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Asian Journal of Plant Science and Research, 2016, 6(4):30-36

ISSN : 2249-7412
CODEN (USA): AJPSKY

Genetical studies on allelopathic activity and yield component in some lines of hybrid rice against barnyardgrass weed under Nitrogen stress

El Shamey E. A. Z.

Rice Research & Training Center, Agricultural Research Center, Egypt
Institute of Food and Agricultural Science, University of Florida, USA

ABSTRACT

Twenty three hybrids were used to know the relationship between rice varieties and weeds (barnyardgrass) under two different nitrogen (N) levels in hydroponics. Some crosses had higher percentage for allelopathic activity as follow; IR69625A × Rikuto Norin 22, IR69625A × Giza 179 and IR69625A × HR 195 and their values were 87, 85 and 85 %, respectively. The varieties Rikuto Norin22, Giza 179 and HR 195 exhibited the highest activity against E. crus-galli during two seasons 2014 and 2015. These results showed highly genetic effect for these traits while the environmental effect is not significant. The additive and dominance genetic variances could be important in the inheritance of allelopathic characters. Rice variety Giza 179 showed high allelopathic activity to suppress grow of barnyardgrass at low N level in culture solution. Although rice variety Giza 179 has restoring fertility but it also exhibited high allelopathic activity under field conditions. Low nutrient growth levels also increased allelopathic activity and allelochemicals (momilactone B) concentrations in rice seedlings. Momilactone B secrate and increase in the culture media through growing barnyardgrass and rice together. We recommended to cultivate the hybrid IR69625A x Giza 179 to increase yield and allelopathic activity.

Key words: allelopathy, allelochemicals, barnyardgrass, weed, and hybrid rice

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INTRODUCTION

In Egypt the averaged national yield level of rice has to be increased by 25 – 30 % to meet the demands of the increasing population this seems difficult considering the narrow gap between yield potential and actual yield (12t/ha) in 2015. However, among available technologies to increase yield above the present ceiling, is the exploitation of heterosis in hybrid rice, which appears to the practical approach for Egypt. The cytoplasmic male sterile (CMS) lines that are utilized for developing the popular "three line" hybrids .The majority of the rice hybrids that are currently under commercial cultivation in the world derive their cytoplasm from the WA source [1].

Allelopathy in crops is one of the hot topics in agricultural ecology and chemoecology. In recent years, rice allelopathy has been extensively studied as it is considered as one of the environment-friendly alternative approaches in weed control. Evidences in earlier studies have indicated that rice allelopathy is a quantitative trait, which is mediated by both genetic effects and environmental conditions. The genetic control of allelopathy in rice has been assessed. Several studies have been reported on mapping quantitative trait loci (QTLs) for allelopathy in rice. Other research [2] identified four QTLs on three chromosomes, which collectively explained 35% of the total phenotypic variation of the allelopathic activity in the population. [3] Also identified several QTLs, among which one QTL on chromosome 6 had the largest effect, explaining 16.1 % of the phenotypic variation.

The allelopathic potential of rice was closely related to the types and concentrations of phenolic compounds especially under stress conditions, in which the activities of related enzymes involved in the phenylpropanoid metabolic pathway in allelopathic rice was significantly increased [4]. Previous studies have indicated that low nitrogen is an imminent compound of phenol in plants and is involved in many physiological reactions as well as signal transduction that trigger off cascade reactions functioning in the induction of systemic acquired resistance (SAR) [5]. It was also shown that low nitrogen could induce the accumulation of phenylalanine ammonia-lyase (PAL) mRNA, resulting in increased enzyme PAL activity in crop plants against pest and weed infection [6].

Application of plant activators to induce resistance has become a new method for weed control and is expected to be an alternative for effective and sustainable management. In this research, we present a study on the effect of low nitrogen at barnyardgrass and show the activity of induced allelopathy in suppression of the target weed and production of hybrid rice have heterosis in allelopathic potential and yield components by traditional plant breeding methods.

MATERIALS AND METHODS

Plant materials and growth conditions

Twenty three genotypes using a line x tester model, which including three CMS lines, five testers and fifteen cross during two summer season 2014 and 2015, were used to study the allelopathic activity and yield components. Observations were taken on three random plants from each plot. The crop was raised following the package of recommendation practices for summer season at the research farm of RRTC. Seeds of rice Giza 179 were surface sterilized in 70% ethanol and grown at 25◦C with a 12-h photoperiod for 10 days in a growth chamber as described by [7]. Seeds of barnyardgrass (*Echinochloa crus-galli* L.) were germinated and grown for 10 days as described above. Uniform rice and barnyardgrass seedlings (50 % of each) were then transferred onto a holed plate that was floated on the medium of 1L Hoagland nutrient solution in plastic container as described by [8]. Only roots of both plants were immersed in the medium. The pH value of the medium was maintained at 6.0. After 10 days incubation at 25◦C with a 12-h photoperiod, rice seedlings were collected for momilactone B determination.

Barnyardgrass bioassay

Four concentration levels of Hoagland solution, 1N (normal strength), 0.3N, 0.1N and 0.03N were prepared for the grow of rice. Rice seedlings (10 g fresh weight) were homogenized in 100 mL 50% (v/v) cold aqueous methanol and the homogenate was filtered through filter paper. The residue was homogenized again with 100 mL of methanol and filtered and the two filtrates were combined [7]. An aliquot of the extract was evaporated to dryness, dissolved in 0.2 mL of methanol and added to a sheet of filter paper in a 3-cm Petri dish. Methanol was evaporated in a draft chamber. Then, the filter paper in the Petri dishes was moistened with 0.8 mL of a 0.05% (v/v) aqueous solution of Tween 20 [9]. The final assay concentration was one rice plant equivalent extract / mL. After germination in the darkness at 25◦C for 120 h, 10 uniform barnyardgrass seedlings were placed into the Petri dishes (Kato-Noguchi et al., 2002). The length of their shoots and roots was measured after 48 h of incubation in the darkness at 25◦C. Inhibitory activity (%) was determined by the formula: [(control plant length−plant length treated with rice extract)/control plant length]×100. Control bioassays did not contain rice extracts. The bioassay was repeated three times using a completely randomized design with 10 plants for each determination. Significant differences were evaluated by Tukey's HSD test.

Determination of momilactone B concentration in rice seedlings

Rice seedlings were homogenized as mentioned above and grow at two different nutrient concentrations (Normal I N and 0.03 N). Then, the residue was loaded onto a column of synthetic polystyrene adsorbent and purified, and momilactone B was quantified as described by [7]. The experiment was repeated three times.

Significant differences were evaluated by Tukey's HSD test for all collected data then subjected to statistical analysis using ANOVA as described by [10]. All statistical analyses were performed using analysis of variance technique by means of "MSTAT" computer soft ware package.

RESULTS AND DISCUSSION

Allelopathic characters

Differences in allelopathic activity were showed among the tested rice genotypes, ranging between 25-87% as shown in Table 1. The parents were crossed with their CMS lines and produced 15 crosses with weed control ranging between 30-87% as shown in Table 2, and eight out of 15 crosses showed allelopathic activity from 81-87% on *E. crus-galli* during 2014 season. The following varieties; Giza 179, HR 195, and Rikuto Norin 22 originated from different countries and showed higher allelopathic activity during 2013 season, their values were 83, 83, and 87 %, respectively as shown in Table 1. [11] Reported that the varieties Shmokita and Rikuto Norin22 provided at least 80 and 70% weed control against duck salad in field experiment at Stuttgart, Arkansas, USA.

On the other hand, among the crosses, there were some crosses had higher percentage for allelopathic activity as follow; IR69625A × Rikuto Norin 22, IR58025A x Rikuto Norin 22, IR69625A × Giza 179 and IR69625A × HR

195 and their value were 87, 85, 85 and 85%, respectively. For radial area character, we found that the parents HR 195, Giza 179, and Rikuto Norin22 had higher values among all parents used during 2014 season as shown in Table 1. These parents were used to be crossed with their CMS lines and produced crosses with values ranging between 5.33–12.33cm, and the high desirable values which were expressed in; IR69625A × Rikuto Norin22, IR70368A × Giza 179, IR69625A x Giza 179 and IR69625A × HR 195 during 2015 season as shown in Table 2.

These results referred to highly genetic effect which controlled these traits since the environmental effect is not significant. In a similar study, [12] found that the cultivars; Giza 182 and Rikuto Norine provided at least 80% weed control in transplanted rice against *E. crus-galli* under Egyptian conditions.

Table 1 Mean performance of radial area and weed control for the parental lines

No.	Parents	Radial area (cm)	Weed control %
1	Giza 179	11.37	83
2	HR 195	11.83	83
3	Giza 178	9.33	44
4	Rikuto Norin 22	11.33	87
5	Sakha 103	2.30	25
6	IR 69625 A	5.33	39
7	IR 70368 A	6.17	46
8	IR 58025 A	7.33	50

Table 2 Mean performance of radial area and weed control for the obtained crosses

No.	Crosses	Radial area (cm)	Weed control %
1	IR69625A × Giza 179	11.33	85
2	IR69625A × HR 195	11.33	85
3	IR69625A × Giza 178	8.17	65
4	IR69625A × Rikuto Norin 22	12.33	87
5	IR69625A × Sakha 103	6.33	30
6	IR70368A × Giza 179	11.83	83
7	IR70368A × HR 195	9.67	68
8	IR70368A × Giza 178	6.33	65
9	IR70368A × Rikuto Norin 22	10.17	81
10	IR70368A × Sakha 103	7.00	30
11	IR58025A × Giza 179	11.17	83
12	IR58025A × HR 195	11.67	81
13	IR58025A × Giza 178	8.83	60
14	IR58025A × Rikuto Norin 22	11.00	85
15	IR58025A × Sakha 103	5.33	30

Yield and its component characters

The mean performance of yield and its component characters i.e., number of panicles per plant, panicle length, panicle weight, grain yield per plant, number of grains per panicle, 1000-grain weight and seed set (%) for the parental lines are presented in Table 3. A wide range for yield and its component traits of the parental lines with their crosses was recorded, the data summarized in Table 4 show that the general mean values of the three CMS lines and the five testers were (17.17, 22.14, 3.35, 28.15, 148.79, 24.39 and 71.38) for number of panicles per plant, panicle length, panicle weight, grain yield per plant, number of grains per panicle, 1000-grain weight and seed set (%), respectively. The decrease of the mean values for grain yield per plant referred to the decrease of out-crossing to the CMS lines. But, the general mean values performance of F1 hybrids for these characters were (17.92, 22.49, 3.74, 34.33, 157.99, 25.35 and 70.61), respectively, as shown in Table 4.

Table 3 Mean performance of yield and its component characters for the parental lines

No.	Parents	No. of panicles / plant	Panicle length	Panicle Weight	Grain Yield/ plant	No. of grains/ panicle	1000-grain weight	Seed set %
1	Giza 179	23.00	24.00	4.20	49.53	176.00	24.10	96.62
2	HR 195	17.00	24.00	5.30	49.00	223.00	27.10	91.94
3	Giza 178	22.00	23.33	4.56	37.67	155.00	21.00	96.57
4	Rikuto Norine 22	10.00	20.17	3.00	23.67	93.33	31.00	98.93
5	Sakha 103	13.33	18.00	3.74	25.33	126.00	24.27	81.00
6	IR 69625 A	16.00	21.00	1.53	10.00	124.00	22.00	32.00
7	IR 70368 A	17.00	23.17	2.30	17.00	128.00	22.67	31.00
8	IR 58025 A	19.00	23.43	2.20	13.00	165.00	23.00	43.00
	Mean	17.17	22.14	3.35	28.15	148.79	24.39	71.38

Table 4 Mean performance of yield and its component characters for the obtained crosses

No.	Crosses	No. of panicles / plant	Panicle length	Panicle Weight	Grain Yield/ plant	No. of grains/ panicle	1000-grain weight	Seed set
1	IR69625A × Giza 179	18.33	23.00	4.80	54.00	175.00	27.00	98.50
2	IR69625A × HR 195	19.00	25.17	5.40	43.33	214.33	26.67	92.60
3	IR69625A × Giza 178	18.33	23.33	4.53	55.67	173.33	27.00	98.80
4	IR69625A × Rikuto Norine 22	15.33	20.67	2.77	18.67	102.67	29.67	18.03
5	IR69625A × Sakha 103	16.67	19.33	2.67	16.33	123.00	24.33	48.43
6	IR70368A × Giza 179	12.00	23.33	4.80	64.33	151.67	25.00	98.33
7	IR70368A × HR 195	16.33	23.83	5.17	40.00	203.33	25.67	91.80
8	IR70368A × Giza 178	12.00	23.00	4.60	65.00	152.33	25.00	98.37
9	IR70368A × Rikuto Norine 22	12.67	20.67	2.83	18.00	102.00	28.67	17.27
10	IR70368A × Sakha 103	14.33	18.50	2.37	15.33	119.33	25.33	46.30
11	IR58025A × Giza 179	28.33	26.00	5.20	66.33	260.33	24.00	90.50
12	IR58025A × HR 195	28.33	25.83	5.57	47.00	222.67	27.67	93.03
13	IR58025A × Giza 178	29.00	26.33	5.27	67.33	262.00	24.00	91.07
14	IR58025A × Rikuto Norine 22	15.33	22.67	3.13	22.33	111.67	31.67	15.30
15	IR58025A × Sakha 103	24.00	20.83	2.73	14.00	134.00	23.00	49.10
	Mean	18.67	22.83	4.12	40.51	167.18	26.31	69.83
	General Mean (Parents and Crosses)	17.92	22.49	3.74	34.33	157.99	25.35	70.61

Analysis of variance in the two seasons showed highly significant differences in allelopathic potential against *E. crus-galli* among the rice genotypes as shown in Table 5. In order to emphasis these results, heritability in broad sense was estimated in weed control and radial area characters and found to be 75.67% and 98.93%, respectively during 2014 season. These characters could be transferred in the genetic background of the varieties by involving such donor variation in the breeding programs. According to the behavior of the screened material, the Five varieties; Giza 179, HR 195, Giza 178, Rikuto Norin 22 and Sakha 103 were used to generate the genetic materials for this study.

Table 5 Mean square estimates of the ordinary analysis for weed control and radial area

S.O.V	df	Radial area	Weed control
Rep	2	0.04 $^{n.s}$	0.001 $^{n.s}$
Treat	22	46.66 **	0.17 **
Crosses	14	4.87 **	0.02 **
Parents	7	56.49 **	0.19 **
P.vs cross	1	1131.13 **	4.02 **
Lines	2	20.53 **	0.04 **
Testers	4	70.75 **	0.25 **
L*T	8	-29.81 **	-0.10 **
Error	44	0.11	0.01
h^2.b.s %		98.93	75.67

n.s: not significant ***: high significant*

Allelopathy in rice is a typical quantitative trait involving several loci and possibly some degrees of epistasis, as well as, the cultivar IAC 165 showed strong and consistent allelopathic activity against barnyardgrass, whereas Co 39 showed weakly allelopathy[13]. The estimate of the broad-sense heritability for allelopathic activity was reasonably high with a value of 0.68.

The results summarized in Table 6 revealed that highly significant differences were recorded among treatments, parents, crosses, parent vs. crosses, lines, testers and line x testers for most of yield and its components characters i.e., panicle length, panicle weight, No. of panicles per plant, number of grains per panicle, 1000-grain weight, grain yield per plant and seed set %. On the contrary, insignificant differences among reps for all yield and its component characters were recorded.

From the above results which are summarized in Table 6, it could be conducted that the pedigree method can be used to improve all traits under study and develop the new hybrid rice verities. While, the mean square values of all characters were highly significant for, treatments, parents, crosses, parent vs. crosses, lines, testers and lines x testers. Similar results were obtained by [14]. He estimated heterosis and combining ability of ten cytoplasmic genetic male sterile lines and five Egyptian testers (restorers) by using line x tester analysis for some agronomic characters and yield and its components to obtain useful information for hybrid rice program in Egypt. Among the ten cytoplasmic male sterile lines (CMS), IR58025A and IR69625A were the best general combiners for grain yield. But, the restorers, Giza 178 and Giza 179 were the best general combiners among the testers for grain yield and most studied characters. In addition, the highly significant mean squares of lines x testers for all traits indicated that they interacted and produced more kindly differences in combining ability effects, indicating the presented wide genetic diversity among the lines and testers.

The interactions of parental lines and the resultant crosses were significant for all traits under investigation, indicating that the average heterosis overall crosses of these traits was inconsistent. It could therefore be concluded that the test of potential parents for the expression of heterosis would be necessarily conducting over a number of environmental conditions. Also, genetic diversity alone would not guarantee the expression of heterosis but the suitability of the environmental conditions would be required.

Table 6 Mean square estimates of the ordinary analysis for yield and its component characters

Source of variance	d.f.	No. of panicles/plant	Panicle length	Panicle Weight	Grain yield/plant	No. of grains/ panicle	1000-grain weight	Seed set
Reps	2	1.03 $^{n.s}$	0.93 $^{n.s}$	0.21 $^{n.s}$	0.82 $^{n.s}$	0.75 $^{n.s}$	0.60 $^{n.s}$	0.67 $^{n.s}$
Entries	22	91.67 **	18.73 **	3.05 **	799.03 **	4946.35 **	91.29 **	828.47 **
Parents	7	11.17 **	2.08 **	0.29 **	80.33 **	574.83 **	11.58 **	40.98 **
Crosses	14	29.76 **	13.39 **	3.73 **	447.50 **	3710.58 **	63.18 **	1758.50 **
P* V.C	1	2939.68 **	524.85 **	72.77 **	23561.6 **	133991.9**	2701.75**	12398.08**
Lines	2	7.00 **	5.34 **	0.52 **	37.00 **	1533.00 **	0.78 **	133.00 **
Testers	4	38.12 **	16.66 **	4.86 **	588.44 **	4606.77 **	84.07 **	2315.11 **
L*T	8	1.84 **	5.57 **	2.02 **	168.91 **	1547.61 **	23.46 **	1106.30 **
Error	44	2.71	0.46	0.02	27.34	149.48	0.44	1.26

Estimation of the genetic components and heritability in broad and narrow senses

The genetic variance components i.e. additive genetic variance and dominance genetic variance were estimated for all traits under study. Also, these components were used to compute the heritability estimates in broad and narrow senses. The results presented in Tables 7 and 8 show the estimated values of genetic components and heritability in broad and narrow senses for allelopathic characters and yield and its component characters, respectively.

Allelopathic characters

As shown in Table 7, the partitioning of genetic variance for allelopathic characters, recorded high estimates of dominance component of variance in comparison with the additive genetic variance and ranged between 0.030 to 9.900 for weed control and radial area, respectively. As well as, for weed control as an example for allelopathic characters, the data in Table 7 showed that the dominance genetic variance as a portion of the total genetic variance was larger than the additive genetic variance. Their respective values were 0.03 and 0.001. These results indicated that, the two genetic variance components might be important in the inheritance of weed control, whereas the dominance genetic variance played the more important role in this case.

Table 7 Estimates of genetic parameters and heritability for the studied allelopathic characters

Source	Radial area	Weed control
Additive gene	0.317	0.001
Dominance gene	9.900	0.030
Environment effect	0.110	0.010
Heritability broad %	98.93	75.67
Heritability narrow %	3.10	2.68

Regarding heritability estimates, Table 7 illustrates that high value 75.67% was recorded in broad sense. On the other hand, very low narrow sense heritability value of 2.68% was estimated. These findings were in agreement with those obtained earlier from the partitioning of genetic variance in this study. These results indicated that this character was influenced by environmental effect. This means that selection for allelopathic characters might be practiced successfully in late generations.

Yield and its component characters

As shown in Table 8, the partitioning of genetic variance for all yield characters, recorded high estimates of dominance component of variance in comparison with the additive genetic variance and ranged between 0.043 to 466.040 for number of panicles per plant and number of grains per panicle, respectively.

In general, panicle length as an example for yield and its component characters, the data in Table 6 showed that, the dominance genetic variance as a portion of the total genetic variance was larger than the additive genetic variance. Their respective values were 1.703 and 0.096. These results indicated that the two genetic variance components might be important in the inheritance of panicle length, whereas the dominance genetic variance played the more important role in this case.

Regarding heritability estimates, Table 6 illustrates that high value of 79.64% was determined in broad sense. On the other hand, very low narrow sense heritability value of 4.25% was estimated. These findings were in agreement with those obtained earlier from the partitioning of genetic variance in this study. These results indicated that, this character was influenced by environmental effect. This means that, selection for panicle length might be practiced successfully in late generations.

Table 8 Estimates of genetic parameters and heritability for yield and its components

Source	No. of panicles / plant	Panicle length	Panicle weight	Grain yield / plant	No. of grains / panicle	1000-grain weight	Seed set
Additive gene	0.364	0.096	0.020	3.464	26.630	0.494	5.410
Dominance gene	0.043	1.703	0.670	47.190	466.040	7.670	368.350
Environment effect	1.710	0.460	0.02	27.340	149.480	0.440	1.260
Heritability broad %	19.23	79.64	97.18	64.95	76.72	94.89	99.66
Heritability narrow %	17.19	4.25	2.82	4.44	4.15	5.74	1.44

Barnyardgrass with the allelopathic potential and momilactone B concentrations

Rice seedlings of Giza 179 variety were growed with (mix-incubation) or without (mono-incubation) barnyardgrass seedlings for 10 days and allelopathic activity of rice was analyzed by barnyardgrass bioassay (Figure 1A). The extracts of mono-incubated rice inhibited the growth of barnyardgrass roots and shoots by 16 and 13%, respectively, and the extracts of mixed-incubated rice inhibited the growth of barnyardgrass roots and shoots by 78 and 74%, respectively. Thus, the mixed-incubation induced 5.1– 6.1-times increases in allelopathic activity in the rice extracts against barnyardgrass roots and shoots, respectively.

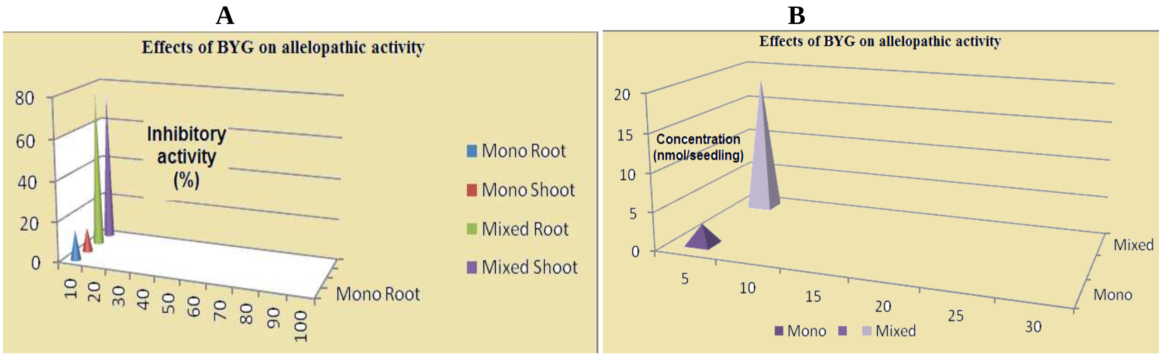

Figure 1. Effects of barnyardgrass on allelopathic activity (A) and momilactone B concentration in rice (B)

The concentration of momilactone B in mono-incubated rice seedlings was 2.8 nmol/seedling and that in mixed-incubated rice seedlings was 18.7 nmol/seedling. Thus, the concentration was 6.7-fold greater in mixed-incubated rice seedlings than in mono-incubated rice seedlings (Figure 1B). This result suggests that the secration of momilactone B in rice seedlings may be increased by the presence of barnyardgrass. Momilactone B suppressed growth of roots and shoots of barnyardgrass at concentration levels more 1µmol/L. The activity of momilactone B on the growth inhibition of rice seedlings themselves was less than 1% of that on the growth inhibition of barnyardgrass [15]. Therefore, the increased concentration levels of momilactone B in rice seedlings under mixed-incubation with barnyardgrass (Figure 1B) associate with increased allelopathic activity of rice seedling (Figure 1A)

Effects of N levels in rice medium on the allelopathic activity and momilactone B concentration in rice

Rice seedlings of Giza 179 were grown without barnyardgrass seedlings in the culture containing two different nutrient concentrations (Normal IN and 0.03 N) for 10 days, and allelopathic activity of the rice extracts was determined by barnyardgrass methods (Figure 2). The allelopathic activity of rice extracts was increased significantly at N levels. The extracts of rice seedlings incubated in 1N culture suppresed the growth of barnyardgrass roots and shoots by 17 and 15%, respectively, whereas the extracts of rice seedlings growed in 0.03N medium inhibited the growth of barnyardgrass roots and shoots by 45 and 47 %, respectively.

These results show the growth conditions under limit nutrient levels may increase allelopathic activity of rice seedlings. It was also reported that phosphate or nitrogen limited growth conditions increased allelopathic activity of rice [4],[8].

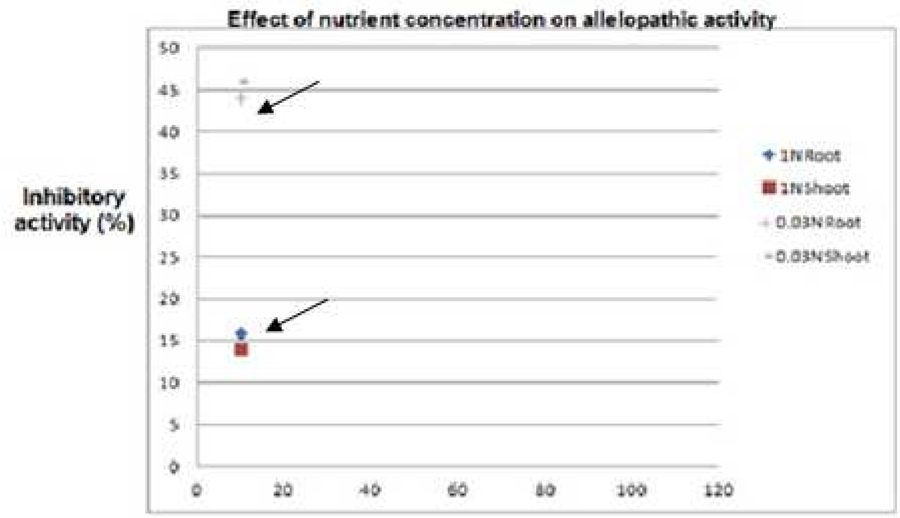

Figure 2. Effect of N levels in medium on allelopathic activity of rice

Acknowledgment

This work was funded by Agricultural Research Center, Field Crops Research Institute, Rice Research & Training Center, Seed Production Dept., many thanks to all friends who's help me and support at the Dept.

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