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Soil Microorganisms and Their Impact on Rice Straw Allelopathic Potential

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Abstract: Allelopathy is the chemical interaction between plants (including microorganisms), and reported as an ecological process structuring plant communities. Bioavailability and persistence of allelochemicals are much affecting by soil microbes. In the same time we find (according to the different studies) growth inhibition in filter paper bioassay enriched with an allelochemical or an extract, such results have not been verifiable in more natural settings with plants growing in soil. We examined *in vitro* the allelopathic potential of rice straw extract incubated 40 and 70 days with different isolates of *Fusarium moniliforme* (F1) and *F. oxysporium* (F2, and F3), besides a combination treatment including them all against seedling root and shoot growth of *Triticum aestivum*, *Raphanus sativus*, *Lactuca sativa* and *Eruca sativa* as bioassay. Rice straw was also incubated under natural conditions of soil (for 40 & 70 days) and the leachates were used in bioassay- plants reaction experiments. A qualitative study on the potential allelopathic chemicals in both decomposed and non-decomposed straw was carried out. Disparate results (swung between increasing and inhibiting growth) were recorded for the acetone extract of the incubated materials with the different isolates regarding their influence on test species. Lettuce seedlings were the most affecting (negatively) in comparison with the others, with a superior activity against root growth rather than on shoot growth. A remarkable inhibition was observed with F1 extract against approximately all tested seeds. No inhibitory effect was noted under soil incubation. The data recorded general stimulation in this regard. Chromatography analysis (using paper chromatography –PC- technique) of the allelochemicals (i.e., phenolics) that suppose to play a role in this regard taking F1 extract (at 40 days) as an example, revealed no phenolic compounds were existed in the extract, in converse the situation with the intact straw extract where six phenolic acids were detected including caffeic, cinnamic, ferulic, *p*-coumaric, *o*-coumaric and *p*-hydroxybenzoic acids. The results revealed the impact of soil microorganisms in affecting rice straw allelpathic activity. However, we can't rely on microorganisms as a sole factor determines rice straw allelopathic activity; there are many other factors that interfere with the process, and those should be taken into consideration.

Key words: allelopathy, allelochemicals, decomposition, rice straw, soil microorganisms/microbes, phytotoxic effect, weeds.

INTRODUCTION

Rice is an important crop worldwide from both the social and the economic point of views. Its straw is a big problem. In Egypt, farmers burn annually of about 4 million tonnes, resulted from 0.64 million hectares (Afify *et al*., 2002). That causes a lot of damages. Little goes to industry and as feedstuff.

Rice (*Oryza sativa* L.) allelopathy has been on the research agenda for a decade. Now, there is a big concern of using rice straw as a source for natural herbicides. Xuan *et al*. (2005) and Seal *et al*. (2005) reported inhibition of several weeds by rice straw residues and leachates. Its content of the allelopathic chemicals is big and diverse (Chung *et al*., 2001; Kong *et al*., 2004; Kato-Noguchi and Ino, 2005; Kong *et al*., 2006; Macías *et al*., 2006; Kato-Noguchi *et al*., 2012). The question most arguing… what is the fate of allelochemicals and allelopathic activity in nature?

Biotic and abiotic factors play a significant role in affecting many of the allelopathic plants. Soil is a very dynamic system. In the same time the decomposition of plant residues provides the largest quantity of allelochemicals that may be added to the rhizosphere, the activity of substances released can be quit transitory, since they are subject to destruction, soil adsorption and inactivation, and transformation by soil microflora (Cheng, 1995; Inderjit and Weiner, 2001). However, this does not preclude their having effects on plant growth during decomposition, and often sustained toxicity may occur as new toxic products are formed in some transformations (Inderjit, 2005). This is further enhanced by the fact that the microorganisms active in decomposition may themselves produce inhibitory allelochemicals (Culter, 1991). We can not omit in this regard the changes in the dynamics of microbial populations and their functional diversities due to allelopathic activities where that can indirectly affect such phenomenon (Allison and Killham, 1988; Bertin *et al*., 2003; Kong *et al*., 2008). Ruiyu *et al*. (2007) found a positive correlation between the total microbial population and

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the inhibition rate on the root length of lettuce owing to the different allelopathic activities of rice cultivars. Important variables in decomposition process are the nature of plant residue, the soil type (including its content of microorganisms), and substrate conditions (Facelli and Pickett, 1991; Inderjit *et al.*, 1996). Depending on conditions substances highly toxic, nontoxic, or stimulatory to plants can be found (Patrick *et al*., 1964). In one of our unpublished researches (El-Shahawy and Abdelhamid) we found no inhibitory effect of *P. vulgaris* extracts after the day five of incubation under soil conditions. Before that, the extracts were extremely toxic. A favorably effect, however, was noted with rice straw decomposed 3 months before sowing in increasing growth and yield in cucumber (El-Shahawy *et al.*, 2006).

Our objective is to study the role of soil microbes in affecting rice straw allelopathic activity. Together with studying the effect of the different isolates of *F. moniliforme* and *F. oxysporium* fungi in decomposing rice straw, a study was conducted to identify the main responsible phytotoxic components in this regard in both decomposed and non-decomposed straw.

MATERIALS AND METHODS

Plant Materials:

Rice (*Oriza sativa* L., cv. Sakha 101) straw waste was collected from the field (Damanhour, El-Behara Governorate, Egypt) and allowed to dry at room temperature for an additional time (20 days). The straw was chopped into (0.5 - 1 cm) lengths. The furnished straw was then transferred to the National Research Centre facilities (Microbial Chemistry and Botany Departments) for the incubation with soil microorganisms and the extraction in a further step.

Microorganisms Preparation:

Three isolates of *F. moniliforme* (F1) and *F. oxysporium* (F2 and F3) were obtained from the soil where rice was grown. The isolates were identified by Plant Pathology Department, National Research Centre, Dokki, Cairo, Egypt.

Growing Fungi with the Straw:

Eight flasks (1000 ml/each), each containing 50 g of the chopped straw, were prepared in this regard. The straw was wetted with a 100 ml solution of $NaNo_3$ 2.0 g/L, $MgSO_4$ 0.5 g/L, KCl 0.5 g/L, $FeSO_4$ 0.0001 g/L, and KH_2SO_4 1.05 g/L, synchronism with the inoculation with the different isolates of *F. moniliforme* (F1) and *F. oxysporium* (F2 and F3). A combination treatment including them all (F1+F2+F3) was also applied. The flasks were incubated under controlled conditions (28 ± 2 °C) for 40 and 70 days.

Extraction Procedure:

After the intended period, the decomposed rice straw was extracted overnight (17 h) with acetone (500 ml/each). After filtration through six layers of cheesecloth (to remove the majority of the cellulose material) and filter paper Whatman No. 1 (to remove particulate matter), the acetone extracts were made up to a 500 ml volume to yield 10% w/v concentration at a dry weight basis. A control was prepared from the intact straw following the same procedure. The extracts were stored at -21 °C until use within 24 h in the bioassay test.

Bioassay Test:

The bioassay test was carried out on four species of crops known by sensitivity to the allelochemicals including wheat (*Triticum aestivum* L., cv. Sakha 61), radish (*Raphanus sativus* L.), lettuce (*Lactuca sativa* L.) and watercress (*Eruca sativa* L.) as reported by many authors (Lydon and Duke, 1989). The test was further done on seedling root and shoot growth because of the high sensitivity of these organs in comparison with the seed germination percentage (Moosavi *et al*., 2011; El-Shahawy, 2012).

The seeds were germinated in 11-cm glass Petri dishes for 2 - 3 days (based on seeds type) until radical growth reaches about 2 mm. At this stage the seedlings are ready for testing. The acetone extracts were added to 7-cm diameter glass Petri dishes (3 ml/each) containing filter paper Whatman No. 1. The dishes were left uncovered overnight until complete dryness. An equal volume of distilled water (3 ml) was added instead of the evaporated solvent. Ten uniform seedlings of the test species were transferred to the dishes, and left to stand (under darkness) at room temperature (18 – 21 °C) for five days. Two controls were prepared in this regard; one with distilled water and the other with the extract obtained from the intact straw. After the intended period, the data on seedling root and shoot growth (cm) were estimated.

Incubating Rice Straw Under Soil Conditions:

An experiment was conducted in this regard to make a comparison between the situation under natural conditions of soil and the controlled conditions of incubation under *F. moniliforme* and *F. oxysporium* regarding the influence on rice straw allelopathic activity. Five kg of silty clay soil were collected in this regard. An equal

amount of sand and gravels was washed very carefully with 2 M HCl to remove adsorbed organic materials that might interfere with the process. The sand and gravels were then rinsed several times with distilled water to remove any traces from the acid. Six plastic columns (10 x 33 cm) were prepared by adding glass wool and 5 cm from sand and gravels (3 + 2 cm, resp.) from the bottom. Two columns were filled with a mixture of sand and soil (500 g/each) and chopped straw (50 g). Two other columns were filled with sand and straw only (1000 + 50 g/each). The remainder two were filled with soil only (1000 g/each) as a blank control. Half of the columns (by taking one from each pair) were incubated for 40 days. The other half was incubated for 70 days. All under lab conditions (18 – 25 °C). The columns were kept wet as much as possible by adding distilled water (50 - 100 ml) from time to time. After the determined period, the columns were eluted with 350 – 500 ml distilled water and the resultant leachates were straighten to a 500 ml volume to yield a 10% w/v concentration (the same that was previously used). The solutions were immediately examined for their biological activities within 24 h.

The bioassay test was done following the same procedure using the same test organisms and under the same circumstances.

Isolation and Identification of the Phytotoxic Agents in the Straw:

Isolation and identification of the phytotoxic phenolic compounds were done on the decomposed and non-decomposed straw. The resultant extract from the incubation with F1 at 40 days was comparatively the most effective, so it was decided to continue with such extract in our our present examination. The F1 acetone extract was evaporated under vacuum (at 50 °C) to dryness. The residue was taken into 50 ml of diethyl ether which was then evaporated and the remainder was re-dissolved in 5 ml 95% ethanol. The ethanol extract was chromatographed one-dimensionally on paper chromatography [(PC), Chrom.-Paper, Sartorius AG, 37070 Goettingen, Germany] with *n*-BuOH-HOAc-H_2O (4:1:5, top layer). Marker solutions of coumarin, caffeic acid, cinnamic acid, ferulic acid, *p*-coumaric acid, gallic acid, 2,5-dihydroxybenzoic acid, *o*-coumaric acid, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldhyde and benzoic acid were signed as controls. The dried chromatograms were sprayed with 1% aqueous ferric chloride, after exposure to UV light at 254 nm. Typical R_f values, and colours under UV and with ferric chloride were determined as a general procedure.

Statistical Analysis:

All experiments were conducted in a randomized complete design with four replications each treatment. ANOVA table was calculated, and LSD test (at 0.01 and 0.05 *probabilities*) was used to compare means (Snedecor and Cochran, 1989). The inhibition percentage on seedling root and shoot growth was also calculated using the equation (data not reported):

(%) Inhibition= 100- (growth in treatment/growth in control×100)

Results:

The effect of aqueous acetone extracts obtained by the incubation with the different isolates of *F. moniliforme* (F1) and *F. oxysporium* (F2 and F3) was illustrated in Table (1). The data encompasses the effect on seedling root and shoot growth of wheat, radish, watercress and lettuce. The effect swung between stimulating and inhibiting growth based on test species. Overall, root growth was more sensitive than shoot growth and lettuce seedlings were the most sensitive amongst all. Radish, in particular, was highly tolerant. A stimulatory response was noted in this regard with the whole extracts, except with F1 extract. The extract from F1 was the most effective in suppressing root and shoot growth if compared with the others. A remarkable inhibition was observed in this regard against all tested species. Little variations were observed between the effectiveness of the extracts obtained at 40 days and those obtained at 70 days.

Soil leachates, on the other hand, slightly affected plant species, irrespective of the date that they were obtained after (Table 2). A diminution in toxicity, or might be more a stimulatory effect was noted in most cases.

Identification of the main responsible components in the extract resulted from the incubation with F1 (at 40 days) using PC technique showed no phenolic compounds were present in the extract, in the same time with 6 phenolics (e.g., caffeic, cinnamic, ferulic, *p*-coumaric, *o*-coumaric and *p*-hydroxybenzoic acids) were reported in the intact straw (Table 3).

Table 1: Effect of aqueous acetone extract of decomposed rice straw incubated 40 and 70 days under controlled conditions (28 ± 2 °C) with different isolates of *F. moniliforme* (F1) and *F. oxysporium* (F2 and F3) on seedling root and shoot growth of certain crop species. The experiment was performed *in vitro* (under Petri dishes conditions) with five days old seedlings and 22 °C temperature in average.

Micro-organisms	Incubation time (day)	Test seeds							
		Wheat		Radish		Watercress		Lettuce	
		Root growth	Shoot growth	Root growth	Shoot growth	Root growth	Shoot growth	Root growth	Shoot growth
		- cm -		- cm -		- cm -		- cm -	
F1	40	2.71	4.43	3.22	3.04	0.34	0.69	0.42	0.55
F2		6.57	4.95	4.32	3.98	2.26	3.107	0.79	1.45
F3		7.06	4.73	5.42	3.38	2.67	3.26	0.83	1.46
F1+2+3		8.20	5.30	4.63	3.26	2.58	3.13	1.21	1.80
F1	70	7.57	5.35	4.56	3.51	2.13	2.66	1.20	1.80
F2		6.55	5.44	4.86	3.86	3.10	2.92	0.88	1.81
F3		8.57	5.60	7.39	3.56	3.10	3.46	1.14	1.79
F1+2+3		8.42	5.66	5.53	3.52	3.07	3.41	1.26	2.05
Intact straw		7.14	4.91	6.30	3.95	1.32	2.45	0.65	1.81
Control (water)		8.44	4.38	4.51	2.92	3.02	2.66	3.07	1.82
LSD 5%		0.89	0.48	0.99	0.55	0.45	0.34	0.22	0.16
1%		1.21	0.69	1.35	0.75	0.60	0.47	0.31	0.22

Table 2: Effect of soil leachates of rice straw incubated 40 and 70 days under lab conditions on seedling root and shoot growth of certain crop species. Incubation was done in a silty clay soil amended with an equal amount of washed sand. Assaying was carried out *in vitro* (under Petri dishes conditions) with five days old seedlings and 22 °C temperature in average.

Incubation conditions	Incubation time (day)	Test seeds							
		Wheat		Radish		Watercress		Lettuce	
		Root growth	Shoot growth	Root growth	Shoot growth	Root growth	Shoot growth	Root growth	Shoot growth
		- cm -		- cm -		- cm -		- cm -	
Sand + straw	40	9.84	6.02	9.25	6.01	2.43	4.42	2.42	2.51
Soil + straw		9.62	5.29	4.80	4.52	2.91	3.69	2.07	2.27
Control (soil alone)		9.28	5.33	4.52	5.11	2.59	3.98	1.66	2.22
Sand + straw	70	9.83	5.72	9.54	5.46	2.09	3.92	1.94	2.33
Soil + straw		10.02	5.34	3.94	4.03	3.02	4.01	2.88	2.63
Control (soil alone)		9.97	5.21	4.14	4.09	2.04	3.68	2.13	2.73
LSD 5%		0.97	0.54	1.18	0.75	0.63	0.50	0.22	0.22
1%		0.91	0.51	1.11	0.71	0.59	0.47	0.20	0.21

Table 3: Phenolics as bioactive agents in decomposed rice straw due to incubation with *Fusarium moniliforme* (F1) for 40 days in comparison with the intact straw.

Phenolics	R_f (x 100) *in* BAW	*Fluorescence in* UV light at 245 nm	*Colour with* 1% ferric chloride	*Presence in*	
				Intact straw	*Decomposed Straw with F. moniliforme*
Coumarin	-	none	none	-	-
Caffeic acid	78.67	bright blue	green	+	-
Cinnamic acid	96.00	blue	yellow	+	-
Ferulic acid	89.33	bright blue	reddish- brown	+	-
p-coumaric acid	92.00	blue	brick red	+	-
Gallic acid	58.00	blue	gray	-	-
2,5-dihydroxybenzoic acid	87.33	bright blue	clear blue	-	-
o-coumaric acid	88.00	bright yellow	orange	+	-
p-hydroxybenzoic acid	93.33	blue	light yellow	+	-
p-hydroxybenzaldhyde	94.66	blue	mauve	-	-
Benzoic acid	-	none	none	-	-

Solvent key: BAW = *n*-BuOH-HOAc-H_2O (4:1:5, top layer)

Discussion:

Soil microbes are determinant of the many of the allelopathic activities. Under fungi decomposition we obtained moderate results in suppressing root and shoot growth. A species- dependant activity was recorded in this regard. A high inhibitory effect was obtained with F1 extract against nearly all tested species and parameters, especially on root growth in comparison with the others. According to the different hypotheses, a lost of activity is expected where the allelochemicals produced by plants (esp. phenolics) can readily be metabolized by microorganisms especially when adequate mineral nutrients are present (Blum and Shafer, 1988), but we can't forget in the same time that the microorganisms themselves are able to produce compounds

with a regulatory growth properties including toxic action (Duke, 1992; Duke *et al*., 2002). That might explain our findings in this portion.

Incubating rice straw under natural conditions of soil showed a different result. A stimulatory response was noted approximately with the whole leachates. That might refer to more than one fact: 1) There are many microorganisms that might interfere with the process, 2) They may act together in strengthen or debilitation the activity, 3) Soil conditions could not be obviated in this regard. For allelopathy to be done in field situations, allelochemicals must be accumulate and persist at phytotoxic level and come in contact with the target plant (Choesin and Boerner, 1991) where that could not be done at the ecological level as processes like retention (sorption), adsorption, transport and transformation breakdown the process (Cheng, 1995; Inderjit *et al*., 2008). Kaur *et al*. (2009) showed that the growth inhibition of a species in filter paper bioassay enriched with a single chemical is always great and used as evidence of allelopathic interaction, but for some of these putative examples, the results have not been verifiable in more natural settings with plants growing in soil. On that basis, the allelopathic chemicals could disappear as they could be consumed by soil microorganisms (Levy and Carmeli, 1995; Inderjit, 1996), transform to more virulence compounds (Nair *et al*., 1992; Inderjit *et al*., 1999), or even to other chemcals with positive properties (Patrick *et al*., 1964). Besides producing phytotoxic substances, nutrients are also released during decomposition (Facelli and Pickett, 1991) and their role as growth motivators is well known. This might also help (beside what we mentioned above) in explaining our results in this regard.

Analyzing the extracts for the existence of the phenolic compounds that could play a role in the observed allelopathic effect in the decomposed straw (taking F1 extract as an example) in comparison with the non-decomposed revealed six phenolic acids were present in the intact straw including caffeic, cinnamic, ferulic, *p*-coumaric, *o*-coumaric and *p*-hydroxybenzoic acids, with no such phenolics in the decomposed one. This might refer to the use of the putative phenolics by soil microorganisms and other phytotoxic substances could then be released and play their role as new phytotoxic agents. That might give an explanation regarding what we obtained from results, especially with F1 extract. However, the matter needs more research to stand on the fact of such (new) phytotoxic agents.

Microorganisms couldn't then be disregarded as an effective element in affecting allelopathic activities in plants. Between losing activity under soil conditions and effectiveness under fungi decomposition, still many absent facts need to be explored.

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