasturi						cv. Pant 11					
Treatments		7th Day			10th Day			7th Day			10th Day		
NaCl (%)	TE (%)	RL	SH	SdDW	RL	SH	SdDW	RL	SH	SdDW	RL	SH	SdDW
0	0	0.099	-0.21	0.16	0.013	-0.09	0.3	-0.09	-0.04	0.11	0.22	0.15	0.1
	2.5	0.03	-0.3	-0.2	-0.01	-0.1	0.13	-0.8	-0.5	0.51	-0.054	-0.033	0.068
	5	0.169	-0.07	-0.01	0.145	-0.02	0	-0.3	-0.23	0.067	0.2	0.2	-0.01
0.5	0	0.11	0.17	0.6	0.13	0.23	0.29	0.06	-0.02	0.09	0.09	0.2	0.38
	2.5	0.63	0.57	0.0549	0.316	0.3	0.28	0.069	0.22	0.43	0.35	0.3	0.589
	5	0.28	0.19	0.43	0.316	0.27	0.286	0	0.09	0.15	0.4	0.34	0.38
1.0	0	0.55	0.6	0.67	0.55	0.64	0.69	0.07	0.421	0.55	0.45	-0.1	0.671
	2.5	0.57	0.63	0.67	0.47	0.57	0.688	0.186	0.47	0.78	0.47	-0.09	0.671
	5	0.5	0.48	0.692	0.526	0.46	0.48	0.186	0.42	0.28	0.47	0.53	0.38

Competitive ratio (CR)

CF for sh in 0, 2.5%TE and 5% TE treatments both at 7th and 10th day. CR shows, kasturi becomes increased its competitiveness at 10th day. CR values suggest under 0.5% (w/v) NaCl+2.5% TE and 1.0% (w/v) NaCl+5% TE treatments basmati was better competitor for root length, seedling height and dry weight than non-basmati cultivar.

Table 4: Relative competitive intensity of rice cultivars at 7th and 10th Day, RL=Root length, SH= Seedling height, SdDW= Seedling dry weight, P=0.01.

CR		cv. Kasturi						cv. Pant 11					
Treatments		7th Day			10th Day			7th Day			10th Day		
NaCl (%)	TE (%)	RL	SH	SdDW	RL	SH	SdDW	RL	SH	SdDW	RL	SH	SdDW
0	0	0.82	1.16	0.94	1.26	1.27	0.77	1.21	0.86	1.06	0.79	0.78	1.28
	2.5	0.55	0.88	2.38	0.96	1.1	0.93	1.81	1.129	0.42	1.04	0.91	1.07
	5	0.6	0.9	1.1	1.07	1.25	0.99	1.6	1.1	0.9	0.93	0.8	1.01
0.5	0	0.59	0.82	0.43	0.95	0.96	1.158	1.7	1.23	2.3	1.04	1.03	0.86
	2.5	0.39	0.54	1.66	1.05	0.99	1.73	2.54	1.829	0.6	0.94	1	0.57
	5	0.71	0.89	0.67	1.15	1.1	1.159	1.39	1.124	1.47	0.86	0.9	0.86
1.0	0	0.48	0.69	0.74	0.81	0.97	0.95	2.06	1.45	1.35	1.24	1.03	1.05
	2.5	0.51	0.7	1.56	0.99	1.07	0.948	1.92	1.42	0.64	1	0.93	1.05
	5	0.6	0.89	0.43	0.89	1.14	0.84	1.65	1.11	2.3	1.11	0.87	1.18

Inhibition ratio %

Mixed culture is stimulatory for 0 treatments for shoot both at 7th & 10 days. Similar interaction effect of phosphorous on biomass accumulation was reported in rice-wild rice mixed cultures [9].

Table 5: Inhibition ratio (IR %) % on shoot and root biomass of rice cultivars at 7th and 10th Day, RL=Root length, SH= Seedling height, SdDW= Seedling dry weight, P=0.01

IR (%)		cv. Kasturi						cv. Pant 11					
Treatments		7th Day			10th Day			7th Day			10th Day		
NaCl (%)	TE (%)	RL	SH	SdDW	RL	SH	SdDW	RL	SH	SdDW	RL	SH	SdDW
0	0	9.85	-21.3	16.48	1.31	-9	29.8	-9.3	-4.2	11.11	21.6	14.7	9.59
	2.5	2.8	-31	-16	-1.3	-13.5	12.99	-77	-47	51.1	-5.4	-3.33	6.84
	5	16.9	-7.4	-1.1	14.4	-2.25	0	-30	-23.2	6.66	20.2	18	-1.37
0.5	0	11.3	16.7	60.4	13.2	22.6	28.6	-51.2	-2.1	8.89	9.46	20	38.4
	2.5	63.3	57.4	5.49	31.5	30	28.57	6.9	22.1	43.11	35.1	30	58.9
	5	28.2	19.4	42.9	31.5	27	28.57	0	9.4	15.56	40.5	34	38.36
1.0	0	54.9	60.2	67	55.3	63.9	68.8	6.9	42.1	55.5	44.59	62.6	67.1
	2.5	57.7	62.9	67	47.3	57.1	68.83	18.6	47.3	78.88	47.29	60	67.1
	5	50.7	48.15	69.2	52.6	46.6	48	18.6	42.1	28.88	47.29	53.3	38.3

Relative interaction index

Table 6: Relative interaction index (RII) on shoot and root biomass of rice cultivars at 7th and 10th Day, P=0.01

RII		cv. Kasturi				cv. Pant 11			
Treatments		7th Day		10th Day		7th Day		10th Day	
NaCl (%)	TE (%)	RDW	SDW	RDW	SDW	RDW	SDW	RDW	SDW
0	0	-0.07	-0.11	-0.1	-0.26	0.043	-0.18	0.03	-0.13
	2.5	0.02	0.13	-0.09	-0.05	-0.5	-0.2	0.117	-0.22
	5	-0.21	0.159	-0.056	0.049	-0.1	0.021	0.029	-0.01
0.5	0	-0.6	-0.3	-0.2	-0.1	0.21	-0.53	-0.1	-0.4
	2.5	-0.179	0.09	-0.13	-0.2	-0.438	-0.15	-0.172	-0.71
	5	-0.35	-0.2	-0.15	-0.18	-0.05	-0.12	-0.13	-0.34
1.0	0	-0.53	-0.48	-0.6	-0.5	-0.66	-0.18	-0.478	-0.53
	2.5	-0.58	-0.43	-0.35	-0.73	-0.629	-0.67	-0.36	-0.66
	5	-0.7	-0.38	-0.27	-0.37	0.063	-0.53	-0.214	-0.26

The relative interaction index showed highly competitive response for accumulating both shoot and root biomass. Negative impact on accumulation of shoot and root biomass in all treatments, however increase in root biomass accumulation under 0.5% (w/v) NaCl. The relative interaction index (RII) showed competitive interaction for accumulating both shoot and root biomass. Root and shoot biomass increased under 0.5% (w/v) NaCl+TE and 0.5% NaCl + 5% TE respectively in basmati and decreased in non-basmati. Shoot biomass increased in 1.0% (w/v) NaCl + 5%TE in both the cultivers.

Negative impact on accumulation of shoot and root biomass in all treatments, however increase in root biomass accumulation under 0.5% (w/v) NaCl. Competitive interaction of two rice (*Oryza sativa L.)* cultivers in aqueous testa extracts of cashew-nut (*Anacardium occidentale L.)* in basmati/non-basmati mixed culture under NaCl salinity stress

Conclusion

The present study suggests, under low concentration of salinity, plant-plant interactions and plant-neighbour interactions have positive impact on early seedling emergence and growth in both the rice cultivers. Presence of allelochemical (TE), competitive ability of basmati (cv. Kasturi) increased under NaCl salinity stress.

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